

Cognitive flexibility in the deer mouse and its response to escitalopram and levetiracetam

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Preface

All progress in science is progress in civilisation, and consequently contributes to the welfare of man. As researchers it is our job not to delay death but improve quality of life.

--Charles Richet

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Congress proceedings

The results of the current investigation were presented at the South African Society for Basic and Clinical Pharmacology (SASBCP) online via Zoom, October 2021. The presenting author is underlined.

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Abstract

Obsessive-compulsive disorder (OCD)¹ causes functional impairment which severely and negatively impacts the lives of affected individuals. As its name suggests, OCD is characterised by both obsessions and compulsions. The former includes intrusive and unwanted thoughts, mental images and urges while the latter consists of overt or covert compulsive mental routines and repetitive behaviours which manifest to attenuate the high degree of obsession-related stress and anxiety. Four main obsession/compulsion symptom dimensions have been described, i.e. contamination/washing, safety/checking, symmetry/ordering, and forbidden or taboo thoughts/covert mental compulsions.

On a neurobiological level, OCD is broadly founded upon dysfunctional cortico-striato-thalamo-cortical (CSTC)² circuit processing. This circuit subdivides into a direct, behaviourally activating and an indirect, behaviourally inactivating, pathway. On a theoretical level, it is often proposed that an overactive direct pathway underlies the expression of compulsive-like behavioural symptoms. This in turn is believed to result from a hyposerotonergic state, which allows the unopposed propagation of dopaminergic signalling by means of limited serotonin heteroreceptor-mediated negative feedback.

Following from the above, OCD can effectively be treated with selective serotonin reuptake inhibitors (SSRIs)³. However, approximately one third of patients do not respond to treatment with SSRIs. In these cases, up to 50 % of SSRI-resistant patients respond to cognitive behavioural therapy (CBT)⁴. Thus, it is likely that some phenotypes of OCD may differentially be associated with uniquely aberrant cognitive processes which may influence their treatment response. As such, valuable insights may be gained from research that explores treatment aimed at addressing deficits in cognitive function. One such potentially useful compound, levetiracetam (LEV)⁵, has been shown to improve neuropsychological and cognitive performance in patients suffering from Alzheimer's disease and epilepsy, but it has not yet been trialled in other

¹ obsessive-compulsive disorder

² cortico-striato-thalamo-cortical

³ selective serotonin reuptake inhibitors

⁴ cognitive behavioural therapy

⁵ levetiracetam

neuropsychiatric disorders that may present with similar deficits in cognitive function. While the exact mechanism of action of this anti-epileptic drug is still unclear, it is apparent that, as opposed to other neural stabilisers, LEV¹ binds to synaptic vesicle glycoprotein 2A (SV2A)², thereby regulating neurotransmitter release and synaptic function. Therefore, instead of targeting a single neurotransmission system in isolation, it is believed that LEV broadly normalises neurotransmission in the central nervous system (CNS)³, thereby restoring brain function in cases of imbalanced signalling.

The deer mouse (*Peromyscus maniculatus bairdii*) model system of compulsive-like behaviour is one of the foremost naturalistic animal models of compulsive like behaviour. The validity of this model is founded upon the naturalistic presentation of stereotypical behaviour, i.e. repetitive vertical jumping and pattern running. Such behaviours vary within the deer mouse population, with 35 – 45 % of male and female deer mice presenting with high-stereotypical (H)⁴ behaviour. Since H behaviour waxes and wanes over the course of a dark cycle, serves no apparent realistic function and has shown response to chronic, high-dose exposure to the SSRI⁵, escitalopram (ESC)⁶, stereotypy in deer mice has been proposed to mimic clinical compulsive behaviour in humans. Further, H animals, but not their normal (low-stereotypical) (N)⁷ behavioural counterparts, present with significantly reduced striatal serotonin transporter (SERT)⁸ density, lending a high degree of construct validity to the model.

That said, since motor stereotypy is also a symptom of several neuropsychiatric disorders which are devoid of direct cognitive involvement, it remains difficult to argue that such behaviours are indeed related to an underlying psychobiological construct. Thus, in this work, we aimed to explore whether motor stereotypy associates with deficits in cognitive flexibility as measured by spontaneous alternation in a T-maze. The T-maze assessment is a popular cognitive task used to assess various cognitive constructs in rodents and to detect cognitive dysfunction in neuropsychological

¹ levetiracetam

² synaptic vesicle glycoprotein 2A

³ central nervous system

⁴ high-stereotypical

⁵ selective serotonin reuptake inhibitor

⁶ escitalopram

⁷ low-stereotypical

⁸ serotonin transporter

diseases. Subsequently, we aimed to investigate whether any potential association between stereotypy and altered spontaneous alternation in H¹ compared to N² animals would respond to ESC³ and the potentially novel cognitive enhancer, LEV⁴. Ultimately, the broad theme of this work was to highlight potentially useful avenues for future anti-compulsive therapeutic research which may be based on targeting cognitive, rather than motor processes in patients with OCD⁵.

Briefly, 124 mice of both sexes (aged 10-12 weeks at the onset of experimentation) were screened for stereotypical behaviour and divided into two behavioural cohorts, i.e. N and H. Since only 35 – 45 % of animals show H behaviour, an initial pool of 124 animals was necessary to yield 31 N and 34 H animals (total $n = 65$ used for experimentation). The 59 animals that have not been selected for further study, were euthanised. After quantification of stereotypical behaviour and stereotypical classification, all N and H mice were subjected to T-maze assessment, in which the number of successive alternate arm choices are calculated, i.e. the higher the number of left-right or right-left alternations, the more flexible an animal was proposed to be. Animals were then divided into the following drug exposure groups i.e. normal water control, ESC (50 mg/kg/day), and LEV (75 mg/kg/day) (both obtained from BLD Pharm®, Shanghai, China). Final group numbers were as follows: N control & ESC: 11 each; N LEV: 9; H control and ESC: 11 each; H LEV: 12. Groups were as far as possible constituted by an equal number of male and female animals. On the second last day of water or drug exposure, all N and H animals were again assessed for alternation behaviour in the T-maze, followed by a single night of stereotypy assessment on the last day of drug exposure. All subjects were then euthanised as per the standard protocol of the Vivarium of the North-West University (NWU)⁶. Prior to the onset of experimentation, the study was approved by the AnimCare Research Ethics Committee of the North-West University (approval number: **NWU-00522-20-A5**).

¹ high-stereotypical

² low-stereotypical

³ escitalopram

⁴ levetiracetam

⁵ obsessive-compulsive disorder

⁶ North-West University

The main findings of this work were that 1) higher stereotypical intensity in deer mice significantly and negatively predicted spontaneous alternation scores in the T-maze, 2) LEV, but not control or ESC exposure, significantly increased the time that H, but not N animals spent engaging in no stereotypical behaviour, and 3) neither of the interventions employed here, affected spontaneous alternation scores in N¹ and H² deer mice.

With respect to our first main finding, we showed that stereotypical motor expression in deer mice negatively predicted spontaneous alternation in a T-maze. That deer mouse stereotypy is associated to some degree with impaired cognitive flexibility as quantified by decreased spontaneous alternation scores in a T-maze, we argue that such behaviour is not simply an artefact of aberrant processes regulating motor control, but that deficits in cognitive ability, e.g. action-outcome-planning, may underlie the expression of such routines. While this notion remains to be explored in future studies, the validity of the deer mouse model is strengthened as a framework in which to investigate the psychobiology of naturalistic compulsive-like behaviours.

Secondly, we showed that LEV³ significantly increased the time that H animals engaged in no stereotypical activity compared to LEV-exposed N animals. The fact that neither ESC⁴ nor LEV influenced the expression of H behaviour per se, was an important result, since such a lack of H adaptation to ESC differs markedly from the results of our previous work. While we provided potential explanations for this unexpected result in this dissertation, future work will be needed to elucidate the exact reasons underlying our present data. It is possible that H behaviour might arise from both anxiety- and compulsive-like processes, for which the appropriate controls, e.g. anxiolytic drug intervention and additional anxiety testing, were not included, since said confounding results were not foreseen from the outset.

Last, neither control nor ESC or LEV intervention influenced T-maze alternation. While this result could have been expected for ESC—if it is argued that ESC targets hyperactive cortico-striatal motor circuits instead of cognitive (read executive)

¹ low-stereotypical

² high-stereotypical

³ levetiracetam

⁴ escitalopram

processes—the LEV result is somewhat confounding. Nevertheless, it is likely that restoration of the cognitive deficits that associate with H behaviour by LEV were masked by the methods followed here and that a different paradigm, i.e. trained, cue-reward contingency T-maze alternation, might have been more informing.

