An investigation of habitual behaviour in deer mice (Peromyscus maniculatus bairdii) displaying motor persistence

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“Trust in the Lord with all your heart and lean not on your own understanding; in all your ways submit to Him, and He will make your paths straight.”
Proverbs 3:5-6

“Wisdom is supreme; therefore get wisdom. Though it cost all you have, get understanding.”
Proverbs 4:7

* * *

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“For the Lord gives wisdom; from His mouth comes knowledge and understanding.”

Proverbs 2:6.

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“For of Him, and through Him, and to Him, are all things: to whom be glory forever. Amen.” Romans 11:36

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If we knew what it was, we were doing, it would not be called research, would it?

-Albert Einstein

Congress proceedings

The results of the current investigation were presented at the South African Neuroscience Society (SANS) meeting, hosted at the 2022 Biological Psychiatry congress at Century City Cape Town, September 2022. The presenting author is underlined.

Hurter B, Wolmarans D, Gourley S. The relationship between distinct compulsive-like behavioural* phenotypes in deer mice and working memory: response to levetiracetam. Annual meeting of the South African Neuroscience Society, Century City Cape Town, September 2022.

The presentation won the first prize at the SANS meeting.

**Spelling as per the submitted congress title*

Abstract

Obsessive-compulsive disorder (OCD)[1] is characterized by thematically specific obsessions, i.e. intrusive thoughts and impulses, and/or compulsions, that is highly repetitive behaviors or mental acts. Obsessions and compulsions are time consuming and persistent (lasting for at least one hour daily) and cause a significant degree of distress in the sufferer. OCD is a heterogeneous disorder, meaning that the symptom phenotypes differ with respect to, among others, theme, content, manifestation, severity and treatment response. Four major symptom phenotypes have been described, i.e. contamination/washing, safety/checking, symmetry/ordering, and intrusive thoughts associated with covert mental routines.

As many as 25 % of adult OCD patients have already shown noticeable signs of OCD by the age of 14 years. Further, more than half of OCD cases is diagnosed between the ages of 6 – 10 years. Juvenile onset OCD is classified as one of the most occurring mental disorders in children with a prevalence rate of up to 3 %. However, this number might be higher, especially since accurate diagnoses of OCD in young age groups are difficult, likely because many behaviors often shown by children, i.e. rituals and habit-like repetition of certain actions, agree with some of the diagnostic criteria of the condition. Further, it is entirely unknown if and how said normal behaviors in children, will predict and contribute to the development of OCD in some, but not others.

In terms of neurobiology, OCD is primarily associated with abnormalities in and dysfunction of the cortico-striatal-thalamo-cortical (CSTC)[2] pathways. These anatomical components and their associated pathways play a crucial role in the planning, execution, and termination of goal-directed actions. It is proposed that in OCD, overactivity in the CSTC circuitry may contribute to the excessive behavioral engagement typical of the condition. Specifically, it is believed that a relative hyposerotonergic state may underlie the excessive propagation of executive signals via the CSTC pathways, explaining the response, albeit suboptimally so, of OCD to high-dose treatment with selective serotonin reuptake inhibitors (SSRIs)[3].

Several neurocognitive theories have been proposed to explain the etiology and development of OCD in different patients. One such theory focuses on the potential role that habit-proneness may play in certain individuals. Broadly viewed, behavior is governed by either goal-directed or habitual processes. Goal-directed behavior usually depends on the value of the specific action's outcome, is carefully planned and executed, and terminated when the outcome is realized. In contrast, habits are learned and thus

[1] obsessive-compulsive disorder
[2] cortico-striatal-thalamo-cortical
[3] selective serotonin reuptake inhibitors

automated, require less attention and fewer cognitive resources. Both response styles are necessary for our normal functioning. However, when the balance between goal-directed and habitual responses becomes dysfunctional, individuals may struggle to switch from habits that are no longer necessary and goal-directed engagement; this is a common neuropsychological endophenotype in OCD[1]. Against this background, the habit hypothesis of OCD implies that individuals with increased habit-proneness may be at a greater risk of developing OCD. This is important, since it is possible that a lack of understanding of how habits progress to OCD, or how habit-like persistence may promote the expression of compulsive symptomology, contribute to the less-than-optimal treatment outcomes seen in OCD. In other words, different obsessive-compulsive symptom phenotypes, might have unique psychobiological foundations that will arguably show unique responses to different interventions.

Considering this, novel treatment options that may target both the cognitive and neurobiological underpinnings of OCD, may be useful. Levetiracetam (LEV)[2], may be a promising target for investigation, since this 5-enantiomer of 5-alpha-ethyl-2-oxo-1-pyrrolidine acetamide, clinically prescribed for the treatment of epilepsy, shows promise as a potentially useful cognitive enhancer in both epileptic and healthy cohorts. In this work, its potential as an anti-compulsive agent in a naturalistic model of compulsive-like persistent behaviors was explored.

Deer mice *(Peromyscus maniculatus bairdii)* that are housed under standard laboratory conditions variably present with two distinct compulsive-like behavioral phenotypes, i.e. large nest building (LNB)[3] behavior and high motor stereotypy (HS)[4]. Both respond to chronic, high-dose SSRI[5] intervention and are expressed by animals of both sexes. However, the neurocognitive underpinnings of these behaviors remain largely unknown. Thus, the broad aim of this work was to explore the potential relationships between these adult behavioral phenotypes and both early-life and adulthood measures of habit-proneness. We further aimed to investigate whether LNB and HS would respond to LEV, and how their potential associations with increased habit-proneness, would respond to the same intervention.

Briefly, 76 juvenile (28-day-old) deer mice of both sexes (ethics approval no.: **NWU-00423-21-A5**) were assessed for habit-proneness in the Barnes maze (BM)[6] and divided into two exposure groups (n = 37-39 per group), i.e. control- (CTRL[7]; normal drinking water) and LEV (75 mg/kg/day; administered in the

[1] obsessive-compulsive disorder
[2] levetiracetam
[3] large nest building
[4] high motor stereotypy
[5] selective serotonin reuptake inhibitor
[6] Barnes maze
[7] control

drinking water). After 56 days of uninterrupted CTRL[1] or LEV[2] exposure, all mice were screened for nesting and stereotypical behavior, and then assessed for working memory in the T-maze.

The main findings of the present work were that 1) performance in the BM[3] of juvenile *P. maniculatus bairdii* mice was not related to LNB[4] or stereotypy in adulthood; 2) chronic LEV exposure from a juvenile age until adulthood decreased LNB expression without affecting overall nesting scores, but increased CR[5] activity; 3) stereotypical expression, notably so CR, and nesting expression were divergent persistent behavioral phenotypes; and 4) LEV exposure generally improved the relationship between working memory and stereotypical engagement.

Keywords

deer mouse; stereotypy, nest building, Barnes maze; T-maze; alternation; working memory

Solemn declaration

I, Bianca Hurter (28555252) herewith declare that this dissertation is my own work and that no part thereof has been copied from any other sources.

[1] control
[2] levetiracetam
[3] Barnes maze
[4] large nest building
[5] patterned cage running

Contents

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List of tables and figures

1 Introduction

1.1 Dissertation approach and layout

This dissertation has been prepared in article format, as specified by the North-West University (NWU)[1], South Africa. The main experimental body of work will be presented in the form of a journal article in **Chapter 3** of the dissertation. Here, the experimental methodology, results, and the main findings of the present investigation, will be reported. This chapter will be submitted for publication in an international accredited, peer-reviewed journal, i.e., *'Psychopharmacology'*.

The complete dissertation will comprise four chapters, i.e. **Chapter 1**, which gives a concise summary of the investigation, including the problem statement, our working hypothesis, study aims and objectives, the experimental layout, and details regarding the ethical approval of this work. In **Chapter 2**, a detailed literature review, focused on the theme of the present work, will lay the foundation for the investigation presented in **Chapter 3**. Lastly, **Chapter 4** provides a broad summary of and conclusion to this work. It also affords attention to the potential shortcomings of the investigation and highlights some key aspects that could be considered in future.

One addendum is included (**Addendum A**) that contains supplementary details regarding the methods followed. These could not be provided in **Chapter 3**, as per the instructions to the authors prescribed by *Psychopharmacology*.

This dissertation is presented in US English, since this is the language prescribed by *Psychopharmacology.* Miss Hurter's co-supervisor, Prof Shannon Gourley, is also employed in the United States of America (Emory University, Atlanta).

* * *

[1] North-West University

1.2 Problem statement

Obsessive-compulsive disorder (OCD)[1] is diagnosed in up to 3 % of children, adolescents, and adults (Ruscio et al. 2010; Walitza et al. 2011). The condition responds suboptimally to current first-line treatment strategies, i.e. high-dose selective serotonin reuptake inhibitors (SSRIs)[2] and psychotherapeutic interventions, e.g. exposure and response prevention (ERP)[3] (Robbins et al. 2019). The diagnosis of obsessions and compulsions in children can be especially difficult, since many of the symptoms that are assessed during evaluation, correspond with behavioral traits that naturally present in most children e.g. highly ritualized and rigid habit-like behaviors (Evans et al. 2002; Leonard et al. 1990). Further, it is uncertain if and how such traits may predict and ultimately contribute to a clinical diagnosis of OCD in some but not others (Hjemdal et al. 2011; Thorsen et al. 2019; van der Straten et al. 2020).

Focusing on habit-proneness, it has been reported that dysregulation in the balance between goal-directed and habitual response styles can contribute to the development and severity of compulsive symptomology (Robbins and Costa 2017). In the same sense, habit-proneness can be regarded as a potential endophenotype in specific cases of OCD (Gillan et al. 2011). However, it is yet unclear whether habits play an equally important role in the psychobiological architecture of the different obsessive-compulsive symptom phenotypes, i.e. contamination/washing, safety/checking, symmetry/ordering, and covert mental rituals.

Considering the less-than-optimal response of OCD to current treatment strategies, novel pharmacotherapeutic agents that may target both the cognitive and neurobiological domains of OCD, may be useful. Against this background, levetiracetam (LEV)[4], which is used for the treatment of epilepsy, may be promising. Levetiracetam has been shown to improve working memory, verbal skill, and visual feedback processing in epilepsy cohorts (Helmstaedter and Witt 2010; Operto et al. 2019; Piazzini et al. 2006), but some results from studies in other clinical cohorts, e.g. patients suffering from Alzheimer's disease (Sanchez et al. 2012), and non-clinical samples have also been informative. For example, after administration of a single dose in healthy volunteers, LEV enhanced executive functioning on the levels of working memory, task planning and decision making, all of which are pivotal for habits to be learned (Magalhães et al. 2015). Interestingly, as opposed to other neuronal stabilizers, e.g. valproate and carbamazepine that act by modulating the actions of voltage-gated ion channels, levetiracetam influences neurotransmitter release via its actions on synaptic vesicle glycoprotein 2A

[1] obsessive-compulsive disorder
[2] selective serotonin reuptake inhibitors
[3] exposure and response prevention
[4] levetiracetam

(SV2A)[1]. Indeed, the fact that LEV[2] does not interact directly with known neurotransmitter systems or influence membrane excitability, may be key to its potential large therapeutic window as a cognitive enhancer (Helmstaedter and Witt 2008; Wu et al. 2009).

Deer mice *(Peromyscus maniculatus bairdii)* that are bred, reared, and housed under standard laboratory conditions variably present with distinct, but equally persistent and repetitive behavioral phenotypes, i.e. large nest building (LNB)[3] behavior and high motor stereotypy (HS)[4] (de Brouwer et al. 2020). These behaviors are non-induced, occur in animals of both sexes, and respond to chronic high-dose oral exposure to the SSRI[5], escitalopram (Scheepers et al. 2018). While the deer mouse model system is regarded as one of the most important naturalistic model systems of compulsivity (Hoffman and Cano-Ramírez 2018), the neurocognitive underpinnings of the different behavioral phenotypes, and thus their potential to expand to our understanding of how unique neurocognitive processes can contribute to heterogeneous compulsive-like behaviors, remain unknown.

To this end, the present work attempted to explore not only the potential longitudinal relationship between habit-proneness in juvenile mice and the expression of compulsive-like behaviors in adulthood, but also how LNB and HS may associate with changes in habitual engagement during adulthood, as assessed in the T-maze. We further investigated if LNB and HS as well as its potential relationships with habit-proneness, would respond to chronic exposure to LEV, administered throughout the rearing period.

1.3 Study questions

In an attempt to address the research problem alluded to above, the following research questions were asked:

1) Will habit-proneness as shown in juvenile mice in a Barnes maze (BM)[6] assessment, be predictive of adult compulsive-like, persistent LNB and HS?
2) Will the adulthood expression of LNB and HS associate with increased habitual response styles as assessed in a T-maze?
3) How will LNB and HS, as well as its relationships with altered T-maze ambulation, respond to chronic LEV exposure, administered throughout the entire rearing period?

[1] synaptic vesicle glycoprotein 2A
[2] levetiracetam
[3] large nest building
[4] high motor stereotypy
[5] selective serotonin reuptake inhibitor
[6] Barnes maze

4) Will such exposure to LEV[1] also modulate any potential longitudinal relationships between juvenile habit-proneness and adulthood compulsive-like expression?

1.4 Research hypothesis

The overarching hypothesis of this work was that the adulthood expression of LNB[2] and HS[3] will uniquely associate with habit-proneness, as assessed in juvenile mice, and thus be longitudinally related to the expression of habits in the juvenile life stage. We further hypothesized that in adulthood, LNB and HS will be differentially associated with habit-proneness as assessed in adult mice, and that such associations would respond uniquely to the potential cognitive enhancer, LEV, administered throughout the rearing period.

1.5 Study aims and objectives

1.5.1 Aims

The main aims of this project were to:

i) Determine whether juvenile deer mice of both sexes (aged 28 days at the onset of experimentation) would separate in terms of habit-proneness as assessed in the BM[4];

ii) Determine whether adult deer mice of both sexes (aged 12 weeks) separate in terms of habit-proneness as assessed in the T-maze;

iii) Determine whether habit-proneness as determined in (i) and (ii), would associate with adulthood behavioral persistence (LNB and/or HS as determined at the age of 12 weeks); and

iv) Establish whether LEV would influence the expression of LNB and HS in adulthood and manipulate the potential relationships between early-life and/or adulthood habitual engagement and the expression of adulthood LNB and HS.

[1] levetiracetam
[2] large nest building
[3] high motor stereotypy
[4] Barnes maze

1.5.2 Objectives

This project had the following objectives:

a. Selecting seventy-six (76) juvenile mice, aged postnatal day (PND)[1] 28 at the time of selection, from the deer mouse colony housed at the vivarium of the NWU[2];

b. Assessing all mice for habit-proneness in the BM[3];

c. After completion of the BM assessment, dividing all mice into two exposure groups, i.e. a normal water control (CTRL)[4] and a LEV[5] group (n = 37 – 39 per group);

d. Rearing all mice, housed in pairs of same-sex, same exposure animals per cage, under standard laboratory conditions, while being exposed to either CTRL or LEV, until the age of 12 weeks; and

e. At reaching the age of 12 weeks, assessing all mice for nest building behavior, stereotypical expression and habit-proneness.

1.6 Project layout

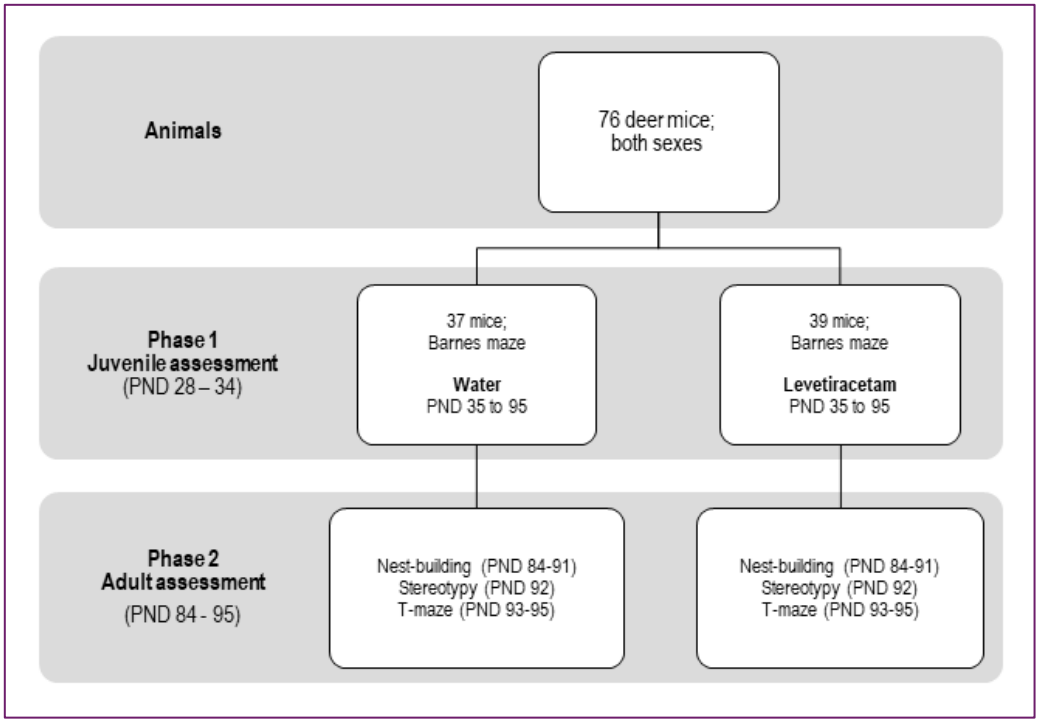

FIGURE 1-1 - SCHEMATIC LAYOUT OF THE PROJECT

[1] postnatal day
[2] North-West University
[3] Barnes maze
[4] control
[5] levetiracetam

1.7 Ethical approval

This investigation has been approved by the NWU[1] AnimCare Research Ethics Committee (approval number: **NWU-00423-21-A5**). Throughout the course of this investigation, the 3 R's as they pertain to animal experimentation, were adhered to (Doke and Dhawale 2015; Ferdowsian and Beck 2011; Hooijmans et al. 2010; Ranganatha and Kuppast 2012). All procedures were performed in accordance with the code of ethics stipulated in the relevant national legislation (South African National Standard for the Care and Use of Animals for Scientific Purposes; SANS 10386:2008).

* * *

[1] North-West University

1.8 References

de Brouwer G, Fick A, Lombaard A, Stein DJ, Harvey BH, Wolmarans DW (2020) Large nest building and high marble-burying: two compulsive-like phenotypes expressed by deer mice (Peromyscus maniculatus bairdii) and their unique response to serotoninergic and dopamine modulating intervention. Behavioural Brain Research: 112794.

Doke SK, Dhawale SC (2015) Alternatives to animal testing: A review. Saudi Pharmaceutical Journal 23: 223-229.

Evans DW, Milanak ME, Medeiros B, Ross JL (2002) Magical beliefs and rituals in young children. Child Psychiatry and Human Development 33: 43-58.

Ferdowsian HR, Beck N (2011) Ethical and scientific considerations regarding animal testing and research. PloS one 6: e24059.

Gillan CM, Papmeyer M, Morein-Zamir S, Sahakian BJ, Fineberg NA, Robbins TW, De Wit S (2011) Disruption in the balance between goal-directed behavior and habit learning in obsessive-compulsive disorder. American Journal of Psychiatry 168: 718-726.

Helmstaedter C, Witt J-A (2008) The effects of levetiracetam on cognition: a non-interventional surveillance study. Epilepsy & Behavior 13: 642-649.

Helmstaedter C, Witt J-A (2010) Cognitive outcome of antiepileptic treatment with levetiracetam versus carbamazepine monotherapy: a non-interventional surveillance trial. Epilepsy & Behavior 18: 74-80.

Hjemdal O, Vogel PA, Solem S, Hagen K, Stiles TC (2011) The relationship between resilience and levels of anxiety, depression, and obsessive–compulsive symptoms in adolescents. Clinical Psychology & Psychotherapy 18: 314-321.

Hoffman KL, Cano-Ramírez H (2018) Lost in translation? A critical look at the role that animal models of obsessive-compulsive disorder play in current drug discovery strategies. Expert Opinion on Drug Discovery 13: 211-220.

Hooijmans CR, Leenaars M, Ritskes-Hoitinga M (2010) A gold standard publication checklist to improve the quality of animal studies, to fully integrate the Three Rs, and to make systematic reviews more feasible. Alternatives to Laboratory Animals 38: 167-182.

Leonard HL, Goldberger EL, Rapoport JL, Cheslow DL, Swedo SE (1990) Childhood rituals: normal development or obsessive-compulsive symptoms? Journal of the American Academy of Child & Adolescent Psychiatry 29: 17-23.

Magalhães JC, Gongora M, Vicente R, Bittencourt J, Tanaka G, Velasques B, Teixeira S, Morato G, Basile LF, Arias-Carrión O (2015) The influence of levetiracetam in cognitive performance in healthy individuals: neuropsychological, behavioral and electrophysiological approach. Clinical Psychopharmacology and Neuroscience 13: 83.

Operto FF, Pastorino GMG, Mazza R, Roccella M, Carotenuto M, Margari L, Verrotti A (2019) Cognitive profile in BECTS treated with levetiracetam: A 2-year follow-up. Epilepsy & Behavior 97: 187-191.

Piazzini A, Chifari R, Canevini MP, Turner K, Fontana SP, Canger R (2006) Levetiracetam: an improvement of attention and of oral fluency in patients with partial epilepsy. Epilepsy research 68: 181-188.

Ranganatha N, Kuppast I (2012) A review on alternatives to animal testing methods in drug development. International Journal of Pharmacy and Pharmaceutical Sciences 4: 28-32.

Robbins TW, Costa RM (2017) Habits. Current Biology 27: R1200-R1206.

Robbins TW, Vaghi MM, Banca P (2019) Obsessive-Compulsive Disorder: Puzzles and Prospects. Neuron 102: 27-47.

Ruscio A, Stein D, Chiu W, Kessler R (2010) The epidemiology of obsessive-compulsive disorder in the National Comorbidity Survey Replication. Molecular psychiatry 15: 53-63.

Sanchez PE, Zhu L, Verret L, Vossel KA, Orr AG, Cirrito JR, Devidze N, Ho K, Yu G-Q, Palop JJ (2012) Levetiracetam suppresses neuronal network dysfunction and reverses synaptic and cognitive deficits in an Alzheimer's disease model. Proceedings of the National Academy of Sciences 109: E2895-E2903.

Scheepers IM, Stein DJ, Harvey BH (2018) Peromyscus maniculatus bairdii as a naturalistic mammalian model of obsessive-compulsive disorder: current status and future challenges. Metabolic brain disease 33: 443-455.

Thorsen AL, de Wit SJ, de Vries FE, Cath DC, Veltman DJ, van der Werf YD, Mataix-Cols D, Hansen B, Kvale G, van den Heuvel OA (2019) Emotion regulation in obsessive-compulsive disorder, unaffected siblings, and unrelated healthy control participants. Biological Psychiatry: Cognitive Neuroscience and Neuroimaging 4: 352-360.

van der Straten A, van Leeuwen W, Denys D, van Marle H, van Wingen G (2020) The effect of distress on the balance between goal-directed and habit networks in obsessive-compulsive disorder. Translational Psychiatry 10: 1-10.

Walitza S, Melfsen S, Jans T, Zellmann H, Wewetzer C, Warnke A (2011) Obsessive-compulsive disorder in children and adolescents. Deutsches Ärzteblatt International 108: 173-9.

Wu T, Chen C-C, Chen T-C, Tseng Y-F, Chiang C-B, Hung C-C, Liou H-H (2009) Clinical efficacy and cognitive and neuropsychological effects of levetiracetam in epilepsy: an open-label multicenter study. Epilepsy & Behavior 16: 468-474.

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END OF CHAPTER ONE

2 Literature review

2.1 Obsessive-compulsive disorder – a clinical summary

Obsessive-compulsive disorder (OCD)[1] is most often characterized by thematically specific obsessions, i.e. intrusive thoughts and impulses relating to specific themes, and compulsions, i.e. persistent and repetitive behaviors (Ersche et al., 2019). Further, these symptoms are normally very rigid, in that they are expressed irrespective of the associated negative outcomes, e.g. occupational and social failure (Torregrossa et al., 2008). Importantly, as opposed to other psychiatric disorders that present with components of delusion, i.e. psychosis and Alzheimer's disease, patients with OCD generally show insight into the absurdity and irrationality of their actions (Loosen and Hauser, 2020). Thus, individuals suffering from OCD attempt to resist the expression of such symptoms (Kohler et al., 2018).

The condition is clinically heterogeneous, meaning that different patients diagnosed with OCD may present with unique symptom profiles (Iervolino et al., 2011). The most common symptom dimensions are related to safety/checking, contamination/washing, or symmetry/ordering. A fourth symptom dimension is characterized by intrusive thoughts about repugnant or shaming themes, e.g. sex, religion, and violence, which are associated with covert mental routines, e.g. excessive praying (Graybiel and Rauch, 2000; Hojgaard et al., 2018). With respect to the symptom profiles seen in the different sexes, men mostly present with intrusive thoughts related to repugnant themes, whereas women often present with contamination/washing OCD (Destree et al., 2020). A possible explanation for this may be that in certain cultures, specific behaviors that show some overlap with OCD symptom dimensions (e.g., washing and house ordering) may partly be seen as behaviors typical to the female domain (Mathis et al., 2011). This may lead to a delayed detection of OCD onset in some (Frydman et al., 2014). Irrespective of symptom phenotype, the diagnostic criteria for OCD require symptoms to last for at least one hour of the day. Also, patients must find the urges of the disorder to be very unpleasant and unwanted, and the obsessions and compulsions must occur rigidly, leading to disrupted daily routines (Abramowitz and Jacoby, 2014). Ultimately, obsessive-compulsive (OC)[2] symptoms cause disturbances in an individual's occupational and social functioning due to the stressful and time-consuming nature thereof. This leads to a decline in the quality of life (Snorrason et al., 2016).

Obsessive-compulsive disorder affects men more than women (male to female ratio of 3:2) (Daniel et al., 2012), with the greatest risk period for developing the condition being between the ages of six and

[1] obsessive-compulsive disorder
[2] obsessive-compulsive

40 years (Rosenberg and Keshavan, 1998). However, OCD[1] is now understood as a condition with a bimodal age of onset with the first peak in new cases seen in the pre-adolescence phase (at around 10 - 12 years of age) and the second in early adulthood (20 - 22 years of age) (Stewart et al., 2017). Childhood onset OCD is more prevalent in male patients and although approximately 80 % of cases develop at an early age (younger than 18 years), only 40 % of these cases continue into adulthood (Kenezloi et al., 2018). Further, individuals that develop OCD at a later age in their lifetime (i.e. after 30 years), usually present with less severe symptoms for a shorter duration of time (Frydman et al., 2014). A complicating factor for the clinical identification and treatment of the condition is the uncertainty whether childhood onset OCD should be diagnosed when symptoms are first reported (or observed), or only when said symptoms begin to cause functional impairment (Frydman et al., 2014). Usually, OCD is only diagnosed from the moment that the compulsions and distress interfere with the normal routine of patients. This theme will, to a certain extent, also be explored in the present body of work.

Although the exact etiology of OCD is still unclear, the condition is borne from both genetic and environmental factors, demonstrating a heritability rate of 30 - 50 % (Grant and Chamberlain, 2020). Unaffected first-degree relatives of OCD patients often have a higher risk to develop OCD in future, compared to non-relatives (Menzies et al., 2008; Peng et al., 2020), while environmental factors and undesirable life events (such as loss of a loved one or a severe injury) also increase the likelihood for an individual to develop OCD (Hofer et al., 2020). Notably, 50 – 70 % of OCD patients experienced at least one severe life event in the six months prior to a clinical OCD diagnosis (Destree et al., 2020). Even if such stressful life events do not directly precipitate OCD, they may intensify the symptom severity and speed up the progression of the disorder (Fineberg et al., 2019). It thus stands to reason that stressful life events may then be applied as useful predictor of future OCD.

Along its developmental trajectory, OCD demonstrates three clinical stages. First, there is the endophenotype stage, during which only mild endophenotypic abnormalities are present. Endophenotypes (which are heritable, state-independent traits, i.e. behavioral tendencies that are not modifiable by changes in circumstance) may be recognized as minor dysfunctions in executive abilities. In the case of compulsivity, this may be habit-proneness, response inhibition deficits, or even physical changes such as grey matter volume disparities (Marzuki et al., 2020; Menzies et al., 2007; Viswanath et al., 2009). Indeed, retrospective research demonstrated a five-to-seven-year period during which sub-threshold OCD symptoms are seen in patients before they proceeded to diagnosable OCD (Coles et al., 2011). The second stage, called the alarming stage, usually involves the onset of diagnosable OCD

[1] obsessive-compulsive disorder

(that is, when the patient begins to feel distressed as a result of his/her symptoms or when the required diagnostic criteria are met). The third and final stage is characterized by major executive dysfunctions, e.g. reward-feedback processing deficits during which time patients exhibit compulsions that are conceptually akin to behavioral addiction (Grassi, 2016).

It is vital to note at this stage that obsessions (and thus the execution of compulsions) are generated in response to implicit fears or feelings of anxiety (Milad et al., 2013). Thus, while patients realize the futility of their actions, they are unable to prevent engaging in neutralizing compulsive rituals. In other words, while it would be normal to express certain behaviors as part of our daily routines, e.g. locking a door, it is theorized that patients with OCD[1] do not find closure after task completion (Zor et al., 2011). In fact, since they respond to implicitly generated fears—in this case concerns about their safety—the mere locking of a door only provides short-term relief. Once the implicit fear is generated again, a repetitive cycle of self-reinforcing compulsive behavior is induced. Such a pattern can be ascribed to several mechanisms that we will discuss below. However, it must be reiterated that in most cases, compulsions are initially expressed in response to (or at least in association with) irrational fears that are self-generated, and which are therefore resistant to normal goal-directed action-outcome feedback closure (Hinds et al., 2012; Krypotos et al., 2015).

2.2 The neurobiology of OCD

2.2.1 Neuroanatomy

From a developmental perspective, it is unclear whether OCD can truly be regarded as a neurodevelopmental condition (Grassi, 2016). However, since approximately 80 % of new OCD diagnoses are recorded in children, this seems to be at least partly true (Geller et al., 2021). Further, among children, OCD shows a high degree of comorbidity with Tourette's disorder and attention-deficit/hyperactivity disorder (ADHD)[2], both of which are recognized neurodevelopmental disorders (Grant and Chamberlain, 2020; Rosenberg and Keshavan, 1998).

Obsessive-compulsive disorder is primarily associated with abnormalities in and dysfunction of executive loops in the cortico-striatal-thalamo-cortical circuits (CSTC)[3] (Peters et al., 2016). The CSTC circuitry plays a pivotal role in processes of cognitive control, notably those that are responsible for regulating sensorimotor, executive, and motivational functions (Kubota et al., 2019; Stein et al., 2019). Thus, it is evident that the CSTC circuitry is also implicated in the control of movement, habit

[1] obsessive-compulsive disorder
[2] attention-deficit/hyperactivity disorder
[3] cortico-striatal-thalamo-cortical

development (by reinforcing certain behaviors or behavioral patterns through continuous executions) and reward-feedback processing (the motivational characteristic of a stimulus that leads to appetitive behaviors) (Radulescu et al., 2017). Perturbations in all of these constructs have been shown in individuals suffering from OCD[1] (Gillan et al., 2016; Ozcan et al., 2016). However, before closer attention can be afforded to these neurocognitive themes against the background of OC[2] symptom expression, a brief overview of the relevant structures that collectively constitute the CSTC[3] pathway, will be provided. At the simplest level, the CSTC circuits coordinate discrete executive (planning-execution-stop) functions via cortico-striatal projections (Haber, 2016). Serving as the main 'go-no-go' gate for behavioral engagement, striato-thalamic afferents relay the signal back to the cortex, where the loop is finally closed and the behavioral action triggered (or prevented) (Griffiths et al., 2014; Lanciego et al., 2012).

With respect to the frontal cortex, it is apparent that more than one distinct neuroanatomical area could be involved in the psychobiological architecture of OCD. First, the anterior cingulate cortex (ACC)[4] is mostly responsible for the planning of executive processes, while research is mostly congruent in that the orbitofrontal and motor cortices are vital for the ultimate execution (or non-execution) of voluntary behaviors (Fried et al., 2017). It is therefore not surprising that hyperactivity of the orbitofrontal cortex (OFC)[5] is proposed as a potential neuroanatomical construct that may underlie OCD (Melloni et al., 2012). Interestingly, it has been shown that patients presenting with OFC hyperactivity demonstrate an intensified response towards 'intimidating' stimuli which may be associated with compulsive behaviors which are purportedly expressed to respond to such stimuli (Amoretti et al., 2002). Notably, as will be discussed later in this chapter, patients with OCD also demonstrate a propensity towards poorer attention, information processing speed, cognitive flexibility, decision making as well as motor inhibition deficits. Such cognitive and motor impairments across different domains have also been linked to perturbations in the medial prefrontal cortex, the right inferior frontal cortex, and the dorsolateral prefrontal cortex (Leisman et al., 2016; Pauwels et al., 2019).

