# STEREOTYPICAL BEHAVIOUR IN THE DEER MOUSE

(*Peromyscus maniculatus bairdii*):

## A pharmacological investigation of the frontal-cortico-striatal serotonergic system

De Wet Wolmarans (B. Pharm.)

POTCHEFSTROOM, SOUTH AFRICA

2011

# Stereotypical behaviour in the deer mouse (*Peromyscus Maniculatus bairdii*): A pharmacological investigation of the frontal-cortico-striatal serotonergic system

De Wet Wolmarans (B. Pharm.)

*Dissertation submitted in partial fulfilment of the requirements for the degree*

**Magister Scientiae**

*in the*

**School of Pharmacy (Pharmacology)**

*at the*

**North-West University (Potchefstroom Campus)**

SUPERVISOR – Prof. Brian H Harvey

CO-SUPERVISOR – Prof. Linda Brand

POTCHEFSTROOM, SOUTH AFRICA

2011

# ABSTRACT

Obsessive-compulsive disorder (OCD) is a psychiatric condition that is characterized by two main symptom cohorts, namely recurrent inappropriate thoughts (obsessions) and seemingly purposeless repetitive motor actions (compulsions). In 70% of cases, the condition only responds to *chronic,* but not sub-chronic, *high dose* treatment with the selective serotonin reuptake inhibitors (SSRIs), such as fluoxetine and escitalopram. This indicates a role for hyposerotonergic functioning in the primary brain areas involved in OCD, namely the components of the cortico-striatal-thalamic-cortical (CSTC) circuit which include the prefrontal cortex, the basal ganglia, and the thalamus. A number of studies have demonstrated a lower serotonin transporter (SERT) availability in OCD patients compared with healthy controls, supporting the hypothesis of a hyposerotonergic state in OCD.

The current study focuses on the validation of the deer mouse *(Peromyscus maniculatus bairdii)* model of OCD and builds on previous work done in our laboratory. Deer mice that are bred and housed in confinement naturally develop two main forms of stereotypical behaviour, namely vertical jumping and pattern running. Furthermore, these behaviours can be categorized into various levels of severity, namely high (HSB), low (LSB) and non-stereotypic (NSB) cohorts. The seemingly purposeless and repetitive nature of these behaviours mimics the compulsions that characterize human OCD and constitutes the basis for the face validity of the model. However, although these two forms of stereotypy seem equally repetitive and persistent, stereotypical pattern runners do not complete the required number of cage revolutions per 30 minutes compared to the amount of jumps executed by stereotypical vertical jumpers. As only one set of criteria for the appraisal of the different topographies of deer mouse stereotypy has been applied in previous studies, the matter of whether pattern runners do in fact generate stereotypical behaviour of the same persistent and severe nature as opposed to the behaviour expressed by vertical jumpers, is problematic.

Therefore, the first objective of the current study was to develop a new classification system for the appraisal of the different forms of behavioural topographies of deer mice and subsequently to evaluate whether pattern runners can indeed be categorized into non-, low- and high stereotypical cohorts. After an eight-week behavioural assessment period, deer mice expressing the two different behavioural topographies could be classified into non-, low- and high stereotypical cohorts (NSB, LSB, and HSB respectively), applying different criteria for each behavioural topography. Based on the weekly mean stereotypy count generated during three 30-minute intervals of highest stereotypical behaviour over the course of a 12-hour assessment

period, HSB pattern runners were found to execute on average 296 cage revolutions per 30 minutes, while HSB vertical jumpers executed an average of 3063 jumps per 30 minutes. This discrepancy between the generated numbers of the different topographies of stereotypy indicates that one classification system for the appraisal of both behavioural topographies is indeed inappropriate, and hence requires re-evaluation and validation.

As patients with OCD present with a lower central SERT availability compared to healthy controls, the second objective of the study was to determine whether a decrease in SERT density could be demonstrated in HSB animals compared to the NSB and LSB controls. After eight weeks of behavioural assessment, animals were sacrificed and frontal-cortical and striatal SERT binding was performed. HSB deer mice presented with significantly lower *striatal*, but not frontal-cortical SERT availability compared to the [NSB/LSB] control animals ($p = 0.0009$). As far as it concerns a lower SERT availability in HSB animals and involvement of the CSTC circuitry, this data is congruent with that demonstrated in human OCD and strengthens the construct validity of the model.

Although previous studies undertaken in our laboratory demonstrated that deer mouse stereotypy is attenuated after chronic (21-day) fluoxetine administration, OCD *only* responds to chronic, *but not sub-chronic* treatment with the SSRIs. The lack of response of deer mouse stereotypy to sub-chronic treatment has not been established and therefore the third study objective was to assess the behavioural effects of sub-chronic (7-day) *and* chronic (28-day) SSRI treatment on expression of deer mouse stereotypy. Chronic, *but not sub-chronic* treatment with oral escitalopram (50 mg/kg/day) significantly increased the number of intervals over a 12-hour assessment period during which no stereotypical behaviour were expressed by HSB deer mice ($p = 0.0241$) and decreased the number of intervals during which high-stereotypical behaviour were executed ($p = 0.0054$). Neither chronic, nor sub-chronic treatment significantly affected the behaviour of animals in the [NSB/LSB] cohort. The fact that the model demonstrates a lack of response to sub-chronic treatment with high dose SSRIs, positively contributes to the predictive validity of the deer mouse model of OCD.

The results from the current study therefore strengthens the construct and predictive validity of the deer mouse model of OCD and confirm the model's status as a prominent animal model of OCD. Not only is hyposerotonergic functioning in the CSTC circuitry implicated in the behaviour of HSB animals, but the model also demonstrates selective response to chronic SSRI-treatment – two core characteristics of human OCD.

**Keywords:** obsessive-compulsive disorder (OCD), deer mouse, behavioural topographies, serotonin transporter (SERT), selective serotonin reuptake inhibitor (SSRI), escitalopram.

# OPSOMMING

Obsessiewe-kompulsiewe siekte (OKS) is 'n psigiatriese toestand wat deur veral twee simptoomkomplekse gekenmerk word, nl. terugkerende, onvanpaste gedagtes (obsessies) en herhalende motoriese bewegings wat op die oog af doelloos voorkom (kompulsies). Die toestand reageer in 70% van gevalle slegs op chroniese (maar nie sub-chroniese), hoë dosis behandeling met die selektiewe serotonien-heropnameremmers (SSHRs), bv. *s*-sitalopram. Dit dui op hiposerotonergiese funksionering in die breinareas wat geassosieer word met die patologie van OKS, nl. die komponente van die kortiko-striatale-talamiese-kortikale (KSTK) bane (insluitend die prefrontale korteks, basale kerne en die talamus). 'n Aantal studies het getoon dat pasiënte met OKS 'n laer serotonien-heropnamereseptor (SHR) beskikbaarheid vertoon, vergeleke met gesonde kontroles, ondersteunend tot die hiposerotonergiese hipotese van OKS.

Die huidige studie handel oor die validering van die *deer*-muis *(Peromyscus maniculatus bairdii)* -model van OKS en bou voort op vorige studies wat in ons laboratorium uitgevoer is. *Deer*-muise wat in aanhouding geteel en gehuisves word, ontwikkel twee vorme van stereotipiese gedrag, nl. vertikale spronge en hardlooppatrone wat as hokomwentelings uitgevoer word. Hierdie gedrag kan volgens ernstigheidsgraad geklassifiseer word, nl. hoë- (HSG), lae- (LSG) en geen- (GSG) stereotipiese gedrag. Die oënskynlike doellose en herhalende wyse van hierdie gedrag, boots die simptome van menslike OKS na en vorm die basis van die model se validering op grond van sigwaarde. Alhoewel albei vorme van stereotipiese gedrag ewe herhalend en aanhoudend uitgedruk word, genereer die diere wat hokomwentelings voltooi, minder stereotipiese tellings per 30 minute, vergeleke met die diere wat vertikaal spring. Gegewe die feit dat slegs een stel kriteria vir die klassifisering van beide tipes stereotipiese gedrag in vorige studies gebruik is, maak dit die klassifisering van diere wat hokomwentelings voltooi problematies omdat dit onseker is of hierdie diere se gedrag van dieselfde ernstigheidsgraad is as dié van diere wat vertikale spronge uitvoer.

Die eerste doelwit van die studie was dus om 'n nuwe klassifiseringsisteem vir die evaluering van die verskillende vorme van *deer*-muis stereotipiese gedrag te ontwikkel en om te bepaal of diere wat hokomwentelings voltooi wel gekategoriseer kan word as GSG, LSG en HSG. Na afloop van 'n agt-weke periode waartydens *deer*-muise se gedrag bestudeer is, kon muise wat onderskeidelik vertikale spronge uitvoer en hokomwentelings voltooi, geklassifiseer word as GSG, LSG en HSG, in ag genome dat twee verskillende stelle kriteria toegepas is vir die beoordeling van die onderskeie tipes stereotipiese gedrag. Data wat gebaseer is op die drie intervalle van 30-minute gedurende die weeklikse 12-uur lange evalueringsperiodes waartydens elke muis die meeste stereotipiese bewegings uitgevoer het, dui daarop dat hoë-stereotipiese diere

wat hokomwentelings voltooi, gemiddeld 296 omwentelings per 30 minute voltooi, teenoor die 3063 gemiddelde aantal spronge per 30 minute wat uitgevoer word deur die hoë-stereotipiese diere wat vertikale spronge uitvoer. Hierdie verskil in die aantal stereotipiese bewegings wat uitgevoer word deur diere wat die onderskeie vorme van gedrag openbaar, dui daarop dat die toepassing van een stel kriteria vir die evaluering van beide tipes gedrag onvoldoende is en daarom herontwerp en hervalideer moes word.

Die feit dat pasiënte met OKS presenteer met 'n laer sentrale SHR-beskikbaarheid vergeleke met gesonde kontroles, het gelei tot die tweede doelwit van die studie, nl. om te bepaal of 'n laer SHR-beskikbaarheid aangetoon kan word in HSG-diere, vergeleke met die GSG- en LSG-kontroles. *Deer*-muise is onthoof en frontale-kortikale en striatale SERT-bindingsdigtheid is bepaal na afloop van 'n agt-weke periode waartydens die gedrag bestudeer is. Daar is bevind dat hoë stereotipiese *deer*-muise met aansienlik minder *striatale,* (maar nie frontale-kortikale) SHR-beskikbaarheid presenteer, vergeleke met die [GSG/LSG]-kontroles ($p$ = 0.0009). Betreffende die laer SHR-bindingsdigtheid en die assosiasie van die KSTK-bane met die gedrag van die HSG muise, stem hierdie data ooreen met wat in pasiënte aangetoon is en word die konstrukte geldigheid van die model dus hierdeur versterk.

Tydens vorige studies wat in ons laboratorium uitgevoer is, is aangetoon dat stereotipiese gedrag wat deur *deer*-muise geopenbaar word, verminder kan word deur die chroniese toediening (21-dae) van fluoksetien. Klinies reageer pasiënte met OKS egter *slegs* op chroniese, *maar nie op sub-chroniese,* behandeling met die SSHRs, 'n verskynsel wat nog nie in die *deer*-muismodel gedemonstreer is nie. Die derde doelwit van die die studie was dus om die effekte van sub-chroniese (7-dae) en chroniese (28-dae) SSHR-behandeling op stereotipiese gedrag te evalueer. Gevolglik kon aangetoon word dat chroniese, *maar nie sub-chroniese*, behandeling met orale *s*-sitalopram (50 mg/kg/dag) gelei het tot 'n aansienlike afname in die aantal intervalle gedurende 'n 12-uur assesseringsperiode waartydens hoë-stereotipiese gedrag deur HSG diere geopenbaar is ($p$ = 0.0054). Verder het die aantal periodes waartydens geen stereotipiese gedrag deur HSG diere geopenbaar is nie, statisties toegeneem ($p$ = 0.0241) na afloop van chroniese, *maar nie sub-chroniese*, behandeling. Nie sub-chroniese of chroniese behandeling het die gedrag van [GSG/LSG] diere beïnvloed nie. Die feit dat die stereotipiese gedrag wat deur *deer*-muise geopenbaar word, *nie verminder na sub-chroniese behandeling* met die SSHRs nie, versterk die voorspellingsgeldigheid van die model.

Die resultate van die huidige studie versterk dus hoofsaaklik die konstrukte en voorspellingsgeldigheid van die *deer*-muis model van OKS en verstewig die model se status as 'n toonaangewende dieremodel vir OKS. Nie net is hiposerotonergiese funksionering in die KSTK-

bane aangetoon nie maar is dit ook aangetoon dat die model slegs reageer op chroniese behandeling met SSHRs – twee kerneienskappe van OKS.

**Sleutelwoorde:** Obsessiewe-kompulsiewe siekte (OKS), *deer*-muis, gedragstopografieë, serotonien-heropnamereseptor (SHR), selektiewe serotonien heropnamereseptor (SSHR), *s*-sitalopram.

# CONGRESS PROCEEDINGS AND PUBLICATIONS

## CONGRESS PROCEEDINGS

ORAL PRESENTATIONS

- *Animal models of obsessive-compulsive disorder: where are we and where are we going?* Presented at the Biological Psychiatry Congress, 28 – 31 May 2009, Arabella Sheraton Hotel, Kleinmond, South Africa. The meeting was held under the auspices of the South African Society of Psychiatrists (SASOP).

- *Current established and putative animal models of obsessive-compulsive disorder: a systematic review.* Presented at the 5th International Conference on Pharmaceutical and Pharmacological Sciences, 23 – 26 September 2009, North-West University Campus, Potchefstroom, South Africa. The meeting was held under the auspices of the South African Society for Basic and Clinical Pharmacology and the Academy of Pharmaceutical Sciences of South Africa.

- *Natural stereotypy in deer mice and its association with frontal-cortical and striatal serotonin transporter (SERT) density: implications for a putative animal model of OCD.* Presented at the 6th International Conference on Pharmaceutical and Pharmacological Sciences, 25 – 27 September 2011, Coastlands on the Ridge, Umhlanga, South Africa. The meeting was held under the auspices of the South African Society for Basic and Clinical Pharmacology and the Academy of Pharmaceutical Sciences of South Africa.

## PUBLICATIONS

- Güldenpfennig, M., Wolmarans, D., Du Preez, J.L., Stein, DJ., Harvey, B.H. 2011. Cortico-striatal oxidative status, dopamine turnover and relation with stereotypy in the deer mouse. *Physiology and Behaviour*, 103:404-411

# ACKNOWLEDGEMENTS


- PROF. BRIAN H HARVEY – *You have laid me the strongest foundations any young researcher can dream of, while your endless encouragement and patience carried me through this project. I have learned so much from you and I hope that the person and researcher growing up under your hands will one day make you proud.*

- PROF. LINDA BRAND – *You are, and always will be my greatest inspiration. Thank you for making the academia, and more specifically Pharmacology my passion, my fun and the second greatest love of my life. If I were to be Harry Potter, you would be my Professor McGonagall.*

- MRS. ANTOINETTE FICK – *Without your guidance on animal behaviour and your endless efforts in maintaining the deer mouse colony at the Animal Research Centre of the North-West University, this study would not have been possible. I appreciate your inputs immensely.*

- MISS. SHARLENE LOWE – *Sharlene, no one could have asked for a better laboratory assistant. Your company in the lab and thorough knowledge of the technicalities of receptor binding studies remains to this date invaluable to me.*

- MY WIFE ANSA, AND TWO GORGEOUS CHILDREN, EWAN AND ALKE – *You are and always will be the greatest loves of my life. The encouragement, love and understanding you gave me, made my studies possible. I will never be able to repay you for what you meant to me during the past four years. Without you, my world is not a world indeed. Without you, I am nothing.*

- MY PARENTS, CORRIE AND HANNEKE WOLMARANS – *How can I say thank you for 30 years of love? How can I thank you for making me the man I am today? How can one ever appreciate enough how much a parent means to his child? I will love you for as long as I live.*

- MY LOVING SISTER, DEIDRÉ – *Boeta will never forget our time together in mom and dad's home. Your voice and laughs warm my heart every time I hear it. Your energy and perseverance are two beacons that guide me in the sometimes-stark dark night.*

- MY PARENTS IN LAW, FREDDIE AND JEANETTE KEMP – *Mom and dad, thank you for always being there and taking care of Ansa and the children during all those weekends they visited so that I could finish what I started four years ago. This is as much your study as it is mine.*

- MY DEAREST FRIENDS, SAREL AND HENK – *You made my time as a Masters student full of stories, music, humour, news and debate. Your company kept me awake through some of the longest days and nights of my life and your friendship provided me with the endless wind blowing through my often-placid sails. To make one best friend is difficult. To make two, is nearly impossible. I have had the opportunity to conquer the impossible. I will be ever thankful for that.*

- RONEL – *Nella, my other best friend, and colleague in the private sector, I love you so much. Thank you for being strong and showing me how much one can appreciate so little.*

- MY FELLOW MASTERS AND DOCTORAL STUDENTS AT PHARMACOLOGY: SAREL, STEPHAN, MADELEIN, MARISA, AND LYLLY – *I thoroughly enjoyed the collaborative fun we shared. You are the best partners in crime anyone can ask for.*

- MY COLLEAGUES AT PHARMACOLOGY: LINDA, BRIAN, TIAAN, MICHELLE, MALIE, RINA, LIANA, AND DAAN – *Always know that I love this subject as a direct result of the effort that you put into teaching it to me eight years ago. It is a privilege to work side by side with such colossal figures in Pharmacology. I will never stop to learn from you.*

* * *

***I dedicate this project to all the parents who completed their degrees before me while balancing their academic responsibilities and the love for their families during the endless months and years it took to complete their studies. When the going got tough, the thought of you inspired me. It is thanks to you that I was never alone.***

# TABLE OF CONTENTS

**CHAPTER 1 – INTRODUCTION** ..... **1**

**1.1 Problem statement** ..... **1**

**1.2 Study questions** ..... **4**

**1.3 Project aims** ..... **4**

**1.4 Project layout** ..... **5**

**1.5 Predicted outcomes** ..... **6**

**CHAPTER 2 – LITERATURE REVIEW** ..... **7**

**2.1 OCD in the clinical environment** ..... **7**

2.1.1 The classification and diagnosis of OCD ..... 7

2.1.2 The symptoms of OCD and its comorbidity with other conditions ..... 8

2.1.3 The treatment of OCD ..... 10

**2.2 The neurobiology of OCD – Boulder hopping across unknown waters** ..... **12**

2.2.1 The neurocircuitry of OCD ..... 13

*2.2.1.1 An explanation of the cortico-striatal-thalamic-cortical (CSTC) pathway ..... 13*

*2.2.1.2 A proposed dysfunction in the CSTC circuitry in patients with OCD ..... 17*

2.2.2 The neurotransmission of OCD ..... 18

*2.2.2.1 Glutamate and excitatory signalling ..... 18*

*2.2.2.2 GABA and inhibitory signalling ..... 21*

*2.2.2.3 Dopamine and the differential regulation of the direct and indirect pathways ..... 23*

*2.2.2.4 Serotonin and the serotonergic system – a balancing act par excellence ..... 29*

*2.2.2.5 Miscellaneous neurotransmitters and biological agents in the pathology of OCD .. 39*

**2.3 Designing animal models of OCD – A constant confrontation with thoughtful repetition ..... 41**

2.3.1 Repetitive behaviour – corner stone for establishing face validity for OCD ..... 41

*2.3.1.1 Stereotypy in humans – a common symptom of many comorbid conditions ..... 42*

*2.3.1.2 Stereotypy in animals – normal biology or abnormal pathology ..... 44*

2.3.2 A favourable response to SSRIs – the mainstay of predictive validity ..... 46

2.3.3 The construct of OCD ..... 46

2.3.4 Current animal models of OCD ..... 47

*2.3.4.1 Animal models based on the natural development of stereotypy ..... 48*

*2.3.4.2 Animal models based on pharmacological or genetic manipulation ..... 50*

*2.3.4.3 An animal model based on behavioural training ..... 51*

**2.4 A review of spontaneous stereotypy in the deer mouse – 1999 – 2011 ..... 53**

2.4.1 A timeline of major developments in the appraisal of deer mouse stereotypy ..... 53

2.4.2 The current validation status of the deer mouse model of OCD ..... 55

**CHAPTER 3 – APPRAISING DEER MOUSE STEREOTYPY AS AN ANIMAL MODEL OF OCD ..... 61**

**3.1 The reappraisal of past methods ..... 61**

3.1.1 How was deer mouse stereotypy assessed in the past? ..... 61

3.1.2 The influence of the different topographies on the classification of stereotypy ..... 62

3.1.3 12-hour assessments influence stereotypy classification ..... 63

**3.2 A review of study objectives ..... 68**

**CHAPTER 4 – THE METHODOLOGICAL BASIS OF THE CURRENT STUDY ..... 70**

**4.1 The study outline ..... 70**

**4.2 Experimental materials and procedures ..... 72**

4.2.1 Animals ..... 72

4.2.2. Drug used and administration ..... 73

4.2.3 Assessing the behavioural topographies of deer mice ..... 75

*4.2.3.1 Generating the behavioural data ..... 75*

*4.2.3.2 Analyzing the behavioural data ..... 79*

4.2.4 Determination of frontal-cortical and striatal SERT density ..... 83

*4.2.4.1 Chemicals and equipment used to determine SERT density ..... 83*

*4.2.4.2 Methodology for the determination of SERT density ..... 84*

**CHAPTER 5 – RESULTS ..... 88**

**5.1 Study Objective I – The development of a new classification system for the appraisal of deer mouse stereotypy ..... 88**

**5.2 Study Objective II – An investigation into frontal-cortical and striatal SERT densities of treatment naive [NSB/LSB] and HSB deer mice ..... 93**

5.2.1 Frontal-cortical and striatal SERT densities in [NSB/LSB] animals ..... 93

5.2.2 Frontal-cortical and striatal SERT densities in HSB animals ..... 94

5.2.3 Comparing frontal-cortical SERT densities of [NSB/LSB] and HSB animals ..... 95

5.2.4 Comparing striatal SERT densities of [NSB/LSB] and HSB animals ..... 96

**5.3 Study Objective III – The effect of sub-chronic and chronic oral escitalopram treatment on deer mouse stereotypy ..... 97**

5.3.1 The effect of escitalopram on stereotypy generated ..... 98

*5.3.1.1 Weekly manifestation of vertical jumping (VBI) expressed by [NSB/LSB] and HSB animals, and response to 1 or 4 weeks of escitalopram treatment ..... 98*

*5.3.1.2 Average VBI in [NSB/LSB] and HSB cohorts before and after sub-chronic (day 28-35) and chronic (day 28-56) escitalopram treatment ..... 99*

*5.3.1.3 Weekly manifestation of the pattern running (HR) executed by [NSB/LSB] and HSB animals, and response to 1 or 4 weeks of escitalopram treatment ..... 100*

*5.3.1.4 Average HR in [NSB/LSB] and HSB cohorts before and after sub-chronic (day 28-35) and chronic (day 28-56) escitalopram treatment ..... 101*

5.3.2 Effect of escitalopram on the weekly amount of rest periods and HSB intervals over the course of 12 hours ..... 103

*5.3.2.1 The average occurrence of rest periods observed in the behaviour of animals from the [NSB/LSB] and HSB cohorts, and response to sub-chronic (day 28-35) and chronic (day 28-56) escitalopram treatment ..... 106*

*5.3.2.2 The average occurrence of intervals of HSB activity observed in animals from the [NSB/LSB] and HSB cohorts, and response to sub-chronic (day 28-35) and chronic (day 28-56) escitalopram treatment ..... 108*

5.3.3 General locomotor activity of [NSB/LSB] and HSB deer mice, and response to sub-chronic and chronic escitalopram treatment ..... 111

**CHAPTER 6 – DISCUSSION ..... 114**

**6.1 Introduction ..... 114**

**6.2 Study Objective I – The development of a new classification system for the appraisal of deer mouse stereotypy ..... 117**

**6.3 Study Objective II – An investigation into frontal-cortical and striatal SERT densities of treatment naive [NSB/LSB] and HSB deer mice ..... 119**

**6.4 Study Objective III – The effect of sub-chronic and chronic oral escitalopram treatment on deer mouse stereotypy ..... 124**

**CHAPTER 7 – CONCLUSION AND FUTURE STUDY QUESTIONS ..... 127**

**REFERENCES** ..... **130**

**ADDENDUM A** ..... **157**

**ADDENDUM B** ..... **176**

**ADDENDUM C** ..... **179**

**ADDENDUM D** ..... **182**

# LIST OF FIGURES

**CHAPTER 2**

Figure 2-1 – The CSTC circuit implicated in OCD ..... 15

**CHAPTER 3**

Figure 3-1 – An example of the quantitative manifestation of pattern running and the chronological variation of the behavioural topographies expressed in deer mice ..... 67

**CHAPTER 4**

Figure 4-1 – A schematic representation of the study outline ..... 71

Figure 4-2 – The time course of the study ..... 71

Figure 4-3 – The Fusion® hardware setup ..... 76

Figure 4-4 – The Fusion® interface during the recording of behaviour ..... 77

Figure 4-5 – An excerpt from The Microsoft® Excel® data sheet exported following the completion of each behavioural screen ..... 78

Figure 4-6 – An excerpt from the summary of the weekly averages of stereotypical behaviour and locomotor activity of the placebo group ..... 82

Figure 4-7 – A graphical representation of a saturation binding assay ..... 85

**CHAPTER 5**

Figure 5-1 – Weekly mean numbers of vertical beam interruptions (VBI) generated during a period of 30 minutes by treatment naive [NSB/LSB] animals expressing vertical stereotypy compared to HSB animals ..... 89

Figure 5-2 – Weekly mean numbers of cage revolutions (HR) executed during a period of 30 minutes by treatment naive [NSB/LSB] animals expressing pattern running compared to HSB animals ..... 90

Figure 5-3 – Frontal-cortical and striatal SERT densities in treatment naive [NSB/LSB] animals ..... 94

Figure 5-4 – Frontal-cortical and striatal SERT densities in treatment naive HSB animals ..... 95

Figure 5-5 – A comparison between frontal-cortical SERT densities of [NSB/LSB] and HSB animals ..... 95

Figure 5-6 – A comparison between striatal SERT densities of [NSB/LSB] and HSB animals ..... 96

Figure 5-7 – Weekly manifestation of vertical jumping (VBI) evident in [NSB/LSB] and HSB cohorts, and response to escitalopram treatment ..... 98

Figure 5-8 – Average VBI generated by animals of the [NSB/LSB] cohort before and after escitalopram treatment ..... 99

Figure 5-9 – Average VBI generated by animals of the HSB cohort, before and after escitalopram treatment ..... 100

Figure 5-10 – Weekly manifestation of pattern running (HR) evident in [NSB/LSB] and HSB cohorts, and response to escitalopram treatment ..... 101

Figure 5-11 – Average HR generated by animals of the [NSB/LSB] cohort before and after escitalopram treatment ..... 102

Figure 5-12 – Average HR generated by animals of the HSB cohort, before and after escitalopram treatment ..... 102

Figure 5-13 – An excerpt from the data sheet used to calculate the weekly amounts of rest periods and intervals of HSB pattern running activity observed in the data generated by individual animals over the course of eight weeks ..... 105

Figure 5-14 – The average weekly occurrence of rest periods observed in the behaviour of animals from the [NSB/LSB] and HSB cohorts, and response to sub-chronic or chronic escitalopram treatment ..... 106

Figure 5-15 – The average occurrence of rest periods observed in the behaviour of animals from the [NSB/LSB] cohort, and response to sub-chronic or chronic escitalopram treatment ..... 107

Figure 5-16 – The average occurrence of rest periods observed in the behaviour of animals from the HSB cohort, and response to sub-chronic or chronic escitalopram treatment ..... 107

Figure 5-17 – The average weekly occurrence of intervals of HSB activity observed in the behaviour of animals from the [NSB/LSB] and HSB cohorts, and response to sub-chronic or chronic escitalopram treatment.....108

Figure 5-18 – The average occurrence of intervals of HSB activity in the behaviour of animals from the [NSB/LSB] cohort, and response to sub-chronic or chronic escitalopram treatment.109

Figure 5-19 –The average occurrence of intervals of HSB activity observed in the behaviour of animals from the HSB cohort, and response to sub-chronic or chronic escitalopram treatment .....110

Figure 5-20 – The average weekly locomotor activity of [NSB/LSB] and HSB deer mice, and response to sub-chronic or chronic escitalopram treatment.....111

Figure 5-21 – The average amounts of horizontal movements generated by animals of the [NSB/LSB] cohort, and response to sub-chronic or chronic escitalopram treatment.....112

Figure 5-22 – The average amounts of horizontal movements generated by animals of the HSB cohort, and response to sub-chronic or chronic escitalopram treatment.....112

# LIST OF TABLES

**CHAPTER 2**

Table 2-1 – Common obsessions and compulsions in patients diagnosed with OCD ..... 8

**CHAPTER 3**

Table 3-1 – The newly defined cut-off values for each cohort as a function of the topography expressed ..... 66

Table 3-2 – A synopsis of the study objectives and rationale ..... 68

**CHAPTER 4**

Table 4-1 – Chemicals and equipment used in determining SERT binding density ..... 83

**CHAPTER 5**

Table 5-1 – The newly defined cut-off values for each cohort as a function of the topography expressed ..... 88

Table 5-2 – The number of animals in each experimental group developing stereotypy ..... 89

Table 5-3 – Weekly differences between the mean numbers of VBI generated by animals classified as [NSB/LSB] and HSB, respectively ..... 90

Table 5-4 – Weekly differences between the mean numbers of HR executed by animals classified as [NSB/LSB] and HSB, respectively ..... 91

Table 5-5 – The weekly mean amounts of VBI generated, and HR executed by HSB vertical jumpers and pattern runners, respectively ..... 91

# CHAPTER 1
# INTRODUCTION

## 1.1. PROBLEM STATEMENT

Animal models of human psychiatric conditions are pivotal instruments that aid in elucidating the neurobiological mechanisms underlying human disorders as well as provide a suitable framework for the development and pre-clinical evaluation of new treatment strategies. The current study follows on previous work undertaken in our laboratory (Güldenpfennig *et al.*, 2011; Korff *et al.*, 2008; Korff *et al.*, 2009) and concerns the validation of spontaneous stereotypy in the deer mouse *(Peromyscus maniculatus bairdii)* as an animal model of obsessive-compulsive disorder (OCD).

In most patients, OCD is characterized by two main symptoms, namely recurrent and intrusive thoughts (obsessions) and rigid repetition of certain motor actions (compulsions) (American Psychiatric Association, 2000). Although OCD is currently classified by the American Psychiatric Association as an anxiety disorder, the prevalence of different OCD endophenotypes has sparked much debate as to whether or not OCD is indeed an anxiety disorder (Bartz and Hollander, 2006; Nestadt *et al.*, 2001; Stein, 2002; Tynes *et al.*, 1990). The DSM-IV clearly stipulates that OCD can be diagnosed in a patient without the presence of obsessive and intrusive thoughts (American Psychiatric Association, 2000). Furthermore, traditional anxiolytics such as the benzodiazepine class of drugs are ineffective in the clinical management of OCD (El Mansari and Blier, 2006; Erzegovesi *et al.*, 2005; Fineberg and Craig, 2007). The fact that OCD can be diagnosed without obsessions being present, has important implications for the development of animal models of OCD, as cognitive disturbances such as obsessions are difficult to demonstrate in animals. OCD also demonstrates high comorbidity with a group of conditions collectively known as the obsessive-compulsive spectrum disorders (Bartz and Hollander, 2006; Nestadt *et al.*, 2001), which includes trichotillomania, compulsive gambling, anorexia and body dysmorphic disorder, none of which responds to traditional anxiolytics.

However, in 70% of cases OCD responds preferentially to high dose, chronic treatment with the selective serotonin reuptake inhibitors (SSRIs) (Blier *et al.*, 1996; El Mansari and Blier, 2006; Fineberg and Craig, 2007). This evidence implicates a role for hyposerotonergic signalling in the brain areas associated with the pathology of OCD, namely the prefrontal cortex (most notably the orbitofrontal and anterior cingulate cortices), the basal ganglia and the thalamus

(Evans *et al.*, 2004; Husted *et al.*, 2006; Maia *et al.*, 2008; Markarian *et al.*, 2010). In fact, a number of studies have demonstrated that patients with OCD present with hyposerotonergic signalling (Delgado and Moreno, 1998; Goddard *et al.*, 2008) and that a decreased availability of serotonin transporters (SERT) is associated with increased symptom severity (Hesse *et al.*, 2005; Reimold *et al.*, 2007; Zitterl *et al.*, 2008). Nevertheless, 30% of OCD patients remain refractive to treatment with SSRIs as monotherapy, in which case augmentation strategies with especially low dose antipsychotics may be followed (El Mansari and Blier, 2006; Erzegovesi *et al.*, 2005; Fineberg and Craig, 2007). These latter drugs act by blocking the $D_2$ receptors in the basal ganglia, located on neuronal pathways responsible for the activation of the cortex via the thalamus (Brown *et al.*, 2006; Denys *et al.*, 2004c; Kempf *et al.*, 2007). Antagonizing these receptors with low dose antipsychotics in combination with serotonin reuptake inhibition has been demonstrated to be effective in most patients that remain refractory to treatment with monotherapy SSRIs (Fineberg and Craig, 2007), thus supporting a dual role for serotonin and dopamine in the neuropathology and treatment of OCD.

Suitable animal models of OCD are necessary to understand the complex neurobiological mechanisms underlying obsessive-compulsive behaviour. That obsessions may play as prominent a role as compulsive-like repetition in the symptomology of OCD, complicates the development of animal models since cognitive abnormalities such as recurrent thoughts and obsessions are impossible to demonstrate in animals. However, by associating compulsive-like repetition of certain motor actions in animals with the fundamental constructs of OCD, certain conclusions can be made that may have direct relevance to the human disorder. Thus, by targeting altered serotonergic and dopaminergic signalling, involvement of the prefrontal-cortex and basal ganglia as well as a favourable response to chronic, but not sub-chronic high dose SSRIs, certain repetitive behaviours in an animal can be distinguished from such behaviours without a confounding cognitive association (Barnard *et al.*, 2002; Langen *et al.*, 2011a; Makki *et al.*, 2008; Rasmussen *et al.*, 1994). This allows the model to distinguish OCD-like behaviour from other illnesses such as autism, Tourette's syndrome and Parkinson's disease.

Much has already been done to validate spontaneous stereotypy in the deer mouse as an animal model for OCD (Güldenpfennig *et al.*, 2011; Korff *et al.*, 2008; Korff *et al.*, 2009). In short, deer mouse stereotypy can be categorized into two main behavioural topographies, namely repetitive vertical jumping and pattern running. These behaviours mimic the rigid repetitive motor actions observed in human OCD and form the basis for the face validity of the model. Korff and colleagues (2008) demonstrated that chronic (21-day) intraperitoneal treatment with 10 and 20 mg/kg/day fluoxetine significantly decreased the expression of spontaneous stereotypy in stereotypical deer mice, a finding that provided the first evidence for the predictive validity of

the model. Furthermore, the authors later presented evidence for an increase in cyclic adenosine monophospate (cAMP) and a decrease in phospodiesterase-4 ($PDE_4$) expression in stereotypical animals as opposed to non-stereotypical animals. Since the SSRIs are known to exert adaptive changes in this second messenger system via indirect actions on serotonin (5-hydroxytryptamine; 5HT) $1_{A/B/D}$ and $5HT_{2C}$ receptors (Barnes and Sharp, 1999; Bergqvist *et al.*, 1999), this observation has important implications for the construct validity of the model.

The fact that chronic treatment with fluoxetine attenuates spontaneous stereotypy in the deer mouse confirms that an altered serotonergic system underscores the expression of stereotypic behaviour in this model. However, this observation must also be considered in the light that OCD does not respond to acute or sub-chronic treatment with high dose SSRIs. The latter finding is a critical observation that is typical of the SSRI response in OCD and that needs to be demonstrated in deer mice. Furthermore, it needs to be established whether there are any differences in the baseline expression of SERT between non-stereotypical controls and high stereotypical animals, as is the finding in healthy human controls compared to patients with OCD (Hesse *et al.*, 2005; Reimold *et al.*, 2007; Zitterl *et al.*, 2008). Such an observation would significantly strengthen the construct validity of the deer mouse model of OCD. Recent evidence from our laboratory (Güldenpfennig *et al.*, 2011; Korff *et al.*, 2008; Korff *et al.*, 2009) has shown that stereotypical pattern runners are almost always excluded from the high stereotypical cohort. These animals do not execute the required number of cage revolutions (the main behavioural manifestation of pattern running) needed to be classified as high stereotypical animals, evaluated using the current classification criteria (for a full discussion on the classification criteria of deer mice stereotypy, refer to Chapter 3, paragraph 3.1.1). Since the expression of pattern running seems just as rigid and repetitive as vertical jumping, the question arises whether the current behavioural classification system is appropriate to accommodate and quantify both behavioural topographies. In fact, it is possible that the inadvertent inclusion of stereotypical pattern runners in the non- or low-stereotypical cohorts instead of the high stereotypical cohort can complicate the analysis of neurochemical data resulting from the inappropriate classification of these animals.

## 1.2. STUDY QUESTIONS

Following from the introduction, the following questions will be addressed in the current study:

a) Given that stereotypical pattern runners are almost never included in the high stereotypical (HSB) cohort, can the current behavioural classification system described by Korff and colleagues (2008) be adapted and improved to more reproducibly and accurately appraise both vertical jumping and pattern running as behavioural topographies expressed by deer mice?

b) Taking its cue from human OCD, can the stereotypical behaviour of deer mice classified as HSB be associated with altered serotonergic signalling as depicted by a decrease in SERT availability compared to their non-stereotypical and low-stereotypical ([NSB/LSB]) controls? Moreover, as in OCD, will any such evidence have a dependency on brain regions of the cortico-striatal thalamic-cortical circuit?

c) If such a difference in SERT density is evident, will HSB deer mice respond to an increase in serotonergic signalling following chronic, *but not sub-chronic*, treatment with high dose escitalopram, a known antagonist of SERT (Owens *et al.*, 2001), as has been demonstrated in humans with OCD?

## 1.3. PROJECT AIMS

To address the study questions laid out in section 1.2, this project will aim to:

- Develop a new classification system for the appraisal of deer mouse stereotypy and investigate whether a clear distinction can be made between stereotypical and non-stereotypical pattern runners.
- Determine the baseline frontal-cortical and striatal SERT density in treatment naive HSB animals and compare these values to animals assigned to the [NSB/LSB] cohort and determine whether high and low stereotypic animals can be differentiated by regional brain differences in SERT binding in the frontal cortex and striatum.
- Assess whether chronic, *but not sub-chronic* treatment with high dose oral escitalopram (50 mg/kg/day) will attenuate deer mouse stereotypy.

## 1.4. PROJECT LAYOUT

The current project will be divided into two sections:

- CONTROL (DRUG-NAIVE) STUDY

Forty deer mice will be studied for eight weeks in order to develop a new classification system for the appraisal of deer mouse stereotypy, as well as to determine frontal-cortical and striatal SERT density in animals from the [NSB/LSB] and HSB cohorts.

- DRUG TREATMENT STUDY

Forty deer mice will be used to determine the effects of sub-chronic (1 week) and chronic treatment (4 weeks) with high dose (50 mg/kg/day) oral escitalopram on the expression of stereotypy by deer mice. The introduction of escitalopram to the drinking water of deer mice will be preceded by four weeks of baseline behavioural assessments to determine the pre-treatment stereotypy score for each animal.

* * *

Animals in both studies will be assessed for stereotypical and locomotor behaviour on a weekly basis for eight weeks. Every behavioural assessment will be performed over a period of 12 hours during the dark cycle of the animals (18:00 – 06:00). After the 8-week behavioural assessment period, animals from the control (drug naive) study will be sacrificed, their behaviour scored and analysed by computer-aided means and the frontal-cortical and striatal SERT densities of the respective cohorts determined. The behaviour of animals in the drug treatment study will be evaluated following the eight weeks of behavioural assessment and the effects of sub-chronic and chronic high dose (50 mg/kg/day) escitalopram on the expression of stereotypy determined.

* * *

Different addenda (A – D) are included at the end of the dissertation and contain examples of the behavioural data generated by deer mice (Addendum A) and the results from pilot studies performed during the course of the main study. The aims of these pilot studies were to assess the normal drinking behaviour of deer mice (Addendum B) and to determine whether the administration of escitalopram in the drinking water alters this behaviour (Addendum C). In Addendum D, a dose-response analysis was also performed in order to establish the appropriate dose of escitalopram for application in the drug treatment study.

## 1.5. PREDICTED OUTCOMES

It is hypothesized that although stereotypical pattern runners do not execute the number of cage revolutions per 30 minutes than the number of jumps executed by stereotypical vertical jumpers, pattern runners can be separated into non-, low- and high stereotypical cohorts applying newly developed classification criteria for the appraisal of deer mouse stereotypy. Furthermore, it is hypothesized that HSB animals will present with significantly lower frontal-cortical and striatal SERT densities compared to animals of the [NSB/LSB] cohort. Chronic (4-week), but not sub-chronic (1-week) treatment with high dose (50 mg/kg/day) oral escitalopram will significantly attenuate the expression of stereotypy in HSB animals, while the behaviour of animals in the [NSB/LSB] groups will remain unchanged.

* * *

In Chapter 2, a thorough review of the current literature on clinical OCD and its neurobiology will be presented, as well as an overview of current animals models for OCD. Chapter 3 will present the methodology that has been developed during prior studies in our laboratory for the appraisal and scoring of deer mouse stereotypy, as well as the manner in which this method of assessment has been adapted for application in the current study.

*END OF CHAPTER 1*

# CHAPTER 2
# LITERATURE REVIEW

## 2.1. OCD IN THE CLINICAL ENVIRONMENT

### *2.1.1. The classification and diagnosis of OCD*

By historical definition OCD is a debilitating psychiatric condition characterized by intrusive and disturbing thoughts (obsessions) leading to mounting anxiety which manifests in repetitive stereotypical behaviour with its only purpose being to relieve the anxiety caused by the obsession (Stein, 2002). Strictly, according to this definition, a patient has to present with both obsessions and compulsions before OCD can be diagnosed, although as explained later, this diagnostic criterion has changed over the past three decades (American Psychiatric Association, 2000). OCD has a lifetime prevalence of between 2.5% and 3% in the general population, making it the fourth most common psychiatric disorder (Pittenger *et al.*, 2006).

OCD is currently classified as an anxiety disorder by the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV), mainly due to the apparent role anxiety plays in the pathogenesis of the condition (American Psychiatric Association, 2000; Tynes *et al.*, 1990). Whether it is appropriate to categorize OCD with anxiety disorders such as phobias, post-traumatic stress disorder (PTSD), and generalized anxiety disorder, is a question that has been much debated during the past two decades (Bartz and Hollander, 2006; Stein *et al.*, 2002). This debate had its origin partly in the realization that obsessions do not necessarily always translate into anxiety and that certain compulsions are in many cases not a direct consequence of either obsessions or the anxiety caused by a certain obsession. Indeed, certain symptoms of OCD share some characteristics with other conditions grouped under the obsessive-compulsive (OC) spectrum of disorders – conditions without anxiety as a pivotal diagnostic criterion (Bartz and Hollander, 2006).

Interestingly, the DSM-IV stated criteria for diagnosing OCD clearly state that either obsessions or compulsions, or a combination of both, may justify the diagnosis of OCD. Although patients can be diagnosed with OCD, irrespective of whether obsessions and compulsions, or only one of the two, are present, the DSM-IV sets certain restrictions to the criteria for diagnosing obsessions and compulsions and eventually OCD. Examples of such restrictions are that the patient must realize that the obsessions or compulsions are senseless or unreasonable, that the obsessions and compulsions must be time consuming and impair the normal day to day functionality of the patient, and that the obsessions or compulsions cannot be attributed to any

other mental or physical condition, or be the direct or indirect consequence of drug usage or abuse (American Psychiatric Association, 2000).

Although more that 95% of OCD patients report both obsessions and compulsions (Foa and Kozak, 1995; Goodman *et al.*, 1989), the fact that only obsessions or compulsions can be present in a patient with OCD, changes the general assumption that anxiety always plays a central role in the pathogenesis of the condition. Compulsions that manifest without the patient expressing either obsessions or anxiety can now be diagnosed as OCD. This diagnostic separation between the two symptoms has important implications with respect to modelling the condition in animals, as imitating the obsessive symptoms of the condition in putative animal models has proven to be especially problematic.

As will be explained in paragraph 2.1.2, OCD is a condition that presents itself in different forms and subtypes with symptoms representing a number of other conditions, from anxiety related psychiatric conditions to impulse-control disorders. Thus, demonstrating the comorbidity of OCD with other psychiatric and motor conditions may aid in the better understanding of the etiology and pathophysiology of the condition and ultimately the improvement of current treatment strategies for patients diagnosed with OCD (Bartz and Hollander, 2006).

### *2.1.2. The symptoms of OCD and its comorbidity with other conditions*

Markarian and colleagues (2010) conclude that at least five main subtypes of OCD can be identified. These are highlighted in the Table 2-1:

| Obsessions | Compulsions |
|---|---|
| Concerns about contamination | Excessive washing |
| Concerns about harming oneself or others | Checking and praying |
| Concerns about symmetry and order | Ordering, arranging and counting |
| Obsessions only *(mainly of sexual, religious or aggressive nature)* | No compulsions prevalent |
| Concerns about waste | Collecting and hoarding |

***TABLE 2-1 – COMMON OBSESSIONS AND COMPULSIONS IN PATIENTS DIAGNOSED WITH OCD***

Of the above five subtypes, the most prevalent obsessions are concerns about contamination (55%), followed by inappropriate aggressive and sexual thoughts (50% and 32% respectively), and concerns about symmetry and order (36%). The most common compulsions are ritualistic checking (80%), cleaning and decontamination rituals (46%) and counting (21%) (Abramowitz *et al.*, 2003). From these statistics, it is also evident that many patients present with symptoms

that span across the different subtypes of OCD, a fact that further complicates the diagnosis of the condition.

In a systematic review by Husted and Shapira (2006), the authors investigated the possible role of disgust in OCD. Disgust normally involves the evaluation of objects and events for their possible role in contamination. Normal individuals have the ability to discount any fears of contamination if it remains below a certain level (Husted *et al.*, 2006), and thus the conclusion could be made that the normal process of fear of contamination and its subsequent extinction may be dysfunctional in patients with OCD. In addition, it could likely be concluded that OCD patients expressing concerns about contamination and subsequently engage in washing rituals, may express a lower threshold for experiencing disgust and fail to perceive a decline in the contagiousness of a contaminated object, hence the expression of washing rituals. Since the earliest definitions that suggested disgust to be the expression of revulsion against taste and other sensory stimuli (Darwin, 1965), the definition has subsequently evolved to include revulsion at the oral incorporation of contaminants (Rozin and Fallon, 1987), as well as disgust arising from abstract concerns like personal appearance, religion, and sexual thought (Rozin *et al.*, 1999). The authors also concluded that the same neurocircuitry implicated in OCD, mainly the cortico-striatal-thalamic-cortical (CSTC) circuit, is also activated in the response to disgust, providing further evidence for a possible role of disgust in the etiology of OCD.

When reviewing the comorbidity of OCD with other mood and anxiety disorders, the general finding is that patients diagnosed with OCD exhibit a higher prevalence rate for major depressive disorder (MDD), social phobia, panic disorder, agoraphobia and generalized anxiety disorder, compared to the general population (LaSalle *et al.*, 2004; Nestadt *et al.*, 2001). Interestingly though, Denys and co-workers (2004b) found that with respect to MDD, OCD typically precedes depression, a finding that suggests that depression does not have an etiological relationship with OCD, but rather results from OCD. Whether or not the same relationship exists between OCD and anxiety disorders, is still not clear (Denys *et al.*, 2004b). Furthermore, it is especially interesting to note at this stage that OCD responds exclusively to drugs that potently inhibit the synaptic reuptake of serotonin, such as the selective serotonin reuptake inhibitors (SSRIs), also the first line drug choice for patients with MDD. However, the traditional anxiolytics, for instance the benzodiazepines, have no clinical effect in patients with OCD, nor do drugs that target the noradrenergic system (Fineberg and Craig, 2007). Consequently, demonstrating comorbidity of OCD with anxiety disorders may not necessarily be an indication that OCD is an anxiety disorder, but simply that patients with OCD are more prone to develop other anxiety disorders, compared to the general population. Indeed, that anxiety is often a co-morbid symptom in patients with MDD re-emphasizes this fact.

As alluded to earlier, OCD shares some characteristics with a cluster of conditions called the obsessive-compulsive (OC) spectrum of disorders (Bartz and Hollander, 2006). Although these conditions cannot be classified as OCD, they present with a distinctly similar range of characteristics that are also found in OCD. Obsessive thinking or compulsive behaviour, though somewhat different in presentation to the typical phenomenology of OCD, is also central to the nature of these conditions. The OC spectrum of disorders can be classified into three main clusters (Bartz and Hollander, 2006): 1) body image/body sensitization/body weight concern disorders; 2) impulse control disorders; and 3) neurological disorders with repetitive behaviours. Like in OCD, the first cluster of disorders are characterized by intense intrusive and anxiety provoking thoughts and include conditions like bulimia nervosa, anorexia and hypochondriasis. The second cluster is characterized by impulsivity, such as compulsive gambling, but unlike in OCD the compulsive behaviour is associated with pleasure. The third cluster includes syndromes like Tourette's syndrome and autism and can be classified as pure neurological disturbances that present with, among others, stereotypical motor behaviour. Generally, patients primarily diagnosed with OCD also have higher lifetime prevalence rates for OC-spectrum disorders compared to the general population (Denys *et al.*, 2004b; du Toit *et al.*, 2001). Moreover, patients primarily diagnosed with an OC-spectrum disorder also have higher prevalence rates for OCD, a relationship that is not consistently shown in comorbidity studies of OCD and mood and anxiety disorders (Bartz and Hollander, 2006; Gunstad and Phillips, 2003; Thornton and Russell, 1997).

### *2.1.3. The treatment of OCD*

It has been widely demonstrated that OCD responds best to drugs that selectively targets serotonergic, but not noradrenergic neurotransmission, especially in the frontal cortex, striatum and thalamus (Fineberg and Craig, 2007; Grados and Riddle, 2001; Jenike, 1993; Stein, 2002; Vythilingum *et al.*, 2000). Clomipramine, a tricyclic antidepressant (TAD) that is particularly effective in inhibiting the presynaptic reuptake of serotonin, was the first drug shown to be consistently effective in the treatment of OCD. This is in direct contrast with desipramine, a TAD mainly inhibiting the presynaptic reuptake of noradrenalin, which has no demonstrable clinical efficacy in OCD (Fineberg and Craig, 2007). However, the development of the SSRIs was an important advance in the treatment of OCD, as these drugs have a better safety and tolerability profile than clomipramine. To date, however, no study has been able to present proof that SSRIs have greater therapeutic effect in treating OCD than clomipramine, although the lack of serious side-effects with the SSRIs (for example cardiotoxicity as seen with clomipramine treatment), may favour the prescribing of SSRIs over clomipramine (Fineberg and Craig, 2007).

Two general traits characterize the treatment of OCD with the SSRIs: 1) Unlike in depression, OCD responds optimally to SSRI treatment only after 4 to 8 weeks of treatment, and 2) a better response can usually be achieved with initial SSRI doses higher than that prescribed for the treatment of depression (Fineberg and Craig, 2007; Stein, 2002). Although it has been shown that some patients with OCD do in fact respond to SSRI doses corresponding to the doses used in depression, relapse using low dose SSRI treatment is common. Moreover, subsequent reinstatement of treatment after such a relapse is associated with a poorer clinical outcome (Maina *et al.*, 2001).

Resistance to SSRI therapy is a major clinical challenge. Even after long-term treatment, approximately 30% of patients remain unresponsive to monotherapy with the SSRIs (Fineberg and Craig, 2007). The treatment of refractory OCD is difficult, with some authors advocating for an increased dose and a longer duration of treatment (Bejerot and Bodlund, 1998; Stein, 2002), while others advise switching treatment to another SSRI (Fineberg *et al.*, 2006; Stein, 2002). A third strategy that may prove to be especially useful in patients that have shown a partial response to a SSRI after 10 – 12 weeks, is to augment the SSRI therapy with a *low dose* antipsychotic (Erzegovesi *et al.*, 2005; Hollander *et al.*, 2003; McDougle *et al.*, 2000). With respect to the latter, it is interesting to note that no clinically significant difference with respect to efficacy has been observed between typical antipsychotics, such as haloperidol, and atypical antipsychotics such as clozapine or risperidone (Fineberg and Craig, 2007).

Although glutamate and *gamma*-amino butyric acid (GABA) are major role players in the functioning of the CSTC circuit (refer to section 2.2), less work has been done in targeting these systems in the treatment of OCD. Nevertheless, GABAergic (Oulis *et al.*, 2009) as well as glutamatergic agents (Coric *et al.*, 2005; Lafleur *et al.*, 2006; Onder *et al.*, 2008; Stewart *et al.*, 2010) have demonstrated their possible usefulness in treating OCD. Because of their acknowledged importance in OCD neurocircuitry, the targeting of these two neurotransmitters and their receptors for the treatment of OCD are under continuous investigation (El Mansari and Blier, 2006).

It is recommended that from the time of diagnosis, treatment should be initiated with a SSRI as first choice and titrated relatively quickly to high doses until remission of symptoms. Furthermore, treatment should be continued for at least 12 weeks before any change in the regime is considered. Once stabilized, treatment should not be interrupted for at least a year (Fineberg and Craig, 2007; Stein, 2002; Maina *et al.*, 2001).

## 2.2. THE NEUROBIOLOGY OF OCD – BOULDER HOPPING ACROSS UNKNOWN WATERS

When reviewing the different texts, articles, and data concerning the neurobiology of OCD currently at our disposal, one finds oneself boulder hopping across a wide and unknown river. Every now and then you can grab onto a steady boulder of knowledge and/or information that is robust and familiar, only to realise that in order to cross the river (and attempt to understand the neurobiology of OCD), you need to take giant leaps to the next boulder, as what lurks in between is neither steady, nor valid enough to allow you to take small confident steps.

Although the etiology and neurobiology of OCD are not yet fully elucidated, there are certain clinical and neurobiological certainties around which the puzzle can be built.

First, it is clear that in most cases OCD is characterized by both cognitive and behavioural abnormalities (den Heuvel *et al.*, 2010; Markarian *et al.*, 2010; Stein, 2002). Different hypotheses exist that attempt to explain the symptomology of OCD, but whether patients diagnosed with OCD express a lower threshold for disgust (Husted *et al.*, 2006) and subsequently present with compulsions like ritualistic hand washing, or simply cannot find closure after a certain task is completed (e.g. ritualistic locking due to obsessions about security), it is clear that an abnormal regulation of goal-directed behaviour is central to the symptomology of OCD. Thus, it is evident that the brain areas implicated in OCD would be among others, those that translate cognitive planning and experiences into motor behaviour, and subsequently mediate goal-directed behaviour. These brain areas include the prefrontal cortex, striatum and thalamic nuclei which communicate with each other via different pathways (Bartz and Hollander, 2006; den Heuvel *et al.*, 2010; Evans *et al.*, 2004; Nambu *et al.*, 2002).

Secondly, certain neurotransmitters have been identified as playing a central role in the pathogenesis of OCD, of which the excitatory amino acid glutamate, the inhibitory neurotransmitter GABA, and the monoamines dopamine and serotonin are the most well studied (El Mansari and Blier, 2006; Markarian *et al.*, 2010; Pittenger *et al.*, 2006; Sareen *et al.*, 2004; Stein, 2002).

The neurobiology of OCD will subsequently be discussed by elaborating on these neuroanatomical and neurochemical theories, thus trying to build stable and tested bridges that will afford us with a greater understanding of the neurobiology and treatment of OCD.

### *2.2.1. The neurocircuitry of OCD*

#### *2.2.1.1. An explanation of the cortico-striatal-thalamic-cortical (CSTC) pathway*

The term 'CSTC circuit' denotes the functional organization of the three distinct brain areas that are purported to be involved in the pathology of OCD *viz.,* the prefrontal cortex, the striatum (and other parts of the basal ganglia), and the thalamus (Stocco *et al.*, 2010). These structures are organized in such a manner that the cortex innervates the striatum, which subsequently influences other parts of the basal ganglia and ultimately exerts feedback via the thalamus to the cortex. As a whole, the CSTC circuit is fundamental in the planning, execution and termination of complex motor behaviour and reward based learning – two major processes that are hypothesized to be dysfunctional in patients with OCD (Stocco *et al.*, 2010).

Many different models have been postulated by different authors to try and conceptualize the functioning of the cortico-striatal-thalamic circuits (Beiser *et al.*, 1997; Bogacz and Larsen, 2011; Bullock *et al.*, 1993; Chakravarthy *et al.*, 2010; Fukai and Tanaka, 1997; Kotter and Wickens, 1995; McHaffie *et al.*, 2005; Wickens *et al.*, 1995). It is also important to note that a number of these circuits coexist and each is hypothesized to have different functions (McHaffie *et al.*, 2005). The model that is generally accepted to fit the phenomenology of OCD describes a circuit that forms a closed loop between these three structures. After being initiated in the prefrontal cortex, the circuit passes through the basal ganglia via a direct and an indirect pathway, continuing through the thalamus, and ends in an anatomically different part of the prefrontal cortex than where the circuit originated (Stocco *et al.*, 2010). This specific model will be explained in more detail in an attempt to simplify the neurobiological understanding of OCD.

* * *

***'C' for cortex.*** For the sake of simplicity, the cortex can be assumed to be the initiator or trigger for the normal functioning of the CSTC circuit (Evans *et al.*, 2004). When a certain action has been planned, the cortex activates the striatum via corticofugal glutamatergic afferent projections (den Heuvel *et al.*, 2010), initiating a number of subsequent events that result in the translation of the impulse. Following transmission and processing of the impulse in the basal ganglia and thalamus, the cortex also functions as the termination stage of the CSTC circuit. Two main cortical regions that are implicated in the pathology of OCD are the orbitofrontal cortex and the anterior cingulate cortex (Baxter Jr. *et al.*, 1992; Evans *et al.*, 2004; Maia *et al.*, 2008; Saxena and Rauch, 2000; Stein, 2002).

The orbitofrontal cortex (OFC) is especially important for the development of reward-based learning (Rolls, 2004). It contains the secondary taste and olfactory cortices in which the re-

ward of taste and smell is represented. It has also been shown that the orbitofrontal cortex is activated by pleasant and painful touch and by more abstract reinforcers such as winning or losing money (Rolls, 2004).

Numerous studies using functional neuroimaging to investigate the brain activity of patients with OCD have demonstrated an increased activity in the anterior cingulate cortex (ACC) compared to healthy controls (Maia *et al.*, 2008; Maina *et al.*, 2001; Maltby *et al.*, 2005; Markarian *et al.*, 2010; Saxena and Rauch, 2000). The main functions of the anterior cingulate cortex include the control of emotional and cognitive behaviour, and the mediation of executive processes (Shim *et al.*, 2009).

***'S' for striatum.*** In order to comprehend the functioning of the striatum and its role in the pathogenesis of OCD, it must be placed in perspective to the rest of the nuclei in the basal ganglia. The basal ganglia are a set of major subcortical nuclei that are located in the midbrain, around the thalamus (Stocco *et al.*, 2010). The following structures form the main components of the basal ganglia:

- striatum (composed of the caudate nucleus, putamen and ventral striatum)
- globus pallidus (GP – consisting of an internal [GPi] and external [GPe] section)
- substantia nigra (SN – divided in two sections *viz.,* the pars compacta [SNc] and the pars reticulata [SNr])
- subthalamic nucleus (STN)

***Note:*** *The GPi and SNr will henceforth be discussed as a single functional entity (GPi/SNr) as their actions in the CSTC circuit are identical (Stocco et al., 2010).*

To simplify the understanding of the functions of the basal ganglia in the CSTC circuit, we must move one level up for a moment and examine the CSTC circuit holistically. The cortex plans and initiates complex motor patterns. In order to execute the relevant motor pattern, the cortex needs to activate the thalamus via the basal ganglia circuitry, which will lead to subsequent feedback to the cortex and the execution of the plan (Stocco *et al.*, 2010). The obvious question is why does the cortex need to convey its plan of action to the thalamus via the basal ganglia and back to the cortex, if the cortex is responsible for both the planning and eventual execution of a relevant motor pattern? Firstly, the prefrontal-cortical area responsible for planning the action (e.g. the anterior cingulate cortex, ACC) is a different cortical region from the one executing the relevant plan and inhibiting irrelevant motor actions (i.e. the orbitofrontal cortex, OFC). These two distinct cortical areas communicate with each other via the basal ganglia and the thalamus (Evans *et al.*, 2004). Secondly, the cortex initiates a number of complex motor pat-

terns at any given time, which need to be filtered before the relevant action can be taken. It is the role of the basal ganglia to select the relevant action and convey the message to the orbitofrontal cortex via the thalamus. The orbitofrontal cortex then suppresses the irrelevant motor patterns and executes only the desired action as selected by the basal ganglia (Stocco *et al.*, 2010).

The striatum functions as the major entry point for cortical input to the basal ganglia, whereas the GPi/SNr functions as the major output nuclei for relaying the relevant messages to the thalamus (Albin *et al.*, 1989; Chesselet and Delfs, 1996). The striatum is connected to the GPi/SNr via two relays, as depicted in Figure 2-1 by the direct (blue) and indirect (orange) pathways.

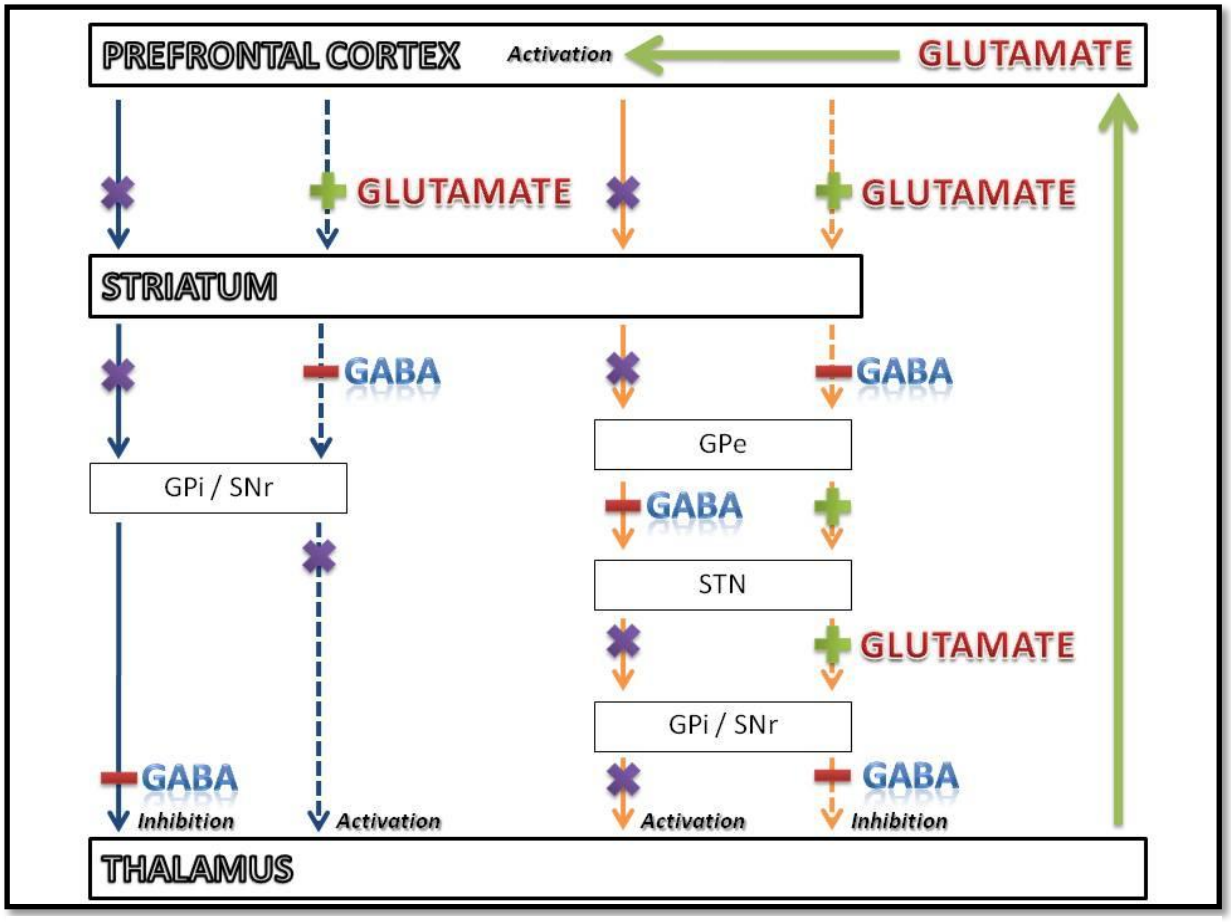



***FIGURE 2-1 – THE CSTC CIRCUIT IMPLICATED IN OCD***

Blue lines, direct pathway; Orange lines, indirect pathway; Solid lines, no cortical activation of pathways; Dotted lines, cortically activated pathways; Purple crosses, no considerable neurotransmitter release; Red minus signs, GABAergic inhibition; Green plus signs, lack of target inhibition / glutamatergic activation; GPi / SNr, globus pallidus interna / substantia nigra pars reticulata; GPe, globus pallidus externa; STN, subthalamic nucleus; GABA, gamma-amino butyric acid.

The striatum mainly consists of GABAergic projection neurons which divide into two subgroups: striato-nigral (SN) neurons *originating in the direct pathway* (projecting to and inhibiting the GPi/SNr), and striato-pallidal (SP) neurons *originating in the indirect pathway* (projecting to and inhibiting the GPe) (Rymar *et al.*, 2004; Yelnik *et al.*, 1991). The striatum also consists of dominating GABAergic interneurons, *which inhibits the functioning of the GABAergic inhibitory projection neurons* under resting conditions (Wilson and Groves, 1981). Furthermore, an increase in striatal dopamine before a certain motor action is to be executed shifts the balance to the direct pathway, leading to an increase in overall motor activity. Thus under resting conditions, the indirect pathway plays the dominant role (Chakravarthy *et al.*, 2010). A more detailed discussion of dopamine and its influence on the CSTC circuit follows later in this paragraph and in paragraph 2.2.2.3.

***Note:*** *As the basal ganglia are composed mostly of GABAergic neurons, the use of the word 'activating' depicts the activation of inhibiting neurons. The net effect following the activation of these neurons will evidently be inhibition of their targets, unless explained otherwise.*

The GPi/SNr mostly consists of tonically active GABAergic neurons (TANs) which inhibit the thalamus and therefore also the OFC under resting conditions. These TANs decrease their firing rate dramatically at the onset of behaviourally significant stimuli from among others, the ACC (Apicella, 2002; Morris *et al.*, 2004).

When activated by cortical glutamatergic afferents, the striatum activates both the direct and indirect GABAergic pathways. When the direct pathway is activated, the striatum exerts inhibitory control *on the usually tonic inhibitory* GPi/SNr-complex, which leads to the activation of the thalamus. Figure 2-1 also depicts the effects of a resting (solid line) and activated (dotted line) indirect pathway. It is evident that the activation of the indirect pathway leads to the inhibition of the thalamus via the indirect activation of the GPi/SNr (Albin *et al.*, 1989; DeLong, 1990; Penney Jr. and Young, 1986).

If the direct pathway favours activation, and the indirect pathway not, and both are activated at the same time, how is a relevant voluntary motor plan ever executed? This contradiction between "yes" and "no" signals are solved by the SNc which produces dopamine. The SN neurons of the direct pathway express dopamine-1 ($D_1$) receptors, which is associated with $G_s$, a GTP binding protein that mediates stimulation of neurotransmitter-receptor signalling. Stimulation of $D_1$ receptor-$G_s$ coupling by dopamine therefore results in elevated cAMP levels and increased GABA release. The net effect is the continuous activation of the direct pathway and subsequently the activation of the thalamus. The SP neurons on the other hand express dopamine-

2 ($D_2$) receptors, which are associated with the $G_i$-protein, a GTP binding protein that mediates inhibition of neurotransmitter-receptor signalling. Stimulation of the $D_2$ receptor thus leads to a decrease in cAMP levels and a decrease in the release of GABA resulting in the inhibition of the indirect inhibitory pathway and the subsequent activation of the thalamus (Gerfen *et al.*, 1990; Tepper and Bolam, 2004). For a more detailed discussion on dopamine, refer to paragraph 2.2.2.3.

To summarize the functions of the striatum and the basal ganglia in the functioning of the CSTC circuit, it can be concluded that under resting conditions the basal ganglia are in a state of inactivity, with the net effect being thalamic suppression. Once cortical input activates the striatum, a cascade of events is triggered in the basal ganglia. Action selection then takes place and irrelevant signalling is suppressed, resulting in decisive feedback to the thalamus. Dopamine can be regarded as an important part of the 'yes-no' switch, as only actions transmitted while dopamine exerts its effect on both of the pathways, are transferred to the thalamus.

***'T' for thalamus.*** The thalamus, located between the cortex and the midbrain, serves as a hub for stimuli from different subcortical brain areas in transit to the cortex, notably also from the basal ganglia (Haber and Calzavara, 2009) and is the main entry point to the cortex for impulses from these brain regions. The thalamic anatomy is comprised of a system of lamellae that separates it into different functional clusters (Jones, 1991). This anatomical organization enables the thalamus to regulate a number of brain functions, for instance the processing of sensory afferents before entering the cortex, the regulation of sleep patterns and especially significant in the case of OCD, the delivery of motor tasks to the cortex as conveyed to the thalamus by the basal ganglia (Steriade and Llinas, 1988). The thalamus then activates the relevant region of the cortex via glutamatergic signalling, resulting in the execution of the plan as originally designed by the cortex.

#### *2.2.1.2. A proposed dysfunction in the CSTC circuitry in patients with OCD*

Given the persistent inability of most OCD patients to find closure after the 'reward of task completion', it is not surprising to find that both the ACC and the OFC are hyperactive in OCD patients as numerous functional neuroimaging studies have demonstrated (Maia *et al.*, 2008; Maltby *et al.*, 2005; Markarian *et al.*, 2010; Saxena and Rauch, 2000; Shim *et al.*, 2009; Stein, 2002). The fact that the ACC is vital in the control of cognitive and behavioural processes and mediates certain executive functions, justifies the hypothesis that in patients with OCD, an overactive ACC maybe plays a role in the instigation of compulsive behaviour. The fact that no 're-

ward after task completion' is established in most OCD patients could suffice to explain the hyperactivity in the OFC.

Furthermore, it is believed that there is a bias in favour of the direct thalamus-activating pathway over the indirect thalamus-inhibiting pathway in the basal ganglia of OCD patients compared to healthy controls (Saxena and Rauch, 2000). This not only results in an overactive OFC, but also increases the activities of both the caudate nucleus and the thalamus. The subsequent hyperactivity in the CSTC circuit as a whole, is believed to be central to the pathological presentation of OCD (Bartz and Hollander, 2006; Saxena and Rauch, 2000).

### *2.2.2. The neurotransmission of OCD*

#### *2.2.2.1. Glutamate and excitatory signalling*

Following the discussion on the neurocircuitry of OCD, it is clear that excitatory glutamatergic signalling via corticofugal afferents initially activates the striatum and that the thalamus functions as the final output of the CSTC circuit, activating the prefrontal cortex by means of the same neurotransmitter. Evidently, a valid question to ask would be whether patients with OCD present with dysfunctional glutamatergic signalling in these two subcortical areas. Recent studies performed on patients with OCD using magnetic resonance spectroscopy (MRS), demonstrated that patients diagnosed with OCD have an increase in caudate, but a decrease in cingulate, levels of 'Glx' compared with the healthy controls (Bolton *et al.*, 2001; Moore *et al.*, 1998; Rosenberg *et al.*, 2000; Rosenberg *et al.*, 2004). The term 'Glx' denotes a number of markers used to determine amino acid neurotransmitter levels in general and cannot be used to represent the levels of glutamate specifically (Pittenger *et al.*, 2006). Due to methodological constraints, the MRS determination of glutamate and GABA as single molecular compounds is difficult. By determining the levels of Glx, which includes glutamate, glutamine, homocaronsine, and GABA, it could be concluded that patients with OCD express abnormal amino acid neurotransmitter levels, but whether it implies an increase in amino acid signalling, or just an increase in the metabolic stores of these compounds, is not clear (Pittenger *et al.*, 2006). Also, the method does not differentiate between glutamate and GABA concentrations, and therefore studies that are more specific need to be done to clear up the meaning of an increased Glx level in the caudate nuclei of OCD patients.

One such a study was done by Chakrabarty and colleagues (Chakrabarty *et al.*, 2005). In an investigation focussing only on central glutamate levels, the authors compared the glutamate concentrations in the cerebrospinal fluid (CSF) of 21 drug naive OCD patients to that of 18 healthy controls (Chakrabarty *et al.*, 2005). The authors found a significant elevation in the glu-

tamate concentrations of the OCD patients compared to healthy controls. This finding may support the MRS data suggesting that patients with OCD present with abnormal caudate glutamate levels (Pittenger *et al.*, 2006).

* * *

Glutamate exerts its effects via ionotropic and metabotropic receptors. The term 'ionotropic' refers to receptors producing rapid ion flux across the post-synaptic membrane resulting in relatively large conductance changes in the post-synaptic neuron. Metabotropic glutamate receptors activate second messengers such as cyclic nucleotides (cAMP and cGMP), inositol triphosphate ($IP_3$) and diacylglycerol (DAG) which ultimately mediate metabolic changes in the post-synaptic cell (Nicoletti *et al.*; Schoepp *et al.*, 1999). The ionotropic group of glutamate receptors include N-methyl-D-aspartate (NMDA), α-amino-3-hydroxy-5-methyl-4-isoxazole-propionate (AMPA) and kainate (KA) receptors, while there are eight metabotropic glutamate receptor types (mGluRs), divided into three groups, and termed mGluR1 – mGluR8 (Galvan *et al.*, 2006). As mentioned earlier, various glutamate active compounds, including memantine, riluzole and N-acetylcysteine have demonstrated usefulness in treating OCD (Coric *et al.*, 2005; Lafleur *et al.*, 2006; Onder *et al.*, 2008; Stewart *et al.*, 2010).

***The ionotropic NMDA and AMPA receptors.*** NMDA and AMPA receptors co-localize post-synaptically at glutamatergic, but not at GABAergic synapses (Galvan *et al.*, 2006). The high density of co-localized NMDA and AMPA receptors at the glutamatergic synapses allows for rapid exposure to synaptic glutamate and thus facilitates fast glutamatergic transmission. NMDA and AMPA receptor mediated effects with a rapid onset of action have been reported in the striatum (Carter and Sabatini, 2004) (which is activated by corticofugal glutamatergic afferents), both the internal and external segments of the GP (Kita *et al.*, 2005), the STN (Nambu *et al.*, 2000) (which activates its targets via glutamatergic signalling), and the SNc (Christoffersen and Meltzer, 1995). As stated earlier, both sections of the GP contain mostly GABAergic neurons (Apicella, 2002; Morris *et al.*, 2004), a fact which renders the demonstration of an increased density in NMDA and AMPA receptors in the GPi and GPe contradictory to the general understanding of the functioning of these two subcortical regions. Kita and co-workers explains this finding by proposing a complex interaction between the STN, GPe, and GPi. Such an interaction is believed to mediate the control of the activities of both the GPe and GPi by the STN (Kita *et al.*, 2005). The fact that the STN activates its targets via glutamatergic signalling, may subsequently explain the increased densities of NMDA and AMPA receptors in the pallidal structures. Furthermore, the glutamatergic neurons of the STN also project to the SNc, which functions as the main dopaminergic regulator of the striatum (Chatha *et al.*, 2000). The glutamatergic afferents

from the STN regulates the release of dopamine from the SNc via both ionotropic NMDA and AMPA receptors and metabotropic mGlu-receptors (Chatha *et al.*, 2000), a functional interaction accounting for the presence of these receptors in the SNc. Although NMDA and AMPA receptors are also found at certain presynaptic locations with the main function of decreasing the release of glutamate, this distribution is not significant enough in the basal ganglia to exert any meaningful physiological effects.

Evidence for the involvement of ionotropic glutamate receptors in OCD have been forthcoming from animal studies. For example, using the marble-burying test in mice, a putative screening test for putative OCD-like behaviour, Egashira and colleagues observed that the non-competitive NMDA antagonists memantine, amantadine and MK801 inhibit marble-burying behaviour, although MK801 also markedly increased locomotor activity (Egashira *et al.*, 2008b). However, the AMPA receptor antagonist NBQX, and the glutamate release inhibitor riluzole showed no effect in this regard. With regard to the AMPA receptor, the AMPA receptor potentiator, CX546, significantly inhibits marble-burying behaviour, while the NR2B subunit-containing NMDA receptor antagonist, Ro25-6981, also reduces marble-burying behaviour in this test, suggesting that AMPA receptor potentiators and NR2B receptor antagonists may be useful in treating OCD (Iijima *et al.*, 2010). In the compulsive lever press model of Joel and co-workers (Joel and Avisar, 2001), systemic administration of the partial NMDA receptor agonist, D-cycloserine, selectively decreased compulsive lever pressing in rats (Albelda *et al.*, 2010).

***The ionotropic KA receptor.*** It has been demonstrated that KA receptors are located both pre-and post-synaptically in among others, the striatum and GP, although only a few studies have been done up to date to obtain information regarding the expression of KA receptors in other parts of the basal ganglia (Galvan *et al.*, 2006). It is believed that the activation of post-synaptic KA receptors inhibit GABAergic transmission indirectly due to the release of adenosine, which acts on $A_{2A}$ receptors (Chergui *et al.*, 2000), while the activation of presynaptic KA receptors in the nucleus accumbens inhibits the release of glutamate and therefore suppresses glutamatergic signalling (Crowder and Weiner, 2002). This finding may predict the same physiological effect of the presynaptic KA receptor in the striatum and GP, but further studies in this regard must still be undertaken.

***Group I metabotropic receptors – mGluRs 1 and 5.*** Group I mGluRs are mainly expressed post-synaptically in the glutamatergic neurons of the basal ganglia, most notably so in the striatum, GP, SN and STN (Conn *et al.*, 2005; Valenti *et al.*, 2002). They mostly exert the excitatory effects of glutamate by activating phospholipase C (PLC) and subsequently increasing intracellular $Ca^{2+}$. As these receptors are located in regions of the basal ganglia predominantly consisting

of GABAergic efferents, it could be hypothesized that the glutamatergic stimulation of the post-synaptic group I mGluRs, will result in an increased GABAergic outflow (Galvan *et al.*, 2006). mGluR1 is also expressed on the dopaminergic neurons of the SNc where it co-facilitates the release of dopamine and enhances dopaminergic signalling in the nigro-striatal pathway (Kosinski *et al.*, 1998). In translational animal model studies, the mGluR5 antagonist, MPEP, has been found to decrease burying behaviour in the marble-burying test (De La Mora *et al.*, 2006; Spooren *et al.*, 2000).

***Group II (mGluRs 2 and 3) and Group III (mGluRs 4, 6, 7, and 8) metabotropic receptors.*** Little is known about the functions of the Group II and III mGluRs, other than that they are expressed presynaptically in the basal ganglia on glutamatergic neurons and mediate a decrease in glutamate release via the negative regulation of adenylyl cyclase and cyclic AMP production. It is thought that Group III mGluRs modulate the release of GABA in the striatum, GP and SN, though it is not yet known for certain what the manner of this modulation may be (Galvan *et al.*, 2006). Group II and III mGluRs are also localized on the neurons of the STN and modulate the release of dopamine from the SNc (Corti *et al.*, 2002).

It must be stressed that up to date, little data have been obtained regarding the role of metabotropic glutamate receptors in the pathology of OCD (Nicoletti *et al.,* In Press). It remains to be elucidated whether targeting mGluRs will result in clinical advances concerning the understanding and treatment of OCD.

#### *2.2.2.2. GABA and inhibitory signalling*

Apart from the glutamatergic outputs of the prefrontal cortex to the striatum, the STN to the GPi / SNr, and the thalamus to the prefrontal cortex, the most notable functions of the CSTC circuit are characterized by the inhibitory actions of GABA (Beiser *et al.*, 1997; Chakravarthy *et al.*, 2010; den Heuvel *et al.*, 2010; Haber and Calzavara, 2009; Husted *et al.*, 2006). Of these functions, probably the most important is the tonic inhibition of the thalamus by the GPi / SNr under normal resting conditions. As explained in paragraph 2.2.1, this effect is attenuated following glutamatergic input in the striatum via corticofugal afferents, a process that may be overactive in patients diagnosed with OCD. Very little evidence has been obtained regarding the direct role of GABAergic neurotransmission in the pathogenesis of OCD. Most computational models of OCD, and studies done in animal models as well as in patients diagnosed with OCD focus on, and demonstrate, dysfunctional glutamatergic and / or dopaminergic signalling which subsequently influences the GABAergic neurotransmission in the basal ganglia (Bolton *et al.*, 2001; Chakrabarty *et al.*, 2005; Chakrabarty *et al.*, 2005; Chakravarthy *et al.*, 2010; Jones *et al.*, 2001; Mark-

arian *et al.*, 2010; Moore *et al.*, 1998; Pittenger *et al.*, 2006; Rosenberg *et al.*, 2000; Rosenberg *et al.*, 2004). The treatment of patients with drugs that target GABAergic signalling, have also been demonstrated to be mostly unsuccessful, either as monotherapy or as an augmentation strategy for SSRI-refractory OCD (El Mansari and Blier, 2006; Fineberg and Craig, 2007; Jenike, 1993). Most authors therefore conclude that dysfunctional glutamatergic regulation of normal GABAergic signalling is at least partly responsible for the typical symptoms of OCD, and is not a primary dysfunction in GABAergic neurotransmission itself. Nevertheless, a recent study using tiagabine has suggested some value in pursuing this direction (Oulis *et al.*, 2009).