In conclusion, we explored whether high motor stereotypy as observed in deer mice, is associated with cognitive rigidity as measured in a T-Maze, which was shown to be so. Further, the current work provided valuable insights into the potential interactions between anxiety- and compulsive-like processes that may act in concert to give rise to naturalistic stereotypy; this will need careful and well-conceptualised future work to clarify. Nevertheless, naturalistic stereotypy in deer mice is innately associated with perturbations in cognitive flexibility. This paves the way for future cognitive and anti-compulsive therapeutic research in this species.

Keywords

deer mouse; T-maze; alternation; cognitive flexibility; obsessive-compulsive disorder; escitalopram; levetiracetam

Solemn declaration

I, HM de Ridder (27055698), herewith declare that this dissertation is my own work and that no part thereof has been copied from other sources.

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1 Introduction

1.1 Dissertation approach and layout

This dissertation is compiled in article format as prescribed and approved by the North-West University (NWU)¹, South Africa. As such, the main body of the dissertation is presented as a single manuscript (Chapter 3), with the experimental work, results, main findings, and conclusions presented in the form of a journal article that has been submitted for publication following input from the examiners to '*Neuroscience Letters*'. However, the dissertation will consist of four chapters. In Chapter 1, the problem statement is provided, while the study questions, aims and objectives, experimental layout and study methodology are also summarised. Chapter 2 comprises a review of literature supporting the current project, while Chapter 3 contains the mentioned journal article. Chapter 4 concludes the dissertation with a broad summary of the work presented here, consideration of shortcomings and suggestions for follow-up studies. Finally, one Addendum is also included. Since all co-authors of this work were involved in the supervision of this study, permission letters to submit this work for examination are not provided. Thus, Addendum A contains supplementary data and additional descriptions of the methods followed which were not described in Chapter 3, as well as detailed methods of animal husbandry, housing and care.

The manuscript was prepared in accordance with the instructions to authors provided by '*Neuroscience Letters*'. This applies to both language (UK English) and referencing style throughout the dissertation.

¹ North-West University

1.2 Problem statement

Obsessive-compulsive disorder (OCD)¹ is a neuropsychiatric disorder which has a global prevalence rate of 1.1 – 1.8% and presents with remarkable functional impairment [5, 27, 44, 46]. OCD is characterised by both obsessions and compulsions, the former being intrusive and unwanted thoughts, images and urges, and the latter repetitive behaviours or mental routines. The characteristic compulsive traits, of which at least four broad symptom dimensions are diagnosed, are believed to manifest as a result of a high degree of underlying obsession-induced stress and anxiety [1, 10].

The implicated neurobiological and pathophysiological architecture by which OCD is described, suggests the cortico-striato-thalamo-cortical circuitry (CSTC)² to be a fundamental target of interest [2]. In OCD, positive correlations between the degree of CSTC circuit activation and symptom severity has been shown [1, 49]. Although research is not clear in terms of whether specific abnormalities, within discrete sub-circuits of the CSTC pathways, underlie symptom dimension-specific cognitive performance only, dysfunctional CSTC circuit connectivity has indeed been demonstrated extensively in clinical [36] and pre-clinical literature [2].

OCD is often described as a condition characterised by hyposerotonergic functioning, since both highly selective serotonin reuptake inhibitors (SSRIs)³, e.g. escitalopram, citalopram, fluvoxamine, fluoxetine, paroxetine, sertraline, and serotonin reuptake inhibiting tricyclic antidepressants (TCAs)⁴, e.g. clomipramine, are generally effective for the treatment of OCD [19]. That said, TCAs that target noradrenalin reuptake, e.g. desipramine [55] and imipramine [21], are mostly not useful in OCD, purportedly due to their predominant actions on the noradrenergic system [20]. Furthermore, several investigations have reported no significant differences in treatment response to SSRIs when compared to clomipramine, although the safety profile of SSRIs result in their favoured use. Still, approximately one third of patients do not respond to first line pharmacotherapy with SSRIs [32], in which case two additive approaches can be

¹ obsessive-compulsive disorder

² cortico-striato-thalamo-cortical

³ selective serotonin reuptake inhibitors

⁴ tricyclic antidepressants

followed, i.e. addition of psychotherapy (most notably cognitive behavioural therapy; CBT¹) or pharmacological augmentation with low-dose anti-dopaminergic therapy [3, 4]. Briefly, psychotherapy comprises cognitive reconstruction of behaviour by means of exposure and response prevention (ERP)² [3]. Roughly 50% of SSRI³-resistant patients respond to CBT. Interestingly patients presenting with excessive saving, symmetry, and contamination symptoms are less responsive to SSRIs [33, 47], but respond relatively well to CBT [34, 43]. With respect to anti-dopaminergic augmentation strategies, data are promising with nearly one-third of patients with treatment refractory OCD⁴ responding to antipsychotic augmentation strategies [8]. That said, discontinuation of effective augmented SSRI therapy causes a relapse in 83.3% of patients within 2 months [31].

Considering the above, it is likely that some phenotypes of OCD may be predominantly associated with aberrant cognitive processes, e.g. cognitive rigidity or impaired action-outcome control, and resultantly show a favourable response to psychobehavioural interventions. Yet, even psychotherapeutic approaches remain ineffective in a significant number of patients [28]. As such, valuable insights can potentially be gained from research which explores novel pharmacotherapies that do not target the serotonergic or dopaminergic systems, but rather act by improving cognitive function. This is a research area that has previously gained much attention in models of Alzheimer's disease and epilepsy [40, 45, 52]. However, given the likely association between OCD and cognitive rigidity alluded to above, this idea will be one of the focuses of this work.

Against this background, levetiracetam (LEV)⁵, clinically used to treat epilepsy, may show some promise. This 5-enantiomer of 5- α -ethyl-2-oxo-1-pyrrolidine acetamide was initially prescribed as a supplementary treatment for epilepsy but has since been shown to improve the neuropsychological and cognitive performance, e.g. working and spatial memory and attention, of affected individuals [30, 35]. Interestingly, as opposed to other neuronal stabilizers, e.g. valproate and

¹ cognitive behavioural therapy

² exposure and response prevention

³ selective serotonin reuptake inhibitor

⁴ obsessive-compulsive disorder

⁵ levetiracetam

carbamazepine that act mostly by modulating the actions of voltage-gated ion channels, LEV influences neurotransmitter release via its actions on synaptic vesicle glycoprotein 2A (SV2A)¹, a neurotransmitter release-regulating transmembrane protein. By binding to SV2A, LEV² modulates synaptic vesicle exocytosis and modifies synaptic function and the levels of excitation and inhibition. Indeed, the fact that LEV does not interact directly with known neurotransmitter systems, but merely plastically modulate the release thereof, may be key to its potential as a cognitive enhancer [25, 52]. Specifically, LEV has been shown to improve task execution on the levels of attention, external stimulus processing and set-shifting ability [52]. In the current work, we will explore the actions of LEV on the behaviour of deer mice (*Peromyscus maniculatus bairdii*)—a subpopulation of which naturalistically expresses compulsive-like motor repetition [14, 51]; see later—that may also present with cognitive inflexibility as assessed in the rodent T-maze.

To frame the theoretical basis of this project against the appropriate background, a summary of the potential overlap between the cognitive deficits in OCD³ patients and T-maze alternation in rodents are needed. To do so, we will first briefly highlight three cognitive domains of human functioning that show abnormalities in OCD. We will then conclude by briefly explaining how T-maze alternation may be appropriate to assess similar constructs in rodents.

Predictive learning, which is underpinned by cortico-striatal processes involved in expectation and outcome valuation, is crucial for our survival [7]. However, such processing is relatively energy intensive and slow. Hence, upon repeated exposure to the same outcome, whether positive or negative, a bias in favour of automated responses begin to emerge [15, 17, 49], whereby low-intensity responses to known circumstances become the dominant response [7, 23]. Called habit, these automated responses quickly reaffirm an appropriate course of action, reducing the need to assess, evaluate and consider different alternatives [23]. That said, adequate cognitive flexibility, e.g. being able to rapidly switch to goal-directed action among others, is vitally important for our normal functioning. Considering the phenotypic

¹ synaptic vesicle glycoprotein 2A

² levetiracetam

³ obsessive-compulsive disorder

presentation of OCD in terms of symptom persistence and insensitivity to goal-directed task-completion, it is likely that impaired goal-oriented action-motivation and a persistent reliance on maladaptive habitual responses, play a role in the psychobiological picture of the condition [22]. Indeed, excessive reliance on habitual responses can impair cognitive flexibility and erode our ability to engage in processes of goal-directed action-motivation [42].