Upon cortical signaling of the striatum, executive signals travel via two distinct pathways through the basal ganglia to the thalamus, i.e. a behaviorally activating direct, and a behaviorally inactivating indirect pathway (Balasubramani et al., 2015; Nambu, 2008). Since both pathways are activated simultaneously, the ultimate 'go' (direct pathway activation) or 'no-go' (indirect pathway activation) direction is determined by dopamine which, upon the anticipation of a positive (or rewarding outcome), is released

[1] obsessive-compulsive disorder
[2] obsessive-compulsive
[3] cortico-striatal-thalamo-cortical
[4] anterior cingulate cortex
[5] orbitofrontal cortex

in the nigro-striatal pathways (Rommelfanger and Wichmann, 2010). While the direct pathway expresses excitatory dopamine-1 (D_1) receptors, the indirect pathway mostly expresses inhibitory dopamine-2 (D_2) receptors. Thus, if a positive outcome is predicted, our present understanding of striatal neurobiology dictates that dopamine release will *inactivate* the behaviorally *inactivating* indirect pathway, thus resulting in a net behaviorally engaging stimulus. In contrast, a decrease in dopamine release upon anticipating a negative outcome, will not inactivate the otherwise tonically activated indirect pathway, thereby maintaining a net inhibitory striato-thalamic output (Rommelfanger and Wichmann, 2010).

Thalamic, and ultimately cortical activation or inhibition is subsequently regulated by the net striato-thalamic signal propagated. The thalamus is broadly responsible for higher level processing in terms of relaying executive signals from the striatum to the cortex. It acts as a relay station for the communication between the striatum and the OFC[1] and therefore serves as the final output node in the CSTC[2] circuit before behavioral signals are terminated (Sherman, 2006). Once signals are relayed from the thalamus to the OFC, behavioral output is terminated, resulting in the deactivation of the applicable CSTC circuits (Fettes et al., 2017).

2.2.2 Neurotransmission

With respect to OC[3] symptom manifestation, conclusive findings regarding neurotransmitter involvement remain largely elusive. That said, considering the aforementioned explanation of how cortico-striatal signaling facilitates voluntary motor behavior, research into dopaminergic and serotonergic signaling yielded significant and valuable advances in our understanding. To explain, a brief overview of the seminal work by Schultz (2002, 2006, 2007a, 2007b), Hashemi et al. (2012), and Seymour, Daw, Roiser, Dayan, and Dolan (2012), is necessary. Collectively, these authors showed that, under circumstances of action-outcome planning and valuation, dopamine and serotonin function in a relationship of opponency, where dopamine is believed to promote reward-engaging approach behavior (i.e. engagement in voluntary goal-directed action), while serotonin will mostly be responsible for behavioral inhibition. In this sense, OCD[4] is believed to be a condition of *hypo*serotonergic signaling, which theoretically facilitates a hyperdopaminergic state in the CSTC circuitry (Lissemore et al., 2018). It is this functional interaction that is believed to explain the treatment response to selective serotonin reuptake inhibitor (SSRI)[5] intervention, alone or in combination with low-dose anti-dopaminergic

[1] orbitofrontal cortex
[2] cortico-striatal-thalamo-cortical
[3] obsessive-compulsive
[4] obsessive-compulsive disorder
[5] selective serotonin reuptake inhibitor

intervention (Fineberg and Craig, 2007; Hollander et al., 2003). Further, once released into the synaptic cleft, serotonin is cleared by among others, the actions of the serotonin transporter (SERT)[1] (Murphy and Lesch, 2008). Patients with OCD have shown a decrease in OFC[2] SERT density (Atmaca et al., 2011), the magnitude of which has been inversely correlated with symptom severity in some studies (Zitterl et al., 2008). These findings support current theories of OCD neurobiology, because reduced SERT concentrations may be linked with blunted serotonergic transmission (Stengler-Wenzke, et al., 2004).

Apart from serotonin and dopamine, the excitatory and inhibitory neurotransmitters, glutamate and γ-aminobutyric acid (GABA)[3] respectively, are also of noteworthy importance (Wu et al., 2012; Zhang et al., 2016). These neurotransmitters broadly regulate neuronal activation and inactivation and play a fundamental role in processes of cognition, memory, and motor activities, all of which are particularly important in OCD (Muller and Roberts 2005; Karthik et al., 2020).

Glutamate (the precursor of GABA) is the main excitatory neurotransmitter found in the mammalian brain. In the CSTC[4] circuits, ionotropic glutamatergic activity promotes rapid executive signal propagation along the cortico-striatal pathways (Galvan et al. Kurebayashi, 2006). In contrast, metabotropic glutamatergic signaling (mainly via the mGluR1 and mGluR5 receptors) maintains indirect pathway activity by counteracting the disinhibiting effects of dopamine (Conn et al., 2005). However, the remaining metabotropic receptors seem to favor direct pathway activity, and hence, a clear role for glutamate's actions in OC[5]-neurobiology, remains largely elusive. Thus, pharmacotherapeutic interventions aimed at glutamatergic targets are not used as often (Kellner, 2010). In turn, GABA, functioning mainly via $GABA_A$ and $GABA_B$ heteroceptors, elicits rapid inhibitory responses (Galvan et al., 2006) on mostly non-GABAergic terminals, including dopaminergic nerve endings (Pickel and Chan, 1990).

[1] serotonin transporter
[2] orbitofrontal cortex
[3] γ-aminobutyric acid
[4] cortico-striatal-thalamo-cortical
[5] obsessive-compulsive

2.3 The pharmacological and psychological treatment of OCD

Obsessions and compulsions mostly respond to a limited series of pharmacological treatments. These include the SSRIs[1] (Lissemore et al., 2018), which are considered as the first-line pharmacological intervention for OCD[2]. Although the response rate remains suboptimal, modest symptom improvement in most patients supports the aforementioned theory of hyposerotonergic functioning in the CSTC[3] circuits as a central neurobiological construct (Reghunandanan et al., 2015). The response of OCD to SSRIs is not unique to this condition. Drugs in this class, e.g. escitalopram, sertraline, fluoxetine, paroxetine and fluvoxamine, are also used for the treatment of several other neuropsychiatric disorders, e.g. major depression (MD)[4] and anxiety (Ghaffari et al., 2020; Strawn et al., 2019). For OCD and anxiety, higher doses than the nominal anti-depressive doses are useful (Janardhan et al., 2017; Kellner, 2010; Ninan et al., 2006). Further, treatment is only effective after a chronic period of time, i.e. 12 weeks or longer (Lissemore et al., 2018), pointing to long-term neuromodulatory mechanisms, rather than rapid changes in serotonin concentrations, as the biological construct of the treatment response. Sadly, 40 - 60 % of patients fail to show a sufficient response to first line OCD treatment strategies. In such SSRI-refractory cases, patients are usually either initiated on a higher dose of the currently used SSRI or switched to another SSRI that has a different receptor binding and/or adverse effect profile (Del Casale et al., 2019). If these approaches fail, augmentation therapy with low-dose anti-dopaminergic drugs, e.g. haloperidol, risperidone, olanzapine, or quetiapine administered in combination with an SSRI, are used (Bandelow et al., 2008; Middleton et al., 2019). In this way, both the hyposerotonergic and dopaminergic theories of compulsive symptom expression are targeted (Albert et al., 2018). As our understanding of OC[5] phenotypes expand, it becomes clearer that treatment resistance in OCD can potentially be ascribed to the underlying neurocognitive differences that may typify the various symptom phenotypes (Miguel et al., 2005); this has also been demonstrated in pre-clinical studies (de Brouwer et al., 2020). This possibility highlights a need for a deeper understanding of the psychobiological processes that perpetuates the different obsessive-compulsive symptom dimensions, a research topic that will also be afforded attention in this work.

Although OCD is no longer classified as an anxiety disorder, anxiety plays a central role in the neuropsychological architecture of the condition (Stein et al., 2010). This will be discussed later in this chapter. However, it stands to reason that most patients experience a high degree of anxiety (which is believed to associate with or trigger the expression of compulsive acts). Therefore, classic anxiolytics,

[1] selective serotonin reuptake inhibitor
[2] obsessive-compulsive disorder
[3] cortico-striatal-thalamo-cortical
[4] major depression
[5] obsessive-compulsive

e.g. the benzodiazepines, should be effective as a treatment to some extent. This is mostly not the case (Starcevic et al., 2016). That said, benzodiazepines can sometimes be useful to attenuate feelings of anxiety in OCD[1] patients with co-occurring mood and anxiety disorders or in cases where obsessions cause a high degree of stress, for example in patients suffering from covert mental obsessions (Brakoulias et al., 2013). Benzodiazepines are also sometimes used in addition to an SSRI[2] until the latter elicits its maximum effect (Kellner, 2010). With respect to the specific agents used in OCD, clonazepam is most often prescribed, followed by bromazepam, alprazolam, and lorazepam (Starcevic et al., 2016).

With a continued effort to better understand the role of sex and endocrine systems on neuropsychiatric outcomes (Guglielmi et al., 2017), and taking the bimodal age of onset of OCD in males and females into account, it is interesting to note that sex does not seem to have an influence on ultimate treatment outcomes (Mathes et al., 2019). While certain nuances in treatment outcomes between sexes are sometimes noted, for example that men may show a higher rate of symptom remission after two years of therapy compared to women (Eisen et al., 2013), or that women show a quicker response to treatment (Eisen et al., 2010) compared to men, general clinical consensus is that men and women diagnosed with the same symptom phenotypes, present with similar long-term treatment outcomes (Iervolino et al., 2011). In this regard, patients diagnosed with contamination/washing OCD, seem to respond better to cognitive behavioral therapy (CBT)[3] and exposure-response prevention (ERP)[4], compared to SSRI treatment (Masi et al., 2009), whereas sexual and aggressive obsessions show a greater response to SSRIs (Thorsen et al., 2018).

With respect to cognitive therapy, CBT and ERP are most often used (Reid et al., 2021). The former is based on the idea that psychobiological disorders are associated with specific cognitive perturbations (see later in this chapter), and that assisting patients to identify and talk through their disrupted beliefs and thoughts that result in the expression of OC[5] symptoms, can not only have a significant and lasting effect on their belief systems, but also slow clinical progression (Leeuwerik et al., 2019). In contrast, while ERP is also based on cognitive theory, it is a more invasive form of psychotherapy in that patients are in actual fact confronted with the content of their OC symptoms, i.e. contaminated surfaces, or unsafe or disordered environments (Reid et al., 2021), so as to evoke an emotional response. Then, while being exposed, patients are prevented from engaging in neutralizing rituals while the therapist discusses the irrationality of said evoked emotions and urges to engage in neutralizing responses. For

[1] obsessive-compulsive disorder
[2] selective serotonin reuptake inhibitor
[3] cognitive behavioral therapy
[4] exposure-response prevention
[5] obsessive-compulsive

apparent reasons, ERP[1] sessions are highly anxiogenic and cause a significant degree of distress (Olatunji et al., 2013). The purpose of ERP is thus to instill a level of control over the experience of obsessions and the expression of compulsions, by assisting patients to form new and realistic associations between specific stimuli and appropriate responses (Ong et al., 2022). While the response rates to CBT[2] and ERP are also modest, indications are that psychotherapy may have a longer lasting and more robust outcome in responders, compared to pharmacotherapy (Skapinakis et al., 2016). Researchers have ascribed such findings to the fact that CBT and ERP assist patients to *understand* the futility of their symptoms and that they thus become equipped to reason their way through potential future scenarios (Leeuwerik et al., 2020).

Considering that the successful outcome of CBT and ERP is, as is true for pharmacotherapy, dependent on patient compliance, cause a significant degree of distress, and that several long sessions are needed to yield a response, patient adherence is a major factor for consideration. Indeed, it has been noted that between eight and 33 % of individuals fail to adhere to therapy in the long run, hence contributing to therapeutic failure (Mancebo et al., 2011). Thus, to decrease the levels of anxiety experienced and expedite the treatment outcome, CBT and ERP are often used in combination with anti-OC[3] pharmacotherapy (Marazziti et al., 2020) and anxiolytics (Del Casale et al., 2019).

Collectively, our knowledge of anti-OC treatment interventions has expanded significantly over the past few decades (Fineberg et al., 2011). Still, while neither pharmacotherapy nor psychotherapy is without shortcomings, a clear need exists for a deeper understanding of the condition, its development, and ultimate clinical outcomes so as to further our current therapeutic successes.

[1] exposure-response prevention
[2] cognitive behavioral therapy
[3] obsessive-compulsive

2.4 Cognitive constructs and theories that attempt to explain OCD

2.4.1 Anxiety remains a key component

Obsessive-compulsive disorder was initially classified as an anxiety disorder in previous versions of the Diagnostic and Statistical Manual of Mental Disorders (DSM)[1]. As alluded to before, anxiety is more often than not associated with the expression of obsessions and compulsions, while anxiety disorders are frequently co-diagnosed in patients with OCD[2] (Stein et al., 2010). Anxiety is an emotion borne from experiencing tension, discomfort or troubled thoughts that develop from the prediction of—or a lower degree of sensitivity to—potential danger (Griffin, 1990; Kurebayashi et al., 2012).

Our initial understanding of the relationship between anxiety and OCD was clouded by uncertainty as to whether anxiety should be seen as a secondary response to the expression of obsessions and compulsions, or whether these experienced anxieties drive the progression of the disorder (Stein et al., 2010). The latter view is supported by the fact that OC[3] symptoms in humans (Raposo-Lima et al., 2022), and similar behaviors in animals (Dias-Ferreira et al., 2009), usually worsen under stressful conditions. Notably however, although anxiety is a key construct of OCD, classic anxiolytics, e.g. the benzodiazepines, are overly ineffective for the management of OCD (Starcevic et al., 2016)—this we referred to earlier. Thus, a causal relationship remains unlikely (Nutt and Malizia, 2006). However, rather than disregarding a role for anxiety in the pathogenesis of OCD, the current diagnostic guideline highlights nuances of this disorder with respect to its presentation and association with other constructs like anxiety (APA, 2013; Black and Grant, 2014). Arguments for this change in classification were based on different findings, i.e. that the long-term expression of repetitive rituals and behaviors is often found to be the dominant clinical feature, even when elevated anxiety levels were absent (Stein et al., 2010). Further, the fact that agents solely aimed at the attenuation of anxiety are ineffective for the management of OCD, also contributed. Irrespective, parallels between OCD and anxiety can be drawn on various levels. This is important since an accurate diagnosis of the primary condition will determine the manner in which the condition is managed.

From an epidemiological and developmental perspective, generalized anxiety disorder (GAD)[4] has a lifetime prevalence rate of 6.2 % (Ruscio et al., 2017), while women are more likely to develop GAD at a younger age compared to men (the opposite is observed in OCD) (Weisberg, 2009). An estimated 25 % of GAD cases are diagnosed prior to the age of 20 years, whereas an additional 50 % of cases

[1] Diagnostic and Statistical Manual of Mental Disorders
[2] obsessive-compulsive disorder
[3] obsessive-compulsive
[4] generalized anxiety disorder

present prior to 47 years of age (Kessler et al., 2005). Given the role of anxiety in OC[1] symptom promulgation, GAD[2] is one of the most common comorbid diagnoses in patients suffering from OCD[3] (Nutt et al., 2006). As is true for OCD, almost 50 % of patients suffering from GAD that are treated with first-line GAD treatments, e.g. SSRIs[4], do not respond to treatment (Ansara, 2020). Comorbidity between OCD and GAD also affect anti-OC treatment outcomes. For example, OCD patients with comorbid GAD usually demonstrate an increased severity of fear-related OCD symptoms, e.g. aggressive thoughts, sexual or religious ideation, or contamination obsessions (Sharma et al., 2021). Comorbid GAD patients usually show a higher degree of avoidance behaviors which often associate with an increased likelihood for suicide (Hansen et al., 2007). These findings indicate that comorbid GAD presenting in patients diagnosed with OCD can contribute to shaping unique nuances in the phenotypic presentation of the disorder (Sharma et al., 2021). Importantly, comorbid GAD and OCD patients are more likely to interrupt their treatment and thus may need increased clinical oversight in the early stages of treatment (Hansen et al., 2007).

In terms of pharmacotherapy, the mere fact that classic anxiolytics like the benzodiazepines are overly ineffective for the treatment of OCD, does not preclude a similar therapeutic signature underlying both conditions. Indeed, as we have referred to before, chronic high-dose SSRI treatment not only has anti-compulsive actions but are also used for the treatment of anxiety disorders (Matsumoto et al., 2007). Thus, the time-dependent response of anxiety to chronic high dose SSRIs vs. its immediate clinical improvement following acute benzodiazepine intervention, highlights a unique role for GABA[5]ergic signaling in the manifestation of anxiety that is arguably not seen in compulsive symptom expression.

In conclusion, it is clear that OCD and GAD share specific psychobiological traits that will arguably inform the diagnosis and treatment of either primary disorder. Further, a clear understanding of the interactions between anxiety- and OC-related processes and how they may interact to yield the specific clinical picture observed, will arguably result in improved management and a more robust long-term therapeutic outcome.

[1] obsessive-compulsive
[2] generalized anxiety disorder
[3] obsessive-compulsive disorder
[4] selective serotonin reuptake inhibitors
[5] γ-aminobutyric acid

2.4.2 A matter of being less flexible

Cognitive flexibility is described as the ability to adapt one's behavior to align with environmental or circumstantial changes (Albertella et al., 2020). Similarly, the term set-shifting refers to the ability to quickly switch between relevant thought routines (Abramovitch and Cooperman, 2015). Both cognitive flexibility and intact set-shifting ability are important for generating proper behavioral responses so that one can act and react to changing circumstances in an effective, appropriate, and beneficial manner (Dajani and Uddin, 2015). Central to flexibility is the ability to learn. Specifically, action-outcome contingency learning (and thus also reversal learning) are vital competencies for being able to cope with sudden changes in a familiar environment (Chamberlain et al., 2007); for this, the OFC[1] is pivotal (Piras et al., 2013). Since perturbations in orbito-frontal functioning are often noted in OCD[2], a potential explanation is provided for the fact that OCD patients often find set-shifting and task-switching difficult, thus persistently engaging in a specific behavioral output (Graybiel and Rauch, 2000).

In extension, another cognitive component, i.e. working memory, comes into play. Briefly, working memory refers to a system of information that is vital to performing complex tasks such as reasoning, comprehension and learning (Cowan, 2014) and is therefore important for cognitive flexibility (Cowan, 2014). Similarly, spatial working memory conserves details of specific locations and environmental cues, which is important for our ability to navigate in and engage with our environments in a successful manner (Broadbent et al., 2004). Thus, when impairments in working or spatial memory are seen, patients have difficulty to express every-day goal-directed actions (Verghese et al., 2017). Against this background, individuals suffering from OCD, often also show deficits in specific aspects of their working memory ability (Shahar et al., 2017). This leads to a disruption in spatial working memory capacity, which in turn may strengthen the expression of inflexible routine-like behaviors as patients struggle to process and implement constantly changing information pertaining to fluid environmental contexts (Heinzel et al., 2021). Thus, in this work, closer attention will be afforded to the concept of cognitive flexibility, as reflected by working memory capacity in an animal model of compulsive-like persistence, so as to gain insights into the potential naturalistic and etiological relationships between such constructs and the parallel manifestation of compulsive-like behavioral persistence.

[1] orbitofrontal cortex
[2] obsessive-compulsive disorder

2.4.3 Intolerance of uncertainty: is it a question of being overly safe or unnecessarily perfectionistic?

The capacity of healthy individuals to tolerate uncertainty is fundamental for our existence in changing and uncertain environments (Hillen et al., 2017). In contrast, an 'intolerance towards (of) uncertainty' (IU)[1], defined as *"a dispositional characteristic that develops due to a negative belief concerning uncertainty and the causes that it may have"* (Boswell et al., 2013), is observed in some. This results in negative emotional, cognitive, and behavioral reactions (Buhr and Dugas, 2009), e.g. excessive worry and inflated emotional and behavioral responses to cope with problems and prepare for any number of different potential outcomes. While a mild degree of IU may be to our benefit (Freeston et al.,1994), excessive worry about uncertain outcomes mostly causes a high degree of anxiety due not feeling in control (Greco and Roger, 2003). In patients presenting with disrupted cognitive processes pertaining to outcome planning and valuation, notably also in those suffering from OCD[2], OC[3] symptoms are often self-reported to be borne from attempts to engender a measure of control over uncertain, albeit unrealistic, circumstances (Steketee and Frost, 2003). However, since the stimuli responsible for engaging in neutralizing behavioral routines are either implicitly generated (or then, implicitly inflated), patients fail to exert proper control over such 'uncertain' circumstances, thus self-reinforcing and perpetuating the expression of compulsive routines. In principle, this is argued by Woody and Szechtman (2011) and Szechtman and Woody (2004), who regard OCD as a disturbance of security-motivation. While not explicitly referring to IU, they argue that since patients respond to implicitly generated concerns about safety, the neutralizing behaviors that they express will not be sufficient to engender feelings of being safe, and thus not provide sufficient or adequate goal-directed feedback. Against this background, IU can be regarded as a driving force that underlie innate security concerns, hence triggering the expression of compulsive rituals.

From a different perspective, IU can also relate to perfectionism, another trait often observed in patients with symmetry/ordering OCD (Pozza et al., 2019). Perfectionism can loosely be defined as holding oneself to almost unrealistic performance standards (Frost et al.,1993). In this sense, perfectionism is also believed to be exercised to maintain a high degree of control over certain outcomes. Specifically, perfectionism is often borne from an inflated sense of responsibility to prevent negative events from occurring, in turn also interfacing with safety/checking or contamination/washing OCD (Moretz and McKay, 2009). Patients reporting a higher degree of IU, will thus arguably also be candidates for compulsions of a perfectionistic nature, since attempts at being perfect can in principle be equated to

[1] intolerance towards (of) uncertainty
[2] obsessive-compulsive disorder
[3] obsessive-compulsive

an active coping response in the face of uncertain and potentially harmful outcomes (Vellozo et al., 2021). With respect to the current investigation, previous work from this laboratory argued that the behavior of large nesting deer mice seemingly represents a form of safety-seeking or perfectionistic behavior. In this work, we will once again afford attention to this behavioral phenotype, seeking to explore the potential associations between said behavior and changes in specific OC[1]-relevant neurocognitive constructs, i.e., working memory.

2.4.4 Impulsivity as opposed to compulsivity

Discussions about OCD[2] are often intertwined and colored with referral to an involvement of impulsive traits in the condition (Robbins et al., 2012; Robbins et al, 2019). Indeed, in addition to OCD, many other neuropsychiatric disorders are characterized by poor goal-directed behavioral regulation due to an inability to suppress other distracting, overriding urges (Snyder et al., 2015). Impulsivity is generally characterized by a lack of planning ability and persistence, thrill seeking, intensified urges to commit unnecessary actions, as well as an inability to distinguish between positive and negative urges (Whiteside and Lynam, 2001). Grant and Chamberlain (2020) also added that impulsivity consists of hasty and risky reactions that lead to unwanted outcomes. While impulsivity may contribute to an individual's risk to develop several neuropsychiatric disorders (Nigg, 2017), its relationship with OCD seems especially intricate. Impulsivity and OCD are often viewed as two conditions on opposing ends of the so-called 'impulsive-compulsive spectrum' (Chamberlain et al., 2016; Grant and Chamberlain, 2020). Within such a framework, compulsivity is seen as a condition that is characterized by overexaggerating potential harm, while impulsivity is related to the underestimation of potential (and often realistic) harm (Hollander and Rosen, 2000). Further, whereas impulsivity is driven by a need to gain reward (a pleasurable outcome), compulsivity is aimed at relieving stress (Hollander and Rosen, 2000), i.e. it is borne from negative or stressful emotion or feelings. In other words, both OCD and impulsivity are characterized by deficits in reward-feedback valuation and processing (Abramovitch and McKay, 2016), albeit of a different nature.

Apart from these distinct differences, OCD and impulsivity also share certain commonalities (Torregrossa et al., 2008), e.g. an inability to delay responses and/or suppressing unnecessary behaviors, repetitiveness, and an inability to stop (Sohn et al., 2014). Interestingly, similar behavioral elements are also shown in patients with addiction (Grassi et al., 2015). In fact, compulsive-like behavior is often seen in both preclinical models and clinical theorems of addiction, whereby the first phases of addiction are characterized by a high-proneness for impulsivity, risk-taking, and a bias towards seeking

[1] obsessive-compulsive
[2] obsessive-compulsive disorder

immediate rewards. Thereafter, behaviors become compulsive when the driving force changes from seeking immediate rewards, to a need for avoiding the negative outcomes associated with behavioral withdrawal (Abramovitch and McKay, 2016). Against this background, studies of addiction provided valuable insights into the potential temporal relationship between impulsivity and compulsivity.

A clear understanding of impulsive vs. compulsive behavior is important for several reasons. First, different phenotypes of OCD[1] may associate uniquely with impulsive processes, thus implicating different start- and stop-mechanism deficits (Benatti et al., 2014). This has implications for the way in which the condition will be managed, since said divergent mechanisms will arguably relate uniquely to other potential co-morbid conditions, e.g. attention-deficit/hyperactivity disorder (ADHD)[2] (characterized by impulsive starting responses) and drug addiction (more related to an inability to stop) (Asherson et al., 2014). Also, since impulsivity and compulsivity involve deficits in reward feedback processing, patients with OCD that also show impulsive traits will respond differently to psychotherapeutic interventions, e.g. ERP[3], compared to patients that are overly harm avoidant and less impulsive (Gondré-Lewis et al., 2020).

More importantly, from an animal modelling perspective, distinguishing between impulsive and compulsive-like behavioral traits is vital, since animal models of impulsivity and compulsivity both rely on the demonstration of repetitive behavioral phenotypes. Thus, a clear understanding of the underlying psychobiological signature that is modelled, is necessary to advance our understanding of both conditions. To this, we will refer in greater detail later in this chapter.

2.4.5 Do all roads lead to habit?

Following from the above, it is clear that OCD is variably characterized by deficits or dysfunctions in a range of neurocognitive domains. Broadly viewed, our behavioral engagement is ultimately guided by either goal-directed or habitual responses. As such, a clear understanding of these processes is important since they can all contribute to an inappropriate switch from goal-directed to overly habitual responses. In turn, it is theorized that an overreliance on habit may ultimately contribute to the prognosis and therapeutic response of OCD symptomology (Gillan et al., 2011). Since this theme is a core focus of the present work, a brief explanation of goal-directed and habitual behavioral responses will be provided, prior to the relationship between goal and habit in OCD being discussed.

[1] obsessive-compulsive disorder
[2] attention-deficit/hyperactivity disorder
[3] exposure-response prevention

Goal-directed, or planned, behavior is aimed at a specific, intended outcomes. Goal-directed behavior can thus either be seen as one of two opposing ideas, i.e. avoiding negative outcomes, or engaging in behaviors that will result in a positive outcome (Neal et al., 2006). Under normal circumstances, goal-directed behavioral response styles determine our ability to succeed on a functional, occupational, and social level (Peng et al., 2020). Goal-directed action-outcome processing is also central to the manner in which we learn from positive and negative feedback, with the former prompting continued behavioral engagement, while the latter facilitates behavioral adaptation to avoid repeated exposure to negative life events or experiences (Ehmer et al., 2020).

In contrast, habitual response mechanisms are mostly automated and well-learned, thus requiring less attention. However, since most habits are also 'aimed' at gaining a specific outcome, e.g. hitting the breaks once a stop-signal turns red, their ultimate execution required previous experience with the particular outcome in question (Ehmer et al., 2020). Habits, defined as *"settled or regular tendencies or practices, especially those that are hard to stop"*, can thus be viewed as *"automatic reactions to a specific situation"* (Robbins and Costa, 2017). Based on three different models, habits form gradually when actions are repeated in the same manner towards a frequently desired outcome. Specifically, behaviors can be repeated in a specific environmental context (*direct-context-cue model*, e.g. taking off ones shoes [behavior] before entering a home [context]), to repeatedly achieve a specific goal (*implicit-goal model*, e.g. brushing teeth), or to constantly seek a specific reward in a specific environment (*motivated-context model*, e.g. picking up a phone to check for notifications) (Neal et al., 2006). Even though habits initially begin as behaviors aimed at reaching a specific goal, constant repetition shifts the executive output to automated responses that may dissociate from the initial goal (Voon et al., 2015). In this way, when specific behaviors are continually repeated in the same manner, the environmental cues linked to the specific activity are stored in memory, making future access and repetition of the same executive output, convenient (Dickinson, 1985). The automation of common actions into habits requires a lesser cognitive load for such behaviors to be expressed, allowing cognitive resources to be reserved for other difficult tasks (Gillan et al., 2016).

On balance then, the formation of habits appears to be a useful process in terms of our daily executive functioning. However, habitual behaviors are inherently rigid and cannot be altered rapidly or with ease when a sudden environmental or contextual change occurs; this may lead to inappropriate (i.e., outdated, non-productive) responses. Given that habit formation is a typical process of healthy functioning individuals, they usually do not disturb our normal day-to-day lives (Graybiel and Rauch, 2000). However, certain habits might become more dominant than others. Interestingly, and as is true for the expression of compulsive behaviors as explained before, stressful environments or anxious emotions can contribute to a rapid shift from goal-directed to habitual response styles (Kim et al., 2001).

Importantly, under such circumstances, habits are often related to avoidance behaviors, i.e. to escape from or prevent unpleasant feelings; remarkably, this is also a feature of compulsive rituals (Gillan and Sahakian, 2015). Against this background, a disrupted balance between goal-directed and habitual responses induces a degree of executive conflict, whereby it may be difficult to switch from habitual engagement to goal-directed action-outcome processing when the need arises (Peng et al., 2020). This is in itself also an artefact of cognitive inflexibility, another neuropsychological endophenotype seen in OCD[1] (Gillan et al., 2011). Indeed, the habit hypothesis of OCD states that individuals presenting with habit-proneness may be at a greater risk of developing OCD (Snorrason et al., 2016). A further perpetuating factor that contributes to the habitual expression of compulsions is dysfunctional goal-directed action-outcome processing seen in individuals diagnosed with the disorder. To explain, dysfunctional goal-seeking behavioral processing can also lead to the senseless repetition of senseless certain behaviors, thus potentially interacting with impairments in cognitive flexibility to further strengthen habit-like OC[2] symptoms (Robbins et al., 2012).

Still, it is challenging to decisively distinguish between what must be defined as functional (i.e. normal) habits and abnormal (or maladaptive) habits that may associate with, or point to OCD. To this end, the best described differentiating characteristic may be that in OCD, habits (which can now be viewed as *compulsions*) are accompanied by an intense drive to respond as quickly as possible to a specific stimulus (either implicit or explicit). Such emotional valence is absent in healthy individuals engaging in everyday habitual responses.

2.5 A neurodevelopmental perspective: OCD from childhood to adulthood

From the above, a potentially useful theory arises. If cognitive constructs, e.g. anxiety sensitivity, cognitive flexibility, intolerance of uncertainty, and goal-directed action-outcome processing, can gradually interact to support the development of OCD, it might arguably be useful to follow children from a young age so as to provide a prospective window on potential future diagnoses. Since this theme will be explored here in a naturalistic animal model system of compulsivity, deeper insights into such a theory, will briefly be highlighted.

Most mental health conditions emerge during early life, with approximately 50 % of cases occurring before the age of 14 and a further 75 % of cases that are diagnosed after this age, presenting before the age of 24 (Kessler et al., 2005). Taking this into consideration and revisiting the epidemiological background of OCD summarized earlier in this chapter, the American Psychiatric Association also reports

[1] obsessive-compulsive disorder
[2] obsessive-compulsive

that 25 % of all OCD[1] patients have already shown noticeable OC[2] symptoms by 14 years of age (Loosen and Hauser, 2020). Notably, approximately 80 % of childhood onset OCD cases are diagnosed in children between the ages of 6 - 10 years (Dell'Osso et al., 2017; Kessler et al., 2005). Clinically viewed, the rate of OC symptom progression to a stage of diagnosis, is defined as the interlude between the age at which OC symptoms are first noticed by a practitioner and the age at which the OC symptoms *begin* to cause suffering (Thompson et al., 2020). In most children, this period spans approximately seven years (Thompson et al., 2020). Interestingly, and in line with the effects of similar events on the expression of compulsivity and habits, the rate at which OCD develops can be accelerated by stressful life events (Rajarethinam et al., 2000), reiterating the complex interaction between anxiety and compulsivity in patients diagnosed with OCD.

Juvenile OCD is one of the most prevalent mental disorders in children and is diagnosed in 1 - 3 % (Walitza et al., 2011) of children globally. Initially, diagnoses of OCD in children were not often made, likely due to OC symptoms in children often being 'hidden' due to the nature of normal behavioral rituals displayed by most children and which may resemble OCD-like behaviors. From as young as two years of age, most children show ritualistic and habit-like behaviors, including highly rigid sleeping or eating patterns, or expressing a strong preference for certain clothes or foods (Evans and Maliken, 2011). Although habits (which may associate with certain compulsions) and rituals in children share many commonalities, they differ based on the value of their outcomes, where habits occur despite an outcome, while rituals are mostly focused on a very specific outcome (Giovagnoli, 2018). Children displaying a high level of ritualistic behavior when growing up, often also demonstrate restrictive, inflexible behaviors when engaging in otherwise normal activities, e.g. getting ready for bedtime (Evans and Maliken, 2011). While this is normal and harmless under most circumstances, obsessions and compulsions also present in this manner in children and are thus often overlooked. However, after the age of seven, these rituals become less frequent, since ageing children learn more strategic ways of coping with their emotions and environments (De Caluwé et al., 2020) and so, diagnoses of OCD become less complicated. Another aspect that complicates the diagnosis of childhood OCD is that children often experience difficulties in describing and identifying obsessions whereas adults can verbalize and recognize their experiences in a more conducive manner (Stein et al., 2019).