* * *

The effects of GABA are mediated by the ionotropic $GABA_A$ and metabotropic $GABA_B$ receptors (Galvan *et al.*, 2006).

***The ionotropic $GABA_A$ receptor.*** The $GABA_A$ receptor is a ligand gated chloride channel and consists of five subunits (Galvan *et al.*, 2006). Different combinations of the eight types of $GABA_A$ receptor subunits already identified are used to form physiologically different receptors, with each different $GABA_A$ receptor type expressing different physiological properties. The most common $GABA_A$ receptor stoichiometry is two alphas (α), two betas (β) and one gamma (γ) subunit (Korpi *et al.*, 2002; Sieghart and Sperk, 2002). The rapid inhibitory actions that characterize GABAergic neurotransmission are mediated by the $GABA_A$ receptor via the opening of the ligand-gated chloride channel, resulting in an inhibitory post-synaptic potential (IPSP). Interestingly though, the highest density of $GABA_A$ receptors in the rat striatum are expressed on terminals that do not present with detectable GABA levels (Fujiyama *et al.*, 2002), suggesting a $GABA_A$ receptor association with non-GABAergic terminals such as on dopaminergic nerve endings (Smith *et al.*, 1994). This association demonstrates a reciprocal relationship between GABA and other neurotransmitters, which could allow for the bi-directional control of interneuron signalling.

***The metabotropic $GABA_B$ receptor.*** $GABA_B$ receptors located post-synaptically mediate slow hyperpolarization while presynaptic receptors inhibit the release of GABA from the presynaptic terminal (Galvan *et al.*, 2006). The greatest proportion of post-synaptic receptors located in the basal ganglia are found intracellularly and show a prominent co-expression with Group I mGluRs (Boyes and Bolam, 2003). Therefore, certain functions of the CSTC circuit do not necessarily involve only the rapid effects caused by the ionotropic glutamatergic and GABAergic receptors, but also the longer lasting actions brought about by the cross talk between glutamate and GABA metabotropic receptors. Interestingly, the glucose isomer and sec-

ond messenger precursor for metabotropic directed PLC signalling, myo-inositol, has demonstrated efficacy in OCD (Harvey *et al.*, 2002). Consequently, it can be expected that metabotropic receptors, be it GABAergic or glutamatergic, that function via this signalling system may have a role to play in the treatment of OCD. In fact, recent clinical evidence suggests that inositol administration exerts its therapeutic benefits in OCD via an alternate neuronal circuitry to the SSRIs (Carey *et al.*, 2004).

In the striatum, presynaptic $GABA_B$ receptors are found in the corticofugal and thalamic afferents (Boyes and Bolam, 2003; Galvan *et al.*, 2006). This indicates that the two main glutamatergic inputs to the striatum can be regulated by GABA. However, due to the absence of controlled studies in humans and animal models, it remains speculation as to whether the stimulation of the striatal presynaptic $GABA_B$ receptor will prove useful in the treatment of OCD.

* * *

*Following the discussion on the CSTC circuit and the two most prominent neurotransmitters directly responsible for the transduction of signals via the direct and indirect pathways, namely, glutamate and GABA, the focus will now be shifted to the neurotransmitters responsible for the functional regulation of the CSTC circuit. The two neurotransmitters that are especially significant in this regard are dopamine and serotonin (Bartz and Hollander, 2006; Biggs et al., 1997; Chakravarthy et al., 2010; Markarian et al., 2010; Mora et al., 2008). Referring to section 2.1.3, it is important to note that drugs found to consistently demonstrate efficacy in the treatment of OCD target these latter two regulatory neurotransmitters, and not glutamatergic and GABAergic signalling as could be expected. Current understanding of the pathology of OCD therefore implies a major dysfunction in the regulatory processes of the CSTC circuitry and not necessarily an abnormal CSTC circuit itself.*

#### *2.2.2.3. Dopamine and the differential regulation of the direct and indirect pathways*

Dopamine clearly plays a prominent, if not critical, role in the regulation of the CSTC circuit and therefore by implication will play a central role in the pathology of OCD. A number of studies have attempted to elucidate the exact functions of the dopaminergic system in the CSTC circuitry (Biggs *et al.*, 1997; Denys *et al.*, 2004c; Hrabovska *et al.*, 2010; Jones *et al.*, 2001; Olver *et al.*, 2009), while others have hypothesized different functions for the basal ganglia and its regulation by dopamine (Albin *et al.*, 1989; Beiser *et al.*, 1997; Bogacz and Larsen, 2011; Bullock *et al.*, 2009; Chakravarthy *et al.*, 2010; Chesselet and Delfs, 1996; DeLong, 1990; den Heuvel *et al.*, 2010; Evans *et al.*, 2004; Gerfen *et al.*, 1990; Husted *et al.*, 2006; Joel and Weiner, 2000; Kotter and Wickens, 1995; McHaffie *et al.*, 2005; Morris *et al.*, 2004; Smith and Kieval, 2000; Tepper

and Bolam, 2004; Wickens *et al.*, 1995). As explained in paragraph 2.2.1.1, $D_1$ receptor stimulation *activates* the direct pathway via an *increase* in GABA release, and $D_2$ receptor stimulation *inhibits* the indirect pathway via a *decrease* in GABA release. Thus, the net effect of dopamine release in the striatum and its actions on SN and SP neurons (Albin *et al.*, 1989; Beiser *et al.*, 1997; Bogacz and Larsen, 2011; Bullock *et al.*, 1993; Bullock *et al.*, 2009; Husted *et al.*, 2006; Joel and Weiner, 2000; Markarian *et al.*, 2010; Stocco *et al.*, 2010) is the activation of the prefrontal cortex, resulting in the expression of complex motor behaviour. The subsequent hypothesis is that patients presenting with OCD symptoms will express a relative increase in striatal dopaminergic signalling and thus, when targeting the dopaminergic system in a patient with OCD, a non-selective $D_1$ / $D_2$ receptor antagonist would theoretically be ideal. Even so, treating OCD with dopaminergic antagonists as monotherapy has proved unsuccessful (El Mansari and Blier, 2006; Erzegovesi *et al.*, 2005; Fineberg and Craig, 2007; Hollander *et al.*, 2003; Sareen *et al.*, 2004), most probably due to the complicated cross talk between dopamine, GABA, and glutamate in the striatum and basal ganglia. The fact that dopaminergic antagonists are useful as augmentation therapy with SSRIs implicates a regulatory role for serotonin in the normal functioning of dopamine in the basal ganglia (see paragraph 2.2.2.4).

Knowing that dopamine plays a role in the functioning of the CSTC circuit raises the question how certain motor programs are executed only when the need arises. Put another way, what exactly triggers dopamine release when a certain motor program needs to be executed? Moreover, can the symptomology of OCD be directly associated with a dysregulated dopaminergic system, or is it a combination of striatal glutamatergic, GABAergic, and dopaminergic mechanisms? To answer these questions, it is necessary to delve deeper into the origins of dopamine in the basal ganglia in order to understand how and when it is released during the execution of complex motor patterns.

* * *

In addition to the intrastriatal organization of dopaminergic neurons and the local release of dopamine in the substantia nigra itself, the dopaminergic neurons in the basal ganglia can be classified into three major projections (Smith and Kieval, 2000). The *nigro-striatal, nigro-pallidal,* and *nigro-subthalamic* projections convey dopaminergic signalling from the SNc to the striatum, GP, and the STN respectively. Although the relevance of the latter two pathways is still to be established in OCD (Smith and Kieval, 2000), it is evident that with respect to the basal ganglia, the SNc functions as the main originator for dopaminergic signalling.

***The dendritic release of dopamine in the substantia nigra.*** The dendritic release of dopamine in the substantia nigra is well established (Araneda and Bustos, 1989; Campusano *et al.*, 2002; Cheramy *et al.*, 1981; Le Boulch *et al.*, 1991; Rosales *et al.*, 1994). Dopamine released locally in the substantia nigra mainly regulates the activity of the nigrofugal dopaminergic outputs to the striatum, GP and STN via the stimulation of pre- and post-synaptic dopamine $D_1$, $D_2$, $D_3$, and $D_4$ receptors (Bouthenet *et al.*, 1991; Tepper *et al.*, 1997). A full description of dopamine $D_1$ – $D_4$ receptors will follow later in this paragraph. In the SNr, dopamine also mediates the release of GABA from striato-nigral afferents (Cameron and Williams, 1993; Waszczak, 1990) which explains the net inhibition of the thalamus via the direct pathway in the absence of striato-nigral activation.

***The nigro-striatal dopaminergic projection.*** The dopaminergic projection from the SNc to the striatum is believed to be the main source of dopamine utilized in the regulation of the direct and indirect pathways in the CSTC circuit (Gerfen *et al.*, 1990; Tepper and Bolam, 2004). Interestingly, the majority of dopaminergic nigro-striatal afferents converge with corticofugal afferents on individual spines of striatal neurons (Smith and Bolam, 1990). This fact provides the pivotal piece of information needed to understand the basic dopaminergic regulation of complex motor behaviour as it demonstrates the co-localization of cortical glutamatergic afferents (which instigates the execution of motor patterns) and nigro-striatal dopaminergic inputs (which in turn is believed to be central to appropriate action selection). Following innervation by corticofugal and nigro-striatal afferents, the striatal efferents divide in two distinct neuronal bundles as explained in paragraph 2.2.1.1. The substance-P-containing SN-neurons of the direct pathway mainly express $D_1$ receptors, whereas the enkephalin-containing SP-neurons of the indirect pathway mostly express $D_2$ receptors (Gerfen *et al.*, 1990).

***Intrastriatal dopaminergic neurons.*** The expression of dopaminergic neurons in the normal striatum is insignificant (Greenamyre, 1997). Interestingly though, their density increases with a decrease in nigrofugal dopaminergic outputs to the striatum as demonstrated in patients with Parkinson's disease (PD) (Greenamyre, 1997).

***The nigro-pallidal and nigro-subthalamic dopaminergic projections.*** Although the functional significance of the nigro-pallidal and nigro-subthalamic projections are not yet fully understood (Smith and Kieval, 2000), it is known that these two projections terminate in the GP and STN respectively and exert dopaminergic effects in both of these structures via $D_1$ and $D_2$ receptors (Hauber and Lutz, 1999).

* * *

Following the discussion on the major dopaminergic pathways that interconnect the different components of the basal ganglia, the question remains as to how the differentiating role of dopamine only comes to play when a certain motor action is to be executed. From that discussed in paragraph 2.2.1.1, it is evident that any given motor task can only be executed if dopamine simultaneously stimulates $D_1$ and $D_2$ receptors in the direct and indirect pathways of the CSTC circuit respectively. However, what exactly triggers dopamine release from the SNc? The answer to this question is complicated, and intertwines many different aspects of both the neurobiological and cognitive functions of the human brain. There is however one factor that is always central to the release of dopamine and that is the concept of "*reward".* Since OCD is postulated to be a condition that is characterized by the dysfunctional appraisal of reward (refer to section 2.1 and paragraph 2.2.1.1), the role of reward in the regulation of nigro-striatal dopaminergic signalling will now be discussed.

The term 'reward' denotes any form of satisfactory feedback, from the pleasant taste of foods and liquids or the response to a pleasant or aversive experience, to the successful completion of a certain task (Schultz, 2002). For the sake of simplicity, it can be assumed that any motor action driven to accomplish a certain task is initiated by the anticipation of a reward that should result from the successful completion of that specific action. For example, the locking of a door is initiated by the anticipated reward of a secure environment. Since this action had been highly prioritized (Schultz, 2002), it led to certain actions being taken which, in this case could be indentifying the correct key, inserting it into the lock and turning the key. Importantly though, under normal conditions, the repetitive experience of any given reward should mediate a process called reward-based learning or 'reward conditioning'. As explained later in this paragraph, this process prevents further reward-seeking behaviour and is pivotal to the normal day-to-day functioning of any human being (Schultz, 2002).

The anticipation and appraisal of reward is closely correlated with dopaminergic signalling in the brain (Ljungberg *et al.*, 1991; Mirenowicz and Schultz, 1994; Schultz *et al.*, 1993). During the initial anticipation of a novel reward, roughly 75% of the dopaminergic neurons in the basal ganglia are activated – most notably those forming the nigro-striatal projection (Ljungberg *et al.*, 1991; Mirenowicz and Schultz, 1994). Over time, with repetitive exposure to the same reward, the brain becomes conditioned to the reward through the complex association thereof with different environmental, physiological, and circumstantial factors. Reward conditioning enables the brain to evaluate future confrontations with the same set of factors it was conditioned to, and to associate it with an applicable reward, even before the reward has been presented (Romo and Schultz, 1990). Once the reward is presented, the subsequent reaction of the brain can manifest as either one of the following: 1) if the reward is greater than that anticipated or

totally unpredicted, dopaminergic signalling is elicited; 2) if the reward has been predicted in full, no dopamine response is elicited, and 3) if the anticipated reward was omitted a suppression in dopaminergic signalling is induced (Schultz *et al.*, 1997).

Following dopamine response after actual exposure to the reward, the basal ganglia codes a 'reward prediction error' – a term used to represent the difference between the predicted and actual reward (Schultz *et al.*, 1997). The reward prediction error is pivotal to the process of reward-based learning, as it is used to compute the changes that must be implemented in future to either keep experiencing the same reward in the case of a positive error, or experiencing a better reward in the case of a negative error. It can be postulated that the lack of a significant dopaminergic response after the manifestation of a fully predicted reward, may account in part for finding closure after task completion, as the lack of a dopamine response will not induce further reward-seeking behaviour.

The effects of dopamine on the various post-synaptic neurons can be separated into tonic and phasic aspects (Schultz, 2002). In the striatum, sustained low extracellular concentrations of dopamine (5 – 10 nM), as regulated in a narrow band by different factors such as reuptake transport, negative feedback control of synthesis, and release via autoreceptors, stimulates the high affinity $D_2$ receptors, preferentially expressed on the neurons originating in the indirect pathway (Richfield *et al.*, 1989). When a reward is predicted though, the striatal release of dopamine increases up to 40 fold (peaking at 150 – 400 nM), which permits the stimulation of the low affinity $D_1$ receptors expressed in the direct pathway (Richfield *et al.*, 1989). This transient increase in dopamine temporarily shifts the balance to the direct pathway, resulting in thalamic-cortical activation.

* * *

Although the matters of dopamine origin in the basal ganglia and the factors triggering dopaminergic signalling are now clearer, it is still not known how only a certain motor task is being executed and how a total increase in general motor activity following the dopaminergic activation of the thalamus, is suppressed. The answer to this question lies in the fact that most nigro-striatal dopaminergic afferents converge with glutamatergic corticofugal afferents on the individual GABAergic neurons of the striatum, as stated earlier in this paragraph. In addition, as it is evident from paragraph 2.2.1.1, the frontal cortex plays a central role in the regulation of cognition, behaviour and reward based learning. For a simplistic explanation of the cross talk of dopamine and glutamate and how it influences striatal GABAergic neurons, it can be assumed that an increase in dopaminergic neuron activity represents the generation of a reward predic-

tion error during the appraisal of a given reward. At the same time, some corticofugal glutamatergic afferents code another aspect of the same reward, for instance its sensory modality, colour, or taste, while other cortical inputs related to different events or experiences not applicable during this specific situation, are inactive (Schultz, 2002). Thus, in the theoretical absence of corticofugal glutamatergic signalling in the striatum, a transient increase in dopamine would have led to a global increase in motor activity due to the thalamic-cortical activation via the direct pathway. However, due to the selective activity of the corticofugal afferents in the striatum in specific reward-related situations, only the striatal neurons simultaneously activated by dopamine *and* glutamate will be influenced (Schultz, 2002), thus engendering a specific and selected motor response.

Patients diagnosed with OCD clearly do not find closure after task completion. As they constantly exhibit repetitive compulsive behaviour of some nature, it can be hypothesized that, concerning the processes of reward appraisal and reward-based learning, a dysfunctional reward system may be central to the pathology of OCD (Husted *et al.*, 2006).

* * *

Dopamine exerts its effects via two main classes of metabotropic receptors, namely $D_1$-like receptors ($D_1$ and $D_5$) and $D_2$-like receptors ($D_2$, $D_3$, and $D_4$) (Vallone *et al.*, 2000).

***The metabotropic $D_1$ and $D_5$ receptors.*** The $D_1$-like receptors generally stimulate $G_s$-coupled membrane receptors resulting in elevated intracellular cAMP and protein kinase A (PKA) levels (Jackson and Westlind-Danielsson, 1994; Monsma Jr. *et al.*, 1990; Sherrington *et al.*, 1993). The $D_1$ receptor is the most widespread of dopaminergic receptors in the brain and $D_1$ receptor mRNA expression has been demonstrated in both the striatum and the thalamus (Dearry *et al.*, 1990; Fremeau Jr. *et al.*, 1991). Although $D_1$ receptors have also been located in the GP and SNr, $D_1$ mRNA has not been detected in these localities, a demonstration of the fact that the $D_1$ receptors expressed in these regions are only present on the GABAergic striatal projections (Le Moine *et al.*, 1991). As explained earlier, the most important function of the $D_1$ receptor with respect to the neurobiology of OCD is the mediation of GABA release from the neurons forming the direct pathway through the CSTC circuit, resulting in thalamic-cortical activation (Gerfen *et al.*, 1990; Tepper *et al.*, 1997). $D_5$ receptors are expressed in a number of brain areas that are not directly implicated in OCD (Jaber *et al.*, 1996), and will therefore not be discussed in more detail.

***The metabotropic $D_2$, $D_3$, and $D_4$ receptors.*** The $D_2$-like receptors are generally associated with the inhibition of adenylyl cyclase and a subsequent decrease in intracellular cAMP levels via the stimulation of $G_i$-coupled membrane receptors (Jaber *et al.*, 1996; Jackson and Westlind-Danielsson, 1994; Vallone *et al.*, 2000). The $D_2$ receptor has a long and a short isomer and although both inhibit the activity of adenylyl cyclase, the short isomer does so to a greater extent and needs a lower dose of agonist to induce half-maximal inhibition (Hayes *et al.*, 1992; Montmayeur and Borrelli, 1991; Montmayeur *et al.*, 1993). Although the inhibition of adenylyl cyclase via the stimulation of $D_3$ and $D_4$ receptors has also been demonstrated in certain cell lines (Jaber *et al.*, 1996), the same effect could not be demonstrated in many others. Whether these two receptors have any role to play in the pathology of OCD remains to be elucidated. The fact that certain atypical antipsychotics like risperidone demonstrate clinical effect when combined with a SSRI in augmentation strategies (Erzegovesi *et al.*, 2005; Fineberg and Craig, 2007; Sareen *et al.*, 2004), do not suffice to answer this question. Most dopaminergic agonists and antagonists, although they demonstrate specificity for either one of the two families, do not clearly discriminate between the different receptor subtypes of the $D_1$-like and $D_2$-like families respectively (Vallone *et al.*, 2000). Concerning the functions of the $D_2$ receptor with respect to OCD, the most notable is its suppression of GABA release from the neurons in the indirect pathway of the CSTC circuit, resulting in the activation of the thalamus (Gerfen *et al.*, 1990; Tepper *et al.*, 1997).

#### *2.2.2.4. Serotonin and the serotonergic system – a balancing act par excellence*

At this point, it is clear that although GABA, glutamate, and dopamine each has a certain putative role to play in the neurobiology of OCD, probably none of them is as clinically important as serotonin (5-hydroxytryptamine, 5-HT). The fact that only drugs targeting the serotonergic system are proven to be consistently successful in alleviating the symptoms of OCD in the majority of patients (Fineberg and Craig, 2007; Grados and Riddle, 2001; Jenike, 1993; Stein, 2002; Vythilingum *et al.*, 2000), implicates a central role for serotonin in the pathology of obsessive-compulsive behaviour. However, whether serotonin (or the physiological shortage thereof) is the etiological instigator of obsessive-compulsive behaviour, is not known (Fineberg and Craig, 2007; Goddard *et al.*, 2008; Husted *et al.*, 2006; Markarian *et al.*, 2010).

When reviewing the available literature on serotonin and its functioning in the central nervous system, it becomes clear that in itself the serotonergic system is very complex. Given the vast distribution of serotonin in the central nervous system (Barnes and Sharp, 1999; Hayes and Greenshaw, 2011), and the relative lack of information regarding its global functioning as a regulatory neurotransmitter (Barnes and Sharp, 1999; Cools *et al.*, 2008; Daw *et al.*, 2002; Hayes

and Greenshaw, 2011), our understanding of this neurotransmitter and its functioning still leaves us with more questions than answers. This void in our knowledge of serotonin can essentially be attributed to four factors. Firstly, unlike GABAergic, glutamatergic, and dopaminergic signalling which is mostly found in certain distinct brain areas only, serotonergic signalling has been demonstrated globally across the central nervous system and thus it is evident that it influences a great number of processes and functions (Barnes and Sharp, 1999; Hayes and Greenshaw, 2011). Secondly, unlike the receptors for GABA, glutamate, and dopamine, of which each can generally be localized on certain nerve bundles only (for example $D_1$ receptors being expressed mainly in, among a few other locations, the direct pathway in the basal ganglia and $D_2$ in the indirect pathway), each serotonin receptor can in many instances be found at a variety of locations (Barnes and Sharp, 1999; Galvan *et al.*, 2006; Gerfen *et al.*, 1990; Jackson and Westlind-Danielsson, 1994; Le Moine *et al.*, 1991; Sherrington *et al.*, 1993; Tepper *et al.*, 1997). Also unlike most of the receptors for GABA, glutamate, and dopamine, certain serotonin receptors can be found at pre- and post-synaptic locations in different regions of the brain, each receptor subsequently being able to exert different location-dependant effects (Barnes and Sharp, 1999). Thirdly, serotonin has been demonstrated to influence the release and transmission of more or less every other major neurotransmitter, including GABA, glutamate, dopamine, acetylcholine, and noradrenalin (Barnes and Sharp, 1999; Hayes and Greenshaw, 2011), which complicates the matter of whether it plays a direct or indirect role in the mediation of certain functions that are currently attributed to be the consequence of serotonergic action. Lastly, also in contrast to most of the other neurotransmitters, fourteen different receptors for serotonin have been identified of which at least twelve have been shown to exert functional effects in physiological systems (Barnes and Sharp, 1999; Hannon and Hoyer, 2008; Hayes and Greenshaw, 2011; Hoyer *et al.*, 2002). In addition, no ligand has been developed for many of these receptors yet (Barnes and Sharp, 1999; Hayes and Greenshaw, 2011). This slow development of highly receptor-selective agents has delayed research regarding the specific neurophysiological effects of each receptor. A more detailed discussion on the serotonergic receptors can be found later in this paragraph.

In this section, a summary of what is known about this neurotransmitter and its functioning is given. This could form a framework to aid in a better understanding of the role that serotonin may play in the pathology of OCD. First, the serotonergic system as a whole as well as its postulated functioning in behavioural and emotional processing will be reviewed. Secondly, the focus will move to the individual serotonergic receptors and the neurobiological and functional significance of each.

* * *

Serotonin has been implicated in a number of behavioural phenomena including impulse control abnormalities, obsessions, anxiety, mood disturbances, hallucinations, and eating disorders (Barnes and Sharp, 1999; Cools *et al.*, 2008; Daw *et al.*, 2002; Deakin *et al.*, 1991; Goddard *et al.*, 2008; Hayes and Greenshaw, 2011; Hoyer *et al.*, 2002). Also, in certain cases of patients diagnosed with impulse control disorders such as aggressive behaviour following alcohol abuse, decreased levels of serotonin have been demonstrated in the CNS (Coccaro, 1989; Evenden, 1999; Linnoila and Virkkunen, 1992). However, contradictory findings have complicated the unravelling of its role in each of these conditions. For example, while the benzodiazepines alleviate the symptoms of anxiety, they also decrease the release of serotonin (Deakin *et al.*, 1991). On the other hand, long-term use of drugs like the SSRIs which increase serotonergic neurotransmission, have also been shown to be effective in the treatment of anxiety, albeit in higher doses than those used for depression (Hollander, 1998).

The fact that behavioural inhibition has been associated with serotonergic neurotransmission (Cools *et al.*, 2008; Daw *et al.*, 2002) provides a possible explanation for the ameliorative effects observed when treating these conditions with drugs enhancing serotonergic functioning. In most of these conditions, dopamine is a major role player and thus a number of authors propose a role for the serotonergic system to act as the behavioural opponent of dopamine (Cools *et al.*, 2008; Daw *et al.*, 2002; Deakin *et al.*, 1991; Fletcher and Korth, 1999; Fletcher *et al.*, 1999). In a comprehensive review of the opponent interactions between serotonin and dopamine (Daw *et al.*, 2002), the authors use the term 'opponency' to describe a situation in which more than one system code for different affective events. As such, it has been demonstrated that the dopaminergic system codes for rewarding stimuli and that it is crucial for reward-based learning (for a review on dopamine and reward, refer to paragraph 2.2.2.3), while serotonin is activated during the experience of aversive stimuli (Daw *et al.*, 2002; Fletcher, 1995; Fletcher and Korth, 1999; Fletcher *et al.*, 1999; Kapur and Remington, 1996). Indeed, work undertaken in our laboratory using an animal model of posttraumatic stress disorder has clearly demonstrated the central role for serotonin in the bio-behavioural response to aversive stimuli (Harvey *et al.*, 2003; Harvey *et al.*, 2006), as well as its response to serotonergic agents (Harvey *et al.*, 2004; Uys *et al.*, 2006). Also, in a number of studies undertaken by Fletcher and colleagues (Fletcher *et al.*, 1993; Fletcher, 1995; Fletcher *et al.*, 1999), the authors have broadly shown that enhancing serotonergic signalling suppresses both conditioned behaviours (such as lever pressing for food) and unconditioned behaviours (such as feeding) which is normally associated with an activation of dopaminergic signalling. Consequently, when antagonizing serotonin or agonizing dopamine, the opposite effect is mediated. This also corresponds with data demonstrating

that serotonin antagonizes the effects of dopamine in the substantia nigra and striatum (Kapur and Remington, 1996).

Daw *et al* (2002) hypothesizes that if dopamine is responsible for approach behaviour, motor excitement, and reward processing, serotonin will be associated with avoidance behaviour, motor suppression, and the processing of aversive or punishing stimuli. In different terms, it can be understood that the balance between reward seeking behaviour and aversive reactions is related to the balance between dopaminergic and serotonergic signalling respectively (Daw *et al.*, 2002; Deakin *et al.*, 1991). A number of studies done in animals support this hypothesis and show that enhancing serotonergic transmission, broadly reduces reward seeking behaviour (Harrison *et al.*, 1999; Harrison and Markou, 2001; Higgins and Fletcher, 2003; Parsons *et al.*, 1998). However, in direct contrast to this hypothesis, it has been postulated that patients with depression express a bias in favour of the processing of negative, instead of positive stimuli as a result of the relative *lack* of sufficient serotonergic signalling demonstrated in this condition (Cools *et al.*, 2008; Murphy *et al.*, 2002). Thus, serotonin may be involved in the mediation of contrary behaviours, namely the preservation of a normal mood (euthymia) and the suppression of reward-based behavioural responses. Deakin and colleagues (1991) attempted to resolve this paradox by explaining that the serotonergic projections from the dorsal and median raphe nuclei respectively, are responsible for different cognitive and behavioural functions. The authors explained this by implicating the serotonergic projection from the dorsal raphe nuclei to the amygdala and basal ganglia to be the opponent of dopamine functioning, while the projection from the median raphe nuclei is postulated to play a role in the pathology of mood disorders such as depression.

In summary, it is possible that serotonin will affect diverse neural systems via different serotonergic projections and a number of different serotonergic receptors. Taken from paragraph 2.2.2.3, an abnormal reward appraisal system may be central to the pathology of OCD. As OCD patients generally do not find closure after task completion, it is hypothesized that the dopaminergic response following the presentation of a given reward, is always of the same magnitude and that reward based learning does therefore not occur (refer to paragraph 2.2.2.3).

* * *

Although much regarding the role of serotonin as a global regulator of cognition and behaviour is still lacking, another way in which to probe its functioning may be to focus on its receptors and their individual physiological effects. The main advantage of studying the serotonin receptors is that it sheds more light on the region-specific functions of the neurotransmitter,

especially in conditions such as OCD which are associated with the abnormal functioning of certain distinct brain areas only, namely the prefrontal cortex and basal ganglia (refer to paragraph 2.2.1).

Serotonin mediates its effects via the stimulation of seven classes of receptors, denoted $5HT_1$ – $5HT_7$. As a result of modern cloning techniques, fourteen different receptors have been identified and grouped based on their structural, transductional, and functional characteristics (Barnes and Sharp, 1999; Hoyer *et al.*, 2002). Currently the Receptor Nomenclature Committee of the International Union of Pharmacology (NC-IUPHAR) classifies the different receptors as reviewed in Table 2-2 (see following page) (Hannon and Hoyer, 2008).

* * *

***The $5HT_1$ receptor class.*** Referring to Table 2-2, five $5HT_1$ receptors have been indentified up to date. With the exception of $5ht_{1e}$ (refer to Table 2-2 for an explanation of lower case letters), all of these receptors have been located in the human brain where they also have shown to exert functional effects. As the $5HT_{1C}$ receptor was found to share more functional and transductional similarities with the $5HT_2$ receptor class it was aptly renamed $5HT_{2C}$ (Hannon and Hoyer, 2008; Hoyer *et al.*, 2002). The $5HT_1$ receptor family mainly functions via coupling of adenylyl cyclase to $G_{i/0}$ thereby inhibiting the formation of cAMP resulting in cellular hyperpolarization (Barnes and Sharp, 1999; Hoyer *et al.*, 2002).

| Serotonin receptor class | Neurophysiological effect | Receptor subtypes |
|---|---|---|
| **$5HT_1$** | $G_{i/0}$ (decrease cAMP) | $5HT_{1A}$<br>$5HT_{1B}$<br>$5HT_{1D}$<br>$5ht_{1e}$<br>$5HT_{1F}$ |
| **$5HT_2$** | $G_q$ (increase $IP_3$/DAG) | $5HT_{2A}$<br>$5HT_{2B}$<br>$5HT_{2C}$ |
| **$5HT_3$** | Opening of cation channels (mainly $Na^+$, $Ca^{2+}$, and $K^+$) | - |
| **$5HT_4$** | $G_s$ (increase cAMP) | - |
| **$5ht_5$** | Probably $G_i$ (decrease cAMP) | $5ht_{5a}$<br>$5ht_{5b}$ |
| **$5HT_6$** | $G_s$ (increase cAMP) | - |
| **$5HT_7$** | $G_s$ (increase cAMP) | - |

***TABLE 2-2 – THE CURRENT CLASSIFICATION OF THE 5HT RECEPTORS***

*(Lower case representation designate receptors for which no physiological functions have been identified yet)*

*$5HT_{1A}$ receptors* are found throughout the brain but are highly expressed in the raphe nuclei, limbic structures such as the hippocampus, and the anterior cingulate cortex (Barnes and Sharp, 1999; Hannon and Hoyer, 2008; Hoyer *et al.*, 2002). In the raphe nuclei, they are located pre-synaptically and function mainly to suppress serotonergic outflow to the cortex. In the frontal cortex and limbic areas they are expressed post-synaptically and their actions result in the inhibition of neuronal activity (Barnes and Sharp, 1999; Sprouse and Aghajanian, 1988). It is not found in the basal ganglia (Barnes and Sharp, 1999). Importantly, it has been shown that administering the selective $5HT_{1A}$ antagonist, WAY 100 635, results in a stimulatory effect on serotonergic cell firing, suggesting that the $5HT_{1A}$ receptor might be under the influence of tonic stimulation (Fornal *et al.*, 1996). In addition, administering $5HT_{1A}$ antagonists, strengthens the effects of the SSRIs, TADs, and venlafaxine on serotonergic neurotransmission, most probably because it prevents the $5HT_{1A}$ induced negative feedback on serotonin release resulting from an acute rise in serotonin following the administration of these drugs (Barnes and Sharp, 1999). However, in a study done by Ceglia and colleagues (2004), the authors demonstrate that chronic treatment with the SSRIs, escitalopram and citalopram, only partially desensitize $5HT_{1A}$ autoreceptors. They conclude by stating that the desensitization of $5HT_{1A}$ autoreceptors may only play a small role in the long-term clinical efficacy of the SSRIs, although it is possible that the administration of $5HT_{1A}$ antagonists may quicken the onset of action of the SSRIs. Curiously though, by acting on heteroreceptors $5HT_{1A}$ agonists exert a stimulatory effect on the release of acetylcholine and noradrenalin, especially in the hippocampus and frontal cortex of rats and guinea pigs

(Done and Sharp, 1994; Wilkinson *et al.*, 1994). The behavioural effects of the $5HT_{1A}$ receptor have been well documented (Barnes and Sharp, 1999; Hannon and Hoyer, 2008; Hayes and Greenshaw, 2011; Heisler *et al.*, 1998; Hoyer *et al.*, 2002; Parks *et al.*, 1998). $5HT_{1A}$ receptor knockout (KO) mice, which exhibit an increase in serotonergic outflow from the raphe nuclei, present with increased levels of anxiety and show antidepressant like behaviour in a number of behavioural paradigms. These findings are in accordance with clinical evidence that demonstrates the same behaviour in humans associated with increased and decreased levels of serotonin respectively (Deakin *et al.*, 1991; Murphy *et al.*, 2002; Murphy *et al.*, 2002). With regards to reward-related behaviour it has been demonstrated that low dose treatment with $5HT_{1A}$ agonists such as 8-OH-DPAT increases behavioural responses to reward, while high doses exert the opposite effect (Harrison and Markou, 2001; Papp and Willner, 1991). The facilitation of reward-related behaviour is postulated to be a consequence of decreasing serotonergic outflow from the raphe nuclei, while the opposite effect observed with high dose treatment is thought to result from the stimulation of post-synaptic $5HT_{1A}$ receptors. Another important set of behaviours associated with the stimulation of *post-synaptic* $5HT_{1A}$ receptors are hyperphagia, hypothermia, altered sexual behaviour and the tail flick response (Lucki, 1992; Millan *et al.*, 1991). In conclusion, the $5HT_{1A}$ receptor may be an important target for future clinical trials in the treatment of OCD, as the slow onset of action of SSRI-treatment in OCD poses a clinical dilemma.

The *$5HT_{1B/D}$ receptors* have originally been regarded as the rodent and non-rodent homologues of the same receptor, as they share 97% amino acid sequence homology (Hartig *et al.*, 1996; Hoyer *et al.*, 2002). Both of these receptors have since been demonstrated in rodents and humans and the receptor nomenclature has been changed to align their animal and human denominations (Hartig *et al.*, 1996). Although the $5HT_{1B}$ receptor is expressed in far greater concentrations than the $5HT_{1D}$ receptor, both are found pre- and post-synaptically in the basal ganglia, striatum, frontal cortex, hippocampus, and hypothalamus and functions to decrease the release of neurotransmitters from nerve terminals and cell bodies in these areas (El Mansari and Blier, 2006; Hoyer *et al.*, 2002). Interestingly, administering $5HT_{1B}$ antagonists does not intensify the increase in prefrontal cortical serotonin levels following the acute administration of SSRIs (Barnes and Sharp, 1999). However, after 8-week treatment with the SSRI paroxetine, an increase in electrically evoked serotonin release from the OFC, but not from the caudate nucleus, has been demonstrated and thought to be the direct result of the desensitization of terminal $5HT_{1D}$ receptors in the OFC (El Mansari and Blier, 2006). The fact that striatal $5HT_{1D}$ receptor sensitivity remained unaltered is interesting. Moreover, $5HT_{1D}$ receptors in the hippocampus and hypothalamus desensitizes only after three weeks of administering SSRI-treatment, implicating different location-specific mechanisms of $5HT_{1D}$ receptor function (Blier *et al.*, 1996; El

Mansari and Blier, 2006). In a study done by Shanahan and colleagues (2009), the authors provide evidence to implicate $5HT_{1B}$ receptor activation in perseverative locomotor paths and that this behaviour is only attenuated after chronic, but not sub-chronic, treatment with SSRIs. These findings could possibly relate to those of Blier (Blier *et al.*, 1996), demonstrating terminal $5HT_{1B}$ autoreceptor desensitization only after 8 weeks of SSRI-treatment, as discussed above. Although the $5HT_{1B}$ receptor is also expressed presynaptically on afferent terminals to the dorsal raphe nuclei, it does not seem to influence the serotonergic outflow from this area (Barnes and Sharp, 1999). Evidence exists that demonstrates a function for the $5HT_{1B}$ receptor as a heteroreceptor in the regulation of acetylcholine, glutamate, GABA, noradrenalin, and dopamine release (Pauwels, 1997). It has been reported that $5HT_{1B}$ agonists indirectly stimulate frontal cortical and nigral dopamine release via the disinhibition of GABAergic innervation of the dopaminergic terminals resulting from a decrease in GABA release (Johnson *et al.*, 1992). Interestingly, $5HT_{1D}$ receptors, but not $5HT_{1B}$ receptors, have also been located on cell bodies that originate in the dorsal raphe nuclei, but due to the low levels of its expression, the functional significance of its actions in this location is unknown (Sanchez-Alavez *et al.*, 2001). $5HT_{1B/D}$ receptors are also expressed on cerebral arteries and sumatriptan, a non-selective $5HT_{1B/D}$ agonist, exerts anti-migraine effects (Hoyer *et al.*, 2002). In the light of a number of studies which showed that administering $5HT_{1B/D}$ agonists at least did not exacerbate the symptoms of OCD (Bergqvist *et al.*, 1999; Boshuisen and Den Boer, 2000; Tsaltas *et al.*, 2005), investigating the role of either non-specific $5HT_{1B/D}$ antagonists or specific $5HT_{1B}$ and $5HT_{1D}$ antagonists in the treatment of OCD could produce valuable results.

Apart from the fact that no selective ligand for the *$5ht_{1e}$ receptor* has been developed yet, and although high expression of the *$5HT_{1F}$ receptor* has been demonstrated in the cortex, caudate nucleus, putamen and the cingulate cortex, very little data exist on the physiological functions of these two receptors (Hannon and Hoyer, 2008), and therefore they will not be discussed in more detail.

***The $5HT_2$ receptor class.*** All of the $5HT_2$ receptors preferentially stimulate $G_q$ resulting in the activation of phospholipase C and an excitatory post-synaptic potential via the increase of intracellular $IP_3$ and DAG (Barnes and Sharp, 1999; Hannon and Hoyer, 2008).

The *$5HT_{2A}$ receptor* is expressed in among other locations, the cerebral cortex, caudate nucleus, nucleus accumbens and hippocampus (López-Giménez *et al.*, 1997). In the cortex it is expressed on GABAergic interneurons while it is also localized on glutamatergic cortico-striatal efferents (Barnes and Sharp, 1999; Sheldon and Aghajanian, 1991). Two characteristic behavioural traits of the $5HT_{2A}$ receptor are the mediation of wet-dog shakes in rats and head twitch-

ing in mice (Schreiber *et al.*, 1995). The $5HT_{2A}$ receptor also probably has hallucinogenic properties as the affinity of $5HT_{2A}$ agonists strongly correlate with their hallucinogenic potency (Glennon, 1990). The fact that most atypical antipsychotics such as olanzapine, quetapine, and risperidone block $5HT_{2A}$ and $D_2$ receptors complicates investigations into the hallucinogenic properties of $5HT_{2A}$ receptor stimulation, as it may exert this effect indirectly via cross-talk with dopamine (Hoyer *et al.*, 2002). Although some authors propose a role for $5HT_{2A}$ receptor stimulation as a mediator of the anti-compulsive effects of the SSRIs (Fineberg *et al.*, 2010), this is controversial as atypical antipsychotics block the $5HT_{2A}$ receptor and are used in augmentation strategies in patients refractory to SSRI monotherapy (El Mansari and Blier, 2006; Fineberg, 2004).

*$5HT_{2B}$ receptors* are mostly localized in the heart, kidneys, and the fundus of the stomach. In the central nervous system, it is expressed in the cerebellum, hypothalamus and amygdala (Hoyer *et al.*, 2002). Direct administration of $5HT_{2B}$ agonists in the amygdala has been associated with anxiolytic effects in rats (Duxon *et al.*, 1997).

Extensive mapping of the *$5HT_{2C}$ receptor* has demonstrated its expression in the prefrontal cortex, limbic system and the basal ganglia, most notably in the substantia nigra and caudate nucleus (Barnes and Sharp, 1999; Sheldon and Aghajanian, 1991). The activation of $5HT_{2C}$ receptors are associated with hypolocomotion, hypophagia, anxiety, and penile erections (Koek *et al.*, 1992). Importantly, $5HT_{2C}$ receptor activation results in a tonic inhibition of dopaminergic, but not serotonergic signalling (Millan *et al.*, 1998). The $5HT_{2C}$ receptor is believed to be the *major mediator* of the opponent actions of the serotonergic system on reward-seeking behaviour (Higgins and Fletcher, 2003) and may pose as a possible target for the development of novel drugs for the treatment of OCD. In line with this, Chou-Green and colleagues (Chou-Green *et al.*, 2003) have demonstrated compulsive-like behaviour in the $5HT_{2C}$ receptor KO mouse.

***The $5HT_3$ receptor class.*** The main difference between the $5HT_3$ receptor and its other serotonergic stable mates is the fact that $5HT_3$ receptors act as non-selective ligand gated cation channels that facilitate rapid depolarization via the influx of $Na^+$ and $Ca^{2+}$ and efflux of $K^+$ ions. $5HT_3$ receptor expression has been demonstrated especially in the hippocampus, the dorsal motor nucleus of the solitary tract, and the area postrema (Hoyer *et al.*, 2002). Although $5HT_3$ receptor activation in the brain leads to increased dopamine release, no clinical trials have been done to assess the behavioural significance of this interaction (Hannon and Hoyer, 2008; Hayes and Greenshaw, 2011). The main functions of the $5HT_3$ receptors seem to be mostly limited to the enteric nervous system where receptor activation results in the conveyance of enteric stimuli to the central nervous system. $5HT_3$ antagonists have since been developed for the treat-

ment of chemotherapy-induced nausea and vomiting (Hannon and Hoyer, 2008; Hoyer *et al.*, 2002).

***The $5HT_{4/6/7}$ receptor classes.*** As these three subtypes of serotonergic receptors all bind preferentially to $G_s$ resulting in increased intracellular cAMP levels, they will be discussed in more detail as one group.

*$5HT_4$ receptors* are mainly expressed in the nigro-striatal and meso-limbic pathways of the brain (Barnes and Sharp, 1999; Hannon and Hoyer, 2008). High expression levels of the $5HT_4$ receptor have been demonstrated on striato-pallidal and striato-nigral efferents and evidence has been presented to indicate that $5HT_4$ receptors, although they are expressed in the striatum, are not present on dopaminergic nerve-terminals but on afferent neurons terminating in the striatum (Ullmer *et al.*, 1996). More specifically, activation of central $5HT_4$ receptors may increase the release of GABA and dopamine in the striatum (Steward *et al.*, 1996). A clinically important observation is that the administration of $5HT_4$ antagonists do not exert any behavioural effect, most probably due to a very low level of endogenous tone on $5HT_4$ receptors (Steward *et al.*, 1996). $5HT_4$ receptors are, like $5HT_3$ receptors, also localized in the enteric nervous system and function to increase smooth muscle tone and gastric emptying following receptor activation (Hoyer *et al.*, 2002).

Recently, the expression of *$5HT_6$ receptors* was mapped and it was subsequently demonstrated that this serotonergic receptor subtype is located in the striatum, amygdala, nucleus accumbens, hippocampus and cortex, with no expression in the peripheral tissues (Hannon and Hoyer, 2008). The main function of the $5HT_6$ receptor seems to be its regulation of cholinergic transmission, as $5HT_6$ antagonists not only increase the release of acetylcholine but also have positive effects on learning and memory (Meneses *et al.*, 2007).

Apart from its extensive localization on vascular and non-vascular smooth muscle (Hannon and Hoyer, 2008), the *$5HT_7$ receptor* is expressed in the central nervous system mainly in the thalamus, hypothalamus, and hippocampus (To *et al.*, 1995). Its main physiological function is believed to be the regulation of sleep and circadian rhythm (Barnes and Sharp, 1999; Hannon and Hoyer, 2008; Hayes and Greenshaw, 2011; Hoyer *et al.*, 2002). One study did however implicate the $5HT_7$ receptor in a model of compulsive-like marble-burying (Hedlund and Sutcliffe, 2007). The authors conclude that antagonizing the $5HT_7$ receptor in this model decreases spontaneous marble-burying and that the $5HT_7$ receptor could be a valuable target for the development of novel drugs for the treatment of OCD.

#### *2.2.2.5. Miscellaneous neurotransmitters and biological agents in the pathology of OCD*

Except for the major neurotransmitters discussed above, evidence has been presented to demonstrate a role for factors such as increased nitric oxide (NO) levels (Atmaca *et al.*, 2005), the circadian effects of the female hormonal cycle (Abramowitz *et al.*, 2003), an altered oxidative status (Güldenpfennig *et al.*, 2011), and decreased nocturnal growth hormone secretion (Kluge *et al.*, 2006) in the pathology of OCD.

Indeed, considering the central role for the glutamate NMDA receptor in the CSTC circuit and in OCD (refer to paragraph 2.2.2.1), it is not surprising that NO, a central sub-cellular messenger for the NMDA receptor (Frade *et al.*, 2009; Zomkowski *et al.*, 2010), has been implicated in OCD. Therapies effective in the management of OCD such as the SSRIs and $D_2$ receptor antagonists also reduce the synthesis of NO (Almeida *et al.*, 2006; Harkin *et al.*, 2004; Nel and Harvey, 2003; Wegener *et al.*, 2003). Furthermore, it has been demonstrated that the cyclic secretion of ovarian hormones may influence the expression of OCD symptomology (Abramowitz *et al.*, 2003), while the administration of GnRH analogues have been associated with an amelioration of OCD symptoms (Eriksson, 2000), most probably due to the decrease in androgenic hormones. A number of studies also implicate a role for oxidative stress in the pathology of OCD (Behl *et al.*, 2010; Chakraborty *et al.*, 2009a; Chakraborty *et al.*, 2009b; Ozdemir *et al.*, 2009). *N*-acetylcysteine (NAC), an antioxidant, has been demonstrated to be effective in some patients with OCD that do not respond to the first-line treatment options (Lafleur *et al.*, 2006). The mechanism of action underlying the ameliorating effects of NAC on OCD symptomology may be twofold. In addition to its antioxidant effects, it also inhibits the release of glutamate via the indirect stimulation of presynaptic mGlu2 and mGlu3 receptors, thereby again implicating the glutamatergic system. One report demonstrated that patients with OCD, secrete substantially less growth hormone during the onset of sleep when compared to healthy controls (Kluge *et al.*, 2006). The authors did not however discuss the basis of the observed association between OCD and growth hormone, and the results have yet to be confirmed in different studies. Myo-inositol (MI) is another biological agent that has been implicated in the pathology of OCD. Four studies have been published reporting conflicting results regarding its successful application as a possible treatment for OCD (refer to Camfield *et al.*, 2011 for a review). Although Harvey and colleagues (2001; 2002) demonstrated that MI may up-regulate both $5HT_2$ and $D_2$ receptor densities in the striatum, the possible mechanisms underlying its therapeutic effects in OCD remains to be elucidated. Interestingly, based on neuroimaging studies, Carey and colleagues (2004) suggest that MI acts via a different pathway within the CSTC circuit than do the SSRIs.

The evidence implicating these miscellaneous neurotransmitters in the pathology of OCD has been mostly generated by initial observations in case reports. While these mechanisms are implicated in OCD, it remains to be confirmed whether these changes are causal or secondary to a more central neurotransmitter dysfunction. As noted earlier, NO is a direct sub-cellular target for glutamate NMDA receptor function so that any change in glutamate activity will very likely result in altered NO levels. The fact that the mainstay of treatment in OCD patients is based on the targeting of the serotonergic and dopaminergic systems indicates that most of these factors are probably being brought into play as a consequence of serotonin, dopamine and/or glutamate dysfunction. Nevertheless it remains imperative that these leads be followed up in order to obtain a more global view of the neurobiology of the disorder and possibly to identify new targets for treatment.

## 2.3. DESIGNING ANIMAL MODELS OF OCD - A CONSTANT CONFRONTATION WITH THOUGHTFUL REPETITION

In sections 2.1 and 2.2 the pieces of the puzzle that will eventually be fitted together to perhaps give a better picture of OCD, was discussed. Cross-referencing to these two sections now become very important and it would be convenient for the reader to bookmark them for quick and easy references for the sections to follow. In short, as explained in the aforementioned two sections OCD is characterized not only by compulsions but also in most cases by disturbances in cognition such as obsessions. In the following paragraphs, it will become clear that although perseverative motor repetition, which may represent human compulsions, is a common behavioural phenotype in animals, it is difficult to associate this behaviour with cognitive deficits. In animal models of OCD this association is very important, as it differentiates normal repetitive stereotypy from compulsive-like motor repetition.

When considering the development of a suitable animal model of OCD, a few important questions need to be borne in mind: First, what are the core symptoms of a condition that the putative animal model is required to display? Second, which treatment options should be considered once the appropriate symptomology has been described? Third, which neurophysiological targets should be scrutinized for similarities between the animal model and the human condition? In essence, these three fundamental questions constitute the foundation of behavioural research in animals and can otherwise be conceptualized as face, predictive and construct validity, respectively (van der Staay, 2006). Subsequently, the importance of each of these validation criterions as they apply to the development of a suitable animal model of OCD will be discussed.

* * *

### *2.3.1. Repetitive behaviour – corner stone for establishing face validity for OCD*

OCD patients typically experience constant recurrent thoughts or compulsive-like repetition of certain actions and as such repetitive (and perseverant) behaviour is central to the manifestation of OCD symptomology. Although the repetition of behavioural sequences forms part of normal human behaviour, under certain conditions and pathological states it may become abnormally frequent, rigid and resistant to change, purposeless, and relatively mono-dimensional – the four principles that collectively define the term stereotypy (Garner and Mason, 2002; Langen *et al.*, 2011b). For example, locking doors and brushing teeth are normal daily behaviour of a ritualistic nature, but in patients with OCD, it may become a pathological manifestation resulting in major functional impairment. Applying the four principles of stereotypy to strengthen the

face validity of a given animal model of OCD seems to be uncomplicated, as compulsive-like repetition of certain behavioural sets is relatively easy to model in animals. Consequently, all the animal models of OCD currently at our disposal are based foremost on the demonstration of some sort of stereotypical behaviour (Albelda and Joel, In Press (a); Fineberg *et al.*, 2010; Joel *et al.*, 2008; Korff and Harvey, 2006; Wang *et al.*, 2009) (refer to paragraph 2.3.4 for a more detailed discussion on the major current animal models for OCD). However, great care is to be taken if stereotypical behaviour forms the only strength of a putative animal model of OCD, as pathological motor repetition is a diagnostic criterion for a number of human disorders as well as a common behavioural manifestation in many different animal species housed under confined circumstances (Eilam *et al.*, 2006). In addition, if motor stereotypy can be described as lower order repetition, most OCD patients also present with higher order repetitions such as recurrent thoughts or obsessions (Abramowitz *et al.*, 2003; Bartz and Hollander, 2006; du Toit *et al.*, 2001; Nestadt *et al.*, 2001; Rasmussen *et al.*, 1994; Stein, 2002). Although it is just as important as the demonstration of motor stereotypy, these types of repetitions involve cognitive abnormalities and are very difficult to model, as this by implication would suggest that an animal is obsessive. An animal model of OCD therefore needs to distinguish between the stereotypy expressed in OCD patients, and the various forms of stereotypy observed in other disorders. In addition, in order to utilize repetitive animal behaviour as a means of establishing face validation in animal models of OCD, the development and manifestations of stereotypy in animals need to be explored (Eilam *et al.*, 2006).

#### *2.3.1.1. Stereotypy in humans – a common symptom of many comorbid conditions*

Motor stereotypy is a diagnostic criterion for a number of human conditions and, although the basal ganglia are involved in the pathogenesis of most forms of stereotypy (Garner and Mason, 2002; Haber and Calzavara, 2009; Joel and Weiner, 2000; Langen *et al.*, 2011a; Langen *et al.*, 2011b; Maia *et al.*, 2008), the various conditions respond differently to the available treatment options. As a result of this, a number of studies have attempted to elucidate the exact role of the basal ganglia in the etiology of the repetitive behaviour observed in conditions such as OCD, Tourette's syndrome, trichotillomania, and autism. Since then, three major functional loops linking the cortex and basal ganglia have been described (Groenewegen *et al.*, 2003; Langen *et al.*, 2011b). In short they are termed the sensorimotor loop (linking the sensory and motor cortices with the putamen), the associative loop (linking the dorsolateral prefrontal cortex with the basal ganglia), and the limbic loop (comprising the anterior cingulate and orbitofrontal cortices). The main function of each loop is believed to be the control or regulation of goal directed behaviour (sensorimotor), cognitive functioning (associative) and motivational behaviour (limbic) (Langen *et al.*, 2011b). If any success is to be gained during the development of an

animal model of OCD, the differences between the neurobiological and behavioural presentation of the stereotypies observed in some of the most prevalent conditions associated with motor repetition, need to be understood. Therefore, in the following few paragraphs a brief discussion on the stereotypies associated with Tourette's syndrome, autism and OCD respectively will be given.

*Tourette's syndrome* is characterized by verbal and motor tics (American Psychiatric Association, 2000). It mostly develops between 5 and 7 years of age, peaks in severity at age 12, and then gradually ameliorates during puberty. An inverse relationship has been demonstrated between symptom severity and the volume of the caudate nucleus and, although the condition is postulated to be of a hyper-dopaminergic nature due to its *favourable* response to dopaminergic antagonists, it can also be treated with the SSRIs (Langen *et al.*, 2011a; Makki *et al.*, 2008; Rasmussen *et al.*, 1994). Caudate involvement implicates the limbic loop and distinguishes the stereotypy observed in Tourette's from pure motor repetition.

Patients with *autism* present with three main symptoms namely stereotypy, non-stereotypical repetitive behaviour, and restricted interests (American Psychiatric Association, 2000). A number of studies have associated autism with abnormalities of the anterior cingulate and posterior parietal cortices (Shafritz *et al.*, 2008) and it has been demonstrated that patients with autism make more mistakes in experimental tasks and fail to distinguish between correct and incorrect responses compared to healthy control subjects (Thakkar *et al.*, 2008). Although the repetitive behaviour expressed in autistic patients respond to some degree to SSRIs (Kolevzon *et al.*, 2006), dopamine antagonists are still the mainstay of therapy (Barnard *et al.*, 2002).

Stereotypical behaviour in most patients with *OCD* involves motor and cognitive repetition (Abramowitz *et al.*, 2003; Bartz and Hollander, 2006; Rasmussen *et al.*, 1994; Stein, 2002; Tynes *et al.*, 1990). As reviewed in section 2.2, OCD has been associated with increased activity in the anterior cingulate and orbitofrontal cortices, and interestingly also in the caudate nucleus (Maia *et al.*, 2008; Maina *et al.*, 2001; Maltby *et al.*, 2005; Markarian *et al.*, 2010; Saxena and Rauch, 2000). The anterior cingulate and orbitofrontal cortices function as the cortical inputs of the limbic loop, while the caudate functions as the striatal entry point for the associative loop, implicating a possible role for the cross-talk between these two major pathways in the pathology of OCD. A number of studies using positron emission tomography (PET) have demonstrated decreased midbrain levels of the serotonin transporter (SERT) in patients with OCD compared to healthy controls (Hesse *et al.*, 2005; Reimold *et al.*, 2007; Zitterl *et al.*, 2008). Although studies done by Simpson (Simpson *et al.*, 2003) and Pogarell (Pogarell *et al.*, 2003) could not repli-

cate the latter findings, they did demonstrate that the pre-treatment SERT density in OCD-patients positively correlated with response to treatment with clomipramine, although this was inversely related to the severity of OCD symptomology (Zitterl *et al.*, 2008). In summary, it seems that the repetitive behaviour of OCD is unique in that it involves motor *and* cognitive repetition and responds mostly to monotherapy with the SSRIs and not atypical antipsychotics. Subsequently, these two parameters are the principle targets under consideration in an animal model of OCD.

#### *2.3.1.2. Stereotypy in animals – normal biology or abnormal pathology?*

Now that certain criteria have been established with respect to stereotypy in animals and how these can be explored for possible application in an animal model of OCD, it is now necessary to evaluate the different forms of stereotypy expressed in animals. Motor stereotypy is a common manifestation in many animal species and usually develops when wild animals are bred or kept in restricted and/or environmentally deprived habitats (Eilam *et al.*, 2006). However, it can also be induced by behavioural training and through pharmacological or genetic manipulation of the neuronal pathways implicated in repetitive behaviour, especially the cortico-striatal circuitry (Berridge *et al.*, 2005; Chou-Green *et al.*, 2003; Egashira *et al.*, 2008b; Joel and Avisar, 2001; Klavir *et al.*, 2009; Langen *et al.*, 2011a; Szechtman *et al.*, 1998; Szechtman *et al.*, 2001; Tsaltas *et al.*, 2005).

Many different theories have been put forward to explain the development of stereotypy resulting from environmental deprivation or so called cage stereotypy, including that a restrictive environment only allows a certain behavioural pattern to be executed (Ridley, 1994) or that the stress resulting from confinement induces abnormal repetitive motor behaviour (Langen *et al.*, 2011b). Cage stereotypy mostly manifests as stationary behaviour, in other words motor repetitions that are being executed only in a few preferred locations, and although it seems senseless (as do the compulsions in human OCD), it is not necessarily the case. In fact a number of studies have reported that while most OCD patients realize the futility of their actions, preventing the completion of these motor routines sometimes results in acute anxiety (Eilam *et al.*, 2006; Goodman *et al.*, 1989; Rapoport, 1989). It can thus be argued that at least in some patients, repetitive behaviour plays an anxiolytic role. Bearing this in mind, it would be valuable to assess the behavioural effects induced by the prevention of cage stereotypy in putative animal models of OCD. Still, most forms of cage stereotypy resemble one another through their rigid repetition of various motor routines. The question thus arises how can these behaviours be distinguished from one another? Referring back to paragraph 2.3.1.1, different forms of stereotypy are central to the diagnosis of each human condition. While rigid motor patterns without any evident cog-

nitive influence forms the core of stereotypical behaviour in conditions such as Tourette's syndrome, tics and autism, compulsive-like stereotypy is exhibited by OCD patients. Eilam and colleagues (2006) propose that compulsive-like repetitions can be distinguished from pure motor stereotypy based on the idea that compulsions are characterized by flexibility and thoughtfulness. They explain that in most cases, patients with OCD will not execute the compulsive behavioural routine if the environmental paradigm triggering it is not present (therefore the behaviour is regarded as flexible) and that the compulsions are mostly the result of obsessions or inappropriate thoughts. These two characteristics can be assessed in animals expressing repetitive behaviour, albeit only to a certain extent, as demonstrated in rats expressing compulsive like behaviour following treatment with quinpirole (a non-selective $D_{2/3}$ agonist) (Szechtman *et al.*, 1998; Szechtman *et al.*, 2001) (for a more detailed discussion on this specific model, refer to paragraph 2.3.4). These two proposed characteristics of compulsions could be employed to distinguish between rigid motor patterns and compulsive-like repetitive behaviour, both of which manifest as motor stereotypy. In summary, the spontaneous development of repetitions such as cage stereotypies can be a valuable way in which to investigate the etiological mechanisms behind the development of OCD.

The genetic manipulation of animals resulting in stereotypy, or the pharmacological modulation of certain receptors implicated in the pathology of stereotypy, provides one major advantage over cage stereotypy in that it induces repetitive behaviour as a direct consequence of the relevant manipulation. Thus, the roles of individual pathways and receptors in the development of stereotypy can be investigated and thus have great importance from a construct point of view. In other words, they can be used to study certain defined neurobiological underpinnings believed to mediate the presenting stereotypy. A major disadvantage of these models is that, as a direct consequence of this predetermined construct, they disregard the role of crosstalk between the different neuronal pathways and neurotransmitters in the development of OCD. For a complete discussion on animal models of OCD that have been developed based on genetic and pharmacological manipulation, refer to paragraph 2.3.4.

To summarize, stereotypical behaviour in itself may strengthen the face validity of an animal model of OCD, provided that it can be associated with either the demonstration of flexibility or thoughtfulness, or linked with good predictive and construct validity (refer to paragraphs 2.3.2 and 2.3.3) which could substantiate the type of stereotypy being expressed. Rigid repetition of specific motor patterns in itself cannot be regarded as compulsive behaviour.

### *2.3.2. A favourable response to SSRIs – the mainstay of predictive validity*

Although all the current animal models of OCD demonstrate various forms of repetitive behaviour, it is evident that these behaviours need to be associated with good predictive and construct validity, as stereotypy in itself is a common behavioural manifestation in animals. As described in section 2.1, it is clear that for an animal model of OCD to be credible, it must demonstrate selective response to the SSRIs, either as monotherapy, or in a combination with low-dose antipsychotics (Fineberg *et al.*, 2006; Grados and Riddle, 2001; Stein, 2002). In addition, the model must be unresponsive to treatment strategies that have no clinical effect in human OCD, for instance drugs targeting the noradrenergic system and the benzodiazepines (Fineberg, 2004; Grados and Riddle, 2001; Stein, 2002).

Various treatment regimens have been utilized in the different animal models of OCD (refer to paragraph 2.3.4 for a more detailed discussion), including acute, sub-chronic and chronic administration of the SSRIs, either as mono-therapy or in a combination with drugs from different classes. However, as explained in paragraph 2.1.3, patients with OCD only respond to *high dose SSRIs* and only after *chronic administration.* The predictive validity of animal models of OCD could thus be strengthened if it could be demonstrated that chronic, *but not acute or sub-chronic*, and high dose (but not nominal or antidepressant doses) treatment with the SSRIs is successful in alleviating stereotypy while drugs ineffective in human OCD exert no response. In addition, it would be interesting to assess whether animals refractory to treatment with the SSRIs, will respond to the augmentation thereof with low dose antipsychotics. Interestingly, OCD symptoms do not exacerbate due to serotonin depletion following cessation of chronic SSRI therapy (Delgado and Moreno, 1998) and it would be a valuable observation if the same effect could be demonstrated in an animal model of OCD.

### *2.3.3. The construct of OCD*

The criteria that must be met to establish construct validity of an animal model of OCD can be summarized from section 2.2. In short, the CSTC circuitry is fundamental in the pathology of OCD and must therefore be implicated in the animal model. As patients with OCD mostly respond selectively to the SSRIs, serotonin is regarded as a major role player, whether direct or indirect. As explained in paragraph 2.2.2.4, post-synaptic $5HT_{2C}$ receptor activation is thought to be the major mediator of the anti-compulsive effects of the SSRIs, while the down regulation of $5HT_{1B/D}$ receptors is hypothesized to be linked to an increase in serotonergic neurotransmission following chronic treatment with the SSRIs. Although the presynaptic $5HT_{1A}$ receptors in the raphe nuclei may suppress the serotonergic outflow to the cortex, it is believed that $5HT_{1A}$

antagonism will only quicken the onset of action of the SSRIs and that the down regulation of the $5HT_{1A}$ receptors during chronic SSRI-treatment is not a contributing factor to the efficacy of the SSRIs (Ceglia *et al.*, 2004).

As explained in paragraph 2.2.1, it is also believed that there is a bias in favour of the activity of the direct pathway through the basal ganglia over that of the indirect pathway. As $D_1$ receptors are predominantly found in the direct pathway and $D_2$ receptors in the indirect pathway, it would be interesting to evaluate the behavioural effects following the administration of various selective dopaminergic stimulating and blocking agents in established and putative animal models of OCD.

Although a number of other factors, such as an altered oxidative status (Güldenpfennig *et al.*, 2011), increased glutamatergic signalling (Pittenger *et al.*, 2006), increased nitric oxide levels (Atmaca *et al.*, 2005), the circadian effects of the female hormonal cycle (Abramowitz *et al.*, 2003), and increased growth hormone levels (Kluge *et al.*, 2006) to name but a few, may contribute to the symptomology of OCD, these are likely to be secondary to changes in serotonin and/or dopamine. Furthermore, most of these markers or mediators of disease are altered in a wide variety of conditions and therefore cannot be used to establish the construct validity of an animal model of OCD. However, these markers can be used to strengthen an already solid foundation built from a combination of good face, predictive and construct validity (refer to Güldenpfennig *et al*., 2011).

Taken together, the construct validity of an animal model of OCD could be strengthened if the stereotypy can be linked to a dysfunctional CSTC circuit, abnormal serotonergic and dopaminergic signalling, differences in the relevant serotonin receptor densities and the activity of their respective second messengers compared to healthy controls, and lastly if changes in stereotypical behaviour pre- and post-treatment can be correlated with altered serotonergic and possibly dopaminergic signalling.

### *2.3.4. Current animal models of OCD*

Although repetitive behaviour that may imitate human motor compulsions may be quite straightforward to demonstrate in animals, demonstrating this seemingly primitive repetition of specific behavioural sets in association with an underlying condition that may represent human OCD is extremely difficult. Considering paragraphs 2.3.1 – 2.3.3, it is evident that motor stereotypy, as exhibited by many animal species, has to be linked with a favourable response to chronic high dose SSRIs or a combination of chronic SSRIs and low-dose antipsychotics. Moreover, this needs to be combined with either an obsessive/thoughtful origin, serotonergic and

dopaminergic abnormalities in the prefrontal cortex and basal ganglia, or both. Although many animal models of OCD have been proposed during the past 35 years (Joel, 2006), it is difficult to discriminate between established and putative models, as none of them can yet be regarded as complete. Each model however, reflects some characteristics of the condition that may be helpful towards novel drug discovery for the treatment of human OCD.

As repetitive behaviour in animals is easy to demonstrate, it provides a solid foundation for the development of most if not all current animal models of OCD. Repetitive behaviour may develop naturally (whether resulting from confinement or not), or due to pharmacological (or genetic) manipulation or behavioural training. Different models fit into either of these categories and in the following paragraphs, a brief summary of the most prominent models of each category will be provided. The current study can be classed in the first category and centres on the natural expression of stereotypical behaviour by deer mice and therefore this model will be reviewed separately in section 2.4.

#### *2.3.4.1. Animal models based on the natural development of stereotypy*

***Spontaneous marble-burying in mice and rats.*** The burying of marbles in bedding material by mice and rats has been postulated to represent OCD symptomology (Gyertyan, 1995) based primarily on the fact that this behaviour responds favourably to treatment with SSRIs (Broekkamp *et al.*, 1986). Furthermore, it has been demonstrated that marble-burying is not anxiety-induced behaviour, but rather reflects repetitive compulsive-like behaviour (Thomas *et al.*, 2009). Much work has since been done to assess the validity of marble-burying as an animal model of OCD. Considering the roles of reward and task completion in the pathology of OCD, Londei and colleagues (1998) postulated that the burying of marbles by rodents begins as a normal investigation of novel objects. As marbles do not have reactionary properties, the authors postulate that a lack of perceptible feedback, that should induce a sense of task-completion, induces compulsive burying. This hypothesis, which implicates a role for thoughtfulness, combined with the compulsive-like burying of marbles, strengthens the face validity of the model. Unfortunately, although it has been demonstrated that the SSRIs, but not the noradrenalin reuptake inhibitor (NRI) desipramine, are successful in the attenuation of marble-burying (Broekkamp *et al.*, 1986; Ichimaru *et al.*, 1995; Takeuchi *et al.*, 2002), the model does not discriminate between the serotonergic drugs and anxiolytics such as the benzodiazepines (Broekkamp *et al.*, 1986). Since OCD does not respond to benzodiazepines, this significantly undermines the predictive validity of the model. The construct validity of the model has not directly been investigated but is incidentally based on the observations that drugs acting via mechanisms that have been proved clinically useful in OCD, also decrease the burying of mar-

bles. These drugs include the NMDA antagonist, memantine (Egashira *et al.*, 2008b), and the atypical antipsychotic, aripiprazole, a partial agonist at $D_2$ , $5HT_{1A}$ and $5HT_{2C}$ receptors and an antagonist at $5HT_{2A}$ receptors (Egashira *et al.*, 2008a). These findings implicate the glutamatergic, dopaminergic, and serotonergic systems in marble burying and may aid in strengthening the construct validity of the model.

***Nest building in mice.*** Although only one report on this model has been published (Greene-Schloesser *et al.*, 2011), the study design and the resulting outcomes are striking and thus well worth mentioning here. In this model, the authors evaluated and classified nest building behaviour of house mice *(Mus musculus)* into BIG and SMALL nest building cohorts. The BIG cohort was regarded as being compulsive while the SMALL cohort was defined as the control group. The authors demonstrated a 40-fold difference between the nest sizes of the BIG and SMALL cohorts, with the behaviour of the BIG cohort possibly correlated with the clinical symptoms of hoarding and concerns about security so often observed in human OCD. Furthermore, the authors also demonstrated that mice from the BIG cohort bury more marbles than mice from the SMALL cohort, thus constituting a link between nest building behaviour and marble-burying, another proposed animal model of OCD (see above). These findings provide the basis for the face validity of the model. The authors also provided evidence that the SSRI fluoxetine and the SRI clomipramine, but not the NRI desipramine, attenuated nest building behaviour of the BIG cohort. Also, nest building behaviour does not return to the baseline values during the four weeks post-treatment, a finding that correlates with studies done in OCD patients that demonstrated the long lasting behavioural effects of chronic SSRI-treatment in patients with OCD following cessation of drug administration (Delgado and Moreno, 1998). Subsequently the model demonstrates good predictive validity. The model has to date not been subjected to testing with respect to its construct validity for OCD. Another recent study has investigated nest building behaviour in rabbits as a model of OCD (Hoffman and Rueda Morales, 2009). This model is relevant to understanding compulsions related to feelings of incompleteness, "just right" sensations, and the perception of task completion and has provided some interesting evidence in support of its face validity for OCD. However, this model still requires investigation with regard to predictive and construct validity and will not be discussed further at this time. In summary, nest building behaviour may be a novel animal model of OCD, providing that the CSTC circuitry and other biological markers associated with OCD can be implicated in its underlying biology.

*2.3.4.2. Animal models based on pharmacological or genetic manipulation*

***8-OH-DPAT-induced decrease in spontaneous alternation.*** Rats have a natural tendency to explore novel places in any given environment (Albelda and Joel, In Press (a)). In this model, originally developed by Yadin and colleagues (1991), the authors demonstrated that administration of the $5HT_{1A}$ agonist, 8-OH-DPAT to rats was associated with a compulsive-like decrease in spontaneous alternating behaviour that provides the foundation for the face validity of the model. A number of studies have also demonstrated that sub-chronic and chronic administration of fluoxetine and clomipramine, but not desipramine, prevented this decrease in spontaneous alternation (Albelda and Joel, In Press (b); Fernández-Guasti *et al.*, 2003; Yadin *et al.*, 1991), thus strengthening the predictive validity of the model. Although the decrease in spontaneous alternation is brought about by targeting the serotonergic system – a fact that may provide the model with some degree of construct validity – this behaviour is also common in conditions such as Parkinson's disease, autism and schizophrenia (Albelda *et al.*, 2010; Langen *et al.*, 2011a), which may somewhat lessen the face validity of the model.

***Quinpirole-induced compulsive checking.*** In this model developed by Szechtman, Sulis and Eilam (1998), compulsive checking behaviour was induced in rats by the administration of the $D_{2/3}$ receptor agonist, quinpirole. Compulsive checking was defined with the demonstration that animals treated with quinpirole preferentially stopped at two specific locations only in a given environment compared to the non-specific exploratory behaviour of saline treated rats. The authors distinguished the compulsive checking behaviour induced by quinpirole from motor stereotypy by implicating a role for thoughtfulness. They demonstrated that in addition to the compulsive checking at the two preferred locations in the environment, rats treated with quinpirole demonstrated shorter return times to these locations compared to the other locations in the cage and performed a certain set of behaviours at each location. These observations markedly increase the strength of the model's face validity, as it not only demonstrates repetitive behaviour, but also links the repetition with cognitive planning. However, chronic clomipramine only partially attenuated the compulsive checking (Szechtman *et al.*, 1998) while no trial with an SSRI has yet been performed. In addition, the model has not been subjected to challenge with drugs known to be ineffective in human OCD, which diminishes its overall predictive validity. While some construct validity may be provided by the implication of $D_2$ receptor involvement, it must be stressed that this model may represent only a part of the clinical picture of OCD, as the behaviour is the result of dopaminergic modulation and therefore does not account for the crosstalk between dopamine and serotonin. This may be the reason why clomipramine only partially attenuates the compulsive checking induced by quinpirole.

***The $5HT_{2C}$-receptor knockout mouse.*** $5HT_{2C}$ receptor KO mice were originally developed to assess the influence of the receptor on feeding behaviour (Nonogaki *et al.*, 1998). However, Chou-Green and colleagues (2003) soon realised that these animals exhibited compulsive traits. It has since been demonstrated that not only do $5HT_{2C}$ receptor KO mice chew on objects and substances without any nutritive value, but do so in a very rigid, neat and organized way. The authors concluded that this type of behaviour may represent the compulsive expression of neatness and symmetry observed in OCD-patients, and as such provides the model with good face validity. Interestingly (as explained in paragraph 2.2.2.4), the $5HT_{2C}$ receptor is thought to be a major mediator of the opponent actions of serotonin on reward-based learning, a fact that may strengthen the face and construct validity of this model. It can be postulated that these animals constantly experience the same level of reward as a result of the compulsive-like and neat chewing of plastic screens. However, no drug treatment trials have been done to assess the effects of drugs known to be effective in human OCD, nor with drugs that are clinically ineffective, such that the model has no recognised predictive validity (Joel, 2006; Wang *et al.*, 2009).

The following models can also be classed in this category, but due to the lack of recent work to further their validation, they are not used and will therefore not be discussed in more detail: $D_1$CT-7 mutant mice, dopamine transporter (DAT) knockdown mice, SAPAP3-mutant mice, oestrogen-deficient mice, and Hoxb8-mutant mice. For a review on these models, see Wang (2009) and Joel (2006).

#### *2.3.4.3. An animal model based on behavioural training*

***The signal-attenuation model.*** This model involves the training of rats to press a lever for reward, in this case food. The presentation of the reward is accompanied by a cue that signals a successful lever press, upon which the food is introduced. Subsequently, the rewarding property of the signal is attenuated by repeatedly presenting the cue without the reward. In short, rats keep pressing the lever in a compulsive manner, even if no reward is gained from their actions. This model has been developed by Joel and Avisar (2001) based on the theory that a dysfunctional reward-based learning system may be central to the pathogenesis of OCD and thus the model demonstrates excellent face and some construct validity. Not only is compulsive-like lever pressing demonstrated, but it is also linked with a dysfunction in reward-based learning that distinguishes lever pressing from rigid motor stereotypy. Furthermore, that the dopaminergic, serotonergic and glutamatergic systems have been implicated in the model further strengthens the construct validity of the model. The authors argue that the model has a solid predictive foundation, as acute treatment with the SSRIs, but not with desipramine or diazepam, have been found to attenuate compulsive lever pressing. However, this aspect somewhat dimin-

ishes the predictive validity of the model since human OCD only responds after chronic and high dose treatment with the SSRIs.

* * *

In summary, it is clear that none of the models discussed above have strong foundations anchored in all three of the validation criterions necessary to establish an animal model as a credible representation of a human condition. In general, natural stereotypy can prove valuable to investigate the etiological development of specific compulsive-like behaviours and shed more light on the crosstalk in the CSTC circuitry, while the pharmacological or genetic manipulation of animals can provide insights into the specific roles of certain receptors and neurotransmitters in the pathology of stereotypical behaviour, whether it may represent human compulsions or not. Lastly, the signal attenuation model is one of the only models available that implicates a role for reward and thus may further our understanding of the role of reward-based learning in the symptomology of OCD.

## 2.4. A REVIEW OF SPONTANEOUS STEREOTYPY IN THE DEER MOUSE – 1999 – 2011

Since stereotypical behaviour in the deer mouse *(Peromyscus maniculatus bairdii)* was first studied in 1999 by Susan Powell and her colleagues at the University of Florida (Powell *et al.*, 1999), much work has been done to investigate the pathogenesis and underlying pathology of these behaviours. Deer mice exhibit three main topographies of stereotypy when housed under normal laboratory conditions, namely repetitive jumping, pattern running and backward somersaulting (Hadley *et al.*, 2006; Korff *et al.*, 2008; Powell *et al.*, 1999). These three behavioural phenotypes have since been under investigation as putative animal models of stereotypical movement disorder (Hadley *et al.*, 2006; Powell *et al.*, 1999; Presti *et al.*, 2004), and more recently, OCD (Güldenpfennig *et al.*, 2011; Korff *et al.*, 2008; Korff *et al.*, 2009). In this section, the history of the model will briefly be reviewed, from its initial development as an animal model of stereotypical movement disorder to the more current investigations concerning OCD. Furthermore, the validation status of the deer mouse model of OCD will be evaluated, focussing on the model's current strengths and shortcomings.

* * *

### *2.4.1. A timeline of major developments in the appraisal of deer mouse stereotypy*

Powell and colleagues was the first group to conceptualize the behaviour expressed in deer mice housed in confinement as being abnormal and of a stereotyped nature (Powell *et al.*, 1999). The main purpose at that time was to evaluate the influence of environmental enrichment on the development of stereotypy and therefore animals were not categorized based on the amount of stereotypy they expressed, but rather according to the percentage time each animal spent exhibiting stereotypy. In addition, the authors demonstrated that 62% of the animals housed in laboratory cages developed stereotypy, a number that has changed somewhat during the course of later studies due to the implementation of newer assessment protocols. Important to note though, is that not all animals develop this behavioural trait, a fact that has not changed over the past years.

At that time, the group did not make use of automated screening and the behavioural categorization was done by means of visual observation (Powell *et al.*, 1999). Due to the time constraints imposed by this method of screening, the authors observed each animal twice weekly for only 5 minutes at a time. Since then the same group has broadened their investigations with the purpose of developing and validating an animal model of stereotypical movement disorder (Powell *et al.*, 1999; Presti *et al.*, 2004). In 2004 the same group introduced automated screen-

ing which opened up the possibility of evaluating the behaviour over longer periods of time (for a full discussion on the methodology of automated screening, refer to Chapter 4). This resulted in the first quantitative assessment of stereotypy scores with the measurement unit being expressed as counts per hour (Presti *et al.*, 2004). The authors classified the animals into one of two cohorts, *viz.* low-stereotypical (LS, less than 300 vertical beam interruptions per hour), and high-stereotypical (HS, more than 1000 vertical beam interruptions per hour) based on the mean stereotypy score calculated following behavioural screening during an eighteen hour-long session over two days (Presti and Lewis, 2005). Although this classification system excluded animals that executed 300 to 1000 jumps per hour, the authors intended to determine whether any differences in the striatal opioid content between the LS and HS cohorts could be demonstrated. It was therefore crucial to exclude the 'grey' middle population of animals from the group (Presti and Lewis, 2005), a rationale that was exploited in the current study as well. In addition, due to the practical constraints imposed by the methodology of striatal micro-dialysis, the pattern running and backward somersaulting topographies were excluded from the study as these types of behaviour interfered with the normal functioning of the micro-dialysis equipment.

In 2005, our laboratory initiated the validation of deer mouse stereotypy as a putative animal model of OCD, with Korff and colleagues (2008) being the first group to comprehensively describe the face and predictive validity of deer mouse stereotypy as a putative animal model of OCD. As pattern running and backward somersaulting were included in this study, the authors realised that the original classification system developed by Presti was insufficient to meet the purposes of the study at that time. The main reason for this was that backward somersaulting resulted in more beams being broken per stereotypical movement than was the case with vertical jumping. Subsequently the authors re-classified the behaviour expressed by deer mice into three cohorts, *viz.* non-stereotypical behaviour (NSB, less than 1000 beam interruptions per hour), low-stereotypical behaviour (LSB, 1000 – 2000 beam interruptions per hour), and high-stereotypical behaviour (HSB, more than 2000 beam interruptions per hour) (Korff *et al.*, 2008) (for an explanation of the rationale followed when applying beam interruptions as a means of behavioural categorization, refer to paragraph 3.1.2 and 3.1.3). Important to note is that during this study, as well as two subsequent studies from this group (Güldenpfennig *et al.*, 2011; Korff *et al.*, 2009), the animals were classified into either one of the cohorts using a mean stereotypy score calculated from the data obtained during three individual one-hour long behavioural screening sessions, each one week apart to exclude the influence of handling stress. This methodology has since been reappraised (see section 3.1).