Secondly, and closely related to goal and habit, is the concept of task-switching. This allows an individual to disengage from a previously executed task set and shift his/her attention and executive processing to a new, unrelated task. Intact task-switching ability also ensures that a new course of action can dominate over other less important or otherwise devalued tasks [6, 13, 23], for example running to turn off a burning stove, instead of finishing the current cleaning task. As is true for alternating between goal-directed and habitual behaviour, executive processing is crucial for our ability to switch between different tasks. As such, compromised executive processing may present in various ways [13, 23, 37, 48]. In OCD¹, such deficits are observed on the levels of set-shifting and response inhibition [39]. Often, OCD patients present with impaired set-shifting ability, which has been shown to correlate with symptom severity [13, 39, 48]. In other words, they struggle to change the rules of engagement which translates to locking a door for a specific number of times or washing their hands in an awfully specific manner, instead of adapting to the requirements of their circumstances, e.g. having to hurry up to prevent being late for an important appointment. On the other hand, response inhibition speaks to *disengagement* from a previous response [13], a cognitive domain that currently gains much attention in OCD research [9]. Indeed, it is believed that the inability of OCD patients to *switch off* certain behaviours, rather than being unable to shift between behaviours, is what may compromise their functioning [18].

Last, excessive and rigid repetition of certain motor patterns is another neurocognitive domain that may show dysfunction in OCD. As opposed to habit, motor rigidity refers to overly involuntary repetition of certain motor responses which are devoid of any goal-directed outcome, a construct exemplified in patients with autism, Tourette's disorder and tic disorder [29, 53]. That said, motor rigidity is also studied in OCD,

¹ obsessive-compulsive disorder

since patients often present with seemingly senseless repetition of actions that cannot be described according to theories of goal-directed action or task-switching. In practical terms, motor rigidity may be a fundamental construct of compulsivity without a clear behavioural outcome, e.g. symmetry and ordering compulsions and counting rituals [48].

In rodents, the T-maze is often applied as a test of cognitive flexibility [12, 16]. In a T-maze, an animal makes several successive arm-choices of which the alternation pattern is quantified. However, since the traverse arm joins the stem in a perpendicular manner, the test requires an animal to be reintroduced to the start box in the stem of the maze between each successive trial. Therefore, the T-maze can be regarded as an intervened choice test. Various theories have been proposed to explain the nature of cognitive processes involved in and assessed with the T-maze [16]. These include overreliance on arm choice habit, instead of goal-directed exploration [38], impaired working memory and set-shifting ability [11], and rigid motor responses devoid of a functional outcome [24]. Suffice it to conclude that T-maze alternation behaviour is a generalised, rather than specific, indicator of cognitive flexibility which can be applied to draw broad conclusions regarding the association between the expression of certain biobehavioural traits, e.g. motor stereotypy, and general deficits in cognitive functioning. Here, such an approach will be followed.

In fact, to expand our understanding of the potential associations between compulsive-like symptomology and cognitive impairment, we will assess the relationship between naturalistic repetitive behaviours and spontaneous alternation behaviour in deer mice. The deer mouse model system is a naturalistic model of compulsive-like behaviours that is founded on the presentation of stereotypical behaviour that develops spontaneously, *viz.* repetitive—read rigid—vertical jumping and pattern running that varies naturally (i.e. without induction) in intensity within a cohort, *viz.* low (N)¹ and high (H)²-stereotypical behaviour [51]. These time-consuming behaviours mimic the rigid repetitive motor actions observed in human OCD³ and form the basis of the face validity of the model, i.e. emulating the symptomology of the human condition [50].

¹ low-stereotypical

² high-stereotypical

³ obsessive-compulsive disorder

Deer mouse stereotypy favourably responds to chronic, but not sub-chronic treatment with high-dose SSRIs¹, a finding which formed the basis of the predictive validity of the model, i.e. in mimicking the response of OCD to pharmacological manipulation [50]. Furthermore, Wolmarans, et al. [51] presented evidence that H deer mice present with a significant decrease in striatal serotonin transporter (SERT)² expression compared to the low-stereotypical controls, making for some construct validity, which is to say physiological similarity between the modelled condition and the model [50]. The latter results are also in line with clinical findings [26, 41, 54]. Here, we will aim to explore the association between said stereotypy and potential alterations in cognitive flexibility as measured in the T-maze.

Taken together, OCD³ is a phenotypically heterogeneous condition that is characterised by noteworthy differences in symptom presentation, psychobiological constructs, and treatment response between patients. Further, it is likely that various neurocognitive processes contribute in distinct ways to the manifestation of symptoms in individuals suffering from the condition. As such, to gain a better understanding of the cognitive processes underlying compulsivity, pre-clinical studies in animal models are crucial. It would be of further value to assess the effects of potentially novel cognitive enhancers, e.g. LEV⁴, on both compulsive-like behavioural expression and on associated markers of cognitive inflexibility, so as to identify potentially novel treatment strategies which are able to modify potential contingencies between motor rigidity and cognitive deficits in at least some subpopulations of OCD sufferers. It is against this background, that the current investigation is framed.

¹ selective serotonin reuptake inhibitors

² serotonin transporter

³ obsessive-compulsive disorder

⁴ levetiracetam

1.3 Study questions

- i) Will N¹ and H² deer mice show distinct patterns of spontaneous alternation as assessed in a T-maze?
- ii) Will escitalopram, a known anti-compulsive intervention which has been shown to reduce spontaneous stereotypy in deer mice and which will thus be used here as a positive anti-stereotypy control, also improve any potential differences in spontaneous T-maze alternation behaviour as shown by N and H mice?
- iii) How will the effects of levetiracetam, a potentially useful cognitive enhancer, compare to that of escitalopram on both spontaneous stereotypy and alternation behaviour?
- iv) Would it be valuable to explore novel treatment strategies that are devoid of direct monoaminergic action for the treatment of compulsive-like traits that may be associated with cognitive impairment?

1.4 Research hypothesis

We hypothesise that, should stereotypy be an artefact of cognitive rigidity, H, but not N deer mice, will present with a higher degree of persistent arm choices in a T-maze and that such reductions in spontaneous alternation will correlate with the degree of stereotypy. Further, since H, but not N behaviour is responsive to chronic, high-dose oral escitalopram, we also hypothesize that, should motor stereotypy and cognitive rigidity show an association, escitalopram will result in improvements across both said domains, while levetiracetam, which has shown promise as a potentially novel cognitive enhancer in clinical trials before, would improve T-maze alternation, irrespective of its effect on stereotypy. However, since clinical research into the relationship between compulsive symptoms and cognitive rigidity delivered discrepant results, it is also possible that stereotypy will not associate with deficits in cognitive flexibility. If this would be emulated in the deer mouse model, we expect escitalopram only to be effective for the treatment of stereotypy and not for the rigid alternation behaviour that may be shown by some mice, irrespective of stereotypical cohort.

¹ low-stereotypical

² high-stereotypical

However, we hypothesize that in such event, levetiracetam would still show some promise as a cognitive enhancer.

1.5 Study aims and objectives

To test our research hypothesis and answer the questions, we aimed to:

- i. Quantify the expression of stereotypy and the degree of T-maze spontaneous alternation in drug-naïve N¹ and H² mice, respectively;
- ii. Expose all mice to either normal tap water (control), escitalopram or levetiracetam over 28 days; and
- iii. Reassess all mice for stereotypy and spontaneous alternation in the T-maze after 28 days of water or drug exposure.

These aims were realised by addressing the following objectives:

- i. 124 mice of both sexes (aged 10 - 12 weeks at the onset of experimentation) were screened for stereotypical behaviour and divided into two behavioural cohorts, viz. N and H. Since only 35 – 45 % [14] of animals show H behaviour, an initial pool of 124 animals were necessary to yield 31 N and 34 H animals (total $n = 65$ used for experimentation). The 59 animals from this pool that were not selected for further investigation, were euthanized.
- ii. After quantification of stereotypical behaviour and stereotypical classification, all 31 N and 34 H mice were subjected to T-maze assessment and the number of arm alternations calculated;
- iii. Animals were then divided into the following drug exposure groups (N control & ESC³: 11 each; N LEV⁴: 9; H control and ESC: 11 each; H LEV: 12, near equally distributed between sexes; refer to Chapter 3) and exposed to said interventions for 28 days:
 - a. Normal tap water (control);
 - b. Escitalopram oxalate (50 mg/kg/day; [51]); and
 - c. Levetiracetam (75 mg/kg/day; [45]).

¹ low-stereotypical

² high-stereotypical

³ escitalopram

⁴ levetiracetam

- iv. On the second last day of water or drug exposure, all N and H animals were again assessed for alternation behaviour in the T-maze.
- v. On the last day of water or drug exposure, all N¹ and H² animals were again assessed for stereotypical behaviour, after which subjects were euthanized as per the ethically approved protocol of the Vivarium of the NWU³.