In terms of potential triggers of childhood OCD, undesirable early-life events increase the risk. Parental affection may act as a protective factor, although research is unclear in terms of what such affection should entail and how much affection is necessary (Rachman, 1998). Parental affection is believed to

[1] obsessive-compulsive disorder
[2] obsessive-compulsive

provide a safe framework in which children can develop and nurture certain coping mechanisms against the effects of severely stressful life events. This may decrease their risk for developing neuropsychological conditions (Hofer et al., 2020). With respect to OCD[1], optimal parental care and fostering engenders the necessary coping skillset in children to become independent, responsible, and able to function in a normal environment (Frydman et al., 2014; Frydman et al., 2020). In other words, by 'not being there while being there', children are exposed to everyday experiences that are often somewhat dangerous, albeit in a safer environment. As such, they learn that dirty hands, for example, do not kill. In this way, the realistic value of everyday occurrences is conveyed, which helps to shape and prepare the brain for future similar scenarios (Vogel and Schwabe, 2016). In contrast, a complete lack of parental care on the one hand (Brander et al., 2016), and overprotectiveness on the other (Hofer et al., 2020), do not convey such skills, since children are either exposed to very severe experiences with real-life consequences, or to none potentially harmful scenarios at all (Wu et al., 2016). In the same sense, placing increased age-inappropriate responsibility on young children, e.g. requiring them to take care of and responsibility for a frail grandparent, can induce an inflated sense of harm prediction and safety concerns in young individuals that may have a detrimental later-life impact (Crittenden and DiLalla, 1988). In fact, OCD regularly develops due to a sudden increase in responsibility, explaining why children observing the world as threatening and feeling incompetent (Crittenden and DiLalla, 1988; Hofer et al., 2020), may develop OC[2] symptoms. These findings agree with theories regarding stress-inoculation (DiCorcia and Tronick, 2011), a theme that for the purposes of time and space, cannot be explored further here.

Given the intricate presentation of and interplay between normal childhood rituals, habits and the underlying psychobiology that may differ in each child, a clearer understanding of habits and rituals in children is vital. The emphasis on diagnosing OCD in children would therefore be on distinguishing between normal routine behaviors and continuous OC-like engagement in such behaviors as they grow older (De Caluwé et al., 2020). In the same sense, it would be fruitful and valuable to study such behaviors from a psychobiological perspective to establish whether certain endophenotypic traits shown by most children, can more accurately predict the risk for developing OCD later in their developmental trajectory.

[1] obsessive-compulsive disorder
[2] obsessive-compulsive

2.6 A potential way forward: exploring cognitive enhancers as a novel therapeutic avenue

Considering the less-than-optimal response of OCD[1] to current treatment strategies, novel treatment options that may target both the maladaptive cognitive and neurobiological mechanisms underlying OCD, may be useful (Middleton et al., 2019). Improving cognitive function and ability is an important overall goal of the broad neuroscientific field, since successful therapy will result in outcomes such as improved attention capacity and executive flexibility, better awareness, more vigilant observational capacity, and more effective information processing, decision making, and planning. Cognitive functioning is a broad term which encompasses all the mental processes that are associated with the achievement of knowledge, its processing, and manipulation, as well as our ability to reason (Kiely, 2014). Cognitive function is negatively affected in many psychiatric disorders, although causal relationships between cognitive impairment and neuropsychiatric symptom manifestation remain unclear (Magalhães et al., 2015). It suffices to say that given the cognitive neuropsychological architecture of OCD, which is related to many proposed alterations in neurocognitive functioning, therapies that are aimed at improving these processes, might be useful. For example, it stands to reason that if OCD is related to impaired cognitive flexibility, increased habit-proneness, poor goal-directed action-outcome processing, or an overestimation of potential, but improbable threat, broad improvements in cognitive functioning might contribute to an improved symptom profile. Moreover, if specific illness endophenotypes that could be predictive of later-life progression to OCD can be targeted at a young age, it might be possible to modify the long-term clinical outcome in susceptible individuals. Against this background, cognitive enhancers were developed as a means to improve specific cognitive impairments in patients living with neuropsychiatric disorders, e.g. dementia (Magalhaes et al., 2015).

From this perspective, levetiracetam (LEV)[2], the 5-enantiomer of 5-alpha-ethyl-2-oxo-1-pyrrolidine acetamide which was initially developed as a supplementary treatment for the management of partial and generalized epilepsy, may be a promising molecule. In fact, LEV treatment has been associated with improved neuropsychological and cognitive performance in individuals suffering from epilepsy, prompting its exploration as a potential cognitive enhancer (Piazzini et al., 2006). However, such potential in this context fundamentally remains confounded by the therapeutic framework. In other words, it is uncertain whether its effects on cognition, are contingent on its anti-epileptic action and as such, numerous investigations are underway to interrogate its neurocognitive actions (Magalhaes et al., 2015; Zhou et al., 2008) in other clinical cohorts. Interestingly, as opposed to other neuronal stabilizers, e.g. valproate and carbamazepine, that act by modulating the activity of voltage-gated ion channels, LEV

[1] obsessive-compulsive disorder
[2] levetiracetam

influences neurotransmitter release via its actions on synaptic vesicle glycoprotein 2A (SV2A)[1], which regulates the rate of synaptic vesicle exocytosis during neurotransmitter release (Brodie et al., 2007). Indeed, the fact that LEV[2] does not interact directly with known neurotransmitter systems, i.e. via receptor-ligand dynamics, or influence membrane excitability, may be important for its potential as a cognitive enhancer with a large therapeutic window (Helmstaedter and Witt, 2008; Wu et al., 2009). Instead, LEV regulates abnormal neurotransmission in an activity-dependent fashion, meaning that its actions are likely state dependent (Meehan et al., 2012).

In studies focusing on the cognitive actions of LEV, focus was mainly placed on behavioral response times and decision making, changes in levels of alertness, memory consolidation and recall, as well as an improved overall quality of life (Magalhaes et al., 2015). As an example, it was shown that patients suffering from epilepsy that took LEV as an add on therapeutic intervention, scored higher on overall quality of life scales, compared to patients from the same illness cohort, but that were not treated with LEV (Zhou et al., 2008). Also, irrespective of being administered as monotherapy or in combination with other anti-epileptics, LEV improves functioning on the levels of attention, external stimulus processing and set-shifting speed (Helmstaedter and Witt, 2008; Helmstaedter and Witt, 2010; Wu et al., 2009). Importantly, LEV also reduced the response time of these patients across all three stages of set-shifting tasks, i.e. stimulus uptake, option evaluation and final motor execution (Magalhães et al., 2015). Lastly, LEV decreases hyperactivity, impulsivity, mood uncertainty, as well as aggressive behavior in autistic children (Rugino and Samsock, 2002), highlighting its therapeutic potential in OCD[3] patients that may show similar impairments.

In this work, we will first explore the potential longitudinal relationship between signs of early-life cognitive impairment and later-life changes in working memory and compulsive-like behavior, i.e. large nest building (LNB)[4] behavior and high motor stereotypy (HS)[5], expressed by deer mice. We will then explore the effects of long-term LEV exposure from a young juvenile age until adulthood on said relationships and the expression of compulsive-like behaviors later in adulthood. Importantly, since LEV is devoid of a deleterious adverse effect profile (Bootsma et al., 2008), and considering that it is prescribed chronically for children and adults suffering from epilepsy (Goldberg-Stern et al., 2013), its potential as a long-term therapeutic strategy in children that present with early-life cognitive impairment that might be predictive of later life compulsivity, cannot be underestimated. To this end, the focus of the present body of research will be to establish whether temporal relationships between cognitive

[1] synaptic vesicle glycoprotein 2A
[2] levetiracetam
[3] obsessive-compulsive disorder
[4] large nest building
[5] high motor stereotypy

deficits and compulsive-like symptom expression can, in theory, prospectively be modified by exposure to a drug that shows broad cognitive-enhancing potential.

2.7 Animal models of OCD

Animal models are experimental systems established in animal species that can be laboratory-housed (mostly rodents or primates) to help us improve our understanding of disorders observed in another species (Alonso et al., 2015). This is also true for OCD[1], for which model systems that can convincingly mimic certain aspects of the human condition, are required. However, to mimic OCD-like behaviors in animals, is not an easy task. Specifically, while demonstrations of the compulsive, i.e. repetitive and persistent, symptoms of the human condition are relatively uncomplicated to replicate in an animal, the obsessional aspects of the disorder are problematic (Wolmarans et al., 2016). In fact, some researchers have expressed doubt as to whether it is at all possible to develop an entirely accurate animal model for OCD that encompasses both the cognitive/obsessive and motor/compulsive symptoms. Apart from 'obsessions' being difficult to show in animal behavior, mimicking the associations between obsessions and compulsions is equally challenging. The primary motivational driver of compulsive symptom expression is understood to be intrusive thoughts (obsessions). These are complex cognitive processes that involve continuous, repetitive, disturbing, and undesirable thoughts that *lead to* or *associate with* feelings of anxiety and that ultimately *instigate* the expression of compulsive symptom expression. Thus, while most preclinical OCD research is based on demonstrations of repetitive motor behaviors as the primary foundation for a model system's face validity (Wolmarans et al., 2016), it would still be difficult to show or argue that such behaviors associate with or stem from an underlying 'obsessional' foundation. Despite this, animal models are arguably required for researching the aspects of clinical OCD that could be studied with much success in rodents.

Irrespective of the type of animal model applied, there are certain requirements that all model systems should meet to be considered as valid research frameworks. There are three types of generally proposed validation criteria against which models can be by assessed, namely face, predictive, and construct validity (Belzung and Lemoine, 2011). A fourth validation criterion advocated for in this laboratory, pertains to the methodological integrity of translational studies, and can in concept be described as methodological validity (Wolmarans et al., 2017). Face validity is demonstrated when certain relevant symptomological and/or trait similarities between animal behaviors and specific symptoms of the human disorder are shown (Belzung and Lemoine, 2011). With regards to OCD, the face validity of an animal model is generally based on the demonstration of compulsive-like, repetitive,

[1] obsessive-compulsive disorder

and persistent behavioral phenotypes (Albelda and Joel, 2012). On the other hand, predictive validity focuses on treatment response in the model, whereby known effective treatments for the disorder also alter the modelled behavior appropriately (i.e. anti-depressant drugs should reduce the expression of depressive behaviors). Conversely, it would be equally valuable to show that treatments that are overly ineffective for the management of the clinical disorder, are also devoid of a contrasting effect in the animal model (Alonso et al., 2015). Thus, in the case of OCD[1], the animal model would ideally demonstrate a significant attenuating response towards chronic, high-dose SSRI[2] treatment, with or without the addition of low-dose anti-dopaminergic drugs. Construct validity is generally based on the psychobiological constructs known to be critically involved in the disease (Alonso et al., 2015). For models of compulsivity, such psychobiological overlap would point to serotonergic and dopaminergic involvement, while demonstrations of cognitive dysfunction (where possible), such as response inhibition deficits, impulsivity, or habitual behavioral persistence, would be equally important (Albelda and Joel, 2012; Wolmarans et al., 2017). Lastly, models are well founded on methodological integrity when the method applied to measure certain human-like behavioral traits in animals, are translationally relevant. Disappointingly, this is not always true, adding to the dilemma of translational 'irrelevance' which contribute to a lack of progress in the field of neuropsychiatry (Al Dahhan et al., 2019; Monteiro and Feng, 2016; Stanford, 2020). For example, in one of the most widely applied tests for 'anti-compulsive' drug action, i.e. the marble burying test, most studies use an acute 30-min pre-test intraperitoneal dose of SSRIs to 'prove' anti-compulsive drug action, as reviewed in-depth by de Brouwer, Fick, Harvey, and Wolmarans (2018). Further, the method applied does not distinguish between marble-directed burying and general burrowing activity, while a single test, instead of repeated tests, is used. While the 'purposelessness' of marble 'burying' is put forward as the principal supporting notion that such behavior might be representative of compulsivity (de Brouwer et al., 2018), very little of the test itself is reminiscent of actual compulsive-like behavior. Thus, the present work will be focused on naturalistic behavioral phenotypes that consider the persistent, repetitive, and non-functionality of compulsivity. These will be explained in greater detail below.

[1] obsessive-compulsive disorder
[2] selective serotonin reuptake inhibitor

As a collective, there are three main classes of animal models that are used in translational research, i.e. behavioral (trained or naturalistic), pharmacological, and genetic animal models. Behavioral models are defined as models that express certain characteristics or symptoms which are natural or learned. In other words, the observed behaviors are not pharmacologically, genetically, or otherwise induced. The model system applied in this work, i.e. HS[1] and LNB[2] in deer mice *(Peromyscus maniculatus bairdii)*, is an example of such a naturalistic, non-trained model. The major advantage of naturalistic models is that they can provide useful and detailed insights into the natural etiopathological processes that may underlie the spontaneous development of compulsive-like behaviors, while prospective investigations can be conducted in which the relationships between compulsive-like behavioral expression and various psychobiological constructs, e.g. cognitive biological and biological disturbances, can be explored. Certain investigations to this end have already been concluded in this laboratory (de Brouwer et al., 2021; de Brouwer et al., 2020; Scheepers et al., 2020). Pharmacological animal models are founded upon drug-induced behavioral alterations that are reminiscent of the clinical symptoms. An illustration of this model class is seen in quinpirole-induced compulsive checking, that focusses on behavioral changes observed in rodents after chronic treatment with quinpirole, a non-selective $D_{2/3}$ receptor agonist (Alonso et al., 2015). Lastly, genetic animal models are developed by either inbreeding for specific behavioral traits, or discrete genetic modifications, such as the knock-out of specific genes to yield characteristic behaviors. An example of such a model is the $5HT_{2C}$ receptor knock out mouse that engages in compulsive-like chewing behaviors (Alonso et al., 2015; Chou-Green et al., 2003).

[1] high motor stereotypy
[2] large nest building

2.7.1 The deer mouse model of OCD

The deer mouse model is an entirely naturalistic, non-induced behavioral model system that is reminiscent of specific aspects of clinical OCD[1]. When bred, reared, and housed under standard laboratory conditions, discrete subpopulations of deer mice spontaneously present with excessive, repetitive, and functionally purposeless behaviors. Further, as is true for clinical compulsivity, the behaviors shown by deer mice are heterogenous in their presentation and present in animals of both sexes (Scheepers et al., 2018). The three most studied phenotypes are HS[2], LNB[3] behavior, and high marble burying (HMB)[4] (Wolmarans et al., 2016; Wolmarans et al., 2013). Since this work will focus on the study of HS and LNB behavior and how both phenotypes might associate with distinct cognitive footprints, these will be reviewed in greater detail in the following few paragraphs.

2.7.1.1 Nest building

Nest building forms part of the normal behavioral repertoire of rodents; however notable variation in nest building behavior has been observed between individual mice of the same species (Lewarch and Hoekstra, 2018). In the natural environment, mice build nests for protection against environmental and predatory stimuli and to provide shelter for breeding and rearing purposes. The same is true for nesting under laboratory conditions. However, under such circumstances, nests are also built to provide shelter from noxious stimuli like bright light, human handling, and noise (Jirkof, 2014). Thus, healthy nesting performance is used to evaluate the overall well-being of rodents in the research setting (Jirkof, 2014). From this perspective, excessive nest building, i.e. behavior that deviates from the 'normal' behavioral phenotype expressed by most rodents in a laboratory-housed population, may be indicative of an aberrant and inflated phenotype borne from a different psychobiology, compared to normal nesting behavior (Wolmarans et al., 2016). Such argument can be maintained, since excessive nests serve no specific purpose within the context of the laboratory where such behaviors are normally explored in single-housed animals that are all maintained under the same conditions. In this laboratory, such behavior is studied from the view that it might represent an inflated coping response aimed at or related to safety- or perfectionism-seeking instincts (de Brouwer et al., 2020; Wolmarans et al., 2022).

[1] obsessive-compulsive disorder
[2] high motor stereotypy
[3] large nest building
[4] high marble burying

Nevertheless, deciding which behaviors are 'normal' and 'abnormal' can be highly subjective. Thus, the classification and description of LNB[1] for the purposes of studying compulsive-like behavioral expression, such behavior is identified and defined on the basis of two core characteristics of clinical OCD[2], i.e. excessiveness and persistence. It follows then that LNB is defined as nesting scores that cluster within the top 25 % of nesting scores generated by all assessed animals, and that show the least degree of between-day variation (Wolmarans et al., 2016). Another aspect of such behavior, but one which is also true for any form of nesting, is that nesting is highly goal-directed in its execution. While some authors argue the contrary, saying that nesting is merely an innate and pre-programmed behavior that is executed without any outcome planning (Prinsloo, 2021), it should be highlighted that nesting is the result of a set of actions involving a variety of muscle systems, featuring activities from the front and hind legs, paws, and mouth to pull cotton wool through the boundaries (or walls) of a container. Cotton wool strands (or another similar material) are then arranged into highly organized structures (Layne, 1969). Further, research from other laboratories indicate nesting to be subject to goal-directed feedback, whereby disturbances in nesting quality are rectified and the nests restored to its initial form (Chou-Green et al., 2003; Hoffman and Morales, 2009). Thus, it can be considered that excessively large nests are an inflated form of goal-directed behavior, in a similar manner to what is true for clinical OCD.

The question thus begs to what extent LNB expression in deer mice is validated as a model of clinical compulsivity. First and foremost, apart from its apparent robust face validity summarized above and elsewhere (Scheepers et al., 2018), LNB-expressing animals, which comprise roughly 30 % of the housed population, show an inflated drive to access nesting material, even under circumstances of contextual punishment, i.e. when they are voluntarily punished for accessing nesting material (de Brouwer et al., 2020). Thus, as is true for OCD when patients fail to disengage from compulsive symptom expression despite the negative consequences of such actions, LNB-expressing deer mice persistently seek nesting material in an entirely voluntary context (Wolmarans et al., 2022). From a predictive perspective, LNB, but not normal nesting behavior (NNB)[3], shows a selective response to chronic, high-dose oral exposure to the SSRI[4], escitalopram (Wolmarans et al., 2022), alone or in combination with low-dose flupentixol, a non-selective $D_{1/2}$ receptor antagonist (Yamada et al., 2018). Specifically, while both NNB and LNB seems to exacerbate over the course of chronic single-housed periods (Lewarch and Hoekstra, 2018), such a response is prevented in escitalopram-exposed mice (Wolmarans et al., 2022). This is important, since it might point to an influence of state anxiety on the

[1] large nest building
[2] obsessive-compulsive disorder
[3] normal nest building
[4] selective serotonin reuptake inhibitor

expression of nesting behavior which deserves continued study, especially since LNB[1]-expressing mice do not seem to present with an inflated degree of trait anxiety (Prinsloo, 2021). In terms of construct validity and the apparent involvement of serotonergic processes aside, LNB, compared to NNB[2], is associated with a unique and entirely naturalistic gut microbiota composition, separating these phenotypes on more than a behavioral or neurochemical level (Scheepers et al., 2020).

2.7.1.2 Spontaneous motor stereotypy

Stereotypy can be defined as *"extreme engagement in certain specific motor acts which are also excessive, purposeless and without any clear endpoint or goal"* (Ridley, 1994). Within the context of clinical OCD, stereotypies are observed in different forms, e.g. in a productive and organizational sense when repetitive behaviors that were initially done to complete certain tasks become exaggerated, or in environmental contexts when other comorbid neuropsychiatric conditions might trigger compulsive avoidance behaviors (Murphy et al., 2010). In animals, confinement-stereotypy develops within enclosures, e.g. laboratory cages, and due to environmental deprivation (Ridley, 1994). Thus, separating cage stereotypies from compulsive-like stereotypical expression, might be problematic.

Approximately 45 % of laboratory-housed deer mice present with continuous and seemingly meaningless repetitive motor stereotypies that can be categorized into backward somersaulting, vertical jumping and horizontal pattern running stereotypies (de Brouwer et al., 2021; Korff and Harvey, 2006). However, such behaviors do not seem to adhere to the normal criteria for cage stereotypy, since it presents in a waxing and waning nature over the course of a complete dark cycle. Thus, it is likely subject to processes of stop-start control, albeit abnormally so, as well as to implicit processes of voluntary action. Indeed, the manner of its presentation may arguably represent bouts of mounting anxiety, which are attenuated by the expression of such behaviors (Abramowitz, 2006), a possibility that is continuously being investigated in this laboratory (Wolmarans et al., 2013). This aspect of its presentation provides the overall basis for the face validity of stereotypy as a model framework for the study of compulsive-like behavioral repetition. The predictive validity of the phenotype is founded upon its response to chronic, high-dose, oral escitalopram exposure (Wolmarans et al., 2013). Also, recent findings indicated HS[3], but not non-stereotypical (NS)[4] behavior, responds to anti-adenosinergic intervention (de Brouwer et al., 2021), and to some extent also to LEV[5] (de Ridder et al., 2022). In terms

[1] large nest building
[2] normal nest building
[3] high motor stereotypy
[4] non-stereotypical
[5] levetiracetam

of construct, HS[1] is associated with a significant reduction in striatal SERT[2] density (Wolmarans et al., 2013), which is indicative of a hyposerotonergic state underlying stereotypical expression (Korff et al., 2008); this is in congruence with clinical theory (Lissemore et al., 2014).

Since HS and LNB[3] behavior are two different and unique persistent behavioral phenotypes expressed by deer mice, they will be investigated in parallel with the overall objective, i.e. drawing potential parallels between cognitive impairment and compulsive-like behavioral expression, in mind. This is important, since research is unclear in terms of the potentially unique associations between various compulsive phenotypes, and specific cognitive aberrations. Importantly, since the context of the present work also focuses on a developmental trajectory, LNB behavior and HS have not yet been studied from a developmental perspective. Currently, it is known that while younger animals may already present with signs of HS (Powell et al., 2000), such behaviors are fully established by the age of 10 – 12 weeks and as such, nesting and stereotypy will only be assessed once animals reached the age of 12 weeks.

2.8 Behavioral tests of cognitive flexibility and habit-proneness in rodents as applied in this work

Since cognition broadly refers to several domains of functioning, as explained earlier in this text, its testing in animal model systems, is mostly guided by the research question asked. In this work, attention will mostly be afforded to cognitive flexibility as reflected by habit-proneness and working memory capacity as a potential associative construct that may longitudinally and simultaneously present in parallel with compulsive-like behavioral repetition. To this end, two tests that are commonly used to study this aspect of cognition, will briefly be reviewed here.

2.8.1 General principles pertaining to habit testing

Most tests of habit in animals are performed by making use of devaluation paradigms to study the intensity of stimulus-response habits (Griffiths et al., 2014). In order for such assessments to be successful and informative, animals first have to undergo a pre-test training procedure to establish a repetitive behavioral pattern that can either be goal-directed in the beginning, or merely trained, e.g. by means of forced entries into a specific arm of a T-maze (Deacon and Rawlins, 2006). The principle of such training is to *over-train* animals so that a behavioral shift in an altered context, i.e. being able to choose any of the arms in which to enter, can accurately be measured in so-called probe trials (the reader is referred to Graybiel (2008) and Smith and Graybiel (2014) for a review). In principle, animals that show a return to their normal pre-training behavioral expression during post-training testing, are

[1] high motor stereotypy
[2] serotonin transporter
[3] large nest building

seen to be more governed by goal-directed processes. On the other hand, animals that persist in the expression of the learned behavior, despite the change in context or outcome, are seen to be driven by habitual responses (Ozcan et al., 2016). However, these tests are unfortunately characterized by a large degree of inter-laboratory variance with different training- and probe-trial protocols and successes reported for the same test (d'Isa et al., 2021; Hieu et al., 2020).

In this work, we will apply two of the aforementioned neurocognitive models of habit, i.e., the motivated-context model and the direct-context-cuing model, to determine prospectively and simultaneously, whether the different compulsive-like behavioral phenotypes shown by deer mice, i.e. LNB[1] behavior and HS[2], are uniquely associated with habit-proneness. For the motivated-context model, juvenile deer mice (aged 28 – 34 days) will be trained to seek and find a potential reward, i.e. a means to escape an aversive environment in the Barnes maze (BM)[3] (O'Leary and Brown, 2012), before changing the context so that no escape opportunity is granted anymore. Then, after mice have been raised until adulthood (12 weeks of age), habit-proneness will once again be assessed within the direct-context-cuing framework, by training mice to enter a single arm of a T-maze only, prior to being offered free choice of entry in the probe trials (d'Isa et al., 2021).

The use of two different paradigms at different life stages, i.e., BM in the young, and T-maze in adulthood, is vitally important, since no previous work in deer mice has investigated this research question and thus, no information is available as to the extent to which previous test experience, irrespective of the duration of the between-test interval, might influence subsequent test performance.

2.8.2 The Barnes maze assessment

Animals inherently apply instinctive navigational skills to explore their environment without getting lost (Vorhees and Williams, 2014). Specifically, they can rely on route-based navigation, where a certain path is memorized as it is cued by environmental detail (Sjolund et al., 2018). While these routes and movements are memorized, they can also become overtrained in such a way that strong habits are developed. For this to occur, animals rely on procedural memory.

[1] large nest building
[2] high motor stereotypy
[3] Barnes maze

The BM[1] assessment, named after Carol Barnes, who first described the procedure, was initially conceptualized as an assessment of spatial memory in rodents (Pitts, 2018); however, since the constructs of spatial and procedural memory in rodents overlap to a large degree (mice need procedural memory to acquire and recall spatial memory), it was selected for use in the present work. The BM setup typically involves a circular, or similarly shaped, platform with several holes that are equally arranged on its perimeter. A movable escape tunnel is attached underneath any of the holes chosen for this purpose, while the other holes remain closed off. The open space of the walking surface as well as the bright light under which the test is conducted, are anxiogenic and aversive and thus, the inherent principle of the assessment relies on mice learning the 'way out' over the course of several training trials that differ according to the procedures of each testing laboratory (Valibeigi et al., Dumas, 2018). After sufficient training experience, the context is altered by the removal of the escape box, and mice are assessed in terms of their return to a normal escape-seeking and/or exploratory response (Koopmans et al., 2003). More methodological detail is provided in **Chapter 3** of this dissertation.

2.8.3 The T-maze assessment

The T-maze consists of a start arm and two lateral left and right arms. When beginning this procedure, mice are placed into a separate starting enclosure and then released into the start arm. Mice can then freely choose any one of the two arms (L or R) to explore. Once the first choice has been made, the chosen arm is closed off and the mice have to stay there for a few seconds before being allowed to return to the starting position for the next trial (d'Isa et al., 2021). After ten trials, the preferred arm of each animal is noted, followed by the animal being forced to enter the opposite arm of the T-maze only. In this sense, mice are spatially trained to enter the other, non-preferred arm of the maze only (d'Isa et al., 2021). This is followed with probe trials, during which habitual entry into the same arm is measured upon being given the choice to once again enter any of the arms.

The T-maze is used to assess cognitive processes related to working and spatial memory and reversal learning, constructs that might show impairment in animals presenting with compulsive-like behavioral phenotypes (de Brouwer et al., 2021; de Ridder et al., 2022), and that can be reflective of habitual responding (Moustgaard and Hau, 2009). Thus, we regard the test as a useful and translationally relevant assessment of habitual responding within the contextual framework of the present investigation.

* * *

END OF CHAPTER TWO

[1] Barnes maze

2.9 References

Abramovitch A, Cooperman A (2015) The cognitive neuropsychology of obsessive-compulsive disorder: A critical review. Journal of Obsessive-Compulsive and Related Disorders 5: 24-36.

Abramovitch A, McKay D (2016) Behavioral Impulsivity in Obsessive-Compulsive Disorder. Journal of Behavioral Addiction 5: 395-7.

Abramowitz JS (2006) The psychological treatment of obsessive—compulsive disorder. The Canadian Journal of Psychiatry 51: 407-416.

Abramowitz JS, Jacoby RJ (2014) Obsessive-compulsive disorder in the DSM-5. Clinical Psychology: Science and Practice 21: 221.

Al Dahhan NZ, De Felice FG, Munoz DP (2019) Potentials and Pitfalls of Cross-Translational Models of Cognitive Impairment. Frontiers in Behavioral Neuroscience 13: 48.

Albelda N, Joel D (2012) Current animal models of obsessive compulsive disorder: an update. Neuroscience 211: 83-106.

Albert U, Marazziti D, Di Salvo G, Solia F, Rosso G, Maina G (2018) A systematic review of evidence-based treatment strategies for obsessive-compulsive disorder resistant to first-line pharmacotherapy. Current Medicinal Chemistry 25: 5647-5661.

Albertella L, Le Pelley ME, Chamberlain SR, Westbrook F, Lee RSC, Fontenelle LF, Grant JE, Segrave RA, McTavish E, Yucel M (2020) Reward-related attentional capture and cognitive inflexibility interact to determine greater severity of compulsivity-related problems. Journal of Behavior Therapy and Experimental Psychiatry 69: 101580.

Alonso P, Lopez-Sola C, Real E, Segalas C, Menchon JM (2015) Animal models of obsessive-compulsive disorder: utility and limitations. Neuropsychiatric Disease and Treatment 11: 1939-55.

American Psychiatric Association (2013) Diagnostic and statistical manual of mental disorders: DSM-5. American Psychiatric Association Washington, DC

Amoretti M, Amsler C, Bonomi G, Bouchta A, Bowe P, Carraro C, Cesar CL, Charlton M, Collier MJT, Doser M, Filippini V, Fine KS, Fontana A, Fujiwara MC, Funakoshi R, Genova P, Hangst JS, Hayano RS, Holzscheiter MH, Jørgensen LV, Lagomarsino V, Landua R, Lindelöf D, Rizzini EL, Macrì M, Madsen N, Manuzio G, Marchesotti M, Montagna P, Pruys H, Regenfus C, Riedler P, Rochet J, Rotondi A, Rouleau G, Testera G, Variola A, Watson TL, van der Werf DP (2002) Production and detection of cold antihydrogen atoms. Nature 419: 456-459.

Ansara ED (2020) Management of treatment-resistant generalized anxiety disorder. Mental Health Clinician 10: 326-334.

Asherson P, Young AH, Eich-Höchli D, Moran P, Porsdal V, Deberdt W (2014) Differential diagnosis, comorbidity, and treatment of attention-deficit/hyperactivity disorder in relation to bipolar disorder or borderline personality disorder in adults. Current Medical Research and Opinion 30: 1657-72.

Atmaca M, Onalan E, Yildirim H, Yuce H, Koc M, Korkmaz S, Mermi O (2011) Serotonin transporter gene polymorphism implicates reduced orbito-frontal cortex in obsessive–compulsive disorder. Journal of Anxiety Disorders 680-685.

Balasubramani PP, Chakravarthy VS, Ravindran B, Moustafa AA (2015) A network model of basal ganglia for understanding the roles of dopamine and serotonin in reward-punishment-risk based decision making. Frontiers in Computational Neuroscience 9: 76.

Bandelow B, Zohar J, Hollander E, Kasper S, Möller HJ, Zohar J, Hollander E, Kasper S, Möller HJ, Bandelow B, Allgulander C, Ayuso-Gutierrez J, Baldwin DS, Buenvicius R, Cassano G, Fineberg N, Gabriels L, Hindmarch I, Kaiya H, Klein DF, Lader M, Lecrubier Y, Lépine JP, Liebowitz MR, Lopez-Ibor JJ, Marazziti D, Miguel EC, Oh KS, Preter M, Rupprecht R, Sato M, Starcevic V, Stein DJ, van Ameringen M, Vega J (2008) World Federation of Societies of Biological Psychiatry (WFSBP) guidelines for the pharmacological treatment of anxiety, obsessive-compulsive and post-traumatic stress disorders - first revision. The World Journal of Biological Psychiatry 9: 248-312.

Belzung C, Lemoine M (2011) Criteria of validity for animal models of psychiatric disorders: Focus on anxiety disorders and depression. Biology of Mood & Anxiety disorders 1: 9.

Benatti B, Dell'Osso B, Arici C, Hollander E, Altamura AC (2014) Characterizing impulsivity profile in patients with obsessive–compulsive disorder. International Journal of Psychiatry in Clinical Practice 18: 156-160.

Black DW, Grant JE (2014) DSM-5® guidebook: the essential companion to the diagnostic and statistical manual of mental disorders. American Psychiatric Publishing Inc.

Bootsma HPR, Ricker L, Diepman L, Gehring J, Hulsman J, Lambrechts D, Leenen L, Majoie M, Schellekens A, de Krom M, Aldenkamp AP (2008) Long-term effects of levetiracetam and topiramate in clinical practice: A head-to-head comparison. Seizure 17: 19-26.

Boswell JF, Thompson-Hollands J, Farchione TJ, Barlow DH (2013) Intolerance of uncertainty: a common factor in the treatment of emotional disorders. Journal of Clinical Psychology 69: 630-45.

Brakoulias V, Starcevic V, Berle D, Milicevic D, Moses K, Hannan A, Sammut P, Martin A (2013) The characteristics of unacceptable/taboo thoughts in obsessive–compulsive disorder. Comprehensive Psychiatry 54: 750-757.

Brander G, Pérez-Vigil A, Larsson H, Mataix-Cols D (2016) Systematic review of environmental risk factors for Obsessive-Compulsive Disorder: A proposed roadmap from association to causation. Neuroscience & Biobehavioral Reviews 65: 36-62.

Broadbent NJ, Squire LR, Clark RE (2004) Spatial memory, recognition memory, and the hippocampus. Proceedings of the National Academy of Sciences of the United States of America 101: 14515-20.

Brodie MJ, Perucca E, Ryvlin P, Ben-Menachem E, Meencke HJ (2007) Comparison of levetiracetam and controlled-release carbamazepine in newly diagnosed epilepsy. Neurology 68: 402-8.