### *2.4.2. The current validation status of the deer mouse model of OCD*

In the first article published on deer mouse stereotypy and its relevance to OCD, Korff and colleagues (2008) demonstrated that deer mouse stereotypy is heterogeneous within a given population of animals. As such, 45% of the animals were classified as HSB, 41% as LSB, and 16% as NSB. Without elaborating on the importance of the diversity of stereotypy expressed, the authors compared the differences in symptom severity in human OCD to the heterogenic distribution of the stereotypy scores of deer mice. Indeed, it must be stressed that these behaviours develop naturally and that a number of deer mice *do not* exhibit stereotypy at all. Although the latter cohort of animals is small compared to the cohorts exhibiting either high or low stereotypical behaviour, it can be postulated that a genetic basis for the expression of stereotypy may exist and that the proposed influences of confinement stress (Langen *et al.*, 2011b) and the restricting role of the environment on normal behavioural motor patterns (Ridley, 1994) may only be triggering factors for the development of stereotypy and not necessarily the etiological origins thereof. Although Powell presented evidence for an ameliorative effect of environmental enrichment on the expression of deer mouse stereotypy (Powell *et al.*, 1999), which is in line with the hypothesis of Ridley (Ridley, 1994), animals in both standard and enriched cages did however develop stereotypical behaviour. The difference was that a smaller percentage of the animals in enriched cages expressed stereotypy, also developing it at a later stage and displaying mostly pattern running instead of vertical jumping or somersaulting (Powell *et al.*, 1999). In addition, the average amount of stereotypy expressed in animals housed in enriched cages was less than the amounts expressed in their fellow subjects housed in the standard cages. These findings were replicated in a study done by Hadley and colleagues (2006). This observation, combined with the evidence that a proportion of animals do not develop any form of stereotypy, would suggest that the environment these animals are kept in is not the determining factor in the expression of stereotypy and that it may only play a modulatory role in the extent to which stereotypy is exhibited. As such, the face validity of the model is based on the demonstration of rigid repetition of certain motor patterns in intensities of a heterogenic nature across a given population and that it is not entirely dependent on environment, but may have a genetic basis. In addition, the demonstration that deer mouse stereotypy cannot be prevented by environmental enrichment, but only alleviated to a certain extent, is in line with the hypothesis that compulsions can be distinguished from rigid motor patterns on the basis of thoughtfulness and flexibility (Eilam *et al.*, 2006). Although this behaviour does not disappear in total, it can be influenced by environmental factors and thus can be regarded as flexible. This hypothesis further strengthens the face validity of the deer mouse model for OCD.

* * *

Evidence in support of the predictive validity of the model has also been established. Korff and colleagues (2008) demonstrated that chronic high dose treatment with the SSRI fluoxetine, but not the NRI desipramine, decreased the amount of stereotypy exhibited by HSB and LSB animals without affecting general locomotor activity. As chronic and high dose treatment with the SSRIs is the mainstay of treatment for human OCD, these findings contribute positively to the predictive validation of deer mouse stereotypy as an animal model of OCD. Based on the hypothesis that a bias in favour of the direct pathway through the basal ganglia over that of the indirect pathway is central to the pathology of OCD, the authors also challenged deer mice with sub-acute quinpirole at a dosage of 5 mg/kg/day. Quinpirole, a non-selective $D_{2/3}$ agonist, should theoretically have inactivated the indirect pathway, leading to an exacerbation of stereotypy (refer to section 2.2.2 for a discussion on the functioning of the direct and indirect pathways in the control of movement). Indeed, Szechtman and colleagues (1998) used quinpirole to induce what they termed 'compulsive checking' in rats, another putative animal model of OCD (see paragraph 2.3.4). However, in the study by Korff and colleagues (2008), quinpirole attenuated the spontaneous stereotypy exhibited by deer mice. Given the number of conflicting reports regarding the effects of dopamine releasers like amphetamine and selective dopaminergic agents in the treatment of OCD (Denys *et al.*, 2004a), the fact that quinpirole decreases the stereotypical behaviour in deer mice implicates a role for dopamine in the pathology of deer mouse stereotypy and thus strengthens the construct of the model, albeit only with regards to the neurotransmitters involved. If one attempts to draw a parallel to human OCD, in contrast to what has been proposed with respect to dopamine's role in OCD, the Korff study suggests that at least the stereotypy component of the OCD spectrum of symptoms may be related to reduced dopaminergic activity within certain CSTC circuits. It should however be reiterated that various studies have failed to demonstrate a hyperdopaminergic state in OCD (Brambilla *et al.*, 2000; Pitchot, 1996). Further, while amphetamine-like drugs (dopamine releasers) are known to exacerbate obsessive-compulsive symptoms, dopamine agonists may also improve symptoms in OCD patients (Denys *et al.*, 2004a). Moreover, while dopamine agonists may precipitate compulsive/stereotypic behaviour in a non-pathological (induced) animal model such as the quinpirole model described by Szechtman and colleagues, they seem to suppress these behaviours in spontaneous stereotypy models (Korff *et al.*, 2008; Vandebroek and Odberg, 1997). These data concur that further study is not only necessary to delineate the role of dopamine in stereotypy as it pertains to OCD, but also to better understand the underlying neurobiology of induced versus natural stereotypy.

Korff and colleagues (2008) also found that the administration of m-chlorophenylpiperazine (mCPP), a non-selective $5HT_{1A/2A/2B/2C}$ agonist, decreased the degree of stereotypy expressed in deer mice. This is in line with data published reporting compulsive-like behaviour in the $5HT_{2C}$ receptor KO mouse (see paragraph 2.3.4) (Chou-Green *et al.*, 2003) and the hypothesis that the stimulation of $5HT_{2C}$ receptors may be an important mediator of the suppressing effects of serotonin on reward-seeking behaviour (Millan *et al.,* 1998). This mechanism is postulated to be central to at least some forms of OCD (refer to section 2.1 and paragraph 2.2.2.3 for a full discussion on the role of reward in the pathology of OCD). However, conflicting clinical reports have been published regarding the effects of mCPP administration in patients with OCD, with some authors reporting an exacerbation of symptoms (Hollander *et al.*, 1991), while others failed to demonstrate the same (Khanna *et al.*, 2001). As mCPP decreased the amount of stereotypy expressed in deer mice, a role for the $5HT_{1A/2C}$ receptors is implicated in the pathology of deer mouse stereotypy. Although the finding does not necessarily strengthen the predictive validity of the model, it certainly is not an undermining factor. As explained in section 2.2, the crosstalk between serotonin, dopamine, glutamate, and GABA in the CSTC circuitry is complex. The manner in which stereotypy develops may shed light on the functioning of the CSTC circuitry and aid in explaining the conflicting results reported concerning dopaminergic and serotonergic drug challenges and their effect on stereotypy.

* * *

As dopamine and serotonin has been implicated in the symptomology of deer mouse stereotypy (Korff *et al.*, 2008), the validity of the model is strongly founded on this construct. It has been demonstrated that the amount of stereotypy expressed in deer mice is positively correlated to frontal-cortical, but not striatal, cAMP levels and inversely related to $PDE_4$-enzyme activity in the frontal cortex but not striatum (Korff *et al.*, 2009). The select involvement of the frontal cortex strongly supports a frontal cortical lesion in OCD (Evans *et al.*, 2004; Husted *et al.*, 2006; Markarian *et al.*, 2010). The authors proposed that, given the fact that an earlier study (Korff *et al.*, 2008) demonstrated reduced stereotypy in HSB mice following administration of a non-selective $5HT_{1A}$ agonist, increased stereotypy would be characterized by reduced $5HT_{1A}$ $G_i$ dependent adenylate cyclase coupling and increased levels of cAMP (Korff *et al.*, 2009). In both brain regions, the authors also found a significant inverse correlation between PDE4 activity and stereotypic behaviour. Since PDE4 selectively hydrolyses cAMP, this negative correlation indicates that the increase in cAMP observed as a function of stereotypy in HSB mice is related to reduced hydrolysis by PDE4 (Korff *et al.*, 2009). Perseverative locomotor paths have been associated with the stimulation of $5HT_{1B/1D}$ receptors (Shanahan *et al.*, 2009) and the desensitization of these receptors is thought to mediate some of the ameliorative effects of the SSRIs on

OCD symptomology (Blier *et al.*, 1996). As the desensitization of frontal-cortical $5HT_{1B/D}$ autoreceptors results in the increased release of frontal-cortical serotonin (which in turn can be associated with the attenuation of stereotypy), the assumption would be that the frontal-cortical cAMP levels in HSB animals, should actually be lower than in the LSB cohort, which is not the case. The opposite observation in deer mice on the one hand can be ascribed to the use of mCPP, a relatively non-selective 5HT agonist, or due to state-dependent differences in the animal models used, such as natural versus induced stereotypy models. Clearly, these findings warrant further investigation into the serotonergic mechanisms underlying the development of spontaneous stereotypy in the deer mouse.

Studies confirming the involvement of the direct and indirect pathways in the development of deer mouse stereotypy (Presti *et al.*, 2004; Presti and Lewis, 2005), together with data implicating the frontal cortex in the pathology of these behaviours strengthens the construct validity of the model.

* * *

In summary, deer mouse stereotypy presents with sufficient face, predictive and construct validity to constitute a solid foundation for its further development as an animal model of OCD. Nevertheless, the face validity of the model can be strengthened by studying the association of high stereotypical behaviour with compulsive-like traits in the animal, such as marble-burying and nest building. Furthermore, the predictive validity of the model can be taken further by assessing the effects of augmenting SSRI treatment with low-dose antipsychotics in animals that are refractory to SSRI monotherapy, and demonstrating that acute administration of the SSRIs does not exert any ameliorative effect in HSB mice. The administration of selective $5HT_{2C}$ agonists as monotherapy and $5HT_{1A/B/D}$ antagonists in combination with the SSRIs may also produce valuable results. Since the pathogenesis of OCD remains a topic of much debate, specifically with respect to the brain regions and neuronal systems and neurotransmitters implicated in OCD, the construct validity of the model is at this point relatively robust. However, studies aimed at strengthening the construct of the model by confirming a role for a dysfunctional reward-based learning system in HSB deer mice would be very valuable. Such a demonstration would be in line with the hypothesis that reward may play a role in the symptomology of OCD (see section 2.1 and paragraph 2.2.2.3) and correlate with findings from the signal-attenuation model of Joel and Avisar (2001).

* * *

Like many other psychiatric conditions, OCD remains an enigma. In the clinical environment the diagnosis of the condition is challenged by many difficulties, including the high prevalence of comorbid disorders (Le Boulch *et al.*, 1991; Nestadt *et al.*, 2001) and the variation in obsessive-compulsive symptomology (Abramowitz *et al.*, 2003; Bartz and Hollander, 2006). The etiological and pathological foundations of the condition have also not yet been fully elucidated (Fineberg *et al.*, 2010; Stein, 2002). However, it is clear that functional abnormalities in the CSTC circuitry are central to the symptomology of OCD (Markarian *et al.*, 2010) and that most patients respond selectively to treatment with SSRIs (Fineberg and Craig, 2007; Vythilingum *et al.*, 2000). Thus, the demonstration of CSTC involvement and a selective response to chronic, high dose SSRIs, at least in 70% of animal subjects, would constitute a solid foundation for the development of an animal model of OCD. However, these cannot be the only strengths of an animal model of OCD, as many psychiatric conditions share the same characteristics. OCD is also characterized by the rigid repetition of certain motor patterns that can be distinguished from simple motor repetition on the basis of thoughtfulness and flexibility (Eilam *et al.*, 2006). Another aim in the development of a model for OCD would thus be to investigate the origins and the characteristics of the different forms of motor stereotypy expressed in animals.

In the following chapters, the current study will be discussed in detail, including a reappraisal of deer mouse stereotypy as a putative animal model of OCD, the objectives of the study, the results, and finally a discussion of the findings and how these relate to our current knowledge of deer mouse stereotypy and, indeed, OCD.

*END OF CHAPTER 2*

# THE CURRENT STUDY

# CHAPTER 3
# APPRAISING DEER MOUSE STEREOTYPY AS AN ANIMAL MODEL OF OCD

During the course of the current study, I drew on ideas from different methods described in our previous studies (Güldenpfennig *et al.*, 2011; Korff *et al.*, 2009) as well as from other published studies (Hadley *et al.*, 2006; Powell *et al.*, 1999) to assess and evaluate the degree of stereotypy expressed in deer mice. In this chapter, I will focus on the critical reappraisal of deer mouse behaviour specifically in the light of its relevance to OCD and shortly discuss the reasoning behind the methodology followed in the current study.

## 3.1. THE REAPPRAISAL OF PAST METHODS

### *3.1.1. How was deer mouse stereotypy assessed in the past?*

In studies based on the discrimination between the different amounts of stereotypy expressed in deer mice, baseline assessment of stereotypy is required in order to classify each animal into one of the three cohorts (HSB, LSB or NSB) before any treatment- and neurochemical-related studies can be initiated. During the previous investigations in our laboratory, this classification was done based on the mean stereotypy score calculated following three behavioural screens, each one hour in duration and one week apart. The apparatus used at that time (Digiscan® Animal Activity Monitoring System, Accuscan Instruments, Columbus, Ohio, USA) only allowed for a maximum of four animals to be screened at a time and did not have the capability to provide the animals with food and water throughout the night for extended behavioural analysis. Animals were then transferred from their home cages to the testing room 30 minutes prior to the behavioural assessment to allow them to habituate in the test cages. Following the one-hour assessment period, the animals were returned to their home cages and the next group of four mice subjected to the habituation protocol. This method allowed screening of 12 animals maximum per night or 48 animals per week. This procedure was repeated for three weeks in order to obtain three stereotypy values for each animal from which a mean value could be calculated. Thereafter the drug administration began and continued for the duration of the treatment period without any intermittent behavioural screening. The last behavioural assessment after the cessation of drug administration was performed as a once-off measurement to determine treatment-associated behavioural changes. This methodology worked well as far as the expression of stereotypy is concerned since the mean value used to determine the baseline stereotypy score mostly excluded any outliers from the post-treatment assessment. However,

the fact that the post-treatment assessment involved a single measurement, posed some significant challenges in the evaluation of the data.

The original classification system used was an adaptation of that developed by Presti (2004) and divided animals precisely into one of the three cohorts. At this stage, it is important to explain how the data generated by the Digiscan® Animal Acitivity Monitoring System were subsequently used to determine the stereotypy scores for each individual animal (for a full description of the mechanics of the activity monitoring system, in particular the newer version, refer to Chapter 4). Briefly, every time an animal moves, it interrupts a light beam at a certain location in a grid of beams spaced 2.5cm apart, organized in a XY-manner (see Figures 4-3 and 4-4). In addition, another set of beams are located 10cm above the bottom grid to record vertical jumping and somersaults, without being influenced by rearing activity. If an animal interrupts the same beam at the same location repeatedly, the software records the repetitions as stereotypy. Behaviour such as head dipping and eating chow can cause this type of data to be recorded and therefore the stereotypy score generated by the software could not be regarded as definitive. Thus, for vertical activity the amount of vertical beam interruptions as a measure of vertical jumping and somersaulting was used. As pattern running by deer mice manifests as running in circles, the amount of cage revolutions recorded by the software was used to determine the pattern running scores, as this is a measurement of the cage circling behaviour of the animals (note that rotating behaviour in one spot is not recorded as cage revolutions). Based on these recordings, the animals were then divided into three cohorts, namely HSB, LSB or NSB, using the same classification criteria for all animals without discriminating between the three behavioural topographies and thus not taking into account the different types of stereotypy expressed by the different animals. Animals expressing 1000 vertical beam interruptions or cage revolutions per hour or less were classified as NSB. If the amount of vertical beam interruptions or cage revolutions generated were between 1000 and 2000 per hour the animal was classified as LSB and if the amount of vertical beam interruptions or cage revolutions equalled 2000 and more per hour, the animal was classified HSB.

### *3.1.2. The influence of the different topographies on the classification of stereotypy*

As stated earlier, Presti and Lewis (2005) excluded the pattern running and backward somersaulting topographies from their studies. As these behaviours are as repetitive and persistent as vertical jumping, these topographies were included in our studies. Subsequently the same classification criteria explained above to categorize the three different cohorts across all forms of stereotypy were used. However, in hindsight this method of discrimination excluded the pattern runners from our experiments as the number of cage revolutions never amounted to more

than 1000 (see Figure 3-1 and Addendum A for supportive data using the Fusion®). The reason for this is that the animal spends much more time completing one revolution compared to completing one vertical jump. Thus, even if the pattern runners ran in circles without end, they would never complete 1000 revolutions. Similarly, it would be even more difficult to attain a score of 2000 needed to be classified as HSB. Nevertheless, the behaviour they express is just as rigid and repetitive as that of the vertical jumpers and backward somersaulters. Consequently, it is possible that in our previous studies, certain pattern runners that executed relatively high numbers of cage revolutions could have been classified inappropriately as NSB animals. It was therefore decided to reappraise the original classification system employed in our laboratory to accommodate the topographical differences in the expression of stereotypy. In general, vertical jumpers and backward somersaulters generate the same amount of beam interruptions per cohort and it is very difficult to discriminate between these two topographies statistically when only studying the behavioural data. Therefore, we now only distinguish between vertical stereotypies and pattern running as the two main topographies. These behavioural observations have resulted in the development of a new classification system that will be explained in the following section.

### *3.1.3. 12-hour assessments influence stereotypy classification*

Since the publication of the first series of validation studies undertaken in our laboratory (Korff *et al.*, 2008; Korff *et al.*, 2009), the original Digiscan® Animal Activity Monitoring System was replaced with a novel more well-appointed model from the same company, called the Fusion® (see Figure 4-3). This apparatus can accommodate eight animals at a time and is capable of continuous behavioural screening of the mice for 12 hours. Furthermore, it has the capability of providing food and water ad lib for the duration of the dark cycle, thus enabling extended and continuous behavioural evaluation. It was therefore decided to screen eight animals at a time, five nights of the week. The total amount of animals screened during a week thus equalled the same amount screened using the old system, except that it has now been possible to increment the data during the night and thereby obtain a mean value of stereotypy for each animal after only *one night* instead of three weeks. Thus, mean stereotypy scores could now be generated for each animal on a weekly basis, providing a much more objective way to assess the behaviour. In addition, behavioural screening can continue throughout the treatment phase of a study, providing us with "snapshots" over time depicting the changes in behaviour as a function of treatment progression. Thus for example, response after 1 and/or 7 days of treatment (i.e. response following acute and/or sub-chronic treatment) with an automatic extension to longer treatment periods, such as 3 – 8 weeks (i.e. chronic treatment) could be considered, allowing a more robust assessment of acute versus chronic drug response (in support of the model's pre-

dictive validity). An interesting consequence of the 12-hour screening protocol was that preliminary observations indicated that many animals do not express the same levels of stereotypy during the whole of the dark-cycle (see Figure 3-1 and Addendum A for supporting data). Instead, there are periods during which they execute a high number of stereotypical repetitions, followed by periods of very little stereotypical activity, this despite the fact that the general locomotor activity stays the same during these periods. A statistical analysis of this behavioural phenomenon could provide interesting insights since stereotypy expressed in this manner could be a functional reaction to anxiety. If such a pattern in the expression of stereotypy can be confirmed, it may be postulated that the execution of stereotypy may play an anxiolytic role, explaining the fluctuating manner in which many animals express this behaviour. Further studies of the chronological expression of stereotypy are therefore warranted. Such studies would have to take into account the different behavioural topographies, the individual chronological variation of stereotypy between animals and the differences in stereotypy response of animals to a wide range of treatment options. Such an analysis was beyond the scope of this study, but will be considered for future investigations in our laboratory.

It is therefore possible that certain animals in our previous studies were classified inappropriately as they were only observed for one hour during the dark cycle. Although each animal was observed during three consecutive weekly sessions, the fact that many animals express stereotypical behaviour of varying intensity during the night (see above), implies that the manifestation of an animal's behaviour during the time of observation could have been misinterpreted. Therefore, in all subsequent work the entire 12-hour assessment period was used to generate data for each animal after each screen. Subsequently, the weekly mean individual stereotypy scores after each assessment was determined from the three 30-minute intervals during which the highest behavioural scores were generated by each animal during a 12-hour screen. This is based on the idea that it does not matter *when* an animal expresses the 'compulsive' behaviour, but that it *does* express it.

Furthermore, the time increments of behavioural assessment were reduced from 1 hour to 30 minutes for reasons that will be explained. Normally an animal generating a vertical stereotypy score of 4000 counts per hour would be classed as an HSB animal. Some animals generate the whole count per hour during the first 10 minutes of the hour, while others execute jumps at a slower rate but still complete the 4000 movements within the hour. For identifying stereotypy, these two animals may be regarded as being the same. However, when reappraising these behaviours with respect to OCD, a very different picture unfolds. If it is assumed that the severity of the 'compulsiveness' of an animal is represented by the amount of stereotypy it expresses and the rate at which it generates these movements (see section 6.2 for an explanation), it can

be postulated that the animal executing 4000 jumps within the first 10 minutes of the hour is more 'compulsive' than the animal executing the same amount of jumps at a slower rate. If the duration of the individual increments is shortened, to 30 minutes in this case, the animal that executes 4000 jumps within the first ten minutes of the hour will still generate a stereotypy score of 4000 counts per 30 minutes, while the animal expressing the behaviour at a slower rate, will generate a lower score.

Interestingly, preliminary observations using this revised method of analysis found that the mean weekly stereotypy scores generated for each animal could differ on a week-to-week basis (See Figure 4-6). On these grounds, it was decided to precede each treatment period with at least four weeks of baseline screens (or five behavioural assessments) in order to generate a reliable pre-treatment stereotypy score to more appropriately classify the animals.

The classification system that resulted from the above initial observations can be summarized as follows. All animals are screened on a weekly basis whereafter a weekly stereotypy score for each animal is calculated from the three highest counts/30 minutes generated during a 12-hour assessment period. Five of these baseline assessments precede each treatment- or neurochemical study. Subsequently, the median value (not the average) of the five weekly mean stereotypy scores is used to determine the baseline stereotypy count and to classify the animals. The median value excludes the influence of any outlier values on the classification of the animals (the median of numbers is defined as that number that represents the physical middle of a range of numbers and therefore reduces the value of outliers). Table 3-1 (next page) summarizes the newly defined cut-off values for each cohort. It must be stressed that in the current study more animals were assessed behaviourally than needed for the neurochemical analysis, a rationale also followed by Presti and Lewis (2005). This ensured a clear separation of the HSB and [NSB/LSB] animals used in the neurochemical analysis.

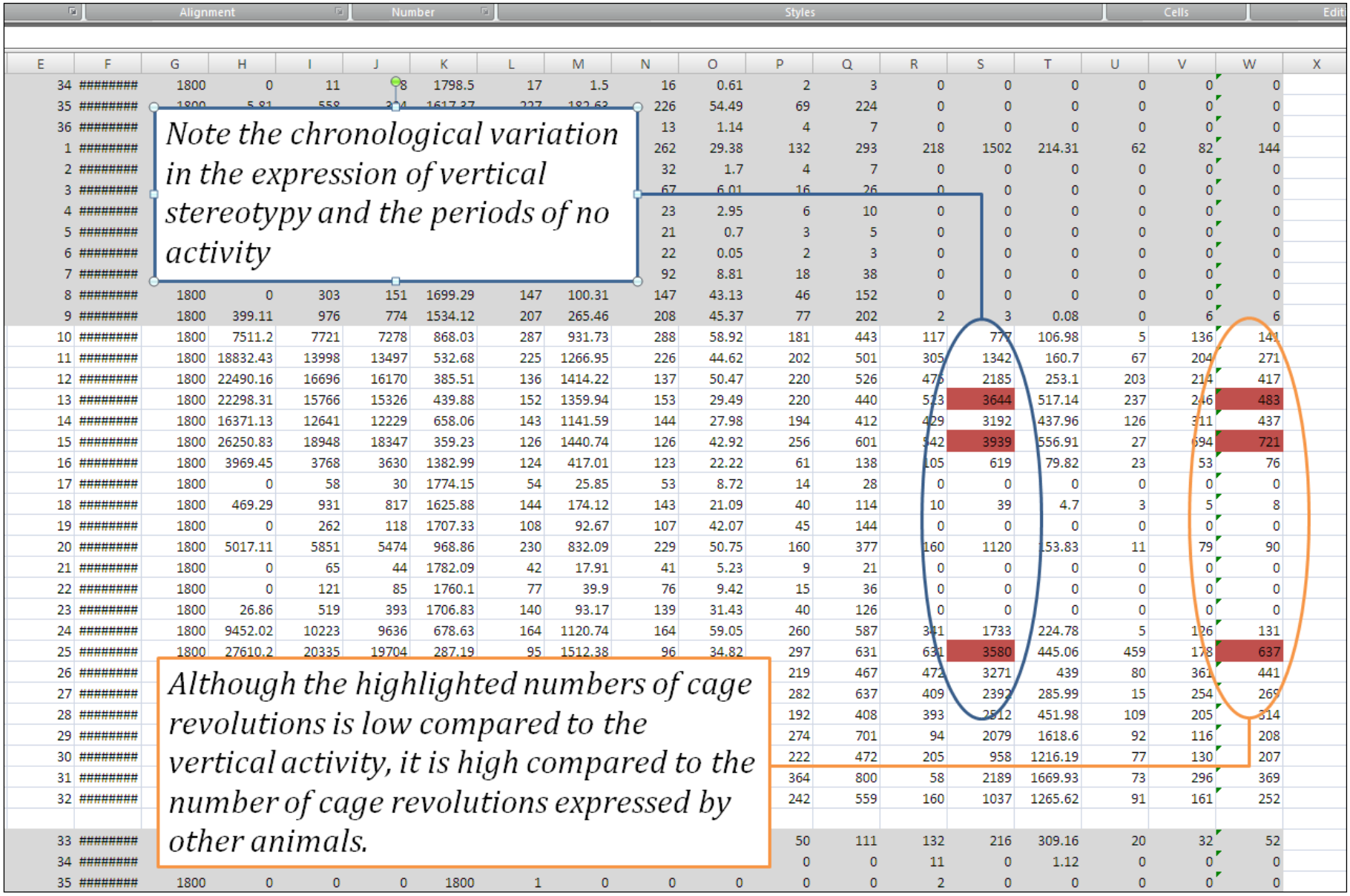
*Note – As the behavioural scores generated by some of the HSB animals are much higher than those generated by animals classified as NSB or LSB, we decided to group the latter two cohorts together.*

* * *

When all of the above is considered, the net result of the changes made to the original methodology has resulted in a more definitive discrimination between the different cohorts. Moreover, it will also ensure a clear-cut separation of the animals for extraction of brain tissue used in the neurochemical analysis.

| Cohort | Vertical counts / 30 minutes *(number of beam interruptions)* | Horizontal revolutions / 30 minutes |
|---|---|---|
| **HSB** | > 2000 | > 200 |
| **LSB** | 500 - 2000 | 150 – 200 |
| **NSB** | < 500 | < 150 |

***TABLE 3-1 – THE NEWLY DEFINED CUT-OFF VALUES FOR EACH COHORT AS A FUNCTION OF THE TOPOGRAPHY EXPRESSED***




| | E | F | G | H | I | J | K | L | M | N | O | P | Q | R | S | T | U | V | W | X |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 34 | | ######## | 1800 | 0 | 11 | 8 | 1798.5 | 17 | 1.5 | 16 | 0.61 | 2 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | |
| 35 | | ######## | 1800 | 5.81 | 558 | 314 | 1617.37 | 227 | 182.63 | 226 | 54.49 | 69 | 224 | 0 | 0 | 0 | 0 | 0 | 0 | |
| 36 | | ######## | | | | | | | | 13 | 1.14 | 4 | 7 | 0 | 0 | 0 | 0 | 0 | 0 | |
| 1 | | ######## | | | | | | | | 262 | 29.38 | 132 | 293 | 218 | 1502 | 214.31 | 62 | 82 | 144 | |
| 2 | | ######## | | | | | | | | 32 | 1.7 | 4 | 7 | 0 | 0 | 0 | 0 | 0 | 0 | |
| 3 | | ######## | | | | | | | | 67 | 6.01 | 16 | 26 | 0 | 0 | 0 | 0 | 0 | 0 | |
| 4 | | ######## | | | | | | | | 23 | 2.95 | 6 | 10 | 0 | 0 | 0 | 0 | 0 | 0 | |
| 5 | | ######## | | | | | | | | 21 | 0.7 | 3 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | |
| 6 | | ######## | | | | | | | | 22 | 0.05 | 2 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | |
| 7 | | ######## | | | | | | | | 92 | 8.81 | 18 | 38 | 0 | 0 | 0 | 0 | 0 | 0 | |
| 8 | | ######## | 1800 | 0 | 303 | 151 | 1699.29 | 147 | 100.31 | 147 | 43.13 | 46 | 152 | 0 | 0 | 0 | 0 | 0 | 0 | |
| 9 | | ######## | 1800 | 399.11 | 976 | 774 | 1534.12 | 207 | 265.46 | 208 | 45.37 | 77 | 202 | 2 | 3 | 0.08 | 0 | 6 | 6 | |
| 10 | | ######## | 1800 | 7511.2 | 7721 | 7278 | 868.03 | 287 | 931.73 | 288 | 58.92 | 181 | 443 | 117 | 777 | 106.98 | 5 | 136 | 141 | |
| 11 | | ######## | 1800 | 18832.43 | 13998 | 13497 | 532.68 | 225 | 1266.95 | 226 | 44.62 | 202 | 501 | 305 | 1342 | 160.7 | 67 | 204 | 271 | |
| 12 | | ######## | 1800 | 22490.16 | 16696 | 16170 | 385.51 | 136 | 1414.22 | 137 | 50.47 | 220 | 526 | 476 | 2185 | 253.1 | 203 | 214 | 417 | |
| 13 | | ######## | 1800 | 22298.31 | 15766 | 15326 | 439.88 | 152 | 1359.94 | 153 | 29.49 | 220 | 440 | 523 | 3644 | 517.14 | 237 | 246 | 483 | |
| 14 | | ######## | 1800 | 16371.13 | 12641 | 12229 | 658.06 | 143 | 1141.59 | 144 | 27.98 | 194 | 412 | 429 | 3192 | 437.96 | 126 | 311 | 437 | |
| 15 | | ######## | 1800 | 26250.83 | 18948 | 18347 | 359.23 | 126 | 1440.74 | 126 | 42.92 | 256 | 601 | 342 | 3939 | 556.91 | 27 | 694 | 721 | |
| 16 | | ######## | 1800 | 3969.45 | 3768 | 3630 | 1382.99 | 124 | 417.01 | 123 | 22.22 | 61 | 138 | 105 | 619 | 79.82 | 23 | 53 | 76 | |
| 17 | | ######## | 1800 | 0 | 58 | 30 | 1774.15 | 54 | 25.85 | 53 | 8.72 | 14 | 28 | 0 | 0 | 0 | 0 | 0 | 0 | |
| 18 | | ######## | 1800 | 469.29 | 931 | 817 | 1625.88 | 144 | 174.12 | 143 | 21.09 | 40 | 114 | 10 | 39 | 4.7 | 3 | 5 | 8 | |
| 19 | | ######## | 1800 | 0 | 262 | 118 | 1707.33 | 108 | 92.67 | 107 | 42.07 | 45 | 144 | 0 | 0 | 0 | 0 | 0 | 0 | |
| 20 | | ######## | 1800 | 5017.11 | 5851 | 5474 | 968.86 | 230 | 832.09 | 229 | 50.75 | 160 | 377 | 160 | 1120 | 153.83 | 11 | 79 | 90 | |
| 21 | | ######## | 1800 | 0 | 65 | 44 | 1782.09 | 42 | 17.91 | 41 | 5.23 | 9 | 21 | 0 | 0 | 0 | 0 | 0 | 0 | |
| 22 | | ######## | 1800 | 0 | 121 | 85 | 1760.1 | 77 | 39.9 | 76 | 9.42 | 15 | 36 | 0 | 0 | 0 | 0 | 0 | 0 | |
| 23 | | ######## | 1800 | 26.86 | 519 | 393 | 1706.83 | 140 | 93.17 | 139 | 31.43 | 40 | 126 | 0 | 0 | 0 | 0 | 0 | 0 | |
| 24 | | ######## | 1800 | 9452.02 | 10223 | 9636 | 678.63 | 164 | 1120.74 | 164 | 59.05 | 260 | 587 | 341 | 1733 | 224.78 | 5 | 126 | 131 | |
| 25 | | ######## | 1800 | 27610.2 | 20335 | 19704 | 287.19 | 95 | 1512.38 | 96 | 34.82 | 297 | 631 | 631 | 3580 | 445.06 | 459 | 178 | 637 | |
| 26 | | ######## | | | | | | | | | | 219 | 467 | 472 | 3271 | 439 | 80 | 361 | 441 | |
| 27 | | ######## | | | | | | | | | | 282 | 637 | 409 | 2392 | 285.99 | 15 | 254 | 269 | |
| 28 | | ######## | | | | | | | | | | 192 | 408 | 393 | 2512 | 451.98 | 109 | 205 | 314 | |
| 29 | | ######## | | | | | | | | | | 274 | 701 | 94 | 2079 | 1618.6 | 92 | 116 | 208 | |
| 30 | | ######## | | | | | | | | | | 222 | 472 | 205 | 958 | 1216.19 | 77 | 130 | 207 | |
| 31 | | ######## | | | | | | | | | | 364 | 800 | 58 | 2189 | 1669.93 | 73 | 296 | 369 | |
| 32 | | ######## | | | | | | | | | | 242 | 559 | 160 | 1037 | 1265.62 | 91 | 161 | 252 | |
| | | | | | | | | | | | | | | | | | | | | |
| 33 | | ######## | | | | | | | | | | 50 | 111 | 132 | 216 | 309.16 | 20 | 32 | 52 | |
| 34 | | ######## | | | | | | | | | | 0 | 0 | 11 | 0 | 1.12 | 0 | 0 | 0 | |
| 35 | | ######## | 1800 | 0 | 0 | 0 | 1800 | 1 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | |

***FIGURE 3-1 – AN EXAMPLE OF THE QUANTITATIVE MANIFESTATION OF PATTERN RUNNING AND THE CHRONOLOGICAL VARIATION OF THE BEHAVIOURAL TOPOGRAPHIES EXPRESSED IN DEER MICE***

## 3.2. A REVIEW OF STUDY OBJECTIVES

Table 3-2 summarizes the study objectives as outlined in Chapter 1 and the literature upon which these objectives have been founded.

| Study objective | Literature that formed the foundation |
|---|---|
| *i. To classify deer mice into [NSB/LSB] and HSB cohorts based on a newly developed classification system accommodating the different manifestations of the two main topographies, viz. pattern running and vertical activity.* | Based on observations and methodologies taken from our earlier work (Güldenpfennig *et al.*, 2011; Korff *et al.*, 2008; Korff *et al.*, 2009), the rationale for this particular study objective is thoroughly explained in section 3.1 and strengthened by the data presented in Addendum A. |
| *ii. Strengthen the construct of the deer mouse model of OCD by determining whether any differences exist between baseline values of frontal-cortical and striatal SERT densities in treatment naive [NSB/LSB] and HSB animals.* | It is postulated that the serotonergic system, which is thought to oppose the behavioural effects of dopamine, is hypo-functioning in OCD (Daw *et al.*, 2002; Goddard *et al.*, 2008; Husted *et al.*, 2006; Markarian *et al.*, 2010; Zitterl *et al.*, 2008) (see paragraphs 2.2.2.4 and 2.3.1.1). Since a number of studies have indicated that patients with OCD have lower densities of SERT than healthy controls (Hesse *et al.*, 2005; Reimold *et al.*, 2007; Zitterl *et al.*, 2008), it can be postulated that the SERT density could be used as a robust measure of serotonergic signalling. |
| *iii. Strengthen the predictive validity of the deer mouse model of OCD by determining the effect of sub-chronic and chronic high-dose oral treatment with the SSRI, escitalopram, on the expression of stereotypy in the deer mouse.* | OCD responds preferentially to chronic high-dose SSRI treatment. Thus, the efficacy of chronic high dose SSRI treatment in attenuating deer mouse stereotypy needs to be determined and whether sub-chronic treatment is indeed ineffective (see paragraphs 2.1.3 and 2.3.2). |

***TABLE 3-2 – A SYNOPSIS OF THE STUDY OBJECTIVES AND RATIONALE***

Although much investigation into the model has been undertaken to date, and while recent reviews on the subject have highlighted the deer mouse model as a leading animal model of relevance for OCD (Albelda and Joel, In Press (a); Albelda and Joel, In Press (b); Hoffman and Rueda Morales, 2009; Joel, 2006; Joel *et al.*, 2008), stereotypy in the deer mouse still needs to be more comprehensively evaluated and validated before it can be regarded as a definitive animal model of OCD. As outlined by Chapter 2, much is still to be elucidated regarding the pathogenesis and underlying pathology of OCD, a fact that not only complicates the understanding and treatment of OCD in humans, but also slows the development of suitable animal models. Since there are many uncertainties regarding the pathogenesis and neurobiology of OCD, such as the role of comorbid disorders and the functioning of certain neurotransmitters, an investigation into a specific form of stereotypical animal behaviour will not be sufficient to ensure adequate

validation of the animal model for OCD. Therefore, the main aim of the current study will be to strengthen the predictive and construct validity of stereotypy in the deer mouse as a putative animal model of OCD. This will be done by more stringently delineating the criteria for assessing stereotypic behaviours in these animals, especially by developing a new method of analysing and scoring stereotypy in deer mice, and by implicating the cortico-striatal serotonergic system (altered SERT density) as a causal neurobiological factor in the genesis of these behaviours.

In Chapter 4, the methods used in an attempt to achieve the above-mentioned behavioural and neurochemical outcomes of the study will be discussed.

*END OF CHAPTER 3*

# CHAPTER 4
# THE METHODOLOGICAL BASIS OF THE CURRENT STUDY

## 4.1. THE STUDY OUTLINE

As the main aim of the study was to strengthen the construct and predictive validity of stereotypical behaviour in the deer mouse as a putative animal model of OCD, the framework of the investigation was designed to support the predictive validation on a platform of behavioural assessment followed by pharmacological treatment with the SSRI escitalopram. Construct validation of the model was based on post-mortem SERT analysis. In order to more accurately observe drug treatment related changes on deer mouse stereotypy, it was necessary to identify specific topographies of stereotypical behaviour in these animals that allowed for more reliable and robust identification and interpretation. After close scrutiny of the escitalopram treated data sheets, a new method of analysing the effects of treatment on the expression of stereotypy by deer mice was developed based on (1) identifying and scoring rest periods between bouts of stereotypical behaviours, and (2) identifying and scoring periods of heightened stereotypy between normal bouts of activity.

In this chapter, the study design, animals, reagents and equipment used, as well as the behavioural and neurochemical methodology followed during the course of this study will be discussed in detail. The study method as will be discussed comprises a number of small pilot studies that were necessary to decide on certain aspects of the study design, how to identify key behaviours within a pattern of data, and how to score these data. The final data are now presented in Chapter 5 as well as in the different addendums.

In order to compare the baseline behaviour and frontal cortical and striatal SERT densities of the [NSB/LSB] cohort with that of the HSB cohort, the 40 animals included in the control study were used. The subsequent outline of the main study is summarized in Figure 4-1, while a graphical representation of the study progress is shown on a timeline in Figure 4-2.

The same behavioural and neurochemical methodology was followed for each of the two studies, the only difference between these two groups being that the control group never received any drug, while the treatment group received daily escitalopram from day 29 (one day after the fifth behavioural screen) until day 56. The progression of the study from day 0 until day 56 is discussed in detail in section 3.2, without discriminating between the two groups except when discussing the administration of treatment.

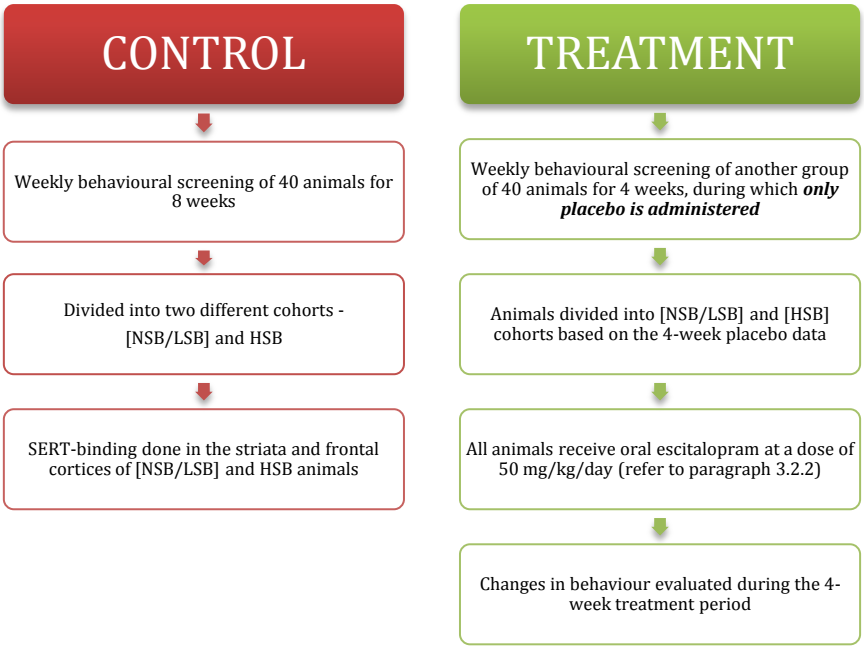



***FIGURE 4-1 – A SCHEMATIC REPRESENTATION OF THE STUDY OUTLINE***

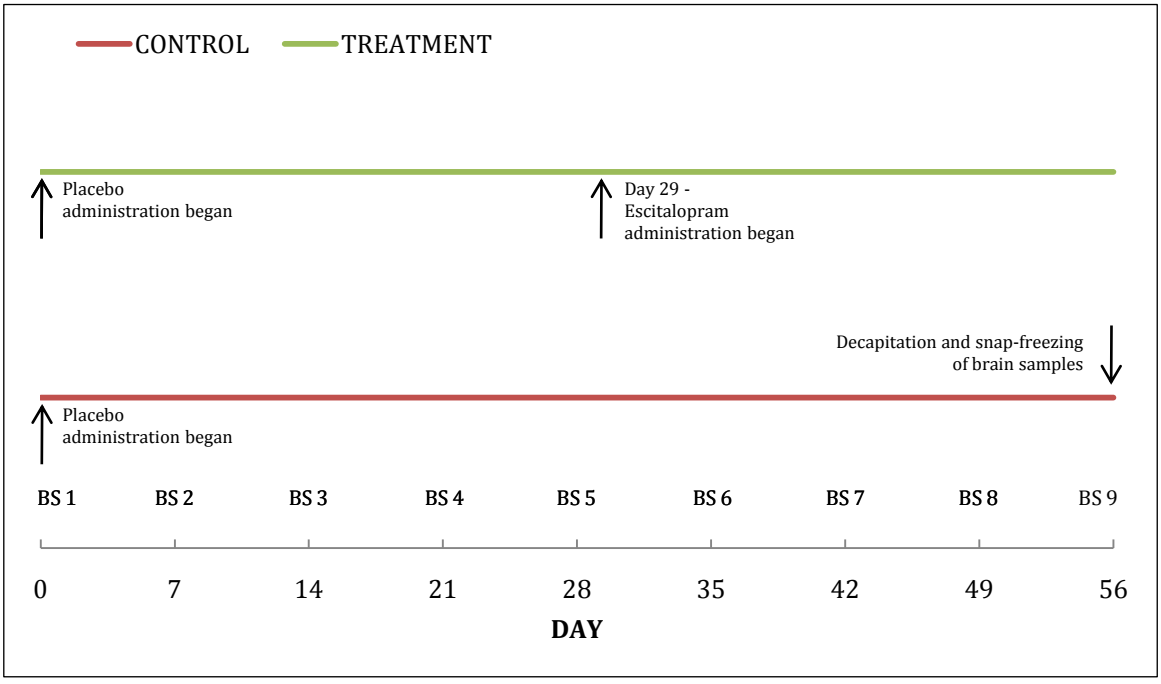



***FIGURE 4-2 – THE TIME COURSE OF THE STUDY***

(BS 1 – 9: Behavioural screens)

## 4.2. EXPERIMENTAL MATERIALS AND PROCEDURES

### *4.2.1. Animals*

Forty (40) deer mice were used during each study and were obtained from the deer mouse colony maintained and housed at the Animal Research Centre of North-West University (NWU), Potchefstroom, South Africa (Ethical Approval Number - NWU-00066-10-S5). As the stereotypical behaviour expressed by deer mice is established by the age of 8 weeks (Korff *et al.*, 2008), only mice between the ages of 10 and 12 weeks were chosen as experimental subjects at the onset of behavioural assessments (day 0). Mice were randomly chosen from different litters, without sex or weight bias with the average weight of a deer mouse being 19 grams. One day before the start of the behavioural assessments (day -1), each animal was allocated individually to an automatically climate-controlled laboratory cage (35cm (l) x 20cm (w) x 13cm (h); Techniplast® S.P.A., Varese, Italy) and maintained at 23°C on a 12-hour light/dark cycle (06:00 to 18:00). Food and water were provided ad lib for the duration of the control study and over the first four weeks of the treatment study. However, from the fifth week of the treatment study, oral drug administration via the drinking water began, with the solutions being replaced every day (for a full discussion on the administration of the drug, refer to paragraph 3.2.2). Cages were cleaned and new bedding material, food and water provided on a weekly basis.

* * *

As the average weight of a deer mouse is 19 grams, the brain tissue from one animal is not sufficient to complete one saturation binding assay for SERT analysis (refer to paragraph 4.2.4 for a discussion on the methodology of saturation binding). In experimental trial runs before the actual study, it was determined that striatal and frontal-cortical tissues from at least 3 animals (or 90mg), classified in the same behavioural cohort, were needed to perform one binding assay. As a minimum of three to four assays are needed to guarantee statistical credibility (previous experience from similar studies), 9 – 12 animals per cohort were needed to determine the average SERT density of each cohort respectively. To compensate for unplanned deaths, and to discriminate as clearly as possible between animals from the different cohorts, it was decided that at least 40 animals were needed in both studies, providing a 'grey' margin of 16 – 22 animals across both topographies in each study. Therefore, after the control study, the striatal and frontal-cortical tissue of the 9 – 12 animals presenting with the highest and the lowest weekly amounts of stereotypy in the saturation binding studies were used. This rationale was also followed by Presti and Lewis (2005) and ensures the generation of optimal neurochemical results.

### *4.2.2. Drug used and administration*

Escitalopram oxalate was a generous gift of H. Lundbeck A/S® (9 Ottiliavej, Valby, Copenhagen, Denmark). Escitalopram for oral administration was prepared by dissolving it in the drinking water. This was done because of the long duration of each study (8 weeks) and to minimize the influence of injection stress on the behaviour, well-being, and survival of the animals. Furthermore, the half-life of escitalopram in rodents is less than one hour (data supplied by H. Lundbeck A/S®), so that at least three daily intraperitoneal injections would be necessary to ensure adequate plasma escitalopram concentrations. As OCD is postulated to be an anxiety disorder (American Psychiatric Association, 2000), all forms of contact with the mice was kept at a minimum to prevent any possible anxiogenic effects that might result from physical handling. For the same reason, it was also decided not to use oral gavage as a route of drug administration.

However, oral treatment in drinking water cannot be regarded as a dosage route free from complications. A number of uncertainties characterize oral dosing when the drug is administered in drinking water, including inter-animal differences in the amount of water ingested, uncertainties about oral bioavailability of drugs in rodents, the inevitable wastage of drug, and the leakage of water bottles filled with very little quantities of liquid (preliminary observations in our laboratory – data not shown). However, these obstacles can be overcome to a large extent by administering a drug in a dose based on a calculation of the average water intake by animals from the same species, and supplying each animal with a freshly prepared drug solution every day. In pilot studies done to assess the normal intake of water by deer mice (see Addendum B), it was established that the average amount of water ingested by a deer mouse equals 0.25 ml/g/day, confirming statistics provided by the Animal Research Centre. Subsequently, the following calculation was applied to determine the amount of drug to be dissolved to ensure that the correct dose would be administered (the calculation shown is applicable to a dose of 30 mg/kg/day):

| | | |
|---|---|---|
| *Average water intake by a deer mouse* | = | *0.25 ml/g/day* |
| *A dose of 30 mg/kg/day* | = | *0.03 mg/g/day* |
| *THUS: Concentration of drug desired* | = | *0.03 mg/0.25 ml* |
| | = | *12 mg/100 ml* |

*(The mass calculated above was converted to the mass of escitalopram oxalate before the solutions were prepared)*

* * *

The main problem with the administration of drug in drinking water is the unnecessary wastage of drug resulting from the regular replacement of drug solution. In an attempt to limit the wastage to as little as possible, each animal was provided with the daily minimum amount of liquid required that would prevent leakage of water during times of no drinking activity, and to satisfy the average required individual intake of liquid. This amount equalled 20ml per animal per day. Unfortunately, this quantity is also associated with increased leakage during times of drinking activity so that daily weighing of water bottles could not be used as a reliable measure of liquid intake. Subsequently, a pilot study was launched to assess the drinking behaviour of deer mice presented with a 4 mg/100ml solution of escitalopram. At this concentration, the average individual daily intake of escitalopram is 10 mg/kg/day (according to the calculation above). The primary purpose of the pilot study was to assess whether deer mice express any aversion to the taste of escitalopram in their drinking water and not to perform any drug-treatment response on behaviour or neurochemistry. Therefore, and in order to conserve the limited amount of drug available, a dose of 10 mg/kg/day rather than higher doses normally recommended for an OCD-related study, eg. 20 - 50 mg/kg/day that would later be applied in the primary study, was administered. During the pilot study, each bottle was filled to full capacity with the escitalopram solution to prevent unnecessary leakage. The daily intake of solution was measured by weighing the water bottles every day between 9:00 and 10:00 AM. This study demonstrated that on average, the animals drank the same amount of escitalopram solution compared to water over the period of evaluation (Addendum C for supportive data).

Once the above series of studies were completed, a dose-response analysis was initiated during which escitalopram was administered in doses of 1, 10, 30, and 40 mg/kg/day respectively over a period of 14 days. These doses were chosen based on previous studies done in our laboratory with fluoxetine, which demonstrated an ameliorative effect on deer mouse stereotypy at an intraperitoneal dose of 20 mg/kg/day (Korff *et al.*, 2008). Although the duration of treatment in the current study was 4 weeks, previous data generated in our laboratory (not published) demonstrated that at least some ameliorative effect on deer mouse stereotypy could be observed after two weeks of intraperitoneal treatment with fluoxetine. Therefore, due to factors such as the availability of animals of appropriate age and the amount of drug at our disposal, it was decided that a dose-response analysis of only two weeks in duration would be deployed. Unfortunately, no behavioural effect could be demonstrated (see Addendum D). In the study published by Greene-Schloesser and colleagues (2011) (see paragraph 2.3.4.1 for a discussion), the authors administered fluoxetine in the drinking water of mice at a dose of 50 mg/kg/day, which resulted in the near maximal inhibition of compulsive-like nest-building be-

haviour. Although both fluoxetine and escitalopram are potent inhibitors of SERT, escitalopram demonstrates even more selectivity for SERT, and inhibits it more potently than fluoxetine (Owens *et al.*, 2001). It was therefore decided to administer escitalopram at a dose of 50 mg/kg/day but over a period of four weeks.

* * *

As described in section 3.1, the treatment study consisted of two parts. During the first four weeks, all animals received only water. Each animal was subsequently classified into either the [NSB/LSB] or HSB cohort based on the median behavioural score calculated from the first five weekly stereotypy scores generated by each individual (refer to paragraph 3.1.3). On day 29, following the fifth behavioural assessment, the administration of escitalopram in the drinking water began. Every day a new solution of escitalopram was prepared at a concentration of 20 mg/100ml, sufficient to provide each animal with 50 mg/kg/day based on an average water intake of 0.25 ml/g/day (see the calculation above), of which 20ml was transferred to each water bottle. The daily replacement of escitalopram solution continued throughout the four weeks of drug administration. Animals that were behaviourally screened during the night, received the same solution during behavioural assessment.

### *4.2.3. Assessing the behavioural topographies of deer mice*

#### *4.2.3.1. Generating the behavioural data*

As stated earlier, all animals underwent weekly behavioural screening during the 8-week period of each study. On any specific assessment day, eight animals were moved from their housing environment to the behavioural screening room. These areas are located 15m from one another on the same floor of the Animal Research Centre and are environmentally controlled with respect to temperature (23°C), humidity, and light-dark cycle. Subsequently each animal was introduced to a test cage (21cm (w) x 21cm (l) x 35cm (h); Accuscan® Instruments, Columbus, Ohio, USA) constructed from clear, translucent Plexiglas®. Bedding material was provided in quantities enough to cover the floor of the test cages, but ensuring that it did not interrupt the light beams and influence the scoring of behavioural data. Food was also provided ad lib on the floor of the cage in the form of course pellets broken from the normal rodent chow. Water and escitalopram solutions were provided through a tiny hole in a wall of each test cage. As some mice have the ability to jump as much as 7 times higher than their own length, the cages were covered with lids but at the same time allowing uninterrupted airflow. The animals were introduced to these environments by 16h00 and habituated for at least two hours before the behavioural assessments started at the onset of the dark cycle. This routine was repeated for five

days of the week, screening a different group of eight animals every time, allowing two nights of the week during which rescreening could be done should power outages or other interruptions have occurred during the week. The test cages were cleaned after each screen using the virucidal, bactericidal, and mycocidal disinfectant, Virkon® (DuPont de Nemours®, Melrose Arch, Sandton, South Africa).

* * *

The behavioural assessment was performed using the Fusion® Animal Activity Monitoring System (Accuscan® Instruments, Columbus, Ohio, USA). As explained briefly in paragraph 3.1.1, the cage presents with a grid of infra-red light beams that cross the cage roughly 2cm above the cage floor along the X- and Y-axes (Figure 4-3), while another set of beams cross the cage 10cm above the bottom grid only along the Y-axis (also termed the Z-axis).

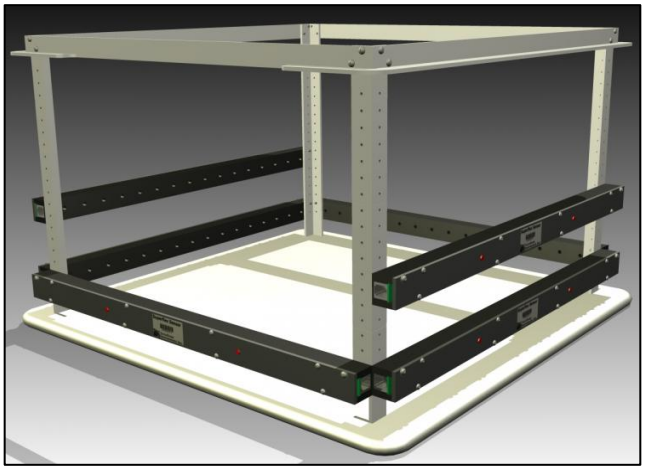


***FIGURE 4-3 – THE FUSION® HARDWARE SETUP***

These beams are spaced 2.5cm apart and record activity every time it is interrupted by movement. Thus, the software can track the movements of the animals and the distances they travel, while also recording different forms of behaviour, such as stereotypy, vertical jumping, and locomotor activity. Each of these hardware units were divided in four quarters, allowing two animals to be screened in two of the four separate compartments, each 21cm (w) x 21cm (l). As explained earlier, the software records the repetitive interruption of the same beam or set of beams in the same location, as stereotypy. When assessing stereotypic behaviour of deer mice, however, these data cannot be used as the forms of stereotypy recorded by the software may also include behaviour such as head dipping and tail flicking. Therefore, only the parameters of circling behaviour and the amount of vertical beam interruptions recorded by the soft-

ware were used to assess the behavioural topographies expressed by deer mice. For a discussion on the criteria applied to assess these three behavioural topographies, refer to section 3.1.

During the recording session, the Fusion® software generates data continuously (Figure 4-4), allowing for experimental playback and the export of behavioural reports the following day. The data reports were subsequently used to calculate the average stereotypy score of a mouse following each behavioural screen.

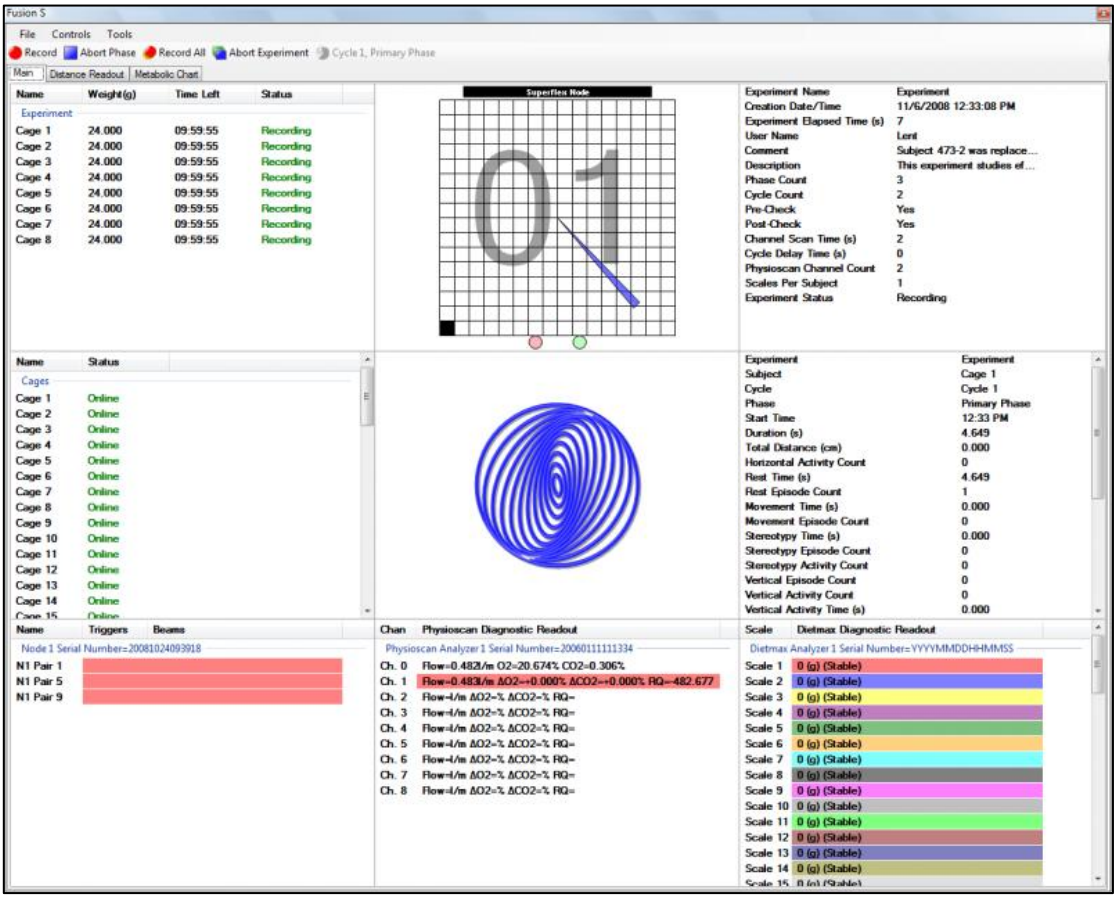
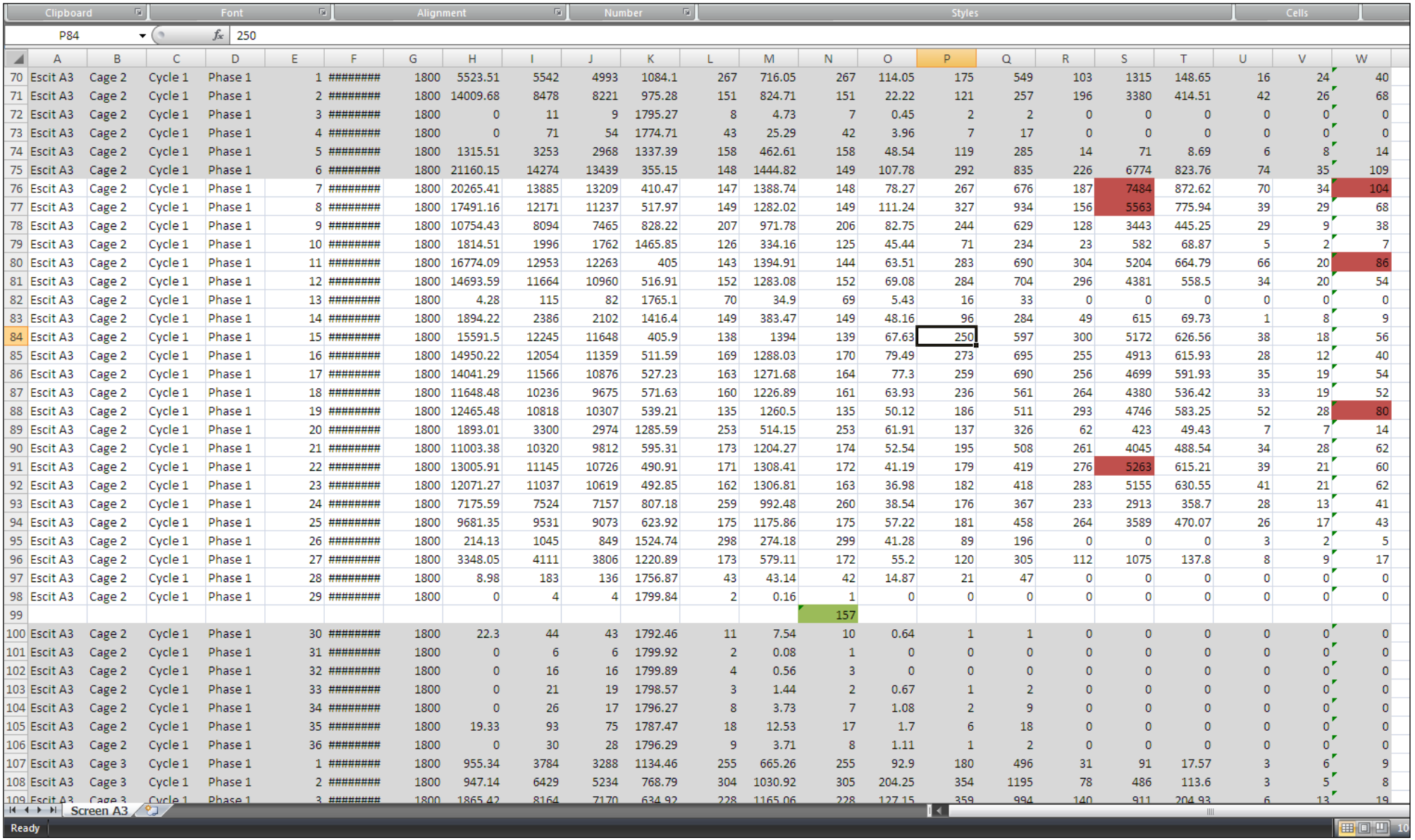



***FIGURE 4-4 – THE FUSION® INTERFACE DURING THE RECORDING OF BEHAVIOUR***

NOTE: Each individual black line drawn in the square represents one beam. Beams are spaced 2.5cm apart. *This figure depicts the cage setup for one animal. This cage had been divided in four quarters to allow for the screening of 2 animals at a time*

As explained in paragraph 3.1.3, the data is incremented in 30-minute intervals during the recording of behaviour. Subsequently, the software generates all behavioural statistics for each of the 24 timeslots incorporated in a 12-hour screening session. This data can subsequently be exported in Microsoft® Excel® format, after the completion of each screening session (Figure 4-5).



| | A | B | C | D | E | F | G | H | I | J | K | L | M | N | O | P | Q | R | S | T | U | V | W |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 70 | Escit A3 | Cage 2 | Cycle 1 | Phase 1 | 1 | ######## | 1800 | 5523.51 | 5542 | 4993 | 1084.1 | 267 | 716.05 | 267 | 114.05 | 175 | 549 | 103 | 1315 | 148.65 | 16 | 24 | 40 |
| 71 | Escit A3 | Cage 2 | Cycle 1 | Phase 1 | 2 | ######## | 1800 | 14009.68 | 8478 | 8221 | 975.28 | 151 | 824.71 | 151 | 22.22 | 121 | 257 | 196 | 3380 | 414.51 | 42 | 26 | 68 |
| 72 | Escit A3 | Cage 2 | Cycle 1 | Phase 1 | 3 | ######## | 1800 | 0 | 11 | 9 | 1795.27 | 8 | 4.73 | 7 | 0.45 | 2 | 2 | 0 | 0 | 0 | 0 | 0 | 0 |
| 73 | Escit A3 | Cage 2 | Cycle 1 | Phase 1 | 4 | ######## | 1800 | 0 | 71 | 54 | 1774.71 | 43 | 25.29 | 42 | 3.96 | 7 | 17 | 0 | 0 | 0 | 0 | 0 | 0 |
| 74 | Escit A3 | Cage 2 | Cycle 1 | Phase 1 | 5 | ######## | 1800 | 1315.51 | 3253 | 2968 | 1337.39 | 158 | 462.61 | 158 | 48.54 | 119 | 285 | 14 | 71 | 8.69 | 6 | 8 | 14 |
| 75 | Escit A3 | Cage 2 | Cycle 1 | Phase 1 | 6 | ######## | 1800 | 21160.15 | 14274 | 13439 | 355.15 | 148 | 1444.82 | 149 | 107.78 | 292 | 835 | 226 | 6774 | 823.76 | 74 | 35 | 109 |
| 76 | Escit A3 | Cage 2 | Cycle 1 | Phase 1 | 7 | ######## | 1800 | 20265.41 | 13885 | 13209 | 410.47 | 147 | 1388.74 | 148 | 78.27 | 267 | 676 | 187 | 7484 | 872.62 | 70 | 34 | 104 |
| 77 | Escit A3 | Cage 2 | Cycle 1 | Phase 1 | 8 | ######## | 1800 | 17491.16 | 12171 | 11237 | 517.97 | 149 | 1282.02 | 149 | 111.24 | 327 | 934 | 156 | 5563 | 775.94 | 39 | 29 | 68 |
| 78 | Escit A3 | Cage 2 | Cycle 1 | Phase 1 | 9 | ######## | 1800 | 10754.43 | 8094 | 7465 | 828.22 | 207 | 971.78 | 206 | 82.75 | 244 | 629 | 128 | 3443 | 445.25 | 29 | 9 | 38 |
| 79 | Escit A3 | Cage 2 | Cycle 1 | Phase 1 | 10 | ######## | 1800 | 1814.51 | 1996 | 1762 | 1465.85 | 126 | 334.16 | 125 | 45.44 | 71 | 234 | 23 | 582 | 68.87 | 5 | 2 | 7 |
| 80 | Escit A3 | Cage 2 | Cycle 1 | Phase 1 | 11 | ######## | 1800 | 16774.09 | 12953 | 12263 | 405 | 143 | 1394.91 | 144 | 63.51 | 283 | 690 | 304 | 5204 | 664.79 | 66 | 20 | 86 |
| 81 | Escit A3 | Cage 2 | Cycle 1 | Phase 1 | 12 | ######## | 1800 | 14693.59 | 11664 | 10960 | 516.91 | 152 | 1283.08 | 152 | 69.08 | 284 | 704 | 296 | 4381 | 558.5 | 34 | 20 | 54 |
| 82 | Escit A3 | Cage 2 | Cycle 1 | Phase 1 | 13 | ######## | 1800 | 4.28 | 115 | 82 | 1765.1 | 70 | 34.9 | 69 | 5.43 | 16 | 33 | 0 | 0 | 0 | 0 | 0 | 0 |
| 83 | Escit A3 | Cage 2 | Cycle 1 | Phase 1 | 14 | ######## | 1800 | 1894.22 | 2386 | 2102 | 1416.4 | 149 | 383.47 | 149 | 48.16 | 96 | 284 | 49 | 615 | 69.73 | 1 | 8 | 9 |
| 84 | Escit A3 | Cage 2 | Cycle 1 | Phase 1 | 15 | ######## | 1800 | 15591.5 | 12245 | 11648 | 405.9 | 138 | 1394 | 139 | 67.63 | 250 | 597 | 300 | 5172 | 626.56 | 38 | 18 | 56 |
| 85 | Escit A3 | Cage 2 | Cycle 1 | Phase 1 | 16 | ######## | 1800 | 14950.22 | 12054 | 11359 | 511.59 | 169 | 1288.03 | 170 | 79.49 | 273 | 695 | 255 | 4913 | 615.93 | 28 | 12 | 40 |
| 86 | Escit A3 | Cage 2 | Cycle 1 | Phase 1 | 17 | ######## | 1800 | 14041.29 | 11566 | 10876 | 527.23 | 163 | 1271.68 | 164 | 77.3 | 259 | 690 | 256 | 4699 | 591.93 | 35 | 19 | 54 |
| 87 | Escit A3 | Cage 2 | Cycle 1 | Phase 1 | 18 | ######## | 1800 | 11648.48 | 10236 | 9675 | 571.63 | 160 | 1226.89 | 161 | 63.93 | 236 | 561 | 264 | 4380 | 536.42 | 33 | 19 | 52 |
| 88 | Escit A3 | Cage 2 | Cycle 1 | Phase 1 | 19 | ######## | 1800 | 12465.48 | 10818 | 10307 | 539.21 | 135 | 1260.5 | 135 | 50.12 | 186 | 511 | 293 | 4746 | 583.25 | 52 | 28 | 80 |
| 89 | Escit A3 | Cage 2 | Cycle 1 | Phase 1 | 20 | ######## | 1800 | 1893.01 | 3300 | 2974 | 1285.59 | 253 | 514.15 | 253 | 61.91 | 137 | 326 | 62 | 423 | 49.43 | 7 | 7 | 14 |
| 90 | Escit A3 | Cage 2 | Cycle 1 | Phase 1 | 21 | ######## | 1800 | 11003.38 | 10320 | 9812 | 595.31 | 173 | 1204.27 | 174 | 52.54 | 195 | 508 | 261 | 4045 | 488.54 | 34 | 28 | 62 |
| 91 | Escit A3 | Cage 2 | Cycle 1 | Phase 1 | 22 | ######## | 1800 | 13005.91 | 11145 | 10726 | 490.91 | 171 | 1308.41 | 172 | 41.19 | 179 | 419 | 276 | 5263 | 615.21 | 39 | 21 | 60 |
| 92 | Escit A3 | Cage 2 | Cycle 1 | Phase 1 | 23 | ######## | 1800 | 12071.27 | 11037 | 10619 | 492.85 | 162 | 1306.81 | 163 | 36.98 | 182 | 418 | 283 | 5155 | 630.55 | 41 | 21 | 62 |
| 93 | Escit A3 | Cage 2 | Cycle 1 | Phase 1 | 24 | ######## | 1800 | 7175.59 | 7524 | 7157 | 807.18 | 259 | 992.48 | 260 | 38.54 | 176 | 367 | 233 | 2913 | 358.7 | 28 | 13 | 41 |
| 94 | Escit A3 | Cage 2 | Cycle 1 | Phase 1 | 25 | ######## | 1800 | 9681.35 | 9531 | 9073 | 623.92 | 175 | 1175.86 | 175 | 57.22 | 181 | 458 | 264 | 3589 | 470.07 | 26 | 17 | 43 |
| 95 | Escit A3 | Cage 2 | Cycle 1 | Phase 1 | 26 | ######## | 1800 | 214.13 | 1045 | 849 | 1524.74 | 298 | 274.18 | 299 | 41.28 | 89 | 196 | 0 | 0 | 0 | 3 | 2 | 5 |
| 96 | Escit A3 | Cage 2 | Cycle 1 | Phase 1 | 27 | ######## | 1800 | 3348.05 | 4111 | 3806 | 1220.89 | 173 | 579.11 | 172 | 55.2 | 120 | 305 | 112 | 1075 | 137.8 | 8 | 9 | 17 |
| 97 | Escit A3 | Cage 2 | Cycle 1 | Phase 1 | 28 | ######## | 1800 | 8.98 | 183 | 136 | 1756.87 | 43 | 43.14 | 42 | 14.87 | 21 | 47 | 0 | 0 | 0 | 0 | 0 | 0 |
| 98 | Escit A3 | Cage 2 | Cycle 1 | Phase 1 | 29 | ######## | 1800 | 0 | 4 | 4 | 1799.84 | 2 | 0.16 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 99 | | | | | | | | | | | | | | 157 | | | | | | | | | |
| 100 | Escit A3 | Cage 2 | Cycle 1 | Phase 1 | 30 | ######## | 1800 | 22.3 | 44 | 43 | 1792.46 | 11 | 7.54 | 10 | 0.64 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| 101 | Escit A3 | Cage 2 | Cycle 1 | Phase 1 | 31 | ######## | 1800 | 0 | 6 | 6 | 1799.92 | 2 | 0.08 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 102 | Escit A3 | Cage 2 | Cycle 1 | Phase 1 | 32 | ######## | 1800 | 0 | 16 | 16 | 1799.89 | 4 | 0.56 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 103 | Escit A3 | Cage 2 | Cycle 1 | Phase 1 | 33 | ######## | 1800 | 0 | 21 | 19 | 1798.57 | 3 | 1.44 | 2 | 0.67 | 1 | 2 | 0 | 0 | 0 | 0 | 0 | 0 |
| 104 | Escit A3 | Cage 2 | Cycle 1 | Phase 1 | 34 | ######## | 1800 | 0 | 26 | 17 | 1796.27 | 8 | 3.73 | 7 | 1.08 | 2 | 9 | 0 | 0 | 0 | 0 | 0 | 0 |
| 105 | Escit A3 | Cage 2 | Cycle 1 | Phase 1 | 35 | ######## | 1800 | 19.33 | 93 | 75 | 1787.47 | 18 | 12.53 | 17 | 1.7 | 6 | 18 | 0 | 0 | 0 | 0 | 0 | 0 |
| 106 | Escit A3 | Cage 2 | Cycle 1 | Phase 1 | 36 | ######## | 1800 | 0 | 30 | 28 | 1796.29 | 9 | 3.71 | 8 | 1.11 | 1 | 2 | 0 | 0 | 0 | 0 | 0 | 0 |
| 107 | Escit A3 | Cage 3 | Cycle 1 | Phase 1 | 1 | ######## | 1800 | 955.34 | 3784 | 3288 | 1134.46 | 255 | 665.26 | 255 | 92.9 | 180 | 496 | 31 | 91 | 17.57 | 3 | 6 | 9 |
| 108 | Escit A3 | Cage 3 | Cycle 1 | Phase 1 | 2 | ######## | 1800 | 947.14 | 6429 | 5234 | 768.79 | 304 | 1030.92 | 305 | 204.25 | 354 | 1195 | 78 | 486 | 113.6 | 3 | 5 | 8 |
| 109 | Escit A3 | Cage 3 | Cycle 1 | Phase 1 | 3 | ######## | 1800 | 1865.42 | 8164 | 7170 | 634.92 | 228 | 1165.06 | 228 | 127.15 | 359 | 994 | 140 | 911 | 204.93 | 6 | 13 | 19 |

***FIGURE 4-5 – AN EXCERPT FROM THE MICROSOFT® EXCEL® DATA SHEET EXPORTED FOLLOWING THE COMPLETION OF EACH BEHAVIOURAL SCREEN***

The higlighted green square represents the average number of horizontal movements per 30 minutes over the course of 24 hours. The first colomn of highlighted red squares represents the highest vertical jumping scores per 30 minutes over 24 hours, while the last column represents the same values for the horizontal revolutions during the same period.

#### *4.2.3.2. Analyzing the behavioural data*

The methodology described in this paragraph was applied to classify deer mice into the different behavioural cohorts (NSB, LSB and HSB respectively), and was used to categorize animals in the control study after 4 and 8 weeks of behavioural assessment respectively, and animals in the treatment study after the first four weeks of pre-treatment baseline assessments. However, after careful scrutiny of the effects of escitalopram on the expression of deer mouse stereotypy, a novel approach was developed to appraise the effects of sub-chronic and chronic drug treatment on the expression of stereotypical behaviour in deer mice. This new technique was developed after rather unexpected negative results were obtained using escitalopram while applying the original method of behavioural assessment described earlier (results presented in paragraph 5.3.1). However, this new approach has been restricted to evaluating drug treatment and its effect on the expression of stereotypy. To keep this in context the method will only be described in more detail in Chpater 5 (see paragraph 5.3.2).

It must be noted that the 40 animals in the drug treatment study were treated as a separate group. The *4-week pre-treatment assessment period for each animal acted as a built-in control* to compare the effects of escitalopram treatment on the expression of deer mouse stereotypy pre- and post-treatment. It was therefore decided not to analyze the behaviour of the 40 animals in the control (treatment naive) group using the revised method of assessment. It must also be kept in mind that the behaviour of this latter group of animals (i.e. treatment naive) were investigated with the intent to develop a new classification system for the appraisal of deer mouse stereotypy and to separate the [NSB/LSB] and HSB cohorts as clearly as possible, and hence the aims and outcomes were not the same. As a built in control was included in the treatment study, it was never the intention to compare behavioural changes manifesting in the control versus drug treatment groups of the study.

* * *

First, all data generated during the light cycle (the horizontal grey bars on the top and bottom of Figure 4-5) were excluded. The numbers in the left-hand side column indicate the individual 30-minute intervals. Thus, for this specific animal, the dark cycle began at the 11$^{th}$ interval, continuing until the 36$^{th}$ interval. Secondly, the general locomotor activity of the animal was recorded. The cell highlighted in green depicts the average amount of movement episodes per 30 minutes (defined as locomotor episodes separated by at least 1 second) generated during the whole of the dark cycle and was used to determine the locomotor activity of the animals. Therefore, these averages should be more or less constant throughout the study, if escitalopram has

not influenced the locomotor activity of the animals. Third, the average amount of vertical beam interruptions were calculated from the three highest 30-minute values generated during the night. These values are highlighted in red (fifth column from the right). These weekly averages were subsequently used for the evaluation of vertical stereotypy. Lastly, the average amount of cage revolutions were calculated also based on the three highest 30-minute values generated during the screening session. All the clockwise and anti-clockwise revolutions (second and third columns from the right, respectively) were summed and used to calculate the average amount of cage revolutions from the three highest 30-minute sum-totals (far-right column), also highlighted in red. As with the vertical stereotypy averages, the weekly cage revolution scores were used for the evaluation of pattern running. In paragraph 3.1.3, the interrupted manner in which deer mice express stereotypical behaviour is described. This can once again be seen in Figure 4-5, depicting the relatively long intervals between the executions of the highest numbers of stereotypy. Although the three highlighted values of the two different topographies are preceded and followed by numbers near enough to the highlighted values, the expression of repetitive behaviour in many animals clearly varies over time. Also, note the periods of very little to no stereotypical activity.

* * *

After 8 weeks of weekly behavioural screening, another data sheet is generated that summarizes the weekly average vertical activity and pattern running scores for each animal, and an example is provided in Figure 4-6. The data shown here have been generated for the control group. The numbers in the far left-hand column depict the individual behavioural assessment numbers. The first assessment (1) was done on day 0 and the last (9) on day 56 for each animal respectively. The numbers in the third row from the top, just above the first vertical activity scores, indicate the individual animals. Median stereotypy scores for both groups (control and treatment) were calculated from the individual averages after the fourth week of baseline assessment and were applied to categorize animals into the different behavioural cohorts ([NSB/LSB] and HSB respectively).

In order to compare the *behavioural manifestation* (study aim 1) of [NSB/LSB] and HSB animals in the control group during the eight-week assessment period and to categorize animals in the treatment study, all animals were classified after the fourth week of behavioural assessment based on the median value of stereotypy calculated as explained above. This discrimination was based exclusively on the criteria described in paragraph 3.1.3. Animals must have demonstrated [NSB/LSB] behaviour in both the vertical and horizontal topographies to be included in

the [NSB/LSB] cohort, although animals classified as being HSB could have demonstrated high stereotypical behaviour over either one or both of the topographies.

For the purposes of the *neurochemical assessment*, [NSB/LSB] and HSB cohorts in the control group were separated according to the median values calculated after the first four weeks, following the explained rationale. The baseline differences in SERT density between the [NSB/LSB] and HSB cohorts were then determined using striatal and frontal-cortical tissue from 9 animals per cohort classified as [NSB/LSB] and HSB after the first five baseline assessments. Also, even if more than 9 animals could theoretically be classified into either one of the cohorts, it was attempted to discriminate as clearly as possible between these cohorts on a neurobiological level (refer to paragraph 3.1.3).