1.6 Graphical representation of study layout

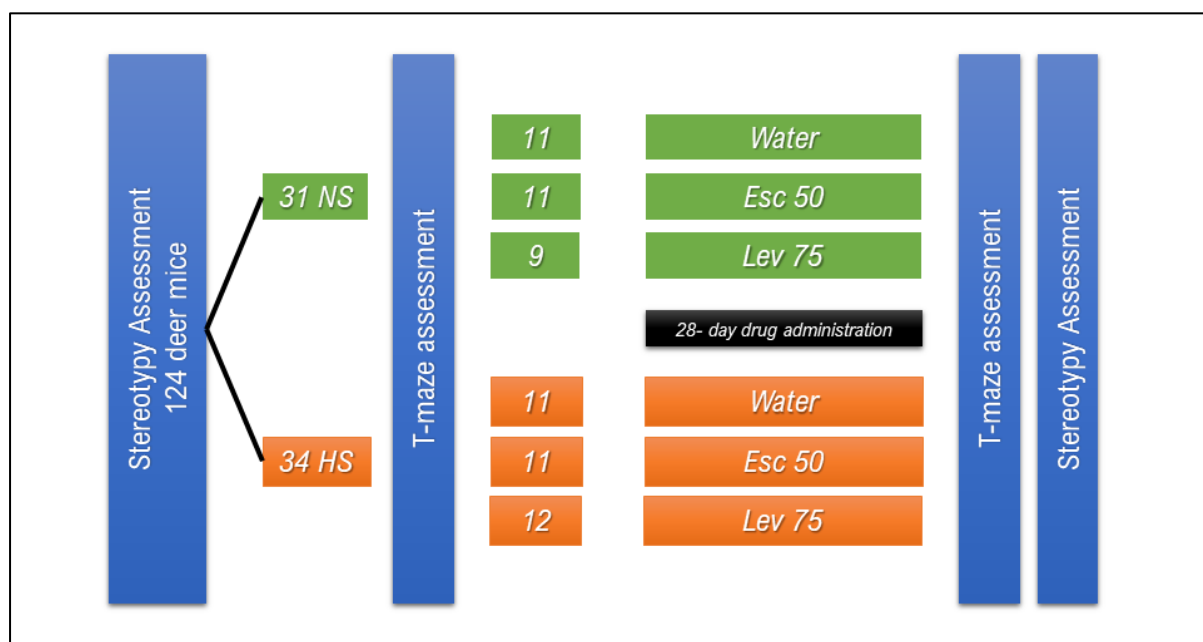


Figure 1-1 Graphical representation of study layout. Post-drug administration T-maze assessment was conducted on day 27 of drug exposure, while stereotypy assessment was concluded on day 28 of drug administration.

NS: low-stereotypical; HS: high-stereotypical; Esc: escitalopram oxalate; Lev: levetiracetam

1.7 Ethical approval

The current investigation has been approved by the AnimCare Research Ethics Committee (NHREC reg. number AREC-130913-015) of the NWU (approval number NWU-00522-20-A5) and has been performed by the researcher, Miss HM de Ridder, under supervision of the project supervisor, Prof PD Wolmarans. All animals were bred and housed, and experimentation performed at the Vivarium of the Preclinical

¹ low-stereotypical

² high-stereotypical

³ North-West University

Drug Development Platform (PCDDP)¹ of the NWU (SAVC reg. number FR15/13458; SANAS3 GLP4 compliance number G0019).

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2 Literature Review

2.1 Obsessive-compulsive disorder – epidemiology, description, and diagnosis

Obsessive-compulsive disorder (OCD)¹ is a chronic and disabling neuropsychiatric disorder that presents with noteworthy functional impairment [18, 70, 118, 119]. The prevalence rate of OCD often varies within communities and study populations; possibly due to limitations across survey methodology and variances in clinical diagnosis [118]. However, consensus has it that 1.1 – 1.8% of individuals globally, suffer from the condition. Quality of life (QOL)², defined as an individual's physical, mental and general well-being, is significantly impaired in OCD. Thus, when assessing and treating a patient with OCD, it is fundamental to not only treat the condition, but also consider the QOL deficits [18, 122].

As the name suggests, OCD is characterised by the presence of obsessions and compulsions. Obsessions are intrusive and unwanted thoughts, (e.g. about symmetry and contamination), mental images (e.g. violent scenes) and urges (e.g. to kill someone) that cause a high degree of anxiety or distress in the affected individual. Importantly, obsessions are not voluntary and are not experienced as enjoyable. In an attempt to attenuate such distress, patients usually engage in overt or covert neutralising compulsions, i.e. repetitive behaviours (e.g. checking or washing) or mental routines (e.g. praying or silently repeating words) [1, 19]. While the expression of compulsive behaviour usually provides some *temporary* relief for the affected individual, it is important to note that neither such relief, nor the experience of obsessions, are pleasurable [1, 11, 19].

Concerning diagnosis, four main symptom dimensions are commonly noted among individuals with OCD, based mainly on the content of said obsessions and compulsions [4]. Obsessions about contamination are associated with neutralising washing and cleaning compulsions. Obsessions about symmetry are associated with repetitive ordering and counting behaviours. A third dimension involves obsessions

¹ obsessive-compulsive disorder

² quality of life

about safety and responsibility whereby individuals feel compelled to prevent harm to themselves or others. Such obsessions are associated with excessive checking behaviour. The fourth phenotype involves obsessions linked with covert mental routines. This phenotype includes experiences of forbidden or taboo thoughts concerning sex, violence and religion which are associated with covert mental compulsive repetition, e.g. excessive praying [11, 66]. The Diagnostic and Statistical Manual of Mental Disorders, 5th edition (DSM-V)¹ [11] stipulates four important criteria for an accurate diagnosis of OCD², i.e., 1) at least one obsession *and/or* compulsion should be present, 2) symptoms must be time consuming (taking more than one hour per day), 3) symptoms must not be ascribed to other medical conditions or be the effect of substance abuse, and 4) symptoms must not be better explained by another neuropsychological disorder.

Historically, OCD was classified as an anxiety disorder. However, with the publication of the fifth edition of the DSM, the condition was reclassified under a new group of disorders, i.e., 'obsessive-compulsive and related disorders' (OCDs)³ [11]. The OCDs include OCD, hoarding disorder, excoriation (skin-picking disorder), body dysmorphic disorder (BDD)⁴, trichotillomania (TTM)⁵, and substance abuse, all of which are characterized by ritualized behaviours that are excessive and persistent [11, 134]. Still, OCD and anxiety share some important traits and often co-occur. Further, since anxiety is known to play a pivotal role in the manifestation of compulsive behaviour, OCD cannot be regarded as a condition devoid of an anxiogenic construct. Rather, its reclassification as an OCD was aimed at aligning OCD with other conditions that are also characterised by similar behavioural traits.

2.2 The neurobiology OCD

2.2.1 Neuroanatomy

The neurobiological underpinnings of OCD remain somewhat unknown. However, the most accepted theories point to involvement of the cortico-striato-thalamo-cortical

¹ Diagnostic and Statistical Manual of Mental Disorders, 5th edition

² obsessive-compulsive disorder

³ obsessive-compulsive and related disorders

⁴ body dysmorphic disorder

⁵ trichotillomania

(CSTC)¹ circuitry [47, 70, 135, 149]. Briefly, the CSTC circuitry comprises several frontal-subcortical circuits which project from the cortex via the striatum to specific parts of the thalamus, ending with thalamo-cortical afferents.

The prefrontal cortex (PFC)², specifically the dorsolateral prefrontal cortex (DLPFC)³, is important for both cognitive and behavioural flexibility, i.e., the ability to quickly switch cognitive/behavioural engagement over to different tasks based on immediate priority. It also plays an important role in the consolidation of short-term memory and is responsible for inhibitory control of behaviour [23, 25, 141]. The orbito-frontal cortex (OFC)⁴ is involved in reward-based learning, social behaviour, and emotional processes [57, 70]. While the anterior cingulate cortex (ACC)⁵ is also involved in different aspects of reward-based behaviours, its major function relates to decision making [70].

Like the frontal cortex, the basal ganglia (BG)⁶, notably the striatum, is also involved in reward-based learning. It is vital for the expression of goal-directed behaviour and critical for learning new and complex behaviours. It can suppress some motor actions while facilitating others [57, 70] and functions as the neural ‘go-no-go’ gate between the cortex and the thalamus. In turn, the thalamus ultimately propagates, or prevents the propagation of, signals from reaching the frontal cortex and closing the executive loops in the frontal cortex [57].