Buhr K, Dugas M (2009) The role of fear of anxiety and intolerance of uncertainty in worry: An experimental manipulation. Behaviour Research and Therapy 47: 215-23.

Chamberlain SR, Fineberg NA, Blackwell AD, Clark L, Robbins TW, Sahakian BJ (2007) A neuropsychological comparison of obsessive-compulsive disorder and trichotillomania. Neuropsychologia 45: 654-62.

Chamberlain SR, Leppink EW, Redden SA, Grant JE (2016) Are obsessive–compulsive symptoms impulsive, compulsive or both? Comprehensive Psychiatry 68: 111-118.

Chou-Green JM, Holscher TD, Dallman MF, Akana SF (2003) Compulsive behavior in the 5-HT2C receptor knockout mouse. Physiology and Behavior 78: 641-649.

Coles ME, Johnson EM, Schubert JR (2011) Retrospective reports of the development of obsessive compulsive disorder: extending knowledge of the protracted symptom phase. Behavioral and Cognitive Psychotherapy 39: 579-89.

Conn PJ, Battaglia G, Marino MJ, Nicoletti FJ (2005) Metabotropic glutamate receptors in the basal ganglia motor circuit. Nature Reviews Neuroscience 6: 787-798.

Cowan N (2014) Working Memory Underpins Cognitive Development, Learning, and Education. Educational Psychology Review 26: 197-223.

Crittenden PM, DiLalla DL (1988) Compulsive compliance: The development of an inhibitory coping strategy in infancy. Journal of Abnormal Child Psychology 16: 585-599.

d'Isa R, Comi G, Leocani L (2021) Apparatus design and behavioural testing protocol for the evaluation of spatial working memory in mice through the spontaneous alternation T-maze. Scientific Reports 11: 21177.

Dajani DR, Uddin LQ (2015) Demystifying cognitive flexibility: Implications for clinical and developmental neuroscience. Trends in Neuroscience 38: 571-8.

Daniel A, Geller MBBS, John M, (CQI): atACoQI, Heather J, Walter MD, Oscar G, Bukstein MD, Co-Chairs a, R S, Benson MD, Allan C, Tiffany R, Farchione MD, John H, Helene K, Joan K, Ulrich S, Matthew S, Saundra S, AACAP liaison: Jennifer M (2012) Practice Parameter for the Assessment and Treatment of Children and Adolescents with Obsessive-Compulsive Disorder. Journal of the American Academy of Child & Adolescent Psychiatry 51.

de Brouwer G, Engelbrecht J, Mograbi DC, Legoabe L, Steyn SF, Wolmarans W (2021b) Stereotypy and spontaneous alternation in deer mice and its response to anti-adenosinergic intervention. Journal of Neuroscience Research 99: 2706-2720.

de Brouwer G, Fick A, Harvey BH, Wolmarans DW (2018) A critical inquiry into marble-burying as a preclinical screening paradigm of relevance for anxiety and obsessive–compulsive disorder: Mapping the way forward. Cognitive, Affective, & Behavioral Neuroscience 19: 1-39.

de Brouwer G, Fick A, Lombaard A, Stein D, Harvey B, Wolmarans DW (2020a) Large nest building and high marble-burying: Two compulsive-like phenotypes expressed by deer mice (Peromyscus maniculatus bairdii) and their unique response to serotoninergic and dopamine modulating intervention. Behavioural Brain Research 393: 112794.

de Brouwer G, Harvey BH, Wolmarans DW (2020b) Naturalistic operant responses in deer mice (Peromyscus maniculatus bairdii) and its response to outcome manipulation and serotonergic intervention. Behavioral Pharmacology 31: 343-358.

De Caluwé E, Vergauwe J, Decuyper M, Bogaerts S, Rettew DC, De Clercq B (2020) The relation between normative rituals/routines and obsessive-compulsive symptoms at a young age: A systematic review. Developmental Review 56.

de Ridder M, Mograbi D, Wolmarans DW (2022) Spontaneous alternation and stereotypical behaviour in deer mice: response to escitalopram and levetiracetam. Behavioural Pharmacology 33: 282-290.

Deacon RM, Rawlins JN (2006) T-maze alternation in the rodent. Nature Protocols 1: 7-12.

Del Casale A, Sorice S, Padovano A, Simmaco M, Ferracuti S, Lamis DA, Rapinesi C, Sani G, Girardi P, Kotzalidis GD (2019a) Psychopharmacological treatment of obsessive-compulsive disorder (OCD). Current Neuropharmacology 17: 710-736.

Dell'Osso B, Benatti B, Nicolini H, Lanzagorta N, Palazzo MC, Marazziti D, Hollander E, Fineberg N, Stein DJ, Pallanti S, Van Ameringen M, Lochner C, Hranov G, Karamustafalioglu O, Hranov L, Menchon JM, Zohar J, Denys D (2017) Childhood, Adolescent and Adult Age at Onset and Related Clinical Correlates in Obsessive-Compulsive Disorder: A Report from the International College of Obsessive-Compulsive Disorders. European Neuropsychopharmacology 27: 610-611.

Destree L, Albertella L, Torres AR, Ferrao YA, Shavitt RG, Miguel EC, Fontenelle LF (2020) Social losses predict a faster onset and greater severity of obsessive-compulsive disorder. Journal of Psychiatric Research 130: 187-193.

Dias-Ferreira E, Sousa JC, Melo I, Morgado P, Mesquita AR, Cerqueira JJ, Costa RM, Sousa N (2009) Chronic stress causes frontostriatal reorganization and affects decision-making. Science 325: 621-5.

Dickinson A (1985) Actions and habits: the development of behavioural autonomy. Philosophical Transactions of the Royal Society 308: 67-78.

DiCorcia JA, Tronick E (2011) Quotidian resilience: Exploring mechanisms that drive resilience from a perspective of everyday stress and coping. Neuroscience & Biobehavioral Reviews 35: 1593-1602.

Ehmer I, Feenstra M, Willuhn I, Denys D (2020) Instrumental learning in a mouse model for obsessive-compulsive disorder: Impaired habit formation in Sapap3 mutants. Neurobiology of Learning and Memory 168: 107162.

Eisen JL, Pinto A, Mancebo MC, Dyck IR, Orlando ME, Rasmussen SA (2010) A 2-year prospective follow-up study of the course of obsessive-compulsive disorder. Journal of Clinical Psychiatry 71: 1033-9.

Eisen JL, Sibrava NJ, Boisseau CL, Mancebo MC, Stout RL, Pinto A, Rasmussen SA (2013) Five-year course of obsessive-compulsive disorder: predictors of remission and relapse. Journal of Clinical Psychiatry 74: 233-9.

Ersche KD, Ward LHE, Lim TV, Lumsden RJ, Sawiak SJ, Robbins TW, Stochl J (2019) Impulsivity and compulsivity are differentially associated with automaticity and routine on the Creature of Habit Scale. Personality and Individual Differences 150: 109493.

Evans DW, Maliken A (2011) Cortical activity and children's rituals, habits and other repetitive behavior: a visual P300 study. Behavioural Brain Research 224: 174-9.

Fettes P, Schulze L, Downar J (2017) Cortico-Striatal-Thalamic Loop Circuits of the Orbitofrontal Cortex: Promising Therapeutic Targets in Psychiatric Illness. Frontiers in Systems Neuroscience 11: 25.

Fineberg NA, Chamberlain SR, Hollander E, Boulougouris V, Robbins TW (2011) Translational approaches to obsessive-compulsive disorder: from animal models to clinical treatment. British Journal of Pharmacology 164: 1044-61.

Fineberg NA, Craig KJ (2007) Pharmacological treatment for obsessive–compulsive disorder. Psychiatry 6: 234-239.

Fineberg NA, Dell'Osso B, Albert U, Maina G, Geller D, Carmi L, Sireau N, Walitza S, Grassi G, Pallanti S, Hollander E, Brakoulias V, Menchon JM, Marazziti D, Ioannidis K, Apergis-Schoute A, Stein DJ, Cath DC, Veltman DJ, Van Ameringen M, Fontenelle LF, Shavitt RG, Costa D, Diniz JB, Zohar J (2019) Early intervention for obsessive compulsive disorder: An expert consensus statement. European Neuropsychopharmacology 29: 549-565.

Freeston MH, Rhéaume J, Letarte H, Dugas MJ, Ladouceur R (1994) Why do people worry? Personality and Individual Differences 17: 791-802.

Fried I, Haggard P, He BJ, Schurger A (2017) Volition and Action in the Human Brain: Processes, Pathologies, and Reasons. Journal of Neuroscience 37: 10842-10847.

Frost RO, Heimberg RG, Holt CS, Mattia JI, Neubauer AL (1993) A comparison of two measures of perfectionism. Personality and Individual Differences 14: 119-126.

Frydman I, do Brasil PE, Torres AR, Shavitt RG, Ferrao YA, Rosario MC, Miguel EC, Fontenelle LF (2014) Late-onset obsessive-compulsive disorder: risk factors and correlates. Journal of Psychiatric Research 49: 68-74.

Frydman I, Mattos P, de Oliveira-Souza R, Yucel M, Chamberlain SR, Moll J, Fontenelle LF (2020) Self-reported and neurocognitive impulsivity in obsessive-compulsive disorder. Comprehensive Psychiatry 97: 152155.

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

Geller DA, Homayoun S, Johnson G (2021) Developmental Considerations in Obsessive Compulsive Disorder: Comparing Pediatric and Adult-Onset Cases. Frontiers in Psychiatry 12: 678538.

Ghaffari Darab M, Hedayati A, Khorasani E, Bayati M, Keshavarz K (2020) Selective serotonin reuptake inhibitors in major depression disorder treatment: an umbrella review on systematic reviews. International Journal of Psychiatry in Clinical Practice 24: 357-370.

Gillan CM, Papmeyer M, Morein-Zamir S, Sahakian BJ, Fineberg NA, Robbins TW, De Wit S (2011) Disruption in the balance between goal-directed behavior and habit learning in obsessive-compulsive disorder. American Journal of Psychiatry 168: 718-726.

Gillan CM, Robbins TW, Sahakian BJ, van den Heuvel OA, van Wingen G (2016) The role of habit in compulsivity. European Neuropsychopharmacology 26: 828-40.

Gillan CM, Sahakian BJ (2015) Which is the driver, the obsessions or the compulsions, in OCD? Neuropsychopharmacology 40: 247-8.

Giovagnoli R (2018) From Habits to Rituals: Rituals as Social Habits. Open Information Science 2: 181-188.

Goldberg-Stern H, Feldman L, Eidlitz-Markus T, Kramer U, Perez S, Pollak L, Phatal-Valevski A (2013) Levetiracetam in children, adolescents and young adults with intractable epilepsy: efficacy, tolerability and effect on electroencephalogram--a pilot study. European Journal of Paediatric Neurology 17: 248-53.

Gondré-Lewis MC, Bassey R, Blum K (2020) Pre-clinical models of reward deficiency syndrome: A behavioral octopus. Neuroscience and Biobehavioral Reviews 115: 164-188.

Grant JE, Chamberlain SR (2020) Impulsive–compulsive problems in young people. Medicine 48: 724-726.

Grassi G (2016) Obsessive-Compulsive Disorder Clinical Staging: From Endophenotype to Behavioral Addiction (a Theoretical Framework). European Neuropsychopharmacology 26: 897.

Grassi G, Pallanti S, Righi L, Figee M, Mantione M, Denys D, Piccagliani D, Rossi A, Stratta P (2015) Think twice: Impulsivity and decision making in obsessive-compulsive disorder. Journal of Behavioral Addictions 4: 263-72.

Graybiel AM (2008) Habits, rituals, and the evaluative brain. Annual Review of Neuroscience 31: 359-87.

Graybiel AM, Rauch SL (2000) Toward a Neurobiology of Obsessive-Compulsive Disorder. Neuron 28: 343-347.

Greco V, Roger D (2003) Uncertainty, stress, and health. Personality and Individual Differences 34: 1057-1068.

Griffin JJ (1990) Anxiety. In: Walker HK, Hall WD, Hurst JW (eds) Clinical Methods: The History, Physical, and Laboratory Examinations. Butterworths (1990) Butterworth Publishers, a division of Reed Publishing., Boston.

Griffiths KR, Morris RW, Balleine BW (2014) Translational studies of goal-directed action as a framework for classifying deficits across psychiatric disorders. Frontiers in Systems Neuroscience 8: 101.

Guglielmi V, Souget J, van Elzelingen W, Willuhn I, Vulink N (2017) Influence of Sex Hormones on OCD. In: Pittenger C, Pittenger C (eds) Obsessive-compulsive disorder: Phenomenology, Pathophysiology, and Treatment. Oxford University Press.

Haber SN (2016) Corticostriatal circuitry. Dialogues in Clinical Neuroscience 18: 7-21.

Hansen B, Vogel PA, Stiles TC, Gotestam KG (2007) Influence of co-morbid generalized anxiety disorder, panic disorder and personality disorders on the outcome of cognitive behavioural treatment of obsessive-compulsive disorder. Cognitive Behavioural Therapy 36: 145-55.

Hashemi P, Dankoski EC, Lama R, Wood KM, Takmakov P, Wightman RM (2012) Brain dopamine and serotonin differ in regulation and its consequences. Proceedings of the National Academy of Sciences of the United States of America 109: 11510-5.

Heinzel S, Bey K, Grützmann R, Klawohn J, Kaufmann C, Lennertz L, Wagner M, Kathmann N, Riesel A (2021) Spatial working memory performance in people with obsessive–compulsive disorder, their unaffected first-degree relatives and healthy controls. The British Journal of Psychiatry 7: e208.

Helmstaedter C, Witt J-A (2008) The effects of levetiracetam on cognition: a non-interventional surveillance study. Epilepsy & Behavior 13: 642-649.

Helmstaedter C, Witt J-A (2010) Cognitive outcome of antiepileptic treatment with levetiracetam versus carbamazepine monotherapy: a non-interventional surveillance trial. Epilepsy & Behavior 18: 74-80.

Hillen MA, Gutheil CM, Strout TD, Smets EMA, Han PKJ (2017) Tolerance of uncertainty: Conceptual analysis, integrative model, and implications for healthcare. Social Science & Medicine 180: 62-75.

Hinds AL, Woody EZ, Van Ameringen M, Schmidt LA, Szechtman H (2012) When too much is not enough: obsessive-compulsive disorder as a pathology of stopping, rather than starting. PLoS One 7: e30586.

Hofer PD, Wahl K, Meyer AH, Miché M, Beesdo-Baum K, Lieb R (2020) Parental emotional warmth interacts with traumatic life events in predicting the onset of obsessive-compulsive disorder. Journal of Obsessive-Compulsive and Related Disorders 26: 100531.

Hoffman KL, Rueda Morales RI (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. Behavioural Brain Research 204: 182-191.

Hojgaard D, Hybel KA, Mortensen EL, Ivarsson T, Nissen JB, Weidle B, Melin K, Torp NC, Dahl K, Valderhaug R, Skarphedinsson G, Storch EA, Thomsen PH (2018) Obsessive-compulsive symptom dimensions: Association with comorbidity profiles and cognitive-behavioral therapy outcome in pediatric obsessive-compulsive disorder. Psychiatry Research 270: 317-323.

Hollander E, Rosen J (2000) Impulsivity. Journal of Psychopharmacology 14: S39-44.

Hollander E, Rossi NB, Sood E, Pallanti S (2003) Risperidone augmentation in treatment-resistant obsessive–compulsive disorder: a double-blind, placebo-controlled study. The International Journal of Neuropsychopharmacology 6: 397-401.

Iervolino AC, Rijsdijk FV, Cherkas L, Fullana MA, Mataix-Cols D (2011) A multivariate twin study of obsessive-compulsive symptom dimensions. Archives of General Psychiatry 68: 637-44.

Janardhan Reddy YC, Sundar AS, Narayanaswamy JC, Math SB (2017) Clinical practice guidelines for obsessive-compulsive disorder. Indian Journal of Psychiatry 59: S74-s90.

Jirkof P (2014) Burrowing and nest building behavior as indicators of well-being in mice. Journal of Neuroscience Methods 234: 139-46.

Kellner M (2010) Drug treatment of obsessive-compulsive disorder. Dialogues in Clinical Neuroscience 12: 187-97.

Kenezloi E, Lakatos K, Horvath EZ, Sasvari-Szekely M, Nemoda Z (2018) A pilot study of early onset obsessive-compulsive disorder: Symptom dimensions and association analysis with polymorphisms of the serotonin transporter gene. Psychiatry Research 268: 388-391.

Kessler RC, Berglund P, Demler O, Jin R, Merikangas KR, Walters EE (2005) Lifetime Prevalence and Age-of-Onset Distributions of DSM-IV Disorders in the National Comorbidity Survey Replication. Archives of General Psychiatry 62: 593-602.

Kiely KM (2014) Cognitive Function. In: Michalos AC (ed) Encyclopedia of Quality of Life and Well-Being Research. Springer Netherlands, Dordrecht, pp 974-978.

Kim JJ, Lee HJ, Han J-S, Packard MG (2001) Amygdala Is Critical for Stress-Induced Modulation of Hippocampal Long-Term Potentiation and Learning. The Journal of Neuroscience 21(14): 5222-5228.

Kohler KC, Coetzee BJ, Lochner C (2018) Living with obsessive-compulsive disorder (OCD): a South African narrative. International Journal of Mental Health Systems 12: 73.

Koopmans G, Blokland A, Vannieuwenhuijzen P, Prickaerts J (2003) Assessment of spatial learning abilities of mice in a new circular maze. Physiology & Behavior 79: 683-693.

Korff S, Harvey BH (2006) Animal models of obsessive-compulsive disorder: rationale to understanding psychobiology and pharmacology. The Psychiatric Clinics of North America 29: 371-90.

Korff S, Stein DJ, Harvey BH (2008) Stereotypic behaviour in the deer mouse: pharmacological validation and relevance for obsessive compulsive disorder. Progress in Neuro-psychopharmacology & Biological Psychiatry 32: 348-55.

Krypotos AM, Effting M, Kindt M, Beckers T (2015) Avoidance learning: a review of theoretical models and recent developments. Frontiers in Behavioral Neuroscience 9: 189.

Kubota Y, Sato W, Kochiyama T, Uono S, Yoshimura S, Sawada R, Toichi M (2019) Corticostriatal-limbic correlates of sub-clinical obsessive-compulsive traits. Psychiatry Research Neuroimaging 285: 40-46.

Kurebayashi LFS, Do Prado JM, Da Silva MJP (2012) Correlations between stress and anxiety levels in nursing students. Journal of Nursing Education and Practice 2: 128.

Lanciego JL, Luquin N, Obeso JA (2012) Functional neuroanatomy of the basal ganglia. Cold Spring Harbor Perspectives in Medicine 2: a009621-a009621.

Layne JN (1969) Nest-Building Behavior in Three Species of Deer Mice, Peromyscus. Behaviour 35: 288-303.

Leeuwerik T, Cavanagh K, Forrester E, Hoadley C, Jones A-M, Lea L, Rosten C, Strauss C (2020) Participant perspectives on the acceptability and effectiveness of mindfulness-based cognitive behaviour therapy approaches for obsessive compulsive disorder. PLoS One 15: e0238845.

Leeuwerik T, Cavanagh K, Strauss C (2019) Patient adherence to cognitive behavioural therapy for obsessive-compulsive disorder: A systematic review and meta-analysis. Journal of Anxiety Disorders 68: 102135.

Leisman G, Moustafa AA, Shafir T (2016) Thinking, Walking, Talking: Integratory Motor and Cognitive Brain Function. Frontiers in Public Health 4: 94.

Lewarch CL, Hoekstra HE (2018) The evolution of nesting behaviour in Peromyscus mice. Animal Behaviour 139: 103-115.

Lissemore JI, Leyton M, Gravel P, Sookman D, Nordahl TE, Benkelfat C (2014) OCD: Serotonergic Mechanisms PET and SPECT in Psychiatry, pp 433-450

Lissemore JI, Sookman D, Gravel P, Berney A, Barsoum A, Diksic M, Nordahl TE, Pinard G, Sibon I, Cottraux J, Leyton M, Benkelfat C (2018) Brain serotonin synthesis capacity in obsessive-compulsive disorder: effects of cognitive behavioral therapy and sertraline. Translational Psychiatry 8: 82.

Loosen AM, Hauser TU (2020) Towards a computational psychiatry of juvenile obsessive-compulsive disorder. Neuroscience and Biobehavioral Reviews 118: 631-642.

Magalhaes JC, Gongora M, Vicente R, Bittencourt J, Tanaka G, Velasques B, Teixeira S, Morato G, Basile LF, Arias-Carrion O, Pompeu FA, Cagy M, Ribeiro P (2015) The influence of levetiracetam in cognitive performance in healthy individuals: neuropsychological, behavioral and electrophysiological approach. Clinical Psychopharmacology and Neuroscience 13: 83-93.

Mancebo MC, Eisen JL, Sibrava NJ, Dyck IR, Rasmussen SA (2011) Patient utilization of cognitive-behavioral therapy for OCD. Behavior Therapy 42: 399-412.

Marazziti D, Pozza A, Avella MT, Mucci F (2020) What is the impact of pharmacotherapy on psychotherapy for obsessive-compulsive disorder? Expert Opinion on Pharmacotherapy 21: 1651-1654.

Marzuki AA, Pereira de Souza A, Sahakian BJ, Robbins TW (2020) Are candidate neurocognitive endophenotypes of OCD present in paediatric patients? A systematic review. Neuroscience and Biobehavioral Reviews 108: 617-645.

Masi G, Millepiedi S, Perugi G, Pfanner C, Berloffa S, Pari C, Mucci M (2009) Pharmacotherapy in Paediatric Obsessive-Compulsive Disorder. CNS Drugs 23: 241-252.

Mathes BM, Morabito DM, Schmidt NB (2019) Epidemiological and Clinical Gender Differences in OCD. Current Psychiatry Reports 21: 36.

Mathis MA, Alvarenga P, Funaro G, Torresan RC, Moraes I, Torres AR, Zilberman ML, Hounie AG (2011) Gender differences in obsessive-compulsive disorder: a literature review. Brazilian Journal of Psychiatry 33: 390-9.

Matsumoto K, Puia G, Dong E, Pinna G (2007) GABA(A) receptor neurotransmission dysfunction in a mouse model of social isolation-induced stress: possible insights into a non-serotonergic mechanism of action of SSRIs in mood and anxiety disorders. Stress 10: 3-12.

Meehan AL, Yang X, Yuan LL, Rothman SM (2012) Levetiracetam has an activity-dependent effect on inhibitory transmission. Epilepsia 53: 469-76.

Melloni M, Urbistondo C, Sedeño L, Gelormini C, Kichic R, Ibanez A (2012) The extended fronto-striatal model of obsessive compulsive disorder: convergence from event-related potentials, neuropsychology and neuroimaging. Frontiers in Human Neuroscience 6: 259.

Menzies L, Achard S, Chamberlain SR, Fineberg N, Chen CH, del Campo N, Sahakian BJ, Robbins TW, Bullmore E (2007) Neurocognitive endophenotypes of obsessive-compulsive disorder. Brain 130: 3223-36.

Menzies L, Chamberlain SR, Laird AR, Thelen SM, Sahakian BJ, Bullmore ET (2008) Integrating evidence from neuroimaging and neuropsychological studies of obsessive-compulsive disorder: the orbitofronto-striatal model revisited. Neuroscience and Biobehavioral Reviews 32: 525-49.

Middleton R, Wheaton MG, Kayser R, Simpson HB (2019) Treatment Resistance in Obsessive-Compulsive Disorder. In: Kim Y-K (ed) Treatment Resistance in Psychiatry: Risk Factors, Biology, and Management. Springer Singapore, Singapore, pp 165-177.

Miguel EC, Leckman JF, Rauch S, do Rosario-Campos MC, Hounie AG, Mercadante MT, Chacon P, Pauls DL (2005) Obsessive-compulsive disorder phenotypes: implications for genetic studies. Molecular Psychiatry 10: 258-75.

Milad MR, Furtak SC, Greenberg JL, Keshaviah A, Im JJ, Falkenstein MJ, Jenike M, Rauch SL, Wilhelm S (2013) Deficits in conditioned fear extinction in obsessive-compulsive disorder and neurobiological changes in the fear circuit. JAMA Psychiatry 70: 608-18.

Monteiro P, Feng G (2016) Learning from animal models of obsessive-compulsive disorder. Biological Psychiatry 79: 7-16.

Moretz MW, McKay D (2009) The role of perfectionism in obsessive-compulsive symptoms: "not just right" experiences and checking compulsions. Journal of Anxiety Disorders 23: 640-4.

Moustgaard A, Hau J (2009) Induction of habits in rats by a forced-choice procedure in T-maze and the effect of pre-test free exploration. Behavioural Processes 82: 104-7.

Murphy DL, Lesch KP (2008) Targeting the murine serotonin transporter: insights into human neurobiology. Nature Reviews Neuroscience 9: 85-96.

Murphy DL, Timpano KR, Wheaton MG, Greenberg BD, Miguel EC (2010) Obsessive-compulsive disorder and its related disorders: a reappraisal of obsessive-compulsive spectrum concepts. Dialogues in Clinical Neuroscience 12: 131-48.

Nambu A (2008) Seven problems on the basal ganglia. Current Opinion in Neurobiology 18: 595-604.

Neal DT, Wood W, Quinn JM (2006) Habits—A repeat performance. Current Directions in Psychological Science E 15: 198-202.

Ngoc Hieu BT, Ngoc Anh NT, Audira G, Juniardi S, Liman RAD, Villaflores OB, Lai YH, Chen JR, Liang ST, Huang JC, Hsiao CD (2020) Development of a Modified Three-Day T-maze Protocol for Evaluating Learning and Memory Capacity of Adult Zebrafish. International Journal of Molecular Science 21.

Nigg JT (2017) Annual Research Review: On the relations among self-regulation, self-control, executive functioning, effortful control, cognitive control, impulsivity, risk-taking, and inhibition for developmental psychopathology. Journal of Child Psychology and Psychiatry; and allied disciplines 58: 361-383.

Ninan PT, Koran LM, Kiev A, Davidson JR, Rasmussen SA, Zajecka JM, Robinson DG, Crits-Christoph P, Mandel FS, Austin C (2006) High-dose sertraline strategy for nonresponders to acute treatment for obsessive-compulsive disorder: a multicenter double-blind trial. Journal of Clinical Psychiatry 67: 15-22.

Nutt D, Argyropoulos S, Hood S, Potokar J (2006) Generalized anxiety disorder: A comorbid disease. European Neuropsychopharmacology 16 Suppl 2: S109-18.

Nutt D, Malizia A (2006) Anxiety and OCD – the chicken or the egg? Journal of Psychopharmacology 20: 729-731.

O'Leary TP, Brown RE (2012) The effects of apparatus design and test procedure on learning and memory performance of C57BL/6J mice on the Barnes maze. Journal of Neuroscience Methods 203: 315-24.

Olatunji BO, Davis ML, Powers MB, Smits JA (2013) Cognitive-behavioral therapy for obsessive-compulsive disorder: a meta-analysis of treatment outcome and moderators. Journal of Psychiatric Research 47: 33-41.

Ong CW, Petersen JM, Terry CL, Krafft J, Barney JL, Abramowitz JS, Twohig MP (2022) The “how” of exposures: Examining the relationship between exposure parameters and outcomes in obsessive-compulsive disorder. Journal of Contextual Behavioral Science 24: 87-95.

Ozcan H, Ozer S, Yagcioglu S (2016) Neuropsychological, electrophysiological and neurological impairments in patients with obsessive compulsive disorder, their healthy siblings and healthy controls: Identifying potential endophenotype(s). Psychiatry Research 240: 110-117.

Pauwels L, Maes C, Hermans L, Swinnen SP (2019) Motor inhibition efficiency in healthy aging: the role of γ-aminobutyric acid. Neural Regeneration Research 14: 741-744.

Peng Z, Xu C, Ma N, Yang Q, Ren P, Wen R, Jin L, Chen J, Wei Z, Verguts T, Chen Q (2020) White Matter Alterations of the Goal-Directed System in Obsessive-Compulsive Disorder Patients and Their Unaffected First-Degree Relatives. Biological Psychiatry: Cognitive Neuroscience and Neuroimaging 6(10), 992–1001.

Peters SK, Dunlop K, Downar J (2016) Cortico-Striatal-Thalamic Loop Circuits of the Salience Network: A Central Pathway in Psychiatric Disease and Treatment. Frontiers in Systems Neuroscience 10: 104.

Piazzini A, Chifari R, Canevini MP, Turner K, Fontana SP, Canger R (2006) Levetiracetam: an improvement of attention and of oral fluency in patients with partial epilepsy. Epilepsy Research 68: 181-188.

Pickel V, Chan J (1990) Spiny neurons lacking choline acetyltransferase immunoreactivity are major targets of cholinergic and catecholaminergic terminals in rat striatum. Journal of Neuroscience Research 25: 263-280.

Piras F, Piras F, Caltagirone C, Spalletta G (2013) Brain circuitries of obsessive-compulsive disorder: A systematic review and meta-analysis of diffusion tensor imaging studies. Neuroscience & Biobehavioral Reviews 37: 2856-2877.

Pitts MW (2018) Barnes Maze Procedure for Spatial Learning and Memory in Mice. Bio-Protocol 8(5): e2744.

Powell SB, Newman HA, McDonald TA, Bugenhagen P, Lewis MH (2000) Development of spontaneous stereotyped behavior in deer mice: effects of early and late exposure to a more complex environment. Developmental Psychobiology: The Journal of the International Society for Developmental Psychobiology 37: 100-108.

Pozza A, Albert U, Dèttore D (2019) Perfectionism and Intolerance of Uncertainty are Predictors of OCD Symptoms in Children and Early Adolescents: A Prospective, Cohort, One-Year, Follow-Up Study. Clinical Neuropsychiatry 16: 53-61.

Prinsloo M (2021) A pharmacological and behavioural investigation of anxiety in the deer mouse model of obsessive-compulsive disorder. M.Sc. Dissertation, North-West University (South-Africa).

Rachman S (1998) A cognitive theory of obsessions: Elaborations. Behaviour Research and Therapy 36: 385-401.

Radulescu A, Herron J, Kennedy C, Scimemi A (2017) Global and local excitation and inhibition shape the dynamics of the cortico-striatal-thalamo-cortical pathway. Scientific Reports 7: 7608.

Rajarethinam RP, James MD, Abelson L, Himle A (2000) Acute onset and remission of obsessions and compulsions following medical illnesses and stress. Depression and Anxiety 12: 238-240.

Raposo-Lima C, Moreira P, Magalhães R, Ferreira S, Sousa N, Picó-Pérez M, Morgado P (2022) Differential patterns of association between resting-state functional connectivity networks and stress in OCD patients. Progress in Neuro-Psychopharmacology and Biological Psychiatry 118: 110563.

Reghunandanan S, Fineberg NA, Stein DJ (2015) Pathogenesis of Obsessive-Compulsive and Related Disorders, in In: Reghunandanan S, Fineberg NA, Stein DJ (eds) 'Obsessive-Compulsive and Related Disorders'. (2015) Oxford University Press.

Reid JE, Laws KR, Drummond L, Vismara M, Grancini B, Mpavaenda D, Fineberg NA (2021) Cognitive behavioural therapy with exposure and response prevention in the treatment of obsessive-compulsive disorder: A systematic review and meta-analysis of randomised controlled trials. Comprehensive Psychiatry 106: 152223.

Ridley RM (1994) The psychology of perseverative and stereotyped behaviour. Progress in Neurobiology 44: 221-231.

Robbins TW, Costa RM (2017) Habits. Current Biology 27: R1200-R1206.

Robbins TW, Gillan CM, Smith DG, de Wit S, Ersche KD (2012) Neurocognitive endophenotypes of impulsivity and compulsivity: towards dimensional psychiatry. Special Issue: Cognition in Neuropsychiatric Disorders 16: 81-91.

Robbins TW, Vaghi MM, Banca P (2019) Obsessive-Compulsive Disorder: Puzzles and Prospects. Neuron 102: 27-47.

Rommelfanger KS, Wichmann T (2010) Extrastriatal dopaminergic circuits of the Basal Ganglia. Frontiers in Neuroanatomy 4: 139.

Rosenberg DR, Keshavan MS (1998) A.E. Bennett Research Award. Toward a neurodevelopmental model of of obsessive--compulsive disorder. Biological Psychiatry 43: 623-40.

Rugino TA, Samsock TC (2002) Levetiracetam in autistic children: an open-label study. Journal of Developmental and Behavioral Pediatrics 23: 225-30.

Ruscio AM, Hallion LS, Lim CCW, Aguilar-Gaxiola S, Al-Hamzawi A, Alonso J, Andrade LH, Borges G, Bromet EJ, Bunting B, Caldas de Almeida JM, Demyttenaere K, Florescu S, de Girolamo G, Gureje O, Haro JM, He Y, Hinkov H, Hu C, de Jonge P, Karam EG, Lee S, Lepine J-P, Levinson D, Mneimneh Z, Navarro-Mateu F, Posada-Villa J, Slade T, Stein DJ, Torres Y, Uda H, Wojtyniak B, Kessler RC, Chatterji S, Scott KM (2017) Cross-sectional Comparison of the Epidemiology of DSM-5 Generalized Anxiety Disorder Across the Globe. JAMA Psychiatry 74: 465-475.