*See Figure 4-6 on the next page, followed by paragraph 4.2.4.*

| VERTICAL ACTIVITY | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **1** | **3** | **5** | **6** | **7** | **9** | **10** | **11** | **12** | **13** | **14** | **15** | **16** | **17** | **18** | **19** | **20** | **21** | **22** | **23** | **25** | **26** | **27** | **28** | **30** | **32** | **33** | **34** | **35** | **36** | **40** |
| **1** | 1372 | 1038 | 124 | 290 | 769 | 1896 | 375 | 2637 | 604 | 290 | 3928 | 721 | 445 | 62 | 139 | 978 | 3026 | 797 | 1102 | 783 | 208 | 533 | 1586 | 2130 | 1044 | 2873 | 689 | 417 | 733 | 2224 | 315 |
| **2** | 376 | 1230 | 1101 | 1224 | 735 | 2118 | 311 | 3898 | 697 | 726 | 3996 | 2658 | 1192 | 29 | 252 | 995 | 3116 | 954 | 716 | 1054 | 390 | 942 | 2139 | 3769 | 991 | 4427 | 161 | 372 | 427 | 3870 | 1783 |
| **3** | 159 | 1791 | 1084 | 1541 | 1223 | 2417 | 290 | 4158 | 945 | 932 | 2398 | 2624 | 1237 | 16 | 225 | 735 | 2652 | 1350 | 1121 | 1557 | 520 | 1265 | 1778 | 4713 | 745 | 3147 | 562 | 237 | 666 | 4289 | 2531 |
| **4** | 866 | 894 | 1006 | 1682 | 1752 | 2151 | 371 | 4563 | 1202 | 895 | 3016 | 2355 | 2763 | 20 | 532 | 939 | 3278 | 1354 | 1030 | 840 | 270 | 771 | 2778 | 5322 | 395 | 3711 | 2166 | 159 | 789 | 5507 | 1477 |
| **5** | 898 | 2623 | 1429 | 2250 | 1317 | 872 | 632 | 3213 | 2656 | 822 | 3484 | 2892 | 1499 | 24 | 396 | 872 | 3652 | 1255 | 1013 | 521 | 472 | 1880 | 2906 | 3959 | 602 | 3367 | 1638 | 138 | 255 | 5805 | 2035 |
| **MED** | **866** | **1230** | **1084** | **1541** | **1223** | **2118** | **371** | **3898** | **945** | **822** | **3484** | **2624** | **1237** | **24** | **252** | **939** | **3116** | **1255** | **1030** | **840** | **390** | **942** | **2139** | **3959** | **745** | **3367** | **689** | **237** | **666** | **4289** | **1783** |
| **6** | 1111 | 2933 | 1372 | 967 | 167 | 1753 | 704 | 3143 | 2590 | 916 | 4701 | 2593 | 3145 | 28 | 541 | 703 | 3693 | 884 | 909 | 236 | 321 | 748 | 4369 | 5412 | 984 | 2013 | 1937 | 174 | 250 | 4406 | 1685 |
| **7** | 657 | 995 | 1339 | 1307 | 909 | 1512 | 867 | 3059 | 1580 | 1257 | 2473 | 1389 | 3190 | 18 | 390 | 1208 | 3058 | 702 | 1082 | 171 | 616 | 1102 | 2943 | 4235 | 573 | 2720 | 1909 | 214 | 592 | 4965 | 1382 |
| **8** | 497 | 1976 | 1689 | 1862 | 189 | 1471 | 593 | 2583 | 1469 | 895 | 3293 | 1669 | 2429 | 66 | 419 | 920 | 4058 | 1609 | 328 | 222 | 580 | 535 | 4086 | 0 | 466 | 1498 | 1841 | 207 | 1248 | 2930 | 1799 |
| **9** | 1480 | 782 | 2075 | 2977 | 374 | 1278 | 954 | 3170 | 2846 | 817 | 3730 | 2711 | 2937 | 160 | 539 | 1535 | 3234 | 1816 | 350 | 307 | 584 | 573 | 3712 | 3747 | 436 | 1083 | 1418 | 103 | 766 | 3014 | 1585 |
| **MED** | **884** | **1486** | **1531** | **1585** | **282** | **1492** | **786** | **3101** | **2085** | **906** | **3512** | **2131** | **3041** | **47** | **479** | **1064** | **3464** | **1247** | **630** | **229** | **582** | **661** | **3899** | **3991** | **520** | **1756** | **1875** | **191** | **679** | **3710** | **1635** |
| REVOLUTIONS | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| **1** | 30 | 12 | 20 | 40 | 96 | 843 | 234 | 74 | 14 | 27 | 75 | 49 | 38 | 35 | 27 | 48 | 27 | 81 | 71 | 88 | 18 | 18 | 14 | 40 | 26 | 46 | 464 | 51 | 77 | 45 | 52 |
| **2** | 13 | 7 | 72 | 50 | 127 | 219 | 143 | 53 | 10 | 61 | 82 | 110 | 28 | 30 | 24 | 40 | 57 | 161 | 31 | 94 | 19 | 13 | 24 | 13 | 25 | 43 | 288 | 20 | 56 | 11 | 34 |
| **3** | 17 | 5 | 94 | 35 | 201 | 343 | 124 | 26 | 9 | 29 | 92 | 67 | 13 | 15 | 26 | 20 | 20 | 167 | 38 | 115 | 11 | 19 | 13 | 7 | 54 | 35 | 340 | 19 | 57 | 15 | 31 |
| **4** | 40 | 5 | 130 | 19 | 258 | 325 | 61 | 32 | 14 | 44 | 45 | 49 | 7 | 11 | 28 | 23 | 18 | 49 | 39 | 73 | 11 | 8 | 6 | 40 | 12 | 15 | 374 | 20 | 64 | 18 | 12 |
| **5** | 77 | 22 | 214 | 15 | 295 | 348 | 44 | 28 | 18 | 77 | 85 | 50 | 11 | 9.6 | 24 | 35 | 19 | 30 | 39 | 32 | 12 | 38 | 69 | 10 | 13 | 29 | 358 | 19 | 67 | 29 | 25 |
| **MED** | **30** | **7** | **94** | **35** | **201** | **343** | **124** | **32** | **14** | **44** | **82** | **50** | **13** | **15** | **26** | **35** | **20** | **81** | **39** | **88** | **12** | **18** | **14** | **13** | **25** | **35** | **358** | **20** | **64** | **18** | **31** |
| **6** | 102 | 15 | 222 | 10 | 167 | 330 | 27 | 38 | 7 | 24 | 71 | 75 | 18 | 18 | 22 | 54 | 41 | 18 | 55 | 25 | 6 | 11 | 51 | 10 | 21 | 8 | 332 | 21 | 93 | 15 | 31 |
| **7** | 21 | 5 | 228 | 8 | 359 | 279 | 41 | 10 | 4 | 23 | 45 | 65 | 7 | 11 | 15 | 62 | 23 | 10 | 48 | 24 | 22 | 25 | 12 | 12 | 51 | 13 | 416 | 19 | 58 | 31 | 15 |
| **8** | 44 | 10 | 159 | 5 | 244 | 273 | 13 | 29 | 3 | 18 | 53 | 76 | 8 | 18 | 20 | 47 | 115 | 24 | 35 | 15 | 19 | 28 | 11 | 1 | 30 | 11 | 348 | 12 | 58 | 23 | 9 |
| **9** | 234 | 14 | 232 | 28 | 303 | 305 | 13 | 51 | 12 | 31 | 77 | 189 | 22 | 26 | 22 | 61 | 94 | 31 | 48 | 24 | 21 | 11 | 11 | 32 | 39 | 21 | 284 | 25 | 66 | 26 | 16 |
| **MED** | **73** | **12** | **225** | **9** | **274** | **292** | **20** | **34** | **6** | **24** | **62** | **76** | **13** | **18** | **21** | **58** | **68** | **21** | **48** | **24** | **20** | **18** | **12** | **11** | **35** | **12** | **340** | **20** | **62** | **25** | **16** |
| HORIZONTAL MOVEMENTS | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| **1** | 51 | 95 | 113 | 111 | 51 | 78 | 200 | 191 | 159 | 189 | 214 | 216 | 209 | 341 | 234 | 268 | 228 | 244 | 214 | 197 | 180 | 212 | 224 | 205 | 234 | 232 | 159 | 249 | 211 | 206 | 295 |
| **2** | 234 | 227 | 229 | 267 | 223 | 117 | 197 | 194 | 180 | 192 | 190 | 190 | 201 | 287 | 283 | 286 | 208 | 215 | 210 | 198 | 185 | 223 | 206 | 196 | 251 | 218 | 164 | 217 | 199 | 219 | 220 |
| **3** | 207 | 214 | 109 | 220 | 187 | 101 | 183 | 202 | 197 | 223 | 169 | 225 | 181 | 252 | 296 | 305 | 225 | 178 | 216 | 189 | 190 | 212 | 197 | 175 | 273 | 217 | 151 | 196 | 233 | 221 | 210 |
| **4** | 209 | 261 | 134 | 278 | 182 | 130 | 177 | 207 | 175 | 211 | 190 | 243 | 161 | 278 | 358 | 330 | 216 | 285 | 218 | 211 | 172 | 218 | 226 | 196 | 221 | 237 | 122 | 245 | 226 | 196 | 179 |
| **5** | 199 | 269 | 163 | 284 | 230 | 97 | 183 | 180 | 166 | 199 | 201 | 232 | 172 | 289 | 308 | 309 | 209 | 306 | 189 | 198 | 202 | 213 | 193 | 238 | 225 | 269 | 137 | 217 | 245 | 169 | 259 |
| **MED** | **207** | **227** | **134** | **267** | **187** | **101** | **183** | **194** | **175** | **199** | **190** | **225** | **181** | **287** | **296** | **305** | **216** | **244** | **214** | **198** | **185** | **213** | **206** | **196** | **234** | **232** | **151** | **217** | **226** | **206** | **220** |
| **6** | 159 | 192 | 148 | 176 | 114 | 127 | 205 | 297 | 198 | 217 | 197 | 218 | 150 | 262 | 331 | 285 | 248 | 248 | 204 | 198 | 182 | 225 | 166 | 165 | 206 | 239 | 144 | 228 | 228 | 192 | 216 |
| **7** | 277 | 218 | 172 | 241 | 176 | 136 | 198 | 199 | 159 | 206 | 176 | 194 | 182 | 285 | 290 | 242 | 209 | 257 | 169 | 200 | 168 | 227 | 204 | 198 | 208 | 263 | 158 | 233 | 258 | 187 | 186 |
| **8** | 188 | 251 | 207 | 235 | 200 | 157 | 195 | 196 | 181 | 223 | 202 | 194 | 196 | 314 | 277 | 260 | 178 | 178 | 162 | 173 | 134 | 214 | 184 | 84 | 238 | 239 | 148 | 240 | 256 | 184 | 244 |
| **9** | 188 | 239 | 160 | 229 | 178 | 137 | 229 | 181 | 184 | 207 | 213 | 224 | 156 | 300 | 288 | 225 | 261 | 186 | 176 | 199 | 164 | 205 | 216 | 137 | 203 | 201 | 155 | 242 | 220 | 184 | 219 |
| **MED** | **188** | **228.5** | **166** | **232** | **177** | **136.5** | **201.5** | **197.5** | **182.5** | **212** | **199.5** | **206** | **169** | **292.5** | **289** | **251** | **228.5** | **217** | **172.5** | **198.5** | **166** | **219.5** | **194** | **151** | **207** | **239** | **151.5** | **236.5** | **242** | **185.5** | **217.5** |

***FIGURE 4-6 – AN EXCERPT FROM THE SUMMARY OF THE WEEKLY AVERAGES OF STEREOTYPICAL BEHAVIOUR AND LOCOMOTOR ACTIVITY OF THE PLACEBO GROUP***

Vertical activity (top), pattern running (middle) and locomotor activity (bottom)

Note the weekly variation in the amount of stereotypy expressed by some animals, for example the vertical activity of animals 1, 7, 11, and 22 and the pattern running of animals 7, 9, and 10.

### *4.2.4. Determination of frontal-cortical and striatal SERT density*

On the day following their last behavioural assessments, the animals were anaesthetized by transferring them to a closed 5 litre container to which 1 ml halothane (acquired from Transfarm® Pharmaceuticals, Johannesburg, South Africa) was added. The mice were subsequently decapitated using a custom-made mouse guillotine provided by the Animal Research Centre. Following the removal of the brains on ice, the frontal cortices and striata were dissected on an ice-cooled dissection slab and snap frozen in liquid nitrogen. All brain samples were stored at –80°C until neurochemical analysis could be performed. Samples were stored for a maximum of 14 days.

#### *4.2.4.1. Chemicals and equipment used to determine SERT density*

| Chemicals and equipment used | Originating company |
|---|---|
| *Warm (radioactive) ligand* | |
| *Paroxetine, [phenyl-6'-$^3$H]* | Perkin-Elmer®; obtained from Separation Scientific®, Sandton, South Africa |
| *Cold ligand* | |
| *Fluoxetine HCl* | Cipla® Pharmaceuticals, Cape Town, South Africa |
| *Tris-HCl Buffer* | |
| *Trizma®-HCl Buffer* | All obtained from Sigma-Aldrich®, Aston Manor, Johannesburg, South Africa |
| *Potassium chloride* | |
| *Sodium chloride* | |
| *Consumables* | |
| *Whatman® GFB glass micro fibre filters* | Whatman® International; obtained from Separation® Scientific |
| *Scintillation vials* | Perkin-Elmer®, obtained from Separation Scientific® |
| *Filter-Count® scintillation liquid* | Perkin-Elmer®, obtained from Separation Scientific® |
| *Eppendorf® 1.5 ml Safe-Lock® tubes* | Eppendorf®; obtained from Merck®, Modderfontein, Johannesburg, South Africa |
| *Gilson® Pipetman® pipettes and tips* | Gilson® Inc.; obtained from Lasec® SA, Centurion, South Africa |
| *Equipment* | |
| *Beckman-Coulter® Optima® L-100 XP ultracentrifuge* | Beckman-Coulter®, Indianapolis, Indiana, USA |
| *Packard® Tri-Carb® 2100 TR liquid scintillation counter* | Packard® Industries, Downers Grove, Illnois, USA |
| *LabSystems® Multiscan® RC microplate reader* | Thermo® Scientific, Waltham, Massachusetts, USA |
| *Kinematica® Polytron* | Kinematica®, Kriens, Luzern, Switzerland |

***TABLE 4-1 – CHEMICALS AND EQUIPMENT USED IN DETERMINING SERT BINDING DENSITY***

#### *4.2.4.2. Methodology for the determination of SERT density*

As this method applies to saturation binding in a tissue homogenate, the theoretical basis upon which this type of assessment is founded, will briefly be explained. All cell membranes contain proteins called receptors that have the ability to interact selectively with certain ligands (McKinney and Raddatz, 2006), for example SERT, which interacts with serotonin and the SSRIs, and $D_1$ and $D_2$receptors that interact with dopamine, dopamine agonists or antagonists. To measure the density of such receptors in tissue homogenates, selective radioactive labelled ligands, which interact with the receptor in question, can be employed. The term saturation binding indicates that a series of radioactive labelled ligand concentrations is prepared and added in equal volumes to different samples of the homogenate. These concentration points are chosen to range from a very small ligand concentration which will result in very little binding, gradually becoming stronger and ending in a concentration point high enough to ensure that a theoretical saturation of all the available receptors in question is established and providing an excess of radioactive labelled ligand in the reaction mixture, not bound to the receptor. As the concentrations of the radioactive labelled ligand increase from low to high in the different samples, saturation of the receptor in question is established at some point. This point could theoretically have been accepted as the $B_{max}$ (a measure of the total amount of receptor in the sample), was it not for the fact that most ligands are not 100% selective for a given receptor. Most radioactive labelled ligands also bind to different types of receptors, certain components of the cell membrane and in general to many cellular and non-cellular components that are not in question (termed the *non-specific binding)*. Therefore, the increased binding of radioactive labelled ligand to the receptors in question as well as to other different components in the homogenate is termed the *total binding*, a value that does not represent the actual amount of receptors in the homogenate.

To determine the actual amount of the receptor in question (termed the *specific binding)*, the non-specific binding must be subtracted from the total binding. As only total binding can be established in a series of homogenates to which only the radioactive ligand has been added, another series of homogenates from the same pool of tissue has to be prepared to determine the non-specific binding. In addition to adding equal volumes of the same concentrations of radioactive ligand as above to this series of points, a non-radioactive (cold) ligand is also added. The cold ligand is usually of the same pharmacological class as the radioactive (warm) ligand and just as selective for the receptor in question, but is not labelled with radioactivity. In this case, fluoxetine was used as cold ligand and tritiated ($H^3$) paroxetine as warm ligand. The principle of establishing non-specific binding is founded upon adding the cold ligand to each sample of homogenate, also containing the warm ligand, in a 100 – 1000 fold excess. This results in competi-

tion between the cold and the warm ligands at the receptor site in question. As the concentration of the cold ligand is much higher than that of the warm ligand, the cold ligand displaces nearly all of the warm ligand from the receptor site in question. The net result is that the warm ligand only binds to the non-specific components in the homogenate, and the generation of radioactive counts from these samples are therefore a true representation of the amount of non-specific binding (McKinney and Raddatz, 2006).

Subsequently, the non-specific binding can be subtracted from the total binding established at each respective concentration point in a different series of homogenates, resulting in the generation of a new concentration curve. This curve can be employed to determine the specific binding and ultimately the actual $B_{max}$ value (see Figure 4-7).

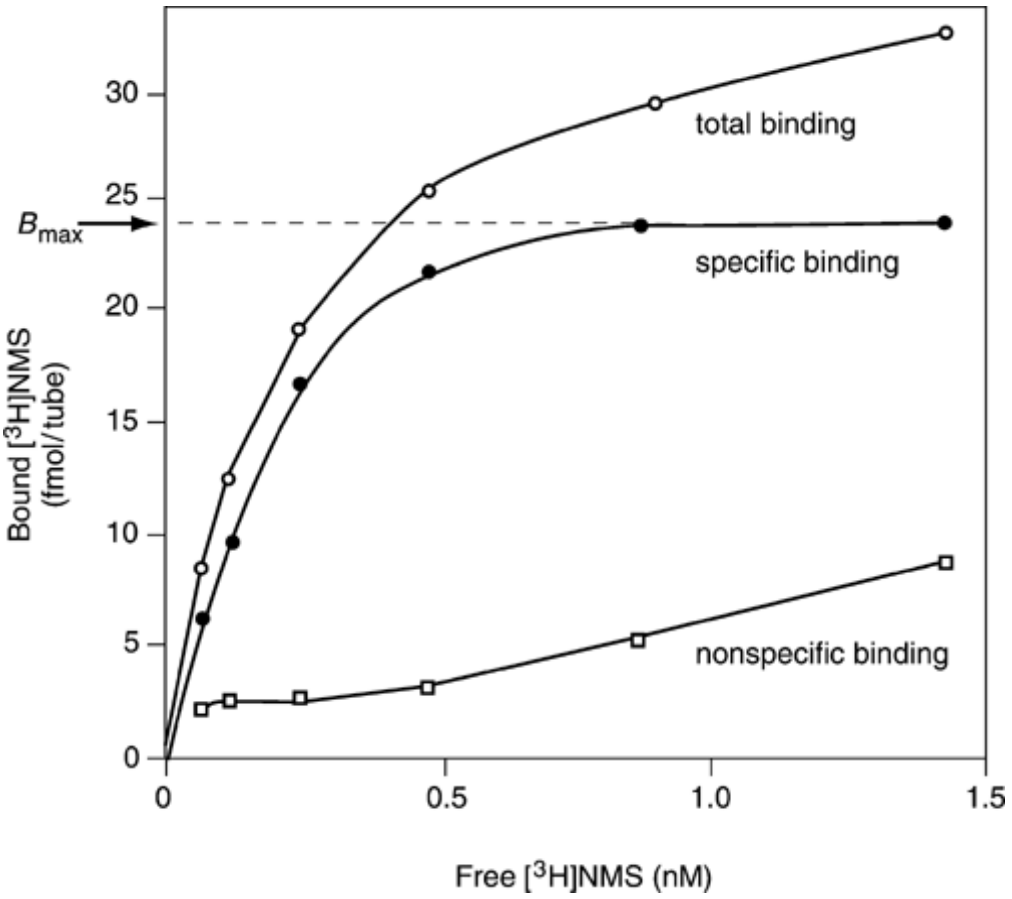



***FIGURE 4-7 – A GRAPHICAL REPRESENTATION OF A SATURATION BINDING ASSAY***

* * *

The method for the determination of SERT density in the current study was adapted from Sato *et al* (2010). At least one but not exceeding two striatal and frontal-cortical assays were performed per day. This allowed both striatal and frontal-cortical assays to be performed simultaneously. At least three assays were performed per brain area.

* * *

First, brain tissue from the identified animals of a given cohort were weighed and pooled in 25ml Erlenmeyer flasks kept on ice. The striata and frontal-cortices from at least 3 animals (or 90mg per brain area) were needed to complete one striatal and one frontal-cortical assay. Depending on the final mass of the pooled tissues, 50 mM Tris-HCl buffer (containing 120 mM NaCl and 5 mM KCl; pH adjusted to 7.4) was added to constitute a 100 volumes dilution of each brain area (90mg tissue per 9ml buffer; 12mg tissue per 120ml buffer, etc.). Subsequently, the pooled tissue was homogenized (5 seconds using a Polytron®, setting 6) (see Table 3-1) and centrifuged at 50 000 x *g* for 10 minutes and the supernatant discarded. The pellets were resuspended in the same amount of new buffer (50 mM Tris-HCl buffer (containing 120 mM NaCl and 5 mM KCl; pH adjusted to 7.4), transferred to glass tubes and homogenized again using a Teflon® drill bit custom-made to fit tightly in the glass tubes. The resultant homogenates were centrifuged again at 50 000 x *g* for 10 minutes. Subsequently, the supernatant was discarded and the last two steps were repeated once more. Thus, each pool of tissue was washed, homogenized and centrifuged three times. After discarding the final volumes of supernatant, the correct amount of buffer was added for a final time and the pellets were homogenized using the Polytron®. All of the above steps took place on ice or in the cold room (temperature, 3°C). The tissue homogenates were subsequently kept on ice until added to the reaction mixtures.

* * *

For the purpose of the current assays, a concentration range of 10 points between 0.1 and 2.0 nM of the radioactive ligand paroxetine-[phenyl-6'-$^{3}$H] (specific activity – 22.9 Ci/mmol) were used. Subsequently, 25µl of each concentration of the radioactive ligand were transferred to separate Eppendorf® Safe-Lock® tubes. The total binding was established by adding 25µl of Tris-HCl buffer to one series of tubes for each of the brain areas. The non-specific binding was determined in the presence of 10µM fluoxetine of which 25µl were added to the remaining series of tubes for each brain area. Thus, two reaction mixtures were used to determine the specific SERT binding in each brain area; one consisted of 25µl radioactive ligand and 25µl Tris-HCl buffer (total binding) and the other 25µl radioactive ligand and 25µl of the 10 µM fluoxetine

(non-specific binding). 450µl of the tissue homogenates were added to each tube, vortexed, and allowed to incubate for one hour at room temperature.

Following the 1-hour incubation period, the content of the tubes were individually and orderly drained via rapid vacuum filtration through Whatman® GFB glass microfiber filters presoaked in ice-cold buffer. Still applying vacuum, the filters were then washed twice with 4ml of ice-cold buffer, inserted into scintillation vials and immersed in 4ml Filter-Count® scintillation liquid. The series of radioactive standards consisted of 25µl of each radioactive concentration added to 4ml Filter-Count® scintillation liquid. The vials were left for two hours after which it was subjected to scintillation counting in the Packard® scintillation counter. The binding data were subsequently analyzed using GraphPad Prism® 5 software (GraphPad® Software, Inc., La Jolla, California, USA) to obtain values for the maximal number of binding sites ($B_{max}$) and the binding affinity ($K_D$).

In Chapter 5, the results of the study will be presented, with an in-depth discussion of these findings and their relevance to OCD presented in Chapter 6. The reader is also referred to the relevant addenda for any additional information when reviewing the results presented in Chapter 5.

*END OF CHAPTER 4*

# CHAPTER 5
# RESULTS

## 5.1. STUDY OBJECTIVE I – THE DEVELOPMENT OF A NEW CLASSIFICATION SYSTEM FOR THE APPRAISAL OF DEER MOUSE STEREOTYPY

The main aim of the first study objective was to develop a new classification system for the appraisal of the different behavioural topographies expressed by deer mice. As mentioned earlier in Chapter 3, these two topographies include pattern running and vertical jumping. Preliminary data from pilot studies done in our laboratory indicated that pattern runners could not be classified as HSB animals when applying the previous criteria (for a full discussion, refer to paragraph 3.1.1). The results from the current study indicate that different criteria can indeed be applied to that previously employed (Güldenpfennig *et al.*, 2011; Korff *et al.*, 2008; Korff *et al.*, 2009) to appraise the different behavioural topographies of deer mice. The newly determined classification criteria for the appraisal of pattern running and vertical jumping indicate a clear separation between the numbers of cage (horizontal) revolutions (HR) and vertical jumps (VBI) executed by animals of the same behavioural cohort expressing either of these two topographies (Table 5-1). The animals could now be separated into [NSB/LSB] and HSB cohorts that allowed sufficient accommodation of both behavioural topographies. This in direct contrast to the data obtained during our previous studies that employed a classification system which only allowed for animals generating 2000 relevant stereotypies per hour or more, to be classified as HSB. A typical example of stereotypy breakdown within a group of animals is illustrated in Table 5-2.

| Cohort | VBI / 30 minutes | HR / 30 minutes |
|---|---|---|
| **HSB** | > 2000 | > 200 |
| **LSB** | 500 - 2000 | 150 – 200 |
| **NSB** | < 500 | < 150 |

***TABLE 5-1 – THE NEWLY DEFINED CUT-OFF VALUES FOR EACH COHORT AS A FUNCTION OF THE TOPOGRAPHY EXPRESSED***

| BEHAVIOURAL COHORT | CONTROL GROUP (*n = 31*) | | TREATMENT GROUP (*n = 39*) | |
|---|---|---|---|---|
| | HSB | [NSB/LSB] | HSB | [NSB/LSB] |
| **Animals expressing vertical stereotypy** | 8 | 20 | 11 | 23 |
| **Animals expressing pattern running stereotypy** | 2 | | 3 | |
| **Animals expressing both behavioural topographies** | 1 | | 2 | |
| **Percentage of Group** | 35% | 65% | 41% | 59% |

***TABLE 5-2 – THE NUMBER OF ANIMALS IN EACH EXPERIMENTAL GROUP DEVELOPING STEREOTYPY***

Of the original 40 animals in the control group at day 1, 31 animals survived until day 56, while 39 out of the original 40 animals in the treatment group survived for the duration of the study. Eleven (35%) animals in the control group (*n* = 31) and sixteen (41%) animals in the treatment group (*n* = 39) could be classified as HSB applying the newly developed classification criteria. This is somewhat less than the 45% of the deer mouse population that were classified as HSB in the study done by Korff and colleagues (2008). This study objective was addressed by assessing the behaviour of the animals in the control group, such that the figures and tables presented henceforth in section 5.1 are representative of data obtained from these animals.

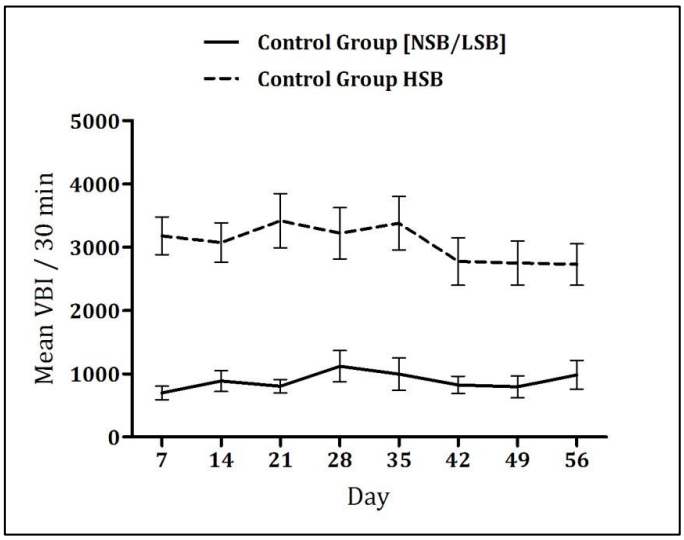



***FIGURE 5-1 –WEEKLY MEAN NUMBERS OF VERTICAL BEAM INTERRUPTIONS (VBI) GENERATED DURING A PERIOD OF 30 MINUTES BY TREATMENT NAIVE [NSB/LSB] ANIMALS EXPRESSING VERTICAL STEREOTYPY COMPARED TO HSB ANIMALS***

Data represent the weekly mean VBI of the 9 animals classified as HSB vertical jumpers and 12 low stereotypy animals classified as ([NSB/LSB]). Data were collected after the fourth week of assessment. The weekly contribution of each animal to the data presented here was calculated as a mean value from three 30-minute intervals over a 12-hour assessment period during which the highest amount of VBI were generated.

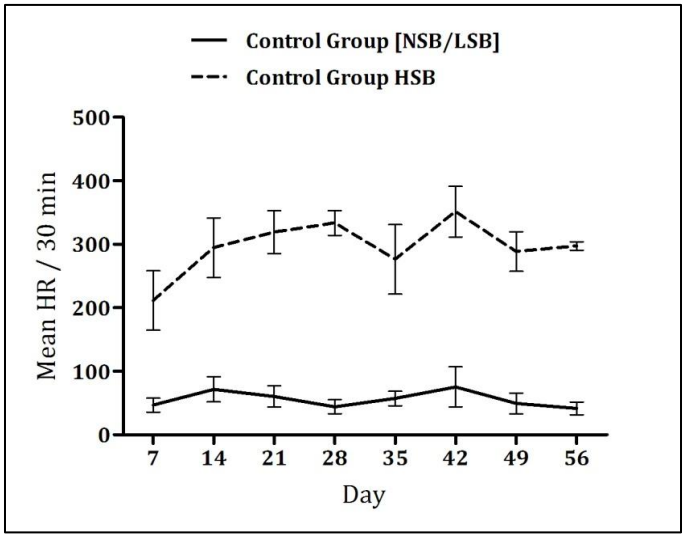



***FIGURE 5-2 – WEEKLY MEAN NUMBERS OF CAGE REVOLUTIONS (HR) EXECUTED DURING A PERIOD OF 30 MINUTES BY TREATMENT NAIVE [NSB/LSB] ANIMALS EXPRESSING PATTERN RUNNING COMPARED TO HSB ANIMALS***

Data represent the weekly mean HR of 3 animals classified as HSB pattern runners and 12 low stereotypy animals classified as ([NSB/LSB]). Data were collected after the fourth week of assessment. The weekly contribution of each animal to the data presented here was calculated as a mean value from three 30-minute intervals over a 12-hour assessment period during which the highest amount of HR were executed.

Table 5-3 lists the weekly differences between the mean amounts of vertical beam interruptions (VBI) generated by [NSB/LSB] and HSB animals respectively, while Table 5-4 lists the same comparative results concerning pattern running (HR). Unpaired t-tests were performed to establish the *p*-values.

| Day | [NSB/LSB] (Mean VBI ± SEM) *n* = 12 | HSB (Mean VBI ± SEM) *n* = 9 | *p* Values (Unpaired t-test) |
|---|---|---|---|
| **7** | 711 ± 114 | 3177 ± 298 | < 0.0001 |
| **14** | 965 ± 183 | 3071 ± 308 | < 0.0001 |
| **21** | 814 ± 126 | 3416 ± 427 | < 0.0001 |
| **28** | 1216 ± 283 | 3219 ± 403 | 0.0007 |
| **35** | 1068 ± 303 | 3377 ± 422 | 0.0003 |
| **42** | 828 ± 152 | 2774 ± 375 | 0.0001 |
| **49** | 817 ± 205 | 2749 ± 349 | 0.0002 |
| **56** | 971 ± 265 | 2726 ± 327 | 0.0006 |
| **AVERAGE** | 923 | 3063 | |

***TABLE 5-3 – WEEKLY DIFFERENCES BETWEEN THE MEAN NUMBERS OF VBI GENERATED BY ANIMALS CLASSIFIED AS [NSB/LSB] AND HSB, RESPECTIVELY***

The weekly contribution of each animal to the data presented here, was calculated as a mean value from three 30-minute intervals over a 12-hour assessment period during which the highest amount of VBI were generated. $p$ values of $< 0.05$ are regarded as significant.

| Day | [NSB/LSB] (Mean HR ± SEM) *n* = 12 | HSB (Mean HR ± SEM) *n* = 3 | *p* Values (Unpaired t-test) |
|---|---|---|---|
| **7** | 46 ± 11 | 211 ± 46 | 0.0001 |
| **14** | 71 ± 19 | 294 ± 46 | 0.0003 |
| **21** | 60 ± 16 | 319 ± 33 | < 0.0001 |
| **28** | 44 ± 11 | 333 ± 19 | < 0.0001 |
| **35** | 57 ± 11 | 276 ± 54 | < 0.0001 |
| **42** | 75 ± 31 | 351 ± 39 | 0.0013 |
| **49** | 49 ± 16 | 288 ± 30 | < 0.0001 |
| **56** | 41 ± 10 | 297 ± 66 | < 0.0001 |
| **AVERAGE** | 55 | 296 | |

***TABLE 5-4 – WEEKLY DIFFERENCES BETWEEN THE MEAN NUMBERS OF HR EXECUTED BY ANIMALS CLASSIFIED AS [NSB/LSB] AND HSB, RESPECTIVELY***

The weekly contribution of each animal to the data presented here was calculated as a mean value from three 30-minute intervals over a 12-hour assessment period during which the highest amount of HR were executed. *p* values of < 0.05 are regarded as significant.

In Table 5-5, the weekly mean numbers of VBI generated by HSB vertical jumpers are compared to the weekly mean HR executed by animals classified as HSB pattern runners.

| Day | HSB Vertical Jumpers (Mean VBI ± SEM) *n* = 9 | HSB Pattern Runners (Mean HR ± SEM) *n* = 3 | *p* Values (Unpaired t-test) |
|---|---|---|---|
| **7** | 3177 ± 298 | 211 ± 46 | 0.0003 |
| **14** | 3071 ± 308 | 294 ± 46 | 0.0006 |
| **21** | 3416 ± 427 | 319 ± 33 | 0.0027 |
| **28** | 3219 ± 403 | 333 ± 19 | 0.0029 |
| **35** | 3377 ± 422 | 276 ± 54 | 0.0025 |
| **42** | 2774 ± 375 | 351 ± 39 | 0.0056 |
| **49** | 2749 ± 349 | 288 ± 30 | 0.0032 |
| **56** | 2726 ± 327 | 297 ± 66 | 0.0023 |
| **AVERAGE** | 3063 | 296 | |

***TABLE 5-5 – THE WEEKLY MEAN AMOUNTS OF VBI GENERATED, AND HR EXECUTED BY HSB VERTICAL JUMPERS AND PATTERN RUNNERS, RESPECTIVELY***

The weekly contribution of each animal to the data presented here was calculated as a mean value from three 30-minute intervals over a 12-hour assessment period during which the highest amount of VBI and HR were executed. *p* values of < 0.05 are regarded as significant.

* * *

The above data indicate that deer mice expressing different behavioural topographies can be classified as [NSB/LSB] or HSB, based on different classification criteria for vertical jumping and pattern running. Furthermore, given the major differences between the amounts of VBI generated by HSB vertical jumpers and HR executed by HSB pattern runners, it is evident that one

behavioural classification system for both topographies is inappropriate, as HSB pattern runners generate much lower levels of stereotypy than do HSB vertical jumpers.

## 5.2. STUDY OBJECTIVE II – AN INVESTIGATION INTO FRONTAL-CORTICAL AND STRIATAL SERT DENSITIES OF TREATMENT NAIVE [NSB/LSB] AND HSB DEER MICE

The second objective of the current study was aimed at strengthening the construct validity of the deer mouse model of OCD by investigating whether any baseline differences in frontal-cortical and striatal SERT densities could be demonstrated in treatment-naive HSB animals as opposed to low stereotypy [NSB/LSB] animals. The data presented here were obtained using tissue from nine animals that were found to consistently generate the highest (HSB) and lowest ([NSB/LSB]) amounts of stereotypy during the first four weeks of the control study, irrespective of the behavioural topography expressed by the animals. Paired t-tests were performed to determine the statistical differences between frontal-cortical and striatal SERT densities in tissues of the same animals (HSB and [NSB/LSB], respectively). In case of comparing the respective frontal-cortical and striatal SERT densities of one cohort of animals (HSB or [NSB/LSB]) with those in the same brain area, but of another cohort, unpaired t-tests were applied.

### *5.2.1. Frontal-cortical and striatal SERT densities in [NSB/LSB] animals*

Frontal-cortical and striatal tissues from nine animals that consistently generated the lowest amount of stereotypy during the first four weeks of the control study were pooled three to an assay, allowing three SERT binding assays to be performed for each of the two brain areas. The mean striatal SERT density in [NSB/LSB] animals was higher than that in the frontal cortex (12.96 ± 1.42 vs. 5.81 ± 1.61 fmol/mg protein), although this difference was not statistically significant ($p$ = 0.1102; Figure 5-3).

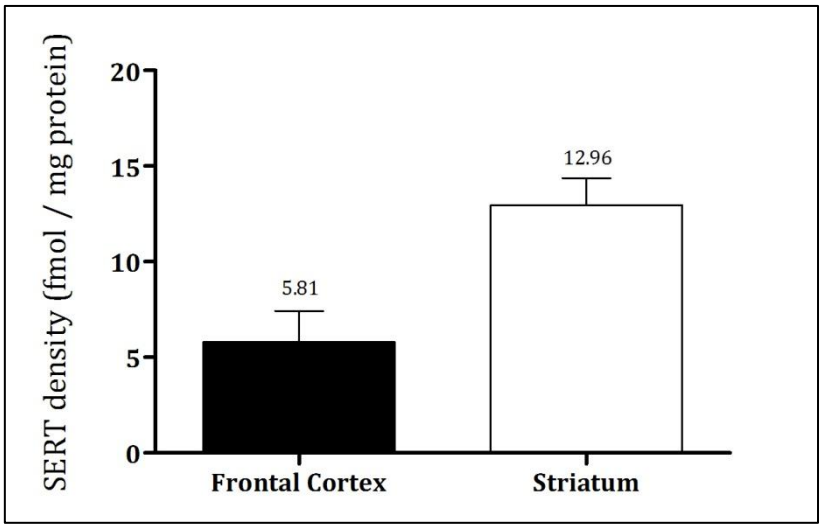



***FIGURE 5-3 – FRONTAL-CORTICAL AND STRIATAL SERT DENSITIES IN TREATMENT NAIVE [NSB/LSB] ANIMALS***

Data represent the mean ± SEM of three assays per brain region. Frontal-cortical and striatal tissues from three animals were pooled to perform one assay. $p > 0.05$ (paired t-test). $B_{max}$ (frontal cortex) = 5.81 ± 1.61 fmol/mg protein; $B_{max}$ (striatum) = 12.96 ± 1.42 fmol/mg protein.

### *5.2.2. Frontal cortical and striatal SERT densities in HSB animals*

Frontal-cortical and striatal tissues from nine animals that consistently generated the highest amount of stereotypy during the first four weeks of the control study, irrespective of the topography expressed, were pooled in the same manner as those obtained from animals in the [NSB/LSB] cohort. The mean frontal-cortical SERT density (4.15 ± 0.25 fmol/mg protein) was found to be significantly higher than that in the striatum (2.3 ± 0.09 fmol/mg protein) ($p$ = 0.0087; Figure 5-4).

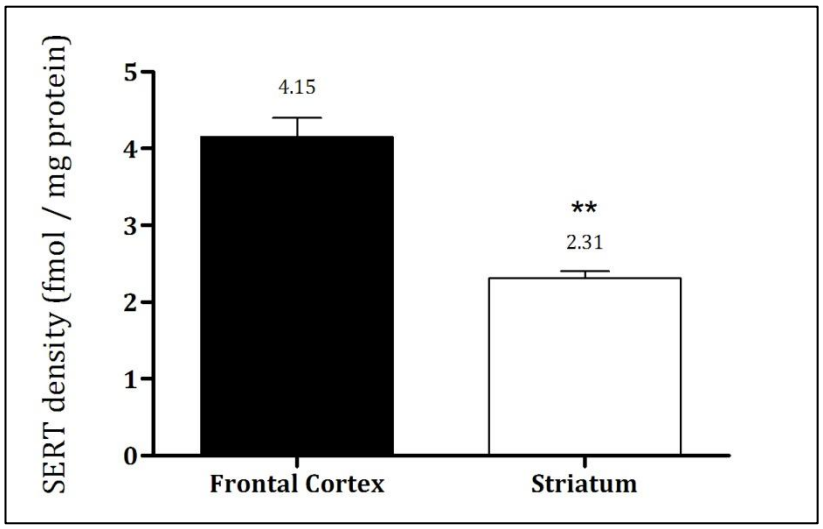



***FIGURE 5-4 – FRONTAL-CORTICAL AND STRIATAL SERT DENSITIES IN TREATMENT NAIVE HSB ANIMALS***

Data represent the mean ± SEM of three assays per brain region. Frontal-cortical and striatal tissues from three animals were pooled to perform one assay. $p > 0.0087$ (paired t-test). $B_{max}$ (frontal cortex) = 4.15 ± 0.25 fmol/mg/protein; $B_{max}$ (striatum) = 2.31 ± 0.09 fmol/mg protein.

### *5.2.3. Comparing frontal-cortical SERT densities of [NSB/LSB] and HSB animals*

Although frontal-cortical SERT density in HSB animals (4.15 ± 0.25 fmol/mg protein) was lower than that in [NSB/LSB] animals (5.81 ± 1.61 fmol/mg protein), these differences were not statistically significant ($p > 0.05$; Figure 5-5).

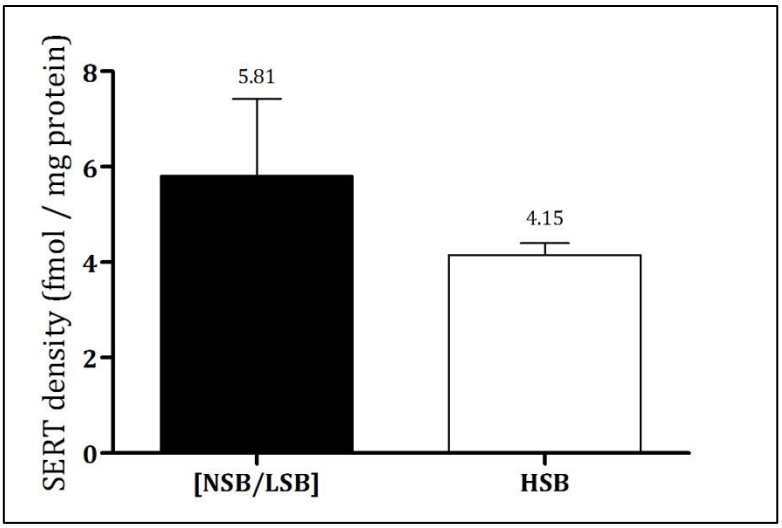



***FIGURE 5-5 – A COMPARISON BETWEEN FRONTAL-CORTICAL SERT DENSITIES OF [NSB/LSB] AND HSB ANIMALS***

Data represent the mean ± SEM of three assays per brain region. Frontal-cortical tissues from three animals were pooled to perform one assay. $p > 0.05$ (unpaired t-test) $B_{max}$ ([NSB/LSB]) = 5.81 ± 1.61 fmol/mg/protein; $B_{max}$ (HSB) = 4.15 ± 0.25 fmol/mg protein.

### *5.2.4. Comparing striatal SERT densities of [NSB/LSB] and HSB animals*

As demonstrated in Figure 5-6, the difference between the striatal SERT densities of treatment naive [NSB/LSB] and HSB animals were found to be highly statistically *significant*. Animals of the [NSB/LSB] cohort (12.96 ± 1.42 fmol/mg protein) presented with much higher striatal SERT concentrations than animals from the HSB cohort (2.3 ± 0.09) ($p$ = 0.0009; unpaired t-test; Figure 5-6).

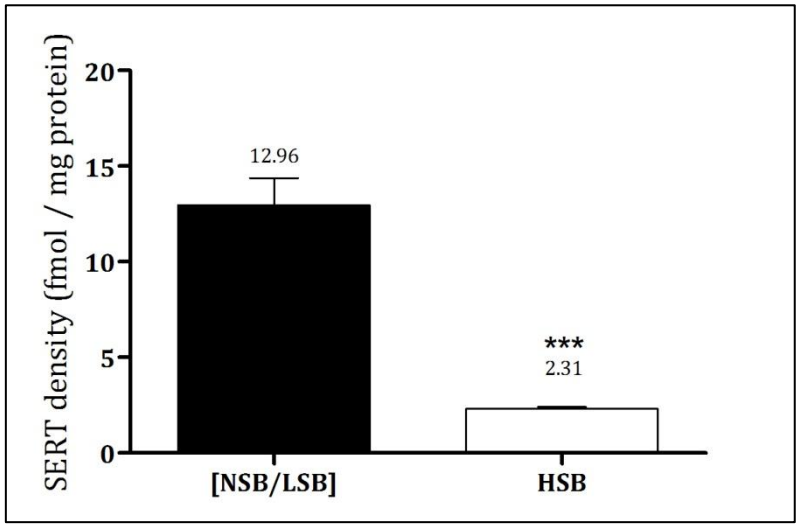



***FIGURE 5-6 – A COMPARISON BETWEEN STRIATAL SERT DENSITIES OF [NSB/LSB] AND HSB ANIMALS***

Data represent the mean ± SEM of three assays per brain region. Frontal-cortical tissues from three animals were pooled to perform one assay. $p$ = 0.0009 (unpaired t-test). $B_{max}$ ([NSB/LSB)] = 12.96 ± 1.42 fmol/mg/protein; $B_{max}$ (HSB) = 2.31 ± 0.09 fmol/mg protein

* * *

In summary, the data presented in Section 5.2 indicate that, while SERT densities between [NSB/LSB] and HSB animals are similar in the frontal cortex, these densities are significantly lower in the striatum of HSB mice compared to their low stereotypic controls ([NSB/LSB]). Although not statistically significant for animals of the ([NSB/LSB]) cohort, the data also indicate an inverse relationship between frontal-cortical and striatal SERT densities in HSB compared to [NSB/LSB] animals. However, more than three assays per brain area will be needed to verify whether this opposite relationship in SERT density between the [NSB/LSB] and HSB cohorts is indeed significant.

## 5.3. STUDY OBJECTIVE III – THE EFFECT OF SUB-CHRONIC AND CHRONIC ORAL ESCITALOPRAM TREATMENT ON DEER MOUSE STEREOTYPY

The third study objective was to determine whether chronic, but not sub-chronic oral treatment with high dose escitalopram (50 mg/kg/day) would result in attenuation of stereotypy expressed by HSB animals. It was also hypothesized that such treatment will not affect the behaviour of animals in the [NSB/LSB] cohort.

In order to clarify the data presented here, a short review of the *intended* methods of assessing deer mouse stereotypy and its response to treatment with high dose escitalopram is necessary. As explained in paragraph 4.2.3.2, animals were classified into either [NSB/LSB] or HSB cohorts based on the median value of stereotypy for each animal after four weeks of baseline behavioural assessment. The weekly mean stereotypy scores for each animal (from which the median value was calculated after four weeks) have been calculated from the three 30-minute intervals over each of the weekly 12-hour screening sessions during which an animal generated the *highest* amount of relevant beam interruptions. It has *originally* been proposed that the weekly mean stereotypy scores generated by each animal should be used to assess the changes in the manifestation of stereotypy as a function of treatment over the course of the 28-day administration of treatment following the four weeks of baseline assessment. Based on this, three weekly mean stereotypy values (day 28, 35 and 56) were used to discriminate between the effects of sub-chronic (day 35; 1 week after the initiation of treatment) and chronic (day 56; 4 weeks after the initiation of treatment) administration of escitalopram on the expression of stereotypy, with day 28 being applied as the pre-treatment control. Data were analyzed using a one-way analysis of variance (ANOVA) followed by Dunnett's test, and are presented in paragraph 5.3.1. In order to exclude the possible effects of escitalopram on the general activity of deer mice, locomotor activity was routinely recorded and is presented in paragraph 5.3.3.

Although the pre-treatment classification of deer mice over 28 days were performed according to the above protocol, *the methodology for evaluating the effects of drug treatment on the expression of deer mouse stereotypy and the statistical methods followed, are explained in paragraph 5.3.2 as this differs drastically from the above mentioned protocol* (refer to paragraph 4.2.3.2 for an explanation).

The data that will be presented in this section will include the results as interpreted following the originally intended methodology (see paragraph 5.3.1), as well as that obtained following the revised protocol, i.e. after changes had been made to the original protocol (see para-

graph 5.3.2). Note that the treatment data presented in this section have been obtained assessing a different group of 40 animals than those included in the control study. Thirty-nine of the 40 animals completed the treatment study, of which 16 animals could be classified as HSB (Table 5-2).

### *5.3.1. The effect of escitalopram on stereotypy generated*

These results are based on the effect of escitalopram (50 mg/kg/day x 1 or 4 weeks) on the weekly manifestation of stereotypy as determined by calculating the highest average stereotypy score for each animal per week. As explained previously, these average individual stereotypy scores were calculated from three 30-minute intervals over a 12-hour period during which an animal executed the highest amounts of stereotypy.

#### *5.3.1.1. Weekly manifestation of vertical jumping (VBI) expressed by [NSB/LSB] and HSB animals, and response to 1 or 4 weeks of escitalopram treatment*

Neither sub-chronic (1 week) nor chronic (4 weeks) treatment with escitalopram (50 mg/kg/day) affected vertical jumping (VBI) in either one of the [NSB/LSB] or HSB cohorts with respect to the highest average VBI generated on a weekly basis (Figure 5-7).

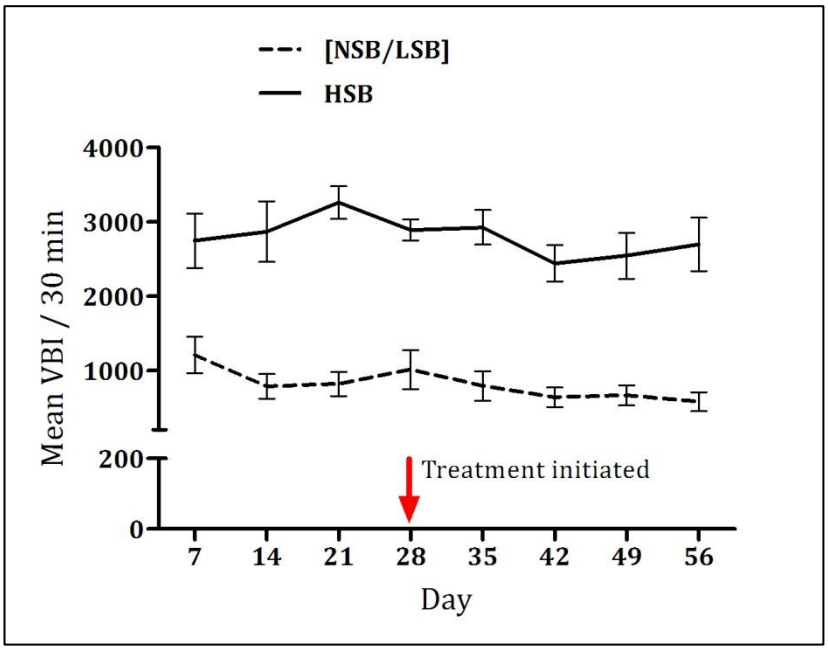



***FIGURE 5-7 – WEEKLY MANIFESTATION OF VERTICAL JUMPING (VBI) EVIDENT IN [NSB/LSB] AND HSB COHORTS, AND RESPONSE TO ESCITALOPRAM TREATMENT***

Data represent the weekly mean VBI of 12 animals that consistently generated the lowest stereotypy scores during the first four weeks of assessment ([NSB/LSB]), as well as nine animals classified as HSB vertical jumpers, after the first four weeks of baseline assessments and following 1 or 4 weeks of escitalopram treatment from day 28. The weekly contribution of each animal to the data presented here was calculated as a mean value from three 30-minute intervals over a 12-hour assessment period during which the highest amount of VBI were executed.

#### *5.3.1.2. Average VBI in [NSB/LSB] and HSB cohorts before and after sub-chronic (day 28-35) and chronic (day 28-56) escitalopram treatment*

No statistical differences could be demonstrated with respect to the average amount of VBI generated by both the [NSB/LSB] or HSB cohorts following treatment with either sub-chronic (1 week) or chronic (4 weeks) treatment with escitalopram (50 mg/kg/day), compared to baseline stereotypy as measured on day 28 (Figures 5-8 and 5-9).

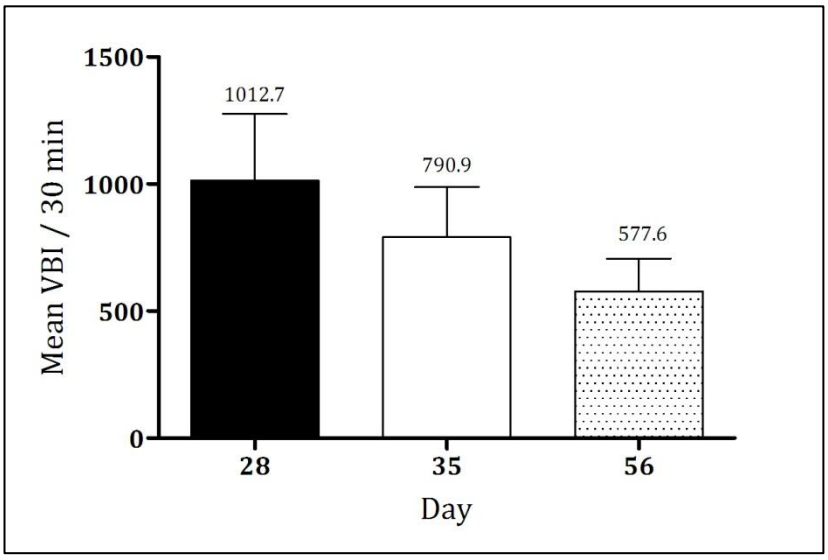



***FIGURE 5-8 – AVERAGE VBI GENERATED BY ANIMALS OF THE [NSB/LSB] COHORT BEFORE AND AFTER ESCITALOPRAM TREATMENT***

Data represent the mean VBI of 12 animals that generated the lowest amount of stereotypy during the first four weeks of assessment (up to day 28), and following treatment with either sub-chronic (1 week) or chronic (4 weeks) treatment with escitalopram. The contribution of each animal to the data presented here was calculated as a mean value from three 30-minute intervals over a 12-hour assessment period during which the highest amount of VBI were generated. No statistical differences could be demonstrated. $p > 0.05$ for both days 35 and 56 compared to day 28 (One-way ANOVA followed by Dunnett's test).

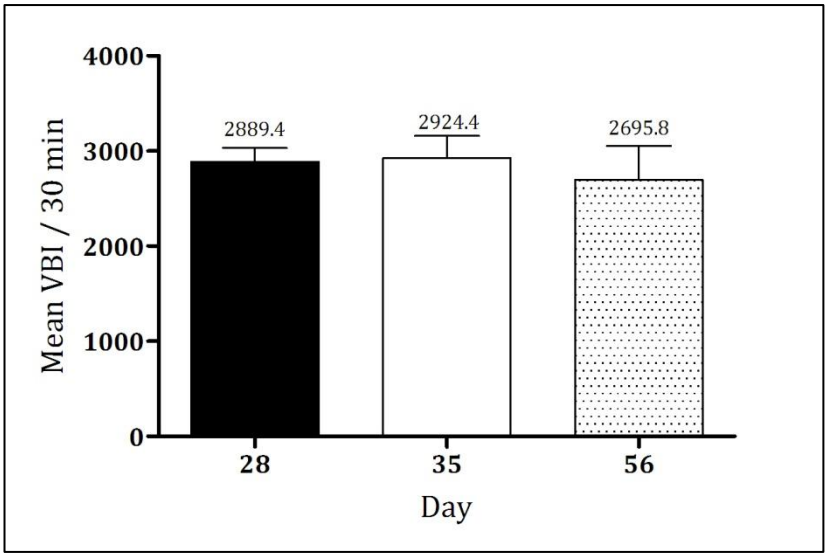



***FIGURE 5-9 – AVERAGE VBI GENERATED BY ANIMALS OF THE HSB COHORT, BEFORE AND AFTER ESCITALOPRAM TREATMENT***

Data represent the weekly mean VBI of 9 animals classified as HSB vertical jumpers after the first four weeks of baseline assessments (up to day 28), and following treatment with either sub-chronic (1 week) or chronic (4 weeks) treatment with escitalopram. The weekly contribution of each animal to the data presented here was calculated as a mean value from three 30-minute intervals over a 12-hour assessment period during which the highest amount of VBI were executed. No statistical differences could be demonstrated. $p > 0.05$ for both days 35 and 56 compared to day 28 (One-way ANOVA followed by Dunnett's test).

#### *5.3.1.3. Weekly manifestation of the pattern running (HR) executed by [NSB/LSB] and HSB animals, and response to 1 or 4 weeks of escitalopram treatment*

As was the case with the effect of escitalopram (50 mg/kg/day) on the expression of vertical jumping in animals of the [NSB/LSB] and HSB cohorts, neither sub-chronic nor chronic treatment with escitalopram resulted in any statistical changes in the weekly expression of pattern running with respect to the highest average stereotypy scores generated (Figure 5-10).

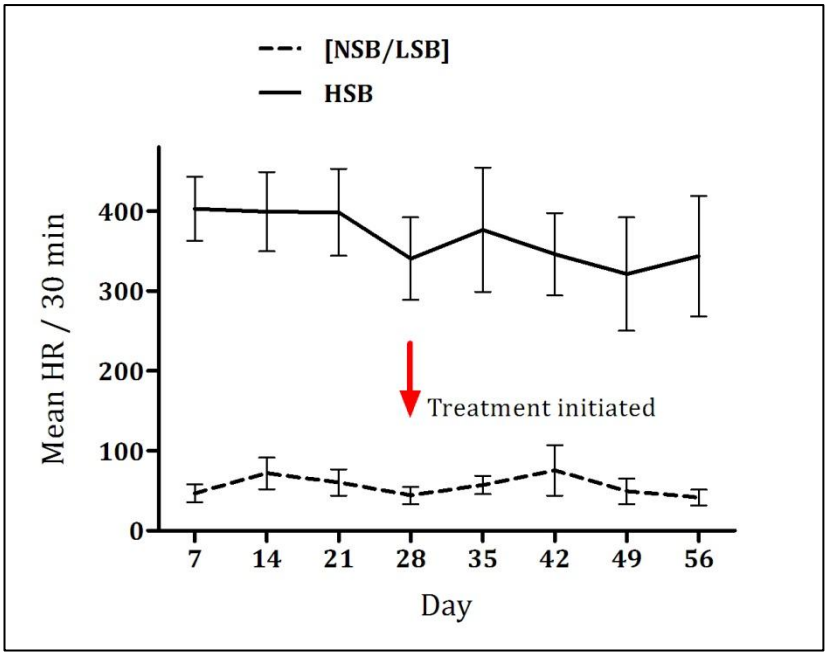



***FIGURE 5-10 – WEEKLY MANIFESTATION OF PATTERN RUNNING (HR) EVIDENT IN [NSB/LSB] AND HSB COHORTS, AND RESPONSE TO ESCITALOPRAM TREATMENT***

Data represent the weekly mean HR of 12 animals classified as ([NSB/LSB]) and five animals classified as HSB pattern runners after the first four weeks of baseline assessments, and following 1 or 4 weeks of escitalopram treatment. The weekly contribution of each animal to the data presented here was calculated as a mean value from three 30-minute intervals over a 12-hour assessment period during which the highest amount of HR were executed.

#### *5.3.1.4. Average HR in [NSB/LSB] and HSB cohorts before and after sub-chronic (day 28-35) and chronic (day 28-56) escitalopram treatment*

Neither sub-chronic nor chronic treatment with oral escitalopram (50 mg/kg/day) altered the manifestation of pattern running behaviour with respect to the highest average HR executed in animals of both the [NSB/LSB] and HSB cohorts (Figures 5-11 and 5-12).

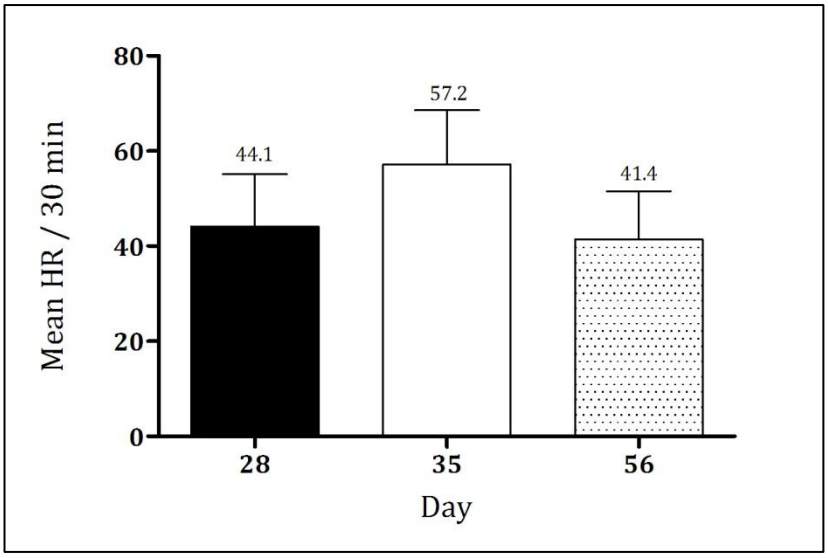



***FIGURE 5-11 – AVERAGE HR GENERATED BY ANIMALS OF THE [NSB/LSB] COHORT BEFORE AND AFTER ESCITALOPRAM TREATMENT***

Data represent the mean HR of 12 animals that generated the lowest amount of stereotypy during the first four weeks of assessment (up to day 28), and following treatment with either sub-chronic (1 week) or chronic (4 weeks) treatment with escitalopram. The contribution of each animal to the data presented here was calculated as a mean value from three 30-minute intervals over a 12-hour assessment period during which the highest amount of HR were generated. No statistical differences could be demonstrated. $p > 0.05$ for both days 35 and 56 compared to day 28 (One-way ANOVA followed by Dunnett's test).

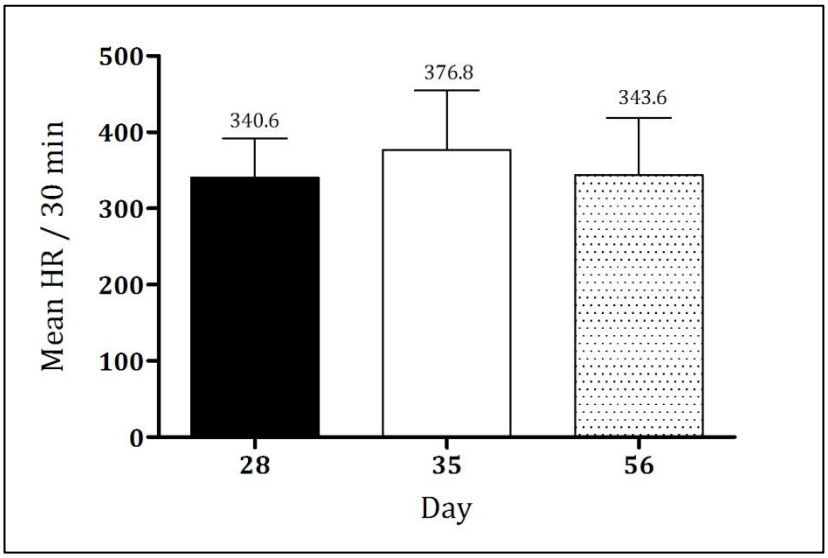



***FIGURE 5-12 – AVERAGE HR GENERATED BY ANIMALS OF THE HSB COHORT, BEFORE AND AFTER ESCITALOPRAM TREATMENT***

Data represent the weekly mean HR of 5 animals classified as HSB pattern runners after the first four weeks of baseline assessments (up to day 28), and following treatment with either sub-chronic (1 week) or chronic (4 weeks) treatment with escitalopram. The weekly contribution of each animal to the data presented here was calculated as a mean value from three 30-minute intervals over a 12-hour assessment period during which the highest amount of HR were executed. No statistical differences could be demonstrated. $p > 0.05$ for both days 35 and 56 compared to day 28 (One-way ANOVA followed by Dunnett's test).

### *5.3.2. Effect of escitalopram on the weekly amount of rest periods and HSB intervals over the course of 12 hours*

According to data presented in Section 5.2, HSB deer mice present with significantly lower striatal SERT densities compared to animals of the [NSB/LSB] cohort. Taking this into account, and given the fact that previous studies (Korff *et al.*, 2008) have demonstrated attenuation of deer mouse stereotypy following 21-day intraperitoneal administration of fluoxetine (10 and 20 mg/kg/day), the results presented in paragraph 5.3.1 seem to contradict these earlier findings. These new data thus oppose the hypothesis that the average amount of stereotypy expressed by HSB animals will decrease after chronic, but not sub-chronic treatment with high dose escitalopram.

In light of this, it was decided to re-evaluate the method of assessing the weekly data generated by each animal following drug treatment. *It must be stressed, however, that the methodology explained here is novel and that no previous studies in our laboratory or by other authors, have assessed the effects of drug treatment on the expression of deer mouse stereotypy applying these new criteria.*

* * *

Instead of focussing only on the three 30-minute intervals during which an animal expresses the highest amounts of stereotypy, all the data generated by an animal over the course of each 12-dark assessment period was now analysed. Two clear trends in the behaviour of deer mice pre- and post drug treatment became obvious, of which a typical data sheet is provided in Figure 5.13. This figure can be explained as follows:

Firstly [NSB/LSB] animals not only present with lower baseline average scores of stereotypy (supported by the data in Addendum A) compared to HSB animals, but they also express more 30-minute intervals over a 12-hour assessment period during which no stereotypy at all are executed. These intervals were defined as *rest periods* and were identified as such if less than 10 repetitions of the respective behavioural topographies were expressed during a 30-minute interval.

Although [NSB/LSB] animals may express high stereotypical behaviour during certain times of the dark cycle, the second trend observed in the behaviour of deer mice was that [NSB/LSB] animals generated significantly less 30-minute intervals during which HSB behaviour were executed as opposed to animals from the HSB cohort. An interval of HSB-activity was defined as a

30-minute interval during which HSB-behaviour was executed, as per the parameters defined in Table 5-1.

The changes in these two parameters following sub-chronic and chronic treatment with escitalopram (50 mg/kg/day) were subsequently applied as a measure of behavioural outcome. To compare the behaviour of deer mice on days 35 (after sub-chronic treatment) and 56 (after chronic treatment) with that of day 28 (the day before treatment was initiated), one-way ANOVA followed by Dunnett's test was performed. An example of the data sheet generated to evaluate the behaviour following this approach is provided in Figure 5-13.

| 22 | | | | | | | | | | 29 | | | | | | | | | | 35 | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 286 | 271 | 242 | 406 | 304 | 345 | 265 | 245 | 261 | | 56 | 43 | 27 | 15 | 24 | 62 | 25 | 21 | 13 | | 2 | 304 | 99 | 264 | 262 | 1 | 74 | 75 | 2 |
| 367 | 284 | 251 | 365 | 231 | 260 | 357 | 192 | 261 | | 46 | 32 | 132 | 25 | 17 | 74 | 35 | 24 | 28 | | 18 | 378 | 458 | 322 | 314 | 164 | 209 | 122 | 118 |
| 481 | 154 | 236 | 268 | 228 | 268 | 275 | 213 | 215 | | 42 | 61 | 132 | 23 | 0 | 116 | 0 | 32 | 13 | | 13 | 338 | 323 | 306 | 108 | 148 | 144 | 0 | 231 |
| 452 | 149 | 226 | 185 | 275 | 216 | 227 | 180 | 257 | | 32 | 11 | 93 | 27 | 3 | 0 | 0 | 0 | 0 | | 81 | 334 | 242 | 271 | 251 | 237 | 0 | 1 | 287 |
| 246 | 165 | 197 | 288 | 219 | 224 | 261 | 0 | 187 | | 12 | 0 | 13 | 36 | 6 | 158 | 7 | 0 | 0 | | 119 | 404 | 277 | 112 | 125 | 48 | 0 | 1 | 217 |
| 404 | 162 | 209 | 209 | 243 | 235 | 112 | 0 | 180 | | 36 | 32 | 0 | 19 | 70 | 124 | 0 | 1 | 1 | | 24 | 403 | 237 | 0 | 0 | 0 | 0 | 0 | 0 |
| 230 | 162 | 217 | 209 | 156 | 252 | 0 | 0 | 233 | | 38 | 49 | 0 | 0 | 140 | 4 | 0 | 0 | 1 | | 1 | 308 | 164 | 193 | 111 | 0 | 1 | 0 | 0 |
| 419 | 182 | 231 | 174 | 377 | 213 | 0 | 64 | 107 | | 56 | 4 | 18 | 4 | 0 | 0 | 0 | 2 | 53 | | 0 | 309 | 0 | 315 | 241 | 0 | 185 | 0 | 136 |
| 264 | 155 | 276 | 113 | 273 | 232 | 60 | 213 | 0 | | 9 | 22 | 19 | 32 | 0 | 4 | 33 | 0 | 16 | | 0 | 306 | 0 | 331 | 186 | 2 | 39 | 123 | 262 |
| 253 | 73 | 263 | 189 | 297 | 239 | 363 | 276 | 0 | | 7 | 50 | 2 | 24 | 0 | 3 | 25 | 7 | 0 | | 175 | 265 | 179 | 345 | 112 | 0 | 0 | 183 | 101 |
| 28 | 190 | 360 | 137 | 220 | 257 | 380 | 299 | 126 | | 50 | 74 | 30 | 44 | 44 | 37 | 180 | 35 | 2 | | 229 | 284 | 332 | 318 | 0 | 58 | 0 | 166 | 0 |
| 310 | 216 | 294 | 202 | 178 | 240 | 224 | 280 | 226 | | 123 | 487 | 26 | 68 | 47 | 28 | 332 | 50 | 160 | | 251 | 466 | 334 | 246 | 122 | 94 | 41 | 0 | 173 |
| 266 | 225 | 206 | 215 | 172 | 262 | 253 | 0 | 217 | | 143 | 420 | 126 | 65 | 0 | 2 | 78 | 65 | 32 | | 330 | 475 | 325 | 325 | 135 | 0 | 209 | 209 | 409 |
| 384 | 207 | 336 | 88 | 255 | 250 | 334 | 264 | 232 | | 119 | 240 | 101 | 26 | 0 | 48 | 36 | 70 | 0 | | 286 | 382 | 275 | 326 | 290 | 200 | 236 | 248 | 224 |
| 322 | 236 | 223 | 185 | 259 | 278 | 383 | 246 | 209 | | 169 | 501 | 87 | 68 | 0 | 198 | 124 | 65 | 21 | | 319 | 390 | 317 | 313 | 277 | 215 | 170 | 294 | 232 |
| 328 | 162 | 286 | 125 | 235 | 213 | 305 | 253 | 222 | | 284 | 502 | 321 | 438 | 10 | 63 | 71 | 79 | 55 | | 435 | 343 | 310 | 301 | 252 | 101 | 231 | 265 | 128 |
| 189 | 213 | 216 | 126 | 292 | 260 | 312 | 321 | 172 | | 164 | 495 | 284 | 85 | 147 | 59 | 104 | 58 | 41 | | 413 | 338 | 302 | 278 | 297 | 167 | 231 | 168 | 119 |
| 280 | 230 | 287 | 183 | 313 | 203 | 280 | 267 | 238 | | 232 | 392 | 327 | 138 | 154 | 64 | 53 | 107 | 39 | | 374 | 285 | 328 | 287 | 327 | 227 | 222 | 235 | 196 |
| 321 | 235 | 298 | 82 | 216 | 215 | 322 | 180 | 181 | | 719 | 402 | 248 | 383 | 40 | 164 | 77 | 153 | 174 | | 337 | 367 | 330 | 301 | 325 | 293 | 272 | 223 | 242 |
| 265 | 189 | 333 | 130 | 255 | 225 | 319 | 248 | 161 | | 528 | 496 | 94 | 140 | 53 | 43 | 13 | 17 | 24 | | 261 | 366 | 311 | 291 | 379 | 331 | 251 | 178 | 160 |
| 227 | 221 | 346 | 186 | 146 | 86 | 317 | 39 | 6 | | 311 | 536 | 27 | 55 | 73 | 3 | 1 | 2 | 7 | | 357 | 405 | 277 | 314 | 422 | 381 | 223 | 191 | 206 |
| 338 | 290 | 343 | 213 | 153 | 0 | 325 | 1 | 0 | | 200 | 536 | 9 | 294 | 115 | 0 | 0 | 0 | 0 | | 325 | 399 | 349 | 256 | 299 | 258 | 251 | 72 | 195 |
| 364 | 319 | 153 | 59 | 211 | 0 | 280 | 0 | 17 | | 121 | 200 | 37 | 223 | 0 | 0 | 0 | 0 | 0 | | 370 | 360 | 253 | 188 | 220 | 17 | 41 | 0 | 58 |
| 0 | 0 | 0 | 0 | 0 | 2 | 2 | 5 | 4 | | 2 | 2 | 4 | 2 | 10 | 9 | 9 | 10 | 10 | | 4 | 0 | 2 | 1 | 2 | 7 | 6 | 8 | 4 |
| 18 | 4 | 12 | 4 | 10 | 9 | 17 | 7 | 3 | | 4 | 10 | 3 | 2 | 0 | 0 | 1 | 0 | 0 | | 12 | 23 | 16 | 14 | 12 | 8 | 3 | 2 | 3 |

***FIGURE 5-13 – AN EXCERPT FROM THE DATA SHEET USED TO CALCULATE THE WEEKLY AMOUNTS OF REST PERIODS AND INTERVALS OF HSB PATTERN RUNNING ACTIVITY OBSERVED IN THE DATA GENERATED BY INDIVIDUAL ANIMALS OVER THE COURSE OF EIGHT WEEKS***

Data in this figure represent the HR executed by animals 22, 29 and 35. All three animals were classified as HSB pattern runners after the first four weeks of assessment. The first behavioural screen for each animal (column indicated at the bottom by a blue rectangle) was excluded from the statistical analysis. The four columns indicated by the red colouration at the bottom represent the weekly data from day 7 until day 28, the day before treatment was initiated. The columns indicated by the green colouring represent the data from the first week after treatment was initiated (day 35) until the termination of treatment (day 56). The numbers written in the coloured rows represent the amount of rest periods in each respective assessment, while the numbers in the row below these indicate the amount of intervals during which an animal expressed HSB behaviour. The numbers in each column represents an individual 30-minute interval. A rest period is defined as a 30-minute interval during which equal to or less than 10 stereotypical movements occurred, irrespective of the topography expressed, while an HSB interval is defined as score of > 2000 VBI / 30 min or > 200 HR / 30 or a combination of both.

*5.3.2.1. The average occurrence of rest periods observed in the behaviour of animals from the [NSB/LSB] and HSB cohorts, and response to sub-chronic (day 28-35) or chronic (day 28-56) escitalopram treatment*

Figure 5-14 demonstrates the average weekly amount of rest periods observed in animals of the [NSB/LSB] and HSB cohorts respectively. Neither sub-chronic, nor chronic treatment with oral escitalopram (50 mg/kg/day) significantly changed the amounts of rest periods observed in the behaviour of [NSB/LSB] animals (Figure 5-15). *However*, a statistically significant *increase* in the amount of rest periods could be observed in the behaviour of animals from the HSB cohort after chronic, but not sub-chronic treatment with escitalopram ($p$ = 0.0241) (Figure 5-16).

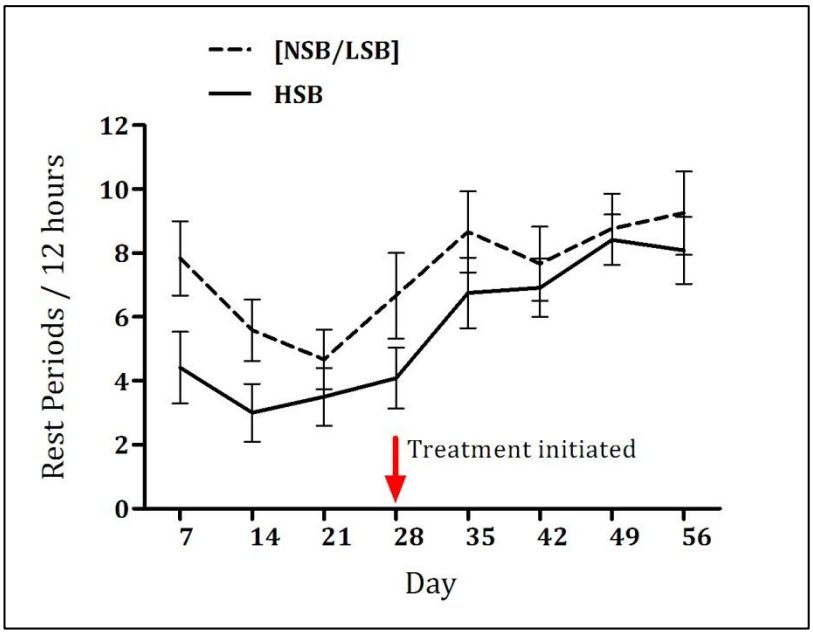



***FIGURE 5-14 – THE AVERAGE WEEKLY OCCURRENCE OF REST PERIODS OBSERVED IN THE BEHAVIOUR OF ANIMALS FROM THE [NSB/LSB] AND HSB COHORTS, AND RESPONSE TO SUB-CHRONIC OR CHRONIC ESCITALOPRAM TREATMENT***

Data representing rest period behaviour of 12 animals classified as ([NSB/LSB)] and HSB during the first four weeks of assessment, and response to 1 or 4 weeks of escitalopram treatment. A rest period is defined as a 30-minute interval during which equal to or less than 10 stereotypical movements were executed.

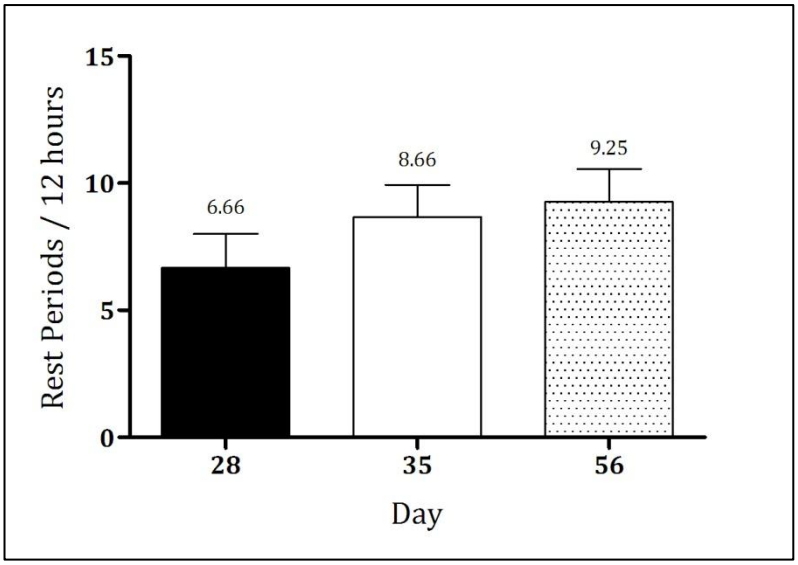



***FIGURE 5-15 – THE AVERAGE OCCURRENCE OF REST PERIODS OBSERVED IN THE BEHAVIOUR OF ANIMALS FROM THE [NSB/LSB] COHORT, AND RESPONSE TO SUB-CHRONIC OR CHRONIC ESCITALOPRAM TREATMENT***

Data represent the rest period behaviour of 12 animals classified as [NSB/LSB] during the first four weeks of assessment, and response to 1 or 4 weeks escitalopram treatment. A rest period is defined as a 30-minute interval during which equal to or less than 10 stereotypical movements were executed. No statistical differences could be demonstrated. $p > 0.05$ for both days 35 and 56 compared to day 28 (One-way ANOVA followed by Dunnett's test).

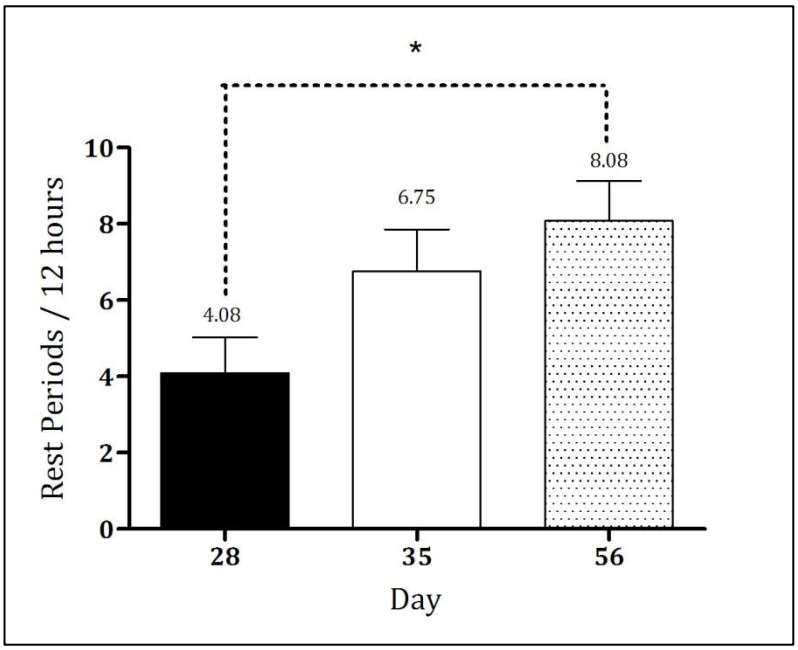



***FIGURE 5-16 – THE AVERAGE OCCURRENCE OF REST PERIODS OBSERVED IN THE BEHAVIOUR OF ANIMALS FROM THE HSB COHORT, AND RESPONSE TO SUB-CHRONIC OR CHRONIC ESCITALOPRAM TREATMENT***

Data represent the rest period behaviour of 12 animals classified as HSB during the first four weeks of assessments, and response to 1 or 4 weeks escitalopram treatment. A rest period is defined as a 30-minute interval during which equal to or less than 10 stereotypical movements were executed. $p = 0.0241$ for day 56 compared to day 28 (One-way ANOVA followed by Dunnett's test).

*5.3.2.2. The average occurrence of intervals of HSB activity observed in animals from the [NSB/LSB] and HSB cohorts, and response to sub-chronic (day 28-56) or chronic (day 28-56) escitalopram treatment*

The weekly occurrence of intervals of HSB activity in both the [NSB/LSB] and HSB cohorts are represented in Figure 5-17. Neither chronic, nor sub-chronic treatment with escitalopram (50 mg/kg/day) significantly altered the amount of HSB-intervals generated by [NSB/LSB] animals ($p$ = 0.3840) (none of the animals in the [NSB/LSB] group expressed HSB behaviour on days 14, 49 and 56) (Figure 5-18). Chronic, but not sub-chronic treatment however, *significantly decreased* the amount of intervals during which HSB animals expressed HSB behaviour ($p$ = 0.0054) (Figure 5-19).