In terms of dynamics, the CSTC⁷ circuitry broadly subdivides into direct (behaviourally activating) and indirect (behaviourally inactivating) pathways. Neurons of the direct pathway primarily express excitatory dopamine-1 (D₁)⁸ dopaminergic receptors, while those of the indirect pathway are typified by the expression of inhibitory dopamine-2 (D₂)⁹ receptors [70, 119]. Briefly, the direct pathway projects via an excitatory glutamatergic relay from the cortex to the striatum which sends an inhibitory gamma-

¹ cortico-striato-thalamo-cortical

² prefrontal cortex

³ dorsolateral prefrontal cortex

⁴ orbito-frontal cortex

⁵ anterior cingulate cortex

⁶ basal ganglia

⁷ cortico-striato-thalamo-cortical

⁸ dopamine-1

⁹ dopamine-2

aminobutyric acid (GABA)¹-ergic signal to the globus pallidus interna/substantia nigra pars reticula (GPi/SNr)² complex, i.e., the main output station of the BG. From here, the signal is relayed to the thalamus which then activates the PFC [70, 90, 119, 135]. Conversely, the indirect pathway projects from the frontal cortex to the striatum, but an inhibitory GABA³ergic signal is then sent to the globus pallidus externa (GPe)⁴ and subsequently the subthalamic nucleus. From there, an excitatory glutamatergic signal is sent to the GPi/SNr⁵ complex which projects GABA releasing neurons that inhibits thalamic activity and downstream prevents cortical activation. In other words, the two pathways functionally oppose one another, while the executive balance between the two pathways, determines the net course of behavioural action, i.e. to propagate or inhibit behavioural engagement [90, 119, 135]. Indeed, goal-directed conduct depends on a flexible equilibrium between the initiation and inhibition of behaviour. When disturbed, behaviour deviates from the 'norm', manifesting as maladaptive behaviours as seen in disorders like OCD⁶ [135]. In OCD, a bias in favour of the direct pathway over the indirect pathway is proposed to underlie the condition, explaining the rigid, focused and excessive engagement in behavioural routines [90, 119].

2.2.2 Neurobiology

A few principal candidate neurotransmitter systems are believed to be dysregulated in OCD. These include the serotonergic, dopaminergic, glutamatergic and GABA-ergic systems [34, 35, 84, 107]. However, only serotonergic and dopaminergic modification presents with extensively evidence-backed therapeutic benefit in OCD to date. Hence, closer attention will be afforded to these monoamines here.

The earliest indication for the involvement of serotonin in OCD was the observation that clomipramine, a mainly serotonergic tricyclic antidepressant (TCA)⁷, was found to be effective for the treatment of OCD in some patients. Serotonin, being a major neurotransmitter in the central nervous system, originates in the midbrain raphe nuclei from where serotonergic neurons project to several cortical and subcortical brain

¹ gamma-aminobutyric acid

² globus pallidus interna/substantia nigra pars reticula

³ gamma-aminobutyric acid

⁴ globus pallidus externa

⁵ globus pallidus interna/substantia nigra pars reticula

⁶ obsessive-compulsive disorder

⁷ tricyclic antidepressant

regions, including the CSTC¹ circuitry. At least 14 different serotonin receptor subtypes have been identified of which a handful, i.e. the serotonin (5HT)²_{1/2} subtypes have been recurrently implicated in the pathophysiology of OCD³. Against this background, it is important to note that blunted cortico-striatal serotonergic action has been implicated in OCD (see below) and that the various receptor subtypes involved in OCD, likely play a lesser role in its pathophysiology.

In addition to the serotonergic system, dopamine has also been implicated in the pathophysiology of OCD. The administration of dopamine agonists generally leads to exacerbated obsessive-compulsive symptomology, while dopamine inhibitors are used to some extent as augmentation therapy in OCD and other related disorders. Since dopamine signalling in the direct and indirect pathways of the CSTC⁴ circuitry regulates signal propagation therein, it stands to reason that altering dopamine actions, could lead to symptom alleviation by addressing hyperactive pathways in the CSTC circuit. However, given that dopamine receptor blockers are overly ineffective as monotherapy, highlights another important mechanism underlying obsessive-compulsive symptomology, i.e. disruption in the balance between serotonin in dopamine. In fact, OCD is commonly referred to as a condition of hyposerotonergia [35, 54]. On a neurobiological level, serotonin and dopamine can be regarded as opponents of each other to some extent. Whereas dopamine and the neural activity thereof is often associated with approach behaviour, reward learning, reward-seeking and goal-directed engagement towards net positive goals, serotonin acts as a suppressor of such actions, encoding cognitive process that cause avoidance and behavioural inhibition, preventing unfavourable outcomes [30]. Thus, apart from the repetitive behaviours characteristic of OCD being associated with a bias in favour of the direct pathway, a relative decrease in the serotonin-dopamine balance also plays a major role. This would likely explain the known anti-compulsive action of high dose selective serotonin reuptake inhibitor (SSRI)⁵ treatment [142].

¹ cortico-striato-thalamo-cortical

² serotonin

³ obsessive-compulsive disorder

⁴ cortico-striato-thalamo-cortical

⁵ selective serotonin reuptake inhibitor

2.3 The cognitive theory of OCD

While the previously discussed efforts to elucidate the proposed neurobiological constructs of OCD, did well to broaden our understanding of the condition, the mere fact that the condition is phenotypically heterogeneous also points to the involvement of highly distinct cognitive processes in the manifestation of the various symptoms. Currently, several theories aim to explain the complex cognitive ‘fingerprints’ of the different compulsive symptoms observed in OCD¹. Primarily founded on executive functioning and cognitive flexibility, such theories include perspectives on differences in task-switching, set-shifting, threat estimation, inflated responsibility, and dysfunctional habit regulation, among others. Since the present investigation is focused on studying the potential associations between compulsive-like behavioural persistence and cognitive flexibility, brief attention will now be afforded to these.

2.3.1 A brief background on executive functioning and cognitive flexibility

Proper executive functioning allows an individual to make decisions to obtain a desired goal. It entails the capacity to think about an action, and to regulate reactions before acting upon certain goal-provoking stimuli [29, 85]. Further, executive functions are also responsible for regulating complex processes pertaining to everyday life, including impulse control, attention, working memory, organising, judgement, decision making, planning, and other functions that also require cognitive flexibility. As an extension of executive functioning [29, 55, 85, 126], cognitive flexibility can be defined as the ability to selectively switch between mental processes to enable an appropriate behavioural response. In constantly changing environments, cognitive flexibility is crucial for our ability to adjust our behaviour according to different outcomes [12, 29]. Several assessments are used to evaluate cognitive flexibility. These include tests of task-switching ability, response control [29, 114], and assessments of goal-directed vs. habitual engagement. These will be afforded some attention in the following paragraphs.

¹ obsessive-compulsive disorder

2.3.2 Task switching

Task-switching (or set-shifting) allows an individual to disengage from a previously executed task and shift his/her attention and executive processes to a new, unrelated or loosely related task which has gained a higher priority in the interim. Thus, intact task-switching ability ensures that a new course of action can dominate over other less important tasks [12, 29]. On a neurobiological level, the PFC¹ in conjunction with the BG² plays a pivotal role in task-switching [56]. Normal task-switching ability is fundamental for cognitive control and is therefore used to measure cognitive flexibility. The rigid mental acts and behaviours seen in patients with OCD have been linked to impaired task-switching ability, since patients often seem to struggle to switch their attention from compulsive engagement to other functionally and occupationally more important tasks, while often being aware of the irrationality of their excessive behaviour to some degree [11]. Importantly, from what we know, such deficits in task-switching ability seem to span only the contextual framework of the obsessive-compulsive theme [102]. In other words, OCD³ patients do not present with impairments in task-switching in general; rather, they find it difficult to disengage from compulsive rituals and behaviours particular to their symptom presentation [56, 114].

2.3.3 Starting, stopping or both – a matter of excessive behavioural engagement

Response inhibition enables an often-dominant response to be deliberately resisted or suppressed to generate a contextually relevant response, for example resisting to check your phone if repeated but not important notifications keep coming through, rather than staying engaged in an important conversation [29, 100]. Inadequate response inhibition, believed to be one of the main propagators of compulsivity [100, 102], is closely interlinked with motivationally driven outcome processing, which is not only necessary to facilitate reward-directed behaviours, but also to protect from potential harm. For example, when a certain context suggests that a potential threat may be expected, preventative behaviours such as checking or washing are motivated. In turn, these actions typically engender negative feedback which terminates the ‘security-motivated’ action. However, OCD patients seem to perform

¹ prefrontal cortex

² basal ganglia

³ obsessive-compulsive disorder

such behaviours in an excessive and repeated manner, suggesting insensitivity to motivational feedback and difficulty in inhibiting their behavioural responses [147]. Against this background, two hypotheses aim to explain OCD, i.e. that compulsivity may be a condition of excessive starting (action initiation), or conversely inadequate stopping ability (action termination) [147, 148]. As for being a starting problem, it is proposed that threat-signalling stimuli trigger certain concerns too intensely, which causes the level of behavioural excitation to be overly strong; this can be described as threat-hypersensitivity, leading in turn to excessive performance of security motivated behaviours [65, 129]. As a stopping problem, it is proposed that potential threats may be processed according to its actual level of severity. However, once activated, related behavioural engagement cannot be terminated, which points to inadequate outcome-feedback insensitivity [65, 127].