Scheepers IM, Cryan JF, Bastiaanssen TFS, Rea K, Clarke G, Jaspan HB, Harvey BH, Hemmings SMJ, Santana L, van der Sluis R, Malan-Müller S, Wolmarans W (2020) Natural compulsive-like behaviour in the deer mouse (Peromyscus maniculatus bairdii) is associated with altered gut microbiota composition. European Journal of Neuroscience 51: 1419-1427.

Scheepers IM, Stein DJ, Harvey BH (2018) Peromyscus maniculatus bairdii as a naturalistic mammalian model of obsessive-compulsive disorder: current status and future challenges. Metabolic brain disease 33: 443-455.

Schultz W (2002) Getting Formal with Dopamine and Reward. Neuron 36: 241-263.

Schultz W (2006) Behavioral theories and the neurophysiology of reward. Annual Review of Psychology 57: 87-115.

Schultz W (2007a) Behavioral dopamine signals. Fifty Years of Dopamine Research 30: 203-210.

Schultz W (2007b) Multiple dopamine functions at different time courses. Annual Review of Neuroscience 30: 259-288.

Seymour B, Daw ND, Roiser JP, Dayan P, Dolan R (2012) Serotonin Selectively Modulates Reward Value in Human Decision-Making. The Journal of Neuroscience 32: 5833.

Shahar N, Teodorescu AR, Anholt GE, Karmon-Presser A, Meiran N (2017) Examining procedural working memory processing in obsessive-compulsive disorder. Psychiatry Research 253: 197-204.

Sharma P, Rosario MC, Ferrao YA, Albertella L, Miguel EC, Fontenelle LF (2021) The impact of generalized anxiety disorder in obsessive-compulsive disorder patients. Psychiatry Research 300: 113898.

Sherman SM (2006) What is the Function of the Thalamus? In: van Hemmen JL, Sejnowski TJ (eds) 23 Problems in Systems Neuroscience. Oxford University Press

Sjolund LA, Kelly JW, McNamara TP (2018) Optimal combination of environmental cues and path integration during navigation. Memory & Cognition 46: 89-99.

Skapinakis P, Caldwell DM, Hollingworth W, Bryden P, Fineberg NA, Salkovskis P, Welton NJ, Baxter H, Kessler D, Churchill R, Lewis G (2016) Pharmacological and psychotherapeutic interventions for management of obsessive-compulsive disorder in adults: a systematic review and network meta-analysis. The Lancet Psychiatry 3: 730-739.

Smith KS, Graybiel AM (2014) Investigating habits: strategies, technologies and models. Frontiers in Behavioral Neuroscience 8: 39.

Snorrason I, Lee HJ, de Wit S, Woods DW (2016) Are nonclinical obsessive-compulsive symptoms associated with bias toward habits? Psychiatry Research 241: 221-3.

Snyder HR, Miyake A, Hankin BL (2015) Advancing understanding of executive function impairments and psychopathology: bridging the gap between clinical and cognitive approaches. Frontiers in Psychology 6: 328-328.

Sohn SY, Kang JI, Namkoong K, Kim SJ (2014) Multidimensional measures of impulsivity in obsessive-compulsive disorder: cannot wait and stop. PLoS One 9: e111739.

Stanford SC (2020) Some Reasons Why Preclinical Studies of Psychiatric Disorders Fail to Translate: What Can Be Rescued from the Misunderstanding and Misuse of Animal 'Models'? Alternatives to Laboratory Animals: 0261192920939876.

Starcevic V, Berle D, do Rosário MC, Brakoulias V, Ferrão YA, Viswasam K, Shavitt R, Miguel E, Fontenelle LF (2016) Use of benzodiazepines in obsessive-compulsive disorder. International Clinical Psychopharmacology 31: 27-33.

Stein DJ, Costa DLC, Lochner C, Miguel EC, Reddy YCJ, Shavitt RG, van den Heuvel OA, Simpson HB (2019) Obsessive-compulsive disorder. Nature Reviews Disease Primers 5: 52.

Stein DJ, Fineberg NA, Bienvenu OJ, Denys D, Lochner C, Nestadt G, Leckman JF, Rauch SL, Phillips KA (2010a) Should OCD be classified as an anxiety disorder in DSM-V? Depression and Anxiety 27: 495-506.

Steketee G, Frost R (2003) Compulsive hoarding: Current status of the research. Clinical Psychology Review 23: 905-927.

Stengler-Wenzke K, Müller U, Angermeyer MC, Sabri O, Hesse S (2004) Reduced serotonin transporter-availability in obsessive-compulsive disorder (OCD). European Archives of Psychiatry and Clinical Neurosciences 254: 252-5.

Stewart SE, Hu YP, Leung A, Chan E, Hezel DM, Lin SY, Belschner L, Walsh C, Geller DA, Pauls DL (2017) A Multisite Study of Family Functioning Impairment in Pediatric Obsessive-Compulsive Disorder. Journal of the American Academy of Child and Adolescent Psychiatry 56: 241-249.e3.

Strawn JR, Poweleit EA, Ramsey LB (2019) CYP2C19-guided escitalopram and sertraline dosing in pediatric patients: a pharmacokinetic modeling study. Journal of Child and Adolescent Psychopharmacology 29: 340-347.

Szechtman H, Woody E (2004) Obsessive-compulsive disorder as a disturbance of security motivation. Psychological Review 111: 111.

Thompson EM, Torres AR, Albertella L, Ferrao YA, Tiego J, Shavitt RG, Conceicao do Rosario M, Miguel EC, Fontenelle LF (2020) The speed of progression towards obsessive-compulsive disorder. Journal of Affective Disorders 264: 181-186.

Thorsen AL, Kvale G, Hansen B, van den Heuvel OA (2018) Symptom dimensions in obsessive-compulsive disorder as predictors of neurobiology and treatment response. Current Treatment Options in Psychiatry 5: 182-194.

Torregrossa MM, Quinn JJ, Taylor JR (2008) Impulsivity, compulsivity, and habit: the role of orbitofrontal cortex revisited. Biological Psychiatry 63: 253-5.

Valibeigi N, McHail DG, Kimball CL, Dumas TC (2018) A Modified Barnes Maze for Juvenile Rats. Bio-Protocol 8(22): e3084.

Vellozo AP, Fontenelle LF, Torresan RC, Shavitt RG, Ferrão YA, Rosário MC, Miguel EC, Torres AR (2021) Symmetry Dimension in Obsessive-Compulsive Disorder: Prevalence, Severity and Clinical Correlates. Journal of Clinical Medicine 10(2): 274.

Verghese J, Lipton R, Ayers E (2017) Spatial navigation and risk of cognitive impairment: A prospective cohort study. Alzheimers & Dementia 13: 985-992.

Viswanath B, Janardhan Reddy YC, Kumar KJ, Kandavel T, Chandrashekar CR (2009) Cognitive endophenotypes in OCD: A study of unaffected siblings of probands with familial OCD. Progress in Neuro-Psychopharmacology and Biological Psychiatry 33: 610-615.

Vogel S, Schwabe L (2016) Learning and memory under stress: implications for the classroom. npj Science of Learning 1: 16011.

Voon V, Derbyshire K, Ruck C, Irvine MA, Worbe Y, Enander J, Schreiber LR, Gillan C, Fineberg NA, Sahakian BJ, Robbins TW, Harrison NA, Wood J, Daw ND, Dayan P, Grant JE, Bullmore ET (2015) Disorders of compulsivity: a common bias towards learning habits. Molecular Psychiatry 20: 345-52.

Vorhees CV, Williams MT (2014) Assessing spatial learning and memory in rodents. Institute for Laboratory Animal Research Journal 55: 310-32.

Walitza S, Melfsen S, Jans T, Zellmann H, Wewetzer C, Warnke A (2011) Obsessive-compulsive disorder in children and adolescents. Deutsches Ärzteblatt International 108: 173-9.

Weisberg RB (2009) Overview of generalized anxiety disorder: epidemiology, presentation, and course. The Journal of Clinical Psychiatry 70 Suppl 2: 4-9.

Whiteside SP, Lynam DR (2001) The Five Factor Model and impulsivity: using a structural model of personality to understand impulsivity. Personality and Individual Differences 30: 669-689.

Wolmarans DW, Stein DJ, Harvey BH (2016) Excessive nest building is a unique behavioural phenotype in the deer mouse model of obsessive-compulsive disorder. Journal of Psychopharmacology 30: 867-74.

Wolmarans DW, Brand L, Stein DJ, Harvey BH (2013) Reappraisal of spontaneous stereotypy in the deer mouse as an animal model of obsessive-compulsive disorder (OCD): Response to escitalopram treatment and basal serotonin transporter (SERT) density. Behavioural Brain Research 256: 545-553.

Wolmarans DW, Prinsloo M, Seedat S, Stein DJ, Harvey BH, de Brouwer G (2022) Escitalopram and lorazepam differentially affect nesting and open field behaviour in deer mice exposed to an anxiogenic environment. Neuroscience Research 177: 85-93.

Wolmarans DW, Stein DJ, Harvey BH (2017) A psycho-behavioral perspective on modelling obsessive-compulsive disorder (OCD) in animals: The role of context. Current Medicinal Chemistry.25(41), 5662–5689.

Woody EZ, Szechtman H (2011) Adaptation to potential threat: the evolution, neurobiology, and psychopathology of the security motivation system. Neuroscience & Biobehavioral Reviews 35: 1019-1033.

Wu K, Hanna GL, Rosenberg DR, Arnold PD (2012) The role of glutamate signaling in the pathogenesis and treatment of obsessive-compulsive disorder. Pharmacology Biochemistry and Behavior 100: 726-35.

Wu MS, McGuire JF, Martino C, Phares V, Selles RR, Storch EA (2016) A meta-analysis of family accommodation and OCD symptom severity. Clinical Psychology Review 45: 34-44.

Wu T, Chen C-C, Chen T-C, Tseng Y-F, Chiang C-B, Hung C-C, Liou H-H (2009) Clinical efficacy and cognitive and neuropsychological effects of levetiracetam in epilepsy: an open-label multicenter study. Epilepsy & Behavior 16: 468-474.

Yamada C, Mogami S, Kanno H, Nishi A, Shimobori C, Hattori T (2018) Ninjinyoeito Ameliorates Anorexia and Decrease in the Nest Building via a Dopamine D2 Receptor-dependent Manner in a Novel Murine Apathy Model. Japanese Pharmacology and Therapeutics 46: 207-216.

Zhang Z, Fan Q, Bai Y, Wang Z, Zhang H, Xiao Z (2016) Brain Gamma-Aminobutyric Acid (GABA) Concentration of the Prefrontal Lobe in Unmedicated Patients with Obsessive-Compulsive Disorder: A Research of Magnetic Resonance Spectroscopy. Shanghai Archives of Psychiatry 28: 263-270.

Zhou B, Zhang Q, Tian L, Xiao J, Stefan H, Zhou D (2008) Effects of levetiracetam as an add-on therapy on cognitive function and quality of life in patients with refractory partial seizures. Epilepsy Behavior 12: 305-10.

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.

Zor R, Szechtman H, Hermesh H, Fineberg NA, Eilam D (2011) Manifestation of incompleteness in obsessive-compulsive disorder (OCD) as reduced functionality and extended activity beyond task completion. PLoS One 6: e25217.

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END OF CHAPTER TWO

3 Journal article

Associations between nesting, stereotypy and working memory in deer mice: response to levetiracetam

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Author contributions

- BH designed the study under the supervision of DWW. BH performed all the pharmacological and behavioral experiments and wrote the first and edited versions of this manuscript.
- SLG was a co-supervisor of this work and assisted with study design and data interpretation. SLG also read the first draft of the manuscript.
- DWW was the supervisor of this work. He conceptualized and designed the study in consultation with BH and SLG. He assisted with behavioral methodology, data interpretation and revision of the first and final versions of this article.

* * *

Abstract

Rationale: Subpopulations of deer mice *(Peromyscus maniculatus bairdii)* exhibit large nest building (LNB)[1] behavior and high motor stereotypy (HS)[2], i.e. vertical jumping activity (VA)[3] and patterned cage running (CR)[4], making these mice a potentially valuable model organism for studying compulsive-like behavior. It remains unknown if and how LNB and HS associate with early-life and adult cognitive functions, and whether such associations respond to levetiracetam (LEV)[5], an antiepileptic drug that appears to enhance cognitive performance in epileptic and non-epileptic cohorts.

Objectives: The objectives of this work were to 1) explore the longitudinal relationship between habitual behavior early in life and the expression of LNB and HS in adulthood, 2) investigate if and how HS and LNB associate with working memory in adulthood, and 3) explore response to chronic LEV exposure.

Methods: 76 juvenile (28-day-old) deer mice of both sexes were assessed for habit-proneness in the Barnes maze (BM)[6] and divided into two exposure groups (n = 37-39 per group), control (CTRL[7]; normal drinking water) and LEV (75 mg/kg/day; administered in the drinking water). After 56 days of uninterrupted exposure, mice were screened for nesting and stereotypical behavior, and then assessed for working memory in the T-maze.

Results and conclusion: Juvenile deer mice overwhelmingly utilize habit-like response strategies, regardless of LNB and HS behavior in adulthood. Further, LNB and HS are unrelated in terms of their expression, while LEV reduces the expression of LNB, but bolsters CR. Lastly, an increased level of control over high stereotypical expression may facilitate improved working memory performance.

Keywords: deer mouse; stereotypy, nest building, Barnes maze; T-maze; alternation; working memory

[1] large nest building
[2] high motor stereotypy
[3] vertical jumping activity
[4] patterned cage running
[5] levetiracetam
[6] Barnes maze
[7] control

3.1 Introduction

Persistent and repetitive behaviors are common in neuropsychiatric conditions, e.g. obsessive-compulsive disorder (OCD)[1] and its other related disorders, autism spectrum disorder, behavioral addictions like gambling disorder, and attention-deficit/hyperactivity disorder (APA 2013). Significant advances have been made to understand the neurocognitive underpinnings of these behaviors. Current research implicates cortico-striatal-thalamo-cortical involvement (Langen et al. 2011; Wood and Ahmari 2015), with cognitive theory variably pointing to dysfunctional processes that regulate, among others, habit formation (Burguière et al. 2015; Graybiel 2008), impulse control (Belin-Rauscent et al. 2016; Boisseau et al. 2012; Robbins et al. 2012), reward feedback processing (Delmonte et al. 2012; Figee et al. 2011; Pinto et al. 2014), and cognitive flexibility (Bradbury et al. 2011; Braem and Egner 2018; Morein-Zamir et al. 2014). The contribution of these and other constructs to the development of persistent and repetitive behaviors are likely unique to different conditions, which clouds our understanding of how these behaviors develop over time.

OCD is diagnosed in up to 3 % of children and adolescents (Walitza et al. 2011). Diagnosis can be especially difficult, since many of the symptoms that are assessed during evaluation correspond with behavioral traits that often present in children, e.g. highly ritualized and rigid habit-like behaviors (Evans et al. 2002; Leonard et al. 1990). Further, it is uncertain if and how such traits may predict and ultimately contribute to a clinical diagnosis of OCD in some but not others (Hjemdal et al. 2011; Thorsen et al. 2019; van der Straten et al. 2020). In terms of clinical progression, neurocognitive processes that regulate the relationship between goal-directed and habitual responses have been proposed to play a role in the obsessive-compulsive symptom manifestation (Gillan et al. 2011). For example, individuals that show a bias towards habitual behavioral engagement (Snorrason et al. 2016) or that present with deficits in goal-directed action-outcome valuation (Ferreira et al. 2017; Peng et al. 2020), both of which may also be markers of impaired cognitive flexibility (Gruner and Pittenger 2017), are at increased risk to develop OCD.

High-dose selective serotonin reuptake inhibitor (SSRI)[2] treatment is the mainstay of current pharmacological treatment approaches (Robbins et al. 2019). Meanwhile, cognitive strategies mainly involve behavioral therapy and exposure-response prevention (Reid et al. 2021). In both instances, treatment responses are less than adequate, highlighting a need for alternative options (Middleton et al. 2019). To this end, early-life pharmacotherapeutic strategies that may target distinct psychobiological processes that can, in some cases, contribute to the development and prognosis of compulsivity, might

[1] obsessive-compulsive disorder
[2] selective serotonin reuptake inhibitor

be fruitful. From this theoretical perspective, levetiracetam (LEV)[1], clinically used for the treatment of partial and generalized epilepsy (Helmstaedter and Witt 2010), shows promise. Although most data that point to the neurocognitive effects of LEV, e.g. improved working memory, verbal skill, and visual feedback processing, have been generated in epilepsy cohorts (Helmstaedter and Witt 2010; Operto et al. 2019; Piazzini et al. 2006), some results from studies in other clinical cohorts, e.g. patients suffering from Alzheimer's disease (Sanchez et al. 2012), and non-clinical samples have also been informative. For example, a single dose of LEV enhances executive functioning on the levels of working memory, task planning and decision making (Magalhães et al. 2015).

Subpopulations of deer mice *(Peromyscus maniculatus bairdii)* that are bred, reared, and housed under standard laboratory conditions variably and spontaneously present with distinct, but equally persistent and repetitive behavioral phenotypes, i.e. large nest building (LNB)[2] behavior and high motor stereotypy (HS)[3] (de Brouwer et al. 2020). HS behavior in turn can be classified into either vertical jumping activity (VA)[4] or patterned cage running (CR)[5]. These behaviors are entirely non-induced, occur in animals of both sexes, and respond to chronic high-dose oral exposure to the SSRI[6], escitalopram (Scheepers et al. 2018), making these mice an interesting model organism for studying compulsive-like behaviors. In this work, we explored the longitudinal relationship between a potential early-life marker of habit-proneness in the Barnes maze (BM)[7] and LNB and HS (on the levels of VA and CR) in adulthood. We further sought to understand if and how these behaviors associate with each other and with working memory, as assessed in the T-maze. Lastly, we investigated if LNB, VA, and CR, as well as their potential relationships with working memory, would respond to chronic LEV exposure, administered throughout the rearing period.

3.2 Materials and methods

3.2.1 Study layout

The present work was divided into two phases, Phase 1 (juvenile) and Phase 2 (adult) (**Fig. 3-1**). First, 76 mice were assessed on the BM from postnatal day (PND)[8] 28-35. Next, mice were divided into two groups, control (CTRL)[9] and LEV (CTRL: n = 37, 20 females, 17 males; LEV: n = 39, 20 females,

[1] levetiracetam
[2] large nest building
[3] high motor stereotypy
[4] vertical jumping activity
[5] patterned cage running
[6] selective serotonin reuptake inhibitor
[7] Barnes maze
[8] postnatal day
[9] control

19 males) and treated until PND[1] 95. From PND 84–95, all mice underwent sequential behavioral testing, i.e. nest building (PND 84-91), stereotypy assessment (PND 92), and T-maze assessment (PND 93-95).

3.2.2 Mice

Peromyscus maniculatus bairdii were acquired from the DSI/NWU Vivarium at North-West University, South Africa (South African Veterinary Council registration number: FR15/13158; AAALAC accreditation file #1717). All procedures were approved by the AnimCare Research Ethics Committee of the North-West University (NWU)[2] (approval number: **NWU-00423-21-A5**) and complied with the South African National Standard for the Care and Use of Animals for Scientific Purposes (SANS 10386:2021).

Experimental subjects were chosen by means of a randomized number system from the litters of at least 20 unique breeding pairs. After selection, animals were ear-tagged for identification purposes and, except if stated otherwise, housed in pairs of same-sex animals throughout the remainder of the investigation. Cages (35 cm (l) x 20 cm (w) x 13 cm (h); Techniplast® S.P.A., Varese, Italy) were automatically climate-controlled and kept ambient at 23 °C, a relative humidity of 60 %, and on a 12-h light/dark cycle (06h00/18h00). Food and water (or drug solution) were provided *ad lib* throughout all phases of the study, while cages were cleaned and new corncob bedding supplied, weekly. Except if stated otherwise, all cages were supplied with paper towel as a form of nesting material and equipped with a piece of white polyvinyl chloride pipe (10 cm (l) x 5 cm (Ø)) as a form of environmental enrichment.

3.2.3 Drug exposure

On the day following the last BM[3] probe trial (PND 35), CTRL[4] (normal tap water) or LEV[5] (BLD Pharma®, Shanghai, China) exposure was initiated via the drinking water and continued through the end of the study period. LEV was prepared at a concentration of 30 mg/100 mL to deliver an approximate 24-hour dose of 75 mg/kg/day; de Ridder et al. (2022); Sanchez et al. (2012). Drug exposure via the drinking water is preferred in this model system, as opposed to intraperitoneal injection or oral gavage, due to the long period of drug exposure. These concentrations are based on the average daily fluid intake of adult deer mice (0.25 mL/g/day; de Brouwer et al. (2020); Wolmarans et al. (2013)). To confirm, the fluid consumption of LEV-exposed mice was recorded daily and compared to that of deer mice that only received water. Fresh drug solutions were prepared every day.

[1] postnatal day
[2] North-West University
[3] Barnes maze
[4] control
[5] levetiracetam

3.2.4 Behavioral assessment – general background

Since 76 mice were tested in behavioral experiments, procedures were timed to enable a sequential experimental approach. Specifically, animals that were born at different time points entered into the behavioral assessment procedures according to their respective ages, as indicated for each assessment in **Fig. 3-1.** This accommodated for the maximum availability of research infrastructure and ensured that each animal went through the same chronological experience, according to age.

3.2.5 Barnes maze assessment

BM[1] experiments were conducted in the dark cycle, between 19:00 and 01:00. To allow animals to fully wake, the first cages were moved to the experimental room at 18:00. After this, animals were moved to the testing room in a sequential manner, on any given night of testing.

A small, walled-off BM, adapted from O'Leary and Brown (2012), was used. The maze was constructed from white Plexiglas® and consisted of an octagon-shaped floor with a diameter of 70 cm, walled off on each side (50 cm high) to prevent the influence of external cues on learning, as far as possible. 16 circular holes (4 cm in diameter) were equally spaced around the perimeter of the maze floor (holes were spaced 3 cm from the walls). The maze was raised to a height of 30 cm above floor level to allow for the placement of a movable escape box (15 cm (l) x 15 cm (w) x 20 cm (h)). The escape box was constructed from black Plexiglas® and connected to a pipe (Ø = 7 cm) that could be attached to any of the holes as a means of escape. Before each trial, the escape box was cleaned with 90 % ethanol, rinsed with normal tap water, and allowed to dry before being prepared with corncob bedding and food taken from the respective home cages of the tested mice. When the escape box was attached to the BM, all other holes were closed off with black lids placed at a depth reaching 2 cm below the floor level of the maze. A separate floorless starting compartment (10 cm x 10 cm x 15 cm) was constructed from black Plexiglas®. This was placed in the center of the BM at the onset of testing. Once experimentation commenced, the starting compartment was removed from the arena, and the mouse left to freely explore. Except if stated otherwise, all phases of the Barnes maze experiment were conducted under bright white light (10 000 lux), while white noise was played in the background at a sound level of 80 dB to bolster escape-related behavior (Rosenfeld and Ferguson 2014).

The BM assessment was conducted as previously described (O'Leary and Brown 2012), with minor modification to allow for the assessment of juvenile deer mice. Briefly, mice were habituated, trained, and tested over three separate stages: **(A)** habituation stage, **(B)** acquisition stage, and **(C)** a probe

[1] Barnes maze

stage. All trials were video recorded to allow for post-test scoring and automated tracking of ambulatory behavior (Ethovision® XT 16; Noldus Information Technology®, Wageningen, The Netherlands).

For the habituation stage **(A)** (experimental day 1, PND[1] 28), mice were introduced to the BM[2] for two 5-min trials spaced 25 min apart. The first habituation was conducted under dim red light without playing white background noise to allow for habituation to the maze under less stressful conditions. Mice were transferred to the BM and placed underneath a 2 L glass beaker located over the hole where the escape burrow was located. The escape box was attached to the hole underneath the glass beaker. Mice were thus able to view the entire spatial environment, but only had access to the hole connected to the escape burrow and box. Next, mice were once again transferred to the BM; however, at this time, mice were placed in the middle of the BM underneath the starting compartment located in the center of the maze and left there for one minute. This session was also conducted under dim red light and in the absence of background noise. After the starting compartment was raised, mice were allowed five minutes to explore the entire maze and find the escape hole with all the other holes still being closed off.

The acquisition stage **(B)** comprised four training days (PND 29-32; two trials per day, spaced 25 min apart). At the beginning of each trial, mice were introduced to the starting compartment located at the center of the maze for 1 min. On the first day, the maze was brightly lit only, with the trials conducted in the absence of white noise. Over the second to fourth days of stage **(B)**, background noise was added to the testing procedure. Again, mice had 5 min to explore the maze during each trial and to find the escape hole, which was still located in the same position as in the habituation phase. Successful target acquisition in each trial was accepted if mice spent at least 5 sec in the proximity of the target hole (within 5 cm of its border) or when mice entered the escape box. Once reaching the escape box, mice were allowed to remain there for 30 sec before being returned to their home cages. Mice that spent time at the escape hole, but that did not enter the burrow, were left in the BM for the full 5-min duration. Irrespective of the number of times said animals returned to the proximity area around the escape hole, learning was counted only once per trial. After completing each trial, the maze and the escape box were cleaned as explained for stage **(A)**. Mice were accepted to have learned the location of the escape hole if they made four or more successful attempts at reaching the hole or entered the burrow over the eight stage **(B)** trials, collectively.

In the probe stage **(C)**, animals were assessed for habit-like behavior, i.e. returning to the proximity area around, or attempting to enter the hole to which the escape box was previously attached, but was now

[1] postnatal day
[2] Barnes maze

closed off. The probe trial phase (PND[1] 33-34; 2 trials per day) was conducted over 2 days of testing. The first probe trail began 24 h after the last stage **(B)** trial. Here, we quantified the number of approaches made towards the target area during each trial. More entries towards the target area in the first trial compared to the last, was regarded as behavioral engagement regulated by goal-directed processing, while a similar number of approaches towards the target area through the final stage **(C)** trial, was regarded as habit-like. These observations were informed by negative and neutral-to-positive trial vs. target approach-slopes generated by each mouse, respectively. Mice generating slopes < -1 were defined as goal-directed because they modified their response strategies when the escape box was closed off, while scores > -1 were considered habit-like because mice persisted in the same routine.

3.2.6 Nesting assessment

All 76 mice were assessed for nest building expression over one week once animals reached the age of 84 days (de Brouwer et al. 2020). For this assessment, each mouse was allocated to its own new home cage, identical to the home cage, for seven days. On each of the seven days of nesting assessment, between 15:00 and 16:00, an excess of sterile and unscented cotton wool was weighed and introduced into the roof of each cage. On every subsequent day, also between 15:00 and 16:00, built nests were removed, discarded and the remaining cotton wool in the roof of the cage weighed and recorded. Where needed, additional cotton wool was supplied. Thus, mice had access to nesting material for 24 h of each day.

After completion of the 7-day nesting assessment, the daily quantity of cotton wool that was utilized by each animal (in grams) was summed and a 7-day total nesting score calculated for each subject (de Brouwer et al. 2020). Identification of normal nesting (NNB)[2] behavior and LNB[3] (where applicable) was based on the extreme ends of the distribution of all total nesting scores generated by mice in the CTRL[4]-exposed group (de Brouwer et al. 2020). Further, a second criterion, that is persistence, was applied to classify LNB. Thus, only mice that generated total nesting scores that clustered within the upper 75th percentile of the distribution, and the lowest quartile of distribution with respect to the variance in the daily nesting scores (as reflected by the percentage coefficient of variance; % CV[5]), were classified as LNB-expressing animals (**Fig. 3-2A**). During periods of nest building analysis, food and water were available as normal, although animals were not provided with any additional form of nesting material.

[1] postnatal day
[2] normal nest building
[3] large nest building
[4] control
[5] coefficient of variance

3.2.7 Stereotypy assessment

On the first day following the conclusion of the nest building assessment, all mice were assessed for stereotypical behavior according to a previously published protocol (Wolmarans et al. 2013), slightly modified for the purposes of the present investigation. Briefly, each mouse underwent a single 12-h stereotypy assessment. At 17:00, one hour prior to the onset of screening, animals were moved in their housing cages to the behavioral screening room located on the same floor of the vivarium. Each animal was then immediately introduced to its own behavioral testing cage (21 cm (l) x 21 cm (w) x 35 cm (h); Accuscan® Inc., Columbus, Ohio, USA), that was constructed from clear Plexiglas® and equipped with a grid of infrared beams that crossed the cage at both 2 cm and 10 cm above floor level. Ground corncob was provided in quantities enough to cover the floor of the test cages, but also ensuring that the scoring of ambulatory activity was not interrupted. Food and water (or drug solutions) were again provided *ad lib* throughout the 12-h assessment session. Cages were cleaned with F10® veterinary cleaner (Health and Hygiene Products Pty. Ltd., Roodepoort, South Africa) after each night of assessment and prepared for the next assessment session.

Infrared beam tracking allows for the quantification of both VA[1] and CR[2]; expressed as the sum of all clockwise and anticlockwise revolutions. Since mice that express jumping activity interrupt more than one beam in the higher infrared grid when making a single jump (Wolmarans et al. 2013), the number of vertical beam interruptions is applied as broad measure of VA. Where applicable, mice were classified as either non-stereotypical (NS)[3] or HS[4] based on two measures, i.e. behavioral intensity and the time spent executing HS behavior. To do this, the 12-h screening session was divided into 24 30-min bouts. HS bouts are defined for each behavioral phenotype as per the cut-off values in **Table 3-1**. Stereotypical intensity was calculated as the average of the three highest 30-min VBI or CR values generated, whereas time spent engaging in stereotypy was calculated as the number of 30-min HS bouts expressed during the 12-h screening session by each mouse, expressed as a percentage. Importantly, HS animals were selected based on the expression of either vertical or horizontal activity, or both.

[1] vertical jumping activity
[2] patterned cage running
[3] non-stereotypical
[4] high motor stereotypy

TABLE 3-1 - CUT-OFF CRITERIA FOR NS AND HS BEHAVIOR ACROSS BOTH BEHAVIORAL PHENOTYPES

Cohort	VBI / 30 min	CR / 30 min
NS	< 500	< 150
HS	> 2000	> 200

NS: non-stereotypical; HS: high stereotypical; VBI: vertical beam interruptions (indicative of vertical jumping activity); CR: patterned cage running (sum of clockwise and anti-clockwise revolutions)

3.2.8 T-maze

Assessment of arm alternation behavior in a T-maze is often used to investigate processes related to working memory (Shoji et al. 2012). Here, we applied a modified version of the test used by Gerlai (1998); however, in this work, the walls of the maze were raised to accommodate for the screening of jumping deer mice. Briefly, the maze, shaped in the form of the letter 'T', was constructed from white, opaque Plexiglas® (stem and arm dimensions 30 cm x 6.5 cm; wall height: 30 cm). When needed (see below), each of the respective arms could be closed off by a manually operated door (Spowart-Manning and van der Staay 2004). A removable divider placed 10 cm from the base of the stem, was used to confine animals to a 'starting area'. The ambulatory activity of mice was analyzed with Ethovision® XT 16 software. Following each T-maze assessment session, the maze was cleaned with 90 % ethanol, rinsed with normal tap water and dried to prevent the possible influence of previously associated odors on the alternation behavior of subsequently assessed mice.

The T-maze assessment was conducted over three stages: **(A)** naturalistic arm preference testing, **(B)** cued forced-arm entry training, and **(C)** probe testing. Stages were separated by 24 h, with stage **(A)** commencing 24 h after the onset of stereotypy assessment. A separate habituation phase was not included as in the BM[1] assessment, since animals were allowed to explore the entire arena during the naturalistic arm preference phase. All T-maze experiments were conducted between 19:00 and 01:00 under dim red light and videotaped. Mice were moved from the housing environment to the testing room on the same floor of the vivarium at least one hour prior to the onset of testing.

Naturalistic arm preference testing was performed on PND[2] 93 (Deacon and Rawlins 2006). Mice were allowed a single 15-min session to explore the maze, or to make a maximum of ten arm entries. The most preferred arm was determined based on the majority arm choice. Animals that failed to make ten arm entries within the 15-min window were not included for assessment in stages **(B)** and **(C)**. For animals that made an equal number of left- or right choices, an arm choice was randomly chosen by the observer for application in stage **(B)**. Stage **(A)** began when an animal was introduced to the enclosed starting area. After 30 sec, the starting area door was raised, and animals were allowed to

[1] Barnes maze
[2] postnatal day

make their first entry into any one of the transverse arms of the maze (Deacon and Rawlins 2006). After having made a choice, the opposite arm was closed off and the animals allowed to freely return to the starting area. Here, mice were confined for 30 sec (Deacon and Rawlins 2006), before being allowed to enter the maze again.

For the cued forced-arm entry stage **(B)**, which was conducted when mice were aged PND[1] 94, animals were forced to enter the least preferred arm (i.e. 'forced arm') as determined in stage **(A)**. In this instance, the forced arm was cued on all three sides with a solid black rectangular mark to promote arm recognition during stage **(C)**. Animals were allowed to make as many arm entries as they could within a single 30-min session. However, animals that completed less than ten arm entries were not included for assessment in stage **(C)**.

During probe test stage **(C)** (PND 95), mice were able to access the entire arena again. The arm that was cued in stage **(B)** remained thus, with the opposite arm being non-cued. Again, mice were allowed to explore the maze and make as many arm choices as they could for 30 min during a single session, with an intertrial session again set at 30 sec. Arm choices were noted and the alternation score, expressed as a percentage of the total arm entries, calculated. Higher, as opposed to lower post-training alternation scores were considered to be indicative of improved working and spatial memory recall.