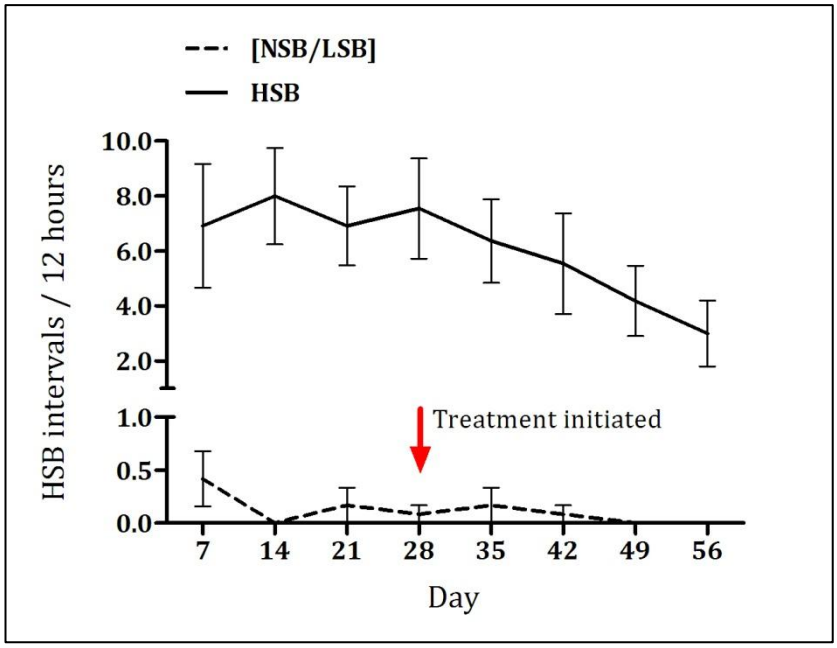



***FIGURE 5-17 – THE AVERAGE WEEKLY OCCURRENCE OF INTERVALS OF HSB ACTIVITY OBSERVED IN THE BEHAVIOUR OF ANIMALS FROM THE [NSB/LSB] AND HSB COHORTS, AND RESPONSE TO SUB-CHRONIC OR CHRONIC ESCITALOPRAM TREATMENT***

Data represent the behaviour of 12 animals classified as ([NSB/LSB]) or HSB during the first four weeks of assessment, and response to 1 or 4 weeks escitalopram treatment. An HSB interval is defined as a score of > 2000 VBI / 30 min or > 200 HR / 30 or a combination of both.

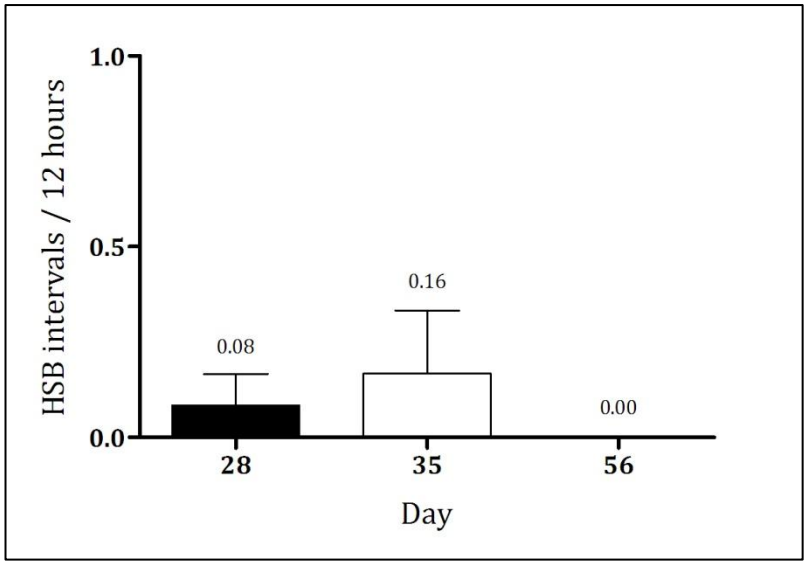



***FIGURE 5-18 – THE AVERAGE OCCURRENCE OF INTERVALS OF HSB ACTIVITY IN THE BEHAVIOUR OF ANIMALS FROM THE [NSB/LSB] COHORT, AND RESPONSE TO SUB-CHRONIC OR CHRONIC ESCITALOPRAM TREATMENT***

Data represent the HSB activity of 12 animals classified as [NSB/LSB] during the first four weeks of assessment, and response to 1 or 4 weeks escitalopram treatment. An HSB interval is defined as score of > 2000 VBI / 30 min or > 200 HR / 30 or a combination of both. None of the animals in the cohort expressed any intervals of HSB activity on day 56. No statistical differences could be demonstrated. $p > 0.05$ for both days 35 and 56 compared to 28 (One-way ANOVA followed by Dunnett's test).

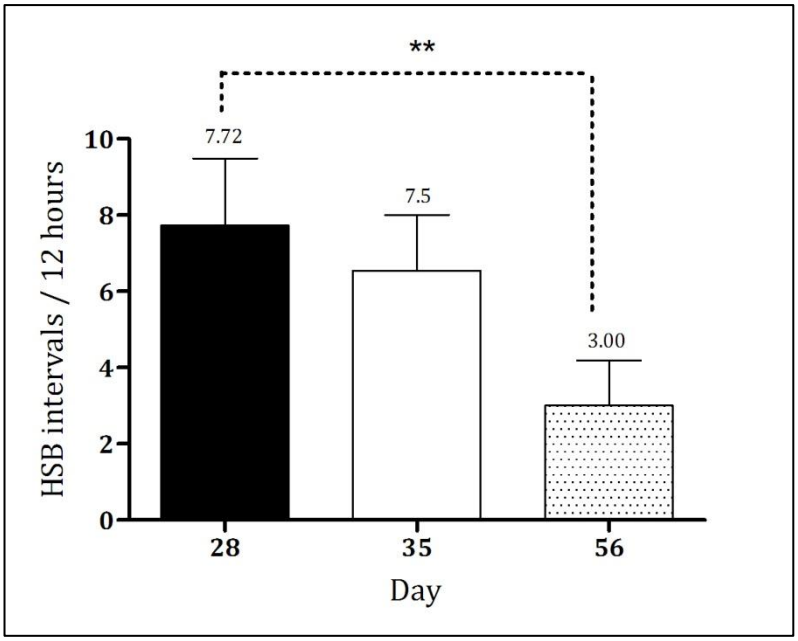



***FIGURE 5-19 – THE AVERAGE OCCURRENCE OF INTERVALS OF HSB ACTIVITY OBSERVED IN THE BEHAVIOUR OF ANIMALS FROM THE HSB COHORT, AND RESPONSE TO SUB-CHRONIC OR CHRONIC ESCITALOPRAM TREATMENT***

Data represent the HSB activity of 12 animals classified as HSB during the first four weeks of assessment, and response to 1 or 4 weeks escitalopram treatment. An HSB interval is defined as a score of > 2000 VBI / 30 min or > 200 HR / 30 or a combination of both. $p = 0.0054$ for day 56 compared to day 28 (One-way ANOVA followed by Dunnett's test).

* * *

In summary, it is evident from these data that although HSB deer mice continuously express high stereotypical behaviour even after long term treatment with high dose escitalopram, the frequency with which these high stereotypical bouts are expressed decreases as a result of treatment. Furthermore, while the average behaviour of treatment naive HSB deer mice are characterized by very little periods of inactivity (4.08 intervals or roughly 2 hours), this level of inactivity doubles after chronic, but not sub-chronic treatment with high dose escitalopram, again emphasizing the susceptibility of both these behaviours to modification by effective SSRI treatment. This new approach to analysing deer mouse stereotypy has thus proved to be particular useful. It has especially helped to delineate certain behavioural characteristics not previously observed in these animals that allows for a more accurate and robust measure of stereotypy, especially following a drug intervention.

### *5.3.3. General locomotor activity of [NSB/LSB] and HSB deer mice, and response to sub-chronic or chronic escitalopram treatment*

In order to exclude the possibility that escitalopram could affect locomotor activity of the animal and subsequently influence stereotypical behavioural, the general locomotor activity of the animals was recorded as horizontal movements. These data are presented in Figure 5-20. A horizontal movement is defined as any beam interruption in the lower grid of light beams, each interruption separated by at least one second. Neither sub-chronic nor chronic treatment with escitalopram significantly influenced the average general locomotor activity of HSB deer mice ($p > 0.05$) (Figure 5-22). However, chronic but not sub-chronic treatment with escitalopram *significantly decreased* the average locomotor activity of the animals in the [NSB/LSB] cohort ($p = 0.0088$; Figure 5-21).

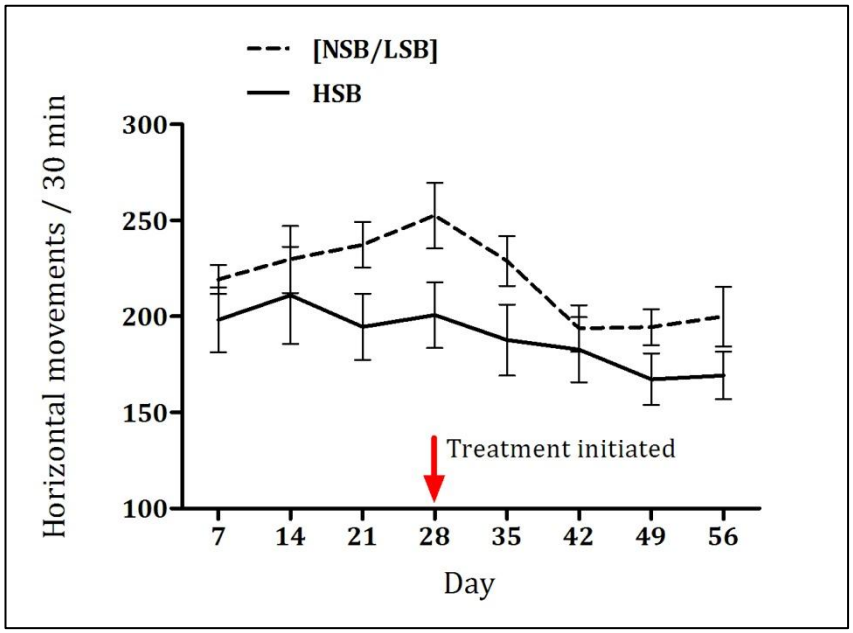



***FIGURE 5-20 – THE AVERAGE WEEKLY LOCOMOTOR ACTIVITY OF [NSB/LSB] AND HSB DEER MICE, AND RESPONSE TO SUB-CHRONIC OR CHRONIC ESCITALOPRAM TREATMENT***

A horizontal movement is defined as a beam interruption in the lower grid of beams separated by at least one second. The contribution of each animal to the data presented here was calculated as the mean number of horizontal movements generated during the whole 12-hour assessment period.

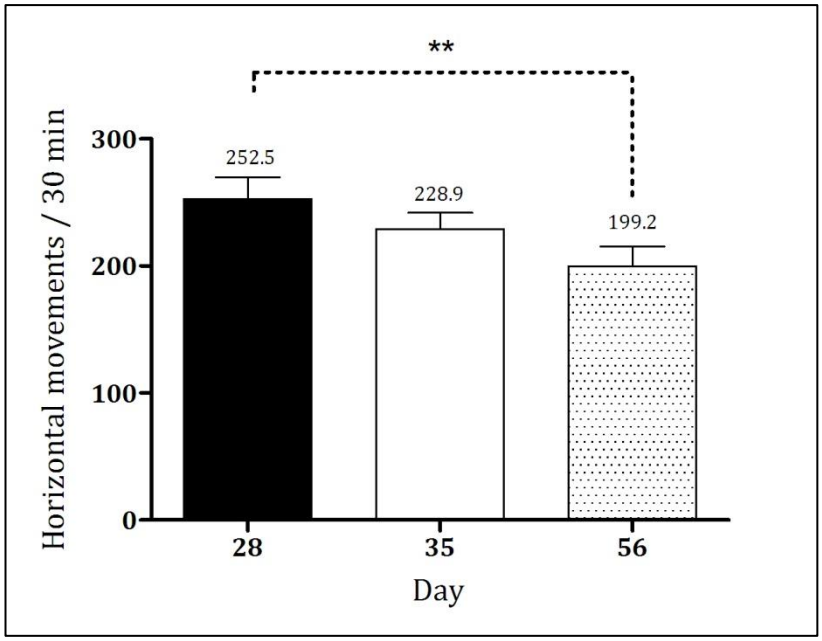



***FIGURE 5-21 – THE AVERAGE AMOUNTS OF HORIZONTAL MOVEMENTS GENERATED BY ANIMALS OF THE [NSB/LSB] COHORT, AND RESPONSE TO SUB-CHRONIC OR CHRONIC ESCITALOPRAM TREATMENT***

A horizontal movement is defined as a beam interruption in the lower grid of beams separated by at least one second. The contribution of each animal to the data presented here was calculated as the mean number of horizontal movements generated during the whole 12-hour assessment period. $p = 0.0088$ for day 56 compared to day 28 (One-way ANOVA followed by Dunnett's test).

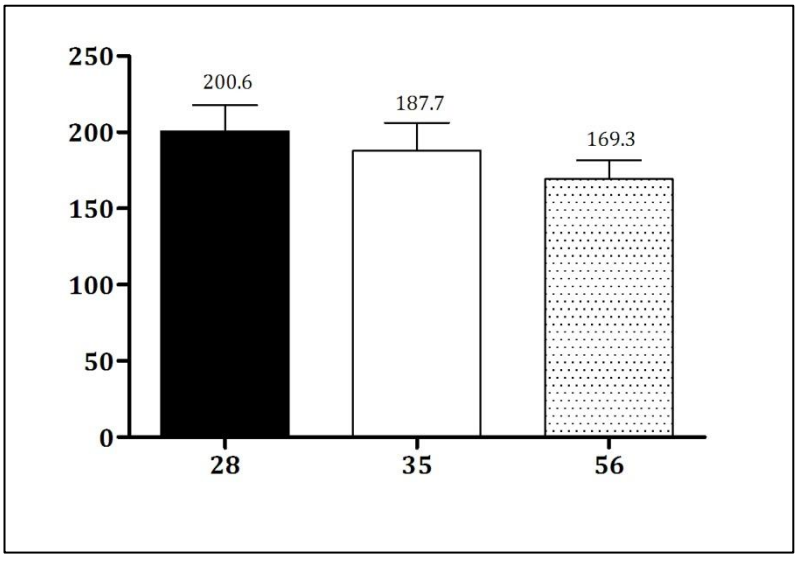



***FIGURE 5-22 - THE AVERAGE AMOUNTS OF HORIZONTAL MOVEMENTS GENERATED BY ANIMALS OF THE HSB COHORT, AND RESPONSE TO SUB-CHRONIC OR CHRONIC ESCITALOPRAM TREATMENT***

A horizontal movement is defined as a beam interruption in the lower grid of beams separated by at least one second. The contribution of each animal to the data presented here was calculated as the mean number of horizontal movements generated during the whole 12-hour assessment period. No statistical difference could be demonstrated. $P > 0.05$ for both days 35 and 56 compared to day 28 (One-way ANOVA followed by Dunnett's test).

*END OF CHAPTER 5*

# CHAPTER 6
# DISCUSSION

## 6.1. INTRODUCTION

The deer mouse model is currently regarded as one of the five leading animal models of OCD (Albelda and Joel, In Press (a); Albelda and Joel, 2012); Hoffman and Rueda Morales, 2009; Joel *et al.*, 2008; Wang *et al.*, 2009) and has been investigated mostly with regard to its predictive and construct validity (Güldenpfennig *et al.*, 2011; Korff *et al.*, 2008; Korff *et al.*, 2009). The predictive validity of the model is strengthened by the demonstration that chronic administration of the SSRI, fluoxetine (10 and 20 mg/kg/day), results in the attenuation of stereotypy (Korff *et al.*, 2008) while the construct validity is founded in the fact that an imbalance between the direct and indirect pathways in the basal ganglia can be associated with the expression of stereotypy (Presti and Lewis, 2005). Furthermore, high stereotypical deer mice express higher prefrontal-cortical and striatal levels of cAMP and lower levels of PDE4 compared to their non-stereotypical controls (Korff *et al.*, 2009), while high stereotypical deer mice also present with evidence of cortico-striatal disturbances in redox balance (Güldenpfennig *et al.*, 2011). It is widely accepted that OCD involves a lesion of the CSTC circuit (Evans *et al.*, 2004; Husted *et al.*, 2006; Maltby *et al.*, 2005), while recent work has noted the presence of a redox imbalance and oxidative stress in patients with OCD (Ersan *et al.*, 2006; Kuloglu *et al.*, 2002; Ozdemir *et al.*, 2009; Selek *et al.*, 2008). These latter findings therefore strengthen the construct of the model as it not only implicates oxidative stress and a cortico-striatal lesion in the expression of deer mouse stereotypy, but also demonstrates an involvement of the second messenger system modulated by serotonergic signalling (Barnes and Sharp, 1999; Bergqvist *et al.*, 1999) in the expression of such stereotypy.

Building on this foundation, the aim of the current study was to strengthen the predictive and construct validity of the model even more. Although Korff and colleagues demonstrated a selective response of deer mouse stereotypy to chronic fluoxetine treatment versus the noradrenergic agent, desipramine, one of the relative shortcomings of the model was that a sub-chronic pharmacological challenge was not performed. As OCD only responds to chronic treatment with the SSRIs (Fineberg *et al.*, 2006; Fineberg and Craig, 2007), one of the aims of the current study was to assess whether the model will also demonstrate a lack of response to a sub-chronic regimen as is the case in human OCD. Furthermore, the fact that deer mouse stereotypy responds to treatment with fluoxetine (Korff *et al.*, 2008) strongly implicates the serotonergic

system in the neurobiology of these behaviours. However, hyposerotonergic functioning is believed to be directly involved in the symptomology of OCD (Cools *et al.*, 2008; Daw *et al.*, 2002; Delgado and Moreno, 1998; Goddard *et al.*, 2008) and has been associated with a decrease in SERT availability, as demonstrated in a number of clinical studies done in OCD patients (Hesse *et al.*, 2005; Reimold *et al.*, 2007; Zitterl *et al.*, 2008). Another aim of the current study therefore was to investigate whether any differences in frontal-cortical and striatal SERT density could be observed in high versus low stereotypic animals, and to confirm whether altered SERT binding (a marker of serotonin signalling) can be demonstrated between stereotypical and non-stereotypical animals.

To compare the behavioural effects of sub-chronic and chronic SSRI treatment on the expression of stereotypy, and to assess the differences between the baseline SERT densities of stereotypical and non-stereotypical animals, a clear behavioural distinction must be made between these two cohorts. To classify deer mice into non-stereotypical (NSB), low-stereotypical (LSB) and high-stereotypical (HSB) cohorts, previous studies done in our laboratory (Güldenpfennig *et al.*, 2011; Korff *et al.*, 2008) employed a classification system that *did not* discriminate between the different behavioural topographies expressed by deer mice, *viz.* pattern running and vertical jumping. In other words, all the topographies were grouped together and applied with reference to the separation criteria. Consequently, since the type of stereotypy an animal expresses is not important in the argument of whether an animal is stereotypical or not, the emphasis was not which type of stereotypy a particular animal expresses, but that it *does* express repetitive behaviour, and that it mimics the rigid motor actions observed in human OCD. Although this is a valid argument, laboratory data from the current study indicated that, based on the above criteria, pattern runners are more than often *not* included in the HSB cohorts, while vertical jumpers are always included. Scrutiny of the data sheets found that even very high stereotypic pattern runners have markedly less episodes of pattern running than the equivalent high stereotypic animal that expresses vertical jumping. However, although the behavioural topography expressed does not have implications for the theoretical basis of the model per se, it does influence the behavioural and neurochemical evaluation of the animals. The behaviour of pattern runners is characterized by the rigid and repetitive completion of cage revolutions, while that of animals expressing vertical stereotypy manifests as repetitive jumping in one of the corners of the cage. If the behaviour of pattern runners seems just as rigid as that of vertical jumpers but results in the generation of less stereotypy counts, the question arises if these animals are indeed classified correctly. This has major implications for the behavioural and neurochemical assessment of deer mice as it is possible that based on the currently employed classification system, HSB pattern runners will be appraised as NSB or LSB animals. This

may result in obtaining data that do not depict the actual behavioural and neurochemical differences between the HSB and [NSB/LSB] cohorts as the behaviour and neurochemistry of potential HSB pattern runners are included in the assessments of otherwise [NSB/LSB] animals. The first study aim was therefore to *provide a stronger foundation for establishing the predictive and construct validation of the model* through a newly developed classification system that differentiates between the respective behavioural topographies.

## 6.2. STUDY OBJECTIVE I – THE DEVELOPMENT OF A NEW CLASSIFICATION SYSTEM FOR THE APPRAISAL OF DEER MOUSE STEREOTYPY

The classification criteria for the evaluation of deer mouse stereotypy that were developed during the course of the current study differ in two main respects from that employed in our previous studies (Güldenpfennig *et al.*, 2011; Korff *et al.*, 2008; Korff *et al.*, 2009). First, the unit of measurement was changed from stereotypy counts per hour to stereotypy counts per 30 minutes and secondly different cut-off values were applied to assess the respective behavioural topographies.

In an attempt to customize the classification criteria to be applied specifically in an animal model of OCD, it was decided to shorten the duration of assessment intervals from one hour to 30 minutes. From paragraph 3.1.3, it is argued that the behaviour of an animal expressing 2000 or more stereotypical movements per hour is less rigid and repetitive than that of an animal generating the same count in 30 minutes as the latter animal executes stereotypy at a faster pace. As per the previous criteria, both animals can be classified as HSB, while only the latter animal would be included in the HSB cohort according to the newly defined cut-off values (Table 5-1). This modification of the previous criteria can be explained as follows. The Yale-Brown Obsessive-Compulsive Scale (Y-BOCS) (Goodman *et al.*, 1989), a widely used clinical rating scale to assess and score the severity of OCD symptoms in humans, rates the severity of obsessions and compulsions on a scale of 0 (no symptoms) to 40 (very severe). In short, patients are presented with a list of 50 obsessions and compulsions from which the clinician rates the five most prominent obsessions and compulsions respectively. The severity of each of these is assessed applying five criteria namely (1) time, (2) interference, (3) distress, (4) resistance, and (5) control. Each obsession and compulsion is then scored (ranging from 0 – 4) from which a final score of severity is calculated (Abramowitz *et al.*, 2008; Abramowitz *et al.*, 2003). Although it is difficult to determine whether deer mice experience distress while they execute stereotypical behaviour, it can be hypothesized that animals executing repetitive behaviour at a more rapid pace exert less resistance to the expression of stereotypy, and have less control over the 'need' to express stereotypical behaviour. Regarding the time factor (1), it cannot be argued that animals expressing 2000 jumps over the course of 30 minutes spend less time expressing stereotypy than do animals expressing the same amount of jumps in one hour, as these animals simply generate more 30-minute intervals during which high stereotypical behaviour is expressed. However, although the time factor has not been employed in the current study as a means of categorizing deer mice into [NSB/LSB] and HSB cohorts, it can be a valuable tool to separate

treatment naive deer mice based on the amount of time an animal spends in the execution of stereotypical behaviour. Nevertheless, in the current study the time factor has indeed been employed to assess the behavioural outcomes of sub-chronic and chronic escitalopram treatment, which will be discussed in section 6.3.

With respect to the different classification criteria employed for vertical jumpers and pattern runners, it was demonstrated that although pattern runners can be divided into HSB and [NSB/LSB] cohorts (Figures 5-1 and 5-2), the HSB animals execute on average significantly less cage revolutions than HSB animals expressing vertical jumping (296 vs. 3063 respectively; Table 5-5; weekly $p$ values < 0.005; unpaired t-tests). The 12 animals that generated the lowest weekly numbers of stereotypy, irrespective of the behavioural topography expressed, executed on average 55 cage revolutions and 923 vertical jumps per 30 minutes, which is significantly less than the stereotypy counts generated by HSB pattern runners and vertical jumpers respectively (Tables 5-3 and 5-4; weekly $p$ values < 0.005; unpaired t-tests).

Applying the newly defined criteria, only 35% of animals in the control group and 41% of animals in the treatment group could be classified as HSB (Table 5-2). This is somewhat less than the 45% of animals classified as HSB by Korff and colleagues (2008). However, this result was expected since the shorter duration of assessment intervals resulted in the exclusion of candidates who would otherwise have been classified as HSB based on the previous unit of measurement (viz. stereotypy counts per hour instead of stereotypy counts per 30 minutes).

* * *

Data from the current study clearly indicate that one classification system for both behavioural topographies is inadequate to accommodate the differences in the respective manifestations of stereotypy. Furthermore, it is evident that although pattern runners can be separated statistically into HSB and [NSB/LSB] cohorts, HSB pattern runners could not have been classified as such, but rather as [NSB/LSB] animals, when applying the previously published criteria. By applying the newly developed criteria for the appraisal of deer mouse stereotypy, a better distinction can be made between animals of the HSB and [NSB/LSB] cohorts, thereby providing a stronger foundation for treatment and neurochemical studies.

## 6.3 STUDY OBJECTIVE II – AN INVESTIGATION INTO FRONTAL-CORTICAL AND STRIATAL SERT DENSITIES OF TREATMENT NAIVE [NSB/LSB] AND HSB DEER MICE

It has been hypothesized that OCD is characterized by hyposerotonergic functioning in the cortico-striatal-thalamic-cortical circuitry (Delgado and Moreno, 1998; Goddard *et al.*, 2008; Hollander, 1998), explaining the ameliorative effects on OCD symptomology following chronic treatment with the SSRIs (Blier *et al.*, 1996; El Mansari and Blier, 2006; Fineberg and Craig, 2007; Vaswani *et al.*, 2003; Vythilingum *et al.*, 2000). Furthermore, a number of studies have investigated the availability of serotonin transporters (SERT) in patients with OCD compared to healthy controls (Hesse *et al.*, 2005; Pogarell *et al.*, 2003; Reimold *et al.*, 2007; Simpson *et al.*, 2003; Zitterl *et al.*, 2008). Most of these studies assessed SERT densities in the thalamus/hypothalamus, midbrain and brain stem (Hesse *et al.*, 2005; Pogarell *et al.*, 2003; Zitterl *et al.*, 2008). Except for two of these reports (Pogarell *et al.*, 2003; Simpson *et al.*, 2003), all demonstrated a 10 – 20% decrease in SERT availability in untreated OCD patients compared to healthy controls. Furthermore, Zitterl and colleagues (Zitterl *et al.*, 2008) also found a significant negative correlation between the severity of OCD symptoms (as measured by the Y-BOCS-scale) and the availability of SERT in OCD patients.

Although a number of studies have attempted to establish a possible link between OCD and polymorphisms of the promoter region in the SERT gene (Bengel *et al.*, 1999; Chabane *et al.*, 2004; Kinnear *et al.*, 2000), meta-analyses of these studies failed to demonstrate any definitive association (Bloch *et al.*, 2008; Hemmings and Stein, 2006). However, it must be kept in mind that studies investigating polymorphisms in the SERT gene target the human genome and not the actual expression of SERT in the different brain areas. Indeed, no studies to date have compared actual frontal-cortical and striatal SERT densities in OCD patients compared to healthy controls. However, as stated above, a number of studies demonstrated a 10 – 20% decrease in midbrain (containing the striatum and basal ganglia) and thalamic SERT availability in OCD patients, compared to healthy controls (Hesse *et al.*, 2005; Pogarell *et al.*, 2003; Zitterl *et al.*, 2008). Furthermore, a number of reports implicate the CSTC circuitry in the pathology of OCD. Baxter and colleagues (Baxter Jr. *et al.*, 1992) demonstrated altered glucose metabolism in the caudate nuclei of patients with OCD compared to healthy controls, while an imbalance between the direct and indirect pathways of the basal ganglia have also been associated with obsessive-compulsive symptoms (den Heuvel *et al.*, 2010; Nambu *et al.*, 2002). Many studies employing positron emission tomography (PET), single photon emission CT (SPECT), magnetic resonance (MR), and functional MR (fMR) have demonstrated involvement of the frontal-subcortical cir-

cuits in the neurobiology of OCD and are reviewed by Saxena and Rauch (2000). Therefore, as hyposerotonergic functioning *and* abnormalities in the CSTC circuitry are implicated in OCD, it was decided to investigate whether HSB deer mice present with differences in frontal-cortical and striatal SERT densities as opposed to that of [NSB/LSB] deer mice.

* * *

Although the HSB cohort demonstrated a lower mean frontal-cortical SERT density compared to the [NSB/LSB] cohort (Figure 5-5), this result was not statistically significant. However, the striatal SERT density of HSB animals was found to be significantly lower compared to that of [NSB/LSB] animals (Figure 5-6; $p = 0.0009$). This data is congruent to that demonstrated in human OCD (Hesse *et al.*, 2005; Pogarell *et al.*, 2003; Zitterl *et al.*, 2008) and implies a common change or perturbation in SERT binding in these two brain regions, albeit more severe in the striatum. In a SPECT study looking at a combined group of patients with different anxiety disorders (OCD, PTSD, and SAD), Carey and colleagues (2004) found that the SERT antagonist, citalopram, resulted in deactivation within the anterior and superior cingulate cortex, the left hippocampus and the right thalamus. Moreover, this response was more marked in treatment responders. Given the postulated role of serotonin in the suppression of complex motor behaviour (Coccaro, 1989; Cools *et al.*, 2008; Daw *et al.*, 2002; Fletcher *et al.*, 1999), these results fit the hypothesis of a hyposerotonergic functioning in the compulsive-like expression of motor patterns (Delgado and Moreno, 1998; Goddard *et al.*, 2008). Linking these behavioural functions of serotonin with the cortico-striatal circuitry implicated in OCD (den Heuvel *et al.*, 2010; Denys *et al.*, 2004c; Nambu *et al.*, 2002; Saxena and Rauch, 2000), these new findings strengthen the construct of the model, as it not only associates hyposerotonergic functioning with increased stereotypic behaviour in deer mice, but do so in the neural circuitry implicated in both OCD and the execution of complex motor patterns.

Animals of the HSB cohort expressed significantly higher SERT values in the frontal-cortex compared to the striatum (Figure 5-4; $p = 0.0087$), while data obtained from the [NSB/LSB] cohort, although not statistically significant, indicate that this relationship tends to be the opposite from that demonstrated in HSB animals (Figure 5-3). This would indicate a decreased serotonergic functioning in the striatum relative to the frontal cortex in high stereotypic animals, as well as provides support for a more pronounced lesion/dysfunction in the striatum over the frontal-cortex that may be responsible for the higher levels of stereotypy evident in HSB mice. Earlier studies in the deer mouse have also highlighted cortico-striatal differences but with respect to other biological parameters, although favouring a greater disturbance in the frontal-cortex (Güldenpfennig *et al.*, 2011; Korff *et al.*, 2009). Nevertheless, these data confirm a differ-

ential role and pathology within the CSTC circuit in deer mouse stereotypy and OCD. Considering the strong interaction between 5HT and DA in the CSTC circuit, and that deer mouse stereotypy involves disturbances in both 5HT and DA (Korff *et al.*, 2008), the findings regarding SERT density described here can also be interpreted with respect to this interaction. As serotonin is postulated to be the behavioural opponent of dopamine within the CSTC circuit (Daw *et al.*, 2002), these results support the hypothesis of hyposerotonergic functioning in the pathology of OCD (Delgado and Moreno, 1998; Goddard *et al.*, 2008; Hollander, 1998). This can be explained as follows:

A bias in favour of the direct pathway over the indirect pathway in the striatum and basal ganglia is postulated to play a role in the pathology of OCD (Bartz and Hollander, 2006; Saxena and Rauch, 2000), while stereotypical behaviour in the deer mouse has also been linked with an overactive direct pathway (Presti and Lewis, 2005). Furthermore, it is known that the simultaneous innervation of both these pathways by dopamine activates the cortex via the thalamus (Nambu *et al.*, 2002; Nambu, 2008; Penney Jr. and Young, 1986) resulting in the execution of complex motor patterns such as the compulsions observed in OCD. It is hypothesized that serotonin plays the role of behavioural opponent to dopamine (Daw *et al.*, 2002; Delgado and Moreno, 1998) therefore attenuating the behavioural effects elicited by the striatal release of dopamine.

One possible mechanism of such an opponent interaction between serotonin and dopamine could be the decrease in caudate dopamine release elicited following the stimulation of $5HT_{2C}$ receptors (Millan *et al.*, 1998). Keeping in mind that SERT density is used as a measure of serotonergic signalling (Delgado and Moreno, 1998; Goddard *et al.*, 2008; Reimold *et al.*, 2007; Zitterl *et al.*, 2008), the results from the current study support the above-described opposing interaction between dopamine and serotonin. This is evident by an increase in striatal serotonergic signalling, as demonstrated in the [NSB/LSB] cohort (Figure 5-3), and can be associated with an increased stimulation of caudate $5HT_{2C}$ receptors and a subsequent decrease in dopamine release.

Interestingly, in a different mouse model of OCD, Shanahan and colleagues demonstrated that stimulation of orbito-frontal $5HT_{1B/D}$-receptors is associated with the expression of rigid hyperlocomotion and perseveration along a certain set of locomotor paths (Shanahan *et al.*, 2011; Shanahan *et al.*, 2009). Although the authors conclude that the activation of orbitofrontal, but not striatal, $5HT_{1B/D}$ receptors is necessary for the expression of OCD-like behaviour, the data from the current study seem to contradict this. As no significant differences between the frontal-cortical SERT densities of [NSB/LSB] and HSB animals could be demonstrated, it is pos-

sible that striatal, *but not frontal-cortical* serotonergic signalling may play a major role in the pathology of deer mouse stereotypy. The model described by Shanahan and colleagues (Shanahan *et al.*, 2011) may differ from the deer mouse model of OCD with respect to the etiological mechanisms underlying compulsive-like behaviour. However, studies comparing the frontal-cortical and striatal $5HT_{1B/D}$ receptor densities of treatment-naive [NSB/LSB] and HSB deer mice would be valuable in determining whether this is indeed the correct explanation, or whether deer mouse stereotypy can be associated with altered frontal-cortical $5HT_{1B/D}$ receptor density. In fact, earlier work has demonstrated that the amount of stereotypy expressed in deer mice is positively correlated to differences in frontal-cortical cAMP levels (Korff *et al.*, 2009), while it is known that changes in intracellular cAMP are directly regulated by $5HT_{1B/1D}$ receptors (Barnes and Sharp, 1999). Moreover, the desensitization of these receptors is thought to mediate some of the ameliorative effects of the SSRIs on OCD symptomology (Blier *et al.*, 1996). Since stereotypy observed in the model described by Shanahan and colleagues is pharmacologically induced, while the deer mouse model is a naturalistic animal model, studies aimed at investigating deer mouse stereotypy and its association with altered frontal-cortical $5HT_{1B/D}$ receptor density will strengthen the construct validities of *both* these animal models. Not only could such an association aid in elucidating the etiological mechanisms underlying deer mouse stereotypy, but the model of Shanahan and colleagues will benefit from the demonstration that natural stereotypy may develop following frontal-cortical $5HT_{1B/D}$ receptor changes. The main drawback of pharmacological models of OCD is that results can only be related to the pharmacological mechanism of the drug used, and that the naturally occurring etiological basis underlying a given disorder cannot be investigated. Consequently, the construct validity of the model developed by Shanahan and colleagues will benefit from the demonstration that the etiological target of their model is congruent with that demonstrated in the natural developing stereotypy expressed by deer mice.

* * *

In summary, the current study demonstrates that striatal but not frontal-cortical SERT availability significantly differed between animals of the [NSB/LSB] and HSB cohorts. This would indicate that the degree of deer mouse stereotypy expressed by HSB animals is associated with a significant decrease in striatal, but not frontal-cortical SERT density, thus reflecting a disturbance in 5HT transmission in this particular brain region. This change may reflect a direct role for altered 5HT signalling or a result of changes in other signalling pathways that in turn affect serotonin function, such as GABAergic and glutamatergic signalling. Further studies in this regard are required. Although no studies have compared the frontal-cortical and striatal SERT densities in patients with OCD (Zitterl *et al.*, 2008), the respective functions of serotonin in

the frontal-cortex and striatum can be delineated by focussing on the regional effects of the serotonergic receptors, such as $5HT_{1B/D}$ and $5HT_{2C}$, as described above. Studies comparing the frontal-cortical and striatal SERT densities of these two serotonergic receptors in deer mice would be valuable in understanding the functional significance of striatal and frontal-cortical serotonergic signalling in the pathology of repetitive behaviour, as demonstrated in [NSB/LSB] animals. Furthermore, results describing reduced SERT density in HSB deer mice are congruent with data from clinical studies in patients with OCD (Hesse *et al.*, 2005; Reimold *et al.*, 2007; Zitterl *et al.*, 2008), and thus contribute favourably to the construct validity of the deer mouse model of OCD.

* * *

Following the investigation on the construct validity of the model, the predictive validity of the model was studied by investigating whether HSB deer mice respond to chronic, but not sub-chronic, treatment with escitalopram.

## 6.4 STUDY OBJECTIVE III – THE EFFECT OF SUB-CHRONIC AND CHRONIC ORAL ESCITALOPRAM TREATMENT ON DEER MOUSE STEREOTYPY

The third aim of the current study was to evaluate the effects of sub-chronic and chronic high dose escitalopram on the manifestation of deer mouse stereotypy. As chronic but not sub-chronic treatment with the SSRIs demonstrates clinical efficacy in at least 70% of OCD patients (Fineberg and Craig, 2007), the existing predictive validity of the deer mouse model of OCD can be strengthened if it demonstrates the same lack of response to sub-chronic treatment with high dose SSRIs, as is the case in human OCD. Up to date, deer mouse stereotypy has not been tested with regard to this type of drug challenge, and hence the reason for its inclusion in the present work together with a chronic treatment regimen.

* * *

Deer mice were screened weekly for four weeks to determine a baseline pre-treatment stereotypy value for each animal. As explained in Chapter 4, data from the three 30-minute intervals during which the highest number of stereotypical movements were executed, were used to calculate a mean stereotypy value for each animal. Subsequently, animals were divided into either HSB or [NSB/LSB] cohorts and their behaviour recorded for the remaining four weeks of treatment.

Interestingly, evaluation of the weekly changes in these values as a measure of the efficacy of 1 or 4 weeks treatment with escitalopram to attenuate these behaviours, did not demonstrate any significant changes in either [NSB/LSB] or HSB deer mice with respect to the amount of vertical jumps (VBI) and cage revolutions (HR) (Figures 5-7 and 5-10). The sub-chronic and chronic effects of escitalopram treatment on the expression of these stereotypy values was further assessed by comparing the highest average individual stereotypy scores on day 28 (pre-treatment control) with that of day 35 (sub-chronic) and day 56 (chronic) (Figures 5-8, 5-9, 5-11 and 5-12). One-way ANOVA followed by Dunnett's test failed to show any significant changes resulting from either sub-chronic or chronic treatment on the expression of VBI and HR as expressed by [NSB/LSB] and HSB deer mice. However, although not statistically significant, escitalopram tended to decrease the VBI of [NSB/LSB] animals as treatment progressed (Figure 5-8). This can possibly be explained by the significant decrease in locomotor activity observed in the [NSB/LSB] cohort following treatment with chronic but not sub-chronic escitalopram. Interestingly, this effect could not be demonstrated in the HSB cohort.

* * *

As previous studies done in our laboratory (Korff *et al.*, 2008; Korff *et al.*, 2009) demonstrated the attenuation of deer mouse stereotypy after chronic treatment with fluoxetine, it was decided to scrutinize the data for changes in the nocturnal behaviour of deer mice over the course of each of the 12-hour screening sessions. As explained in paragraph 5.3.2, two trends with respect to their behaviour could be identified.

First, the baseline behaviour of [NSB/LSB] deer mice during the four pre-treatment weeks was characterized by significantly fewer intervals of high stereotypical activity on day 28 compared to that of HSB animals, irrespective of the behavioural topography expressed (Figure 5-17). An interval of HSB activity was defined as a 30-minute interval during which high stereotypical behaviour of any topography, as defined in Table 5-1, was expressed. Secondly, although no statistical differences could be demonstrated between the baseline amounts of rest periods (defined as a 30-minute interval during which less than 10 stereotypical movements occurred) expressed by [NSB/LSB] and HSB deer mice, [NSB/LSB] deer mice consistently generated more rest periods than did animals from the HSB cohort (Figure 5-14). In the light of the apparent failed response following chronic escitalopram treatment, the behavioural data were re-analysed using this new method of assessment and scoring (see paragraph 5.3.2).

Comparing the effects of sub-chronic (day 28-35) and chronic (day 28-56) treatment with escitalopram on the manifestation of the above two new parameters, an interesting pattern became apparent. Not only did chronic, but *not sub-chronic* escitalopram significantly *increase* the amount of rest periods observed in the behaviour of HSB *but not [NSB/LSB]* animals (Figures 5-15 and 5-16; $p$ value for HSB day 28-56 = 0.0241; one-way ANOVA followed by Dunnett's test), but it also *decreased* the amount of intervals during which HSB animals generated high stereotypical behaviour, again only evident after chronic *but not sub-chronic* treatment (Figures 5-18 and 5-19; $p$ value for HSB day 28-56 = 0.0054; one-way ANOVA followed by Dunnett's test).

It is therefore proposed that data from the current study indicate that, although HSB deer mice persist in the expression of HSB behaviour even after chronic treatment with high dose escitalopram, significantly less time is spent executing HSB behaviour compared to pre-treatment values after chronic, but not sub-chronic treatment. Furthermore, significantly more periods of resting activity was evident following chronic but not sub-chronic escitalopram treatment in HSB but not [NSB/LSB] animals.

These new data have important implications for the predictive and face validity of the deer mouse model of OCD. First, chronic but not sub-chronic treatment with high dose escitalopram effectively attenuates HSB deer mouse stereotypy, as measured by the amount of time spent executing HSB behaviour. Secondly, since time spent executing obsessive-compulsive behaviour in an OCD patient is one of the diagnostic criteria of the Y-BOCS scale (refer to section 6.2) (Goodman *et al.*, 1989), the fact that effective drug treatment in the deer mouse model was able to reduce time spent expressing stereotypical behaviour is a valuable observation. It can thus be hypothesized that chronic, but not sub-chronic, treatment with high dose escitalopram contributes positively to the face validity of the model, as HSB animals spent less time executing stereotypical behaviour as a result of said treatment. Although these two parameters were only used to evaluate the behavioural effects resulting from treatment with escitalopram, these parameters would also be valuable in categorizing deer mice into HSB and [NSB/LSB] cohorts. However, this would require additional study to confirm this assumption.

Instead of only focussing on the *amount* of stereotypy generated during three predetermined time slots, it is recommended that future studies investigating stereotypical behaviour in deer mice must assess the behaviour of these animals over a longer period of time (12 hours, instead of one hour) and take into account the different behavioural effects that could manifest resulting from drug treatment.

*END OF CHAPTER 6*

# CHAPTER 7
# CONCLUSION AND FUTURE STUDY QUESTIONS

In the current study, the construct and predictive validity of the deer mouse model of OCD has been strengthened by implicating a decreased striatal serotonergic functioning in HSB animals compared to the [NSB/LSB] controls, while the model also demonstrated selective response to chronic, *but not sub-chronic*, treatment with high dose escitalopram.

These results were realised following the successful development of a new classification system for the appraisal of deer mouse stereotypy that accurately discriminates between the different behavioural topographies expressed by deer mice. We propose that the comprehensive accommodation of *both* vertical and horizontal behavioural topographies in the assessment of deer mouse stereotypy contributed to the success of this study, as for the first time a distinct separation between HSB and [NSB/LSB] animals expressing vertical jumping and pattern running respectively could be established. This methodology ensured appropriate classification of deer mice, providing a stronger foundation for the neurochemical and behavioural comparison of HSB and [NSB/LSB] deer mice in this study, and indeed for future studies.

Furthermore, a new method of analyzing the behavioural data generated by deer mice has been developed in this study. Importantly, this new system of scoring stereotypy more closely represents the evaluation criteria employed by clinicians for the diagnosis of OCD. In direct contrast to previous studies that have investigated deer mouse stereotypy (Güldenpfennig *et al.*, 2011; Korff *et al.*, 2008; Korff *et al.*, 2009; Powell *et al.*, 1999; Presti *et al.*, 2004; Presti and Lewis, 2005), this study has demonstrated that "the time spent executing stereotypical behaviour", as well as "the rest-time between episodes of stereotypy", are also to be considered in the evaluation of drug treatment effects on deer mouse stereotypy. This improved system of analysis and scoring opens up a new area of validation that is now possible for the model, as briefly highlighted below.

* * *

Scrutiny of the weekly behavioural data generated by both HSB and [NSB/LSB] deer mice (examples of data sheets presented in Addendum A) highlighted a distinct pattern in the manifestation of deer mouse stereotypy, namely the chronological variation in the severity of stereotypy expressed over the course of 12 hours. Given the role of anxiety in the initiation of compulsive-like behaviour in OCD and the subsequent anxiolytic effects following the expression of compulsions (Bartz and Hollander, 2006; Nestadt *et al.*, 2001; Rasmussen *et al.*, 1994), future

studies investigating an association between the expression of deer mouse stereotypy and an associated anxiolytic-like effect would further strengthen the face validity of the model. Such a result could prove useful to distinguish deer mouse stereotypy from repetitive motor routines without a cognitive basis. The face validity of the model could further be strengthened by determining whether HSB animals demonstrate compulsive-like behaviour if challenged with situations that evoke compulsive-like repetition, such as excessive lever pressing (Joel and Avisar, 2001), marble-burying (Egashira *et al.*, 2008a), and nest-building (Greene-Schloesser *et al.*, 2011; Hoffman and Rueda Morales, 2009).

* * *

The construct validity of the model could further be addressed by assessing whether changes in SERT density occurs following chronic treatment with the SSRIs, as has been demonstrated in patients with OCD (Zitterl *et al.*, 2008). Determining these changes, together with the expression of $5HT_{1B/D}$ and $5HT_{2C}$ receptors in HSB and [NSB/LSB] animals, could prove valuable to our understanding of the mechanisms behind serotonin-directed control of repetitive behaviour. A limitation of the current study was the relatively small number of binding assays that could be performed per brain region. As saturation-binding studies involve the preparation of relatively large amounts of tissue homogenate, tissue from three animals had to be pooled to provide sufficient quantities to perform each assay. Different techniques of neurochemical assessment, such as homogenous time-resolved fluorescence (HTRF®) that enables the analysis of smaller quantities of tissue, could provide valuable data during future investigations into SERT and serotonin, dopamine, glutamate and GABA receptor density changes in HSB and [NSB/LSB] animals. Given the SERT data generated in the current study, further receptor binding studies on the aforementioned transmitter systems are urgently needed.

* * *

The demonstration of a selective response of deer mouse stereotypy to chronic, *but not sub-chronic*, escitalopram treatment has significantly bolstered the predictive validity of the model. As only 70% of patients respond favourably to chronic SSRI treatment, with some patients benefitting from augmentation with atypical antipsychotic agents (Fineberg and Craig, 2007), it would be valuable to assess whether certain animals remain refractory to SSRI treatment, and to investigate the behavioural effects of antipsychotic augmentation on such a group of animals.

* * *

In conclusion, the results from the current study contribute positively to the already strong validation status of the deer mouse model of OCD. This, and the fact that it represents a non-inducible, naturalistic animal model, confirms its status as one of the foremost animal models of OCD. As the construct and predictive validity of the model has received much attention in the current and earlier studies undertaken in our laboratory, the next phase of the model's development should focus on strengthening the face validity of the model by associating the seemingly mono-dimensional motor stereotypy expressed by deer mice with the compulsive-like behaviour of human OCD that invariably presents with a strong cognitive basis.

***--- END ---***

# REFERENCES


Abramowitz, J. S., Schwartz, S.A., Moore, K.M. & Luenzmann, K.R. (2003) Obsessive-compulsive symptoms in pregnancy and the puerperium: A review of the literature. J. Anxiety Disord., **17**, 461-478.

Abramowitz, J. S., Wheaton, M.G. & Storch, E.A. (2008) The status of hoarding as a symptom of obsessive–compulsive disorder. Behav. Res. Ther., **46**, 1026-1033.

Abramowitz, J. S., Franklin, M.E., Schwartz, S.A. & Furr, J.M. (2003) Symptom presentation and outcome of cognitive-behavioral therapy for obsessive-compulsive disorder. J. Consult. Clin. Psychol., **71**, 1049-1057.

Albelda, N., Bar-On, N. & Joel, D. (2010) The role of NMDA receptors in the signal attenuation rat model of obsessive-compulsive disorder. Psychopharmacology, **210**, 13-24.

Albelda, N. & Joel, D. (a) Current animal models of obsessive compulsive disorder: An update. Neuroscience, **In Press, Uncorrected Proof.**

Albelda, N. & Joel, D. (2012) Animal models of obsessive-compulsive disorder: Exploring pharmacology and neural substrates. Neuroscience & Biobehavioral Reviews, **36 (1)**, 47-63.

Albin, R. L., Young, A.B. & Penney, J.B. (1989) The functional anatomy of basal ganglia disorders. Trends Neurosci., **12**, 366-375.

Almeida, R. C., Felisbino, C.S., López, M.G., Rodrigues, A.L.S. & Gabilan, N.H. (2006) Evidence for the involvement of L-arginine-nitric oxide-cyclic guanosine monophosphate pathway in the antidepressant-like effect of memantine in mice. Behav. Brain Res., **168**, 318-322.

American Psychiatric Association (2000) Diagnostic and statistical manual of mental disorders. 4th edition, American Psychiatric Press, 417-423.

Apicella, P. (2002) Tonically active neurons in the primate striatum and their role in the processing of information about motivationally relevant events. Eur. J. Neurosci., **16**, 2017-2026.

Araneda, R. & Bustos, G. (1989) Modulation of dendritic release of dopamine by N-methyl-D-aspartate receptors in rat substantia nigra. J. Neurochem., **52**, 962-970.

Atmaca, M., Tezcan, E., Kuloglu, M. & Ustundag, B. (2005) Plasma nitrate values in patients with obsessive-compulsive disorder. Psychiatry Clin. Neurosci., **59**, 621-623.

Barnard, A. L., Young, A.H., Pearson, A.D.J., Geddes, J. & O'Brien, G. (2002) A systematic review of the use of atypical antipsychotics in autism. Journal of Psychopharmacology, **16**, 93-101.

Barnes, N. M. & Sharp, T. (1999) A review of central 5-HT receptors and their function. Neuropharmacology, **38**, 1083-1152.

Bartz, J. A. & Hollander, E. (2006) Is obsessive–compulsive disorder an anxiety disorder? Prog. Neuro-Psychopharmacol. Biol. Psychiatry, **30**, 338-352.

Baxter Jr., L. R., Schwartz, J.M., Bergman, K.S., Szuba, M.P., Guze, B.H., Mazziotta, J.C., Alazraki, A., Selin, C.E., Ferng, H.-., Munford, P. & Phelps, M.E. (1992) Caudate glucose metabolic rate changes with both drug and behavior therapy for obsessive-compulsive disorder. Arch. Gen. Psychiatry, **49**, 681-689.

Behl, A., Swami, G., Sircar, S.S., Bhatia, M.S. & Banerjee, B.D. (2010) Relationship of possible stress-related biochemical markers to oxidative/antioxidative status in obsessive-compulsive disorder. Neuropsychobiology, **61**, 210-214.

Beiser, D. G., Hua, S.E. & Houk, J.C. (1997) Network models of the basal ganglia. Curr. Opin. Neurobiol., **7**, 185-190.

Bejerot, S. & Bodlund, O. (1998) Response to high doses of citalopram in treatment-resistant obsessive- compulsive disorder. Acta Psychiatr. Scand., **98**, 423-424.

Bengel, D., Greenberg, B.D., Corá-Locatelli, G., Altemus, M., Heils, A., Li, Q. & Murphy, D.L. (1999) Association of the serotonin transporter promoter regulatory region polymorphism and obsessive-compulsive disorder. Mol. Psychiatry, **4**, 463-466.

Bergqvist, P. B. F., Bouchard, C. & Blier, P. (1999) Effect of long-term administration of antidepressant treatments on serotonin release in brain regions involved in obsessive-compulsive disorder. Biol. Psychiatry, **45**, 164-174.

Berridge, K. C., Aldridge, J.W., Houchard, K.R. & Zhuang, X. (2005) Sequential super-stereotypy of an instinctive fixed action pattern in hyper-dopaminergic mutant mice: A model of obsessive compulsive disorder and tourette's. BMC Biology, **3**.

Biggs, C. S., Fowler, L.J., Whitton, P.S. & Starr, M.S. (1997) Extracellular levels of glutamate and aspartate in the entopeduncular nucleus of the rat determined by microdialysis: Regulation by striatal dopamine D2 receptors via the indirect striatal output pathway? Brain Res., **753**, 163-175.

Blier, P., EI Mansari, M., Ducharme, V. & Bouchard, C. (1996) Role of the terminal 5-HT autoreceptor in the orbitofrontal cortex in the mechanism of action of 5-HT reuptake inhibitors in obsessive compulsive disorder. European Neuropsychopharmacology, **6**, 91-91.

Bloch, M. H., Landeros-Weisenberger, A., Sen, S., Dombrowski, P., Kelmendi, B., Coric, V., Pittenger, C. & Leckman, J.F. (2008) Association of the serotonin transporter polymorphism and obsessive-compulsive disorder: Systematic review. American Journal of Medical Genetics, Part B: Neuropsychiatric Genetics, **147**, 850-858.

Bogacz, R. & Larsen, T. (2011) Integration of reinforcement learning and optimal decision-making theories of the basal ganglia. Neural Comput., **23**, 817-851.

Bolton, J., Moore, G.J., MacMillan, S., Stewart, C.M. & Rosenberg, D.R. (2001) Case study: Caudate glutamatergic changes with paroxetine persist after medication discontinuation in pediatric OCD. J. Am. Acad. Child Adolesc. Psychiatry, **40**, 903-906.

Boshuisen, M. L. & Den Boer, J.A. (2000) Zolmitriptan (a 5-HT(1B/1D) receptor agonist with central action) does not increase symptoms in obsessive compulsive disorder. Psychopharmacology (Berl. ), **152**, 74-79.

Bouthenet, M. -., Souil, E., Martres, M.-., Sokoloff, P., Giros, B. & Schwartz, J.-. (1991) Localization of dopamine D3 receptor mRNA in the rat brain using in situ hybridization histochemistry: Comparison with dopamine D2 receptor mRNA. Brain Res., **564**, 203-219.

Boyes, J. & Bolam, J.P. (2003) The subcellular localization of GABAB receptor subunits in the rat substantia nigra. Eur. J. Neurosci., **18**, 3279-3293.

Brambilla, F., Perna, G., Bussi, R. & Bellodi, L. (2000) Dopamine function in obsessive compulsive disorder: Cortisol response to acute apomorphine stimulation. Psychoneuroendocrinology, **25**, 301-310.

Broekkamp, C. L., Rijk, H.W., Joly-Gelouin, D. & Lloyd, K.L. (1986) Major tranquillizers can be distinguished from minor tranquillizers on the basis of effects on marble burying and swim-induced grooming in mice. Eur. J. Pharmacol., **126**, 223-229.

Brown, P., Chen, C.C., Wang, S., Kühn, A.A., Doyle, L., Yarrow, K., Nuttin, B., Stein, J. & Aziz, T. (2006) Involvement of human basal ganglia in offline feedback control of voluntary movement. Current Biology, **16**, 2129-2134.

Bullock, D., Tan, C.O. & John, Y.J. (2009) Computational perspectives on forebrain microcircuits implicated in reinforcement learning, action selection, and cognitive control. Neural Networks, **22**, 757-765.

Bullock, D., Grossberg, S. & Mannes, C. (1993) A neural network model for cursive script production. Biol. Cybern., **70**, 15-28.

Cameron, D. L. & Williams, J.T. (1993) Dopamine D1 receptors facilitate transmitter release. Nature, **366**, 344-347.

Camfield, D. A., Sarris, J. & Berk, M. (2011) Nutraceuticals in the treatment of obsessive compulsive disorder (OCD): A review of mechanistic and clinical evidence. Prog. Neuro-Psychopharmacol. Biol. Psychiatry, **35**, 887-895.

Campusano, J. M., Abarca, J., Forray, M.I., Gysling, K. & Bustos, G. (2002) Modulation of dendritic release of dopamine by metabotropic glutamate receptors in rat substantia nigra. Biochem. Pharmacol., **63**, 1343-1352.

Carey, P. D., Warwick, J., Harvey, B.H., Stein, D.J. & Seedat, S. (2004) Single photon emission computed tomography (SPECT) in obsessive-compulsive disorder before and after treatment with inositol. Metab. Brain Dis., **19**, 125-134.

Carter, A. G. & Sabatini, B.L. (2004) State-dependent calcium signaling in dendritic spines of striatal medium spiny neurons. Neuron, **44**, 483-493.

Ceglia, I., Acconcia, S., Fracasso, C., Colovic, M., Caccia, S. & Invernizzi, R.W. (2004) Effects of chronic treatment with escitalopram or citalopram on extracellular 5-HT in the prefrontal cortex of rats: Role of 5-HT1A receptors. Br. J. Pharmacol., **142**, 469-478.

Chabane, N., Millet, B., Delorme, R., Lichtermann, D., Mathieu, F., Laplanche, J.L., Roy, I., Mouren, M.C., Hankard, R., Maier, W., Launay, J.M. & Leboyer, M. (2004) Lack of evidence for association between serotonin transporter gene (5-HTTLPR) and obsessive-compulsive disorder by case control and family association study in humans. Neurosci. Lett., **363**, 154-156.

Chakrabarty, K., Bhattacharyya, S., Christopher, R. & Khanna, S. (2005) Glutamatergic dysfunction in OCD. Neuropsychopharmacology, **30**, 1735-1740.

Chakraborty, S., Dasgupta, A., Das, H.N., Singh, O.P., Mandal, A.K. & Mandal, N. (2009a) Study of oxidative stress in obsessive compulsive disorder in response to treatment with fluoxetine. Indian Journal of Clinical Biochemistry, **24**, 194-197.

Chakraborty, S., Singh, O.P., Dasgupta, A., Mandal, N. & Das, H.N. (2009b) Correlation between lipid peroxidation-induced TBARS level and disease severity in obsessive-compulsive disorder. Prog. Neuro-Psychopharmacol. Biol. Psychiatry, **33**, 363-366.

Chakravarthy, V. S., Joseph, D. & Bapi, R.S. (2010) What do the basal ganglia do? A modeling perspective. Biol. Cybern., **103**, 237-253.

Chatha, B. T., Bernard, V., Streit, P. & Bolam, J.P. (2000) Synaptic localization of ionotropic glutamate receptors in the rat substantia nigra. Neuroscience, **101**, 1037-1051.

Cheramy, A., Leviel, V. & Glowinski, J. (1981) Dendritic release of dopamine in the substantia nigra. Nature, **289**, 537-542.

Chergui, K., Bouron, A., Normand, E. & Mulle, C. (2000) Functional GluR6 kainate receptors in the striatum: Indirect downregulation of synaptic transmission. Journal of Neuroscience, **20**, 2175-2182.

Chesselet, M. -. & Delfs, J.M. (1996) Basal ganglia and movement disorders: An update. Trends Neurosci., **19**, 417-422.

Chou-Green, J. M., Holscher, T.D., Dallman, M.F. & Akana, S.F. (2003) Compulsive behavior in the 5-HT2C receptor knockout mouse. Physiology and Behavior, **78**, 641-649.

Christoffersen, C. L. & Meltzer, L.T. (1995) Evidence for N-methyl-D-aspartate and AMPA subtypes of the glutamate receptor on substantia nigra dopamine neurons: Possible preferential role for N-methyl-D-aspartate receptors. Neuroscience, **67**, 373-381.

Coccaro, E. F. (1989) Central serotonin and impulsive aggression. British Journal of Psychiatry, **155**, 52-62.

Conn, P. J., Battaglia, G., Marino, M.J. & Nicoletti, F. (2005) Metabotropic glutamate receptors in the basal ganglia motor circuit. Nature Reviews Neuroscience, **6**, 787-798.

Cools, R., Roberts, A.C. & Robbins, T.W. (2008) Serotoninergic regulation of emotional and behavioural control processes. Trends Cogn. Sci. (Regul. Ed. ), **12**, 31-40.

Coric, V., Taskiran, S., Pittenger, C., Wasylink, S., Mathalon, D.H., Valentine, G., Saksa, J., Wu, Y.-., Gueorguieva, R., Sanacora, G., Malison, R.T. & Krystal, J.H. (2005) Riluzole augmentation in treatment-resistant obsessive-compulsive disorder: An open-label trial. Biol. Psychiatry, **58**, 424-428.

Corti, C., Aldegheri, L., Somogyi, P. & Ferraguti, F. (2002) Distribution and synaptic localisation of the metabotropic glutamate receptor 4 (mGluR4) in the rodent CNS. Neuroscience, **110**, 403-420.

Crowder, T. L. & Weiner, J.L. (2002) Functional characterization of kainate receptors in the rat nucleus accumbens core region. J. Neurophysiol., **88**, 41-48.

Darwin, C. (1965) The expression of emotion in man and animals. Chicago University Press, Chicago.

Daw, N. D., Kakade, S. & Dayan, P. (2002) Opponent interactions between serotonin and dopamine. Neural Networks, **15**, 603-616.

De La Mora, M. P., Lara-García, D., Jacobsen, K.X., Vázquez-García, M., Crespo-Ramírez, M., Flores-Gracia, C., Escamilla-Marvan, E. & Fuxe, K. (2006) Anxiolytic-like effects of the selective metabotropic glutamate receptor 5 antagonist MPEP after its intra-amygdaloid microinjection in three different non-conditioned rat models of anxiety. Eur. J. Neurosci., **23**, 2749-2759.

Deakin, J. F. W., Graeff, F.G., Rodgers, R.J., Broekkamp, C.L., Handley, S.L., McBlane, J.W., Everitt, B., Robbins, T., Shepherd, J.K., Blanchard, D.C., De Padua Carobrez, A., Blanchard, R.J., Wetzler, S., Asnis, G.M., Van Praag, H.M., Geyer, M.A. & Panksepp, J. (1991) Critique: 5-HT and mechanisms of defence. Journal of Psychopharmacology, **5**, 305-341.

Dearry, A., Gingrich, J.A., Falardeau, P., Fremeau Jr., R.T., Bates, M.D. & Caron, M.G. (1990) Molecular cloning and expression of the gene for a human D1 dopamine receptor. Nature, **347**, 72-76.

Delgado, P. L. & Moreno, F.A. (1998) Different roles for serotonin in anti-obsessional drug action and the pathophysiology of obsessive-compulsive disorder. British Journal of Psychiatry, **173**, 21-25.

DeLong, M. R. (1990) Primate models of movement disorders of basal ganglia origin. Trends Neurosci., **13**, 281-285.

den Heuvel, O. A. v., der Werf, Y.D.v., Verhoef, K.M.W., de Wit, S., Berendse, H.W., Wolters, E.C., Veltman, D.J. & Groenewegen, H.J. (2010) Frontal–striatal abnormalities underlying behaviours in the compulsive–impulsive spectrum. J. Neurol. Sci., **289**, 55-59.

Denys, D., Zohar, J. & Westenberg, H.G.M. (2004a) The role of dopamine in obsessive-compulsive disorder: Preclinical and clinical evidence. J. Clin. Psychiatry, **65**, 11-17.

Denys, D., Tenney, N., van Megen, H.J.G.M., de Geus, F. & Westenberg, H.G.M. (2004b) Axis I and II comorbidity in a large sample of patients with obsessive–compulsive disorder. J. Affect. Disord., **80**, 155-162.

Denys, D., van der Wee, N., Janssen, J., De Geus, F. & Westenberg, H.G.M. (2004c) Low level of dopaminergic D2 receptor binding in obsessive-compulsive disorder. Biol. Psychiatry, **55**, 1041-1045.

Done, C. J. G. & Sharp, T. (1994) Biochemical evidence for the regulation of central noradrenergic activity by 5-HT(1A) and 5-HT2 receptors: Microdialysis studies in the awake and anaesthetized rat. Neuropharmacology, **33**, 411-421.

du Toit, P. L., van Kradenburg, J., Niehaus, D. & Stein, D.J. (2001) Comparison of obsessive-compulsive disorder patients with and without comorbid putative obsessive-compulsive spectrum disorders using a structured clinical interview. Compr. Psychiatry, **42**, 291-300.

Duxon, M. S., Kennett, G.A., Lightowler, S., Blackburn, T.P. & Fone, K.C.F. (1997) Activation of 5-HT(2B) receptors in the medial amygdala causes anxiolysis in the social interaction test in the rat. Neuropharmacology, **36**, 601-608.

Egashira, N., Okuno, R., Matsushita, M., Abe, M., Mishima, K., Iwasaki, K., Nishimura, R., Oishi, R. & Fujiwara, M. (2008a) Aripiprazole inhibits marble-burying behavior via 5-hydroxytryptamine (5-HT)1A receptor-independent mechanisms. Eur. J. Pharmacol., **592**, 103-108.

Egashira, N., Okuno, R., Harada, S., Matsushita, M., Mishima, K., Iwasaki, K., Nishimura, R., Oishi, R. & Fujiwara, M. (2008b) Effects of glutamate-related drugs on marble-burying behavior in mice: Implications for obsessive–compulsive disorder. Eur. J. Pharmacol., **586**, 164-170.

Eilam, D., Zor, R., Szechtman, H. & Hermesh, H. (2006) Rituals, stereotypy and compulsive behavior in animals and humans. Neuroscience & Biobehavioral Reviews, **30**, 456-471.

El Mansari, M. & Blier, P. (2006) Mechanisms of action of current and potential pharmacotherapies of obsessive-compulsive disorder. Prog. Neuro-Psychopharmacol. Biol. Psychiatry, **30**, 362-373.

Eriksson, T. (2000) Antiandrogenic treatment for obsessive-compulsive disorder [4]. Am. J. Psychiatry, **157**, 483.

Ersan, S., Bakir, S., Erdal Ersan, E. & Dogan, O. (2006) Examination of free radical metabolism and antioxidant defence system elements in patients with obsessive-compulsive disorder. Prog. Neuro-Psychopharmacol. Biol. Psychiatry, **30**, 1039-1042.

Erzegovesi, S., Guglielmo, E., Siliprandi, F. & Bellodi, L. (2005) Low-dose risperidone augmentation of fluvoxamine treatment in obsessive-compulsive disorder: A double-blind, placebo-controlled study. European Neuropsychopharmacology, **15**, 69-74.

Evans, D. W., Lewis, M.D. & Iobst, E. (2004) The role of the orbitofrontal cortex in normally developing compulsive-like behaviors and obsessive–compulsive disorder. Brain Cogn., **55**, 220-234.

Evenden, J. L. (1999) Varieties of impulsivity. Psychopharmacology, **146**, 348-361.

Fernández-Guasti, A., Ulloa, R.E. & Nicolini, H. (2003) Age differences in the sensitivity to clomipramine in an animal model of obsessive-compulsive disorder. Psychopharmacology, **166**, 195-201.

Fineberg, N. A., Potenza, M.N., Chamberlain, S.R., Berlin, H.A., Menzies, L., Bechara, A., Sahakian, B.J., Robbins, T.W., Bullmore, E.T. & Hollander, E. (2010) Probing compulsive and impulsive behaviors, from animal models to endophenotypes: A narrative review. Neuropsychopharmacology, **35**, 591-604.

Fineberg, N. A., Nigam, A. & Sivakumaran, T. (2006) Pharmacologic strategies for treatment-resistant OCD: A review of the evidence. Psychiatric Annals, **36**, 464-473.

Fineberg, N. A. & Craig, K.J. (2007) Pharmacological treatment for obsessive–compulsive disorder. Psychiatry, **6**, 234-239.

Fineberg, N. A. (2004) Pharmacological treatment for obsessive-compulsive disorder. Psychiatry, **3**, 72-76.

Fletcher, P. J. & Korth, K.M. (1999) Activation of 5-HT1B receptors in the nucleus accumbens reduces amphetamine-induced enhancement of responding for conditioned reward. Psychopharmacology, **142**, 165-174.

Fletcher, P. J., Korth, K.M. & Chambers, J.W. (1999) Selective destruction of brain serotonin neurons by 5,7- dihydroxytryptamine increases responding for a conditioned reward. Psychopharmacology, **147**, 291-299.

Fletcher, P. J. (1995) Effects of combined or separate 5,7-dihydroxytryptamine lesions of the dorsal and median raphe nuclei on responding maintained by a DRL 20s schedule of food reinforcement. Brain Res., **675**, 45-54.

Fletcher, P. J., Ming, Z.-. & Higgins, G.A. (1993) Conditioned place preference induced by microinjection of 8-OH-DPAT into the dorsal or median raphe nucleus. Psychopharmacology, **113**, 31-36.

Foa, E. B. & Kozak, M.J. (1995) DSM-IV field trial: Obsessive-compulsive disorder. Am. J. Psychiatry, **152**, 90-96.

Fornal, C. A., Metzler, C.W., Gallegos, R.A., Veasey, S.C., Mccreary, A.C. & Jacobs, B.L. (1996) WAY-100635, a potent and selective 5-hydroxytryptamine1A antagonist, increases serotonergic neuronal activity in behaving cats: Comparison with (S)-WAY-100135. J. Pharmacol. Exp. Ther., **278**, 752-762.

Frade, J. G., Barbosa, R.M. & Laranjinha, J. (2009) Stimulation of NMDA and AMPA glutamate receptors elicits distinct concentration dynamics of nitric oxide in rat hippocampal slices. Hippocampus, **19**, 603-611.

Fremeau Jr., R. T., Duncan, G.E., Fornaretto, M.-., Dearry, A., Gingrich, J.A., Breese, G.R. & Caron, M.G. (1991) Localization of D1 dopamine receptor mRNA in brain supports a role in cognitive, affective, and neuroendocrine aspects of dopaminergic neurotransmission. Proc. Natl. Acad. Sci. U. S. A., **88**, 3772-3776.

Fujiyama, F., Stephenson, F.A. & Bolam, J.P. (2002) Synaptic localization of GABAA receptor subunits in the substantia nigra of the rat: Effects of quinolinic acid lesions of the striatum. Eur. J. Neurosci., **15**, 1961-1975.

Fukai, T. & Tanaka, S. (1997) A simple neural network exhibiting selective activation of neuronal ensembles: From winner-take-all to winners-share-all. Neural Comput., **9**, 77-97.

Galvan, A., Kuwajima, M. & Smith, Y. (2006) Glutamate and GABA receptors and transporters in the basal ganglia: What does their subsynaptic localization reveal about their function? Neuroscience, **143**, 351-375.

Garner, J. P. & Mason, G.J. (2002) Evidence for a relationship between cage stereotypies and behavioural disinhibition in laboratory rodents. Behav. Brain Res., **136**, 83-92.

Gerfen, C. R., Engber, T.M., Mahan, L.C., Susel, Z., Chase, T.N., Monsma Jr., F.J. & Sibley, D.R. (1990) D1 and D2 dopamine receptor-regulated gene expression of striatonigral and striatopallidal neurons. Science, **250**, 1429-1432.

Glennon, R. A. (1990) Do classical hallucinogens act as 5-HT2 agonists or antagonists? Neuropsychopharmacology, **3**, 509-517.

Goddard, A. W., Shekhar, A., Whiteman, A.F. & McDougle, C.J. (2008) Serotoninergic mechanisms in the treatment of obsessive–compulsive disorder. Drug Discov. Today, **13**, 325-332.

Goodman, W. K., Price, L.H., Rasmussen, S.A., Mazure, C., Delgado, P., Heninger, G.R. & Charney, D.S. (1989) The yale-brown obsessive compulsive scale. II. validity. Arch. Gen. Psychiatry, **46**, 1012-1016.

Grados, M. A. & Riddle, M.A. (2001) Pharmacological treatment of childhood obsessive-compulsive disorder: From theory to practice. Journal of Clinical Child and Adolescent Psychology, **30**, 67-79.

Greenamyre, J. T. (1997) Dopaminergic neurons intrinsic to the primate striatum. Journal of Neuroscience, **17**, 6761-6768.

Greene-Schloesser, D. M., Van der Zee, E.A., Sheppard, D.K., Castillo, M.R., Gregg, K.A., Burrow, T., Foltz, H., Slater, M. & Bult-Ito, A. (2011) Predictive validity of a non-induced mouse model of compulsive-like behavior. Behav. Brain Res., **221**, 55-62.

Groenewegen, H. J., Van Den Heuvel, O.A., Cath, D.C., Voorn, P. & Veltman, D.J. (2003) Does an imbalance between the dorsal and ventral striatopallidal systems play a role in tourette's syndrome? A neuronal circuit approach. Brain and Development, **25**, S3-S14.

Güldenpfennig, M., Wolmarans, D.W., du Preez, J.L., Stein, D.J. & Harvey, B.H. (2011) Corticostriatal oxidative status, dopamine turnover and relation with stereotypy in the deer mouse. Physiol. Behav., **103**, 404-411.

Gunstad, J. & Phillips, K.A. (2003) Axis I comorbidity in body dysmorphic disorder. Compr. Psychiatry, **44**, 270-276.

Gyertyan, I. (1995) Analysis of the marble burying response: Marbles serve to measure digging rather than evoke burying. Behav. Pharmacol., **6**, 24-31.

Haber, S. N. & Calzavara, R. (2009) The cortico-basal ganglia integrative network: The role of the thalamus. Brain Res. Bull., **78**, 69-74.

Hadley, C., Hadley, B., Ephraim, S., Yang, M. & Lewis, M.H. (2006) Spontaneous stereotypy and environmental enrichment in deer mice (peromyscus maniculatus): Reversibility of experience. Appl. Anim. Behav. Sci., **97**, 312-322.

Hannon, J. & Hoyer, D. (2008) Molecular biology of 5-HT receptors. Behav. Brain Res., **195**, 198-213.

Harkin, A., Connor, T.J., Burns, M.P. & Kelly, J.P. (2004) Nitric oxide synthase inhibitors augment the effects of serotonin re-uptake inhibitors in the forced swimming test. European Neuropsychopharmacology, **14**, 274-281.

Harrison, A. A. & Markou, A. (2001) Serotonergic manipulations both potentiate and reduce brain stimulation reward in rats: Involvement of serotonin-1A receptors. J. Pharmacol. Exp. Ther., **297**, 316-325.

Harrison, A. A., Parsons, L.H., Koob, G.F. & Markou, A. (1999) RU 24969, a 5-HT(1A/1B) agonist, elevates brain stimulation reward thresholds: An effect reversed by GR 127935, a 5-HT(1B/1D) antagonist. Psychopharmacology, **141**, 242-250.

Hartig, P. R., Hoyer, D., Humphrey, P.P.A. & Martin, G.R. (1996) Alignment of receptor nomenclature with the human genome: Classification of 5-HT1B and 5-HT1D receptor subtypes. Trends Pharmacol. Sci., **17**, 103-105.

Harvey, B. H., Brand, L., Jeeva, Z. & Stein, D.J. (2006) Cortical/hippocampal monoamines, HPA-axis changes and aversive behavior following stress and restress in an animal model of post-traumatic stress disorder. Physiology and Behavior, **87**, 881-890.

Harvey, B. H., Naciti, C., Brand, L. & Stein, D.J. (2004) Serotonin and stress: Protective or malevolent actions in the biobehavioral response to repeated trauma? Annals of the New York Academy of Sciences, **1032**, 267-272.

Harvey, B. H., Naciti, C., Brand, L. & Stein, D.J. (2003) Endocrine, cognitive and hippocampal/cortical 5HT1A/2A receptor changes evoked by a time-dependent sensitisation (TDS) stress model in rats. Brain Res., **983**, 97-107.

Harvey, B. H., Brink, C.B., Seedat, S. & Stein, D.J. (2002) Defining the neuromolecular action of myo-inositol: Application to obsessive-compulsive disorder. Prog. Neuro-Psychopharmacol. Biol. Psychiatry, **26**, 21-32.

Harvey, B. H., Scheepers, A., Brand, L. & Stein, D.J. (2001) Chronic inositol increases striatal D2 receptors but does not modify dexamphetamine-induced motor behavior - relevance to obsessive-compulsive disorder. Pharmacology Biochemistry and Behavior, **68**, 245-253.

Hauber, W. & Lutz, S. (1999) Dopamine D1 or D2 receptor blockade in the globus pallidus produces akinesia in the rat. Behav. Brain Res., **106**, 143-150.

Hayes, D. J. & Greenshaw, A.J. (2011) 5-HT receptors and reward-related behaviour: A review. Neurosci. Biobehav. Rev., **35**, 1419-1449.

Hayes, G., Biden, T.J., Selbie, L.A. & Shine, J. (1992) Structural subtypes of the dopamine D2 receptor are functionally distinct: Expression of the cloned D2(A) and D2(B) subtypes in a heterologous cell line. Molecular Endocrinology, **6**, 920-926.

Hedlund, P. B. & Sutcliffe, J.G. (2007) The 5-HT7 receptor influences stereotypic behavior in a model of obsessive-compulsive disorder. Neurosci. Lett., **414**, 247-251.

Heisler, L. K., Chu, H.-., Brennan, T.J., Danao, J.A., Bajwa, P., Parsons, L.H. & Tecott, L.H. (1998) Elevated anxiety and antidepressant-like responses in serotonin 5-HT1A receptor mutant mice. Proc. Natl. Acad. Sci. U. S. A., **95**, 15049-15054.

Hemmings, S. M. J. & Stein, D.J. (2006) The current status of association studies in obsessive-compulsive disorder. Psychiatr. Clin. North Am., **29**, 411-444.

Hesse, S., Müller, U., Lincke, T., Barthel, H., Villmann, T., Angermeyer, M.C., Sabri, O. & Stengler-Wenzke, K. (2005) Serotonin and dopamine transporter imaging in patients with obsessive-compulsive disorder. Psychiatry Research - Neuroimaging, **140**, 63-72.

Higgins, G. A. & Fletcher, P.J. (2003) Serotonin and drug reward: Focus on 5-HT2C receptors. Eur. J. Pharmacol., **480**, 151-162.

Hoffman, K. L. & Rueda Morales, R.I. (2009) Toward an understanding of the neurobiology of "just right" perceptions: Nest building in the female rabbit as a possible model for compulsive behavior and the perception of task completion. Behav. Brain Res., **204**, 182-191.

Hollander, E., Rossi, N.B., Sood, E. & Pallanti, S. (2003) Risperidone augmentation in treatment-resistant obsessive-compulsive disorder: A double-blind, placebo-controlled study. International Journal of Neuropsychopharmacology, **6**, 397-401.

Hollander, E. (1998) Treatment of obsessive-compulsive spectrum disorders with SSRIs. The British journal of psychiatry, **Supplement**, 7-12.

Hollander, E., DeCaria, C., Gully, R., Nitescu, A., Suckow, R.F., Gorman, J.M., Klein, D.F. & Liebowitz, M.R. (1991) Effects of chronic fluoxetine treatment on behavioral and neuroendocrine responses to meta-chlorophenylpiperazine in obsesive-compulsive disorder. Psychiatry Res., **36**, 1-17.

Hoyer, D., Hannon, J.P. & Martin, G.R. (2002) Molecular, pharmacological and functional diversity of 5-HT receptors. Pharmacology Biochemistry and Behavior, **71**, 533-554.

Hrabovska, A., Farar, V., Bernard, V., Duysen, E.G., Brabec, J., Lockridge, O. & Myslivecek, J. (2010) Drastic decrease in dopamine receptor levels in the striatum of acetylcholinesterase knock-out mouse. Chem. Biol. Interact., **183**, 194-201.

Husted, D. S., Shapira, N.A. & Goodman, W.K. (2006) The neurocircuitry of obsessive–compulsive disorder and disgust. Prog. Neuro-Psychopharmacol. Biol. Psychiatry, **30**, 389-399.

Ichimaru, Y., Egawa, T. & Sawa, A. (1995) 5-HT(1A)-receptor subtype mediates the effect of fluvoxamine, a selective serotonin reuptake inhibitor, on marble-burying behavior in mice. Jpn. J. Pharmacol., **68**, 65-70.

Iijima, M., Kurosu, S. & Chaki, S. (2010) Effects of agents targeting glutamatergic systems on marble-burying behavior. Neurosci. Lett., **471**, 63-65.

Jaber, M., Robinson, S.W., Missale, C. & Caron, M.G. (1996) Dopamine receptors and brain function. Neuropharmacology, **35**, 1503-1519.

Jackson, D. M. & Westlind-Danielsson, A. (1994) Dopamine receptors: Molecular biology, biochemistry and behavioural aspects. Pharmacology and Therapeutics, **64**, 291-369.

Jenike, M. A. (1993) Obsessive-compulsive disorder: Efficacy of specific treatments as assessed by controlled trials. Psychopharmacol. Bull., **29**, 487-498.

Joel, D. & Weiner, I. (2000) The connections of the dopaminergic system with the striatum in rats and primates: An analysis with respect to the functional and compartmental organization of the striatum. Neuroscience, **96**, 451-474.

Joel, D., J. Stein, D. & Schreiber, R. (2008) Animal models of Obsessive–Compulsive disorder: From bench to bedside via endophenotypes and biomarkers. In Robert A. McArthur, PhD, Franco Borsini & PhD (eds), *Animal and Translational Models for CNS Drug Discovery.* Academic Press, San Diego, pp. 133-164.

Joel, D. (2006) Current animal models of obsessive compulsive disorder: A critical review. Prog. Neuro-Psychopharmacol. Biol. Psychiatry, **30**, 374-388.