Both theories are currently the conceptual basis of continuing OCD¹ research; indeed, it is likely that neither can be proven in isolation, but rather that they both contribute in unique ways to the manifestation of certain phenotypes of OCD symptomology [43, 65, 99, 140]. This is especially true, since not all obsessive-compulsive phenotypes are (seemingly) associated with themes of threat, i.e. symmetry and ordering.

2.3.4 Goal vs. habit in OCD

Another cognitive theory proposed to explain compulsivity is based on the study of how habit can contribute to dysfunctional—or in this case, compulsive—goal-directed action-outcome engagement [50, 51, 132].

Habitual actions are extensively practiced and automated behaviours which are driven by prior established stimulus-response associations. These behaviours are relatively independent of the value of the outcome and are commonly elicited by specific environmental stimuli [16, 49, 131, 140]. Habits can arise when there is either a failure in goal-directed control or when simple responses are over-trained or when habits simply offer ideal responses to commonly encountered choices or other recurring daily functions [49]. Taken from above, the balance between goal-directed action and habit can be explained by the ‘dual system theory’ which posits that either goal-directed or habitual actions can support a certain course of action in response to environmental

¹ obsessive-compulsive disorder

demands. When behaviours are performed in a goal-directed manner, any action which is performed is *aimed* at either achieving a desirable goal or avoiding an undesirable outcome. However, when a specific action is performed multiple times, the habitual system can begin to dominate, as the action begins to be performed in an automated fashion. Both systems are necessary for our optimal performance. Whereas goal-directed responses allow us to engage in highly complex and often novel tasks, the habitual system facilitates the execution of repetitive, generally useful behavioural routines, e.g., opening the curtains upon waking, brushing teeth before bed, locking the doors of our homes at night, and packing lunch before work. Though important, these tasks should be performed every day without hesitation and hence should ideally require little cognitive processing. It is in this context where habitual behaviours are thought to be useful, that is to say that important behaviours which retain both long term desirability and stability are carried out with minimal cognitive effort. Importantly, and in extension to the explanation of cognitive switching, our ability to flexibly switch between the goal-directed and habitual systems enables us to make successful occupational and other relevant decisions. However, under certain circumstances, the habitual system can contribute to increased behavioural inflexibility [16, 51]. For example, in OCD¹, flexible goal-directed action engagement is compromised, while the habitual system exerts dominance [51, 52, 132, 140], possibly accounting for the rigid performance of very specific behavioural routines in OCD patients.

2.3.5 Compulsivity vs motor rigidity – a matter of cognitive involvement

Repetitive behaviour, as also observed in OCD, is a salient component of a variety neuropsychiatric and neurological disorders. The term ‘repetitive behaviour’ however includes a broad range of repetitive phenotypes and thus groups together behaviours that are relatively heterogeneous in presentation. Thus, by categorising these behaviours into lower-level basic motor, and higher level cognitive repetitive behaviours, we may highlight important differences between the various forms of behaviour [80, 81, 97]. This will be illustrated here against the background compulsive symptomology.

¹ obsessive-compulsive disorder

In addition to the involvement of the direct and indirect behaviourally gating pathways within the CSTC¹ circuitry, the cortico-striatal loops can also functionally be divided into distinct, parallel macro- and micro feedback circuits. The macro-circuits, with its main cortico-striatal inputs, are typically grouped into the sensorimotor circuit, the associative (or cognitive) circuit, and the limbic circuit. Micro-circuits on the other hand subserve certain functions in the broader functional domain.

Within the sensorimotor circuit, the motor and oculomotor loops (micro-circuits), innervate the motor- and pre-motor cortex and regulate different aspects of motorised movement, and can thus be regarded as 'lower-order' functions since motor behaviour itself is mostly devoid from a cognitive construct. On the other hand, the associative circuit has dorsolateral prefrontal micro-circuits that innervate the DLPFC²; these primarily control cognitive functions. The limbic circuit, which is implicated in emotional-motivational behavioural control, is micro-regulated by lateral orbitofrontal and anterior cingulate micro-circuits. These innervate the lateral OFC³ and ACC⁴, respectively [80, 81].

Interestingly, disruption in any of these macro- and micro-circuits can result in stereotyped or repetitive behaviour. Considering the combination of motor and cognitive symptoms in OCD⁵, the question arises whether psychiatric illnesses like OCD involve both lower and higher level cortico-striatal processing [17, 73, 106]. Also, it can be asked whether the compulsive symptom expression arises from rigid cognitive routines, or vice versa. The answers to these questions are likely yes, since the characteristic presentation of 'goal-directed' motor rigidity in OCD, e.g. excessive door locking, ordering, or cleaning, though it can be conceptualised as a restricted behavioural pattern, arises from an underlying cognitive construct, e.g. action-outcome motivation or inflated fear or anxiety specific to a certain theme. Indeed, the phenotypic presentation of obsessive-compulsive symptoms suggests an impaired ability to modify both aberrant cognitive processes and the top-down influence thereof on the execution of associated motor behaviour [21, 22, 39, 73, 75, 83, 106, 116]. This

¹ cortico-striato-thalamo-cortical

² dorsolateral-prefrontal cortex

³ orbito-frontal cortex

⁴ anterior cingulate cortex

⁵ obsessive-compulsive disorder

broadly distinguishes compulsive motor routines, rigid or otherwise, from the repetitive behaviours seen in many other conditions, i.e. Tourette's disorder, autism spectrum disorder and attention-deficit hyperactivity disorder.

2.4 The treatment of OCD

First line treatment for OCD includes pharmacotherapy or specialised psychotherapy, the former usually comprising SSRIs¹ and the latter cognitive behavioural therapy (CBT)². These can be used as mono-therapeutic interventions, though a combined approach is often shown to be superior [76, 110, 120].

2.4.1 Pharmacotherapy

In the early 1980s the TCA³, clomipramine, showed promise in the treatment of OCD⁴ [130]. Primarily acting as a serotonin reuptake inhibitor (SRI)⁵, its effectiveness in treating compulsivity sparked research into the role of the serotonergic system in the condition and research to conceptualize OCD as a condition characterised by hyposerotonergia [76]. However, it's overly unfavourable side-effect profile, which includes weight gain, sedation, dry mouth, constipation, and cardiotoxicity [110, 120], limits it's use in the modern era. Today, highly SSRIs⁶ are the mainstay of treatment. SSRIs, being potent inhibitors of the serotonin transporter (SERT)⁷, have a preferable side-effect profile to TCA-class drugs and can be used in children and adults in high doses for chronic periods of time without significant detrimental effect. Among the different SSRIs, no evidence exists to show that one compound is overly superior to another [46, 110]. Importantly, for SSRIs to be maximally effective, they should be used without interruption in high doses for a minimum period of 10 – 12 weeks [46, 76, 110]. Still, standard guidelines for the pharmacological treatment of OCD proposes the consecutive use of three SSRIs if one or more of the compounds fail to induce a therapeutic effect. Only once all monotherapeutic SSRI options have been exhausted, i.e., after a third trial of either changing the compound or increasing the dose of the

¹ selective serotonin reuptake inhibitors

² cognitive behavioural therapy

³ tricyclic antidepressant

⁴ obsessive-compulsive disorder

⁵ serotonin reuptake inhibitor

⁶ selective serotonin reuptake inhibitors

⁷ serotonin transporter

currently used SSRI, second-line pharmacotherapy is considered. This includes augmentation with low-dose anti-dopaminergic agents, i.e. haloperidol or risperidone [42].

2.4.2 Cognitive behavioural therapy

CBT¹ refers to a group of interventions that are based on the assumption that mental disorders are driven to a great degree by cognitive components or—by implication—that dysfunctional cognitive processing underlie behavioural problems and emotional distress. Therefore, the aim of psychological therapy is to alter said dysfunctional processing so as to improve symptoms [67]. Although often used when pharmacotherapeutic trials fail, CBT is also used as a first-line therapeutic strategy, or in combination with SSRI therapy [74, 92, 121].