3.2.9 Statistical analyses

Statistical analyses were performed, and graphs drawn with GraphPad® Prism® version 9. Associations between the probe trial behavior of juvenile mice (trial vs. target approach) and the other behavioral outcomes of this investigation (nesting expression, stereotypy, and T-maze alternation, etc.) were explored by multiple Spearman's correlations. The same analysis was used to determine the association between total nesting score and the percentage coefficient of variance with respect to daily nesting scores (an indicator of behavioral persistence over subsequent testing days) in adult mice exposed to either CTRL[2] or LEV[3]. Descriptive statistics were applied to determine the upper 75th and the lower 25th quartiles of distribution for each parameter, respectively. This was used to compare the expression of LNB[4] in CTRL and LEV-exposed mice. For comparisons of the total nesting scores, stereotypical intensity and time spent engaging in HS[5] behavior, and T-maze alternation scores generated by CTRL-

[1] postnatal day
[2] control
[3] levetiracetam
[4] large nest building
[5] high motor stereotypy

and LEV[1]-exposed mice, unpaired Mann-Whitney *U*-tests were run. Significance was regarded as $p < 0.05$.

3.3 Results

3.3.1 Summary of animals that completed the respective assessments

Since this study followed a longitudinal approach, some animals failed to complete the entire battery of assessments. As such, statistical analyses are based on the data of animals that completed the respective assessments (**Table 3-2**), while the correlational data are only reflective of animals that generated data for both of the relevant parameters. **Tables 3-3** & **3-4** report the behavioral measures for the CTRL[2] and LEV groups, respectively.

TABLE 3-2 - OVERVIEW OF ANIMALS IN THE CTRL- AND LEV-EXPOSED GROUPS THAT COMPLETED THE RESPECTIVE ASSESSMENTS

Behavioral Assessment	CTRL *n*	LEV *n*
Barnes maze	37	39
Nesting	37	39
Stereotypy*	31	38
T-maze	33	26

CTRL: control; LEV: levetiracetam; *Sample numbers are lower due to an electrical malfunction of the screening apparatus on the night of testing. Animals could not be reassessed for reasons pertaining to uniformity of methodology.

[1] levetiracetam
[2] control

TABLE 3-3 - BEHAVIORAL OUTPUT GENERATED BY TREATMENT-NAÏVE MICE SELECTED FOR FUTURE CTRL-EXPOSURE ACROSS ALL BEHAVIORAL ASSESSMENTS

Mouse	BM % success training	BM Slope	T-maze % alternation	Nest-building		VA		HR	
				Total	% CV	Ave high	% Time	Ave high	% Time
1	37.5	0	20.0	34.4	31.0	795.3	0	50.0	0
2	100	**-2.3**	43.3	28.8	58.0	1243	0	19.7	0
3	62.5	0.9	37.5	27.9	55.7	989.7	0	16.3	0
4	62.5	2.1	42.9	29.4	30.9	1497.7	0	6.3	0
5	50	**-8.1**	30.6	17.2	55.5	2430.3	16.7	241.7	16.7
6	62.5	**-1**	62.5	35.0	46.2	784.3	0	5.7	0
7	87.5	-0.4	42.9	1.8	66.6	673	0	20.7	0
8	87.5	0.7	53.8	12.8	48.7	1281.3	0	34.0	0
9	50	0.4	30.4	10.7	40.8	2152.7	8.3	37.7	0
10	50	0	18.2	16.3	43.4	1585	0	8.3	0
11	50	0	44.0	11.0	47.7	2127.3	8.3	126.0	0
12	75	-0.5	39.3	21.6	32.9	1519.3	0	18.7	0
13	100	**-3.8**	20.0	9.8	61.2	793	0	38.0	0
14	75	0.3	36.0	6.7	57.8	544.3	0	18.3	0
15	50	0.9	-	19.0	41.3	2228	8.3	8.7	0
16	62.5	0.3	38.1	29.8	50.9	936.3	0	6.3	0
17	100	-0.8	5.9	38.5	31.1	1550.3	0	7.3	0
18	50	0.4	-	20.4	28.7	5066.3	45.8	58.3	0
19	50	1	33.3	30.3	28.7	621	0	9.0	0
20	50	-0.1	41.2	14.5	30.1	-	-	-	-
21	87.5	**-2.2**	35.7	39.4	27.1	-	-	-	-
22	50	0.6	33.3	13.6	45.2	-	-	-	-
23	50	0.2	58.3	44.4	12.3	-	-	-	-
24	50	0.6	65.6	30.3	61.6	-	-	-	-
25	50	1	33.3	33.6	27.1	-	-	-	-
26	50	0.8	43.6	36.7	25.6	432.3	0	32.7	0
27	50	1	55.0	23.0	42.8	2449.3	8.3	6.3	0
28	50	0.3	-	19.4	44.0	4483	33.3	5.7	0
29	75	0	37.5	34.6	35.5	1370	0	12.0	0
30	50	-0.5	60.0	12.7	47.8	684.3	0	20.7	0
31	50	0.1	25.0	49.2	22.3	960.3	0	12.3	0
32	37.5	0.4	28.6	10.4	53.0	9041	58.3	8.7	0
33	50	**-1.8**	23.8	4.6	73.1	3586.3	25	68.0	0
34	50	-0.9	21.6	20.0	52.3	2467.3	12.5	89.0	0
35	0	1.2	-	15.1	49.2	2216.3	12.5	44.3	0
36	50	0.2	68.8	22.7	63.0	1178	0	13.3	0
37	37.5	0.1	9.8	18.5	58.7	1451.3	0	9.0	0

CTRL: control; BM: Barnes maze; CV: coefficient of variance; VA: vertical jumping activity; CR: patterned cage running

TABLE 3-4 - BEHAVIORAL OUTPUT GENERATED BY TREATMENT-NAÏVE MICE SELECTED FOR FUTURE LEV-EXPOSURE ACROSS ALL BEHAVIORAL ASSESSMENTS

Mouse	BM % success training	BM Slope	T-maze % alternation	Nest-building		VA		CR	
				Total	% CV	Ave high	% Time	Ave high	% Time
38	75	-0.9	80.0	21.4	48.1	-	-	-	-
39	50	0	34.5	39.0	36.6	1084.3	0	43.7	0
40	50	0	11.8	28.1	32.5	1392.7	0	19.7	0
41	12.5	-0.3	-	22.8	31.5	209.7	0	33.3	0
42	50	0.5	-	20.8	48.1	240.3	0	364.7	41.7
43	50	0.4	45.2	46.8	24.2	177.3	0	113.3	0
44	100	-0.1	51.9	21.5	27.9	1250	0	16.3	0
45	50	-0.8	17.6	28.7	36.5	1677	4.2	38.0	0
46	50	0	-	25.6	41.3	921	0	14.3	0
47	25	0.3	-	0.5	327.0	2718	29.2	258.0	8.3
48	50	0.1	44.0	14.8	30.3	859.7	0	4.0	0
49	37.5	0.6	25.0	10.8	28.7	1075.3	0	16.7	0
50	50	0.4	47.2	22.9	39.2	649.7	0	48.3	0
51	37.5	0	-	33.6	53.4	339.7	0	10.7	0
52	50	-0.6	-	16.7	40.2	979	0	27.0	0
53	12.5	-0.3	-	15.3	26.0	1349.3	0	162.7	4.2
54	87.5	0.7	-	20.9	32.8	1863.7	4.2	36.0	0
55	87.5	-0.7	20.0	19.0	22.4	633.3	0	30.7	0
56	50	-0.5	21.2	20.6	51.3	996	0	34.7	0
57	87.5	-0.2	46.7	27.3	15.8	1534	0	17.7	0
58	50	0.3	-	14.6	29.7	2080.7	4.2	34.7	0
59	62.5	0.4	46.2	16.0	36.6	763	0	18.7	0
60	62.5	-0.8	33.3	15.4	47.8	2025.7	8.3	20.3	0
61	37.5	-0.1	11.8	22.2	45.0	2390.3	12.5	6.3	0
62	50	0.1	31.4	18.3	37.6	795.7	0	566.7	80.3
63	50	0	19.4	17.3	44.9	1196.3	0	12.7	0
64	62.5	-0.1		24.3	27.3	1332	0	11.7	0
65	62.5	-0.4	24.0	11.9	47.2	182.3	0	72.3	0
66	50	-0.5		18.2	39.9	2265.7	8.3	31.3	0
67	62.5	0.4	16.7	18.1	56.0	2808.7	16.7	66.0	0
68	50	-0.5	43.5	7.0	68.1	1101	0	161.0	0
69	50	-0.1	28.6	18.6	44.4	3962	29.2	66.0	0
70	50	-0.4	60.0	5.6	75.7	1234.7	0	125.0	0
71	87.5	0.5		1.6	128.2	402	0	74.7	0
72	50	0.6	42.4	11.6	58.4	1795	4.2	47.7	0
73	62.5	-0.9	29.0	31.9	39.6	937.3	0	14.0	0
74	50	0.1		4.2	62.4	3132	16.7	378.0	50
75	50	0.8	56.3	4.6	71.4	715.7	0	24.7	0
76	50	1.1	55.6	32.9	20.8	652	0	88.3	0

LEV: levetiracetam; BM: Barnes maze; CV: coefficient of variance; VA: vertical jumping activity; CR: patterned cage running

3.3.2 Barnes maze assessment

Of the 76 juvenile mice assessed for BM[1] behavior, 10 mice failed to satisfy the criteria for successful learning (meaning, they failed to locate the escape port more than 50 % of the time). Thus, the probe test data were not considered. Of the remaining 66 mice, only 6 mice fulfilled the criterium for goal-directed responding (colored lines; **Table 3-3**), with the remaining mice all generating neutral to positive

[1] Barnes maze

slope scores (meaning, they persisted in familiar strategies even when they were not productive). Of these, multiple Spearman's correlations failed to reveal significant relationships with any of the parameters assessed in adulthood, or with the number of successful training trials prior to the test (not shown).

3.3.3 Nesting behavior of CTRL- and LEV-exposed mice

Total nesting scores correlated negatively with the variance in daily nesting scores in both CTRL[1]- and LEV[2]-exposed mice (**Fig. 3-2A**, CTRL: r_S = -0.60; p < 0.0001; LEV: r_S = -0.44; p = 0.006). Based on the statistical cut-off criteria used to identify LNB[3]-expressing mice in the CTRL-exposed group, eight LNB-expressing mice were identified from the pool of 37 (22 %), while only two mice in the LEV-exposed group (5 %) fulfilled the same criteria.

In terms of the average total nesting scores generated by CTRL- and LEV-exposed mice (**Fig. 3-2B**), no significant difference between the median values of the different groups was shown (CTRL: 20.4 g vs. LEV: 18.80 g, p = 0.32, 95CI: -8.1; 2.5, U = 608.5).

3.3.4 Stereotypical behavior of CTRL- and LEV-exposed mice

The median values of the highest average VA[4] scores generated by mice in the CTRL- and LEV-exposed groups were similar (**Fig. 3-3A**; CTRL: 1451.0 vs. LEV: 1093.0, p = 0.12, 95CI: -734.0; 92.0, U = 461.0). In contrast, LEV exposure significantly increased the expression of CR[5] activity, compared to that of CTRL-exposed mice (**Fig. 3-3B;** 35.0 vs.18.0, p = 0.0078, 95CI: 4.00; 29.0, U = 370.5).

In terms of the time that NS[6] and HS[7] animals spent engaging in HS expression, LEV-exposure had no effect on the result (p = 0.92, U = 581.5) (not shown).

3.3.5 T-maze alternation behavior of CTRL- and LEV-exposed mice

Alternation scores generated by CTRL- and LEV-exposed mice completed the assessment, did not differ significantly (p = 0.92; U = 331.5).

[1] control
[2] levetiracetam
[3] large nest building
[4] vertical jumping activity
[5] patterned cage running
[6] non-stereotypical
[7] high motor stereotypy

3.3.6 Associations between nesting, stereotypy, and T-maze alternation in adult mice

To determine whether any relationships exist between nesting behavior, stereotypical expression, and T-maze alternation in adult mice, the following Spearman's correlations were run: **(i)** total nesting scores vs. stereotypical expression (both intensity and time spent executing HS[1] behavior across both VA[2] and CR[3]), **(ii)** total nesting scores vs. alternation scores, and **(iii)** stereotypical expression (both intensity and time spent executing HS behavior across both VA and CR) vs. alternation scores. Statistics are provided in **Table 3-5**.

TABLE 3-5 - SPEARMAN'S CORRELATIONS BETWEEN THE INDICATED MEASURES COLLECTED IN ADULTHOOD

Correlation	Group	Spearman's *r* [CI]	*P*	Pairs *n*
Total nesting score (g) vs. VA intensity	CTRL	-0.246 [-0.597; 0.295]	0.182	31
	LEV	-0.2196 [-0.511; 0.117]	0.185	38
Total nesting score (g) vs. CR intensity	CTRL	-0.4236 [-0.68; -0.07]	**0.018***	31
	LEV	-0.3063 [-0.577; 0.025]	0.062	38
Total nesting score (g) vs. % time spent HS	CTRL	-0.3916 [-0.661; -0.032]	**0.029***	31
	LEV	-0.2411 [-0.528; 0.095]	0.145	38
Total nesting score (g) vs. % T-maze alternation	CTRL	0.086 [-0.269; 0.421]	0.627	34
	LEV	0.017 [-0.383; 0.412]	0.935	26
VA intensity vs. % T-maze alternation	CTRL	-0.282 [-0.606; 0.121]	0.154	27
	LEV	-0.349 [-0.661; 0.066]	0.087	25
CR intensity vs. % T-maze alternation	CTRL	-0.089 [-0.463; 0.312]	0.658	27
	LEV	0.210 [-0.213; 0.568]	0.313	25
% Time spent HS vs. % T-maze alternation	CTRL	-0.144 [-0.506; 0.261]	0.474	27
	LEV	-0.396 [-0.691; 0.011]	**0.049***	25

CTRL: control; LEV: levetiracetam; VA: vertical jumping activity; CR: patterned cage running; HS: high motor stereotypy

[1] high motor stereotypy
[2] vertical jumping activity
[3] patterned cage running

3.4 Discussion

The main findings of the present work are 1) performance in the BM[1] of juvenile *P. maniculatus bairdii* mice is not related to LNB[2] or stereotypy in adulthood; 2) chronic LEV[3] exposure from a juvenile age until adulthood decreases LNB expression without affecting overall nesting scores, but increases CR[4] activity; 3) stereotypical expression, notably so CR, and nesting expression are divergent persistent behavioral phenotypes; and 4) LEV exposure generally improves the relationship between working memory and stereotypical engagement.

One aim of this work was to explore the potential longitudinal relationship between habit-like behavior early in life and compulsive-like behavior in adulthood. We used a BM task, reasoning that repeated exposure to an aversive environment, paired with a means to escape, may be an appropriate method to facilitate habit learning, as reflected by continued approach to the escape location even when it was no longer available. If we accept this premise, our results indicate that nearly all mice in this large study utilized habit-like behavior. Consistent with this notion, probe test behavior was overwhelmingly uncoupled from initial escape acquisition, suggesting that it was driven by factors other than escape learning such as behavioral automaticity. In operant conditioning procedures, young rats favor habit-based response strategies even under conditions that induce goal-seeking behavior in adults, suggesting that young organisms are more prone to use habit-based action strategies (Naneix et al. 2012). Allen et al. (2022) capitalized on this propensity to identify highly habit-prone mice for later selective breeding for habit bias. Relatedly, Moin Afshar et al. (2020) revealed that the capacity for goal-based action strategies develops across juvenile and adolescent development, crystalizing only in young adulthood (Moin Afshar et al. 2020). Thus, the response strategies observed here may reflect the strong tendency of young animals to utilize habit-based behaviors. Juvenile *P. maniculatus bairdii* may be a valuable tool to understand the neurobiology of habit formation and adherence in young developing mammals. This topic is grossly under-studied, as the vast majority of investigations instead focus on the development of competing goal-directed response systems. That said, a second consideration that needs attention relates to prior work using the Morris water maze, which revealed mice to postpone or refrain from active responding in aversive environments, since they tend to freeze (D'Hooge and De Deyn 2001). Such a manner of responding could well have influenced training success in the present paradigm. Not all mice entered the escape box, and we ultimately regarded the time spent in the vicinity of the escape

[1] Barnes maze
[2] large nest building
[3] levetiracetam
[4] patterned cage running

hole as successful training. Thus, what remains unknown is whether the results reported here are borne from such methodological constraints, or whether it is indeed reflective of habitual responding.

The second result of this work was informing. LEV[1], administered throughout the entire rearing period, resulted in fewer animals presenting with LNB[2] behavior, as evaluated against the incidence of LNB in the CTRL[3]-exposed mice. In fact, only two animals in the LEV group, compared to 8 in the CTRL group, satisfied the criteria for LNB expression. Broadly, we regard nesting expression as a highly goal-directed behavior (Chou-Green et al. 2003; Hoffman and Rueda Morales 2009; Wolmarans et al. 2016). Thus, LNB is seen as an inflated goal-directed response that might be founded on any number of neurocognitive constructs, e.g. deficits in outcome feedback processing (Wolmarans et al. 2022). Here, we employed LEV[4] to establish whether its putative beneficial effects on cognitive processes discussed in the Introduction could be explored further in the present model system. It was proposed that if compulsive-like behavior is associated with neurobiological perturbations in the cortico-striatal areas (as has been shown before; Korff et al. (2009); Presti et al. (2003)) that have also been associated with impaired working memory (De Brouwer et al. 2021; de Ridder et al. 2022), LEV may be a valuable drug to investigate. We have shown before that LEV engenders a level of control over the expression of HS[5] behavior (de Ridder et al. 2022), i.e. decreasing the time spent engaging in stereotypy (however, see later in this work). Here, we extend these findings to the expression of LNB. Importantly, this result could not have been borne from negative effects of LEV on executive ability or motor expression since the average expression of nesting behavior was not affected (**Fig. 3-2B**). Overall, this finding paves the way for continued research into the potential neurobiological mechanisms that may underlie the naturalistic development of persistent behaviors in deer mice. That LEV broadly influences synaptic vesicle exocytosis, without modulating the effects of a specific neurotransmitters, e.g. serotonin or dopamine, only, provides a useful point of departure for future work, especially considering the third major result of this investigation.

In contrast to effects on LNB, LEV bolstered CR[6] (**Fig. 3-3B**), without affecting the VA[7] scores of mice. This finding is important because it illustrates for the first time in this model system that VA and CR, as shown by a distinct subpopulation of deer mice, diverge in terms of its sensitivity for and response to pharmacological intervention, thereby potentially being founded on unique neurobiological mechanisms. Further, support for a separation of these phenotypes on the level of cognition is also provided here,

[1] levetiracetam
[2] large nest building
[3] control
[4] levetiracetam
[5] high motor stereotypy
[6] patterned cage running
[7] vertical jumping activity

since CR[1], but not VA[2] intensity, correlates negatively with nesting expression (**Table 3-5**). Interestingly, this result is likely dependent on the period of drug exposure and the age at which LEV[3]-exposure was initiated. In our earlier work, where LEV was administered to adult mice for four weeks (de Ridder et al. 2022), we showed that LEV engendered a level of control over the expression of HS[4] behavior, although at the time, no distinction was made between VA and CR. While our present findings are in line with the previous report in as far as indicating that animals exposed to LEV continues to engage in the expression of HS bouts, although spending less time doing so, we show here that early intervention with LEV which is continued through the entire rearing period, bolsters the expression of CR. Interestingly, in prior clinical work, which explored the use of LEV for pediatric migraine, the drug caused hyperactivity in some patients (Miller 2004). Hyperactivity, like compulsivity and impulsivity, is associated with perturbations in cortico-striatal processes, which in turn are also responsible for motor control (Peters et al. 2016). While the exact mechanisms underlying the present effect of LEV on CR are yet unknown, it is possible that CR, but not VA or LNB[5], shares a similar biological construct to that which may cause LEV to elicit hyperactivity.

Lastly, our results pertaining to the T-maze alternation behavior of deer mice did not highlight distinct differences between the alternation scores of animals in the normal, LNB and HS (irrespective of VA or CR) groupings (**Table 3-5**). We did however show that the relationship between alternation and the time spent engaging in stereotypical behavior, was positively affected in LEV-exposed animals. Although conclusions of causality cannot be drawn from these data, this result seems related to the effect of LEV on the expression of VA. Although narrowly missing statistical significance, VA scores of LEV-exposed mice also trended to correlate negatively with alternation scores (**Table 3-5**). Whereas these correlations were smaller and non-significant in CTRL[6]-exposed animals, LEV-exposure revealed an interesting notion, i.e. that improved cognitive performance, i.e. enhanced working memory, may be seen when stereotypical engagement is positively affected. That this result was not shown for LNB-expressing mice, not only supports the view that stereotypy and nesting expression diverge on the level of neurocognitive architecture, but also shows that improvements in the expression of LNB scores do not contribute to working memory recall as assessed in the T-maze in the manner it was applied here.

In conclusion, we show that specific persistent behavioral phenotypes, e.g. LNB, VA and CR, are divergent in terms of their neurocognitive underpinnings and that chronic LEV administered throughout

[1] patterned cage running
[2] vertical jumping activity
[3] levetiracetam
[4] high motor stereotypy
[5] large nest building
[6] control

the entire rearing period may be of benefit to some phenotypes, e.g. LNB[1], but not others (CR)[2]. We also show that an increased level of control over the expression of stereotypy may facilitate improved working memory performance. These results are informative and pave the way for continued investigation in the deer mouse model system. Specifically, studies into the neurocognitive basis of different persistent behavioral phenotypes expressed by deer mice might be useful for our understanding of how heterogenous, but equally repetitive, behaviors spontaneously develop in specific animals of a single species only.

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[1] large nest building
[2] patterned cage running

3.5 References

Allen AT, Heaton EC, Shapiro LP, Butkovich LM, Yount ST, Davies RA, Li DC, Swanson AM, Gourley SL (2022) Inter-individual variability amplified through breeding reveals control of reward-related action strategies by Melanocortin-4 Receptor in the dorsomedial striatum. Communnications Biology 5: 116.

APA (2013) Diagnostic and statistical manual of mental disorders. American Psychiatric Association, Washington, DC

Belin-Rauscent A, Daniel M, Puaud M, Jupp B, Sawiak S, Howett D, McKenzie C, Caprioli D, Besson M, Robbins T (2016) From impulses to maladaptive actions: the insula is a neurobiological gate for the development of compulsive behavior. Molecular psychiatry 21: 491-499.

Boisseau CL, Thompson-Brenner H, Caldwell-Harris C, Pratt E, Farchione T, Barlow DH (2012) Behavioral and cognitive impulsivity in obsessive–compulsive disorder and eating disorders. Psychiatry research 200: 1062-1066.

Bradbury C, Cassin SE, Rector NA (2011) Obsessive beliefs and neurocognitive flexibility in obsessive-compulsive disorder. Psychiatry Research 187: 160-5.

Braem S, Egner T (2018) Getting a grip on cognitive flexibility. Current Directions in Psychological Science 27: 470-476.

Burguière E, Monteiro P, Mallet L, Feng G, Graybiel AM (2015) Striatal circuits, habits, and implications for obsessive-compulsive disorder. Current Opinion in Neurobiology 30: 59-65.

Chou-Green JM, Holscher TD, Dallman MF, Akana SF (2003) Compulsive behavior in the 5-HT2C receptor knockout mouse. Physiology and Behavior 78: 641-649.

D'Hooge R, De Deyn PP (2001) Applications of the Morris water maze in the study of learning and memory. Brain research reviews 36: 60-90.

De Brouwer G, Engelbrecht J, Mograbi D, Legoabe LJ, Steyn S, Wolmarans DW (2021) Stereotypy and spontaneous alternation in deer mice and its response to anti-adenosinergic intervention. Journal of Neuroscience Research In Press.

de Brouwer G, Fick A, Lombaard A, Stein DJ, Harvey BH, Wolmarans DW (2020) Large nest building and high marble-burying: two compulsive-like phenotypes expressed by deer mice (Peromyscus maniculatus bairdii) and their unique response to serotoninergic and dopamine modulating intervention. Behavioural Brain Research: 112794.

de Ridder M, Mograbi D, Wolmarans DW (2022) Spontaneous alternation and stereotypical behaviour in deer mice: response to escitalopram and levetiracetam. Behavioural Pharmacology 33: 282-290.

Deacon RM, Rawlins JN (2006) T-maze alternation in the rodent. Nature Protocols 1: 7-12.

Delmonte S, Balsters JH, McGrath J, Fitzgerald J, Brennan S, Fagan AJ, Gallagher L (2012) Social and monetary reward processing in autism spectrum disorders. Molecular autism 3: 7.

Evans DW, Milanak ME, Medeiros B, Ross JL (2002) Magical beliefs and rituals in young children. Child Psychiatry & Human Development 33: 43-58.

Ferreira GM, Yucel M, Dawson A, Lorenzetti V, Fontenelle LF (2017) Investigating the role of anticipatory reward and habit strength in obsessive-compulsive disorder. CNS Spectrums 22: 295-304.

Figee M, Vink M, De Geus F, Vulink N, Veltman DJ, Westenberg H, Denys D (2011) Dysfunctional reward circuitry in obsessive-compulsive disorder. Biological Psychiatry 69: 867-874.

Gerlai R (1998) A new continuous alternation task in T-maze detects hippocampal dysfunction in mice. Behavioural Brain Research 95: 91-101.

Gillan CM, Papmeyer M, Morein-Zamir S, Sahakian BJ, Fineberg NA, Robbins TW, De Wit S (2011) Disruption in the balance between goal-directed behavior and habit learning in obsessive-compulsive disorder. American Journal of Psychiatry 168: 718-726.

Graybiel AM (2008) Habits, rituals, and the evaluative brain. Annual review of neuroscience 31: 359-87.

Gruner P, Pittenger C (2017) Cognitive inflexibility in Obsessive-Compulsive Disorder. Neuroscience 345: 243-255.

Helmstaedter C, Witt J-A (2010) Cognitive outcome of antiepileptic treatment with levetiracetam versus carbamazepine monotherapy: a non-interventional surveillance trial. Epilepsy & Behavior 18: 74-80.

Hjemdal O, Vogel PA, Solem S, Hagen K, Stiles TC (2011) The relationship between resilience and levels of anxiety, depression, and obsessive–compulsive symptoms in adolescents. Clinical Psychology & Psychotherapy 18: 314-321.

Hoffman KL, Rueda Morales RI (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. Behavioural Brain Research 204: 182-191.

Korff S, Stein DJ, Harvey BH (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.

Langen M, Durston S, Kas MJ, van Engeland H, Staal WG (2011) The neurobiology of repetitive behavior:… and men. Neuroscience & Biobehavioral Reviews 35: 356-365.

Leonard HL, Goldberger EL, Rapoport JL, Cheslow DL, Swedo SE (1990) Childhood rituals: normal development or obsessive-compulsive symptoms? Journal of the American Academy of Child & Adolescent Psychiatry 29: 17-23.

Magalhães JC, Gongora M, Vicente R, Bittencourt J, Tanaka G, Velasques B, Teixeira S, Morato G, Basile LF, Arias-Carrión O (2015) The influence of levetiracetam in cognitive performance in healthy individuals: neuropsychological, behavioral and electrophysiological approach. Clinical Psychopharmacology and Neuroscience 13: 83.

Middleton R, Wheaton MG, Kayser R, Simpson HB (2019) Treatment Resistance in Obsessive-Compulsive Disorder. In: Kim Y-K (ed) Treatment Resistance in Psychiatry: Risk Factors, Biology, and Management. Springer Singapore, Singapore, pp 165-177

Miller GS (2004) Efficacy and safety of levetiracetam in pediatric migraine. Headache: The Journal of Head and Face Pain 44: 238-243.

Moin Afshar N, Keip AJ, Taylor JR, Lee D, Groman SM (2020) Reinforcement Learning during Adolescence in Rats. Journal of Neuroscience 40: 5857-5870.

Morein-Zamir S, Papmeyer M, Pertusa A, Chamberlain SR, Fineberg NA, Sahakian BJ, Mataix-Cols D, Robbins TW (2014) The profile of executive function in OCD hoarders and hoarding disorder. Psychiatry research 215: 659-667.

Naneix F, Marchand AR, Di Scala G, Pape JR, Coutureau E (2012) Parallel maturation of goal-directed behavior and dopaminergic systems during adolescence. Journal of Neuroscience 32: 16223-32.

O'Leary TP, Brown RE (2012) The effects of apparatus design and test procedure on learning and memory performance of C57BL/6J mice on the Barnes maze. Journal of Neuroscience Methods 203: 315-24.

Operto FF, Pastorino GMG, Mazza R, Roccella M, Carotenuto M, Margari L, Verrotti A (2019) Cognitive profile in BECTS treated with levetiracetam: A 2-year follow-up. Epilepsy & Behavior 97: 187-191.

Peng Z, Xu C, Ma N, Yang Q, Ren P, Wen R, Jin L, Chen J, Wei Z, Verguts T, Chen Q (2020) White Matter Alterations of the Goal-Directed System in Obsessive-Compulsive Disorder Patients and Their Unaffected First-Degree Relatives. Biological Psychiatry: Cognitive Neuroscience and Neuroimaging.

Peters SK, Dunlop K, Downar J (2016) Cortico-Striatal-Thalamic Loop Circuits of the Salience Network: A Central Pathway in Psychiatric Disease and Treatment. Frontiers in Systems Neuroscience 10: 104.

Piazzini A, Chifari R, Canevini MP, Turner K, Fontana SP, Canger R (2006) Levetiracetam: an improvement of attention and of oral fluency in patients with partial epilepsy. Epilepsy research 68: 181-188.

Pinto A, Steinglass JE, Greene AL, Weber EU, Simpson HB (2014) Capacity to delay reward differentiates obsessive-compulsive disorder and obsessive-compulsive personality disorder. Biological Psychiatry 75: 653-659.

Presti MF, Mikes HM, Lewis MH (2003) Selective blockade of spontaneous motor stereotypy via intrastriatal pharmacological manipulation. Pharmacology Biochemistry and Behavior 74: 833-839.

Reid JE, Laws KR, Drummond L, Vismara M, Grancini B, Mpavaenda D, Fineberg NA (2021) Cognitive behavioural therapy with exposure and response prevention in the treatment of obsessive-compulsive disorder: A systematic review and meta-analysis of randomised controlled trials. Comprehensive psychiatry 106: 152223.

Robbins TW, Gillan CM, Smith DG, de Wit S, Ersche KD (2012) Neurocognitive endophenotypes of impulsivity and compulsivity: towards dimensional psychiatry. Special Issue: Cognition in Neuropsychiatric Disorders 16: 81-91.

Robbins TW, Vaghi MM, Banca P (2019) Obsessive-Compulsive Disorder: Puzzles and Prospects. Neuron 102: 27-47.

Rosenfeld CS, Ferguson SA (2014) Barnes maze testing strategies with small and large rodent models. JoVE (Journal of Visualized Experiments): e51194.

Sanchez PE, Zhu L, Verret L, Vossel KA, Orr AG, Cirrito JR, Devidze N, Ho K, Yu G-Q, Palop JJ (2012) Levetiracetam suppresses neuronal network dysfunction and reverses synaptic and cognitive deficits in an Alzheimer's disease model. Proceedings of the National Academy of Sciences 109: E2895-E2903.

Scheepers IM, Stein DJ, Harvey BH (2018) Peromyscus maniculatus bairdii as a naturalistic mammalian model of obsessive-compulsive disorder: current status and future challenges. Metabolic brain disease 33: 443-455.

Shoji H, Hagihara H, Takao K, Hattori S, Miyakawa T (2012) T-maze forced alternation and left-right discrimination tasks for assessing working and reference memory in mice. Journal of visualized experiments : JoVE: 3300.

Snorrason I, Lee HJ, de Wit S, Woods DW (2016) Are nonclinical obsessive-compulsive symptoms associated with bias toward habits? Psychiatry Research 241: 221-3.

Spowart-Manning L, van der Staay FJ (2004) The T-maze continuous alternation task for assessing the effects of putative cognition enhancers in the mouse. Behavioral Brain Research 151: 37-46.

Thorsen AL, de Wit SJ, de Vries FE, Cath DC, Veltman DJ, van der Werf YD, Mataix-Cols D, Hansen B, Kvale G, van den Heuvel OA (2019) Emotion regulation in obsessive-compulsive disorder, unaffected siblings, and unrelated healthy control participants. Biological Psychiatry: Cognitive Neuroscience and Neuroimaging 4: 352-360.

van der Straten A, van Leeuwen W, Denys D, van Marle H, van Wingen G (2020) The effect of distress on the balance between goal-directed and habit networks in obsessive-compulsive disorder. Translational psychiatry 10: 1-10.

Walitza S, Melfsen S, Jans T, Zellmann H, Wewetzer C, Warnke A (2011) Obsessive-compulsive disorder in children and adolescents. Deutsches Ärzteblatt Int 108: 173-9.

Wolmarans DW, Brand L, Stein DJ, Harvey BH (2013) Reappraisal of spontaneous stereotypy in the deer mouse as an animal model of obsessive-compulsive disorder (OCD): response to escitalopram treatment and basal serotonin transporter (SERT) density. Behavioural Brain Research 256: 545-53.

Wolmarans DW, Prinsloo M, Seedat S, Stein DJ, Harvey BH, de Brouwer G (2022) Escitalopram and lorazepam differentially affect nesting and open field behaviour in deer mice exposed to an anxiogenic environment. Neuroscience Research 177: 85-93.