Joel, D. & Avisar, A. (2001) Excessive lever pressing following post-training signal attenuation in rats: A possible animal model of obsessive compulsive disorder? Behav. Brain Res., **123**, 77-87.

Johnson, S. W., Mercuri, N.B. & North, R.A. (1992) 5-hydroxytryptamine(1B) receptors block the GABA(B) synaptic potential in rat dopamine neurons. Journal of Neuroscience, **12**, 2000-2006.

Jones, E. A., Wang, J.Q. & McGinty, J.F. (2001) Intrastriatal GABAA receptor blockade does not alter dopamine D1/D2 receptor interactions in the intact rat striatum. Neuroscience, **102**, 381-389.

Jones, E. G. (1991) Chapter 3 the anatomy of sensory relay functions in the thalamus. In G. Holstege (ed), *Progress in Brain Research.* Elsevier, pp. 29-52.

Kapur, S. & Remington, G. (1996) Serotonin-dopamine interaction and its relevance to schizophrenia. Am. J. Psychiatry, **153**, 466-476.

Kempf, F., Brücke, C., Kühn, A.A., Schneider, G., Kupsch, A., Chen, C.C., Androulidakis, A.G., Wang, S., Vandenberghe, W., Nuttin, B., Aziz, T. & Brown, P. (2007) Modulation by dopamine of human basal ganglia involvement in feedback control of movement. Current Biology, **17**, R587-R589.

Khanna, S., John, J.P. & Lakshmi Reddy, P. (2001) Neuroendocrine and behavioral responses to mCPP in obsessive-compulsive disorder. Psychoneuroendocrinology, **26**, 209-223.

Kinnear, C. J., Niehaus, D.J.H., Moolman-Smook, J.C., Du Toit, P.L., Van Kradenberg, J., Weyers, J.B., Potgieter, A., Marais, V., Emsley, R.A., Knowles, J.A., Corfield, V.A., Brink, P.A. & Stein, D.J. (2000)

Obsessive-compulsive disorder and the promoter region polymorphism (5-HTTLPR) in the serotonin transporter gene (SLC6A4): A negative association study in the afrikaner population. International Journal of Neuropsychopharmacology, **3**, 327-331.

Kita, H., Tachibana, Y., Nambu, A. & Chiken, S. (2005) Balance of monosynaptic excitatory and disynaptic inhibitory responses of the globus pallidus induced after stimulation of the subthalamic nucleus in the monkey. Journal of Neuroscience, **25**, 8611-8619.

Klavir, O., Flash, S., Winter, C. & Joel, D. (2009) High frequency stimulation and pharmacological inactivation of the subthalamic nucleus reduces 'compulsive' lever-pressing in rats. Exp. Neurol., **215**, 101-109.

Kluge, M., Schüssler, P., Weikel, J., Dresler, M., Zuber, V., Querfurt, F., Yassouridis, A. & Steiger, A. (2006) Altered nocturnal growth hormone (GH) secretion in obsessive compulsive disorder. Psychoneuroendocrinology, **31**, 1098-1104.

Koek, W., Jackson, A. & Colpaert, F.C. (1992) Behavioral pharmacology of antagonists at 5-HT2/5-HT(1C) receptors. Neurosci. Biobehav. Rev., **16**, 95-105.

Kolevzon, A., Mathewson, K.A. & Hollander, E. (2006) Selective serotonin reuptake inhibitors in autism: A review of efficacy and tolerability. J. Clin. Psychiatry, **67**, 407-414.

Korff, S., J. Stein, D. & H. Harvey, B. (2008) Stereotypic behaviour in the deer mouse: Pharmacological validation and relevance for obsessive compulsive disorder. Prog. Neuro-Psychopharmacol. Biol. Psychiatry, **32**, 348-355.

Korff, S., Stein, D.J. & Harvey, B.H. (2009) Cortico-striatal cyclic AMP-phosphodiesterase-4 signalling and stereotypy in the deer mouse: Attenuation after chronic fluoxetine treatment. Pharmacology Biochemistry and Behavior, **92**, 514-520.

Korff, S. & Harvey, B.H. (2006) Animal models of obsessive-compulsive disorder: Rationale to understanding psychobiology and pharmacology. Psychiatr. Clin. North Am., **29**, 371-390.

Korpi, E. R., Gründer, G. & Lüddens, H. (2002) Drug interactions at GABAA receptors. Prog. Neurobiol., **67**, 113-159.

Kosinski, C. M., Standaert, D.G., Testa, C.M., Penney Jr., J.B. & Young, A.B. (1998) Expression of metabotropic glutamate receptor 1 isoforms in the substantia nigra pars compacta of the rat. Neuroscience, **86**, 783-798.

Kotter, R. & Wickens, J. (1995) Interactions of glutamate and dopamine in a computational model of the striatum. J. Comput. Neurosci., **2**, 195-214.

Kuloglu, M., Atmaca, M., Tezcan, E., Gecici, Ö., Tunckol, H. & Ustundag, B. (2002) Antioxidant enzyme activities and malondialdehyde levels in patients with obsessive-compulsive disorder. Neuropsychobiology, **46**, 27-32.

Lafleur, D. L., Pittenger, C., Kelmendi, B., Gardner, T., Wasylink, S., Malison, R.T., Sanacora, G., Krystal, J.H. & Coric, V. (2006) N-acetylcysteine augmentation in serotonin reuptake inhibitor refractory obsessive-compulsive disorder. Psychopharmacology, **184**, 254-256.

Langen, M., Durston, S., Kas, M.J.H., van Engeland, H. & Staal, W.G. (2011a) The neurobiology of repetitive behavior: ...and men. Neurosci. Biobehav. Rev., **35**, 356-365.

Langen, M., Kas, M.J.H., Staal, W.G., van Engeland, H. & Durston, S. (2011b) The neurobiology of repetitive behavior: Of mice... Neurosci. Biobehav. Rev., **35**, 345-355.

LaSalle, V. H., Cromer, K.R., Nelson, K.N., Kazuba, D., Justement, L. & Murphy, D.L. (2004) Diagnostic interview assessed neuropsychiatric disorder comorbidity in 334 individuals with obsessive-compulsive disorder. Depress. Anxiety, **19**, 163-173.

Le Boulch, N. L., Truong-Ngoc, N.A. & Gauchy, C. (1991) Role of dendritic dopamine of the substantia nigra in the modulation of nigrocollicular γ-aminobutyric acid release: In vivo studies in the rat. J. Neurochem., **57**, 1080-1083.

Le Moine, C., Normand, E. & Bloch, B. (1991) Phenotypical characterization of the rat striatal neurons expressing the D1 dopamine receptor gene. Proc. Natl. Acad. Sci. U. S. A., **88**, 4205-4209.

Linnoila, V. M. I. & Virkkunen, M. (1992) Aggression, suicidality, and serotonin. J. Clin. Psychiatry, **53**, 46-51.

Ljungberg, T., Apicella, P. & Schultz, W. (1991) Responses of monkey midbrain dopamine neurons during delayed alternation performance. Brain Res., **567**, 337-341.

Londei, T., Valentini, A.M.V. & G. Leone, V. (1998) Investigative burying by laboratory mice may involve non-functional, compulsive, behaviour. Behav. Brain Res., **94**, 249-254.

López-Giménez, J. F., Mengod, G., Palacios, J.M. & Vilaró, M.T. (1997) Selective visualization of rat brain 5-HT(2A) receptors by autoradiography with [3H]MDL 100,907. Naunyn Schmiedebergs Arch. Pharmacol., **356**, 446-454.

Lucki, I. (1992) 5-HT1 receptors and behavior. Neurosci. Biobehav. Rev., **16**, 83-93.

Maia, T. V., Cooney, R.E. & Peterson, B.S. (2008) The neural bases of obsessive - compulsive disorder in children and adults. Dev. Psychopathol., **20**, 1251-1283.

Maina, G., Albert, U. & Bogetto, F. (2001) Relapses after discontinuation of drug associated with increased resistance to treatment in obsessive-compulsive disorder. Int. Clin. Psychopharmacol., **16**, 33-38.

Makki, M. I., Behen, M., Bhatt, A., Wilson, B. & Chugani, H.T. (2008) Microstructural abnormalities of striatum and thalamus in children with tourette syndrome. Movement Disorders, **23**, 2349-2356.

Maltby, N., Tolin, D.F., Worhunsky, P., O'Keefe, T.M. & Kiehl, K.A. (2005) Dysfunctional action monitoring hyperactivates frontal-striatal circuits in obsessive-compulsive disorder: An event-related fMRI study. Neuroimage, **24**, 495-503.

Markarian, Y., Larson, M.J., Aldea, M.A., Baldwin, S.A., Good, D., Berkeljon, A., Murphy, T.K., Storch, E.A. & McKay, D. (2010) Multiple pathways to functional impairment in obsessive–compulsive disorder. Clin. Psychol. Rev., **30**, 78-88.

McDougle, C. J., Epperson, C.N., Pelton, G.H., Wasylink, S. & Price, L.H. (2000) A double-blind, placebo-controlled study of risperidone addition in serotonin reuptake inhibitor-refractory obsessive-compulsive disorder. Arch. Gen. Psychiatry, **57**, 794-801.

McHaffie, J. G., Stanford, T.R., Stein, B.E., Coizet, V. & Redgrave, P. (2005) Subcortical loops through the basal ganglia. Trends Neurosci., **28**, 401-407.

McKinney, M., Raddatz, R. (2006) Practical aspects of radioligand binding. In Enna, S. J., Williams, M. (eds), Current Protocols in Pharmacology. John Wiley & Sons, U.S.A, 1.3.1-1.3.3.

Meneses, A., Manuel-Apolinar, L., Castillo, C. & Castillo, E. (2007) Memory consolidation and amnesia modify 5-HT6 receptors expression in rat brain: An autoradiographic study. Behav. Brain Res., **178**, 53-61.

Millan, M. J., Dekeyne, A. & Gobert, A. (1998) Serotonin (5-HT)(2C) receptors tonically inhibit dopamine (DA) and noradrenaline (NA), but not 5-HT, release in the frontal cortex in vivo. Neuropharmacology, **37**, 953-55.

Millan, M. J., Bervoets, K. & Colpaert, F.C. (1991) 5-hydroxytryptamine (5-HT)(1A) receptors and the tail-flick response. I. 8-hydroxy-2-(di-n-propylamino) tetralin HBr-induced spontaneous tail-flicks in the rat as an in vivo model of 5-HT(1A) receptor-mediated activity. J. Pharmacol. Exp. Ther., **256**, 973-982.

Mirenowicz, J. & Schultz, W. (1994) Importance of unpredictability for reward responses in primate dopamine neurons. J. Neurophysiol., **72**, 1024-1027.

Monsma Jr., F. J., Mahan, L.C., McVittie, L.D., Gerfen, C.R. & Sibley, D.R. (1990) Molecular cloning and expression of a D1 dopamine receptor linked to adenylyl cyclase activation. Proc. Natl. Acad. Sci. U. S. A., **87**, 6723-6727.

Montmayeur, J. -., Guiramand, J. & Borrelli, E. (1993) Preferential coupling between dopamine D2 receptors and G-proteins. Molecular Endocrinology, **7**, 161-170.

Montmayeur, J. -. & Borrelli, E. (1991) Transcription mediated by a cAMP-responsive promoter element is reduced upon activation of dopamine D2 receptors. Proc. Natl. Acad. Sci. U. S. A., **88**, 3135-3139.

Moore, G. J., MacMaster, F.P., Stewart, C. & Rosenberg, D.R. (1998) Case study: Caudate glutamatergic changes with paroxetine therapy for pediatric obsessive-compulsive disorder. J. Am. Acad. Child Adolesc. Psychiatry, **37**, 663-667.

Mora, F., Segovia, G. & del Arco, A. (2008) Glutamate–dopamine–GABA interactions in the aging basal ganglia. Brain Res. Rev., **58**, 340-353.

Morris, G., Arkadir, D., Nevet, A., Vaadia, E. & Bergman, H. (2004) Coincident but distinct messages of midbrain dopamine and striatal tonically active neurons. Neuron, **43**, 133-143.

Murphy, F., Smith, K., Cowen, P., Robbins, T. & Sahakian, B. (2002) The effects of tryptophan depletion on cognitive and affective processing in healthy volunteers. Psychopharmacology (Berl. ), **163**, 42-53.

Nambu, A., Tokuno, H., Hamada, I., Kita, H., Imanishi, M., Akazawa, T., Ikeuchi, Y. & Hasegawa, N. (2000) Excitatory conical inputs to pallidal neurons via the subthalamic nucleus in the monkey. J. Neurophysiol., **84**, 289-300.

Nambu, A. (2008) Seven problems on the basal ganglia. Curr. Opin. Neurobiol., **18**, 595-604.

Nambu, A., Tokuno, H. & Takada, M. (2002) Functional significance of the cortico–subthalamo–pallidal 'hyperdirect' pathway. Neurosci. Res., **43**, 111-117.

Nel, A. & Harvey, B.H. (2003) Haloperidol-induced dyskinesia is associated with striatal NO synthase suppression: Reversal with olanzapine. Behav. Pharmacol., **14**, 251-255.

Nestadt, G., Samuels, J., Riddle, M.A., Liang, K.-., Bienvenu, O.J., Hoehn-Saric, R., Grados, M. & Cullen, B. (2001) The relationship between obsessive-compulsive disorder and anxiety and affective disorders: Results from the johns hopkins OCD family study. Psychol. Med., **31**, 481-487.

Nicoletti, F., Bockaert, J., Collingridge, G.L., Conn, P.J., Ferraguti, F., Schoepp, D.D., Wroblewski, J.T. & Pin, J.P. Metabotropic glutamate receptors: From the workbench to the bedside. Neuropharmacology, **In Press, Corrected Proof**.

Nonogaki, K., Strack, A.M., Dallman, M.F. & Tecott, L.H. (1998) Leptin-independent hyperphagia and type 2 diabetes in mice with a mutated serotonin 5-HT(2C) receptor gene. Nat. Med., **4**, 1152-1156.

Olver, J. S., O'Keefe, G., Jones, G.R., Burrows, G.D., Tochon-Danguy, H.J., Ackermann, U., Scott, A. & Norman, T.R. (2009) Dopamine D1 receptor binding in the striatum of patients with obsessive–compulsive disorder. J. Affect. Disord., **114**, 321-326.

Onder, E., Tural, U. Gokbakan, M. (2008) Does gabapentin lead to early symptom improvement in obsessive-compulsive disorder? Eur. Arch. Psychiatry Clin. Neurosci., **258**, 319-323.

Oulis, P., Masdrakis, V.G., Karapoulios, E., Karakatsanis, N.A., Kouzoupis, A.V., Konstantakopoulos, G. & Soldatos, C.R. (2009) Tiagabine augmentation to fluvoxaminerisperidone combination in the treatment of obsessive-compulsive disorder. World Journal of Biological Psychiatry, **10**, 953-955.

Owens, M. J., Knight, D.L. & Nemeroff, C.B. (2001) Second-generation SSRIs: Human monoamine transporter binding profile of escitalopram and R-fluoxetine. Biol. Psychiatry, **50**, 345-350.

Ozdemir, E., Cetinkaya, S., Ersan, S., Kucukosman, S. & Ersan, E.E. (2009) Serum selenium and plasma malondialdehyde levels and antioxidant enzyme activities in patients with obsessive-compulsive disorder. Prog. Neuro-Psychopharmacol. Biol. Psychiatry, **33**, 62-65.

Papp, M. & Willner, P. (1991) 8-OH-DPAT-induced place preference and place aversion: Effects of PCPA and dopamine antagonists. Psychopharmacology, **103**, 99-102.

Parks, C. L., Robinson, P.S., Sibille, E., Shenk, T. & Toth, M. (1998) Increased anxiety of mice lacking the serotonin1A receptor. Proc. Natl. Acad. Sci. U. S. A., **95**, 10734-10739.

Parsons, L. H., Weiss, F. & Koob, G.F. (1998) Serotonin(1B) receptor stimulation enhances cocaine reinforcement. Journal of Neuroscience, **18**, 10078-10089.

Pauwels, P. J. (1997) 5-HT(1B/D) receptor antagonists. Gen. Pharmacol., **29**, 293-303.

Penney Jr., J. B. & Young, A.B. (1986) Striatal inhomogeneities and basal ganglia function. Movement disorders : official journal of the Movement Disorder Society, **1**, 3-15.

Pitchot, W. (1996) Growth hormone response to apomorphine in obsessive-compulsive disorder. Journal of Psychiatry and Neuroscience, **21**, 343-345.

Pittenger, C., Krystal, J.H. & Coric, V. (2006) Glutamate-modulating drugs as novel pharmacotherapeutic agents in the treatment of obsessive-compulsive disorder. NeuroRX, **3**, 69-81.

Pogarell, O., Hamann, C., Pöpperl, G., Juckel, G., Choukèr, M., Zaudig, M., Riedel, M., Möller, H.-, Hegerl, U. & Tatsch, K. (2003) Elevated brain serotonin transporter availability in patients with obsessive-compulsive disorder. Biol. Psychiatry, **54**, 1406-1413.

Powell, S. B., Newman, H.A., Pendergast, J.F. & Lewis, M.H. (1999) A rodent model of spontaneous stereotypy: Initial characterization of developmental, environmental, and neurobiological factors. Physiol. Behav., **66**, 355-363.

Presti, M. F. & Lewis, M.H. (2005) Striatal opioid peptide content in an animal model of spontaneous stereotypic behavior. Behav. Brain Res., **157**, 363-368.

Presti, M. F., Watson, C.J., Kennedy, R.T., Yang, M. & Lewis, M.H. (2004) Behavior-related alterations of striatal neurochemistry in a mouse model of stereotyped movement disorder. Pharmacology Biochemistry and Behavior, **77**, 501-507.

Rapoport, J. L. (1989) The biology of obsessions and compulsions. Sci. Am., **260**, 82-89.

Rasmussen, S. A., Eisen, J.L., Jenike, McElroy, Dominguez, Pigott, Greist & Shahady (1994) The epidemiology and differential diagnosis of obsessive compulsive disorder. J. Clin. Psychiatry, **55**, 5-14.

Reimold, M., Smolka, M.N., Zimmer, A., Batra, A., Knobel, A., Solbach, C., Mundt, A., Smoltczyk, H.U., Goldman, D., Mann, K., Reischl, G., Machulla, H.-., Bares, R. & Heinz, A. (2007) Reduced availability of serotonin transporters in obsessive-compulsive disorder correlates with symptom severity - A [11C]DASB PET study. J. Neural Transm., **114**, 1603-1609.

Richfield, E. K., Penney, J.B. & Young, A.B. (1989) Anatomical and affinity state comparisons between dopamine D1 and D2 receptors in the rat central nervous system. Neuroscience, **30**, 767-777.

Ridley, R. M. (1994) The psychology of perseverative and stereotyped behaviour. Prog. Neurobiol., **44**, 221-231.

Rolls, E. T. (2004) The functions of the orbitofrontal cortex. Brain Cogn., **55**, 11-29.

Romo, R. & Schultz, W. (1990) Dopamine neurons of the monkey midbrain: Contingencies of responses to active touch during self-initiated arm movements. J. Neurophysiol., **63**, 592-606.

Rosales, M. G., Flores, G., Hernandez, S., Martinez-Fong, D. & Aceves, J. (1994) Activation of subthalamic neurons produces NMDA receptor-mediated dendritic dopamine release in substantia nigra pars reticulata: A microdialysis study in the rat. Brain Res., **645**, 335-337.

Rosenberg, D. R., Mirza, Y., Russell, A., Tang, J., Smith, J.M., Banerjee, S.P., Bhandari, R., Rose, M., Ivey, J., Boyd, C. & Moore, G.J. (2004) Reduced anterior cingulate glutamatergic concentrations in childhood OCD and major depression versus healthy controls. J. Am. Acad. Child Adolesc. Psychiatry, **43**, 1146-1153.

Rosenberg, D. R., Macmaster, F.P., Keshavan, M.S., Fitzgerald, K.D., Stewart, C.M. & Moore, G.J. (2000) Decrease in caudate glutamatergic concentrations in pediatric obsessive-compulsive disorder patients taking paroxetine. J. Am. Acad. Child Adolesc. Psychiatry, **39**, 1096-1103.

Rozin, P., Lowery, L., Imada, S. & Haidt, J. (1999) The CAD triad hypothesis: A mapping between three moral emotions (contempt, anger, disgust) and three moral codes (community, autonomy, divinity). J. Pers. Soc. Psychol., **76**, 574-586.

Rozin, P. & Fallon, A.E. (1987) A perspective on disgust. Psychol. Rev., **94**, 23-41.

Rymar, V. V., Sasseville, R., Luk, K.C. & Sadikot, A.F. (2004) Neurogenesis and stereological morphometry of calretinin-immunoreactive GABAergic interneurons of the neostriatum. J. Comp. Neurol., **469**, 325-339.

Sanchez-Alavez, M., Gallegos, R.A., Kalafut, M.A., Games, D., Henriksen, S.J. & Criado, J.R. (2001) Interaction of serotonin autoreceptor antagonists in the rat dorsal raphe nucleus: An in vitro fast cyclic voltammetry study. Neurosci. Lett., **300**, 45-48.

Sareen, J., Kirshner, A., Lander, M., Kjernisted, K.D., Eleff, M.K. & Reiss, J.P. (2004) Do antipsychotics ameliorate or exacerbate obsessive compulsive disorder symptoms?: A systematic review. J. Affect. Disord., **82**, 167-174.

Sato, H., Skelin, I. & Diksic, M. (2010) Chronic buspirone treatment decreases 5-HT1B receptor densities and the serotonin transporter but increases the density of 5-HT2A receptors in the bulbectomized rat model of depression: An autoradiographic study. Brain Res., **1345**, 28-44.

Saxena, S. & Rauch, S.L. (2000) Functional neuroimaging and the neuroanatomy of obsessive-compulsive disorder. Psychiatr. Clin. North Am., **23**, 563-586.

Schoepp, D. D., Jane, D.E. & Monn, J.A. (1999) Pharmacological agents acting at subtypes of metabotropic glutamate receptors. Neuropharmacology, **38**, 1431-1476.

Schreiber, R., Brocco, M., Audinot, V., Gobert, A., Veiga, S. & Millan, M.J. (1995) (1-(2,5-dimethoxy-4 iodophenyl)-2-aminopropane)-induced head-twitches in the rat are mediated by 5-hydroxytryptamine (5-HT) (2A) receptors: Modulation by novel 5-HT(2A/2C) antagonists, D1 antagonists and 5-HT(1A) agonists. J. Pharmacol. Exp. Ther., **273**, 101-112.

Schultz, W., Dayan, P. & Montague, P.R. (1997) A neural substrate of prediction and reward. Science, **275**, 1593-1599.

Schultz, W., Apicella, P. & Ljungberg, T. (1993) Responses of monkey dopamine neurons to reward and conditioned stimuli during successive steps of learning a delayed response task. Journal of Neuroscience, **13**, 900-913.

Schultz, W. (2002) Getting formal with dopamine and reward. Neuron, **36**, 241-263.

Selek, S., Herken, H., Bulut, M., Ceylan, M.F., Celik, H., Savas, H.A. & Erel, O. (2008) Oxidative imbalance in obsessive compulsive disorder patients: A total evaluation of oxidant-antioxidant status. Prog. Neuro-Psychopharmacol. Biol. Psychiatry, **32**, 487-491.

Shafritz, K. M., Dichter, G.S., Baranek, G.T. & Belger, A. (2008) The neural circuitry mediating shifts in behavioral response and cognitive set in autism. Biol. Psychiatry, **63**, 974-980.

Shanahan, N. A., Velez, L.P., Masten, V.L. & Dulawa, S.C. (2011) Essential role for orbitofrontal serotonin 1B receptors in obsessive-compulsive disorder-like behavior and serotonin reuptake inhibitor response in mice. Biol. Psychiatry, **In Press, Uncorrected Proof**.

Shanahan, N. A., Holick Pierz, K.A., Masten, V.L., Waeber, C., Ansorge, M., Gingrich, J.A., Geyer, M.A., Hen, R. & Dulawa, S.C. (2009) Chronic reductions in serotonin transporter function prevent 5-HT1B-induced behavioral effects in mice. Biol. Psychiatry, **65**, 401-408.

Sheldon, P. W. & Aghajanian, G.K. (1991) Excitatory responses to serotonin (5-HT) in neurons of the rat piriform cortex: Evidence for mediation by 5-HT1(C) receptors in pyramidal cells and 5-HT2 receptors in interneurons. Synapse, **9**, 208-218.

Sherrington, R., Mankoo, B., Attwood, J., Kalsi, G., Curtis, D., Buetow, K., Povey, S. & Gurling, H. (1993) Cloning of the human dopamine D5 receptor gene and identification of a highly polymorphic microsatellite for the DRD5 locus that shows tight linkage to the chromosome 4p reference marker RAF1P1. Genomics, **18**, 423-425.

Shim, G., Jung, W.H., Choi, J.-., Jung, M.H., Jang, J.H., Park, J.-., Choi, C.-., Kang, D.-. & Kwon, J.S. (2009) Reduced cortical folding of the anterior cingulate cortex in obsessive-compulsive disorder. Journal of Psychiatry and Neuroscience, **34**, 443-449.

Sieghart, W. & Sperk, G. (2002) Subunit composition, distribution and function of GABA(A) receptor subtypes. Current topics in medicinal chemistry, **2**, 795-816.

Simpson, H. B., Lombardo, I., Slifstein, M., Huang, H.Y., Hwang, D.-., Abi-Dargham, A., Liebowitz, M.R. & Laruelle, M. (2003) Serotonin transporters in obsessive-compulsive disorder: A positron emission tomography study with [11C]McN 5652. Biol. Psychiatry, **54**, 1414-1421.

Smith, A. D. & Bolam, J.P. (1990) The neural network of the basal ganglia as revealed by the study of synaptic connections of identified neurones. Trends Neurosci., **13**, 259-265.

Smith, Y., Bennett, B.D., Bolam, J.P., Parent, A. & Sadikot, A.F. (1994) Synaptic relationships between dopaminergic afferents and cortical or thalamic input in the sensorimotor territory of the striatum in monkey. J. Comp. Neurol., **344**, 1-19.

Smith, Y. & Kieval, J.Z. (2000) Anatomy of the dopamine system in the basal ganglia. Trends Neurosci., **23**, S28-S33.

Spooren, W. P. J. M., Vassout, A., Neijt, H.C., Kuhn, R., Gasparini, F., Roux, S., Porsolt, R.D. & Gentsch, C. (2000) Anxiolytic-like effects of the prototypical metabotropic glutamate receptor 5

antagonist 2-methyl-6-(phenylethynyl)pyridine in rodents. J. Pharmacol. Exp. Ther., **295**, 1267-1275.

Sprouse, J. S. & Aghajanian, G.K. (1988) Responses of hippocampal pyramidal cells to putative serotonin 5-HT(1A) and 5-HT(1B) agonists: A comparative study with dorsal raphe neurons. Neuropharmacology, **27**, 707-715.

Stein, D. J. (2002) Obsessive-compulsive disorder. Lancet, **360**, 397-405.

Stein, D. J., Westenberg, H.G.M. & Liebowitz, M.R. (2002) Social anxiety disorder and generalized anxiety disorder: Serotonergic and dopaminergic neurocircuitry. J. Clin. Psychiatry, **63**, 12-19.

Steriade, M. & Llinas, R.R. (1988) The functional states of the thalamus and the associated neuronal interplay. Physiol. Rev., **68**, 649-742.

Steward, L. J., Ge, J., Stowe, R.L., Brown, D.C., Bruton, R.K., Stokes, P.R.A. & Barnes, N.M. (1996) Ability of 5-HT4 receptor ligands to modulate rat striatal dopamine release in vitro and in vivo. Br. J. Pharmacol., **117**, 55-62.

Stewart, S. E., Jenike, E.A., Hezel, D.M., Stack, D.E., Dodman, N.H., Shuster, L. & Jenike, M.A. (2010) A single-blinded case-control study of memantine in severe obsessive-compulsive disorder. J. Clin. Psychopharmacol., **30**, 34-39.

Stocco, A., Lebiere, C. & Anderson, J.R. (2010) Conditional routing of information to the cortex: A model of the basal ganglia's role in cognitive coordination. Psychol. Rev., **117**, 541-574.

Szechtman, H., Eckert, M.J., Tse, W.S., Boersma, J.T., Bonura, C.A., McClelland, J.Z., Culver, K.E. & Eilman, D. (2001) Compulsive checking behavior of quinpirole-sensitized rats as an animal model obsessive-compulsive disorder(OCD): Form and control. BMC Neuroscience, **2**.

Szechtman, H., Sulis, W. & Eilam, D. (1998) Quinpirole induces compulsive checking behavior in rats: A potential animal model of obsessive-compulsive disorder (OCD). Behav. Neurosci., **112**, 1475-1485.

Takeuchi, H., Yatsugi, S.-. & Yamaguchi, T. (2002) Effect of YM992, a novel antidepressant with selective serotonin re-uptake inhibitory and 5-HT2A receptor antagonistic activity, on a marble-burying behavior test as an obsessive-compulsive disorder model. Jpn. J. Pharmacol., **90**, 197-200.

Tepper, J. M. & Bolam, J.P. (2004) Functional diversity and specificity of neostriatal interneurons. Curr. Opin. Neurobiol., **14**, 685-692.

Tepper, J. M., Sun, B.-., Martin, L.P. & Creese, L. (1997) Functional roles of dopamine D2 and D3 autoreceptors on nigrostriatal neurons analyzed by antisense knockdown in vivo. Journal of Neuroscience, **17**, 2519-2530.

Thakkar, K. N., Polli, F.E., Joseph, R.M., Tuch, D.S., Hadjikhani, N., Barton, J.J.S. & Manoach, D.S. (2008) Response monitoring, repetitive behaviour and anterior cingulate abnormalities in autism spectrum disorders (ASD). Brain, **131**, 2464-2478.

Thomas, A., Burant, A., Bui, N., Graham, D., Yuva-Paylor, L.A. & Paylor, R. (2009) Marble burying reflects a repetitive and perseverative behavior more than novelty-induced anxiety. Psychopharmacology (Berl. ), **204**, 361-373.

Thornton, C. & Russell, J. (1997) Obsessive compulsive comorbidity in the dieting disorders. Int. J. Eat. Disord., **21**, 83-87.

To, Z. P., Bonhaus, D.W., Eglen, R.M. & Jakeman, L.B. (1995) Characterization and distribution of putative 5-ht7 receptors in guinea-pig brain. Br. J. Pharmacol., **115**, 107-116.

Tsaltas, E., Kontis, D., Chrysikakou, S., Giannou, H., Biba, A., Pallidi, S., Christodoulou, A., Maillis, A. & Rabavilas, A. (2005) Reinforced spatial alternation as an animal model of obsessive-compulsive disorder (OCD): Investigation of 5-HT2C and 5-HT1D receptor involvement in OCD pathophysiology. Biol. Psychiatry, **57**, 1176-1185.

Tynes, L. L., White, K. & Steketee, G.S. (1990) Toward a new nosology of obsessive compulsive disorder. Compr. Psychiatry, **31**, 465-480.

Ullmer, C., Engels, P., Abdel'Al, S. & Lübbert, H. (1996) Distribution of 5-HT4 receptor mRNA in the rat brain. Naunyn Schmiedebergs Arch. Pharmacol., **354**, 210-212.

Uys, J. D. K., Muller, C.J.F., Marais, L., Harvey, B.H., Stein, D.J. & Daniels, W.M.U. (2006) Early life trauma decreases glucocorticoid receptors in rat dentate gyrus upon adult re-stress: Reversal by escitalopram. Neuroscience, **137**, 619-625.

Valenti, O., Jeffrey Conn, P. & Marino, M.J. (2002) Distinct physiological roles of the gq-coupled metabotropic glutamate receptors co-expressed in the same neuronal populations. J. Cell. Physiol., **191**, 125-137.

Vallone, D., Picetti, R. & Borrelli, E. (2000) Structure and function of dopamine receptors. Neurosci. Biobehav. Rev., **24**, 125-132.

van der Staay, F. J. (2006) Animal models of behavioral dysfunctions: Basic concepts and classifications, and an evaluation strategy. Brain Res. Rev., **52**, 131-159.

Vandebroek, I. & Odberg, F.O. (1997) Effect of apomorphine on the conflict-induced jumping stereotypy in bank voles. Pharmacology Biochemistry and Behavior, **57**, 863-868.

Vaswani, M., Linda, F.K. & Ramesh, S. (2003) Role of selective serotonin reuptake inhibitors in psychiatric disorders: A comprehensive review. Prog. Neuro-Psychopharmacol. Biol. Psychiatry, **27**, 85-102.

Vythilingum, B., Cartwright, C. & Hollander, E. (2000) Pharmacotherapy of obsessive-compulsive disorder: Experience with the selective serotonin reuptake inhibitors. Int. Clin. Psychopharmacol., **15**, S7-S13.

Wang, L., Simpson, H.B. & Dulawa, S.C. (2009) Assessing the validity of current mouse genetic models of obsessive-compulsive disorder. Behav. Pharmacol., **20**, 119-133.

Waszczak, B. L. (1990) Differential effects of D1 and D2 dopamine receptor agonists on substantia nigra pars reticulata neurons. Brain Res., **513**, 125-135.

Wegener, G., Volke, V., Harvey, B.H. & Rosenberg, R. (2003) Local, but not systemic, administration of serotonergic antidepressants decreases hippocampal nitric oxide synthase activity. Brain Res., **959**, 128-134.

Wickens, J. R., Kotter, R. & Alexander, M.E. (1995) Effects of local connectivity on striatal function: Simulation and analysis of a model. Synapse, **20**, 281-298.

Wilkinson, L. O., Middlemiss, D.N. & Hutson, P.H. (1994) 5-HT(1A) receptor activation increases hippocampal acetylcholine efflux and motor activity in the guinea pig: Agonist efficacy influences functional activity in vivo. J. Pharmacol. Exp. Ther., **270**, 656-664.

Wilson, C. J. & Groves, P.M. (1981) Spontaneous firing patterns of identified spiny neurons in the rat neostriatum. Brain Res., **220**, 67-80.

Yadin, E., Friedman, E. & Bridger, W.H. (1991) Spontaneous alternation behavior: An animal model for obsessive-compulsive disorder? Pharmacology Biochemistry and Behavior, **40**, 311-315.

Yelnik, J., Francois, C., Percheron, G. & Tande, D. (1991) Morphological taxonomy of the neurons of the primate striatum. J. Comp. Neurol., **313**, 273-294.

Zitterl, W., Aigner, M., Stompe, T., Zitterl-Eglseer, K., Gutierrez-Lobos, K., Wenzel, T., Zettinig, G., Hornik, K., Pirker, W. & Thau, K. (2008) Changes in thalamus-hypothalamus serotonin transporter availability during clomipramine administration in patients with obsessive-compulsive disorder. Neuropsychopharmacology, **33**, 3126-3134.

Zomkowski, A. D. E., Engel, D., Gabilan, N.H. & Rodrigues, A.L.S. (2010) Involvement of NMDA receptors and l-arginine-nitric oxide-cyclic guanosine monophosphate pathway in the antidepressant-like effects of escitalopram in the forced swimming test. European Neuropsychopharmacology, **20**, 793-801.

# ADDENDUM A

## EXAMPLES OF THE RAW DATA GENERATED ON A WEEKLY BASIS BY FOUR DEER MICE OVER THE COURSE OF EIGHT WEEKS

The main purpose of the work presented in this addendum is to demonstrate the behavioural manifestations in deer mice that have prompted us to devise a new method of analysing and scoring deer mouse stereotypy, as described in Chapter 5. Important aspects of deer mouse stereotypy that will be addressed here can be summarized as:

a) the relative low stereotypy scores generated by pattern runners in contrast to the scores generated by animals expressing vertical stereotypy
b) the chronological variation in the expression of stereotypy during the dark cycle

Previous studies done in our laboratory using the Digiscan® Animal Activity Monitor (Güldenpfennig *et al.*, 2011; Korff *et al.*, 2008; Korff *et al.*, 2009) used the following protocol to appraise the behaviour of deer mice. To establish a baseline stereotypy score, animals were screened on a weekly basis for three weeks by assessing the behaviour of each animal for one hour during the dark cycle. The mean value of these three 1-hour screens was used to classify the animals in the different cohorts (NSB, LSB and HSB). Thereafter, treatment was initiated and the behavioural and neurochemical effects thereof assessed using a once-off final screen on the last day of treatment. During the validation of the new Fusion® Animal Activity Monitor for this study, which allows for automated screening over the course of the dark cycle, it became clear that the stereotypy scores generated by deer mice vary during the course of a 12-hour dark period. Therefore, we decided not to use a pre-determined timeslot during the night to evaluate the behaviour of deer mice, but instead to evaluate *all* the data generated by each animal on a weekly basis. The data in this addendum demonstrate these variances in the expression of stereotypy and are highlighted on the relevant sheets.

Furthermore, the fact that HSB pattern runners generate lower stereotypy values as opposed to those generated by HSB vertical jumpers necessitated the development of a new classification system. This has also been one of the objectives of the current study. Based on the classification system of Korff and colleagues (2008) an animal had to execute at least 2000 stereotypies per hour (or 1000 per 30 minutes) to be classified as HSB, irrespective of the behavioural topography the animal expressed. Data from the current study indicate that, although certain animals clearly express higher pattern running behaviour compared to animals from the [NSB/LSB] cohort, none of the pattern runners complete enough cage revolutions per 30 minutes to comply with this criterion. It was therefore necessary to reappraise pattern running as a behavioural topography in deer mice and to set new criteria for the classification of animals expressing this

behaviour. The data presented in this addendum demonstrate these quantitative differences in stereotypy clearly and are highlighted on the relevant sheets.

To meet the purposes of this addendum, the weekly data sheets of three HSB animals (animals 1 – 3) and one [NSB/LSB] animal (animal 4) are included, reflecting the weekly manifestation in stereotypy. All four animals had been participants in the *control study*. The three HSB animals have been chosen based on the type of stereotypy they expressed:

- ANIMAL 1 - Vertical jumper
- ANIMAL 2 - Pattern runner
- ANIMAL 3 - Both a vertical jumper and a pattern runner

Eight sheets (on four separate pages) are presented for each animal, generated weekly from the first until the eighth week of behavioural assessments. The first behavioural data generated for each animal on day 0 are not included. When studying the data sheets, the following data are emphasised:

- The numbers in the far-left column each represents an individual 30-minute interval during the dark cycle. Data from the light cycle have been excluded and are indicated by the horizontal grey colouring.
- The numbers in the fifth column from the right each represents the amount of vertical beam interruptions in a period of 30 minutes of which the three highest values have been highlighted in grey.
- The numbers in the far right hand column each represents the total amount of horizontal cage revolutions an animal executed during 30 minutes, of which the three highest values have been highlighted in grey.

Animals 1 – 3 can be compared according to the quantitative differences in the expression of vertical and horizontal stereotypies, while the chronological variation in the expression of stereotypy can be observed in all animals across both behavioural topographies. The relative differences between the stereotypy scores of HSB and [NSB/LSB] animals across both topographies can be observed when comparing animal 4 with animals 1 – 3.

*NOTE – the chronological variation in the expression of stereotypy during a 12-hour dark cycle can be seen in every table, irrespective of the topography highlighted.*

## ANIMAL 1 – HSB Vertical Jumper

*(The 3 highest weekly vertical activity scores of animal 1 are highlighted on the following eight sheets using a black oval.)*

BEHAVIOURAL SCREENS: WEEKS ***1*** AND ***2***

*Numbers in the first column indicate the individual 30-minute intervals during the dark cycle.*

*HSB vertical jumpers generate negligible amounts of cage revolutions compared to HSB runners such as animal 2.*

*HSB vertical jumpers express high levels of vertical stereotypy compared to animals from the NSB cohort, as demonstrated by this column in each table. The three highest 30-minute intervals during a dark cycle, are indicated by the grey colouring.*

| | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 9 | ######## | 18 | | | | | | | 174 | 42.( | | | | | | 3 | 2 | 5 |
| 10 | ######## | 18 | | | | | | | 127 | 29.1 | | | | | | 0 | 6 | 6 |
| 11 | ######## | 1800 | 1712.34 | 4359 | 3757 | 1194.4 | 303 | 605.42 | 304 | 109.54 | 177 | 602 | 63 | 488 | 87.08 | 15 | 11 | 26 |
| 12 | ######## | 1800 | 3879.44 | 8037 | 7564 | 806.78 | 303 | 993.17 | 304 | 50.89 | 177 | 473 | 262 | 2221 | 383.88 | 19 | 39 | 58 |
| 13 | ######## | 1800 | 1265.27 | 6286 | 5476 | 879.4 | 304 | 920.54 | 304 | 112.95 | 262 | 810 | 228 | 2685 | 467.32 | 8 | 8 | 16 |
| 14 | ######## | 1800 | 12.82 | 73 | 64 | 1779.14 | 33 | 20.86 | 32 | 1.31 | 5 | 9 | 0 | 0 | 0 | 0 | 0 | 0 |
| 15 | ######## | 1800 | 20.82 | 251 | 145 | 1728.38 | 110 | 71.62 | 109 | 19.28 | 26 | 106 | 0 | 0 | 0 | 0 | 0 | 0 |
| 16 | ######## | 1800 | 44.25 | 245 | 207 | 1724.27 | 100 | 75.73 | 99 | 6.65 | 23 | 38 | 0 | 0 | 0 | 0 | 0 | 0 |
| 17 | ######## | 1800 | 47.8 | 216 | 162 | 1742.43 | 74 | 56.63 | 74 | 13.71 | 19 | 54 | 0 | 0 | 0 | 0 | 0 | 0 |
| 18 | ######## | 1800 | 0 | | | | | | 9 | | 27 | 73 | 0 | 0 | 0 | 0 | 0 | 0 |
| 19 | ######## | 1800 | 72.64 | | | | | | 3 | | 93 | 353 | 0 | 0 | 0 | 0 | 1 | 1 |
| 20 | ######## | 1800 | 1122.68 | | | | | | 1 | | 159 | 510 | 172 | 1083 | 203.35 | 7 | 11 | 18 |
| 21 | ######## | 1800 | 2788.52 | | | | | | 7 | | 180 | 481 | 322 | 2918 | 565.72 | 20 | 21 | 41 |
| 22 | ######## | 1800 | 909.85 | | | | | | 2 | | 135 | 428 | 1[illegible] | 1709 | 296.08 | 4 | 5 | 9 |
| 23 | ######## | 1800 | 113.93 | | | | | | 7 | | 29 | 95 | 0 | 0 | 0 | 1 | 0 | 1 |
| 24 | ######## | 1800 | 1305.96 | | | | | | 6 | | 183 | 583 | 191 | 1889 | 341.38 | 8 | 5 | 13 |
| 25 | ######## | 1800 | 1940.79 | | | | | | 3 | | 150 | 428 | 247 | 3111 | 596.77 | 11 | 12 | 23 |
| 26 | ######## | 1800 | 413.81 | | | | | | 1 | | 78 | 268 | 63 | 507 | 79.12 | 2 | 2 | 4 |
| 27 | ######## | 1800 | 1313.61 | | | | | | 9 | | 190 | 508 | 284 | 3439 | 735.63 | 2 | 4 | 6 |
| 28 | ######## | 1800 | 938.95 | | | | | | 1 | | 243 | 668 | 219 | 4607 | 796.26 | 1 | 4 | 5 |
| 29 | ######## | 1800 | 741.47 | 6278 | 5798 | 809.6 | 264 | 990.39 | 265 | 62 | 188 | 480 | 166 | 3649 | 698.81 | 1 | 3 | 4 |
| 30 | ######## | 1800 | 710.97 | 4609 | 4197 | 1007.85 | 308 | 791.68 | 309 | 68.27 | 169 | 412 | 56 | 2077 | 390.55 | 4 | 0 | 4 |
| 31 | ######## | 1800 | 4078.83 | 5955 | 5543 | 886.42 | 280 | 913.54 | 281 | 66.7 | 146 | 412 | 21[illegible] | 2956 | 539.23 | 56 | 5 | 61 |
| 32 | ######## | 1800 | 3368.05 | 5452 | 5005 | 981.09 | 287 | 818.9 | 287 | 58.64 | 160 | 447 | 133 | 2257 | 448.96 | 27 | 5 | 32 |
| 33 | ######## | 1800 | 506.22 | 2600 | 2304 | 1337.73 | 188 | 462.27 | 187 | 55.06 | 110 | 296 | 36 | 951 | 169.43 | 2 | 4 | 6 |
| 34 | ######## | 1800 | 0 | 17 | 15 | 1794.46 | 19 | 5.54 | 18 | 0 | 2 | 2 | 0 | 0 | 0 | 0 | 0 | 0 |
| 35 | ######## | 1800 | 116.75 | 408 | 357 | 1727.51 | 42 | 72.49 | 41 | 8.14 | 20 | 51 | 2 | 6 | 1.53 | 0 | 2 | 2 |
| 36 | ######## | 1800 | 0 | 8 | 4 | 1799.13 | 5 | 0.87 | 4 | 0.31 | 1 | 4 | 0 | 0 | 0 | 0 | 0 | 0 |
| | | | | | | | | | 194.4231 | | | | | | | | | |

| | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 11 | ######## | 1800 | 447.47 | 2163 | 1635 | 1351.12 | 220 | 448.57 | 220 | 120.17 | 148 | 528 | 23 | 27 | 3.32 | 1 | 4 | 5 |
| 12 | ######## | 1800 | 1586.98 | 7215 | 6609 | 737.54 | 316 | 1061.83 | 317 | 95.69 | 213 | 606 | 258 | 2117 | 396.04 | 2 | 15 | 17 |
| 13 | ######## | 1800 | 513.5 | 3380 | 3011 | 1218.48 | 242 | 581.51 | 242 | 56.53 | 144 | 369 | 90 | 1725 | 322.8 | 2 | 3 | 5 |
| 14 | ######## | 1800 | 309.91 | 1921 | 1400 | 1402.97 | 200 | 396.59 | 200 | 114.81 | 133 | 521 | 45 | 539 | 104.2 | 0 | 4 | 4 |
| 15 | ######## | 1800 | 1087.95 | 7603 | 6780 | 550.19 | 275 | 1249.72 | 275 | 129.38 | 300 | 823 | 26[illegible] | 4341 | 811.84 | 3 | 5 | 8 |
| 16 | ######## | 1800 | 217.44 | 1782 | 1580 | 1461.58 | 173 | 338.42 | 172 | 36.77 | 82 | 202 | 38 | 1103 | 188.62 | 1 | 0 | 1 |
| 17 | ######## | 1800 | 22.21 | 177 | 149 | 1735.94 | 82 | 64.06 | 81 | 6.1 | 18 | 28 | 0 | 0 | 0 | 0 | 0 | 0 |
| 18 | ######## | 1800 | 20.06 | 123 | 106 | 1763.9 | 70 | 36.1 | 69 | 4.68 | 9 | 17 | 0 | 0 | 0 | 0 | 0 | 0 |
| 19 | ######## | 1800 | 12.73 | 110 | 87 | 1759.38 | 56 | 40.62 | 55 | 5.99 | 13 | 23 | 0 | 0 | 0 | 0 | 0 | 0 |
| 20 | ######## | 1800 | 663.79 | 3691 | 2863 | 1081.33 | 275 | 718.46 | 275 | 193.33 | 200 | 828 | 85 | 1242 | 224.58 | 3 | 5 | 8 |
| 21 | ######## | 1800 | 331.42 | 2306 | 2028 | 1365.15 | 184 | 434.68 | 184 | 48.48 | 102 | 278 | 72 | 1211 | 211.19 | 0 | 0 | 0 |
| 22 | ######## | 1800 | 5.43 | 161 | 99 | 1747.16 | 69 | 52.84 | 68 | 12.98 | 16 | 62 | 0 | 0 | 0 | 0 | 0 | 0 |
| 23 | ######## | 1800 | 1075.31 | 4420 | 4035 | 1097.11 | 275 | 702.81 | 275 | 60.95 | 146 | 385 | 137 | 1247 | 259.76 | 4 | 11 | 15 |
| 24 | ######## | 1800 | 951.13 | 6839 | 6151 | 654.68 | 317 | 1145.18 | 318 | 97.35 | 247 | 688 | 268 | 4432 | 770.5 | 3 | 4 | 7 |
| 25 | ######## | 1800 | 1160.39 | 6405 | 5936 | 840.76 | 314 | 959.21 | 315 | 77.27 | 171 | 469 | 204 | 2812 | 525.09 | 2 | 9 | 11 |
| 26 | ######## | 1800 | 356.68 | 2589 | 2236 | 1318.12 | 202 | 481.72 | 203 | 67.66 | 122 | 353 | 69 | 1081 | 198.42 | 2 | 3 | 5 |
| 27 | ######## | 1800 | 787.47 | 3857 | 3419 | 1147.25 | 234 | 652.43 | 235 | 79.92 | 137 | 438 | 128 | 1156 | 213.63 | 5 | 2 | 7 |
| 28 | ######## | 1800 | 1531.95 | 6936 | 5972 | 857.82 | 329 | 942.17 | 330 | 136.93 | 266 | 964 | 208 | 3673 | 603.69 | 10 | 5 | 15 |
| 29 | ######## | 1800 | 2331.65 | 8302 | 7583 | 670.88 | 295 | 1129.08 | 295 | 83.63 | 243 | 719 | 224 | 3702 | 562.87 | 22 | 8 | 30 |
| 30 | ######## | 1800 | 495.67 | 2233 | 1898 | 1445.29 | 217 | 354.69 | 217 | 73.09 | 94 | 335 | 37 | 64[illegible] | 95.3 | 2 | 2 | 4 |
| 31 | ######## | 1800 | 3160.57 | 7569 | 7077 | 759.39 | 316 | 1040.41 | 317 | 66.27 | 195 | 492 | 201 | 3478 | 396.99 | 19 | 14 | 33 |
| 32 | ######## | 1800 | 909.6 | 6309 | 5829 | 850.38 | 298 | 948.89 | 299 | 51.35 | 207 | 480 | 148 | 3274 | 522.8 | 2 | 5 | 7 |
| 33 | ######## | 1800 | 317.49 | 2737 | 2415 | 1296.59 | 251 | 503.41 | 250 | 55.02 | 132 | 322 | 49 | 1402 | 214.71 | 0 | 1 | 1 |
| 34 | ######## | 1800 | 32.94 | 304 | 253 | 1710.09 | 110 | 89.91 | 109 | 11.22 | 27 | 51 | 0 | 0 | 0 | 0 | 0 | 0 |
| 35 | ######## | 1800 | 12.8 | 86 | 64 | 1775.77 | 46 | 24.23 | 45 | 3.57 | 15 | 22 | 0 | 0 | 0 | 0 | 0 | 0 |
| 36 | ######## | 1800 | 0 | 27 | 24 | 1789.64 | 26 | 10.36 | 25 | 1.81 | 2 | 3 | 0 | 0 | 0 | 0 | 0 | 0 |
| | | | | | | | | | 202.25 | | | | | | | | | |

## ANIMAL 1 – HSB Vertical Jumper

BEHAVIOURAL SCREENS: WEEKS ***3*** AND ***4***

| | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 6 | ######## | 1800 | 119.86 | 956 | 682 | 1577.62 | 168 | 222.38 | 167 | 66.64 | 85 | 274 | 6 | 8 | 2.5 | 0 | 1 | 1 |
| 7 | ######## | 1800 | 384.07 | 2086 | 1704 | 1415.03 | 211 | 384.92 | 211 | 83.55 | 117 | 382 | 38 | 414 | 58.58 | 1 | 2 | 3 |
| 8 | ######## | 1800 | 1533.37 | 7274 | 6469 | 767.85 | 352 | 1032 | 352 | 143.7 | 235 | 805 | 270 | 2728 | 436.09 | 7 | 9 | 16 |
| 9 | ######## | 1800 | 1277.93 | 7550 | 6794 | 672.21 | 289 | 1127.74 | 290 | 104.17 | 254 | 756 | 28 | 4544 | 723.45 | 6 | 6 | 12 |
| 10 | ######## | 1800 | 2575.42 | 6264 | 4615 | 743.6 | 301 | 1055.65 | 302 | 248.55 | 373 | 1649 | 265 | 4355 | 646.13 | 20 | 8 | 28 |
| 11 | ######## | 1800 | 541.9 | 3497 | 3292 | 1296.24 | 164 | 503.76 | 164 | 26.04 | 82 | 205 | 26 | 2131 | 319.8 | 4 | 2 | 6 |
| 12 | ######## | 1800 | 0 | 124 | 124 | 1799.27 | 5 | 0.73 | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 13 | ######## | 1800 | 5.16 | 95 | 86 | 1788.94 | 31 | 11.06 | 30 | 2.73 | 6 | 9 | 0 | 0 | 0 | 0 | 0 | 0 |
| 14 | ######## | 1800 | 8.55 | 125 | 117 | 1781.3 | 45 | 18.7 | 44 | 3.12 | 6 | 8 | 0 | 0 | 0 | 0 | 0 | 0 |
| 15 | ######## | 1800 | 701.96 | 2861 | 2315 | 1312.1 | 229 | 487.38 | 229 | 116.67 | 131 | 546 | 69 | 663 | 110.14 | 6 | 4 | 10 |
| 16 | ######## | 1800 | 519.8 | 3402 | 3136 | 1284.84 | 211 | 515.09 | 211 | 37.26 | 122 | 266 | 122 | 1586 | 250.36 | 1 | 4 | 5 |
| 17 | ######## | 1800 | 47.64 | 294 | 242 | 1726.28 | 86 | 73.72 | 85 | 8.89 | 23 | 52 | 0 | 0 | 0 | 1 | 0 | 1 |
| 18 | ######## | 1800 | 153.8 | 1433 | 700 | 1408.13 | 267 | 391.76 | 267 | 196.12 | 153 | 733 | 5 | 34 | 4.9 | 0 | 0 | 0 |
| 19 | ######## | 1800 | 2586.64 | 7923 | 7440 | 655.17 | 328 | 1144.16 | 329 | 64.32 | 191 | 483 | 333 | 4329 | 667.96 | 11 | 20 | 31 |
| 20 | ######## | 1800 | 937.68 | 7218 | 6615 | 704.06 | 319 | 1095.05 | 320 | 82.36 | 235 | 603 | 259 | 4790 | 747.09 | 3 | 3 | 6 |
| 21 | ######## | 1800 | 50.24 | 325 | 285 | 1733.34 | 42 | 66.4 | 42 | 7.13 | 16 | 40 | 6 | 85 | 16.58 | 0 | 0 | 0 |
| 22 | ######## | 1800 | 242.9 | 962 | 749 | 1581.69 | 195 | 218.08 | 195 | 44.99 | 83 | 213 | 7 | 8 | 0.5 | 1 | 3 | 4 |
| 23 | ######## | 1800 | 2348.8 | 6629 | 5835 | 928.34 | 368 | 871.3 | 369 | 120.37 | 218 | 794 | 267 | 3010 | 565.21 | 11 | 13 | 24 |
| 24 | ######## | 1800 | 1766.92 | 5318 | 4497 | 1241.37 | 366 | 558.52 | 366 | 113.59 | 196 | 821 | 228 | 2200 | 435.88 | 11 | 10 | 21 |
| 25 | ######## | 1800 | 647.44 | 1245 | 971 | 1621.4 | 141 | 178.15 | 142 | 59.92 | 65 | 274 | 39 | 373 | 69.51 | 5 | 2 | 7 |
| 26 | ######## | 1800 | 1366.26 | 6094 | 5430 | 883.68 | 345 | 916.32 | 344 | 93.36 | 217 | 664 | 260 | 3486 | 544.49 | 7 | 9 | 16 |
| 27 | ######## | 1800 | 546.22 | 3708 | 3297 | 1090.58 | 185 | 709.42 | 184 | 62.91 | 163 | 411 | 137 | 2704 | 474.27 | 1 | 1 | 2 |
| 28 | ######## | 1800 | 1366.84 | 2496 | 2127 | 1346.76 | 218 | 453.14 | 218 | 70.16 | 122 | 369 | 76 | 1121 | 183.11 | 9 | 2 | 11 |
| 29 | ######## | 1800 | 3201.75 | 5126 | 4274 | 987.78 | 324 | 811.63 | 325 | 120.95 | 241 | 852 | 186 | 2163 | 373.51 | 22 | 15 | 37 |
| 30 | ######## | 1800 | 879.8 | 1912 | 1721 | 1532.13 | 175 | 268.71 | 174 | 33.36 | 80 | 191 | 39 | 455 | 67.75 | 6 | 1 | 7 |
| 31 | ######## | 1800 | 9.05 | 44 | 36 | 1794.48 | 6 | 5.52 | 5 | 1.19 | 3 | 8 | 0 | 0 | 0 | 0 | 0 | 0 |
| | | | | | | | | | 207.9583 | | | | | | | | | |

| | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 9 | ######## | 1800 | 51.71 | 385 | 220 | 1681.76 | 111 | 118.21 | 111 | 42.04 | 44 | 165 | 0 | 0 | 0 | 0 | 0 | 0 |
| 10 | ######## | 1800 | 200.28 | 1523 | 1013 | 1443.37 | 200 | 356.37 | 201 | 101.59 | 126 | 510 | 9 | 13 | 2.14 | 0 | 1 | 1 |
| 11 | ######## | 1800 | 1664.29 | 5587 | 4954 | 950.71 | 304 | 848.84 | 304 | 111.61 | 207 | 633 | 179 | 892 | 177.2 | 7 | 12 | 19 |
| 12 | ######## | 1800 | 627.44 | 2372 | 2232 | 1482.43 | 116 | 317.49 | 116 | 17.59 | 65 | 140 | 84 | 623 | 115.5 | 4 | 1 | 5 |
| 13 | ######## | 1800 | 12.1 | 310 | 165 | 1722.29 | 124 | 77.71 | 123 | 20.89 | 35 | 145 | 1 | 2 | 0.48 | 0 | 0 | 0 |
| 14 | ######## | 1800 | 30.75 | 233 | 166 | 1726.49 | 93 | 73.51 | 92 | 19.7 | 26 | 67 | 0 | 0 | 0 | 0 | 1 | 1 |
| 15 | ######## | 1800 | 837.35 | 4857 | 4097 | 1058.58 | 265 | 741.31 | 265 | 155.22 | 214 | 760 | 142 | 1338 | 267.71 | 5 | 0 | 5 |
| 16 | ######## | 1800 | 2189.44 | 8308 | 7635 | 653.21 | 318 | 1146.64 | 319 | 79.88 | 214 | 673 | 319 | 3467 | 640.02 | 20 | 18 | 38 |
| 17 | ######## | 1800 | 135.02 | 890 | 807 | 1665.12 | 86 | 134.8 | 86 | 15.68 | 26 | 83 | 33 | 437 | 72.2 | 1 | 1 | 2 |
| 18 | ######## | 1800 | 28.42 | 186 | 153 | 1764.92 | 66 | 35.08 | 65 | 7.19 | 14 | 33 | 0 | 0 | 0 | 0 | 1 | 1 |
| 19 | ######## | 1800 | 15.17 | 225 | 158 | 1743.7 | 80 | 56.3 | 79 | 11.87 | 19 | 67 | 0 | 0 | 0 | 0 | 0 | 0 |
| 20 | ######## | 1800 | 1490.7 | 5059 | 4268 | 1018.18 | 352 | 781.82 | 351 | 140.01 | 254 | 791 | 119 | 511 | 129.51 | 4 | 12 | 16 |
| 21 | ######## | 1800 | 1040.12 | 4602 | 4217 | 1180.28 | 204 | 619.72 | 203 | 51.18 | 145 | 385 | 177 | 1265 | 282.98 | 7 | 9 | 16 |
| 22 | ######## | 1800 | 0 | 10 | 9 | 1797.36 | 13 | 2.64 | 12 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| 23 | ######## | 1800 | 0.9 | 62 | 55 | 1777.85 | 55 | 22.15 | 54 | 2.12 | 5 | 7 | 0 | 0 | 0 | 0 | 0 | 0 |
| 24 | ######## | 1800 | 462.53 | 2946 | 2279 | 1229.67 | 273 | 570.17 | 273 | 158.12 | 177 | 667 | 61 | 638 | 193.84 | 3 | 1 | 4 |
| 25 | ######## | 1800 | 1603 | 8008 | 7451 | 692.2 | 350 | 1106.07 | 351 | 67.47 | 197 | 557 | 339 | 3467 | 665.02 | 1 | 14 | 15 |
| 26 | ######## | 1800 | 381 | 1528 | 1275 | 1505.71 | 216 | 294.21 | 216 | 51.51 | 93 | 253 | 38 | 141 | 29.09 | 2 | 2 | 4 |
| 27 | ######## | 1800 | 30.05 | 720 | 320 | 1622.83 | 141 | 176.55 | 141 | 71.91 | 80 | 400 | 1 | 1 | 0 | 0 | 0 | 0 |
| 28 | ######## | 1800 | 647.05 | 3503 | 3205 | 1278.59 | 246 | 520.57 | 247 | 41.68 | 120 | 298 | 125 | 975 | 160.95 | 5 | 3 | 8 |
| 29 | ######## | 1800 | 1264.66 | 5922 | 5399 | 931.98 | 280 | 868 | 280 | 89.16 | 167 | 523 | 212 | 2112 | 392.64 | 9 | 5 | 14 |
| 30 | ######## | 1800 | 2333.17 | 6643 | 6071 | 863.8 | 337 | 935.47 | 338 | 81.46 | 214 | 572 | 272 | 2707 | 493.45 | 17 | 11 | 28 |
| 31 | ######## | 1800 | 924.97 | 5838 | 5271 | 844.92 | 343 | 955.08 | 342 | 81.99 | 220 | 567 | 193 | 2470 | 459.41 | 3 | 1 | 4 |
| 32 | ######## | 1800 | 412.63 | 2117 | 1808 | 1400.68 | 286 | 399.32 | 285 | 61.56 | 123 | 309 | 51 | 198 | 43.73 | 3 | 2 | 5 |
| 33 | ######## | 1800 | 62.97 | 427 | 325 | 1691.7 | 118 | 108.3 | 117 | 20.79 | 41 | 102 | 2 | 6 | 2.12 | 0 | 0 | 0 |
| 34 | ######## | 1800 | 0 | 9 | 7 | 1798.28 | 13 | 1.72 | 12 | 0.17 | 1 | 2 | 0 | 0 | 0 | 0 | 0 | 0 |
| 35 | ######## | 1800 | 12.93 | 32 | 30 | 1793.26 | 20 | 6.74 | 19 | 0 | 2 | 2 | 0 | 0 | 0 | 0 | 0 | 0 |
| 36 | ######## | 1800 | 0 | 2 | 2 | 1800 | 4 | 0 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | | | | | | | | | 180.5 | | | | | | | | | |

## ANIMAL 1 – HSB Vertical Jumper

BEHAVIOURAL SCREENS: WEEKS ***5*** AND ***6***

| | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 5 | ######## | 1800 | 46.96 | 446 | 293 | 1650.05 | 160 | 149.95 | 159 | 39.35 | 53 | 153 | 0 | 0 | 0 | 0 | 0 | 0 |
| 6 | ######## | 1800 | 38.98 | 656 | 328 | 1590.11 | 185 | 209.89 | 184 | 89.29 | 86 | 328 | 3 | 3 | 0.08 | 0 | 0 | 0 |
| 7 | ######## | 1800 | 610.44 | 3309 | 2843 | 1243.05 | 282 | 556.79 | 282 | 89.34 | 147 | 466 | 117 | 552 | 91.26 | 0 | 2 | 2 |
| 8 | ######## | 1800 | 878.23 | 7417 | 6615 | 713.86 | 337 | 1085.89 | 337 | 122.01 | 267 | 802 | 324 | 3661 | 670.65 | 6 | 1 | 7 |
| 9 | ######## | 1800 | 695.5 | 5549 | 4981 | 907.91 | 360 | 892.09 | 359 | 91.49 | 196 | 568 | 269 | 3367 | 535.6 | 4 | 4 | 8 |
| 10 | ######## | 1800 | 279.24 | 2369 | 1996 | 1344.84 | 297 | 455.07 | 297 | 71.19 | 125 | 373 | 103 | 1111 | 172.41 | 3 | 1 | 4 |
| 11 | ######## | 1800 | 466.32 | 4574 | 4102 | 1069.41 | 324 | 730.59 | 323 | 79.46 | 172 | 472 | 230 | 2403 | 408.21 | 1 | 2 | 3 |
| 12 | ######## | 1800 | 13.37 | 223 | 164 | 1725.84 | 117 | 74.16 | 116 | 17.6 | 26 | 59 | 0 | 0 | 0 | 0 | 0 | 0 |
| 13 | ######## | 1800 | 301.96 | 2975 | 2232 | 1190.94 | 327 | 608.63 | 327 | 180.09 | 166 | 743 | 111 | 1209 | 194.88 | 0 | 0 | 0 |
| 14 | ######## | 1800 | 435.17 | 4251 | 3871 | 1118.71 | 275 | 681.48 | 274 | 59.14 | 125 | 380 | 263 | 2203 | 593.66 | 3 | 3 | 6 |
| 15 | ######## | 1800 | 0.9 | 71 | 50 | 1780.3 | 53 | 19.7 | 52 | 4.23 | 10 | 21 | 54 | 0 | 19.7 | 0 | 0 | 0 |
| 16 | ######## | 1800 | 490.02 | 3578 | 3072 | 1203.82 | 348 | 596.18 | 347 | 97.43 | 167 | 506 | 319 | 1064 | 669.52 | 4 | 0 | 4 |
| 17 | ######## | 1800 | 218.28 | 2221 | 1818 | 1412.28 | 238 | 387.49 | 238 | 79.93 | 105 | 403 | 217 | 642 | 469.62 | 1 | 0 | 1 |
| 18 | ######## | 1800 | 901.12 | 6145 | 5480 | 840.87 | 336 | 959.13 | 335 | 115.52 | 193 | 665 | 260 | 2204 | 1124.62 | 4 | 3 | 7 |
| 19 | ######## | 1800 | 1043.48 | 3512 | 3116 | 1296.9 | 228 | 502.57 | 228 | 65.68 | 119 | 396 | 205 | 937 | 565.37 | 7 | 8 | 15 |
| 20 | ######## | 1800 | 161.93 | 1833 | 1398 | 1418.03 | 226 | 381.97 | 225 | 96.91 | 118 | 435 | 215 | 490 | 401.69 | 0 | 1 | 1 |
| 21 | ######## | 1800 | 2449.76 | 7409 | 6640 | 917.92 | 377 | 882.08 | 376 | 107.76 | 218 | 769 | 287 | 1067 | 1105.77 | 16 | 14 | 30 |
| 22 | ######## | 1800 | 1772.46 | 3930 | 3529 | 1255.04 | 361 | 544.22 | 362 | 58.7 | 152 | 401 | 338 | 510 | 688.91 | 15 | 8 | 23 |
| 23 | ######## | 1800 | 1573.35 | 5029 | 4648 | 1106.76 | 316 | 692.42 | 316 | 55.35 | 144 | 381 | 264 | 777 | 896 | 11 | 16 | 27 |
| 24 | ######## | 1800 | 538.26 | 2882 | 2663 | 1328.08 | 371 | 470.77 | 372 | 38.91 | 104 | 219 | 335 | 339 | 546.02 | 0 | 6 | 6 |
| 25 | ######## | 1800 | 1494.91 | 5868 | 5357 | 967.79 | 364 | 832.5 | 364 | 72.06 | 209 | 511 | 262 | 564 | 990.43 | 6 | 9 | 15 |
| 26 | ######## | 1800 | 1028.47 | 4713 | 4301 | 1156.34 | 383 | 643.63 | 383 | 74.12 | 136 | 412 | 320 | 396 | 771.52 | 3 | 7 | 10 |
| 27 | ######## | 1800 | 2077.27 | 4819 | 4340 | 1134.71 | 385 | 664.94 | 385 | 68.64 | 158 | 479 | 327 | 557 | 805.52 | 6 | 17 | 23 |
| 28 | ######## | 1800 | 3868.97 | 8946 | 8293 | 821.15 | 343 | 978.17 | 344 | 77.03 | 230 | 653 | 228 | 1124 | 1223.22 | 18 | 39 | 57 |
| 29 | ######## | 1800 | 2061.23 | 4376 | 3915 | 1278.54 | 234 | 521.23 | 234 | 62.8 | 147 | 461 | 184 | 615 | 651.19 | 10 | 16 | 26 |
| 30 | ######## | 1800 | 598.04 | 3114 | 2820 | 1304.84 | 274 | 495.14 | 274 | 52.83 | 115 | 294 | 265 | 467 | 514.14 | 3 | 0 | 3 |
| | | | | | | | | | 297.9167 | | | | | | | | | |

| | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 10 | ######## | 1800 | 158.4 | 1232 | 806 | 1536.76 | 186 | 262.49 | 186 | 95.74 | 115 | 426 | 11 | 19 | 6.15 | 0 | 0 | 0 |
| 11 | ######## | 1800 | 1317.61 | 6698 | 6109 | 831.93 | 325 | 968.07 | 324 | 87.63 | 217 | 589 | 242 | 1431 | 250.02 | 0 | 10 | 10 |
| 12 | ######## | 1800 | 1121.27 | 7462 | 6892 | 842.43 | 362 | 957.54 | 362 | 79.31 | 217 | 570 | 317 | 2729 | 472.58 | 7 | 2 | 9 |
| 13 | ######## | 1800 | 797.17 | 4891 | 4408 | 1046.48 | 319 | 753.34 | 319 | 78.87 | 158 | 483 | 240 | 2335 | 331.24 | 4 | 3 | 7 |
| 14 | ######## | 1800 | 26.05 | 209 | 117 | 1750.24 | 87 | 49.76 | 86 | 10.81 | 13 | 92 | 0 | 0 | 0 | 0 | 0 | 0 |
| 15 | ######## | 1800 | 15.58 | 120 | 97 | 1756.3 | 64 | 43.7 | 63 | 4.74 | 17 | 23 | 0 | 0 | 0 | 0 | 0 | 0 |
| 16 | ######## | 1800 | 31.95 | 197 | 126 | 1728.35 | 88 | 71.65 | 87 | 16.3 | 30 | 71 | 0 | 0 | 0 | 0 | 0 | 0 |
| 17 | ######## | 1800 | 33.52 | 339 | 214 | 1713.9 | 79 | 86.1 | 78 | 18.78 | 25 | 125 | 0 | 0 | 0 | 0 | 0 | 0 |
| 18 | ######## | 1800 | 966.45 | 5580 | 4910 | 970.83 | 395 | 828.99 | 395 | 127.61 | 210 | 670 | 198 | 1341 | 233.3 | 1 | 4 | 5 |
| 19 | ######## | 1800 | 237.66 | 1861 | 1133 | 1334.87 | 275 | 464.18 | 276 | 178.11 | 187 | 728 | 18 | 84 | 14.94 | 0 | 1 | 1 |
| 20 | ######## | 1800 | 869.06 | 4921 | 4668 | 1084.61 | 236 | 714.96 | 236 | 36.74 | 113 | 253 | 223 | 1975 | 339.44 | 1 | 4 | 5 |
| 21 | ######## | 1800 | 43.9 | 324 | 217 | 1707.07 | 97 | 92.93 | 96 | 27.07 | 42 | 107 | 0 | 0 | 0 | 0 | 0 | 0 |
| 22 | ######## | 1800 | 696.15 | 2494 | 2188 | 1426.27 | 224 | 373.73 | 223 | 52.18 | 113 | 306 | 79 | 594 | 98.97 | 1 | 6 | 7 |
| 23 | ######## | 1800 | 1336.49 | 8351 | 7851 | 653.42 | 319 | 1146.1 | 320 | 76.83 | 179 | 500 | 349 | 2687 | 547.57 | 5 | 7 | 12 |
| 24 | ######## | 1800 | 272.36 | 1422 | 1273 | 1527.71 | 147 | 272.27 | 147 | 31.82 | 56 | 149 | 48 | 516 | 88.58 | 1 | 0 | 1 |
| 25 | ######## | 1800 | 384.6 | 2128 | 1684 | 1363.67 | 234 | 436.33 | 233 | 100.91 | 127 | 444 | 46 | 377 | 67.63 | 2 | 1 | 3 |
| 26 | ######## | 1800 | 1530.67 | 8598 | 8242 | 512.08 | 288 | 1287.47 | 289 | 46.52 | 153 | 356 | 428 | 3465 | 621.52 | 3 | 4 | 7 |
| 27 | ######## | 1800 | 1011.62 | 5270 | 4837 | 927.5 | 262 | 872.44 | 262 | 77.34 | 148 | 433 | 225 | 2222 | 377.95 | 1 | 4 | 5 |
| 28 | ######## | 1800 | 115.43 | 917 | 488 | 1544 | 230 | 255.64 | 230 | 106.63 | 100 | 429 | 0 | 0 | 0 | 0 | 0 | 0 |
| 29 | ######## | 1800 | 1309.09 | 6964 | 6602 | 707.84 | 315 | 1091.55 | 316 | 52.08 | 143 | 362 | 301 | 2984 | 538.46 | 3 | 4 | 7 |
| 30 | ######## | 1800 | 720.92 | 4397 | 3919 | 1041.88 | 301 | 758.8 | 301 | 67.82 | 178 | 478 | 179 | 1631 | 272.27 | 1 | 2 | 3 |
| 31 | ######## | 1800 | 804.22 | 4339 | 3882 | 1078.54 | 262 | 721.11 | 262 | 88.27 | 160 | 457 | 137 | 1300 | 243.51 | 5 | 5 | 10 |
| 32 | ######## | 1800 | 322.02 | 2029 | 1846 | 1403.06 | 203 | 396.52 | 203 | 33.11 | 84 | 183 | 54 | 630 | 122.84 | 0 | 1 | 1 |
| 33 | ######## | 1800 | 12.27 | 83 | 60 | 1772.03 | 38 | 27.97 | 37 | 8.14 | 6 | 23 | 0 | 0 | 0 | 0 | 0 | 0 |
| 34 | ######## | 1800 | 59.42 | 621 | 415 | 1632.6 | 159 | 167.4 | 158 | 41.68 | 61 | 206 | 1 | 1 | 0.5 | 0 | 0 | 0 |
| 35 | ######## | 1800 | 0 | 4 | 2 | 1799.92 | 6 | 0.08 | 5 | 0.03 | 1 | 2 | 0 | 0 | 0 | 0 | 0 | 0 |
| 36 | ######## | 1800 | 0 | 16 | 10 | 1793.29 | 13 | 6.71 | 12 | 0.36 | 4 | 6 | 0 | 0 | 0 | 0 | 0 | 0 |
| | | | | | | | | | 199.84 | | | | | | | | | |

## ANIMAL 1 – HSB Vertical Jumper

BEHAVIOURAL SCREENS: WEEKS ***7*** AND ***8***

| | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 3 | ######## | 1800 | 247.89 | 1444 | 1012 | 1468.88 | 245 | 330.72 | 245 | 93.88 | 120 | 432 | 16 | 25 | 6.82 | 1 | 0 | 1 |
| 4 | ######## | 1800 | 87.61 | 554 | 380 | 1632.18 | 108 | 167.78 | 108 | 47.96 | 57 | 174 | 3 | 4 | 1.44 | 0 | 0 | 0 |
| 5 | ######## | 1800 | 1703.36 | 7719 | 7253 | 774.48 | 358 | 1025.17 | 358 | 68.98 | 182 | 466 | 329 | 2177 | 388.18 | 15 | 5 | 20 |
| 6 | ######## | 1800 | 1548.06 | 8734 | 8209 | 623.87 | 314 | 1175.95 | 315 | 66.4 | 196 | 525 | 39 | 2957 | 527.39 | 4 | 5 | 9 |
| 7 | ######## | 1800 | 1311.87 | 4581 | 4238 | 1127.05 | 277 | 672.94 | 277 | 60.8 | 124 | 343 | 197 | 1767 | 281.38 | 10 | 10 | 20 |
| 8 | ######## | 1800 | 8.14 | 104 | 72 | 1772.91 | 49 | 27.09 | 48 | 9.17 | 11 | 32 | 0 | 0 | 0 | 0 | 0 | 0 |
| 9 | ######## | 1800 | 9.42 | 179 | 106 | 1742.28 | 92 | 57.63 | 92 | 18.55 | 26 | 73 | 0 | 0 | 0 | 0 | 0 | 0 |
| 10 | ######## | 1800 | 31.63 | 301 | 209 | 1697.98 | 121 | 101.67 | 121 | 25.6 | 37 | 92 | 0 | 0 | 0 | 0 | 0 | 0 |
| 11 | ######## | 1800 | 15.86 | 124 | 91 | 1763.28 | 90 | 36.4 | 90 | 3.79 | 15 | 33 | 0 | 0 | 0 | 0 | 0 | 0 |
| 12 | ######## | 1800 | 347.69 | 2500 | 1635 | 1274.42 | 266 | 524.92 | 266 | 193.58 | 192 | 865 | 53 | 328 | 64.04 | 0 | 0 | 0 |
| 13 | ######## | 1800 | 679.32 | 3798 | 3500 | 1223.96 | 158 | 575.73 | 158 | 37.2 | 123 | 298 | 176 | 1153 | 402.98 | 3 | 1 | 4 |
| 14 | ######## | 1800 | 31.3 | 206 | 170 | 1755.81 | 69 | 44.19 | 68 | 7.28 | 18 | 36 | 0 | 0 | 0 | 0 | 0 | 0 |
| 15 | ######## | 1800 | 261.62 | 2045 | 1595 | 1464.41 | 235 | 335.15 | 235 | 96.34 | 111 | 450 | 57 | 439 | 112.74 | 2 | 1 | 3 |
| 16 | ######## | 1800 | 367.62 | 3570 | 3120 | 1197.11 | 207 | 602.89 | 206 | 57.11 | 180 | 450 | 252 | 1122 | 342.23 | 1 | 1 | 2 |
| 17 | ######## | 1800 | 11.88 | 108 | 84 | 1773.78 | 55 | 26.23 | 54 | 6.35 | 11 | 24 | 65 | 0 | 34.4 | 0 | 0 | 0 |
| 18 | ######## | 1800 | 1932.52 | 6154 | 4736 | 789.73 | 301 | 1010.27 | 300 | 203.54 | 370 | 1418 | 270 | 1847 | 1180.57 | 12 | 14 | 26 |
| 19 | ######## | 1800 | 44.86 | 163 | 154 | 1792.62 | 18 | 7.38 | 17 | 2.05 | 4 | 9 | 46 | 0 | 33.03 | 0 | 1 | 1 |
| 20 | ######## | 1800 | 966.33 | 3575 | 3109 | 1339.02 | 211 | 460.98 | 210 | 57.53 | 137 | 466 | 107 | 725 | 333.99 | 8 | 2 | 10 |
| 21 | ######## | 1800 | 2830.68 | 5800 | 4980 | 895.7 | 349 | 904.3 | 348 | 105.84 | 253 | 820 | 203 | 2516 | 455.97 | 22 | 8 | 30 |
| 22 | ######## | 1800 | 5502.37 | 4517 | 3897 | 1033.24 | 206 | 766.31 | 207 | 72.6 | 203 | 620 | 178 | 2279 | 414.47 | 22 | 16 | 38 |
| 23 | ######## | 1800 | 579.68 | 1994 | 1759 | 1485.77 | 151 | 313.86 | 152 | 29.35 | 98 | 235 | 71 | 761 | 122.15 | 3 | 4 | 7 |
| 24 | ######## | 1800 | 697.46 | 4383 | 3520 | 1053.33 | 310 | 746.41 | 310 | 113.87 | 199 | 863 | 87 | 1176 | 205.07 | 1 | 6 | 7 |
| 25 | ######## | 1800 | 443.64 | 3445 | 2941 | 1254.42 | 355 | 545.58 | 354 | 98.39 | 170 | 504 | 82 | 348 | 139.7 | 1 | 3 | 4 |
| 26 | ######## | 1800 | 437.14 | 7411 | 3236 | 895.64 | 289 | 904.91 | 288 | 477.79 | 401 | 4175 | 15 | 23 | 5.44 | 1 | 1 | 2 |
| 27 | ######## | 1800 | 0 | 103 | 56 | 1768.82 | 50 | 31.18 | 49 | 12.03 | 14 | 47 | 0 | 0 | 0 | 0 | 0 | 0 |
| | | | | | | | | | 196.6522 | | | | | | | | | |

| | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 8 | ######## | 1800 | 12.26 | 296 | 163 | 1709.62 | 109 | 90.38 | 108 | 30.22 | 42 | 133 | 0 | 0 | 0 | 0 | 0 | 0 |
| 9 | ######## | 1800 | 128.62 | 1392 | 1021 | 1432.81 | 250 | 367.18 | 250 | 85.09 | 134 | 371 | 6 | 9 | 2.79 | 0 | 1 | 1 |
| 10 | ######## | 1800 | 360.34 | 1858 | 1507 | 1415.97 | 240 | 383.7 | 240 | 77.1 | 131 | 351 | 32 | 118 | 27.51 | 1 | 0 | 1 |
| 11 | ######## | 1800 | 1116.36 | 8864 | 8166 | 685.84 | 285 | 1114.16 | 284 | 95.42 | 264 | 698 | 314 | 3130 | 571.17 | 1 | 4 | 5 |
| 12 | ######## | 1800 | 986.95 | 8103 | 7152 | 621.4 | 308 | 1177.92 | 309 | 118.64 | 301 | 951 | 334 | 4402 | 751.11 | 5 | 3 | 8 |
| 13 | ######## | 1800 | 59.1 | 923 | 640 | 1610.39 | 137 | 189.56 | 137 | 59.23 | 73 | 283 | 78 | 310 | 51.22 | 0 | 0 | 0 |
| 14 | ######## | 1800 | 0 | 38 | 23 | 1792.41 | 13 | 7.6 | 12 | 4.1 | 5 | 15 | 0 | 0 | 0 | 0 | 0 | 0 |
| 15 | ######## | 1800 | 19.87 | 125 | 79 | 1763.48 | 39 | 36.52 | 38 | 12.84 | 12 | 46 | 0 | 0 | 0 | 0 | 0 | 0 |
| 16 | ######## | 1800 | 4.91 | 143 | 103 | 1772.37 | 33 | 27.63 | 32 | 11.16 | 12 | 40 | 0 | 0 | 0 | 0 | 0 | 0 |
| 17 | ######## | 1800 | 6.02 | 212 | 92 | 1730.13 | 92 | 69.37 | 92 | 42.89 | 27 | 120 | 0 | 0 | 0 | 0 | 0 | 0 |
| 18 | ######## | 1800 | 670.97 | 4508 | 3640 | 1226.41 | 317 | 572.75 | 317 | 139.1 | 222 | 868 | 150 | 1537 | 285.95 | 3 | 4 | 7 |
| 19 | ######## | 1800 | 3829.18 | 9440 | 8762 | 720.66 | 311 | 1078.41 | 312 | 78.84 | 227 | 678 | 308 | 3436 | 575.12 | 18 | 29 | 47 |
| 20 | ######## | 1800 | 5209.91 | 6210 | 5681 | 977.21 | 328 | 822.79 | 327 | 74.86 | 190 | 529 | 242 | 3245 | 454.6 | 28 | 51 | 79 |
| 21 | ######## | 1800 | 7.6 | 535 | 382 | 1740.28 | 96 | 59.72 | 95 | 27.36 | 42 | 153 | 0 | 0 | 0 | 0 | 0 | 0 |
| 22 | ######## | 1800 | 30.54 | 70 | 61 | 1783.53 | 20 | 16.54 | 19 | 1.31 | 4 | 9 | 0 | 0 | 0 | 0 | 0 | 0 |
| 23 | ######## | 1800 | 52.99 | 247 | 232 | 1783.53 | 12 | 16.47 | 11 | 2.87 | 9 | 15 | 0 | 0 | 0 | 0 | 0 | 0 |
| 24 | ######## | 1800 | 466.03 | 3009 | 2769 | 1392.39 | 235 | 407.61 | 234 | 27.97 | 109 | 240 | 97 | 940 | 161.72 | 6 | 0 | 6 |
| 25 | ######## | 1800 | 664.37 | 5900 | 5445 | 982.9 | 290 | 817.1 | 289 | 89.5 | 161 | 455 | 296 | 2736 | 429.97 | 5 | 3 | 8 |
| 26 | ######## | 1800 | 405.71 | 3747 | 3488 | 1303.14 | 219 | 496.61 | 219 | 33.2 | 105 | 259 | 127 | 2098 | 268.7 | 1 | 1 | 2 |
| 27 | ######## | 1800 | 481.27 | 3631 | 3344 | 1270.88 | 269 | 528.33 | 269 | 40.39 | 121 | 287 | 169 | 1523 | 206.69 | 2 | 2 | 4 |
| 28 | ######## | 1800 | 3711.06 | 8145 | 7534 | 777.01 | 341 | 1022.97 | 341 | 70.83 | 192 | 611 | 285 | 4674 | 647.31 | 12 | 16 | 28 |
| 29 | ######## | 1800 | 420.2 | 3068 | 2800 | 1346.22 | 244 | 453.71 | 244 | 45.78 | 113 | 268 | 76 | 1192 | 186.6 | 1 | 3 | 4 |
| 30 | ######## | 1800 | 271.6 | 1349 | 1221 | 1516.3 | 199 | 283.7 | 198 | 16.99 | 65 | 128 | 20 | 168 | 26.35 | 1 | 2 | 3 |
| 31 | ######## | 1800 | 49.46 | 923 | 692 | 1595.73 | 161 | 204.27 | 160 | 39.33 | 85 | 231 | 0 | 0 | 0 | 0 | 0 | 0 |
| 32 | ######## | 1800 | 0 | 11 | 11 | 1797.63 | 7 | 2.37 | 6 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | | | | | | | | | 181.9565 | | | | | | | | | |