CBT² generally involves two components, i.e., behavioural intervention in the form of exposure and response prevention (ERP)³, and cognitive restructuring or cognitive therapeutic (CT)⁴ strategies. ERP and CT can be utilized either jointly or as separate monotherapeutic interventions [66, 103]. ERP refers to the prolonged and repeated exposure of patients to stimuli that usually give rise to their obsessional fear. Said exposure is followed by response prevention in the form of self-restraint from engaging in neutralising compulsive rituals. Patients are therefore compelled to remain in an anxiety-provoking situation until habituation occurs. The idea is that response prevention conditions patients to the realisation that the obsessional fears they develop, are not truly threatening and that a sense of context-related anxiety will subside on its own regardless of the compulsive ‘neutralising’ ritual not being executed [2, 66, 121]. On the other hand, CT does not involve exposure. Rather, its goal is to correct the dysfunctional thoughts and beliefs that underlie obsessions by means of dialogue and education. Patients are gradually assisted to realise that their thinking patterns are dysfunctional. While the general objective of CBT is to improve patient functioning, only a few clinicians are trained to perform CBT, while the intervention

¹ cognitive behavioural therapy

² cognitive behavioural therapy

³ exposure and response prevention

⁴ cognitive therapy

itself is highly time-consuming. Further, since patients often experience therapeutic sessions as stressful and triggering, adherence is less than adequate [2, 66, 67].

2.4.3 Treatment resistance

Irrespective of the approach followed, treatment resistance remains a clinical dilemma [6, 110, 120]. Only 40-60% of patients show a satisfactory response which leaves a significant percentage of patients who are non-responsive. Pharmacotherapeutic treatment resistance in OCD¹ can be defined as not responding to or achieving a satisfactory level of improvement after at least two trials with SSRI² treatment at the maximum dose tolerated [120]. Anti-obsessive-compulsive treatment response is not generally defined as full remission from the symptoms, but rather as a decline in clinical symptom scores. Typically, illness severity and subsequent treatment-induced improvement is measured using the Yale-Brown Obsessive-Compulsive Scale (Y-BOCS)³ [6, 69]. According to the International Treatment Refractory OCD⁴ Consortium, successful treatment is defined as a reduction of 35% or more in the baseline Y-BOCS score [5, 6, 69]. As alluded to before, several augmentation-, or switching-strategies are employed in the case of treatment resistance, e.g. augmentation with anti-dopaminergic agents. However, these also show modest efficacy, being useful in approximately 30% of treatment resistant patients [66]. Several drugs that enhance the serotonergic activity of SRIs⁵/SSRIs⁶ have been considered as augmentation strategies for OCD treatment. These include L-tryptophan, buspirone, fenfluramine and clonazepam [5, 94], all characterised by inconsistent efficacy. Recently, anti-glutamatergic agents gained interest since some pre-clinical and clinical data suggest that OCD patients might present with excessive levels of extra-synaptic glutamate [110]. Hence, the glutamate antagonists, memantine, ketamine and riluzole have been investigated as augmentative strategies in the treatment of resistant OCD [5, 110]. As a last resort, neurological therapy, e.g.,

¹ obsessive-compulsive disorder

² selective serotonin reuptake inhibitor

³ Yale-Brown Obsessive-Compulsive Scale

⁴ obsessive-compulsive disorder

⁵ serotonin reuptake inhibitors

⁶ selective serotonin reuptake inhibitors

electroconvulsive therapy (ECT)¹ and deep brain stimulation (DBS)² can be considered [46, 94, 120].

2.5 A new way forward – understanding obsessive-compulsive symptom dimensions as unique targets for therapeutic intervention

2.5.1 Evidence for neuroanatomical differences

Given the heterogeneity of obsessive-compulsive symptomology, several functional and structural connectivity studies have been conducted to investigate the possibility that different symptom phenotypes might be founded upon unique biological constructs [10, 40, 115]. Although the data remain somewhat inconclusive, some advancements in understanding were made. For example, contamination/washing symptoms show a greater degree of activation in among others, brain areas that are involved in fear processing, e.g. the amygdala [91, 108, 123, 136]. In patients diagnosed with symmetry/ordering symptoms, it is likely that smaller sensorimotor [59], but larger medial frontal-cortical and anterior cingulate cortex volumes might be prominent [101, 133]. Last, harm obsessions and neutralising compulsions are associated with smaller amygdala size [59], but increased temporal and orbitofrontal cortical activity [26].

Although such neuroanatomical separation of different obsessive-compulsive symptom phenotypes has not yet translated into alternative and targeted treatments, it assists psychiatric research to identify potentially novel neurocognitive domains that might represent promising therapeutic targets. For example, that contamination/washing OCD³ responds better to ERP⁴ compared to covert obsession (see below; [20, 31, 44, 45, 89, 111]), points to a unique role for fear-related outcome-feedback processing in the former phenotype; this notion is supported by findings relating to pronounced amygdalar activation in this cohort of patients, as alluded to above. Thus, separation of obsessive-compulsive phenotypes on the level of neurobiology, informs research into shared psychobiological processes between OCD

¹ electroconvulsive therapy

² deep brain stimulation

³ obsessive-compulsive disorder

⁴ exposure and response prevention

and other neurocognitively-related conditions. This in turn might improve our overall understanding of the condition and influence current treatment approaches.

2.5.2 Evidence for neuropsychological differences

A dimensional approach might also help us to understand the intricate processes that potentially contribute to the manifestation of different obsessive-compulsive symptoms. In extension, the conceptualisation of targeted treatment plans based on the neuropsychological profiles of patients could also result in more effective therapeutic outcomes [60, 72, 105]. In line with evidence showing neurobiological differences between different obsessive-compulsive symptom phenotypes, data also point to distinct cognitive differences. For example, patients suffering from contamination/washing OCD were found to perform better in tests of response inhibition [112] and episodic and working memory [60], compared to patients with checking rituals. Conversely, in other studies, contamination/washing OCD sufferers performed worse than those with symmetry/ordering OCD in tasks related to attention, visuospatial memory and set-shifting [105]. Symmetry/ordering OCD patients in turn seem to perform poorly in tasks assessing visuospatial and verbal memory [72], and present with impaired decision-making [38], set-shifting, and response inhibition abilities [60, 82].

While an exhaustive list of these and other findings related to similar studies falls beyond the scope of the current dissertation, it suffices to say that patients are not similar in terms of neuropsychological performance, which begs the question to what extent underlying cognitive differences contribute to and maintain compulsive behaviour. The answer might be elusive since neuropsychological disturbances are characteristic of many psychiatric disorders and comorbidities likely also cloud the interpretation of data. To this extent, pre-clinical models of naturalistic compulsivity may provide a useful avenue for investigation, given that animals selected for certain behavioural phenotypes can be studied against the background of specific research questions. For example, in this work we will select animals according to different levels of stereotypical behaviour expression [143]. We will then set out to determine whether different behavioural cohorts of animals would present with distinct patterns of cognitive performance as measured in two validated rodent tests. Such research may be valuable to not only distinguish not only between 'normal' and 'abnormal' behaviour,

but also shed light on the cognitive mechanisms that may trigger, promulgate and sustain persistent behavioural phenotypes like compulsivity.

2.5.3 Evidence for unique treatment response

Following from the above, increasing evidence has been presented over the past few decades that different obsessive-compulsive phenotypes respond uniquely to various treatment approaches [61, 93]. For example, patients suffering from contamination/washing OCD¹ respond better to ERP² [20, 31, 44, 45, 89, 111] than to pharmacological treatment [113, 125]. Conversely, symmetry and ordering OCD and covert obsessive thoughts responds more to first line SSRI³ therapy, but less so to ERP [61, 79, 88, 125]. In contrast, safety/checking OCD responds well to both ERP [31, 44, 45, 89] and SSRIs [79]. Hence, considering that a one-treatment-for-all approach seems inadequate and improbable, a better understanding of the unique psychobiological perturbations that may contribute to and underlie the various obsessive-compulsive (OC)⁴ symptom phenotypes, would be important. Indeed, this may contribute to markedly improved treatment [89].