Wolmarans DW, Stein DJ, Harvey BH (2016) Excessive nest building is a unique behavioural phenotype in the deer mouse model of obsessive–compulsive disorder. Journal of Psychopharmacology 30: 867-874.

Wood J, Ahmari SE (2015) A framework for understanding the emerging role of corticolimbic-ventral striatal networks in OCD-associated repetitive behaviors. Frontiers in systems neuroscience 9: 171.

Figure captions

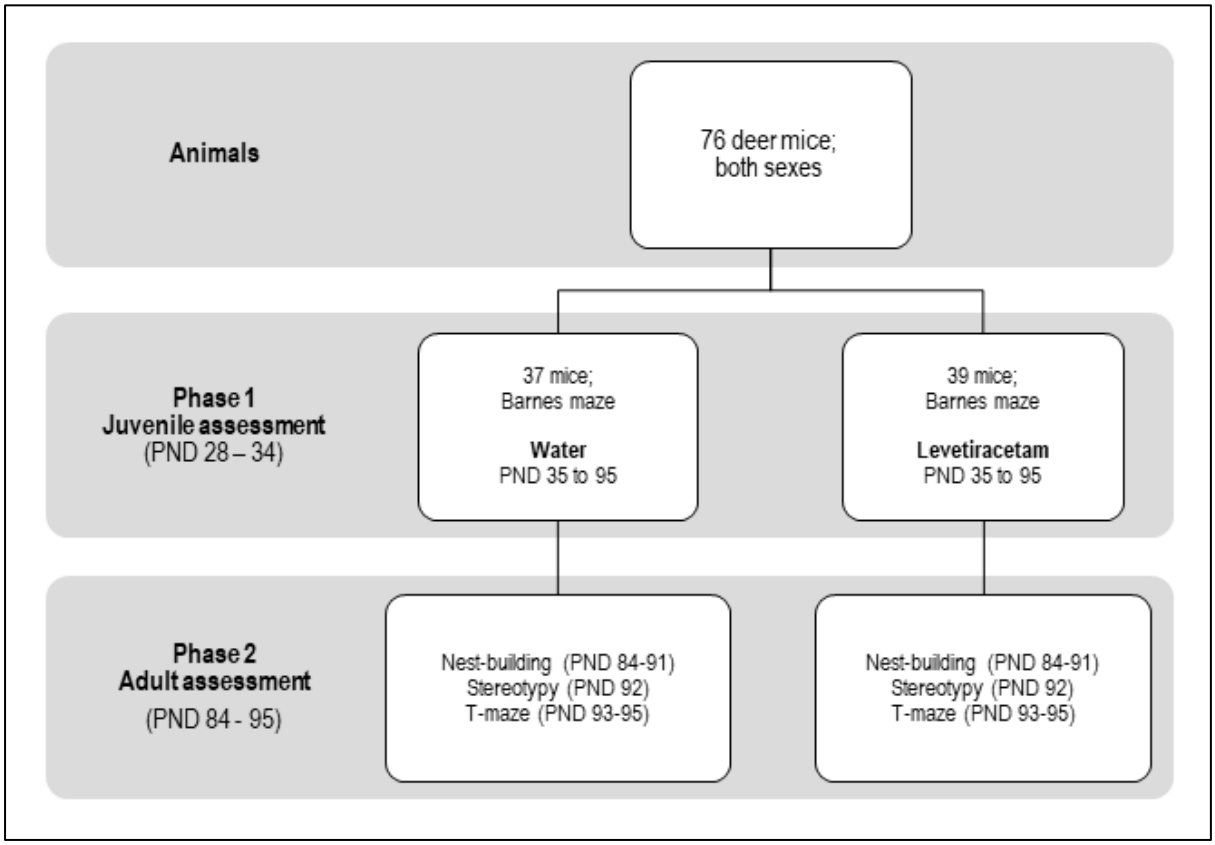

FIGURE 3-1 – STUDY LAYOUT
PND: POSTNATAL DAY

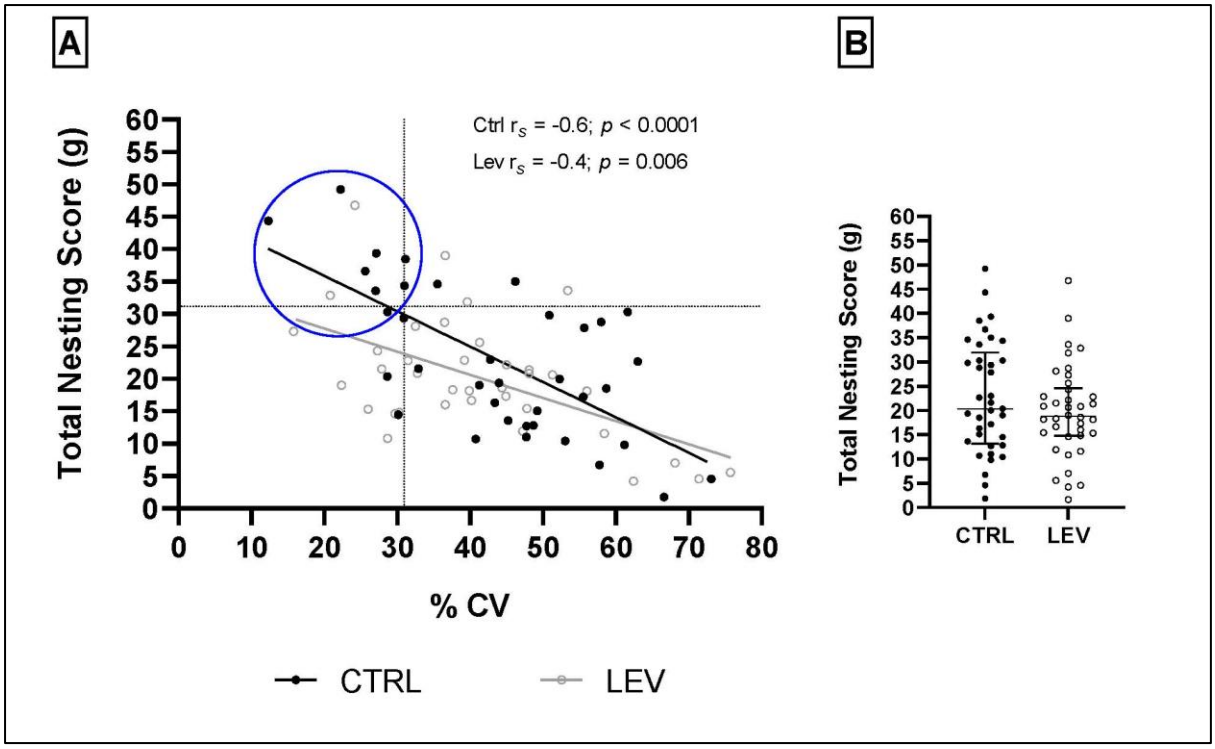

FIGURE 3-2: – (A) GRAPH OF LNB COHORT SELECTION (IN THE BLUE OVAL), FOR THE CONTROL (CTRL)- AND LEVETIRACETAM (LEV)-EXPOSED MICE, RESPECTIVELY BASED ON THE TOTAL NESTING SCORES IN GRAM, COMPARED TO THE % COEFFICIENT OF VARIANCE (CV) PERTAINING TO THE DAILY NESTING SCORES; **(B)** TOTAL NESTING SCORES IN GRAM, FOR CTRL AND LEV-EXPOSED GROUPS.
SPEARMAN'S CORRELATION; DOTTED LINES INDICATE THE 75TH AND 25TH QUARTILES OF DISTRIBUTION FOR THE TOTAL NESTING SCORES AND % CV, RESPECTIVELY CTRL: n = 37, LEV: n = 39

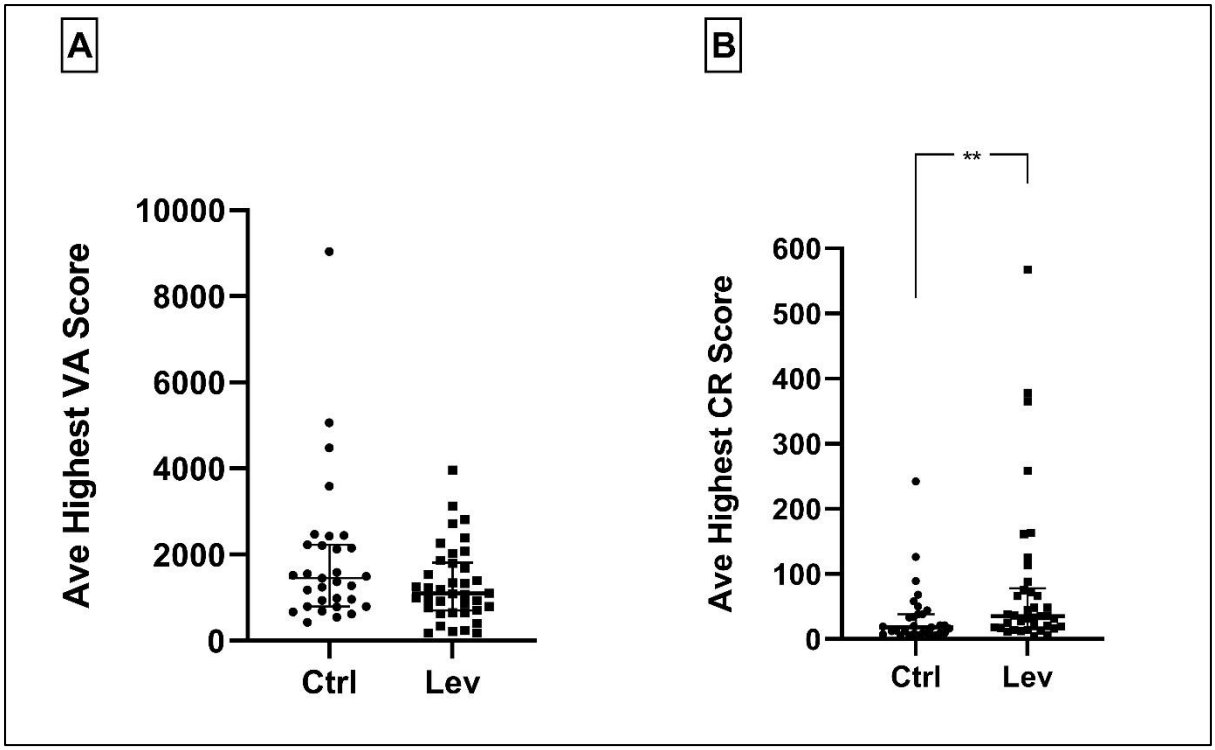

FIGURE 3-3: (A) – AVERAGE HIGHEST VERTICAL JUMPING ACTIVITY SCORES FOR THE CONTROL (CTRL)- AND LEVETIRACETAM (LEV)-EXPOSED GROUPS. **(B)** - AVERAGE HIGHEST NUMBER OF, PATTERNED CAGE RUNNING EXPRESSED AS (CR) FOR THE CTRL AND LEV GROUPS.
MANN-WHITNEY *U*-TEST; ***P* <0.01; CTRL: *n* = 27, LEV: *n* = 25

* * *

END OF CHAPTER THREE

4 Conclusion

Cognitive theories of obsessive-compulsive disorder (OCD)[1] implicate, among others, habit-proneness and deficits in working memory as underlying endophenotypes in some symptom subtypes of the disorder (Gillan et al. 2011). Also, drugs that may act as so-called cognitive enhancers, e.g. levetiracetam (LEV)[2], may be promising treatment alternatives (Magalhães et al. 2015; Operto et al. 2019). The work presented in this research dissertation broadly attempted to expand our understanding of the potential neurocognitive basis of two persistent and compulsive-like behavioral phenotypes in deer mice *(Peromyscus maniculatus bairdii)*, i.e. large nest building (LNB)[3] and high motor stereotypy (HS)[4], with the latter separated on the level of vertical and horizontal activity. Specifically, we aimed to determine whether any longitudinal relationships would exist between habit-proneness in juvenile mice and the expression of LNB and HS in adulthood. Lastly, we explored the notion that LNB and HS might be differentially associated with deficits in working memory and habit-proneness as assessed in adulthood, and whether said behaviors and its potential associations with such cognitive perturbations would respond to chronic LEV exposure, administered throughout the rearing period.

To investigate this research theme, 76 juvenile (28-day-old) deer mice of both sexes were assessed for habit-proneness in the Barnes maze (BM)[5] (Gawel et al. 2019). Mice were then divided into two exposure groups (n = 37-39 per group), i.e. control- (CTRL[6]; normal drinking water) and LEV (75 mg/kg/day; administered in the drinking water; Sanchez et al. (2012)). After 56 days of uninterrupted CTRL or LEV exposure, all mice were screened for nesting and stereotypical behavior, and then assessed for habit-proneness and working memory capacity in the T-maze.

* * *

[1] obsessive-compulsive disorder
[2] levetiracetam
[3] large nest building
[4] high motor stereotypy
[5] Barnes maze
[6] control

The main findings of the present work were 1) performance in the BM[1] of juvenile *P. maniculatus bairdii* mice is not related to LNB[2] or stereotypy in adulthood; 2) chronic LEV[3] exposure from a juvenile age until adulthood decreased LNB expression without affecting overall nesting scores, but increased CR[4] activity; 3) stereotypical expression, notably so CR, and nesting expression are divergent persistent behavioral phenotypes; and 4) LEV exposure generally improves the relationship between working memory and stereotypical engagement.

* * *

One aim of this work was to explore the potential longitudinal relationship between habit-like behavior early in life and compulsive-like behavior in adulthood. To this end, we found probe test behavior to be overwhelmingly uncoupled from initial escape acquisition, suggesting that it was driven by factors other than escape learning such as behavioral automaticity. This result is in line with previous research in operant conditioning procedures, that showed that young rats favor habit-based response strategies even under conditions that induce goal-seeking behavior in adults (Naneix et al. 2012). Thus, the response strategies observed here may reflect the strong tendency of young animals to utilize habit-based behaviors. Juvenile *P. maniculatus bairdii* may be a valuable tool to understand the neurobiology of habit formation and adherence in young developing mammals. This topic is grossly under-studied and needs further scrutiny.

* * *

The second result of this work, pertaining to the effects of LEV on persistent behavioral expression was informing. Specifically, LEV resulted in fewer animals presenting with LNB behavior, as evaluated against the yield of LNB-expressing adult animals in the CTRL[5]-exposed group. Broadly, we regard nesting expression as a highly goal-directed behavior (Chou-Green et al. 2003; Hoffman and Rueda Morales 2009; Wolmarans et al. 2016) and are therefore of the view that LNB is as an inflated goal-directed response that might be founded on any number of neurocognitive constructs, i.e. deficits in outcome feedback processing (Wolmarans et al. 2022). Thus, our result pertaining to the effect of LEV on the expression of LNB points to some degree of non-serotonergic—likely cognitive and developmental—involvement underlying its expression and paves the way for continued research into the potential neurobiological mechanisms that may underlie the naturalistic development of persistent behaviors in deer mice.

[1] Barnes maze
[2] large nest building
[3] levetiracetam
[4] patterned cage running
[5] control

* * *

In contrast to its effect on the expression of LNB[1], LEV[2] bolstered the expression of CR[3], without affecting the VA[4] scores of mice. This finding is vitally important because it illustrates for the first time in this model system that VA and CR, as shown by distinct subpopulations of deer mice, diverge in terms of its sensitivity for and response to pharmacological intervention. VA and CR can thus likely be founded on unique neurobiological mechanisms. Moreover, we showed here that early intervention with LEV, which is continued through the entire rearing period, bolstered the expression of CR. We proposed in this work that CR, but not VA and LNB might be related to the same processes that govern hyperactivity and impulsivity, since clinical data indicated LEV to cause such activating responses in a subpopulation of children who is treated with LEV for pediatric migraine (Miller 2004). Hyperactivity, like compulsivity and impulsivity, is also associated with perturbations in cortico-striatal processes, which in turn are responsible for motor control (Peters et al. 2016). Nevertheless, further work is needed to expand the meaning of this result.

* * *

Lastly, while our results pertaining to the T-maze alternation behavior of deer mice did not highlight differences between the alternation scores (and thus also working memory performance) of animals in the normal, LNB, and HS[5] groupings, we showed that the relationship between alternation and the time spent engaging in stereotypical behavior benefited from LEV exposure. Although narrowly missing statistical significance, the VA scores of LEV-exposed mice also trended to correlate negatively with alternation scores. Whereas these correlations were smaller and non-significant in CTRL[6]-exposed animals, LEV-exposure highlighted an interesting possibility, i.e. that improved cognitive performance may be seen when stereotypical engagement is positively affected. That this result was not shown for LNB-expressing mice, supports the view that stereotypy and nesting expression diverge on the level of neurocognitive architecture.

* * *

In conclusion, this body of work is valuable. We show for the first time that LNB, VA and CR are fundamentally different, though equally persistent and repetitive, behavioral phenotypes that diverge in terms of their neurocognitive basis. We also show that potential cognitive enhancers, e.g. LEV, are

[1] large nest building
[2] levetiracetam
[3] patterned cage running
[4] vertical jumping activity
[5] high motor stereotypy
[6] control

promising alternatives for specific cases of compulsive-like persistence, and that such drugs may be useful to highlight the potential associations between specific compulsive-like phenotypes and other neurocognitive processes. Thus, our findings provide proof-of-concept for continued study into the potential etiological and neurocognitive mechanisms that might underlie the naturalistic expression of phenotypically heterogeneous compulsive-like behaviors.

* * *

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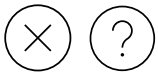
4.1 Final summary of study aims and outcomes

In **Table 4-1**, we summarize the study questions that were posed for this investigation as well as the respective answers to these that were realized during the course of this work.

TABLE 4-1 - SUMMARY OF HYPOTHESIZED OUTCOMES AND FINAL OUTCOMES

Study Question	Outcome
Will habit-proneness as shown in juvenile mice in a BM[1] assessment, be predictive of adult compulsive-like, persistent LNB[2] and HS[3]?	 No. Habit-prone behavioral engagement as assessed in the BM did not predict or correlate adult compulsive-like behavioral persistence. Most juvenile mice overwhelmingly relied on habitual responding, which did not predict adult behavioral output.
Will the adulthood expression of LNB and HS associate with habitual response styles as assessed in a T-maze?	 No. Neither LNB nor HS were found to be related to broad impairments in working memory and increased habit-proneness as assessed in the T-maze. However, since we did not employ a baited T-maze, this result might have been different and similar research should be conducted in future to gain a clear answer.
Will LNB and HS, as well as its relationships with altered T-maze ambulation, respond to chronic LEV[4] exposure, administered throughout the entire rearing period?	 Yes. LEV, administered throughout the entire rearing period, resulted in fewer animals presenting with LNB behavior compared to CTRL[5]-exposed mice. Further, our results showed that the relationship between alternation and the time spent engaging in stereotypical behavior was positively affected in LEV-exposed animals.
Will exposure to LEV also modulate any potential longitudinal relationships between juvenile habit-proneness and adulthood compulsive-like expression?	 This question could not be answered, since most juvenile mice showed habitual responding. It thus remains difficult to answer this question, and future study is needed.

[1] Barnes maze
[2] large nest building
[3] high motor stereotypy
[4] levetiracetam
[5] control

4.2 Study shortcomings and future directions

This work was not without some noteworthy shortcomings that should be afforded attention to inform future investigations in the model system.

The assessment of habit-proneness in juvenile deer mice proved to be more difficult than expected. Since we failed to separate the (relatively large) group of deer mice employed in this investigation into habit-prone and more goal-orientated groupings, it was difficult to draw conclusions regarding the relationship between habit-prone tendencies and compulsive-like behavioral expression. To this end, alternative methods for the study of habit should be explored in future. For example, it is possible that juvenile and adult deer mice, might separate more clearly in experimental paradigms that employ rewarded (or baited) training protocols that are employed over a longer duration of experimentation (Hussein et al. 2018; Meunier et al. 1991).

Another potential shortcoming of the present work was that we did not employ an adult-only LEV[1] administration protocol. As such, we could not draw conclusions regarding the temporality of LEV's effect. In other words, it could not be established whether the observed effects of LEV in this work, were dependent on the administration thereof during the developmental period, or whether similar changes would have been elicited if it was administered during adulthood only. An answer to this question is paramount, since the purpose of this work was also to discern if early-life intervention with a safe pro-cognitive alternative, could prospectively modulate the expression of compulsive-like behaviors. This question remains to be answered in future study.

Lastly, all studies into naturalistic behavioral expressions are unfortunately typified by a very specific shortcoming, which is that the potential prospective yield of abnormal behaviors within a specific population cannot be accurately determined, as is possible in pharmacological and genetic models. Thus, determinations of sample size are highly dependent on prior research, while the actual yield of LNB[2]- and HS[3]-expressing animals (in this case) might be fewer or more than originally envisaged. Since animals are also bred and reared in successive batches, it remains difficult to exclude batch effect from the data obtained. Hence, large numbers of animals are used in these studies. Looking at the eventual yield of LNB- and CR[4]-expressing animals and the relatively small number of animals that expressed these behaviors in the CTRL[5]- and LEV-exposed groups respectively, larger group numbers

[1] levetiracetam
[2] large nest building
[3] high motor stereotypy
[4] patterned cage running
[5] control

than those employed here, would arguably have provided an even more robust view on the current research scenario.

4.3 References

Chou-Green JM, Holscher TD, Dallman MF, Akana SF (2003) Compulsive behavior in the 5-HT2C receptor knockout mouse. Physiology and Behavior 78: 641-649.

Gawel K, Gibula E, Marszalek-Grabska M, Filarowska J, Kotlinska JH (2019) Assessment of spatial learning and memory in the Barnes maze task in rodents-methodological consideration. Naunyn Schmiedebergs Archives Pharmacology 392: 1-18.

Gillan CM, Papmeyer M, Morein-Zamir S, Sahakian BJ, Fineberg NA, Robbins TW, De Wit S (2011) Disruption in the balance between goal-directed behavior and habit learning in obsessive-compulsive disorder. American Journal of Psychiatry 168: 718-726.

Hoffman KL, Rueda Morales RI (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. Behavioural Brain Research 204: 182-191.

Hussein AM, Bezu M, Korz V (2018) Evaluating working memory on a T-maze in male rats. Bio-protocol 8: e2930-e2930.

Magalhães JC, Gongora M, Vicente R, Bittencourt J, Tanaka G, Velasques B, Teixeira S, Morato G, Basile LF, Arias-Carrión O (2015) The influence of levetiracetam in cognitive performance in healthy individuals: neuropsychological, behavioral and electrophysiological approach. Clinical Psychopharmacology and Neuroscience 13: 83.

McHail DG, Valibeigi N, Dumas TC (2018) A Barnes maze for juvenile rats delineates the emergence of spatial navigation ability. Learning & Memory 25: 138-146.

Meunier M, Jaffard R, Destrade C (1991) Differential involvement of anterior and posterior cingulate cortices in spatial discriminative learning in a T-maze in mice. Behavioural Brain Research 44: 133-143.

Miller GS (2004) Efficacy and safety of levetiracetam in pediatric migraine. Headache: The Journal of Head and Face Pain 44: 238-243.

Naneix F, Marchand AR, Di Scala G, Pape JR, Coutureau E (2012) Parallel maturation of goal-directed behavior and dopaminergic systems during adolescence. J Neurosci 32: 16223-32.

Operto FF, Pastorino GMG, Mazza R, Roccella M, Carotenuto M, Margari L, Verrotti A (2019) Cognitive profile in BECTS treated with levetiracetam: A 2-year follow-up. Epilepsy & Behavior 97: 187-191.

Peters SK, Dunlop K, Downar J (2016) Cortico-Striatal-Thalamic Loop Circuits of the Salience Network: A Central Pathway in Psychiatric Disease and Treatment. Frontiers in Systems Neuroscience 10: 104.

Sanchez PE, Zhu L, Verret L, Vossel KA, Orr AG, Cirrito JR, Devidze N, Ho K, Yu G-Q, Palop JJ (2012) Levetiracetam suppresses neuronal network dysfunction and reverses synaptic and cognitive deficits in an Alzheimer's disease model. Proceedings of the National Academy of Sciences of the United States of America109: E2895-E2903.

Wolmarans DW, Prinsloo M, Seedat S, Stein DJ, Harvey BH, de Brouwer G (2022) Escitalopram and lorazepam differentially affect nesting and open field behaviour in deer mice exposed to an anxiogenic environment. Neuroscience Research 177: 85-93.

Wolmarans DW, Stein DJ, Harvey BH (2016) Excessive nest building is a unique behavioural phenotype in the deer mouse model of obsessive–compulsive disorder. Journal of Psychopharmacology 30: 867-874.

* * *

END OF CHAPTER FOUR

Addendum A

In this addendum, additional detail pertaining to the experimental methodology that was followed in this work and examples of results are provided. This detail was considered excessive for the purposes of the journal article in **Chapter 3** and hence, is provided here.

Daily routine during the behavioral investigation

- Throughout the course of this study, the welfare of animals was monitored between 08:00 and 09:00 each morning, and the bottles containing water or drug solution weighed to track fluid consumption. Fresh drug solutions—for the levetiracetam (LEV)[1] group—were provided every day. Apart from the normal weekly cage maintenance routines (**see below**), the mice were left undisturbed for the rest of the day, except during periods of experimentation after 18:00.
- In general, the following parameters were checked every day: 1) humidity, 2) temperatures of the housing and experimental rooms, and 3) food and water (or drug) availability.
- The cages were cleaned once a week, with fresh corncob and clean paper towel provided. Cages were cleaned with F10® Veterinary Disinfectant Solution (Reg No.: G3073).

Animals

- Prior to the onset of study, all mice used in this investigation were bred in the North-West University (NWU)[2] vivarium, Potchefstroom, South Africa (SAVC Reg no.: FR15/13458; SANAS GLP4 compliance no.: G0019; AAALAC5 accreditation file: 1717), by the researcher herself.
- A total of 76 juvenile deer mice of both sexes *(Peromyscus Maniculatus bairdii)*, were acquired from the DSI/NWU Vivarium.
- Experimental subjects were chosen through randomization from a variety of litters without sex and weight bias. Two same-sex animals were housed per cage throughout the investigation, unless stated otherwise, e.g. when nest building is assessed.
- At postnatal day (PND)[3] 21, mice were weaned, and group-housed for one week in cages of six same-sex animals per cage. The ages of mice within a specific rearing cage did not differ with more than six days. Mice were housed as such until PND 28.
- Cages (35 cm (l) x 20 cm (w) x 13 cm (h); Techniplast S.P.A® .,Varese, Italy) were automatically climate-controlled and kept ambient at 23 ˚C on a 12-h light/dark cycle (06h00/18h00).

[1] levetiracetam
[2] North-West University
[3] postnatal day

Throughout the duration of the study, food and water were provided *ad lib*, while cages were cleaned and new bedding supplied, weekly.

- During the study, mice were supplied with piece of paper towel as a form of nesting material and a small polyvinyl chloride (PVC)[1] pipe (as a form of environmental enrichment), except during periods of nest building assessment. During this time, mice only had access to the unscented cotton wool used in the experiment (see **Chapter 3**).
- The behavioral component of this study was performed in successive batches of mice, since the deer mice included in this investigation were bred gradually. All mice had to reach the age of postnatal day PND[2] 28 prior to study onset.
- At PND 28, mice were randomly selected from different group-housed housed cages (also taking litter into account) ear-tagged for identification purposes and reallocated to new home cages in groups of two same sex mice per cage.

Additional information pertaining to the Barnes maze assessment

- In this work, we used the small walled Barnes maze (BM)[3] (**Fig. A-1 and Fig. A-4G**).
- Briefly, BM training and assessment consisted of a habituation phase, an acquisition phase, and a probe trial phase.
- On any of the days on which any BM procedure took place, mice were transferred to the testing room at 18:00 and left to habituate until 19:00.
- All experimental procedures started only at 19:00, so as to ensure that animals were fully awake by the time when experimentation began.
- The procedure was followed as explained in **Chapter 3.**
- Between each BM procedure (or trial), mice were gently relocated to their respective home cages and moved back to the housing room. Here they resided for a 25- min inter-trial interval until the second assessment of the same animal was conducted.

[11] polyvinyl chloride
[2] postnatal day
[3] Barnes maze

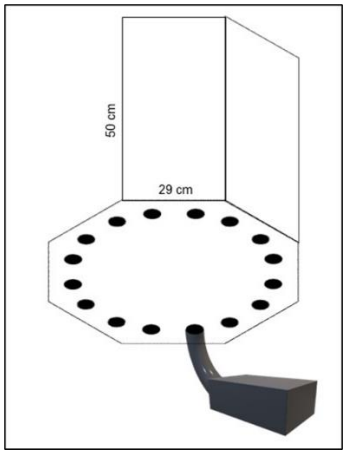


FIGURE A-1 - SCHEMATIC REPRESENTATION OF THE BARNES MAZE
A BLACK FLOORLESS STARTING BOX WILL BE PLACED AT THE CENTER OF THE MAZE AND LIFTED AT THE ONSET OF EXPERIMENTATION

Additional information pertaining to drug administration

- Levetiracetam was orally administered in the drinking water at a dosage of 75 mg/kg/day. The average water consumption of a single deer mouse is 0.25 mL/g/day, as previously determined (de Brouwer et al., 2020). Thus, LEV[1] solutions were prepared at a concentration of 30 mg/100 mL. Preparation of solutions involved the daily provision of 40 mL of the relevant drug solutions to the subjects in their respective group housing cages (or 20 mL to each single housed mouse in the nest-building assessment period). This concentration ensured that each mouse received the needed dosage within a narrow margin (**Fig. A-2**).
- Considering the long duration of drug exposure employed here, alternative routes of administration, such as oral gavage or parenteral routes were not considered, as they may pose serious health risks to the mice.
- To prepare drug solutions, the correct amount of LEV powder was weighed on a microbalance, dissolved in equal amounts of tap water and Milli-Q® ultrapure water and sonicated for two minutes to ensure a homogenous solution with complete dissolution of the drug.
- Fresh drug mixtures were prepared every day. Any remaining water or drug mixtures left from the previous day were discarded in appropriately marked disposal containers for later disposal.
- The fluid intake of mice, receiving treatment (LEV solution), was tracked every day by weighing to confirm fluid intake (**Fig. A-2**). The fluid intake of deer mice was not influenced by the addition of the LEV, in correspondence with previous data.

[1] levetiracetam

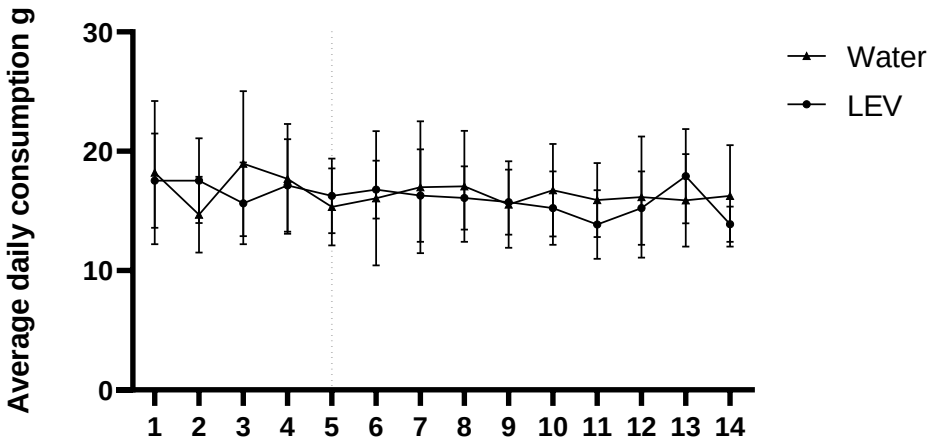


FIGURE A-2 - THE AVERAGE DAILY FLUID INTAKE. DATA IS REPRESENTATIVE OF THE AVERAGE DAILY FLUID INTAKE OF SIX CAGES (TWO MICE PER CAGE, AGED 12 WEEKS) THAT RECEIVED THE LEVETIRACETAM (LEV) SOLUTION, COMPARED TO SIX CAGES OF MICE (TWO MICE PER CAGE, AGED 12 WEEKS) IN OUR LABORATORY THAT RECEIVED NORMAL DRINKING WATER

Additional information pertaining to nest building assessment

- Each day, between 15:00 and 16:00, an excess of pre-weighed white, and unscented cosmetic cotton wool was introduced into the roof of each home cage (**See Fig A-4D**)
- On each following day between 15:00 and 16:00, the unused cotton wool in the roof was weighed to calculate the quantity of cotton wool that was used for nest building during the preceding 24-h cycle.
- If needed, the cotton wool in the roof of the cage was then replenished, and the built nests discarded.
- This process was repeated every day for 7 full days so that 7 x 24-h cycles worth of data were generated (**See Fig A-6**)

Additional information pertaining to stereotypy screening

- When stereotypy assessment took place, the mice were transferred from the normal housing room to the experimental rooms at 17:00.
- Since mice are nocturnal animals and the light cycle in the Vivarium is set at 06:00/18:00 (on/off), all mice were habituated in the experimental Accuscan® cages for at least 60 min prior to the onset of testing, which started at 18:00 (**Fig. A-4E**).
- The mice were then left in the Accuscan® for a full 12-h dark cycle and returned to their home cages between 07:00 and 08:00 the following morning.
- Animals had access to excess food and water (or LEV[1], in the case of the treatment group) throughout the night.