## ANIMAL 2 – HSB Pattern Runner

*(The 3 highest weekly pattern running scores of animal 2 are highlighted on the following eight sheets using a black oval.)*

BEHAVIOURAL SCREENS: WEEKS ***1*** AND ***2***

*HSB pattern runners generate high amounts of cage revolutions compared to animals from the NSB cohort. Again the three highest 30-minute values during a 12-hour dark cycle period, are indicated by the grey colouring. These values are indicated on each of the following eight tables by a black oval.*

*HSB pattern runners generate very low vertical activity scores compared to HSB vertical jumpers, such as animal 1.*

| | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 9 | ######## | 1800 | 0 | 45 | 37 | 1780.92 | 32 | 19.08 | | | | | | | | | 0 | 0 |
| 10 | ######## | 1800 | 0 | 54 | 42 | 1783.35 | 54 | 16.65 | | | | | | | | | 0 | 0 |
| 11 | ######## | 1800 | 0 | 35 | 25 | 1789.02 | 31 | 10.98 | | | | | | | | | 0 | 0 |
| 12 | ######## | 1800 | 517.71 | 1729 | 1428 | 1356.7 | 254 | 443.3 | | | | | | | | | 0 | 4 |
| 13 | ######## | 1800 | 2710.99 | 4648 | 4262 | 1138.7 | 215 | 661.3 | | | | | | | | | 1 | 47 |
| 14 | ######## | 1800 | 0 | 92 | 52 | 1777.69 | 49 | 22.31 | | | | | | | | | 0 | 0 |
| 15 | ######## | 1800 | 7.75 | 558 | 309 | 1589.68 | 191 | 209.61 | | | | | | | | | 0 | 0 |
| 16 | ######## | 1800 | 6823.33 | 8248 | 7573 | 905.55 | 276 | 894.39 | | | | | | | | | 4 | 110 |
| 17 | ######## | 1800 | 21637.88 | 18307 | 17375 | 412.04 | 172 | 1387.56 | 173 | 72.75 | 375 | 932 | 21 | 34 | 0.75 | 235 | 52 | 267 |
| 18 | ######## | 1800 | 21447.91 | 18052 | 17186 | 420.84 | 186 | 1378.7 | 186 | 84.16 | 343 | 866 | 6 | 7 | 0.24 | 175 | 50 | 225 |
| 19 | ######## | 1800 | 19987.44 | 18093 | 17241 | 421.34 | 146 | 1378.19 | 146 | 69.11 | 351 | 852 | 9 | 11 | 0.34 | 212 | 43 | 255 |
| 20 | ######## | 1800 | 19440.74 | 20620 | 19690 | 335.76 | 155 | 1463.69 | 156 | 77.12 | 387 | 930 | 9 | 10 | 0.37 | 252 | 36 | 288 |
| 21 | ######## | 1800 | 16881.26 | 18870 | 17920 | 452.25 | 174 | 1347.66 | 174 | 83.51 | 357 | 950 | 22 | 34 | 0.95 | 226 | 7 | 233 |
| 22 | ######## | 1800 | 13879.44 | 17583 | 16862 | 552.56 | 123 | 1247.44 | 122 | 55.86 | 306 | 721 | 12 | 12 | 0.25 | 223 | 6 | 229 |
| 23 | ######## | 1800 | 2056.01 | 3903 | 3564 | 1221.03 | 247 | 578.97 | 246 | 59.28 | 148 | 339 | 0 | 0 | 0 | 32 | 1 | 33 |
| 24 | ######## | 1800 | 0.9 | 347 | | | | | | | | 229 | 0 | 0 | 0 | 0 | 0 | 0 |
| 25 | ######## | 1800 | 15912.88 | 16336 | | | | | | | | 684 | 13 | 15 | 0.56 | 220 | 16 | 236 |
| 26 | ######## | 1800 | 20648.63 | 19457 | | | | | | | | 1079 | 16 | 20 | 0.78 | 255 | 32 | 287 |
| 27 | ######## | 1800 | 20797.57 | 20727 | | | | | | | | 914 | 41 | 78 | 2.75 | 209 | 43 | 252 |
| 28 | ######## | 1800 | 15443.55 | 17530 | | | | | | | | 857 | 44 | 77 | 1.89 | 203 | 21 | 224 |
| 29 | ######## | 1800 | 17232.62 | 20461 | | | | | | | | 945 | 16 | 27 | 0.83 | 213 | 29 | 242 |
| 30 | ######## | 1800 | 18642.02 | 21170 | 20025 | 290.41 | 141 | 1508.99 | 142 | 89.51 | 461 | 1145 | 55 | 107 | 5.24 | 241 | 50 | 291 |
| 31 | ######## | 1800 | 17654.91 | 19628 | 18411 | 359.8 | 144 | 1440.05 | 145 | 100.93 | 451 | 1217 | 23 | 137 | 4.73 | 211 | 56 | 267 |
| 32 | ######## | 1800 | 13734.06 | 18434 | 17605 | 441.54 | 182 | 1357.91 | 183 | 95.47 | 336 | 829 | 77 | 182 | 7.12 | 185 | 23 | 208 |
| 33 | ######## | 1800 | 12337.54 | 19050 | 18205 | 410.7 | 179 | 1389.36 | 179 | 96.89 | 363 | 845 | 25 | 44 | 1.34 | 181 | 13 | 194 |
| 34 | ######## | 1800 | 15919.95 | 19974 | 19005 | 400.22 | 143 | 1398.9 | 144 | 86.47 | 404 | 969 | 39 | 50 | 1.76 | 204 | 27 | 231 |
| 35 | ######## | 1800 | 20686.68 | 22561 | 21651 | 173.96 | 97 | 1626.03 | 98 | 71.54 | 365 | 910 | 33 | 166 | 9.36 | 199 | 53 | 252 |
| | | | | | | | | | 164.6 | | | | | | | | | |

| | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 4 | ######## | 1800 | 1.9 | 20 | 17 | 1794.96 | 17 | 5.04 | 16 | 0.52 | 1 | 3 | 0 | 0 | 0 | 0 | 0 | 0 |
| 5 | ######## | 1800 | 0 | 17 | 11 | 1793.98 | 17 | 6.02 | 16 | 1.98 | 4 | 6 | 0 | 0 | 0 | 0 | 0 | 0 |
| 6 | ######## | 1800 | 1619.18 | 2712 | 2415 | 1400.27 | 145 | 399.73 | 144 | 56.03 | 119 | 297 | 0 | 0 | 0 | 26 | 1 | 27 |
| 7 | ######## | 1800 | 14468.15 | 16823 | 15965 | 508.71 | 136 | 1291.16 | 136 | 75.04 | 352 | 858 | 38 | 83 | 3.1 | 217 | 6 | 223 |
| 8 | ######## | 1800 | 21105.47 | 22719 | 21786 | 247.21 | 93 | 1552.64 | 94 | 77.49 | 392 | 933 | 96 | 234 | 10.12 | 360 | 4 | 364 |
| 9 | ######## | 1800 | 18723.29 | 22037 | 21048 | 241.71 | 105 | 1558.11 | 106 | 79.59 | 414 | 989 | 194 | 636 | 25.02 | 335 | 1 | 336 |
| 10 | ######## | 1800 | 20621.37 | 23722 | 22625 | 213.29 | 97 | 1586.66 | 97 | 76.04 | 459 | 1097 | 131 | 428 | 17.05 | 292 | 9 | 301 |
| 11 | ######## | 1800 | 536.11 | 1384 | 1125 | 1505.08 | 204 | 294.92 | 203 | 62.35 | 98 | 259 | 0 | 0 | 0 | 6 | 0 | 6 |
| 12 | ######## | 1800 | 73.34 | 636 | 408 | 1623.72 | 146 | 176.28 | 145 | 49.33 | 64 | 228 | 0 | 0 | 0 | 0 | 0 | 0 |
| 13 | ######## | 1800 | 1410.42 | 2616 | 2066 | 1379.98 | 202 | 419.65 | 202 | 126.12 | 143 | 550 | 6 | 15 | 0.41 | 25 | 2 | 27 |
| 14 | ######## | 1800 | 16091.89 | 18381 | 17568 | 331.7 | 124 | 1468.24 | 125 | 66.71 | 353 | 813 | 154 | 568 | 23.38 | 247 | 8 | 255 |
| 15 | ######## | 1800 | 16214.84 | 18241 | 17452 | 477.14 | 120 | 1322.84 | 120 | 61.53 | 335 | 789 | 169 | 483 | 21.03 | 197 | 31 | 228 |
| 16 | ######## | 1800 | 24.64 | 79 | 79 | 1783.11 | 36 | 16.89 | 35 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 17 | ######## | 1800 | 414.08 | 1038 | 861 | 1591.87 | 157 | 208.13 | 156 | 44.24 | 62 | 177 | 0 | 0 | 0 | 6 | 1 | 7 |
| 18 | ######## | 1800 | 17.37 | 77 | 72 | 1773.92 | 48 | 26.08 | 47 | 1.4 | 4 | 5 | 0 | 0 | 0 | 0 | 0 | 0 |
| 19 | ######## | 1800 | 2967.76 | 4905 | 4259 | 1184.78 | 309 | 615.18 | 309 | 118.67 | 193 | 646 | 18 | 52 | 1.37 | 43 | 1 | 44 |
| 20 | ######## | 1800 | 19484.73 | 23123 | 22093 | 197.76 | 82 | 1602.12 | 83 | 67.31 | 434 | 1030 | 118 | 334 | 12.03 | 313 | 8 | 321 |
| 21 | ######## | 1800 | 18101.2 | 21163 | 20284 | 278.23 | 118 | 1521.21 | 119 | 72.85 | 352 | 879 | 115 | 280 | 11.89 | 278 | 9 | 287 |
| 22 | ######## | 1800 | 14548.74 | 19616 | 18679 | 365.23 | 151 | 1434.66 | 152 | 75.56 | 400 | 937 | 54 | 125 | 5.4 | 221 | 9 | 230 |
| 23 | ######## | 1800 | 16960.46 | 22184 | 21148 | 275 | 117 | 1524.94 | 118 | 89.12 | 427 | 1036 | 85 | 217 | 6.3 | 249 | 11 | 260 |
| 24 | ######## | 1800 | 12904.91 | 16876 | 16022 | 535.83 | 152 | 1263.99 | 153 | 77.64 | 341 | 854 | 47 | 126 | 5.21 | 175 | 4 | 179 |
| 25 | ######## | 1800 | 15379.75 | 18917 | 18100 | 452.28 | 161 | 1347.53 | 161 | 64.37 | 346 | 817 | 102 | 253 | 14.57 | 221 | 9 | 230 |
| 26 | ######## | 1800 | 9013.38 | 11837 | 11077 | 754.49 | 251 | 1044.72 | 252 | 96.75 | 302 | 760 | 46 | 109 | 6.39 | 136 | 4 | 140 |
| 27 | ######## | 1800 | 14075.32 | 18132 | 17208 | 528.23 | 182 | 1271.6 | 182 | 86.17 | 380 | 924 | 110 | 305 | 10.61 | 207 | 7 | 214 |
| 28 | ######## | 1800 | 10720.15 | 15388 | 14502 | 607.43 | 214 | 1191.98 | 215 | 91.47 | 339 | 886 | 45 | 120 | 4.06 | 163 | 6 | 169 |
| 29 | ######## | 1800 | 7242.4 | 12053 | 11362 | 769.4 | 275 | 1030.21 | 276 | 84.8 | 288 | 691 | 25 | 52 | 1.77 | 118 | 2 | 120 |
| | | | | | | | | | 151.25 | | | | | | | | | |

## ANIMAL 2 – HSB Pattern Runner

BEHAVIOURAL SCREENS: WEEKS ***3*** AND ***4***

| | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 2 | ######## | 1800 | 0 | 31 | 27 | 1786.76 | 14 | 12.53 | 14 | 0.38 | 3 | 4 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3 | ######## | 1800 | 61.36 | 297 | 243 | 1718.77 | 90 | 80.18 | 91 | 14.21 | 22 | 54 | 0 | 0 | 0 | 1 | 0 | 1 |
| 4 | ######## | 1800 | 5224.87 | 7943 | 7247 | 819.83 | 216 | 980 | 217 | 120.42 | 234 | 696 | 5 | 5 | 0.05 | 89 | 1 | 90 |
| 5 | ######## | 1800 | 322.64 | 1105 | 721 | 1516.03 | 155 | 283.58 | 155 | 104.91 | 92 | 384 | 0 | 0 | 0 | 5 | 0 | 5 |
| 6 | ######## | 1800 | 8392.97 | 9950 | 8792 | 654.93 | 257 | 1144.12 | 258 | 217.72 | 338 | 1158 | 19 | 66 | 2.53 | 157 | 2 | 159 |
| 7 | ######## | 1800 | 15524.92 | 17030 | 16042 | 346.93 | 147 | 1453.06 | 147 | 103.05 | 400 | 988 | 32 | 76 | 3.36 | 286 | 5 | 291 |
| 8 | ######## | 1800 | 22295.41 | 20084 | 19081 | 209.24 | 96 | 1590.71 | 96 | 75.63 | 400 | 1003 | 237 | 706 | 53.02 | 293 | 41 | 334 |
| 9 | ######## | 1800 | 24190.48 | 19680 | 18416 | 255.59 | 107 | 1544.4 | 108 | 108.66 | 467 | 1264 | 360 | 1199 | 109.11 | 286 | 49 | 335 |
| 10 | ######## | 1800 | 29576.63 | 23894 | 22786 | 128.14 | 49 | 1671.79 | 50 | 62.42 | 451 | 1108 | 444 | 1695 | 113.62 | 265 | 114 | 379 |
| 11 | ######## | 1800 | 27630.04 | 22280 | 21028 | 159.47 | 65 | 1640.46 | 66 | 80.88 | 491 | 1252 | 419 | 1502 | 91.8 | 247 | 102 | 349 |
| 12 | ######## | 1800 | 22219.91 | 19249 | | | | | | | | 943 | 401 | 1716 | 128.44 | 224 | 80 | 304 |
| 13 | ######## | 1800 | 154.62 | 297 | | | | | | | | 17 | 0 | 0 | 0 | 2 | 0 | 2 |
| 14 | ######## | 1800 | 12435.2 | 11042 | | | | | | | | 560 | 108 | 322 | 12.5 | 156 | 21 | 177 |
| 15 | ######## | 1800 | 27287.17 | 20169 | | | | | | | | 1277 | 438 | 1789 | 146.07 | 258 | 122 | 380 |
| 16 | ######## | 1800 | 24632.19 | 19364 | | | | | | | | 1199 | 476 | 2397 | 206.95 | 196 | 133 | 329 |
| 17 | ######## | 1800 | 21839.04 | 18105 | | | | | | | | 1503 | 427 | 2051 | 146.84 | 238 | 87 | 325 |
| 18 | ######## | 1800 | 26202.24 | 20211 | | | | | | | | 1204 | 421 | 1915 | 117 | 228 | 136 | 364 |
| 19 | ######## | 1800 | 21407.35 | 17099 | | | | | | | | 1103 | 342 | 1507 | 77.71 | 182 | 110 | 292 |
| 20 | ######## | 1800 | 24795.16 | 20308 | | | | | | | | 1425 | 454 | 2051 | 126.98 | 202 | 121 | 323 |
| 21 | ######## | 1800 | 22226.22 | 20384 | | | | | | | | 1391 | 401 | 1880 | 111.41 | 231 | 80 | 311 |
| 22 | ######## | 1800 | 20840.89 | 18359 | | | | | | | | 1221 | 360 | 1656 | 102.19 | 226 | 74 | 300 |
| 23 | ######## | 1800 | 19402.13 | 17316 | | | | | | | | 1377 | 246 | 797 | 48.51 | 196 | 49 | 245 |
| 24 | ######## | 1800 | 10483.41 | 12930 | | | | | | | | 714 | 46 | 97 | 3.54 | 170 | 10 | 180 |
| 25 | ######## | 1800 | 10333.81 | 13108 | 12255 | 591.8 | 225 | 1208.12 | 226 | 107.72 | 349 | 853 | 33 | 59 | 2.09 | 146 | 3 | 149 |
| | | | | | | | | | 122.0455 | | | | | | | | | |

*Note the sudden increase in the weekly amount of vertical stereotypy executed by animal 2. This animal is an example of an animal excluded from the HSB vertical jumping cohort, as the amount of vertical stereotypy it executed fluctuated dramatically, as can be seen comparing these values with those of weeks 1 and 2 above.*

| | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 5 | ######## | 1800 | 0 | 17 | 17 | 1794.31 | 21 | 5.69 | 20 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 6 | ######## | 1800 | 0 | 82 | 35 | 1770.5 | 37 | 29.5 | 36 | 14.59 | 10 | 47 | 0 | 0 | 0 | 0 | 0 | 0 |
| 7 | ######## | 1800 | 2512.24 | 3757 | 3361 | 1313.31 | 113 | 486.61 | 113 | 73.06 | 122 | 396 | 1 | 1 | 0 | 35 | 0 | 36 |
| 8 | ######## | 1800 | 18008.81 | 17603 | 16600 | 406.04 | 163 | 1393.59 | 164 | 115.57 | 370 | 1003 | 77 | 148 | 7.16 | 297 | 4 | 301 |
| 9 | ######## | 1800 | 21244.46 | 21767 | 20671 | 270.15 | 90 | 1529.81 | 91 | 94.67 | 449 | 1096 | 163 | 450 | 24.17 | 358 | 1 | 359 |
| 10 | ######## | 1800 | 21546.01 | 21916 | 20751 | 248.93 | 119 | 1550.78 | 120 | 84.85 | 481 | 1165 | 287 | 1343 | 99.74 | 352 | 6 | 358 |
| 11 | ######## | 1800 | 27847.58 | 20513 | 19443 | 330.27 | 109 | 1469.5 | 110 | 66.6 | 386 | 1070 | 305 | 1534 | 115.26 | 251 | 105 | 356 |
| 12 | ######## | 1800 | 21415.63 | 18902 | 17975 | 410.17 | 157 | 1389.71 | 158 | 72.14 | 400 | 927 | 289 | 1548 | 106.2 | 246 | 38 | 284 |
| 13 | ######## | 1800 | 20430.55 | 17023 | 16092 | 560.19 | 128 | 1238.85 | 129 | 65.97 | 369 | 931 | 210 | 973 | 56.52 | 236 | 55 | 291 |
| 14 | ######## | 1800 | 23984.46 | 19184 | 18141 | 406.86 | 127 | 1392.83 | 128 | 64.74 | 419 | 1043 | 226 | 1057 | 73.33 | 261 | 81 | 342 |
| 15 | ######## | 1800 | 21212.26 | 19956 | 18909 | 433.9 | 130 | 1366.02 | 131 | 65.03 | 405 | 1047 | 176 | 647 | 28.19 | 254 | 55 | 309 |
| 16 | ######## | 1800 | 16320.85 | 13980 | 13143 | 740.95 | 183 | 1058.99 | 183 | 93.68 | 290 | 837 | 106 | 416 | 20.8 | 153 | 26 | 179 |
| 17 | ######## | 1800 | 0 | 114 | 73 | 1778.64 | 41 | 21.36 | 40 | 13.48 | 8 | 41 | 0 | 0 | 0 | 0 | 0 | 0 |
| 18 | ######## | 1800 | 221.72 | 786 | 546 | 1622.14 | 182 | 177.84 | 182 | 70.58 | 55 | 240 | 0 | 0 | 0 | 0 | 0 | 0 |
| 19 | ######## | 1800 | 20728.31 | 24161 | 23148 | 260.36 | 98 | 1539.55 | 99 | 57.36 | 449 | 1013 | 248 | 1041 | 73.79 | 345 | 4 | 349 |
| 20 | ######## | 1800 | 27583.81 | 26966 | 25741 | 136.41 | 63 | 1663.39 | 64 | 77.4 | 487 | 1225 | 350 | 1645 | 96.61 | 268 | 89 | 357 |
| 21 | ######## | 1800 | 22283.4 | 21142 | 19841 | 371.29 | 115 | 1427.56 | 116 | 86.25 | 501 | 1301 | 276 | 1275 | 73.74 | 260 | 41 | 301 |
| 22 | ######## | 1800 | 24520.57 | 25077 | 23718 | 249.8 | 100 | 1550.12 | 100 | 93.06 | 542 | 1359 | 330 | 1721 | 90.03 | 219 | 38 | 257 |
| 23 | ######## | 1800 | 18265.66 | 20999 | 19833 | 427.46 | 155 | 1372.53 | 156 | 90.47 | 456 | 1166 | 248 | 1213 | 58.74 | 240 | 10 | 250 |
| 24 | ######## | 1800 | 20543.75 | 21126 | 19896 | 410.36 | 131 | 1389.5 | 131 | 78.41 | 466 | 1230 | 221 | 894 | 48.25 | 227 | 28 | 255 |
| 25 | ######## | 1800 | 17422.53 | 22502 | 21366 | 339.36 | 123 | 1460.62 | 123 | 80.92 | 457 | 1136 | 236 | 953 | 51.9 | 263 | 13 | 276 |
| 26 | ######## | 1800 | 17053.44 | 17975 | 16954 | 523.26 | 167 | 1276.7 | 168 | 95.16 | 401 | 1021 | 176 | 668 | 38.48 | 178 | 20 | 198 |
| 27 | ######## | 1800 | 18349.69 | 16921 | 15992 | 566.03 | 195 | 1233.77 | 195 | 80.87 | 356 | 929 | 177 | 652 | 39.81 | 153 | 32 | 185 |
| 28 | ######## | 1800 | 11675.95 | 14571 | 13739 | 657.47 | 224 | 1142.43 | 224 | 88.48 | 329 | 832 | 114 | 447 | 23.37 | 161 | 9 | 170 |
| 29 | ######## | 1800 | 10115.74 | 15384 | 14609 | 573.43 | 223 | 1226.45 | 224 | 70.34 | 334 | 775 | 87 | 258 | 9.46 | 153 | 2 | 155 |
| 30 | ######## | 1800 | 13778.07 | 18593 | 17713 | 344.67 | 140 | 1455.18 | 141 | 74.29 | 388 | 880 | 119 | 377 | 18.34 | 223 | 4 | 227 |
| | | | | | | | | | 137.0833 | | | | | | | | | |

## ANIMAL 2 – HSB Pattern Runner

BEHAVIOURAL SCREENS: WEEKS ***5*** AND ***6***

| | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 5 | ######## | 1800 | 0 | 9 | 8 | 1796.91 | 9 | 3.09 | 8 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| 6 | ######## | 1800 | 47.04 | 333 | 262 | 1688.08 | 141 | 111.92 | 140 | 17.8 | 40 | 71 | 0 | 0 | 0 | 0 | 0 | 0 |
| 7 | ######## | 1800 | 301.98 | 637 | 502 | 1650.3 | 156 | 149.64 | 156 | 29.33 | 54 | 135 | 0 | 0 | 0 | 7 | 0 | 7 |
| 8 | ######## | 1800 | 15953.04 | 19084 | 18336 | 308.57 | 104 | 1491.4 | 105 | 47.78 | 359 | 748 | 8 | 22 | 0.65 | 310 | 2 | 312 |
| 9 | ######## | 1800 | 19250.03 | 22856 | 21748 | 236.49 | 97 | 1563.48 | 98 | 98.53 | 441 | 1108 | 16 | 30 | 1 | 337 | 3 | 340 |
| 10 | ######## | 1800 | 22013.34 | 19308 | 18444 | 423.42 | 126 | 1376.55 | 127 | 78.44 | 363 | 864 | 118 | 181 | 6.92 | 264 | 49 | 313 |
| 11 | ######## | 1800 | 22315.73 | 21853 | 20854 | 312.2 | 106 | 1487.52 | 107 | 82.75 | 415 | 999 | 220 | 467 | 18.49 | 295 | 19 | 314 |
| 12 | ######## | 1800 | 20659.52 | 22407 | 21391 | 314.79 | 124 | 1485.19 | 124 | 82.87 | 420 | 1016 | 223 | 476 | 22.82 | 330 | 12 | 342 |
| 13 | ######## | 1800 | 19911.47 | 21129 | 20014 | 355.95 | 129 | 1443.09 | 130 | 83.61 | 469 | 1115 | 197 | 542 | 32.06 | 269 | 33 | 302 |
| 14 | ######## | 1800 | 17178.93 | 20724 | 19781 | 315.65 | 133 | 1483.78 | 134 | 85.34 | 392 | 943 | 242 | 966 | 77.71 | 288 | 4 | 292 |
| 15 | ######## | 1800 | 13089.91 | 12720 | 12124 | 839.76 | 157 | 959.44 | 157 | 54.35 | 250 | 596 | 167 | 787 | 60.13 | 156 | 38 | 194 |
| 16 | ######## | 1800 | 440.59 | 917 | 796 | 1624.56 | 163 | 175.05 | 164 | 23.31 | 53 | 121 | 0 | 0 | 0 | 7 | 1 | 8 |
| 17 | ######## | 1800 | 29.33 | 464 | 318 | 1675.1 | 146 | 124.66 | 146 | 33.97 | 51 | 146 | 0 | 0 | 0 | 0 | 0 | 0 |
| 18 | ######## | 1800 | 4710.41 | 6455 | 5916 | 1105.6 | 215 | 694.08 | 215 | 89.81 | 196 | 539 | 42 | 92 | 8.85 | 75 | 0 | 75 |
| 19 | ######## | 1800 | 16081.16 | 19839 | 19045 | 342.9 | 132 | 1457.05 | 133 | 63.16 | 362 | 794 | 178 | 698 | 56.07 | 246 | 10 | 256 |
| 20 | ######## | 1800 | 18152.9 | 19686 | 18699 | 365.87 | 146 | 1433.78 | 147 | 81.08 | 400 | 987 | 224 | 911 | 60.22 | 217 | 27 | 244 |
| 21 | ######## | 1800 | 20233.12 | 19299 | 18272 | 328.48 | 134 | 1471.48 | 134 | 92.32 | 392 | 1027 | 302 | 1559 | 111.62 | 220 | 58 | 278 |
| 22 | ######## | 1800 | 24397.89 | 19879 | 18902 | 326.2 | 142 | 1473.63 | 142 | 70.4 | 348 | 977 | 368 | 1842 | 129.85 | 248 | 55 | 303 |
| 23 | ######## | 1800 | 23653.53 | 22486 | 21468 | 210.9 | 88 | 1587.84 | 89 | 68.21 | 413 | 1018 | 405 | 2026 | 151.91 | 229 | 63 | 292 |
| 24 | ######## | 1800 | 20665.97 | 21421 | 20382 | 247.79 | 99 | 1552.16 | 99 | 83.15 | 434 | 1039 | 381 | 1945 | 226.72 | 244 | 57 | 301 |
| 25 | ######## | 1800 | 16744.42 | 19601 | 18829 | 385.14 | 148 | 1414.33 | 149 | 69.63 | 352 | 772 | 315 | 1568 | 148.58 | 242 | 17 | 259 |
| 26 | ######## | 1800 | 14509.51 | 15154 | 14254 | 542.52 | 194 | 1256.84 | 195 | 93.32 | 339 | 900 | 187 | 1024 | 76.33 | 163 | 22 | 185 |
| 27 | ######## | 1800 | 13878.08 | 16266 | 15347 | 530.09 | 188 | 1269.18 | 189 | 86.05 | 378 | 919 | 197 | 1029 | 59.96 | 196 | 4 | 200 |
| 28 | ######## | 1800 | 19401.74 | 21080 | 20020 | 267.45 | 112 | 1532.49 | 113 | 101.78 | 454 | 1060 | 338 | 1705 | 145.18 | 253 | 27 | 280 |
| 29 | ######## | 1800 | 10785.09 | 14095 | 13334 | 585.8 | 220 | 1213.64 | 221 | 66.14 | 310 | 761 | 157 | 764 | 57.67 | 138 | 17 | 155 |
| 30 | ######## | 1800 | 11317.22 | 15883 | 15103 | 443.07 | 193 | 1356.85 | 194 | 73.66 | 341 | 780 | 116 | 504 | 30.44 | 167 | 1 | 168 |
| | | | | | | | | | 144.5 | | | | | | | | | |

| | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 4 | ######## | 1800 | 19.05 | 166 | 124 | 1745.21 | 75 | 54.79 | 74 | 13.45 | 15 | 42 | 0 | 0 | 0 | 0 | 0 | 0 |
| 5 | ######## | 1800 | 34.33 | 592 | 240 | 1627.13 | 137 | 172.87 | 136 | 85.71 | 72 | 352 | 0 | 0 | 0 | 0 | 0 | 0 |
| 6 | ######## | 1800 | 1271.33 | 2512 | 2144 | 1351.05 | 251 | 448.95 | 250 | 77.74 | 129 | 368 | 2 | 2 | 0.02 | 17 | 0 | 17 |
| 7 | ######## | 1800 | 11393.41 | 13120 | 12407 | 558.9 | 204 | 1240.6 | 204 | 78.42 | 300 | 713 | 154 | 308 | 19.68 | 184 | 4 | 188 |
| 8 | ######## | 1800 | 18553.73 | 20484 | 19559 | 287.57 | 123 | 1511.48 | 124 | 74.18 | 387 | 925 | 264 | 451 | 22.99 | 313 | 9 | 322 |
| 9 | ######## | 1800 | 17041.8 | 15232 | 14506 | 627.5 | 199 | 1172.36 | 200 | 72.49 | 317 | 726 | 230 | 327 | 16.35 | 205 | 17 | 222 |
| 10 | ######## | 1800 | 11837.86 | 12052 | 11310 | 859.28 | 196 | 940.71 | 196 | 96.84 | 252 | 742 | 130 | 228 | 13.4 | 174 | 8 | 182 |
| 11 | ######## | 1800 | 3958.38 | 4609 | 4303 | 1370.01 | 160 | 429.29 | 160 | 42.35 | 111 | 306 | 49 | 77 | 4.4 | 62 | 3 | 65 |
| 12 | ######## | 1800 | 25989.42 | 19966 | 19205 | 326.61 | 125 | 1473.24 | 126 | 53.42 | 319 | 761 | 301 | 662 | 49.7 | 264 | 63 | 327 |
| 13 | ######## | 1800 | 25139.77 | 22222 | 21194 | 245.77 | 112 | 1553.92 | 113 | 68.43 | 384 | 1028 | 380 | 1397 | 143.32 | 248 | 75 | 323 |
| 14 | ######## | 1800 | 22720.13 | 20000 | 18886 | 405.79 | 160 | 1393.85 | 161 | 80.18 | 387 | 1114 | 300 | 930 | 79.31 | 263 | 30 | 293 |
| 15 | ######## | 1800 | 28828.44 | 23364 | 22362 | 232.94 | 85 | 1566.97 | 85 | 64.01 | 370 | 1002 | 381 | 1451 | 149.42 | 292 | 68 | 360 |
| 16 | ######## | 1800 | 22952.84 | 19771 | 18765 | 431.98 | 139 | 1368 | 139 | 73.01 | 395 | 1006 | 260 | 1113 | 125.24 | 199 | 67 | 266 |
| 17 | ######## | 1800 | 23652.45 | 21713 | 20824 | 268.59 | 103 | 1530.82 | 104 | 64.22 | 357 | 889 | 319 | 1527 | 175.36 | 255 | 62 | 317 |
| 18 | ######## | 1800 | 28662.06 | 20895 | 20119 | 339.27 | 121 | 1459.75 | 122 | 57.55 | 303 | 776 | 318 | 1383 | 142.78 | 245 | 106 | 351 |
| 19 | ######## | 1800 | 19382.33 | 15337 | 14691 | 619.07 | 167 | 1180.87 | 167 | 76.24 | 239 | 646 | 225 | 1204 | 148.49 | 181 | 76 | 257 |
| 20 | ######## | 1800 | 2665.31 | 4237 | 3964 | 1271.08 | 203 | 528.92 | 202 | 38.83 | 130 | 273 | 24 | 62 | 4.01 | 27 | 1 | 28 |
| 21 | ######## | 1800 | 1435.02 | 2426 | 2217 | 1484.26 | 175 | 315.74 | 174 | 41.97 | 80 | 209 | 2 | 2 | 0.03 | 23 | 1 | 24 |
| 22 | ######## | 1800 | 27028.2 | 21862 | 21126 | 268.06 | 113 | 1531.93 | 113 | 51.34 | 295 | 736 | 297 | 1097 | 109.15 | 324 | 53 | 377 |
| 23 | ######## | 1800 | 32987.16 | 25496 | 24578 | 187.86 | 76 | 1612.1 | 77 | 49.31 | 347 | 918 | 411 | 2013 | 243.73 | 370 | 86 | 456 |
| 24 | ######## | 1800 | 23828.05 | 20864 | 20053 | 365.49 | 152 | 1434.43 | 153 | 68.49 | 293 | 811 | 371 | 1719 | 198.97 | 277 | 65 | 342 |
| 25 | ######## | 1800 | 28152.15 | 24270 | 23409 | 218.45 | 92 | 1580.63 | 93 | 55.14 | 345 | 861 | 377 | 1997 | 266.42 | 339 | 78 | 417 |
| 26 | ######## | 1800 | 20036.39 | 17110 | 16391 | 530.88 | 174 | 1268.43 | 174 | 66.58 | 285 | 719 | 230 | 1131 | 138.64 | 223 | 48 | 271 |
| 27 | ######## | 1800 | 14660.61 | 15148 | 14507 | 563.97 | 207 | 1236.02 | 207 | 73.48 | 270 | 641 | 167 | 656 | 78.19 | 204 | 24 | 228 |
| 28 | ######## | 1800 | 15024.37 | 15966 | 15275 | 479.24 | 195 | 1320.39 | 196 | 74.56 | 294 | 691 | 173 | 549 | 46.62 | 224 | 9 | 233 |
| 29 | ######## | 1800 | 7419.18 | 8576 | 8211 | 968.37 | 252 | 831.59 | 252 | 49.87 | 161 | 365 | 120 | 637 | 67.91 | 118 | 5 | 123 |
| | | | | | | | | | 158 | | | | | | | | | |

## ANIMAL 2 – HSB Pattern Runner

BEHAVIOURAL SCREENS: WEEKS ***7*** AND ***8***

| | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 5 | ######## | 1800 | 7.71 | 41 | 35 | 1784.04 | 25 | 15.96 | 24 | 1.92 | 3 | 6 | 0 | 0 | 0 | 0 | 0 | 0 |
| 6 | ######## | 1800 | 18.74 | 260 | 182 | 1698.38 | 123 | 101.62 | 122 | 21.67 | 35 | 78 | 0 | 0 | 0 | 0 | 0 | 0 |
| 7 | ######## | 1800 | 2123.45 | 4142 | 3611 | 1143.59 | 199 | 656.3 | 199 | 118.42 | 164 | 531 | 27 | 39 | 1.11 | 26 | 1 | 27 |
| 8 | ######## | 1800 | 14928.4 | 18368 | 17471 | 312.92 | 138 | 1487.05 | 139 | 77.53 | 386 | 897 | 160 | 257 | 6.7 | 256 | 3 | 259 |
| 9 | ######## | 1800 | 17526.66 | 17584 | 16622 | 474.85 | 193 | 1325.08 | 194 | 85.96 | 402 | 962 | 224 | 302 | 9.81 | 311 | 4 | 315 |
| 10 | ######## | 1800 | 11706.19 | 14493 | 13668 | 617.74 | 201 | 1182.11 | 202 | 85.41 | 350 | 825 | 148 | 212 | 4.24 | 199 | 3 | 202 |
| 11 | ######## | 1800 | 16039.14 | 16524 | 15611 | 538.05 | 195 | 1261.95 | 195 | 82.83 | 372 | 913 | 171 | 220 | 4.42 | 241 | 9 | 250 |
| 12 | ######## | 1800 | 18321.63 | 17317 | 16481 | 553.88 | 130 | 1246.12 | 130 | 70.47 | 348 | 836 | 228 | 371 | 20 | 259 | 19 | 278 |
| 13 | ######## | 1800 | 589.17 | 790 | 660 | 1679.07 | 145 | 120.93 | 144 | 35.91 | 39 | 130 | 0 | 0 | 0 | 4 | 2 | 6 |
| 14 | ######## | 1800 | 20340.93 | 20479 | 19532 | 336.95 | 133 | 1462.92 | 133 | 78.36 | 415 | 947 | 263 | 582 | 45.98 | 274 | 25 | 299 |
| 15 | ######## | 1800 | 28499.14 | 22853 | 21963 | 194.15 | 83 | 1605.59 | 84 | 56.77 | 366 | 890 | 438 | 1327 | 123.12 | 285 | 71 | 356 |
| 16 | ######## | 1800 | 21357.05 | 18599 | 17716 | 360.75 | 129 | 1439.02 | 130 | 69.14 | 360 | 883 | 381 | 1408 | 146.87 | 186 | 92 | 278 |
| 17 | ######## | 1800 | 24297.11 | 20216 | 19336 | 248.45 | 110 | 1551.43 | 110 | 59.97 | 344 | 880 | 389 | 1456 | 179.82 | 239 | 81 | 320 |
| 18 | ######## | 1800 | 25203.13 | 20856 | 20045 | 365.54 | 118 | 1433.27 | 119 | 65.1 | 343 | 811 | 334 | 1094 | 135.5 | 254 | 73 | 327 |
| 19 | ######## | 1800 | 29399.95 | 21529 | 20649 | 268.37 | 86 | 1531.1 | 87 | 54.32 | 386 | 880 | 394 | 1586 | 242.11 | 212 | 149 | 361 |
| 20 | ######## | 1800 | 25772.98 | 19243 | 18505 | 326.17 | 147 | 1473.77 | 148 | 58.91 | 317 | 738 | 386 | 1606 | 222.7 | 189 | 131 | 320 |
| 21 | ######## | 1800 | 17705.26 | 15549 | 14632 | 530.27 | 130 | 1269.72 | 130 | 66.38 | 360 | 917 | 348 | 1120 | 148.66 | 123 | 85 | 208 |
| 22 | ######## | 1800 | 4173.71 | 4187 | 3832 | 1412.1 | 120 | 387.41 | 120 | 29.84 | 130 | 355 | 77 | 163 | 12.01 | 51 | 16 | 67 |
| 23 | ######## | 1800 | 24344.26 | 20186 | 18929 | 177.52 | 91 | 1622.2 | 92 | 88.19 | 510 | 1257 | 467 | 1730 | 241.57 | 210 | 114 | 324 |
| 24 | ######## | 1800 | 22675.71 | 19272 | 18311 | 333.95 | 128 | 1465.69 | 129 | 79.26 | 398 | 961 | 388 | 1825 | 305.21 | 171 | 118 | 289 |
| 25 | ######## | 1800 | 25878.52 | 19877 | 19041 | 340.78 | 146 | 1459.16 | 147 | 74.46 | 360 | 836 | 409 | 1945 | 312.81 | 226 | 99 | 325 |
| 26 | ######## | 1800 | 25337.92 | 19924 | 19122 | 328.38 | 137 | 1471.62 | 137 | 54.6 | 359 | 802 | 386 | 1755 | 273.43 | 187 | 105 | 292 |
| 27 | ######## | 1800 | 20426.55 | 17044 | 16353 | 464.37 | 185 | 1335.62 | 185 | 54.46 | 306 | 691 | 302 | 1069 | 384.82 | 187 | 76 | 263 |
| 28 | ######## | 1800 | 15839.73 | 12737 | 12209 | 725.37 | 236 | 1074.63 | 235 | 53.81 | 223 | 528 | 252 | 594 | 62.81 | 123 | 47 | 170 |
| 29 | ######## | 1800 | 15534.05 | 12854 | 12271 | 659.93 | 225 | 1140.06 | 225 | 72.55 | 259 | 583 | 202 | 469 | 64.97 | 144 | 48 | 192 |
| 30 | ######## | 1800 | 9121.1 | 10555 | 10019 | 931.03 | 138 | 868.76 | 138 | 48.78 | 221 | 536 | 143 | 353 | 46.98 | 131 | 13 | 144 |
| | | | | | | | | | 148 | | | | | | | | | |

| | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 3 | ######## | 1800 | 0.9 | 89 | 75 | 1764.34 | 66 | 35.66 | 65 | 3.1 | 10 | 14 | 0 | 0 | 0 | 0 | 0 | 0 |
| 4 | ######## | 1800 | 22.62 | 490 | 241 | 1636.4 | 162 | 163.6 | 161 | 67.05 | 58 | 249 | 0 | 0 | 0 | 0 | 0 | 0 |
| 5 | ######## | 1800 | 6382.42 | 8859 | 8316 | 768.89 | 212 | 1030.43 | 212 | 72.54 | 229 | 543 | 49 | 56 | 0.84 | 98 | 1 | 99 |
| 6 | ######## | 1800 | 15430.12 | 18233 | 17325 | 354.48 | 144 | 1444.82 | 145 | 86.24 | 367 | 908 | 117 | 144 | 3.07 | 249 | 1 | 250 |
| 7 | ######## | 1800 | 15784.1 | 16518 | 15688 | 491.56 | 170 | 1307.86 | 171 | 82.37 | 358 | 830 | 159 | 232 | 4.48 | 272 | 7 | 279 |
| 8 | ######## | 1800 | 10097.35 | 13736 | 12848 | 696.18 | 231 | 1102.65 | 232 | 108.08 | 326 | 888 | 109 | 164 | 3.75 | 177 | 1 | 178 |
| 9 | ######## | 1800 | 12328.84 | 16285 | 15420 | 503.89 | 188 | 1295.25 | 189 | 88.37 | 362 | 865 | 149 | 195 | 3.12 | 186 | 9 | 195 |
| 10 | ######## | 1800 | 17784.21 | 17786 | 17061 | 464.19 | 187 | 1335.79 | 188 | 73.3 | 292 | 725 | 174 | 259 | 7.14 | 239 | 23 | 262 |
| 11 | ######## | 1800 | 10447.23 | 9353 | 8903 | 989.18 | 188 | 810.78 | 189 | 47.44 | 168 | 450 | 82 | 112 | 2.89 | 126 | 24 | 150 |
| 12 | ######## | 1800 | 18504.15 | 16798 | 16062 | 469.92 | 175 | 1329.69 | 176 | 63.18 | 280 | 736 | 122 | 178 | 4.96 | 253 | 28 | 281 |
| 13 | ######## | 1800 | 49.94 | 606 | 492 | 1694.34 | 134 | 105.43 | 134 | 23.79 | 37 | 114 | 0 | 0 | 0 | 0 | 0 | 0 |
| 14 | ######## | 1800 | 21737.76 | 18913 | 17890 | 428.75 | 139 | 1371.09 | 139 | 68.69 | 361 | 1023 | 190 | 250 | 11.67 | 224 | 69 | 293 |
| 15 | ######## | 1800 | 18154.47 | 21336 | 19782 | 351.69 | 105 | 1448.09 | 106 | 89.54 | 451 | 1554 | 281 | 371 | 27.01 | 91 | 111 | 202 |
| 16 | ######## | 1800 | 16853.46 | 21257 | 19036 | 265.92 | 87 | 1534.02 | 88 | 121.99 | 625 | 2221 | 460 | 775 | 96.12 | 122 | 82 | 204 |
| 17 | ######## | 1800 | 19407.32 | 18345 | 17098 | 357.66 | 174 | 1442.29 | 175 | 88.56 | 392 | 1247 | 351 | 692 | 83.53 | 148 | 74 | 222 |
| 18 | ######## | 1800 | 20468 | 17508 | 16326 | 411.22 | 194 | 1388.72 | 195 | 81.23 | 392 | 1182 | 393 | 718 | 61.65 | 156 | 62 | 218 |
| 19 | ######## | 1800 | 21044.01 | 18733 | 17535 | 384.05 | 168 | 1415.76 | 169 | 84.49 | 373 | 1198 | 395 | 660 | 51.39 | 153 | 85 | 238 |
| 20 | ######## | 1800 | 8900.8 | 15083 | 12871 | 484.41 | 119 | 1315.49 | 120 | 155.56 | 588 | 2212 | 552 | 1344 | 181.22 | 53 | 46 | 99 |
| 21 | ######## | 1800 | 19336.56 | 19564 | 17731 | 232.15 | 96 | 1567.18 | 97 | 118.34 | 551 | 1833 | 639 | 1661 | 237.05 | 128 | 79 | 207 |
| 22 | ######## | 1800 | 18276.5 | 17088 | 15470 | 342.64 | 124 | 1457.29 | 125 | 136.14 | 512 | 1618 | 557 | 1250 | 138.87 | 149 | 71 | 220 |
| 23 | ######## | 1800 | 16109.26 | 18448 | 16799 | 406.1 | 130 | 1393.62 | 131 | 104.01 | 488 | 1649 | 532 | 1188 | 125.73 | 85 | 65 | 150 |
| 24 | ######## | 1800 | 18605.78 | 16901 | 15894 | 593.97 | 183 | 1205.95 | 184 | 82.36 | 339 | 1007 | 386 | 661 | 32.05 | 125 | 89 | 214 |
| 25 | ######## | 1800 | 20011.75 | 17629 | 16661 | 543.54 | 174 | 1256.37 | 174 | 77.39 | 392 | 968 | 264 | 421 | 33.25 | 162 | 84 | 246 |
| 26 | ######## | 1800 | 23475 | 19353 | 18448 | 427.43 | 150 | 1372.51 | 150 | 66.9 | 345 | 905 | 216 | 312 | 31.07 | 220 | 80 | 300 |
| 27 | ######## | 1800 | 9919.22 | 9717 | 8898 | 737.27 | 264 | 1062.67 | 265 | 119.12 | 300 | 819 | 28 | 32 | 0.83 | 77 | 24 | 101 |
| 28 | ######## | 1800 | 4152.89 | 5897 | 5630 | 1285.32 | 117 | 514.68 | 117 | 21.97 | 122 | 267 | 40 | 58 | 4.04 | 67 | 5 | 72 |
| 29 | ######## | 1800 | 0 | 37 | 13 | 1786.65 | 16 | 13.35 | 15 | 7.13 | 6 | 24 | 0 | 0 | 0 | 0 | 0 | 0 |
| | | | | | | | | | 155.44 | | | | | | | | | |

## ANIMAL 3 – HSB Vertical Jumper and Pattern Runner

*(The 3 highest weekly vertical activity AND pattern running scores of animal 3 are highlighted on the following eight sheets using two black ovals.)*

BEHAVIOURAL SCREENS: WEEKS ***1*** AND ***2***

*HSB animals expressing pattern running AND vertical jumping generate high amounts of cage revolutions AND vertical activity compared to animals from the NSB cohort. Again the three highest 30-minute values during a 12-hour dark cycle period, are indicated by the grey colouring. These values are indicated on each of the following eight tables by black ovals.*

| | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 9 | ######## | 1800 | 27.11 | 321 | | | | | | | | 0 | 0 | 0 | 0 | 0 | 0 | |
| 10 | ######## | 1800 | 6.92 | 283 | | | | | | | | 0 | 0 | 0 | 0 | 0 | 0 | |
| 11 | ######## | 1800 | 5814.08 | 9897 | | | | | | | | 159 | 477 | 39.48 | 23 | 45 | 68 | |
| 12 | ######## | 1800 | 19520.71 | 18097 | | | | | | | | 316 | 1152 | 124.57 | 62 | 147 | 209 | |
| 13 | ######## | 1800 | 28990.58 | 21839 | | | | | | | | 213 | 676 | 37.08 | 82 | 29 | 379 | |
| 14 | ######## | 1800 | 20453.44 | 22909 | | | | | | | | 243 | 875 | 40.08 | 43 | 194 | 237 | |
| 15 | ######## | 1800 | 27056.22 | 23455 | | | | | | | | 233 | 893 | 42.71 | 129 | 255 | 384 | |
| 16 | ######## | 1800 | 21160.49 | 21544 | | | | | | | | 281 | 1077 | 53.95 | 70 | 184 | 254 | |
| 17 | ######## | 1800 | 8076.06 | 16259 | | | | | | | | 342 | 1478 | 75.83 | 32 | 54 | 86 | |
| 18 | ######## | 1800 | 15502.86 | 16991 | | | | | | | | 230 | 842 | 37.75 | 61 | 144 | 205 | |
| 19 | ######## | 1800 | 13428.1 | 14570 | 13553 | 563.9 | 106 | 1236.47 | 106 | 89.63 | 384 | 1017 | 152 | 548 | 26.32 | 58 | 116 | 174 |
| 20 | ######## | 1800 | 24666.84 | 20207 | 19364 | 321.49 | 106 | 1478.46 | 107 | 53.03 | 342 | 843 | 278 | 1246 | 75.64 | 94 | 183 | 277 |
| 21 | ######## | 1800 | 20626.23 | 18623 | 17749 | 325.38 | 137 | 1474.61 | 138 | 65.87 | 344 | 874 | 308 | 1316 | 84.91 | 87 | 173 | 260 |
| 22 | ######## | 1800 | 22079.86 | 17603 | 16797 | 551.23 | 116 | 1248.77 | 116 | 57.86 | 294 | 806 | 208 | 820 | 43.76 | 107 | 162 | 269 |
| 23 | ######## | 1800 | 12032.96 | 9504 | 9000 | 946.36 | 128 | 853.5 | 128 | 46.83 | 201 | 504 | 55 | 144 | 7.11 | 51 | 118 | 169 |
| 24 | ######## | 1800 | 16314.72 | 12020 | 11571 | 900.9 | 108 | 899.07 | 109 | 36.82 | 168 | 449 | 101 | 307 | 15.54 | 75 | 142 | 217 |
| 25 | ######## | 1800 | 29319.22 | 23384 | 22368 | 232.25 | 86 | 1567.28 | 87 | 65.28 | 385 | 1016 | 309 | 1179 | 68.22 | 122 | 224 | 346 |
| 26 | ######## | 1800 | 29357.45 | 23920 | 22688 | 188.79 | 83 | 1611.17 | 84 | 78.7 | 408 | 1232 | 405 | 1727 | 103.92 | 90 | 253 | 343 |
| 27 | ######## | 1800 | 27974.6 | 23311 | 22453 | 269.18 | 99 | 1530.79 | 100 | 55.4 | 329 | 858 | 483 | 2151 | 153.38 | 116 | 223 | 339 |
| 28 | ######## | 1800 | 27221.42 | 23256 | 22388 | 255.25 | 89 | 1544.69 | 90 | 57.66 | 301 | 868 | 392 | 1933 | 150.28 | 108 | 181 | 289 |
| 29 | ######## | 1800 | 27421.56 | 21191 | 20565 | 435.79 | 100 | 1364.2 | 101 | 35.81 | 254 | 626 | 442 | 1876 | 152.93 | 120 | 204 | 324 |
| 30 | ######## | 1800 | 23933.89 | 21618 | 20513 | 301.36 | 113 | 1498.63 | 114 | 80.69 | 360 | 1105 | 471 | 1827 | 140.81 | 91 | 211 | 302 |
| 31 | ######## | 1800 | 19732.43 | 20871 | 19834 | 289.43 | 116 | 1510.33 | 117 | 76.36 | 393 | 1037 | 349 | 2166 | 207.8 | 66 | 149 | 215 |
| 32 | ######## | 1800 | 23806.47 | 20534 | 19560 | 334.77 | 134 | 1465.05 | 135 | 75.46 | 368 | 974 | 327 | 2037 | 192.35 | 93 | 188 | 281 |
| 33 | ######## | 1800 | 14167.79 | 13242 | 12418 | 620.86 | 200 | 1178.95 | 201 | 73.41 | 330 | 824 | 114 | 401 | 22.51 | 50 | 97 | 147 |
| 34 | ######## | 1800 | 25580.8 | 24312 | 23306 | 152.75 | 80 | 1647.22 | 81 | 86.32 | 396 | 1006 | 225 | 811 | 54.26 | 79 | 223 | 302 |
| 35 | ######## | 1800 | 12668.12 | 11201 | 10691 | 907.39 | 244 | 892.56 | 244 | 57.32 | 216 | 510 | 116 | 596 | 66.32 | 63 | 82 | 145 |
| 36 | ######## | 1800 | 8.19 | 147 | 114 | 1742.33 | 110 | 57.67 | 109 | 6.93 | 20 | 33 | 0 | 0 | 0 | 0 | 0 | 0 |
| | | | | | | | | | 117.5385 | | | | | | | | | |

| | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 11 | ######## | 1800 | 19.71 | 762 | 310 | 1553.73 | 235 | 246.26 | 235 | 120.76 | 113 | 452 | 0 | 0 | 0 | 0 | 0 | 0 |
| 12 | ######## | 1800 | 18647.16 | 21913 | 21009 | 201.12 | 76 | 1598.84 | 77 | 68.45 | 398 | 904 | 159 | 400 | 20.13 | 32 | 230 | 262 |
| 13 | ######## | 1800 | 21903.09 | 23931 | 22901 | 116.2 | 58 | 1683.58 | 59 | 65.47 | 460 | 1030 | 207 | 673 | 42.97 | 32 | 272 | 304 |
| 14 | ######## | 1800 | 22888.67 | 23238 | 22107 | 182.77 | 67 | 1617.2 | 68 | 69.98 | 466 | 1131 | 216 | 719 | 44.17 | 34 | 221 | 255 |
| 15 | ######## | 1800 | 27156.41 | 23117 | 22028 | 180.96 | 79 | 1618.84 | 80 | 74.18 | 450 | 1089 | 218 | 714 | 40.37 | 93 | 213 | 306 |
| 16 | ######## | 1800 | 25841.34 | 20300 | 19168 | 267.63 | 111 | 1532.05 | 112 | 70.68 | 386 | 1132 | 235 | 939 | 66.28 | 55 | 267 | 322 |
| 17 | ######## | 1800 | 23945.5 | 19426 | 18342 | 302.35 | 134 | 1497.39 | 135 | 81.32 | 387 | 1084 | 194 | 690 | 42.59 | 61 | 246 | 307 |
| 18 | ######## | 1800 | 33263.4 | 22704 | 21665 | 238.04 | 102 | 1561.87 | 103 | 65.07 | 383 | 1039 | 313 | 1453 | 122.25 | 130 | 237 | 367 |
| 19 | ######## | 1800 | 33955.82 | 24402 | 23272 | 198.86 | 95 | 1601.07 | 96 | 66.97 | 393 | 1130 | 354 | 1848 | 161.28 | 136 | 237 | 373 |
| 20 | ######## | 1800 | 24383.58 | 25386 | 24628 | 194.8 | 71 | 1604.95 | 72 | 48.7 | 342 | 758 | 366 | 2131 | 186.7 | 57 | 237 | 294 |
| 21 | ######## | 1800 | 25525.54 | 26050 | 25286 | 144.65 | 63 | 1655.06 | 64 | 45.11 | 322 | 764 | 386 | 2042 | 188.31 | 61 | 212 | 273 |
| 22 | ######## | 1800 | 23789.18 | 23870 | 23215 | 217.91 | 91 | 1581.95 | 92 | 42.2 | 284 | 655 | 358 | 2050 | 184.57 | 66 | 200 | 266 |
| 23 | ######## | 1800 | 29498.25 | 24148 | 23242 | 184.86 | 83 | 1615.04 | 84 | 60.41 | 368 | 906 | 370 | 2176 | 194.72 | 118 | 189 | 307 |
| 24 | ######## | 1800 | 28728.11 | 24032 | 23229 | 241.82 | 98 | 1558.11 | 99 | 47.87 | 345 | 803 | 364 | 2004 | 180.25 | 97 | 216 | 313 |
| 25 | ######## | 1800 | 24274.56 | 26500 | 25819 | 135.89 | 61 | 1663.19 | 62 | 50.3 | 311 | 681 | 394 | 2079 | 175.93 | 47 | 223 | 270 |
| 26 | ######## | 1800 | 20059.51 | 23826 | 23067 | 246.74 | 99 | 1553.25 | 100 | 61.03 | 323 | 759 | 338 | 1784 | 144.8 | 38 | 217 | 255 |
| 27 | ######## | 1800 | 14570.27 | 17753 | 17152 | 528.67 | 158 | 1271.3 | 159 | 64.07 | 265 | 601 | 275 | 1332 | 114.81 | 30 | 145 | 175 |
| 28 | ######## | 1800 | 23928.18 | 26142 | 25516 | 108.5 | 70 | 1691.4 | 71 | 39.97 | 297 | 626 | 370 | 2378 | 228.33 | 46 | 221 | 267 |
| 29 | ######## | 1800 | 33945.64 | 24761 | 24081 | 195.9 | 87 | 1604.04 | 87 | 46.86 | 281 | 680 | 389 | 2523 | 240.14 | 150 | 173 | 323 |
| 30 | ######## | 1800 | 27773.18 | 26389 | 25734 | 170.24 | 80 | 1628.96 | 81 | 38.72 | 304 | 655 | 334 | 2143 | 174.67 | 74 | 251 | 325 |
| 31 | ######## | 1800 | 30800.03 | 25244 | 24536 | 170.12 | 81 | 1629.76 | 82 | 39.46 | 288 | 708 | 395 | 2261 | 193.05 | 116 | 221 | 337 |
| 32 | ######## | 1800 | 23391.04 | 23407 | 22769 | 225.69 | 97 | 1573.99 | 98 | 46.55 | 267 | 638 | 363 | 2201 | 194.47 | 50 | 222 | 272 |
| 33 | ######## | 1800 | 26156.02 | 23017 | 22350 | 230.97 | 106 | 1568.94 | 107 | 48.92 | 276 | 667 | 304 | 2351 | 226.85 | 96 | 186 | 282 |
| 34 | ######## | 1800 | 11170.31 | 14915 | 14440 | 527.97 | 212 | 1271.95 | 213 | 49.32 | 235 | 475 | 174 | 860 | 75.33 | 16 | 118 | 134 |
| 35 | ######## | 1800 | 12620.4 | 16590 | 16043 | 450.03 | 145 | 1349.85 | 146 | 56.95 | 254 | 547 | 181 | 995 | 81.7 | 19 | 145 | 164 |
| 36 | ######## | 1800 | 376.46 | 1143 | 1063 | 1552.26 | 174 | 247.7 | 174 | 18.91 | 46 | 80 | 1 | 1 | 0.11 | 1 | 2 | 3 |
| | | | | | | | | | 101.8333 | | | | | | | | | |

# ANIMAL 3 – HSB Vertical Jumper and Pattern Runner

## BEHAVIOURAL SCREENS: WEEKS *3* AND *4*

| | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 6 | ######## | 1800 | 14.94 | 423 | 214 | 1687.73 | 98 | 112.27 | 97 | 48.45 | 47 | 209 | 0 | 0 | 0 | 0 | 0 | 0 |
| 7 | ######## | 1800 | 5.33 | 427 | 167 | 1659.53 | 114 | 139.76 | 114 | 70.31 | 67 | 260 | 0 | 0 | 0 | 0 | 0 | 0 |
| 8 | ######## | 1800 | 9553.92 | 13032 | 12217 | 368.43 | 183 | 1431.35 | 184 | 109.89 | 327 | 815 | 258 | 1024 | 93.11 | 26 | 89 | 115 |
| 9 | ######## | 1800 | 18265.09 | 20145 | 19210 | 187.1 | 80 | 1612.5 | 81 | 62.81 | 429 | 935 | 186 | 555 | 32.89 | 46 | 211 | 257 |
| 10 | ######## | 1800 | 20678.3 | 19899 | 18917 | 225.75 | 92 | 1574.21 | 92 | 68.02 | 446 | 982 | 308 | 1145 | 88.91 | 53 | 192 | 245 |
| 11 | ######## | 1800 | 24257.99 | 22068 | 20900 | 177.41 | 81 | 1622.28 | 82 | 74.39 | 417 | 1168 | 351 | 1414 | 119.9 | 27 | 218 | 245 |
| 12 | ######## | 1800 | 27771.08 | 21568 | 19953 | 210.63 | 106 | 1588.75 | 107 | 85.94 | 482 | 1615 | 345 | 1281 | 92.66 | 47 | 241 | 288 |
| 13 | ######## | 1800 | 24898.81 | 19112 | 17809 | 365.59 | 126 | 1434.35 | 126 | 76.79 | 383 | 1303 | 271 | 886 | 53.99 | 62 | 241 | 303 |
| 14 | ######## | 1800 | 22970.26 | 18737 | 17745 | 341.04 | 105 | 1458.72 | 106 | 64.74 | 333 | 992 | 222 | 627 | 37.27 | 48 | 23 | 282 |
| 15 | ######## | 1800 | 26461.29 | 21112 | 20076 | 241.93 | 97 | 1558.06 | 98 | 63.11 | 365 | 1036 | 356 | 1252 | 94.37 | 53 | 292 | 345 |
| 16 | ######## | 1800 | 25185.61 | 19872 | 18993 | 318.92 | 92 | 1480.99 | 93 | 56.37 | 342 | 879 | 331 | 1315 | 109.4 | 53 | 264 | 317 |
| 17 | ######## | 1800 | 20242.07 | 17258 | 16367 | 375.87 | 130 | 1424.12 | 130 | 68.64 | 385 | 891 | 270 | 946 | 65.3 | 36 | 215 | 251 |
| 18 | ######## | 1800 | 11803.25 | 11874 | 11211 | 804.97 | 194 | 995.02 | 194 | 95.61 | 255 | 663 | 194 | 62 | 35.7 | 22 | 110 | 132 |
| 19 | ######## | 1800 | 25057.95 | 23322 | 22422 | 161.41 | 73 | 1638.5 | 74 | 58.3 | 397 | 900 | 40 | 1714 | 143.92 | 54 | 235 | 289 |
| 20 | ######## | 1800 | 23290.7 | 20723 | 19786 | 284.15 | 102 | 1515.82 | 103 | 60.61 | 398 | 937 | 3 | 1886 | 183.6 | 43 | 230 | 273 |
| 21 | ######## | 1800 | 20943.7 | 20347 | 19466 | 267.68 | 105 | 1532.18 | 106 | 72.95 | 380 | 881 | 7 | 1798 | 167.54 | 36 | 226 | 262 |
| 22 | ######## | 1800 | 23664.9 | 20905 | 20073 | 205.7 | 102 | 1593.79 | 103 | 55.34 | 352 | 832 | 7 | 1745 | 152.46 | 69 | 246 | 315 |
| 23 | ######## | 1800 | 21087.13 | 18894 | 17870 | 278.17 | 112 | 1520.74 | 113 | 75.23 | 346 | 1024 | 40 | 2108 | 206.61 | 33 | 197 | 230 |
| 24 | ######## | 1800 | 19454.59 | 18993 | 18114 | 275.41 | 156 | 1524.48 | 157 | 66.25 | 319 | 879 | 4 | 2461 | 239.45 | 27 | 185 | 216 |
| 25 | ######## | 1800 | 22771.68 | 17994 | 17109 | 417.91 | 114 | 1381.17 | 115 | 68.23 | 316 | 885 | 3 | 1776 | 163.96 | 72 | 183 | 25 |
| 26 | ######## | 1800 | 26254.44 | 21125 | 20246 | 270.13 | 124 | 1529.85 | 125 | 66.01 | 367 | 879 | 38 | 1 | 172.56 | 95 | 193 | 288 |
| 27 | ######## | 1800 | 15918.64 | 17961 | 17325 | 456.34 | 157 | 1343.66 | 157 | 45.66 | 289 | 636 | 337 | 1899 | 171.27 | 48 | 134 | 182 |
| 28 | ######## | 1800 | 15317.45 | 17283 | 16507 | 404.27 | 160 | 1395.68 | 160 | 69.82 | 313 | 776 | 325 | 1666 | 140.17 | 40 | 132 | 172 |
| 29 | ######## | 1800 | 10997.06 | 12710 | 12116 | 583.25 | 162 | 1216.75 | 162 | 59.55 | 282 | 594 | 224 | 1014 | 67.07 | 17 | 146 | 163 |
| 30 | ######## | 1800 | 10185.24 | 12026 | 11339 | 636.71 | 207 | 1162.8 | 208 | 67.19 | 293 | 687 | 183 | 795 | 57.14 | 28 | 113 | 141 |
| 31 | ######## | 1800 | 4745.21 | 6634 | 6170 | 962.34 | 256 | 837.38 | 257 | 52.55 | 200 | 464 | 55 | 234 | 29.61 | 14 | 48 | 62 |
| | | | | | | | | | 130.5417 | | | | | | | | | |

| | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 9 | ######## | 1800 | 1.9 | 51 | 38 | 1785.63 | 50 | 14.37 | 49 | 2.97 | 6 | 13 | 0 | 0 | 0 | 0 | 0 | 0 |
| 10 | ######## | 1800 | 45.78 | 508 | 290 | 1630.13 | 209 | 169.88 | 208 | 59.05 | 61 | 218 | 0 | 0 | 0 | 0 | 0 | 0 |
| 11 | ######## | 1800 | 986.64 | 2956 | 2459 | 1287.43 | 247 | 512.37 | 247 | 106.28 | 160 | 497 | 53 | 168 | 23.45 | 3 | 2 | 5 |
| 12 | ######## | 1800 | 13417.08 | 16742 | 15908 | 222.09 | 102 | 1577.82 | 103 | 67.4 | 361 | 834 | 82 | 154 | 14.49 | 10 | 175 | 185 |
| 13 | ######## | 1800 | 18253.5 | 20218 | 19210 | 162.92 | 69 | 1636.79 | 70 | 69 | 409 | 1008 | 26 | 38 | 1.16 | 19 | 287 | 306 |
| 14 | ######## | 1800 | 19604.98 | 21706 | 20806 | 130.99 | 57 | 1668.77 | 58 | 57.5 | 396 | 900 | 34 | 68 | 2.05 | 11 | 260 | 271 |
| 15 | ######## | 1800 | 20288.89 | 23328 | 22253 | 103.9 | 48 | 1695.94 | 49 | 63.95 | 476 | 1075 | 57 | 115 | 4.09 | 29 | 239 | 268 |
| 16 | ######## | 1800 | 18336.99 | 23443 | 22291 | 148.28 | 66 | 1651.65 | 67 | 68.79 | 531 | 1152 | 102 | 246 | 7.87 | 23 | 214 | 237 |
| 17 | ######## | 1800 | 19893.58 | 25906 | 24830 | 123.76 | 55 | 1676.21 | 56 | 62.19 | 494 | 1076 | 151 | 368 | 12.08 | 15 | 233 | 248 |
| 18 | ######## | 1800 | 23724.74 | 24769 | 23703 | 170.19 | 54 | 1629.76 | 55 | 60.72 | 463 | 1066 | 132 | 352 | 12 | 33 | 250 | 283 |
| 19 | ######## | 1800 | 30610.02 | 27151 | 25961 | 106.7 | 48 | 1693.05 | 49 | 63.45 | 518 | 1190 | 80 | 154 | 7.55 | 62 | 278 | 340 |
| 20 | ######## | 1800 | 20161.4 | 25686 | 24697 | 173.77 | 74 | 1626.22 | 75 | 59.26 | 461 | 989 | 122 | 278 | 16.64 | 18 | 210 | 237 |
| 21 | ######## | 1800 | 28347.28 | 25649 | 24487 | 132.79 | 55 | 1666.9 | 56 | 71.38 | 437 | 1162 | 294 | 833 | 63.1 | 73 | 206 | 339 |
| 22 | ######## | 1800 | 24629.24 | | | | | | | | | 1000 | 201 | 557 | 38.55 | 74 | 237 | 311 |
| 23 | ######## | 1800 | 31042.61 | | | | | | | | | 1141 | 231 | 579 | 34.39 | 65 | 292 | 357 |
| 24 | ######## | 1800 | 25714.82 | | | | | | | | | 1110 | 283 | 768 | 48.47 | 45 | 287 | 332 |
| 25 | ######## | 1800 | 21588.94 | | | | | | | | | 1138 | 182 | 549 | 30.4 | 18 | 244 | 262 |
| 26 | ######## | 1800 | 29657.71 | | | | | | | | | 1044 | 249 | 810 | 61.52 | 78 | 272 | 350 |
| 27 | ######## | 1800 | 27507.39 | | | | | | | | | 1290 | 216 | 627 | 39.41 | 87 | 245 | 332 |
| 28 | ######## | 1800 | 29792.18 | | | | | | | | | 1064 | 228 | 564 | 41.33 | 117 | 207 | 324 |
| 29 | ######## | 1800 | 28048.73 | | | | | | | | | 959 | 181 | 819 | 53.11 | 66 | 243 | 309 |
| 30 | ######## | 1800 | 21062.64 | | | | | | | | | 905 | 145 | 852 | 68.41 | 54 | 188 | 242 |
| 31 | ######## | 1800 | 16918.66 | | | | | | | | | 925 | 126 | 947 | 68.77 | 49 | 142 | 191 |
| 32 | ######## | 1800 | 11052.84 | 14953 | 14136 | 502.53 | 203 | 1297.41 | 203 | 71.04 | 380 | 817 | 109 | 428 | 35.23 | 32 | 103 | 135 |
| 33 | ######## | 1800 | 3016.23 | 5585 | 5225 | 872.89 | 307 | 926.79 | 308 | 59.16 | 175 | 360 | 3 | 10 | 0.33 | 6 | 47 | 53 |
| 34 | ######## | 1800 | 8111.48 | 11729 | 11130 | 483.67 | 193 | 1316.19 | 194 | 63.49 | 266 | 599 | 42 | 79 | 3.82 | 10 | 84 | 94 |
| 35 | ######## | 1800 | 1686.88 | 2719 | 2610 | 1521.03 | 111 | 278.87 | 111 | 21.48 | 50 | 109 | 3 | 9 | 0.28 | 5 | 19 | 24 |
| 36 | ######## | 1800 | 0 | 27 | 25 | 1789.54 | 23 | 9.97 | 23 | 0.17 | 2 | 2 | 0 | 0 | 0 | 0 | 0 | 0 |
| | | | | | | | | | 97.73077 | | | | | | | | | |

*Note the sudden decrease in the weekly amount of vertical stereotypy executed by animal 3. It is due to these fluctuations in the expression of stereotypy, that a four week pre-treatment assessment is necessary to establish a baseline stereotypy score for each animal.*

## ANIMAL 3 – HSB Vertical Jumper and Pattern Runner

BEHAVIOURAL SCREENS: WEEKS ***5*** AND ***6***

| | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 5 | ######## | 1800 | 4.02 | 130 | 67 | 1761.45 | 72 | 38.55 | 71 | 8.24 | 21 | 63 | 0 | 0 | 0 | 0 | 0 | 0 |
| 6 | ######## | 1800 | 26.31 | 320 | 244 | 1708.62 | 107 | 91.38 | 106 | 13.84 | 25 | 76 | 0 | 0 | 0 | 0 | 0 | 0 |
| 7 | ######## | 1800 | 255.4 | 1314 | 970 | 1512.28 | 185 | 287.67 | 185 | 77.48 | 102 | 344 | 2 | 3 | 0.97 | 0 | 1 | 1 |
| 8 | ######## | 1800 | 11355.75 | 18646 | 17874 | 219.54 | 102 | 1580.2 | 103 | 61.14 | 333 | 772 | 61 | 110 | 8.74 | 11 | 129 | 140 |
| 9 | ######## | 1800 | 11085.17 | 17985 | 17231 | 310.4 | 102 | 1489.59 | 103 | 59.75 | 318 | 754 | 48 | 102 | 4.71 | 21 | 88 | 109 |
| 10 | ######## | 1800 | 14322.88 | 22070 | 21037 | 131.49 | 63 | 1668.43 | 64 | 72.9 | 414 | 1033 | 41 | 65 | 3.4 | 32 | 102 | 134 |
| 11 | ######## | 1800 | 16095.33 | 20511 | 19654 | 204.21 | 70 | 1595.76 | 71 | 75.86 | 366 | 857 | 37 | 73 | 2.17 | 20 | 159 | 179 |
| 12 | ######## | 1800 | 18241.77 | 21579 | 20915 | 129.66 | 62 | 1670.25 | 63 | 44.06 | 284 | 664 | 46 | 82 | 1.73 | 11 | 205 | 216 |
| 13 | ######## | 1800 | 17866.11 | 19736 | 18946 | 197.92 | 94 | 1602.07 | 94 | 68.01 | 320 | 790 | 128 | 382 | 12.38 | 30 | 225 | 255 |
| 14 | ######## | 1800 | 19261.3 | 17968 | 17261 | 292.99 | 123 | 1507.01 | 122 | 58.21 | 298 | 707 | 323 | 1173 | 58.96 | 26 | 184 | 210 |
| 15 | ######## | 1800 | 19252.39 | 16665 | 16020 | 304.89 | 136 | 1494.86 | 137 | 59.97 | 262 | 645 | 343 | 1251 | 64.67 | 58 | 148 | 206 |
| 16 | ######## | 1800 | 16010.16 | 14409 | 13852 | 506.31 | 116 | 1293.63 | 117 | 48.53 | 246 | 557 | 290 | 1156 | 57.75 | 21 | 200 | 221 |
| 17 | ######## | 1800 | 18963.13 | 18616 | 17914 | 246.66 | 107 | 1553.28 | 108 | 54.29 | 259 | 702 | 381 | 1524 | 91.47 | 48 | 220 | 268 |
| 18 | ######## | 1800 | 20844.54 | 18616 | 17794 | 320.47 | 128 | 1479.26 | 129 | 53.99 | 299 | 822 | 372 | 1666 | 123.81 | 47 | 251 | 298 |
| 19 | ######## | 1800 | 24580.52 | 21802 | 20637 | 272.95 | 104 | 1527 | 105 | 74 | 418 | 1165 | 316 | 1349 | 77.72 | 53 | 301 | 354 |
| 20 | ######## | 1800 | 22277.45 | 20901 | 19565 | 170.21 | 83 | 1629.71 | 84 | 94.53 | 483 | 1336 | 337 | 1460 | 68.52 | 44 | 281 | 325 |
| 21 | ######## | 1800 | 21841.2 | 19731 | 18713 | 349.51 | 113 | 1450.48 | 113 | 75.03 | 405 | 1018 | 328 | 1579 | 101.58 | 44 | 245 | 289 |
| 22 | ######## | 1800 | 22535.71 | 21830 | 20871 | 224.06 | 104 | 1575.94 | 104 | 62.32 | 349 | 959 | 310 | 1939 | 143.64 | 50 | 261 | 311 |
| 23 | ######## | 1800 | 20893.15 | 20401 | 19253 | 257 | 101 | 1542.96 | 102 | 84.89 | 429 | 1148 | 342 | 1674 | 96.63 | 42 | 264 | 306 |
| 24 | ######## | 1800 | 23751.86 | 22950 | 21753 | 221.94 | 88 | 1577.95 | 88 | 74.12 | 463 | 1197 | 354 | 1646 | 99.04 | 55 | 251 | 306 |
| 25 | ######## | 1800 | 16796.04 | 18455 | 17556 | 444.79 | 135 | 1355.15 | 135 | 73.11 | 366 | 899 | 282 | 1228 | 55.81 | 31 | 183 | 214 |
| 26 | ######## | 1800 | 21820.37 | 21666 | 20676 | 261.8 | 124 | 1538.13 | 125 | 71.5 | 401 | 990 | 353 | 1540 | 84.69 | 68 | 210 | 278 |
| 27 | ######## | 1800 | 21485.98 | 22624 | 21480 | 220.3 | 102 | 1579.7 | 102 | 75.82 | 442 | 1144 | 301 | 1282 | 69.71 | 38 | 247 | 285 |
| 28 | ######## | 1800 | 16900.2 | 16227 | 15346 | 492.88 | 183 | 1306.58 | 183 | 71 | 332 | 881 | 214 | 950 | 57.21 | 40 | 178 | 218 |
| 29 | ######## | 1800 | 6400.01 | 9751 | 9084 | 708.01 | 293 | 1091.7 | 294 | 87.11 | 289 | 667 | 86 | 284 | 14.31 | 8 | 69 | 77 |
| 30 | ######## | 1800 | 1216.08 | 3168 | 2818 | 1113.95 | 336 | 685.06 | 336 | 71.55 | 168 | 350 | 2 | 2 | 0.14 | 4 | 12 | 16 |
| | | | | | | | | | 127.7917 | | | | | | | | | |

| | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 10 | ######## | 1800 | 3.91 | 309 | 169 | 1692.85 | 157 | 106.99 | 157 | 37.96 | 42 | 140 | 0 | 0 | 0 | 0 | 0 | 0 |
| 11 | ######## | 1800 | 5299.56 | 9413 | 8786 | 766.11 | 187 | 1033.66 | 188 | 82.93 | 260 | 627 | 63 | 116 | 8.35 | 27 | 33 | 60 |
| 12 | ######## | 1800 | 10543.04 | 17834 | 16897 | 327.12 | 104 | 1472.68 | 104 | 66.55 | 405 | 937 | 23 | 38 | 1.98 | 30 | 49 | 79 |
| 13 | ######## | 1800 | 13248.86 | 19088 | 18190 | 230.13 | 97 | 1568.9 | 98 | 76.97 | 373 | 898 | 17 | 30 | 1.09 | 23 | 116 | 139 |
| 14 | ######## | 1800 | 11255.34 | 16557 | 15822 | 306.12 | 115 | 1493.18 | 116 | 56.3 | 314 | 735 | 11 | 13 | 0.64 | 16 | 104 | 120 |
| 15 | ######## | 1800 | 12985.53 | 17569 | 16848 | 314.85 | 121 | 1485.05 | 122 | 59.07 | 324 | 721 | 11 | 21 | 1.34 | 19 | 123 | 142 |
| 16 | ######## | 1800 | 16792.06 | 19430 | 18553 | 180.94 | 96 | 1618.72 | 97 | 63.18 | 376 | 877 | 64 | 163 | 5.41 | 18 | 225 | 243 |
| 17 | ######## | 1800 | 16268.62 | 16601 | 15691 | 312.03 | 129 | 1487.94 | 130 | 64.55 | 383 | 910 | 109 | 400 | 13.17 | 22 | 245 | 267 |
| 18 | ######## | 1800 | 16054.65 | 15098 | 14287 | 336.52 | 124 | 1463.39 | 125 | 72.32 | 330 | 811 | 164 | 748 | 25 | 17 | 204 | 221 |
| 19 | ######## | 1800 | 14919.11 | 16961 | 15922 | 290.1 | 136 | 1509.8 | 137 | 87.29 | 462 | 1039 | 185 | 799 | 37.17 | 23 | 167 | 190 |
| 20 | ######## | 1800 | 20936.61 | 17610 | 16683 | 283.86 | 157 | 1515.99 | 157 | 74.1 | 376 | 927 | 257 | 1096 | 50.64 | 83 | 122 | 205 |
| 21 | ######## | 1800 | 14351.38 | 15745 | 14848 | 380.24 | 156 | 1419.76 | 156 | 89.76 | 383 | 897 | 216 | 947 | 39.67 | 24 | 134 | 158 |
| 22 | ######## | 1800 | 13955.41 | 12955 | 12385 | 651.54 | 144 | 1147.98 | 144 | 40.13 | 234 | 570 | 210 | 894 | 44.07 | 32 | 149 | 181 |
| 23 | ######## | 1800 | 16604.88 | 16236 | 15282 | 327.55 | 158 | 1472.45 | 158 | 77.38 | 382 | 954 | 247 | 1122 | 53.55 | 20 | 237 | 257 |
| 24 | ######## | 1800 | 8037.87 | 8037 | 7476 | 965.05 | 176 | 834.9 | 176 | 64.15 | 240 | 561 | 110 | 415 | 19.32 | 13 | 105 | 118 |
| 25 | ######## | 1800 | 1013.57 | 1945 | 1694 | 1477.08 | 129 | 322.89 | 129 | 39.85 | 95 | 251 | 7 | 4 | 0.17 | 1 | 10 | 11 |
| 26 | ######## | 1800 | 14204.18 | 17286 | 16381 | 259.09 | 125 | 1540.78 | 126 | 70.57 | 409 | 905 | 144 | 787 | 38.75 | 9 | 208 | 217 |
| 27 | ######## | 1800 | 21997.13 | 18979 | 18192 | 276.68 | 119 | 1523.31 | 120 | 57.77 | 332 | 787 | 257 | 1474 | 100.25 | 58 | 235 | 293 |
| 28 | ######## | 1800 | 19797.89 | 17318 | 16326 | 284.97 | 109 | 1514.98 | 110 | 79.7 | 408 | 992 | 215 | 1486 | 85.62 | 34 | 254 | 288 |
| 29 | ######## | 1800 | 17889.28 | 17632 | 16654 | 338.74 | 140 | 1461.25 | 141 | 79.28 | 409 | 978 | 181 | 1374 | 83.2 | 43 | 222 | 265 |
| 30 | ######## | 1800 | 17996.57 | 15003 | 13976 | 526.86 | 184 | 1273.05 | 184 | 83.24 | 355 | 1027 | 273 | 1576 | 130.85 | 65 | 168 | 233 |
| 31 | ######## | 1800 | 3625.72 | 3610 | 3359 | 1255.38 | 264 | 544.62 | 263 | 40.11 | 100 | 251 | 32 | 175 | 9 | 12 | 33 | 45 |
| 32 | ######## | 1800 | 848.86 | 1902 | 1676 | 1406.26 | 311 | 393.55 | 311 | 51.57 | 94 | 226 | 0 | 0 | 0 | 2 | 11 | 13 |
| 33 | ######## | 1800 | 943.39 | 1975 | 1696 | 1432.39 | 223 | 367.51 | 223 | 53.97 | 100 | 279 | 2 | 3 | 1.17 | 1 | 15 | 16 |
| 34 | ######## | 1800 | 16.59 | 207 | 137 | 1767.18 | 59 | 31.84 | 59 | 16.27 | 16 | 70 | 0 | 0 | 0 | 0 | 1 | 1 |
| 35 | ######## | 1800 | 0 | 15 | 15 | 1795.38 | 14 | 4.62 | 13 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 36 | ######## | 1800 | 0 | 24 | 23 | 1792.36 | 16 | 7.65 | 15 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| | | | | | | | | | 136.56 | | | | | | | | | |

## ANIMAL 3 – HSB Vertical Jumper and Pattern Runner

BEHAVIOURAL SCREENS: WEEKS ***7*** AND ***8***

| | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 3 | ######## | 1800 | 2.27 | 198 | 128 | 1718.04 | 90 | 81.96 | 89 | 20.33 | 30 | 70 | 0 | 0 | 0 | 0 | 0 | 0 |
| 4 | ######## | 1800 | 55.67 | 593 | 431 | 1618.72 | 256 | 181.28 | 255 | 31.7 | 75 | 162 | 0 | 0 | 0 | 0 | 0 | 0 |
| 5 | ######## | 1800 | 5097.86 | 8674 | 7933 | 719.88 | 233 | 1080.06 | 233 | 115.49 | 268 | 741 | 66 | 195 | 11.38 | 2 | 54 | 56 |
| 6 | ######## | 1800 | 4723.04 | 9205 | 8574 | 719.23 | 236 | 1080.01 | 236 | 92.5 | 244 | 631 | 30 | 77 | 4.38 | 11 | 25 | 36 |
| 7 | ######## | 1800 | 9474.61 | 17873 | 16967 | 284.26 | 101 | 1515.26 | 102 | 72.03 | 372 | 906 | 10 | 28 | 1.2 | 11 | 50 | 61 |
| 8 | ######## | 1800 | 9440.22 | 17699 | 16837 | 327.99 | 102 | 1471.93 | 103 | 63.6 | 372 | 862 | 4 | 12 | 0.3 | 19 | 58 | 77 |
| 9 | ######## | 1800 | 9430.72 | 15398 | 14495 | 368.62 | 118 | 1431.24 | 119 | 96.34 | 356 | 903 | 5 | 8 | 0.33 | 11 | 89 | 100 |
| 10 | ######## | 1800 | 10460.33 | 15548 | 14689 | 325.48 | 114 | 1474.51 | 115 | 63.68 | 351 | 859 | 7 | 12 | 0.44 | 8 | 116 | 124 |
| 11 | ######## | 1800 | 14342.1 | 17204 | 16208 | 297.56 | 92 | 1502.38 | 93 | 67.92 | 437 | 996 | 16 | 26 | 0.48 | 18 | 227 | 245 |
| 12 | ######## | 1800 | 15753.74 | 19024 | 18103 | 279.63 | 101 | 1520.12 | 102 | 71.52 | 405 | 921 | 77 | 171 | 11.73 | 13 | 270 | 283 |
| 13 | ######## | 1800 | 13260 | 13822 | 13150 | 585.72 | 153 | 1213.58 | 154 | 61.82 | 286 | 672 | 94 | 172 | 16.99 | 9 | 176 | 185 |
| 14 | ######## | 1800 | 18895.52 | 18895 | 18142 | 247.22 | 87 | 1552.77 | 87 | 47.06 | 311 | 753 | 215 | 751 | 48.25 | 17 | 259 | 276 |
| 15 | ######## | 1800 | 16927.8 | 15949 | 15419 | 437.23 | 125 | 1362.77 | 125 | 36.45 | 231 | 530 | 226 | 904 | 72.69 | 14 | 227 | 241 |
| 16 | ######## | 1800 | 19292.19 | 19250 | 18476 | 273.84 | 85 | 1526.14 | 86 | 62.91 | 321 | 774 | 302 | 1419 | 138.01 | 24 | 236 | 260 |
| 17 | ######## | 1800 | 18161.37 | 17669 | 16963 | 266.75 | 138 | 1533.08 | 138 | 62.93 | 297 | 706 | 171 | 1205 | 92.97 | 30 | 219 | 249 |
| 18 | ######## | 1800 | 11325.8 | 10941 | 10425 | 804.51 | 149 | 995.29 | 150 | 44.26 | 209 | 516 | 170 | 754 | 59.88 | 11 | 152 | 163 |
| 19 | ######## | 1800 | 15662.11 | 13244 | 12625 | 634.93 | 135 | 1164.93 | 135 | 68.24 | 252 | 619 | 56 | 1148 | 72.38 | 20 | 191 | 211 |
| 20 | ######## | 1800 | 16623.61 | 15970 | 15222 | 390.62 | 151 | 1409.31 | 151 | 57.91 | 288 | 748 | 234 | 1357 | 88.86 | 37 | 203 | 240 |
| 21 | ######## | 1800 | 18130.16 | 17233 | 16229 | 348.58 | 178 | 1450.82 | 179 | 79.58 | 355 | 1004 | 356 | 1639 | 120.72 | 46 | 209 | 255 |
| 22 | ######## | 1800 | 7191 | 7552 | 6995 | 961.33 | 227 | 838.57 | 227 | 82.71 | 200 | 557 | 112 | 424 | 30.53 | 28 | 60 | 88 |
| 23 | ######## | 1800 | 2154.41 | 3531 | 3131 | 1204.1 | 225 | 595.87 | 225 | 64 | 165 | 400 | 7 | 19 | 0.67 | 4 | 32 | 36 |
| 24 | ######## | 1800 | 8916.17 | 12417 | 11659 | 512.9 | 172 | 1287.08 | 173 | 70.43 | 310 | 758 | 72 | 234 | 10.36 | 15 | 132 | 147 |
| 25 | ######## | 1800 | 6209.12 | 8721 | 8223 | 693.95 | 251 | 1106.03 | 251 | 60.11 | 222 | 498 | 79 | 307 | 12.83 | 10 | 64 | 74 |
| 26 | ######## | 1800 | 586.54 | 1577 | 1215 | 1382.29 | 291 | 417.71 | 290 | 90.78 | 128 | 362 | 0 | 0 | 0 | 1 | 6 | 7 |
| 27 | ######## | 1800 | 550.37 | 1185 | 1054 | 1569.79 | 149 | 230.21 | 148 | 22.56 | 64 | 131 | 1 | 1 | 0.08 | 0 | 5 | 5 |
| | | | | | | | | | 157.4783 | | | | | | | | | |

| | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 8 | ######## | 1800 | 2.69 | 83 | 54 | 1767.69 | 51 | 32.31 | 50 | 10.52 | 13 | 29 | 0 | 0 | 0 | 0 | 0 | 0 |
| 9 | ######## | 1800 | 52.62 | 627 | 383 | 1600.07 | 212 | 199.93 | 211 | 59.74 | 77 | 244 | 0 | 0 | 0 | 0 | 0 | 0 |
| 10 | ######## | 1800 | 7837.35 | 11904 | 11105 | 491.6 | 193 | 1307.6 | 194 | 89.5 | 330 | 799 | 81 | 255 | 16.9 | 14 | 60 | 74 |
| 11 | ######## | 1800 | 9417.87 | 17103 | 16254 | 277.78 | 129 | 1522.17 | 130 | 72.78 | 353 | 849 | 24 | 52 | 3.12 | 27 | 72 | 99 |
| 12 | ######## | 1800 | 7401.33 | 15221 | 14415 | 295.92 | 130 | 1504.04 | 131 | 76.9 | 337 | 806 | 7 | 10 | 0.45 | 21 | 43 | 64 |
| 13 | ######## | 1800 | 9565.33 | 16107 | 15365 | 315.15 | 133 | 1484.81 | 133 | 75.1 | 306 | 742 | 7 | 12 | 0.44 | 17 | 90 | 107 |
| 14 | ######## | 1800 | 9203.33 | 11620 | 10793 | 547.43 | 190 | 1252.16 | 190 | 97.42 | 319 | 827 | 3 | 5 | 0.16 | 15 | 72 | 87 |
| 15 | ######## | 1800 | 30268.23 | 19948 | 19061 | 193.93 | 71 | 1606.06 | 71 | 53.67 | 346 | 887 | 19 | 31 | 0.83 | 91 | 245 | 336 |
| 16 | ######## | 1800 | 32561.12 | 21696 | 20909 | 215.62 | 70 | 1584.29 | 71 | 41.38 | 322 | 787 | 29 | 62 | 1.73 | 131 | 157 | 288 |
| 17 | ######## | 1800 | 31637.09 | 20658 | 19890 | 174.79 | 68 | 1625.14 | 69 | 51.73 | 332 | 768 | 130 | 406 | 11.99 | 178 | 113 | 291 |
| 18 | ######## | 1800 | 22880.97 | 19580 | 18613 | 200.27 | 84 | 1599.7 | 85 | 63 | 423 | 967 | 138 | 441 | 14.26 | 99 | 131 | 230 |
| 19 | ######## | 1800 | 11975.55 | 16440 | 15616 | 356.52 | 122 | 1443.34 | 123 | 80.32 | 352 | 824 | 128 | 454 | 19.16 | 17 | 108 | 125 |
| 20 | ######## | 1800 | 20800.37 | 18803 | 18010 | 195.15 | 97 | 1604.42 | 98 | 61.36 | 351 | 793 | 242 | 827 | 48.37 | 68 | 147 | 215 |
| 21 | ######## | 1800 | 20721.58 | 18715 | 17779 | 206.45 | 88 | 1593.49 | 89 | 69.75 | 398 | 936 | 204 | 1329 | 108.06 | 48 | 191 | 239 |
| 22 | ######## | 1800 | 18728.64 | 17206 | 16362 | 352.08 | 102 | 1447.74 | 103 | 64.98 | 340 | 844 | 165 | 1265 | 121.21 | 34 | 201 | 235 |
| 23 | ######## | 1800 | 18668.66 | 17536 | 16584 | 292.02 | 148 | 1507.93 | 149 | 73.87 | 361 | 952 | 165 | 1242 | 112.85 | 34 | 210 | 244 |
| 24 | ######## | 1800 | 18759.98 | 17559 | 16729 | 427.66 | 156 | 1372.29 | 157 | 53.27 | 304 | 830 | 275 | 1239 | 96.87 | 51 | 231 | 282 |
| 25 | ######## | 1800 | 15283.38 | 16139 | 15333 | 469.93 | 172 | 1329.31 | 173 | 72.92 | 327 | 806 | 184 | 734 | 45.17 | 42 | 148 | 190 |
| 26 | ######## | 1800 | 17399.52 | 16239 | 15487 | 439.12 | 133 | 1360.88 | 132 | 62.76 | 295 | 752 | 200 | 817 | 61.6 | 24 | 199 | 223 |
| 27 | ######## | 1800 | 22623.18 | 20657 | 19779 | 230.35 | 114 | 1569.6 | 114 | 58.54 | 334 | 878 | 300 | 1386 | 113.34 | 42 | 258 | 300 |
| 28 | ######## | 1800 | 19498.26 | 18016 | 17409 | 421.17 | 141 | 1378.46 | 142 | 53.49 | 214 | 607 | 277 | 1235 | 95.48 | 50 | 228 | 278 |
| 29 | ######## | 1800 | 17356.79 | 16961 | 16246 | 380.25 | 154 | 1419.35 | 155 | 70.46 | 286 | 715 | 217 | 883 | 51.48 | 38 | 192 | 230 |
| 30 | ######## | 1800 | 8765.1 | 12264 | 11599 | 614.4 | 221 | 1185.26 | 222 | 72.36 | 303 | 665 | 95 | 394 | 25.14 | 15 | 82 | 97 |
| 31 | ######## | 1800 | 4751.96 | 7281 | 6693 | 818.13 | 240 | 981.83 | 241 | 74.96 | 231 | 588 | 17 | 74 | 4.63 | 6 | 40 | 46 |
| 32 | ######## | 1800 | 203.88 | 897 | 656 | 1555.88 | 185 | 243.95 | 185 | 56.75 | 73 | 241 | 0 | 0 | 0 | 0 | 3 | 3 |
| | | | | | | | | | 137.2609 | | | | | | | | | |

## ANIMAL 4 – NSB

*(The 3 highest pattern running AND vertical activity scores of animal 4 are highlighted on the following eight sheets using two black ovals.)*

BEHAVIOURAL SCREENS: WEEKS ***1*** AND ***2***

| | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 7 | ######## | 1800 | 1.9 | 107 | 80 | 1759.53 | 64 | 40.47 | 63 | 8.63 | 13 | 27 | 0 | 0 | 0 | 0 | 0 | 0 |
| 8 | ######## | 1800 | 89.94 | 960 | 598 | 1486.1 | 234 | 313.56 | 234 | 101.08 | 115 | 362 | 0 | 0 | 0 | 0 | 1 | 1 |
| 9 | ######## | 1800 | 1626.7 | 5460 | 4498 | 899.41 | 351 | 900.34 | 352 | 180.05 | 344 | 962 | 10 | 10 | 0.38 | 8 | 17 | 25 |
| 10 | ######## | 1800 | 1643.82 | 4868 | 4110 | 1006.82 | 338 | 792.93 | 338 | 116.09 | 282 | 758 | 8 | 9 | 0.83 | 6 | 11 | 16 |
| 11 | ######## | 1800 | 745.93 | 3547 | 2707 | 1168.4 | 423 | 631.6 | 422 | 147.51 | 312 | 840 | 3 | 3 | 0.03 | 0 | 7 | 7 |
| 12 | ######## | 1800 | 998.11 | 3691 | 2870 | 1208.04 | 457 | 591.39 | 457 | 145.6 | 315 | 821 | 5 | 7 | 0.83 | 3 | 13 | 16 |
| 13 | ######## | 1800 | 45.19 | 528 | 353 | 1629.73 | 172 | 170.27 | 171 | 44.59 | 60 | 175 | 0 | 0 | 0 | 0 | 0 | 0 |
| 14 | ######## | 1800 | 415.96 | 1708 | | | | | | | | | | 5 | 0.03 | 0 | 3 | 3 |
| 15 | ######## | 1800 | 45.41 | 717 | | | | | | | | | | 0 | 0 | 0 | 0 | 0 |
| 16 | ######## | 1800 | 5.02 | 111 | | | | | | | | | | 0 | 0 | 0 | 0 | 0 |
| 17 | ######## | 1800 | 626.05 | 2182 | | | | | | | | | | 2 | 0 | 1 | 7 | 8 |
| 18 | ######## | 1800 | 871.64 | 3231 | | | | | | | | | | 1 | 0 | 0 | 4 | 4 |
| 19 | ######## | 1800 | 231.51 | 1133 | | | | | | | | | | 1 | 0.06 | 2 | 1 | 3 |
| 20 | ######## | 1800 | 761.35 | 2548 | | | | | | | | | | 6 | 0.23 | 2 | 9 | 11 |
| 21 | ######## | 1800 | 893.23 | 2726 | | | | | | | | | | 3 | 1.5 | 1 | 6 | 7 |
| 22 | ######## | 1800 | 671.83 | 2095 | | | | | | | | | | 1 | 0 | 0 | 1 | 1 |
| 23 | ######## | 1800 | 1575.52 | 3629 | | | | | | | | | | 15 | 0.41 | 5 | 14 | 19 |
| 24 | ######## | 1800 | 923.28 | 2555 | | | | | | | | | | 4 | 0.28 | 0 | 7 | 7 |
| 25 | ######## | 1800 | 1112.47 | 2926 | | | | | | | | | | 1 | 0.02 | 0 | 12 | 12 |
| 26 | ######## | 1800 | 1088.36 | 2920 | 2638 | 1391.75 | 266 | 408.25 | 265 | 50.1 | 113 | 282 | 1 | 1 | 0 | 2 | 5 | 7 |
| 27 | ######## | 1800 | 1454.35 | 3654 | 3152 | 1221.06 | 347 | 578.94 | 346 | 106.89 | 169 | 502 | 8 | 11 | 1.7 | 3 | 14 | 17 |
| 28 | ######## | 1800 | 877.71 | 2383 | 2122 | 1398.66 | 274 | 401.28 | 274 | 49.12 | 101 | 261 | 1 | 1 | 0 | 1 | 8 | 9 |
| 29 | ######## | 1800 | 1349.67 | 3404 | 3140 | 1333.58 | 231 | 465.73 | 232 | 43.04 | 112 | 264 | 3 | 8 | 0.67 | 3 | 11 | 14 |
| 30 | ######## | 1800 | 2528.13 | 6696 | 6031 | 807.88 | 310 | 991.72 | 310 | 114.51 | 250 | 665 | 21 | 28 | 2.08 | 11 | 18 | 29 |
| 31 | ######## | 1800 | 2488.79 | 5573 | 4783 | 978.63 | 345 | 821.37 | 344 | 134.32 | 283 | 790 | 23 | 41 | 3.9 | 20 | 1 | 37 |
| 32 | ######## | 1800 | 2063.36 | 5231 | 4601 | 1026.18 | 331 | 773.02 | 331 | 107.1 | 243 | 630 | 18 | 19 | 1.06 | 5 | 13 | 18 |
| | | | | | | | | | 287.875 | | | | | | | | | |

*Animals classified as NSB, express negligible amounts of stereotypy over both of the cohorts as compared to their HSB counterparts. This is demonstrated by the black ovals on the following eight sheets. Note that animals had to demonstrate NSB behaviour over both of the topographies to be included in the NSB cohort.*

| | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 3 | ######## | 1800 | 0.9 | 134 | 88 | 1752.48 | 74 | 46.79 | 74 | 11.36 | 19 | 46 | 0 | 0 | 0 | 0 | 0 | 0 |
| 4 | ######## | 1800 | 1630.14 | 4284 | 3764 | 1184.4 | 301 | 615.42 | 301 | 81.76 | 196 | 520 | 6 | 6 | 0.19 | 3 | 13 | 16 |
| 5 | ######## | 1800 | 982.14 | 2760 | 2402 | 1363.25 | 228 | 436.66 | 229 | 69.75 | 113 | 358 | 7 | 8 | 0.83 | 0 | 4 | 4 |
| 6 | ######## | 1800 | 1276.99 | 3196 | 2864 | 1395.8 | 198 | 404.2 | 197 | 56.59 | 117 | 332 | 2 | 2 | 0.09 | 1 | 15 | 16 |
| 7 | ######## | 1800 | 1499.74 | 3588 | 3361 | 1376.47 | 193 | 423.31 | 193 | 27.77 | 96 | 227 | 7 | 8 | 0.41 | 1 | 11 | 12 |
| 8 | ######## | 1800 | 634.39 | 2515 | 2044 | 1348.2 | 250 | 451.8 | 249 | 89.94 | 155 | 471 | 2 | 2 | 0 | 0 | 6 | 6 |
| 9 | ######## | 1800 | 27.35 | 406 | 241 | 1688.45 | 157 | 111.55 | 156 | 36.17 | 38 | 165 | 0 | 0 | 0 | 0 | 0 | 0 |
| 10 | ######## | 1800 | 853.44 | 2206 | 1963 | 1480.19 | 185 | 319.8 | 185 | 38.64 | 94 | 243 | 3 | 6 | 1.06 | 1 | 9 | 10 |
| 11 | ######## | 1800 | 1197.89 | 3167 | 2834 | 1346.81 | 224 | 453.18 | 224 | 55.04 | 133 | 333 | 6 | 6 | 0.23 | 0 | 12 | 12 |
| 12 | ######## | 1800 | 182.79 | 813 | 681 | 1613.07 | 166 | 186.93 | 165 | 33.83 | 60 | 132 | 1 | 1 | 0.05 | 0 | 1 | 1 |
| 13 | ######## | 1800 | 669.94 | 2406 | 1895 | 1346.41 | 283 | 453.26 | 283 | 107.18 | 180 | 511 | 6 | 6 | 0.84 | 1 | 8 | 9 |
| 14 | ######## | 1800 | 628.85 | 2356 | 1895 | 1330.58 | 308 | 469.42 | 307 | 108.52 | 158 | 461 | 1 | 1 | 0.31 | 2 | 6 | 8 |
| 15 | ######## | 1800 | 196.09 | 914 | 677 | 1571.04 | 199 | 228.96 | 198 | 58.49 | 75 | 237 | 1 | 1 | 0.05 | 0 | 0 | 0 |
| 16 | ######## | 1800 | 221.05 | 1387 | 1006 | 1516.69 | 224 | 283.31 | 223 | 73.68 | 114 | 381 | 0 | 0 | 0 | 0 | 3 | 3 |
| 17 | ######## | 1800 | 235.69 | 1546 | 1300 | 1527.1 | 224 | 272.9 | 223 | 59.55 | 96 | 246 | 1 | 1 | 0.11 | 0 | 2 | 2 |
| 18 | ######## | 1800 | 1410.58 | 4252 | 3775 | 1172.91 | 328 | 627.09 | 327 | 77.23 | 196 | 477 | 16 | 25 | 1.45 | 9 | 9 | 18 |
| 19 | ######## | 1800 | 1043.5 | 3344 | 2861 | 1243.53 | 363 | 556.2 | 363 | 88.19 | 186 | 483 | 7 | 7 | 0.34 | 4 | 9 | 13 |
| 20 | ######## | 1800 | 406.11 | 1656 | 1454 | 1558.54 | 215 | 241.32 | 215 | 44.85 | 72 | 202 | 3 | 3 | 0.05 | 0 | 5 | 5 |
| 21 | ######## | 1800 | 509.7 | 1804 | 1574 | 1528.14 | 213 | 271.15 | 213 | 35.74 | 90 | 230 | 0 | 0 | 0 | 0 | 3 | 3 |
| 22 | ######## | 1800 | 649.06 | 2234 | 1972 | 1416.21 | 264 | 383.5 | 264 | 45.88 | 109 | 262 | 1 | 1 | 0 | 0 | 6 | 6 |
| 23 | ######## | 1800 | 346.05 | 1874 | 1484 | 1428.47 | 282 | 371.52 | 282 | 91.37 | 138 | 390 | 2 | 5 | 0.26 | 0 | 0 | 0 |
| 24 | ######## | 1800 | 871.03 | 2894 | 2314 | 1227.51 | 308 | 572.24 | 308 | 120.67 | 194 | 580 | 2 | 4 | 0.19 | 1 | 4 | 5 |
| 25 | ######## | 1800 | 955.69 | 3466 | 2885 | 1143.31 | 409 | 656.58 | 410 | 105.84 | 202 | 581 | 11 | 14 | 1.01 | 4 | 7 | 11 |
| 26 | ######## | 1800 | 855.95 | 2641 | 2233 | 1308.04 | 288 | 491.96 | 287 | 87.15 | 142 | 408 | 10 | 11 | 1.14 | 3 | 6 | 9 |
| 27 | ######## | 1800 | 292.22 | 1561 | 1285 | 1401.73 | 303 | 398.06 | 303 | 60.34 | 115 | 276 | 2 | 2 | 0.56 | 0 | 3 | 3 |
| 28 | ######## | 1800 | 787.64 | 2815 | 2525 | 1359.43 | 265 | 440.57 | 264 | 64.26 | 105 | 290 | 4 | 4 | 0.3 | 4 | 7 | 11 |
| | | | | | | | | | 252.8333 | | | | | | | | | |

## ANIMAL 4 – NSB

BEHAVIOURAL SCREENS: WEEKS ***3*** AND ***4***

| | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | ######## | 1800 | 366.66 | 1673 | 1275 | 1329.48 | 260 | 469.71 | 260 | 105.83 | 133 | 398 | 1 | 1 | 0 | 1 | 1 | 1 |
| 2 | ######## | 1800 | 66.56 | 589 | 439 | 1615.37 | 165 | 184.63 | 164 | 35.07 | 55 | 150 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3 | ######## | 1800 | 1016.08 | 3504 | 2948 | 1200.44 | 292 | 598.72 | 293 | 103 | 203 | 556 | 14 | 16 | 1.79 | 6 | 8 | 11 |
| 4 | ######## | 1800 | 819.4 | 2360 | 2158 | 1426.95 | 255 | 372.18 | 255 | 21.85 | 92 | 202 | 3 | 3 | 0.05 | 0 | 5 | 6 |
| 5 | ######## | 1800 | 916.29 | 2983 | 2356 | 1197.46 | 332 | 601.4 | 333 | 142.52 | 184 | 627 | 16 | 17 | 1.65 | 5 | 2 | 7 |
| 6 | ######## | 1800 | 962.01 | 3302 | 2725 | 1098.55 | 368 | 700.94 | 368 | 119.32 | 210 | 577 | 11 | 16 | 1.86 | 1 | 8 | 9 |
| 7 | ######## | 1800 | 1.9 | 38 | 35 | 1783.42 | 29 | 16.58 | 28 | 0.27 | 3 | 3 | 0 | 0 | 0 | 0 | 0 | 0 |
| 8 | ######## | 1800 | 650.46 | 2083 | 1596 | 1339.38 | 285 | 460.62 | 284 | 103.21 | 142 | 487 | 4 | 5 | 0.8 | 0 | 4 | 4 |
| 9 | ######## | 1800 | 491.19 | 1725 | 1394 | 1418.76 | 295 | 381.24 | 294 | 56.79 | 130 | 331 | 5 | 7 | 1.66 | 1 | 5 | 6 |
| 10 | ######## | 1800 | 402.04 | 1375 | 1098 | 1522.82 | 201 | 276.76 | 201 | 64.86 | 105 | 277 | 8 | 9 | 0.56 | 0 | 4 | 4 |
| 11 | ######## | 1800 | 1191.28 | 3236 | 2911 | 1269.55 | 304 | 530.95 | 303 | 52.72 | 141 | 325 | 13 | 14 | 1.95 | 1 | 9 | 10 |
| 12 | ######## | 1800 | 61.69 | 823 | 482 | 1519.7 | 235 | 279.38 | 235 | 91.44 | 116 | 341 | 0 | 0 | 0 | 0 | 1 | 1 |
| 13 | ######## | 1800 | 737.8 | 2543 | 2116 | 1296.98 | 290 | 502.66 | 290 | 85.7 | 155 | 427 | 16 | 24 | 2.56 | 0 | 2 | 2 |
| 14 | ######## | 1800 | 393.88 | 1786 | 1461 | 1461.82 | 306 | 338.06 | 306 | 46.67 | 110 | 325 | 4 | 7 | 2.26 | 0 | 4 | 4 |
| 15 | ######## | 1800 | 351.72 | 1050 | 829 | 1606.15 | 189 | 193.75 | 189 | 53.52 | 70 | 221 | 4 | 7 | 1.05 | 0 | 4 | 4 |
| 16 | ######## | 1800 | 1065.78 | 2678 | 2418 | 1369.68 | 277 | 430.3 | 278 | 46.27 | 114 | 260 | 9 | 17 | 1.84 | 0 | 8 | 8 |
| 17 | ######## | 1800 | 1188.25 | 3338 | 2943 | 1217.74 | 313 | 582.23 | 313 | 80.59 | 164 | 395 | 9 | 16 | 2.09 | 2 | 10 | 12 |
| 18 | ######## | 1800 | 625.17 | 1844 | 1647 | 1491.28 | 250 | 308.6 | 250 | 30.48 | 89 | 197 | 5 | 5 | 0.14 | 2 | 1 | 4 |
| 19 | ######## | 1800 | 594.04 | 1395 | 1266 | 1579.43 | 209 | 220.37 | 209 | 26.99 | 58 | 129 | 2 | 2 | 0.16 | 0 | 4 | 4 |
| 20 | ######## | 1800 | 894.3 | 2038 | 1832 | 1479.41 | 243 | 319.71 | 243 | 32.64 | 98 | 206 | 3 | 3 | 0.05 | 1 | 8 | 9 |
| 21 | ######## | 1800 | 995.38 | 2752 | 2383 | 1348.32 | 293 | 451.56 | 293 | 70.34 | 148 | 369 | 13 | 20 | 1.4 | 2 | 7 | 9 |
| 22 | ######## | 1800 | 800.83 | 2345 | 2031 | 1320.11 | 379 | 479.89 | 378 | 55.75 | 130 | 314 | 5 | 8 | 2.06 | 0 | 10 | 10 |
| 23 | ######## | 1800 | 789.31 | 2356 | 1976 | 1339.12 | 285 | 460.55 | 285 | 77.84 | 145 | 380 | 9 | 13 | 1.34 | 1 | 2 | 3 |
| 24 | ######## | 1800 | 598 | 2321 | 1911 | 1220.4 | 446 | 579.53 | 446 | 90.62 | 171 | 410 | 3 | 3 | 0.05 | 2 | 4 | 6 |
| 25 | ######## | 1800 | 448.67 | 1580 | 1249 | 1452.18 | 282 | 347.76 | 283 | 64.58 | 113 | 331 | 2 | 3 | 0.03 | 2 | 3 | 5 |
| 26 | ######## | 1800 | 196.53 | 1085 | 907 | 1498.36 | 315 | 301.58 | 315 | 43.17 | 85 | 178 | 2 | 2 | 0.33 | 0 | 1 | 1 |
| | | | | | | | | | 278 | | | | | | | | | |

| | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 7 | ######## | 1800 | 0 | 202 | 86 | 1736.01 | 72 | 63.99 | 71 | 30.34 | 29 | 116 | 0 | 0 | 0 | 0 | 0 | 0 |
| 8 | ######## | 1800 | 117.34 | 771 | 510 | 1548.51 | 228 | 250.18 | 229 | 82.12 | 79 | 261 | 1 | 1 | 0 | 0 | 1 | 1 |
| 9 | ######## | 1800 | 995.94 | 3872 | 3101 | 1120.99 | 351 | 678.9 | 351 | 130.45 | 261 | 771 | 11 | 15 | 2.5 | 3 | 9 | 12 |
| 10 | ######## | 1800 | 688.01 | 2154 | 1866 | 1436.66 | 239 | 363.03 | 239 | 47.94 | 132 | 288 | 4 | 5 | 0.33 | 3 | 4 | 7 |
| 11 | ######## | 1800 | 949.22 | 2734 | 2300 | 1360.59 | 278 | 439.19 | 278 | 72.4 | 146 | 434 | 13 | 14 | 1.19 | 1 | 5 | 6 |
| 12 | ######## | 1800 | 433.5 | 1527 | 1277 | 1466.8 | 267 | 333.2 | 266 | 52.42 | 94 | 250 | 3 | 6 | 1.15 | 0 | 3 | 3 |
| 13 | ######## | 1800 | 309.72 | 2458 | 1414 | 1211.35 | 354 | 588.58 | 354 | 176.94 | 221 | 1044 | 5 | 5 | 0.41 | 0 | 1 | 1 |
| 14 | ######## | 1800 | 80.44 | 571 | 423 | 1690.93 | 110 | 108.71 | 110 | 27.28 | 46 | 148 | 1 | 1 | 0.06 | 0 | 1 | 1 |
| 15 | ######## | 1800 | 565.04 | 1734 | 1446 | 1414.45 | 287 | 385.55 | 286 | 53.57 | 122 | 288 | 3 | 3 | 0.17 | 2 | 3 | 5 |
| 16 | ######## | 1800 | 46.59 | 589 | 295 | 1620.02 | 188 | 179.73 | 188 | 73.59 | 67 | 294 | 0 | 0 | 0 | 1 | 0 | 1 |
| 17 | ######## | 1800 | 543.54 | 1895 | 1508 | 1393.63 | 289 | 406.25 | 289 | 71.58 | 149 | 387 | 2 | 3 | 0.08 | 0 | 3 | 3 |
| 18 | ######## | 1800 | 638.56 | 2012 | 1665 | 1376.98 | 320 | 422.52 | 320 | 55.72 | 128 | 347 | 2 | 2 | 0.16 | 0 | 4 | 4 |
| 19 | ######## | 1800 | 1046.42 | 3010 | 2544 | 1276.09 | 300 | 523.91 | 299 | 93.92 | 170 | 466 | 5 | 5 | 0.19 | 2 | 3 | 5 |
| 20 | ######## | 1800 | 53.52 | 826 | 430 | 1533 | 241 | 267 | 240 | 109.68 | 116 | 396 | 0 | 0 | 0 | 0 | 0 | 0 |
| 21 | ######## | 1800 | 530.08 | 2334 | 1719 | 1331.96 | 317 | 467.86 | 317 | 99.36 | 143 | 615 | 1 | 1 | 0.33 | 1 | 4 | 5 |
| 22 | ######## | 1800 | 594.51 | 1917 | 1668 | 1432.34 | 290 | 367.02 | 291 | 31.7 | 116 | 249 | 3 | 3 | 0.17 | 1 | 3 | 4 |
| 23 | ######## | 1800 | 1387.63 | 4307 | 3696 | 1003 | 374 | 796.44 | 375 | 134.31 | 229 | 611 | 20 | 32 | 5.8 | 3 | 6 | 9 |
| 24 | ######## | 1800 | 440.26 | 3499 | 2587 | 1165.11 | 400 | 634.74 | 400 | 175.52 | 248 | 912 | 5 | 6 | 0.94 | 1 | 2 | 3 |
| 25 | ######## | 1800 | 447.35 | 1708 | 1345 | 1452.26 | 281 | 347.37 | 281 | 80.09 | 120 | 363 | 3 | 3 | 0.39 | 1 | 5 | 6 |
| 26 | ######## | 1800 | 569.93 | 1898 | 1681 | 1442.46 | 260 | 357.54 | 259 | 46.38 | 100 | 217 | 6 | 6 | 0.25 | 1 | 2 | 3 |
| 27 | ######## | 1800 | 946.66 | 3034 | 2550 | 1261.5 | 282 | 538.39 | 282 | 116.14 | 147 | 484 | 22 | 27 | 3.76 | 1 | 8 | 9 |
| 28 | ######## | 1800 | 420.89 | 1850 | 1617 | 1466.35 | 283 | 333.65 | 282 | 43.48 | 105 | 233 | 1 | 2 | 0.06 | 0 | 4 | 4 |
| 29 | ######## | 1800 | 457.69 | 1886 | 1647 | 1507.61 | 257 | 292.39 | 256 | 35.48 | 99 | 239 | 3 | 5 | 0.13 | 1 | 4 | 5 |
| 30 | ######## | 1800 | 576.01 | 2044 | 1815 | 1436.49 | 261 | 363.54 | 260 | 38.23 | 102 | 229 | 9 | 14 | 1.37 | 1 | 6 | 7 |
| 31 | ######## | 1800 | 775.33 | 3072 | 2646 | 1200.55 | 432 | 599.29 | 432 | 81.03 | 181 | 426 | 7 | 14 | 2.05 | 1 | 7 | 8 |
| | | | | | | | | | 289.3478 | | | | | | | | | |

## ANIMAL 4 – NSB

BEHAVIOURAL SCREENS: WEEKS ***5*** AND ***6***

| | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 3 | ######## | 1800 | 135.57 | 1024 | 663 | 1505.94 | 211 | 294.06 | 210 | 79.79 | 120 | 361 | 5 | 5 | 0.72 | 0 | 1 | 1 |
| 4 | ######## | 1800 | 209.61 | 1384 | 895 | 1470.46 | 170 | 329.19 | 170 | 106.51 | 150 | 489 | 7 | 10 | 1.59 | 0 | 1 | 1 |
| 5 | ######## | 1800 | 710.07 | 3779 | 2968 | 1242.82 | 225 | 557.04 | 226 | 94.21 | 251 | 811 | 9 | 9 | 0.98 | 0 | 6 | 6 |
| 6 | ######## | 1800 | 447.04 | 1878 | 1684 | 1506.28 | 234 | 293.63 | 234 | 32.12 | 88 | 194 | 6 | 8 | 0.91 | 2 | 2 | 4 |
| 7 | ######## | 1800 | 864.2 | 2291 | 2064 | 1471.49 | 205 | 326.95 | 206 | 34.59 | 86 | 227 | 3 | 3 | 0.08 | 1 | 8 | 9 |
| 8 | ######## | 1800 | 727.34 | 2246 | 1980 | 1444.81 | 216 | 355.18 | 216 | 52.06 | 105 | 266 | 5 | 5 | 0.59 | 1 | 8 | 9 |
| 9 | ######## | 1800 | 910.12 | 2565 | 2301 | 1411.59 | 274 | 388.41 | 274 | 41.98 | 120 | 264 | 8 | 10 | 1.37 | 1 | 7 | 8 |
| 10 | ######## | 1800 | 677.93 | 2388 | 2020 | 1391.79 | 253 | 408.21 | 252 | 76.01 | 116 | 368 | 3 | 3 | 0.19 | 0 | 4 | 4 |
| 11 | ######## | 1800 | 742.21 | 2841 | 2117 | 1245.15 | 320 | 554.79 | 320 | 167.83 | 188 | 724 | 9 | 15 | 2.06 | 1 | 6 | 7 |
| 12 | ######## | 1800 | 572.56 | 1570 | 1374 | 1579.46 | 70 | 220.41 | 70 | 26.36 | 71 | 196 | 5 | 7 | 1.92 | 2 | 4 | 6 |
| 13 | ######## | 1800 | 4.24 | 109 | 72 | 1778.53 | 42 | 20.75 | 42 | 12.75 | 8 | 37 | 0 | 0 | 0 | 0 | 0 | 0 |
| 14 | ######## | 1800 | 762.28 | 2382 | 2026 | 1361.75 | 306 | 438.25 | 305 | 78.76 | 110 | 356 | 7 | 7 | 0.44 | 1 | 5 | 6 |
| 15 | ######## | 1800 | 810.62 | 2274 | 2058 | 1449.06 | 277 | 350 | 277 | 35.02 | 81 | 216 | [illegible] | 8 | 0.58 | 1 | 4 | 5 |
| 16 | ######## | 1800 | 998.9 | 3016 | 2383 | 1301.23 | 291 | 498.77 | 290 | 136.56 | 159 | 633 | 13 | 21 | 2.58 | 1 | 5 | 6 |
| 17 | ######## | 1800 | 560.66 | 2262 | 1893 | 1358.94 | 310 | 440.41 | 310 | 64.26 | 144 | 369 | 6 | 8 | 1.08 | 0 | 5 | 5 |
| 18 | ######## | 1800 | 1261.09 | 3376 | 2974 | 1291.54 | 344 | 508.46 | 343 | 59.01 | 158 | 402 | 10 | 16 | 0.76 | 6 | 2 | 8 |
| 19 | ######## | 1800 | 1151.6 | 2700 | 2282 | 1330.79 | 322 | 469.19 | 322 | 84.3 | 151 | 418 | 12 | 20 | 4.51 | 6 | 8 | 14 |
| 20 | ######## | 1800 | 761.82 | 2178 | 1831 | 1420.5 | 286 | 379.15 | 286 | 51.95 | 113 | 347 | 12 | 16 | 1.65 | 2 | 4 | 6 |
| 21 | ######## | 1800 | 1498.48 | 3995 | 3434 | 1191.81 | 308 | 607.74 | 309 | 109.45 | 168 | 561 | 14 | 45 | 3.62 | 2 | 12 | 14 |
| 22 | ######## | 1800 | 1073.92 | 2133 | 1707 | 1402.43 | 311 | 397.57 | 310 | 84.93 | 149 | 426 | [illegible] | 16 | 1.37 | 9 | 6 | 15 |
| 23 | ######## | 1800 | 1804.11 | 2269 | 2066 | 1521.3 | 257 | 278.54 | 257 | 32.53 | 79 | 203 | 10 | 18 | 1.18 | 11 | 15 | 26 |
| 24 | ######## | 1800 | 889.67 | 1559 | 1312 | 1552.59 | 293 | 246.83 | 294 | 43.33 | 85 | 247 | 3 | 4 | 0.87 | 6 | 8 | 14 |
| 25 | ######## | 1800 | 1236.12 | 1993 | 1720 | 1495.72 | 327 | 304.06 | 327 | 46.8 | 112 | 273 | 3 | 4 | 0.42 | 4 | 6 | 10 |
| 26 | ######## | 1800 | 658.18 | 1950 | 1639 | 1454.47 | 340 | 345.53 | 339 | 63.62 | 114 | 311 | 3 | 3 | 0.2 | 1 | 7 | 8 |
| 27 | ######## | 1800 | 223.34 | 965 | 680 | 1590.01 | 238 | 209.99 | 237 | 58.51 | 65 | 285 | 1 | 1 | 0 | 1 | 1 | 2 |
| | | | | | | | | | 262.8696 | | | | | | | | | |

| | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 2 | ######## | 1800 | 32.22 | 446 | 264 | 1665.65 | 148 | 134.35 | 147 | 34.77 | 56 | 182 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3 | ######## | 1800 | 454.17 | 1956 | 1414 | 1393.33 | 234 | 405.92 | 234 | 106.9 | 152 | 542 | 10 | 11 | 1.06 | 3 | 2 | 5 |
| 4 | ######## | 1800 | 970.3 | 4060 | 3198 | 1220.64 | 321 | 579.06 | 322 | 104.8 | 267 | 862 | 9 | 10 | 1.2 | 2 | 8 | 10 |
| 5 | ######## | 1800 | 1101.68 | 2679 | 2251 | 1393.94 | 311 | 405.82 | 311 | 72.33 | 145 | 428 | 11 | 13 | 2.57 | 4 | 12 | 16 |
| 6 | ######## | 1800 | 729.79 | 2717 | 2356 | 1339.04 | 311 | 459.5 | 312 | 69.15 | 141 | 361 | 8 | 8 | 0.55 | 2 | 6 | 8 |
| 7 | ######## | 1800 | 349.04 | 1363 | 1160 | 1534.34 | 206 | 264.91 | 206 | 40.03 | 83 | 203 | [illegible] | 13 | 2.84 | 0 | [illegible] | 2 |
| 8 | ######## | 1800 | 301.95 | 1485 | 977 | 1408.33 | 295 | 391.67 | 294 | 129.69 | 134 | 508 | 3 | 3 | 0 | 2 | 3 | 5 |
| 9 | ######## | 1800 | 99.77 | 532 | 382 | 1636.23 | 146 | 163.55 | 146 | 43.36 | 50 | 150 | 0 | 0 | 0 | 0 | 1 | 1 |
| 10 | ######## | 1800 | 1038.12 | 2943 | 2552 | 1350.29 | 265 | 449.63 | 265 | 71.2 | 135 | 391 | 13 | 18 | 2.31 | 1 | 7 | 8 |
| 11 | ######## | 1800 | 629.07 | 2130 | 1756 | 1420.21 | 263 | 379.79 | 262 | 69.25 | 150 | 374 | 7 | 11 | 2 | 1 | 3 | 4 |
| 12 | ######## | 1800 | 30.66 | 499 | 381 | 1628.09 | 197 | 171.91 | 196 | 31.06 | 52 | 118 | 0 | 0 | 0 | 0 | 0 | 0 |
| 13 | ######## | 1800 | 542.52 | 1733 | 1320 | 1407.47 | 316 | 392.53 | 315 | 77.51 | 138 | 413 | 5 | 5 | 0.39 | 3 | 2 | 5 |
| 14 | ######## | 1800 | 277 | 1366 | 893 | 1466.18 | 239 | 333.79 | 239 | 111.27 | 151 | 473 | 3 | 4 | 1.09 | 0 | 3 | 3 |
| 15 | ######## | 1800 | 287.17 | 1905 | 1243 | 1325.75 | 375 | 474.24 | 375 | 151.08 | 177 | 662 | 3 | 3 | 0.44 | 0 | 3 | 3 |
| 16 | ######## | 1800 | 662.81 | 1945 | 1663 | 1470.6 | 246 | 329.4 | 245 | 48.46 | 109 | 282 | 7 | 9 | 1.05 | 1 | 4 | 5 |
| 17 | ######## | 1800 | 0 | 141 | 100 | 1754.04 | 65 | 45.96 | 64 | 8.44 | 18 | 41 | 0 | 0 | 0 | 0 | 0 | 0 |
| 18 | ######## | 1800 | 638.47 | 3803 | 2672 | 1313.28 | 360 | 487.22 | 359 | 121.56 | 221 | 1131 | 10 | 11 | 0.81 | 0 | 4 | 4 |
| 19 | ######## | 1800 | 540.07 | 2110 | 1798 | 1347.53 | 357 | 452.16 | 357 | 61.12 | 137 | 312 | 9 | 12 | 1.67 | 1 | 4 | 5 |
| 20 | ######## | 1800 | 569.75 | 2520 | 1975 | 1293.25 | 379 | 506.03 | 379 | 94.48 | 153 | 545 | 9 | 11 | 1.67 | 3 | 3 | 6 |
| 21 | ######## | 1800 | 355.83 | 1346 | 1159 | 1508.05 | 251 | 291.95 | 250 | 46.69 | 83 | 187 | 3 | 3 | 0.11 | 1 | 3 | 4 |
| 22 | ######## | 1800 | 864.4 | 2787 | 2492 | 1345.3 | 350 | 453.87 | 351 | 46.81 | 135 | 295 | 13 | 25 | 1.01 | 4 | 3 | 7 |
| 23 | ######## | 1800 | 406.69 | 1796 | 1620 | 1582.57 | 295 | 216.63 | 295 | 33.92 | 73 | 176 | 5 | [illegible] | 0.65 | 0 | 4 | 4 |
| 24 | ######## | 1800 | 677.87 | 2141 | 1912 | 1469.49 | 318 | 330.51 | 317 | 46.54 | 86 | 229 | 3 | 8 | 0.17 | 2 | 6 | 8 |
| 25 | ######## | 1800 | 494.75 | 1917 | 1717 | 1494.5 | 347 | 305.5 | 346 | 30.05 | 80 | 200 | 8 | 10 | 1.64 | 0 | 5 | 5 |
| 26 | ######## | 1800 | 758.48 | 2262 | 1977 | 1386.7 | 354 | 413.07 | 354 | 58.38 | 115 | 285 | 5 | 6 | 0.64 | 1 | 6 | 7 |
| | | | | | | | | | 285.2174 | | | | | | | | | |

## ANIMAL 4 – NSB

BEHAVIOURAL SCREENS: WEEKS ***7*** AND ***8***

| | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 7 | ######## | 1800 | 0 | 231 | 125 | 1726.71 | 119 | 73.29 | 118 | 26.41 | 27 | 106 | 0 | 0 | 0 | 0 | 0 | 0 |
| 8 | ######## | 1800 | 53.53 | 591 | 354 | 1593.77 | 184 | 206.23 | 183 | 70.63 | 72 | 237 | 0 | 0 | 0 | 0 | 0 | 0 |
| 9 | ######## | 1800 | 1058.5 | 2681 | 2303 | 1310.21 | 303 | 489.51 | 303 | 62.34 | 151 | 378 | 11 | 11 | 0.76 | 5 | 4 | 9 |
| 10 | ######## | 1800 | 1344.01 | 2957 | 2693 | 1365.15 | 260 | 434.26 | 260 | 32.14 | 108 | 264 | 18 | 30 | 2.48 | 2 | 10 | 12 |
| 11 | ######## | 1800 | 1689.77 | 3473 | 3181 | 1288.28 | 280 | 511.7 | 280 | 42.36 | 128 | 292 | 13 | 14 | 0.7 | 0 | 20 | 20 |
| 12 | ######## | 1800 | 1064.04 | 2935 | 2603 | 1351.46 | 276 | 448.54 | 275 | 65.05 | 122 | 332 | 23 | 35 | 1.87 | 0 | 6 | 6 |
| 13 | ######## | 1800 | 651.02 | 2191 | 1863 | 1451.15 | 266 | 348.85 | 265 | 60.92 | 112 | 328 | 9 | 11 | 0.85 | 1 | 8 | 9 |
| 14 | ######## | 1800 | 133.57 | 751 | 455 | 1615.45 | 227 | 184.55 | 226 | 76.38 | 74 | 296 | 0 | 0 | 0 | 0 | 1 | 1 |
| 15 | ######## | 1800 | 519.05 | 1921 | 1442 | 1392.39 | 291 | 406.94 | 291 | 110.01 | 152 | 479 | 2 | 3 | 0.61 | 3 | 4 | 7 |
| 16 | ######## | 1800 | 365.95 | 1377 | 1175 | 1505.78 | 224 | 293.48 | 224 | 40.59 | 80 | 202 | 8 | 12 | 0.95 | 1 | 1 | 2 |
| 17 | ######## | 1800 | 461.45 | 2241 | 1675 | 1324.61 | 408 | 474.76 | 408 | 121.74 | 171 | 566 | 5 | 5 | 0.45 | 0 | 2 | 2 |
| 18 | ######## | 1800 | 1119.42 | 3315 | 2832 | 1251.17 | 311 | 548.57 | 311 | 87.52 | 167 | 483 | 22 | 49 | 6.08 | 3 | 10 | 13 |
| 19 | ######## | 1800 | 113.26 | 521 | 428 | 1652.24 | 189 | 147.76 | 188 | 21.06 | 39 | 93 | 0 | 0 | 0 | 0 | 0 | 0 |
| 20 | ######## | 1800 | 414.96 | 2734 | 2195 | 1231.69 | 393 | 568.3 | 393 | 99.34 | 190 | 539 | 0 | 0 | 0 | 2 | 3 | 5 |
| 21 | ######## | 1800 | 667.18 | 3085 | 2516 | 1266.13 | 370 | 532.86 | 371 | 74.68 | 168 | 569 | 1 | 18 | 3.5 | 4 | 3 | 7 |
| 22 | ######## | 1800 | 1480.13 | 3312 | 2922 | 1250.44 | 330 | 549.26 | 330 | 71.03 | 155 | 390 | 31 | 72 | 7.22 | 8 | 11 | 19 |
| 23 | ######## | 1800 | 501.09 | 1462 | 1228 | 1488.75 | 279 | 311.14 | 279 | 55.15 | 105 | 234 | 2 | 2 | 0.09 | 1 | 6 | 7 |
| 24 | ######## | 1800 | 403.33 | 1661 | 1394 | 1450.7 | 230 | 349.17 | 230 | 45.07 | 111 | 267 | 8 | 8 | 0.25 | 1 | 3 | 4 |
| 25 | ######## | 1800 | 1311.36 | 3750 | 2844 | 1113.07 | 359 | 686.4 | 359 | 172.53 | 231 | 906 | 30 | 75 | 6.08 | 3 | 10 | 13 |
| 26 | ######## | 1800 | 1562.9 | 3719 | 3281 | 1186.39 | 337 | 613.47 | 337 | 80.04 | 161 | 438 | 20 | 53 | 4.16 | 7 | 9 | 16 |
| 27 | ######## | 1800 | 480.11 | 1978 | 1610 | 1370.48 | 346 | 428.79 | 346 | 81.5 | 138 | 368 | 3 | 8 | 0.28 | 0 | 1 | 1 |
| 28 | ######## | 1800 | 1048.95 | 3788 | 2714 | 1124.1 | 397 | 675.35 | 397 | 188.76 | 244 | 1074 | 24 | 51 | 7.22 | 4 | 8 | 12 |
| 29 | ######## | 1800 | 1053.27 | 3220 | 2710 | 1256.75 | 397 | 541.71 | 398 | 92.67 | 200 | 510 | 3 | 4 | 0.45 | 3 | 10 | 13 |
| 30 | ######## | 1800 | 919.66 | 3018 | 2553 | 1218.57 | 386 | 581.43 | 385 | 90.29 | 180 | 465 | 3 | 5 | 1.51 | 2 | 7 | 9 |
| 31 | ######## | 1800 | 451.91 | 1967 | 1446 | 1274.86 | 380 | 525.14 | 379 | 135.97 | 167 | 521 | 0 | 0 | 0 | 4 | 1 | 5 |
| | | | | | | | | | 314.5652 | | | | | | | | | |

| | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 4 | ######## | 1800 | 50.96 | 598 | 364 | 1607.77 | 204 | 192.23 | 203 | 54.1 | 80 | 234 | 2 | 2 | 0.2 | 0 | 0 | 0 |
| 5 | ######## | 1800 | 372.94 | 2316 | 1706 | 1381.55 | 229 | 417.92 | 229 | 94 | 155 | 610 | 3 | 4 | 0.41 | 0 | 1 | 1 |
| 6 | ######## | 1800 | 1383.88 | 4801 | 4042 | 1104.39 | 319 | 695.61 | 318 | 117.7 | 233 | 759 | 23 | 34 | 3.12 | 2 | 9 | 11 |
| 7 | ######## | 1800 | 1303.01 | 3561 | 3215 | 1241.97 | 284 | 558.03 | 283 | 50.64 | 155 | 346 | 30 | 55 | 7.52 | 1 | 6 | 7 |
| 8 | ######## | 1800 | 553.54 | 2003 | 1762 | 1485.44 | 220 | 314.56 | 219 | 50.03 | 92 | 241 | 4 | 5 | 0.75 | 0 | 8 | 8 |
| 9 | ######## | 1800 | 714.42 | 2386 | 2125 | 1434.91 | 290 | 365.09 | 289 | 43.32 | 109 | 261 | 6 | 8 | 1.76 | 2 | 7 | 9 |
| 10 | ######## | 1800 | 823.75 | 3022 | 2741 | 1307.52 | 295 | 491.62 | 296 | 38.67 | 129 | 281 | 10 | 16 | 4.37 | 2 | 2 | 4 |
| 11 | ######## | 1800 | 332.83 | 1856 | 1520 | 1399.9 | 314 | 399.38 | 314 | 68.02 | 127 | 336 | 6 | 8 | 2.89 | 0 | 1 | 1 |
| 12 | ######## | 1800 | 498.17 | 2269 | 1805 | 1338.9 | 346 | 461.05 | 346 | 81.98 | 158 | 464 | 6 | 9 | 0.72 | 2 | 1 | 3 |
| 13 | ######## | 1800 | 556.06 | 2281 | 2009 | 1397.81 | 347 | 401.21 | 348 | 40.68 | 124 | 272 | 8 | 12 | 1.86 | 4 | 2 | 6 |
| 14 | ######## | 1800 | 536.5 | 2185 | 1822 | 1363.38 | 305 | 435.95 | 305 | 71.55 | 132 | 363 | 6 | 13 | 0.97 | 1 | 1 | 2 |
| 15 | ######## | 1800 | 489.55 | 2257 | 1917 | 1360.19 | 334 | 439.18 | 334 | 61.95 | 141 | 340 | 9 | 19 | 2 | 2 | 1 | 3 |
| 16 | ######## | 1800 | 296.62 | 1872 | 1576 | 1451.67 | 350 | 347.97 | 350 | 46.22 | 126 | 296 | 3 | 4 | 0.78 | 3 | 2 | 5 |
| 17 | ######## | 1800 | 677.33 | 2581 | 2234 | 1382.01 | 292 | 417.85 | 292 | 54.71 | 139 | 347 | 12 | 32 | 5.65 | 2 | 4 | 6 |
| 18 | ######## | 1800 | 786.19 | 2956 | 2577 | 1286.3 | 314 | 513.32 | 314 | 69.37 | 159 | 379 | 14 | 29 | 3.6 | 1 | 2 | 3 |
| 19 | ######## | 1800 | 1349.37 | 3125 | 2895 | 1407.42 | 247 | 392.57 | 247 | 26.49 | 104 | 230 | 21 | 37 | 4.68 | 2 | 3 | 5 |
| 20 | ######## | 1800 | 507.09 | 1821 | 1549 | 1482.85 | 279 | 316.99 | 279 | 48.92 | 102 | 272 | 1 | 9 | 1.26 | 3 | 2 | 5 |
| 21 | ######## | 1800 | 2650.22 | 4411 | 4046 | 1230.75 | 326 | 569.25 | 325 | 54.24 | 151 | 365 | 19 | 216 | 15.19 | 9 | 15 | 34 |
| 22 | ######## | 1800 | 940.07 | 2563 | 2328 | 1479.7 | 291 | 320.3 | 290 | 34.33 | 103 | 235 | 17 | 50 | 5.31 | 3 | 4 | 7 |
| 23 | ######## | 1800 | 1992.98 | 4194 | 3667 | 1214.64 | 289 | 584.85 | 289 | 87.95 | 165 | 527 | 38 | 128 | 12.15 | 8 | 14 | 22 |
| 24 | ######## | 1800 | 611.48 | 2330 | 1975 | 1402.31 | 337 | 397.53 | 337 | 69.95 | 121 | 355 | 9 | 13 | 0.62 | 0 | 4 | 4 |
| 25 | ######## | 1800 | 574.36 | 1932 | 1739 | 1528.44 | 255 | 271.56 | 254 | 27.29 | 88 | 193 | 7 | 18 | 1.95 | 3 | 2 | 5 |
| 26 | ######## | 1800 | 1655.97 | 2983 | 2806 | 1466.43 | 248 | 333.23 | 248 | 29.45 | 90 | 177 | 17 | 136 | 12.01 | 6 | 18 | 24 |
| 27 | ######## | 1800 | 937.69 | 2661 | 2409 | 1416.01 | 306 | 383.87 | 306 | 40.41 | 118 | 252 | 4 | 6 | 0.61 | 7 | 3 | 10 |
| 28 | ######## | 1800 | 1087.09 | 3282 | 2978 | 1327.77 | 317 | 472.2 | 317 | 44.46 | 137 | 304 | 23 | 38 | 6.21 | 3 | 9 | 12 |
| | | | | | | | | | 300 | | | | | | | | | |

# ADDENDUM B

## ASSESSMENT OF WATER CONSUMPTION IN DEER MICE TO PREDICT THE CONCENTRATION OF ESCITALOPRAM OXALATE FOR A DRUG TREATMENT STUDY

## INTRODUCTION

As explained in Chapter 3, it was decided to administer the escitalopram oxalate orally in the drinking water. Deer mice are small and would be subjected to injection stress twice daily for 4 weeks during the treatment phase of the study. As OCD is classified as an anxiety disorder (American Psychiatric Association, 2000) it is more desirable to limit the stress of handling to an absolute minimum. In order to establish the mean concentration of escitalopram oxalate to be dissolved in the drinking water, it was necessary then to assess the average individual intake of water by deer mice. This was done in an appropriate pilot study, the data of which are presented in this addendum.

## METHODS

To assess the normal water intake of deer mice, 16 animals were grouped two per cage in three cages, with three mice per cage in two cages and four per cage in one cage. This was done to assess whether any differences in the *average* amount of water consumption could be demonstrated. The water bottles were weighed on a daily basis for two weeks and the changes in weight recorded. Subsequently, the data were analyzed and the results are presented on the following page.

## RESULTS

In congruence with the data supplied by the Animal Research Centre of the North-West University, deer mice consume water at an average rate of 0.25 ml/g/24 hours. These averages did not differ as a function of the number of animals per cage with a standard deviation between the different cages of only 0.03 ml/g/24 hours, as can be seen in Table B1.

## CONCLUSION

As the average water consumption of deer mice has been established as 0.25 ml/g/24 hours, it was now possible to calculate the mean concentration of escitalopram oxalate to be dissolved in the drinking water. It must be stressed, however, that although the individual water consumption of deer mice may differ, this pilot study demonstrated that *on average* the animals consume the same amounts of water. In an experimental group of 40 animals, certain individuals will differ from the larger group with respect to drinking behaviour. Unfortunately, these animals must be assimilated in the larger group and it can therefore not be guaranteed that every animal ingested the optimal amount of escitalopram during the treatment study.

## Table B1 –

## THE NORMAL WATER CONSUMPTION OF DEER MICE

*These numbers indicate the average amount of water consumed by the animals in each cage per 24 hours over the course of two weeks*

| *Cage (Amount)* | *Average water / 24 hrs (ml* | *Combined animal weight (g)* | *Water (ml) / Gram.24hr* | | |
|---|---|---|---|---|---|
| *A (2)* | 6.28 | 32.8 | 0.19 | | |
| *B (2)* | 9.46 | 36.4 | 0.26 | | |
| *C (2)* | 8.79 | 34.3 | 0.26 | | |
| *D (3)* | 10.96 | 47.8 | 0.23 | | |
| *E (3)* | 12.12 | 47.8 | 0.25 | | |
| *A (4)* | 13.28 | 60.3 | 0.22 | | Std Dev: 0.03 |

*The numbers in brackets indicate the number of animals in each cage.*

*The average individual water consumption of deer mice per gram of body mass as calculated after two weeks.*

# ADDENDUM C

## ORAL ADMINISTRATION OF ESCITALOPRAM OXALATE INTRODUCED INTO THE DRINKING WATER DOES NOT CHANGE THE AVERAGE FLUID INTAKE OF DEER MICE

## INTRODUCTION

Once it had been established that deer mice consume water at an average rate of 0.25 ml/g/24 hours, it was necessary to assess whether the animals expressed any taste aversion to escitalopram as this could possibly have changed their fluid consumption behaviour, resulting in sub-clinical dosaging. The pilot study described in this addendum has been initiated to address this question.

## METHODS

To establish whether the fluid consumption of deer mice changes following the administration of escitalopram in the drinking water, 24 animals housed *two per cage* were used in this pilot study. Twelve animals (six cages) received only water throughout the three-week duration of the pilot, while the remaining twelve received water for one week and thereafter escitalopram solution for two weeks *(i.e. 2 weeks treatment with escitalopram)*. An escitalopram dose of 10 mg/kg/day (4mg / 100ml) (refer to paragraph 4.2.2) was prepared in a solution sufficient to supply each cage with 100ml. Solutions were replaced every two days, and the start and end weight of each bottle were recorded on a daily basis. The animals in cages 1 – 6 received water for one week, followed by escitalopram solution for two weeks, while the animals allocated to cages 7 – 12 received only water for three weeks. The results of this study are now presented. In each of the presented figures, water administration is indicated by blue colouring, while the administration of escitalopram is indicated by green.

## RESULTS

Figure A represents the daily amounts of water consumed by the two animals allocated to each cage. Figure B represents the average amount of water consumed by both groups after one week and again after three weeks. No statistical differences in the amounts of water consumed could be demonstrated for either of the groups over a 3-week period ($p > 0.05$; paired t-test).

## CONCLUSION

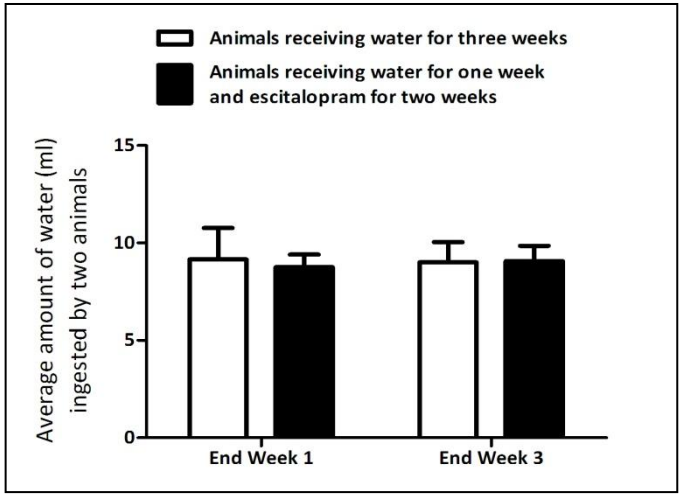
This pilot study has demonstrated that deer mice do not express any taste aversion to escitalopram oxalate and that no changes in the water consumption behaviour of deer mice could be demonstrated following the introduction of escitalopram to the drinking water.

## (A) – THE DAILY AMOUNT OF WATER AND ESCITALOPRAM SOLUTION (ml) (10 mg/kg/day) INGESTED BY DEER MICE

| Cage | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | 25 | 26 | 27 | 28 | 29 | 30 | 31 | 1 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 9.6 | 9.9 | 10 | 11.1 | 10 | 10.8 | 9.5 | 11.4 | 9.4 | 11.3 | 11.7 | 11.6 | 10.3 | 10.3 | 9.5 | 12.4 | 11.9 | 9.7 | 10.6 | 9.4 | 9.9 | 9.9 | 9.2 | 10.4 |
| 2 | 6.8 | 5 | 7.6 | 8.4 | 0.4 | 9 | 8 | 4.8 | 5.1 | 5.4 | 5.3 | 6.4 | 5.6 | 6 | 5 | 5.8 | 7.7 | 7.9 | 6.9 | 5.9 | 6.7 | 6.1 | 6.5 | 7.1 |
| 3 | 9.2 | 9.5 | 8.9 | 10.2 | 8.4 | 9.3 | 8.7 | 9.6 | 8.8 | 9.1 | 8.5 | 11.3 | 9.8 | 9.5 | 9.7 | 10.8 | 10.2 | 16.5 | 11.2 | 9 | 11 | 9.3 | 9.6 | 9.5 |
| 4 | 8.8 | 9.6 | 9.5 | 3.4 | 7.3 | 6 | 7.1 | 11.2 | 10.8 | 9.3 | 9.8 | 12.2 | 9.7 | 10.4 | 8.2 | 10.8 | 10.3 | 10.8 | 11.4 | 9.6 | 10.7 | 10.1 | 9.8 | 9.9 |
| 5 | 10.1 | 11.4 | 11.3 | 11 | 10.4 | 10 | 11 | LEAKED | 10 | 10.8 | 9.7 | 12.3 | 10.8 | 10.5 | 10.3 | 10.5 | 9 | 10 | 10.6 | 9.4 | 10.4 | 9.3 | 9.6 | 9.3 |
| 6 | 8.8 | 9.4 | 8.5 | 9 | 7.9 | 8.6 | 8.1 | 6.8 | 7.3 | 6.1 | 7.1 | 7.8 | 7.5 | 7.6 | 6.7 | 7.1 | 8.9 | 7 | 6.6 | 4 | 8.7 | 7.6 | 7.1 | 6.4 |
| 7 | 9.9 | 10.3 | 10.1 | 10.8 | 10.1 | 9.6 | 10.3 | 7.8 | 9.3 | 8.4 | 9.3 | 10.5 | 9.6 | 9.6 | 8 | 9.4 | 10 | 10.4 | 9.2 | 8.4 | 10.3 | 9.3 | 8.9 | 8.6 |
| 8 | 11.6 | 10.8 | 11 | 12 | 11.3 | 10.1 | 10.9 | 9.3 | 10.1 | 9.8 | 9.8 | 10.3 | 9.5 | 10.6 | 8.6 | 9.4 | 11.6 | 10.2 | 9.6 | 9.1 | 9.9 | 9.9 | 9.1 | 9.6 |
| 9 | 1.2 | 0.8 | 0.7 | 2.8 | 1.3 | 2.5 | 1.8 | 1.4 | 4.2 | 3.4 | 4.6 | 5.1 | 4.1 | 4.7 | 4.1 | 4.9 | 5.5 | 4.7 | 4.7 | 4.4 | 5.3 | 5.4 | 4.3 | 4.7 |
| 10 | 8 | 8.1 | 8.3 | 9.4 | 8.8 | 9.2 | 8.7 | 8.1 | 8.4 | 8.5 | 8.8 | 10 | 8 | 9.5 | 8 | 9.2 | 9 | 9 | 9.2 | 7.2 | 8.2 | 8.2 | 7.1 | 9 |
| 11 | 9.7 | 10 | 11.5 | 10.4 | 10.4 | 10.5 | 10.4 | 8.8 | 10.2 | 10.9 | 10.2 | 11.2 | 9.3 | 10.3 | 10 | 10.6 | 11.5 | 9.3 | 9.9 | 9.6 | 8.9 | 9.5 | 8.8 | 9.3 |
| 12 | 12.5 | 13.3 | 11.9 | 13.6 | 13.6 | 13.6 | 12.5 | 12.1 | 11.8 | 12.6 | 12.2 | 12.8 | 11.5 | 12.5 | 10.6 | 12.7 | 13.7 | 12.3 | 13 | 11.6 | 12.3 | 11.7 | 11.2 | 11.5 |

## (B) – THE DIFFERENCES BETWEEN THE AVERAGE AMOUNT OF WATER AND ESCITALOPRAM SOLUTION INGESTED BY DEER MICE

| Cage | Aver (ml) (up to 15 March) | Aver (ml) (from 16 March) |
|---|---|---|
| 1 | 10.1 | 10.5 |
| 2 | 6.5 | 6.1 |
| 3 | 9.2 | 10.2 |
| 4 | 7.4 | 10.3 |
| 5 | 10.7 | 10.2 |
| 6 | 8.6 | 7.1 |
| 7 | 10.2 | 9.2 |
| 8 | 11.1 | 9.8 |
| 9 | 1.6 | 4.4 |
| 10 | 8.6 | 8.6 |
| 11 | 10.4 | 9.9 |
| 12 | 13.0 | 12.1 |



# ADDENDUM D

THE RESPONSE OF DEER MOUSE STEREOTYPY TO DIFFERENT DOSES OF ESCITALOPRAM OXALATE –

A pilot study to establish the appropriate dose of escitalopram for application in a drug treatment study

## INTRODUCTION

As explained in paragraph 4.2.2, previous studies done in our laboratory employed fluoxetine at an intraperitoneal dose of 20 mg/kg/day as a drug treatment to attenuate deer mouse stereotypy (Güldenpfennig *et al.*, 2011; Korff *et al.*, 2008; Korff *et al.*, 2009) . Based on this work, and that escitalopram is a more potent SERT inhibitor than fluoxetine (Owens *et al.*, 2001), it was decided that the final dose of escitalopram to be dissolved in the drinking water for oral administration would be between 20 and 40 mg/kg/day. Subsequently, this pilot study was designed to confirm and establish this dose.

## METHODS

The same rationale and methodology as described in the main study (refer to Chapter 4) was followed. In short, each animal was screened on a weekly basis for four weeks. The first two weeks of assessment (or five assessments, 1 – 5) provided a baseline value during a placebo phase, while escitalopram was introduced in the last two weeks (or three assessments, 6 – 8) following the placebo administration. Escitalopram was administered orally at doses between 1 and 40mg/kg/day.

## RESULTS

Table D1 represents the data of the 1 and 10 mg/kg/day dosages and Table D2 that of the 30 and 40 mg/kg/day dosages. The numbers in the left-hand column indicate the individual weekly screen numbers while the numbers in the top row indicate the different animals. On each table, vertical activity is indicated in the top row of data, and horizontal revolutions by the bottom row of data. No statistical difference between the median amounts of stereotypy before and after treatment could be demonstrated for any of the doses tested.

## CONCLUSION

The data from the current pilot study could not provide evidence that either one of these doses was effective in the attenuation of deer mouse stereotypy. We subsequently based our final dose of 50 mg/kg/day on a study by Greene-Schloesser and colleagues (2011) in which fluoxetine was dissolved in the drinking water of animals at a dose of 50 mg/kg/24 hours and which resulted in near-maximal inhibition of compulsive-like nest-building behaviour in mice. This pilot study also prompted us to re-visit our method of identifying and quantifying appropriate stereotypic behaviours in deer mouse following drug treatment. This inherent shortcom-

ing led to the development of a new set of parameters and criteria based on rest periods between bouts of stereotypy, as well as periods of heightened stereotypy between bouts of normal activity. This new set of criteria and their application in the study are described in Chapter 5.

## Table D1 –

## THE VERTICAL ACTIVITY AND HORIZONTAL REVOLUTIONS OF ANIMALS TREATED WITH ESCITALOPRAM

***GREEN*** *– 1 mg/kg/day;* ***BLUE*** *– 10 mg/kg/day*

*Median values of stereotypy following 5 base-line assessments*

| VERTICAL ACTIVITY | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | **10** | **11** | **12** | **13** | **14** | **15** | **16** |
| **1** | 280 | 1545 | 184 | 2528 | 2792 | 1081 | 108 | 832 | 1162 | 2093 | 1243 | 860 | 1732 | 309 | 1704 |
| **2** | 259 | 1490 | 62 | 2367 | 2804 | 795 | 193 | 898 | 925 | 2817 | 1440 | 1252 | 1840 | 397 | 921 |
| **3** | 151 | 2663 | 86 | 3152 | 3408 | 428 | 67 | 1451 | 1562 | 2742 | 2042 | 1220 | 2152 | 814 | 1506 |
| **4** | 209 | 1486 | 142 | 2756 | 3774 | 695 | 293 | 873 | 1357 | 2708 | 2466 | 810 | 2206 | 1845 | 1250 |
| **5** | 149 | 1520 | 1683 | 3006 | 3392 | 1024 | 639 | 801 | 1225 | 2675 | 1866 | 868 | 2329 | 1452 | 1522 |
| **MED** | **209** | **1520** | **142** | **2756** | **3392** | **795** | **193** | **873** | **1225** | **2708** | **1866** | **868** | **2152** | **814** | **1506** |
| **6** | 191 | 1452 | 1102 | 2219 | 3610 | 952 | 84 | 719 | 1017 | 2811 | 1627 | 3522 | 2170 | 1879 | 1598 |
| **7** | 427 | 1352 | 875 | 1941 | 4806 | 1444 | 84 | 367 | 1192 | 2239 | 2518 | 2876 | 1963 | 2129 | 1817 |
| **8** | 1848 | 1828 | 1691 | 1177 | 3533 | 1550 | 96 | 635 | 1115 | 1919 | 1809 | 3272 | 2128 | 2567 | 1287 |
| **9** | | | | | | | | | | | | | | | |
| **MED** | **427** | **1452** | **1102** | **1941** | **3610** | **1444** | **84** | **635** | **1115** | **2239** | **1809** | **3272** | **2128** | **2129** | **1598** |
| REVOLUTIONS | | | | | | | | | | | | | | | |
| **1** | 23 | 67 | 35 | 19 | 23 | 138 | 9 | 22 | 35 | 28 | 42 | 24 | 37 | 52 | 34 |
| **2** | 6 | 15 | 24 | 18 | 45 | 120 | 17 | 16 | 13 | 14 | 17 | 11 | 47 | 54 | 49 |
| **3** | 4 | 79 | 52 | 20 | 27 | 39 | 8 | 17 | 74 | 17 | 50 | 10 | 46 | 87 | 111 |
| **4** | 5 | 10 | 26 | 22 | 21 | 163 | 10 | 18 | 11 | 10 | 7 | 14 | 21 | 31 | 130 |
| **5** | 4 | 5 | 19 | 23 | 35 | 181 | 20 | 17 | 14 | 14 | 8 | 15 | 14 | 33 | 90 |
| **MED** | **5** | **15** | **26** | **20** | **27** | **138** | **10** | **17** | **14** | **14** | **17** | **14** | **37** | **52** | **90** |
| **6** | 5 | 10 | 10 | 17 | 16 | 132 | 10 | 13 | 45 | 9 | 11 | 17 | 13 | 42 | 44 |
| **7** | 8 | 12 | 11 | 20 | 11 | 259 | 19 | 15 | 15 | 13 | 66 | 22 | 11 | 22 | 73 |
| **8** | 13 | 8 | 9 | 31 | 22 | 297 | 13 | 13 | 71 | 11 | 15 | 21 | 27 | 8 | 108 |
| **9** | | | | | | | | | | | | | | | |
| **MED** | **8** | **10** | **10** | **20** | **16** | **259** | **13** | **13** | **45** | **11** | **15** | **21** | **13** | **22** | **73** |

*Median values of stereotypy following the three assessments done during the treatment phase of the current pilot study.*

## Table D2 –

## THE VERTICAL ACTIVITY AND HORIZONTAL REVOLUTIONS OF ANIMALS TREATED WITH ESCITALOPRAM

***PURPLE*** *– 30 mg/kg/day;* ***PINK*** *– 40 mg/kg/day*

| VERTICAL ACTIVITY | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **17** | **18** | **19** | **22** | **23** | **24** | **26** | **27** | **28** | **29** | **30** | **31** | **32** |
| **1** | 1748 | 420 | 3674 | 2244 | 2072 | 1602 | 2708 | 786 | 932 | 3723 | 522 | 1258 | 2831 |
| **2** | 1517 | 718 | 2388 | 3656 | 2691 | 1143 | 2655 | 999 | 763 | 1903 | 223 | 1375 | 1492 |
| **3** | 2393 | 990 | 1629 | 4143 | 2622 | 1561 | 5475 | 797 | 1769 | 2250 | 299 | 2080 | 3677 |
| **4** | 3240 | 916 | 2384 | 4071 | 5201 | 1748 | 2464 | 1019 | 562 | 2549 | 167 | 1206 | 2497 |
| **5** | 2906 | 1355 | 1936 | 3735 | 8172 | 1515 | 5546 | 1093 | 469 | 1606 | 350 | 1470 | 1334 |
| **MED** | **2393** | **916** | **2384** | **3735** | **2691** | **1561** | **2708** | **999** | **763** | **2250** | **299** | **1375** | **2497** |
| **6** | 3142 | 898 | 2103 | 3938 | 6856 | 646 | 4540 | 813 | 1017 | 1538 | 493 | 1361 | 1788 |
| **7** | 2931 | 1061 | 1940 | 4124 | 7574 | 1264 | 5184 | 879 | 407 | 1764 | 492 | 1403 | 1536 |
| **8** | 2295 | 1683 | 3156 | 3463 | 6756 | 2074 | 3114 | 1441 | 951 | 1425 | 552 | 1449 | 2795 |
| **9** | | | | | | | | | | | | | |
| **MED** | **2931** | **1061** | **2103** | **3938** | **6856** | **1264** | **4540** | **879** | **951** | **1538** | **493** | **1403** | **1788** |
| REVOLUTIONS | | | | | | | | | | | | | |
| **1** | 315 | 30 | 449 | 62 | 65 | 37 | 38 | 13 | 206 | 17 | 17 | 15 | 12 |
| **2** | 250 | 12 | 693 | 54 | 43 | 36 | 12 | 6 | 212 | 55 | 9 | 28 | 19 |
| **3** | 138 | 14 | 426 | 20 | 25 | 39 | 10 | 5 | 86 | 21 | 20 | 41 | 5 |
| **4** | 233 | 19 | 543 | 39 | 26 | 28 | 8 | 6 | 93 | 33 | 21 | 9 | 8 |
| **5** | 120 | 14 | 392 | 51 | 73 | 27 | 69 | 5 | 54 | 39 | 16 | 12 | 16 |
| **MED** | **233** | **14** | **449** | **51** | **43** | **36** | **12** | **6** | **93** | **33** | **17** | **15** | **12** |
| **6** | 496 | 23 | 323 | 23 | 16 | 25 | 4 | 4 | 52 | 130 | 23 | 13 | 6 |
| **7** | 185 | 22 | 281 | 11 | 14 | 53 | 11 | 4 | 93 | 47 | 58 | 14 | 8 |
| **8** | 262 | 24 | 646 | 41 | 12 | 39 | 4 | 6 | 105 | 37 | 15 | 8 | 17 |
| **9** | | | | | | | | | | | | | |
| **MED** | **262** | **23** | **323** | **23** | **14** | **39** | **4** | **4** | **93** | **47** | **23** | **13** | **8** |