Additional information pertaining to T-maze assessment

- On days of T-maze testing, mice were transferred to the testing room at 18:00 and left to habituate until 19:00.
- Experimentation began at 19:00. Mice were individually placed in the start box of the T-maze and left for 30 sec. Then, the start box door was raised for the first time (**Chapter 3**).
- All assessments were conducted under dim red light (40 lux).
- Between each T-maze assessment, i.e., between assessments of different mice, animals were gently relocated to their respective home cages and moved back to the housing room.

[1] levetiracetam

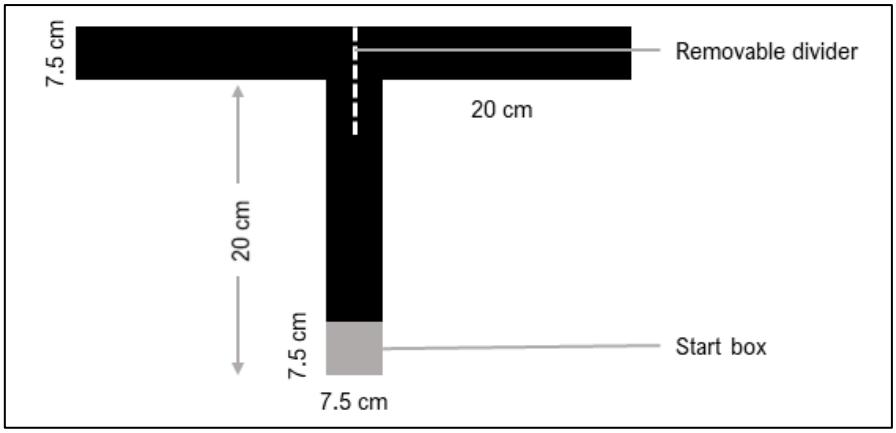


FIGURE A-3 - SCHEMATIC LAYOUT OF T-MAZE APPARATUS

Supplementary images pertaining to animal housing, husbandry, and care

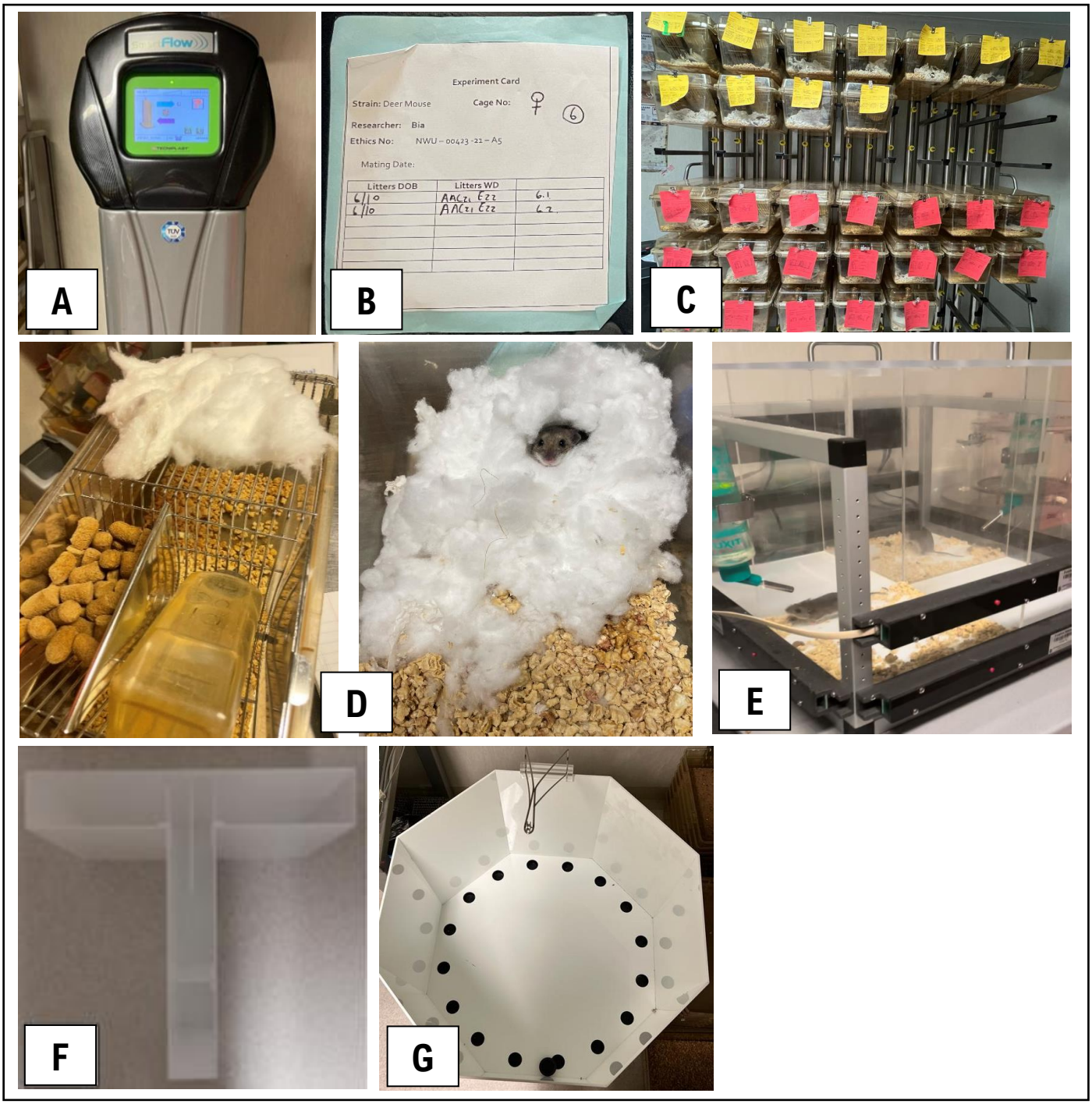


FIGURE A-4 - (A-G): (A) TECHNIPLAST® SMART FLOW FOR THE MONITORING OF VENTILATION, HUMIDITY, AND TEMPERATURE IN THE CAGES; **(B)** EXPERIMENTAL CAGES HOUSING CARDS; **(C)** DEER MICE HOUSING SYSTEM (CAGES: 35 CM (L) X 20 CM (W) X 13 (H) CM; TECHNIPLAST® S.P.A., VARESE, ITALY), YELLOW: BREEDING CAGES, RED: WEANED CAGES; **(D)** NEST BUILDING ASSESSMENT; **(E)** ACCUSCAN®, ILLUSTRATING LASERS THAT CAPTURE VERTICAL AND HORIZONTAL STEREOTYPICAL MOTOR PATTERNS; **(F)** T-MAZE; **(G)** BARNES MAZE.

Examples of raw data exports from Ethovision® for BM probe trial assessments

			Independent Variable	In zone	In zone
			Independent Variable	Goal Quadrant / center-point	Goal Quadrant / center-point
			Animal ID	Frequency	Latency to First
					s
Result 1	Trial	1	11	2	12,32
Result 1	Trial	2	12	1	2,48
Result 1	Trial	3	13	1	35,44
Result 1	Trial	4	14	0	-
Result 1	Trial	5	41	1	4,32
Result 1	Trial	6	42	2	13,04
Result 1	Trial	7	43	2	23,84
Result 1	Trial	8	44	8	44,56
Result 1	Trial	9	21	8	0
Result 1	Trial	10	22	13	0
Result 1	Trial	11	23	8	0
Result 1	Trial	12	24	2	23,44
Result 1	Trial	13	31	2	59,76
Result 1	Trial	14	32	1	92,16
Result 1	Trial	15	33	4	46,32
Result 1	Trial	16	34	4	7,2
Result 1	Trial	17	51	27	5,92
Result 1	Trial	18	52	2	10,8
Result 1	Trial	19	53	2	9,84
Result 1	Trial	20	54	0	-

FIGURE A-5 - EXAMPLE OF RAW DATA OUTPUT AS GENERATED BY ETHOVISION® BEHAVIORAL TRACKER FOR BARNES MAZE PROBE TRIALS. DATA ARE REPRESENTATIVE (FROM THE TOP) OF ALL 4 PROBE TRAIL SESSIONS (1 - 4) OF 5 MICE, EXPRESSED AS THE FREQUENCY SPENT IN THE GOAL QUADRANT AS WELL AS THE LATENCY TO THE FIRST TIME OBSERVED IN THE GOAL QUADRANT

Examples of raw data from nest-building screening

NB data	Day 1			Day 2			Day 3			Day 4			Day 5			Day 6			Day 7			Day 8						
	IN	OUT	DIFF	IN	OUT	DIFF	IN	OUT	DIFF	IN	OUT	DIFF	IN	OUT	DIFF	IN	OUT	DIFF	IN	OUT	DIFF	IN	OUT	DIFF	TOTAL	AVE	STD DEV	% CV
1	9,69	4,12	5,57	8,16	4,02	4,14	8,26	5,32	2,94	8,34	2,74	5,6	8,14	2,67	5,47	8,22	6,32	1,9	8,53	4,36	4,17	8,35	3,75	4,6	34,4	4,3	1,3	31,0
2	8,98	3,93	5,05	8,43	2,82	5,61	8,57	4,71	3,86	8,21	1,48	6,73	8,95	5,93	3,02	8,7	6,58	2,12	8,08	6,62	1,46	8,67	7,77	0,9	28,8	3,6	2,1	58,0
3	9,12	1,83	7,29	8,65	4,05	4,6	8,43	5,53	2,9	8,16	5,03	3,13	8,75	4,4	4,35	8,49	7,36	1,13	8,05	6,42	1,63	8,03	5,19	2,84	27,9	3,5	1,9	55,7
4	9,39	3,6	5,79	8,7	4,88	3,82	8,14	4,94	3,2	8,23	5,93	2,3	8,89	6,27	2,62	8,23	5,23	3	8,41	4,06	4,35	8,07	3,8	4,27	29,4	3,7	1,1	30,9
5	9,55	4,82	4,73	8,46	7,73	0,73	8,55	6,63	1,92	8,37	6,56	1,81	8,82	7,11	1,71	8,48	7,09	1,39	8,2	5,44	2,76	8,45	6,29	2,16	17,2	2,2	1,2	55,5
6	9,46	4,14	5,32	8,93	5,82	3,11	8,78	6,3	2,48	8,11	3,37	4,74	8,74	0	8,74	9,17	6,31	2,86	8,01	3,62	4,39	8,1	4,74	3,36	35,0	4,4	2,0	46,2
7	9,49	9,4	0,09	9,45	9,4	0,05	9,4	9,16	0,24	9,25	9,15	0,1	9,15	9,01	0,14	9,01	8,59	0,42	8,59	8,23	0,36	8,23	7,84	0,39	1,8	0,2	0,1	66,6
10	9,11	5,91	3,2	8,58	7,18	1,4	8,16	7,12	1,04	8,08	6,92	1,16	8,58	6,72	1,86	8,27	7,02	1,25	8,25	6,1	2,15	8,38	7,6	0,78	12,8	1,6	0,8	48,7
11	9,8	7,97	1,83	9,1	8,27	0,83	8,27	6,98	1,29	8,22	6,45	1,77	8,37	6,33	2,04	8,1	7,19	0,91	8,67	8,16	0,51	8,16	6,61	1,55	10,7	1,3	0,5	40,8
12	9,42	6,32	3,1	8,76	7,73	1,03	8,41	5,93	2,48	8,18	7,26	0,92	8,46	5,32	3,14	8,13	6,3	1,83	8,01	6,65	1,36	8,32	5,87	2,45	16,3	2,0	0,9	43,4
13	9,69	8,76	0,93	8,76	7,41	1,35	9,24	8,09	1,15	8,09	6,7	1,39	8,04	6,93	1,11	8,56	7,69	0,87	8,96	7,67	1,29	8,28	5,34	2,94	11,0	1,4	0,7	47,7
14	9,39	5,66	3,73	8,33	5,14	3,19	8,39	7,16	1,23	8,2	4,59	3,61	8,7	7,08	1,62	8,43	5,84	2,59	8,12	5,44	2,68	8,2	5,3	2,9	21,6	2,7	0,9	32,9
15	9,57	7,14	2,43	8,15	7,3	0,85	8,31	7,6	0,71	8,18	7,8	0,38	8,67	6,54	2,13	9,09	7,86	1,23	8,47	7,92	0,55	8,45	6,91	1,54	9,8	1,2	0,8	61,2
16	8,14	6,38	1,76	8,88	8,21	0,67	8,2	7,96	0,24	8,78	8,32	0,46	8,32	7,35	0,97	8,72	7,83	0,89	8,53	7,33	1,2	8,14	7,63	0,51	6,7	0,8	0,5	57,8
17	8,03	5,25	2,78	8,7	7,09	1,61	8,11	6,17	1,94	8,47	4,73	3,74	8,25	6,71	1,54	8,5	6,23	2,27	8,6	4,77	3,83	8,48	7,16	1,32	19,0	2,4	1,0	41,3
18	8,27	3,46	4,81	8,14	7,54	0,6	8,07	2,15	5,92	8,17	6,52	1,65	8,52	5,83	2,69	8,33	4,44	3,89	8,19	3,25	4,94	8,1	2,79	5,31	29,8	3,7	1,9	50,9
19	8,13	0,23	7,9	8,64	4,78	3,86	8,39	3,94	4,45	8,65	5,46	3,19	8,42	4,17	4,25	8,38	2,66	5,72	8,03	4,26	3,77	8,27	2,93	5,34	38,5	4,8	1,5	31,1
20	8,22	5,84	2,38	8,17	5,51	2,66	8,38	6,82	1,56	8,46	6,03	2,43	8,17	4,09	4,08	8,24	5,95	2,29	8,43	6,32	2,11	8,22	5,38	2,84	20,4	2,5	0,7	28,7
21	8,26	4,4	3,86	8,38	4,08	4,3	8,37	4,81	3,56	8,29	5,86	2,43	8,38	3,43	4,95	8,64	6,01	2,63	8,82	5,72	3,1	8,63	3,12	5,51	30,3	3,8	1,1	28,7
22	8,92	6,6	2,32	8,31	6,45	1,86	8,56	6,25	2,31	8,22	6,76	1,46	8,27	7,1	1,17	8,4	7,28	1,12	8,08	6,39	1,69	8,55	6	2,55	14,5	1,8	0,5	30,1
23	8,41	4,43	3,98	8,17	4,87	3,3	8,86	5,48	3,38	8,21	1,88	6,33	8,16	3,61	4,55	8,39	1,59	6,8	8,12	2,95	5,17	8,52	2,66	5,86	39,4	4,9	1,3	27,1
24	8,39	6,19	2,2	8,3	6,34	1,96	8,52	7,48	1,04	8,38	6,79	1,59	8,38	5,36	3,02	8,26	7,74	0,52	8,79	6,84	1,95	8,35	7,05	1,3	13,6	1,7	0,8	45,2
25	8,53	3,35	5,18	8,13	1,32	6,81	8,16	2,81	5,35	8,89	2,86	6,03	8,47	3,14	5,33	8,46	3,25	5,21	8,3	3,75	4,55	8,22	2,33	5,89	44,4	5,5	0,7	12,3
26	8,14	4,64	3,5	8,81	5,12	3,69	8,6	6,32	2,28	8,03	7,52	0,51	8,38	3,24	5,14	8,57	0	8,57	8,08	4,79	3,29	8,39	5,03	3,36	30,3	3,8	2,3	61,6
27	8,02	3,65	4,37	8,25	4,93	3,32	8,53	4,41	4,12	8,01	3,45	4,56	8,62	6,31	2,31	8,65	4,87	3,78	8,18	3,17	5,01	8,11	1,99	6,12	33,6	4,2	1,1	27,1
28	8,11	2,37	5,74	8,59	5,07	3,52	8,36	3,76	4,6	8,04	3,77	4,27	8,14	3,52	4,62	8,41	3,83	4,58	8,26	5,48	2,78	8,39	1,85	6,54	36,7	4,6	1,2	25,6
29	8,19	5,98	2,21	8,83	4,01	4,82	8,32	7,16	1,16	8,13	5,57	2,56	8,2	6,03	2,17	8,58	4,79	3,79	8,41	6,24	2,17	8,26	4,18	4,08	23,0	2,9	1,2	42,8
30	8,06	5,56	2,5	8,21	7,99	0,22	8,43	4,44	3,99	8,22	6,19	2,03	8,89	6,16	2,73	8,34	5,29	3,05	8,18	5,67	2,51	8,56	6,19	2,37	19,4	2,4	1,1	44,0
31	8,16	3,45	4,71	8,26	5,46	2,8	8,29	3,34	4,95	8,33	6,27	2,06	8,64	3,82	4,82	8,51	1,47	7,04	8,47	3,7	4,77	8,09	4,62	3,47	34,6	4,3	1,5	35,5
32	8,39	5,67	2,72	8,41	6,58	1,83	8,54	7,41	1,13	8,02	5,83	2,19	8,32	7,34	0,98	8,46	8,15	0,31	8,15	6,2	1,95	8,32	6,69	1,63	12,7	1,6	0,8	47,8
33	8,64	2,23	6,41	8,48	3,47	5,01	8,1	2,7	5,4	8,18	2,1	6,08	8,49	0,55	7,94	8,56	3,2	5,36	8,39	0	8,39	8,25	3,63	4,62	49,2	6,2	1,4	22,3

FIGURE A-6 - DAILY MEASUREMENTS WITH RESPECT TO NEST BUILDING ANALYSIS. DAILY QUANTITIES OF COTTON WOOL USED EXPRESSED FOR 31 MICE, FOR A SEVEN-DAY CYCLE. THE TOTAL NEST BUILDING SCORE IS CALCULATED AS WELL AS THE AVERAGE OVER THE SEVEN DAYS, THE STANDARD DEVIATION AND THE % COEFFICIENT OF VARIANCE (CV) [(STANDARD DEVIATION/AVERAGE) X 100] FOR EACH MOUSE.

Examples of raw data exports from stereotypical assessments

- 1 Cage 2	Cycle 1	Phase 1	7	5:27:05 pm	1800.00	256.37	643	440	1662.68	171	137.32	170	46.51	60	203	0	0	0.00	1	3	4
- 1 Cage 2	Cycle 1	Phase 1	8	5:57:05 pm	1800.00	1301.09	3453	2854	1343.45	281	456.55	280	106.15	157	599	115	1088	196.37	17	5	22
- 1 Cage 2	Cycle 1	Phase 1	9	6:27:05 pm	1800.00	2554.38	4517	3637	996.59	312	803.33	312	163.31	265	880	196	1741	316.97	18	15	33
- 1 Cage 2	Cycle 1	Phase 1	10	6:57:05 pm	1800.00	749.39	2528	2103	1388.21	262	411.58	263	73.96	131	425	72	413	68.33	4	2	6
- 1 Cage 2	Cycle 1	Phase 1	11	7:27:05 pm	1800.00	1020.27	2260	1788	1425.70	155	374.25	155	73.32	154	472	84	697	166.25	8	5	13
- 1 Cage 2	Cycle 1	Phase 1	12	7:57:05 pm	1800.00	77.31	304	255	1730.88	92	69.26	91	7.99	22	49	0	0	0.00	0	1	1
- 1 Cage 2	Cycle 1	Phase 1	13	8:27:05 pm	1800.00	141.22	783	501	1600.58	212	199.42	211	74.10	90	282	0	0	0.00	0	0	0
- 1 Cage 2	Cycle 1	Phase 1	14	8:57:05 pm	1800.00	312.98	1027	916	1626.67	166	173.85	165	27.58	45	111	21	40	7.82	0	0	0
- 1 Cage 2	Cycle 1	Phase 1	15	9:27:05 pm	1800.00	20.42	84	65	1782.88	35	17.12	34	1.68	9	19	0	0	0.00	0	0	0
- 1 Cage 2	Cycle 1	Phase 1	16	9:57:05 pm	1800.00	67.27	137	115	1772.20	38	27.80	37	5.74	7	22	0	0	0.00	1	0	1
- 1 Cage 2	Cycle 1	Phase 1	17	10:27:05 pm	1800.00	140.41	960	875	1726.41	80	73.43	80	18.86	25	85	0	0	0.00	1	3	4
- 1 Cage 2	Cycle 1	Phase 1	18	10:57:05 pm	1800.00	597.29	1042	854	1609.32	134	190.18	134	33.10	56	188	28	134	26.62	2	2	4
- 1 Cage 2	Cycle 1	Phase 1	19	11:27:05 pm	1800.00	62.60	282	235	1731.18	125	68.24	125	9.96	20	47	0	0	0.00	0	0	0
- 1 Cage 2	Cycle 1	Phase 1	20	11:57:05 pm	1800.00	35.87	116	97	1771.06	46	29.47	45	5.49	12	19	0	0	0.00	0	0	0
- 1 Cage 2	Cycle 1	Phase 1	21	12:27:05 am	1800.00	80.81	373	287	1722.41	129	77.59	128	18.73	33	86	0	0	0.00	0	0	0
- 1 Cage 2	Cycle 1	Phase 1	22	12:57:05 am	1800.00	543.86	1192	1020	1601.12	193	198.89	192	42.29	64	172	31	169	32.71	4	3	7
- 1 Cage 2	Cycle 1	Phase 1	23	1:27:05 am	1800.00	79.15	499	380	1703.98	111	96.02	110	29.58	28	119	0	0	0.00	0	2	2
- 1 Cage 2	Cycle 1	Phase 1	24	1:57:05 am	1800.00	1796.33	2606	2200	1397.83	184	402.17	183	69.39	110	406	108	1046	189.70	20	9	29
- 1 Cage 2	Cycle 1	Phase 1	25	2:27:05 am	1800.00	1056.01	2591	2077	1283.12	299	516.30	299	120.42	163	514	90	775	136.92	10	14	24
- 1 Cage 2	Cycle 1	Phase 1	26	2:57:05 am	1800.00	1985.25	3377	2891	1326.94	245	472.89	245	82.03	100	486	190	1083	213.25	13	22	35
- 1 Cage 2	Cycle 1	Phase 1	27	3:27:05 am	1800.00	571.10	2682	2145	1489.04	261	310.96	260	98.26	118	537	78	781	162.41	4	5	9
- 1 Cage 2	Cycle 1	Phase 1	28	3:57:05 am	1800.00	2455.71	4969	4247	1158.35	405	642.12	404	122.34	189	722	276	2005	429.05	22	18	40
- 1 Cage 2	Cycle 1	Phase 1	29	4:27:05 am	1800.00	806.13	1659	1417	1511.55	176	288.07	176	47.56	89	242	74	616	137.60	6	5	11
- 1 Cage 2	Cycle 1	Phase 1	30	4:57:05 am	1800.00	1520.81	3167	2459	1104.10	170	695.62	170	119.83	272	708	99	1845	374.26	11	15	26
- 1 Cage 2	Cycle 1	Phase 1	31	5:27:05 am	1800.00	202.45	707	558	1655.84	166	143.54	166	32.61	50	149	0	0	0.00	2	0	2
- 1 Cage 2	Cycle 1	Phase 1	32	5:57:05 am	1800.00	217.03	643	448	1605.81	177	194.19	176	45.67	80	195	0	0	0.00	1	0	1
- 1 Cage 3	Cycle 1	Phase 1	1	2:27:05 pm	1800.00	1629.59	3587	3053	1341.60	234	458.27	234	86.83	156	534	48	95	14.87	8	12	20
- 1 Cage 3	Cycle 1	Phase 1	2	2:57:05 pm	1800.00	476.72	1599	1253	1543.52	134	256.48	133	59.78	108	346	21	49	11.70	1	7	8
- 1 Cage 3	Cycle 1	Phase 1	3	3:27:05 pm	1800.00	145.80	632	568	1727.04	54	72.65	54	13.62	28	64	0	0	0.00	1	0	1

FIGURE A-7 - EXAMPLE OF RAW DATA OUTPUT AS GENERATED BY THE ACCUSCAN® BEHAVIORAL TRACKER FOR A SINGLE ANIMAL OVER A SINGLE 12-HOUR SESSION (VALUES IN WHITE ARE THE EXPERIMENTAL DATA; VALUES IN GREY ARE DATA EITHER PRIOR TO OR AFTER THE DARK CYCLE). VERTICAL JUMPING IS INDICATED IN RED. PATTERNED CAGE RUNNING IS INDICATED IN GREEN. VALUES STACKED INTO EACH COLUMN REPRESENT THE BEHAVIORAL EXPRESSION OF A SINGLE MOUSE DURING A 30-MIN INTERVAL. DATA ARE REPRESENTATIVE OF A SINGLE 12-H SCREENING SESSION.

1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	76	0	0	0	0	0	0	0	0	0	0	0	0
3	1	0	0	0	3	0	0	0	158	0	0	0	0	0	0	0	0	0	1	0	0
4	2	0	0	0	6	0	0	0	381	0	221	0	0	0	0	0	0	0	2	0	0
5	23	0	2	0	65	0	0	0	530	0	296	0	0	0	0	0	0	731	10	1	0
6	26	0	6	0	107	0	0	15	588	0	468	0	31	1	0	0	0	857	29	5	0
7	36	0	8	0	160	0	0	51	610	0	476	0	72	4	2	0	0	858	55	6	0
8	38	0	323	0	193	0	2	51	652	3	631	0	106	13	118	0	0	924	90	11	0
9	52	0	329	1	215	0	86	70	658	15	635	0	108	17	120	0	0	1433	106	32	0
10	62	0	354	6	268	0	144	71	751	49	683	0	157	103	124	0	1	1581	232	77	0
11	66	128	375	7	616	0	198	114	753	206	692	0	158	189	216	0	18	1739	305	111	0
12	68	294	403	9	647	0	243	117	791	241	793	9	211	285	445	1	70	1765	353	116	2
13	74	450	412	323	920	0	247	194	852	324	926	19	266	294	741	2	81	1908	389	161	4
14	115	508	483	462	1163	34	278	260	889	512	935	72	321	331	801	5	303	2486	391	265	403
15	118	521	506	689	1257	72	304	317	925	625	991	235	346	349	814	7	554	2696	407	269	469
16	119	524	513	720	1324	78	390	352	938	790	1018	285	365	365	860	23	561	2747	410	273	678
17	123	580	520	834	1423	238	426	413	1045	911	1051	331	365	375	943	244	563	2861	460	300	728
18	179	599	574	866	1438	301	484	441	1136	958	1105	368	536	401	978	364	636	3381	460	312	922
19	331	601	576	1021	1908	371	497	468	1157	1071	1186	382	620	411	1048	483	1049	3450	482	364	1211
20	361	705	584	1036	1938	404	513	513	1213	1221	1522	868	659	438	1623	578	1061	3902	517	387	1227
21	466	805	851	1130	2168	449	531	729	1518	1297	1609	873	680	491	1673	688	1174	4207	521	399	1635
22	606	1129	919	1298	2177	626	533	1004	1971	1460	1934	1262	752	500	1701	912	1362	4596	530	414	1955
23	859	1264	981	1340	2186	808	648	1141	2220	1525	2170	1360	768	508	2428	933	1484	4652	584	435	2036
24	921	1336	1069	1855	2928	919	838	1699	2267	1770	2278	1936	859	625	2555	964	1805	5951	749	448	3357
Average time	0	0	0	0	16,67	0	0	0	8,33	0	8,33	0	0	0	8,33	0	0	45,833	0	0	8,33
Average high	795,3333	1243	989,6667	1497,667	2430,333	784,3333	673	1281,333	2152,667	1585	2127,333	1519,333	793	544,3333	2228	936,3333	1550,333	5066,333	621	432,3333	2449,333

FIGURE A-8 - EXCERPT FROM THE COLLATED VERTICAL JUMPING DATA FOR EACH ANIMAL (ONE COLUMN REPRESENTS ONE ANIMAL) AFTER A SINGLE STEREOTYPY SCREENING SESSION (SEE **CHAPTER 3** FOR DETAIL), SORTED FROM LOW (TOP) TO HIGH (BOTTOM). DATA OF ALL 24 INTERVALS (30-MIN BOUTS) OF 21 ANIMALS ARE REFLECTED HERE. VALUES EXCEEDING THE CUT-OFF CRITERIA FOR VERTICAL JUMPING, I.E. 2000 BEAM BREAKS PER 30 MINUTES, ARE HIGHLIGHTED IN RED. 'VA' STEREOTYPICAL INTENSITY WAS CALCULATED AS FOLLOWS: AVERAGE OF THE 3 HIGHEST BOUTS. TIME SPENT EXPRESSING STEREOTYPY WAS CALCULATED AS FOLLOWS: (THE AMOUNT OF HIGHLIGHTED RED BOUTS OF HS BEHAVIOR / TOTAL OF 24 BOUTS X 100).

Examples of raw data from T-maze assessments

	Trial 1	Trial 2	Trial 3	Training Trials	Alterations	Total Probe	Alteration %	Arm Ent / Tr Trials	% Alt / Trials
1	L, L, R, L, L, L, L, L, L, L	R, R, R, R, R, R, R, R, R, R	L, L, L, L, L, R, R, L, L, L, L, R, L, L, L, L, R, L, L, L, L, L, L, L, L, L, L, L, L, L, L, L, L, L, R, L, L, L,	24	8	40	20,0	1,67	0,83
2	L, R, L, R, L, R, L, R, R, R	L, L, L, L, L, L, L, L, L, L	R, R, L, R, R, R, L, R, L, L, R, R, L, R, R, R, L, R, R, R, R, R, R, L, L, R, R, R, R, L	37	13	30	43,3	0,81	1,17
3	R, L, L, L, L, L, R, R, R, R	R, R, R, R, R, R, R, R, R, R	L, L, R, L, L, R, L, L, L, L, L, L, L, L, L, L, R, L, L, L, R, R, L, R	33	9	24	37,5	0,73	1,14
4	L, R, L, R, R, L, R, R, L, L	L, L, L, L, L, L, L, L, L, L	R, L, R, L, R, R, R, L, R, R, R, R, R, R, R, R, R, R, R, L, R, L, R, R, R, L, R, R	24	12	28	42,9	1,17	1,79
5	R, L, L, L, R, L, L, R, L, R	R, R, R, R, R, R, R, R, R	L, R, L, L, R, R, R, R, R, R, R, R, R, R, R, L, L, L, R, R, L, L, R, L, L, L, L, L, L, L, R, R, L, R, R, R	40	11	36	30,6	0,90	0,76
6	L, L, R, L, R, L, L, R, R, R	L, L, L, L, L, L, L, L, L, L	L, R, R, R, L, R, L R	14	5	8	62,5	0,57	4,46
7	L, L, L, R, L, R, L, L, L, L	R, R, R, R, R, R, R, R, R, R, R, R, R, R, R, R, R, R, R	L, L, L, R, L, L, R, R, L, L, R, L, L, R, L, L, L, L, L, L. R. L, L, R, L, L, L, L	19	12	28	42,9	1,47	2,26
10	L, R, L, R, L, R, L, R, L, R	L, L, L, L, L, L, L, L, L, L, L, L, L, L, L, L, L, L, L, L, L, L, L	L, R, L, L, L, R, L, R, R, R, L, R, R, R, R, L, R, R, L, L, R, R, R, L, R, L	23	14	26	53,8	1,13	2,34
11	L, L, R, L, R, L, L, R, L, L	R, R, R, R, R, R, R, R, R, R, R, R, R, R, R, R, R, R, R, R, R	L, L, R, L, L, L, R, L, L, L, L, L, LR, L, L, L, L, R, R, R, R, R	21	7	23	30,4	1,10	1,45
12	L, L, R, L, R, L, L, L, R, R	R, R, R, R, R, R, R, R, R, R, R, R, R, R, R, R, R, R, R, R, R	R, L, L , L, L, L, L, L, L, L, L, L, L, L, L, L, L, L, L, L, L, R, L, L, R, R, R, L, L, L, R, L, L	21	6	33	18,2	1,57	0,87
13	R, L, R, L, R, L, L, R, R, R	L, L, L, L, L, L, L, L, L, L, L, L, L, L, L, L, L	L, R, R, L, L, R, R, R, R, L, L, R, L, L, R, R, L, R, R, L, L, L, R, R, R	17	11	25	44,0	1,47	2,59
14	R, L, L, L, R, R, R, L, R, L	L, L, L, L, L, L, L, L, L, L, L, L, L, L, L, L, L, L, L, L, L, L, L, L, L, L	L, L, R, R, R, R, L, L, L, R, R, R, L, R, R, L, R, L, R, R, R, R, R, R, R	26	11	28	39,3	1,08	1,51
15	R, L, L, L, R, R, L, R, L, L	R, R, R, R, R, R, R, R, R, R, R, R, R, R, R, R, R, R, R, R, R, R, R,	L, L, L, L, R, L, R, R, L, L, L, L, L, L, L, L, L, L, L, L, L, L, R, L, L, L, L, L, L, L, L, L, L, R, L, L, L, L, L,	34	8	40	20,0	1,18	0,59
16	R, L, L, R, L, R, R, L, R, L	L, L, L, L, L, L, L, L, L, L, L, L, L, L	R, R, R, L, R, L, L, R, R, R, R, R, R, R, R. L, R, R, R, R, R, L, R, L, L	14	9	25	36,0	1,79	2,57

Figure A-9 - Example of the completed data output of 16 mice which completed the T-Maze assessment For animal 16 the alternation score was calculated as follows: 9 (L)-left-(R)-right or R-L alternations made / 25 arm choices made x 100.

References

de Brouwer, G., Harvey, B. H., & Wolmarans, W. (2020). Naturalistic operant responses in deer mice (Peromyscus maniculatus bairdii) and its response to outcome manipulation and serotonergic intervention. Behavioural Pharmacology*, 31*(4), 343-358. doi: 10.1097/fbp.0000000000000536

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END OF ADDENDUM A