2.6 Pharmacologically bolstered cognitive processes – a new direction for the treatment of OCD

2.6.1 Synaptic vesicle glycoprotein 2A and levetiracetam – A potentially novel way forward

Synaptic vesicle glycoprotein 2 (SV2)⁵ is an integral 12-transmembrane glycoprotein which is found in most central synaptic neuron terminals [13, 27, 64, 138]. Three SV2 genes have been identified: synaptic vesicle glycoprotein 2A (SV2A)⁶, 2B (SV2B)⁷ and 2C (SV2C)⁸ [96]. SV2A is the most abundant and widely expressed in the brain. It is abundantly expressed in most brain areas, except for the trigeminal and facial nerve nuclei [64]. Cerebellar, hippocampal and cortical observations demonstrated SV2A

¹ obsessive-compulsive disorder

² exposure and response prevention

³ selective serotonin reuptake inhibitor

⁴ obsessive-compulsive

⁵ synaptic vesicle glycoprotein 2

⁶ synaptic vesicle glycoprotein 2A

⁷ synaptic vesicle glycoprotein 2B

⁸ synaptic vesicle glycoprotein 2C

further to be located at both excitatory and inhibitory synapses [14, 36, 64, 96, 138]. The proposed molecular action of SV2A in nerve terminals is that it regulates and modulates synaptic vesicle exocytosis, i.e. neurotransmitters release. Further, SV2A also plays a role in the homeostasis and transportation of other synaptic vesicle constituents such as calcium and adenosine triphosphate (ATP)¹. In particular, SV2A interacts with the presynaptically-expressed protein, synaptotagmin, a calcium sensing protein, which in turn regulates calcium-dependant neurotransmitter release [36, 104]. SV2A knockout (KO)² mice present with presynaptic calcium accumulation which destabilizes synaptic circuits due to abnormal neurotransmitter release. Further, Venkatesan, et al. [139] demonstrated SV2 KO animals to present with decreased frequency and amplitude of spontaneous inhibitory postsynaptic currents and an imbalance between glutamatergic and GABA³ergic neurotransmission. Since GABA and glutamate play pivotal roles in CSTC⁴-functioning, this finding might provide a valuable research construct for future investigation, especially in light of the fact that current drugs targeting these systems in isolation, is overtly ineffective for the treatment of OCD⁵ [69, 90, 120, 136, 139].

SV2A⁶ was identified as the molecular and therapeutic target for levetiracetam (LEV)⁷, a relatively novel antiepileptic drug [13, 96, 138]. This pyrrolidine derivative is also researched for the off-label treatment of non-epileptic conditions, i.e. neuropathic pain, migraine, movement disorders and even certain psychiatric disorders, e.g. BDD⁸ and bipolar disorder [27, 37, 41]. In one clinical trial, most patients suffering from BDD tolerated LEV well, while 60% of the study group showed symptom improvement [53, 87]. Several putative mechanisms by which LEV may be effective in BDD and other psychiatric conditions, have been proposed. For example, by interacting with SV2A, LEV inhibits presynaptic calcium signalling, thereby reducing neurotransmitter release into the synaptic cleft [53, 87] and stabilising neuronal firing. Importantly, especially with respect to the potential effect it might have on compulsive-like behavioural

¹ adenosine triphosphate

² knockout

³ gamma-aminobutyric acid

⁴ cortico-striato-thalamo-cortical

⁵ obsessive-compulsive disorder

⁶ synaptic vesicle glycoprotein 2A

⁷ levetiracetam

⁸ body dysmorphic disorder

expression, LEV also modulates abnormal motor movement by decreasing hypersynchrony, i.e. excessive rhythmic neuronal discharge, of the neurons in the basal ganglia [27].

For several years, research has attempted to achieve a more productive and efficient human mind. Expanding our mental capacity for attention, decision-making, planning, alertness, memory, information integration and cognitive flexibility, could arguably contribute to an overall improvement in the wellbeing of the human race. Integral to this research theme is the search for novel drugs that may be of interest as so-called cognitive enhancers or nootropic drugs. To this extent, LEV has been trialled in numerous clinical studies to determine if it may be of benefit for the treatment of cognitive impairment often observed in patients suffering from psychiatric illness [8, 53, 87]. In patients suffering from epilepsy, LEV resulted in improved attention and verbal fluency, findings that were not attributable to its concomitant successful seizure control [48, 68, 77, 109]. The effect of LEV on these functions also seem to be superior to those of other established anti-convulsants, e.g. carbamazepine [95]. On an executive level, LEV has also been associated with improved cognitive performance in tasks related to goal-outcome action planning [87], while it also improved psychomotor processing speed [62, 95], visual short-term memory [24], and working memory [86, 95]. In children with autism spectrum disorder [117] as well as adults suffering from Alzheimer's disease [15], LEV also improved performance across various cognitive measures, indicating a unique effect of LEV¹ on cognition which is not related to its established clinical use as an anti-epileptic drug.

Thus, in this work, we will explore the potential association between motor stereotypy and a measure of cognitive rigidity in deer mice (*Peromyscus maniculatus bairdii*). Given the potential of LEV as a cognitive enhancer, we will then assess its effect on the said relationship in order to shed light on the potential underlying psychobiological architecture of behavioural repetition in this species.

¹ levetiracetam

2.7 The deer mouse model of obsessive-compulsive disorder

The use of animal models is of both theoretical and translational importance in exploratory studies of human-related behavioural phenomena. Not only can animal research help us to understand the etiopathological mechanisms of the corresponding human condition, but it can also contribute to efforts aimed at developing improved treatment strategies [9, 28, 143]. Here, we will briefly review the deer mouse model of OCD¹, the model organism that will be employed in the present work.

2.7.1 General background, validity, and translational relevance

Much work has already been done to validate spontaneous behavioural repetition in deer mice. e.g. stereotypical motor behaviour, as an animal model for OCD [144] and as such the model is now regarded as one of the foremost naturalistic animal models of compulsive-like behaviour [7, 98].

In short, the validity of the deer mouse behavioural phenotype studied in this work as a model of compulsivity, is founded on the spontaneous presentation of stereotypical behaviour, that is repetitive vertical jumping/somersaulting and pattern running (clockwise and counter clockwise cage revolutions) performed to excess. In some cases, a single mouse can perform more than 2000 backflips in a 30-minute period. The manifestation of these behaviours varies in intensity between animals within a standard laboratory-housed colony, and individuals can broadly be classified into low (N)²- and high (H)³- stereotypical groups on the basis of their behaviour [143]. These time-consuming behaviours mimic the rigid repetitive motor actions observed in human OCD⁴ and form the basis of the face validity of the model, i.e. emulating the symptomology of the human condition [137]. It was also demonstrated that chronic (21-day) intraperitoneal (IP)⁵ treatment with 10 and 20 mg/kg/day fluoxetine, an SSRI⁶, significantly decreased the expression of spontaneous stereotypy in stereotypical deer mice [78]. Furthermore, such a response was not achieved with desipramine, thus being an outcome congruent with clinical literature. In a follow-up study [143], it was

¹ obsessive-compulsive disorder

² low-stereotypical

³ high-stereotypical

⁴ obsessive-compulsive disorder

⁵ intraperitoneal

⁶ selective serotonin reuptake inhibitor

demonstrated that the response of deer mouse stereotypy is furthermore selective to chronic, but not sub-chronic treatment with high-dose orally administered SSRIs. The results of these two investigations form the basis of the robust predictive validity of the model, i.e. resembling the response of clinical OCD to pharmacological manipulation [137]. Furthermore, Wolmarans, et al. [143] presented evidence that H deer mice present with a significant decrease in striatal SERT¹ expression compared to the N controls. The latter results are in line with clinical findings [63]. Taken together with the findings of Korff, et al. [77], robust construct (i.e. neurobiological similarity or overlap) validity is also implied.

2.7.2 Spontaneous stereotypy – motor rigidity with or without cognitive involvement?

Repetitive behaviour is a natural phenomenon that is observed in many animal species, including rodents, e.g. grooming, barbering, and digging. When observed in a laboratory, such behaviour could be reminiscent of a number of human conditions, including autism, Tourette's disorder, attention-deficit/hyperactivity disorder (ADHD)², and compulsivity [81]. However, what distinguishes compulsivity from the other conditions mentioned, is the degree of cognitive involvement and goal-directedness underlying the expression of the repetitive routines. While the repetitive behavioural patterns that characterise the former conditions are mostly devoid of an underlying cognition, i.e., patients do not necessarily have insight into why or how such behaviour manifests, patients with OCD express repetitive behaviours with a goal-directed outcome in mind, e.g., *to decontaminate*, *to lock*, *to prevent harm*, *to order*, *to avoid eternal damnation*, etc. It is irrelevant whether such behaviours have *actual value* in a real-world setting—for the patient, they are goal-directed. In other words, what sets compulsivity apart from other repetitive behaviours, is that compulsivity represents planned responses in the majority of cases [3, 146]. While the time-consuming and waxing-waning presentation of spontaneous stereotypy, i.e., repetitive jumping and pattern running, in the deer mouse model seems akin to the persistent expression of compulsivity in humans [143], the question remains whether such behaviour is founded exclusively upon a motor construct, or whether animals express such behaviour in response to an underlying cognitive trigger. Hence, since animals cannot

¹ serotonin transporter

² attention-deficit/hyperactivity disorder

be interrogated to ascertain why and how such behaviour are expressed, research can only gain such insight by making indirect inferences from studies that are aimed at divulging the roles of other potential processes that may have cognitive underpinnings. Here, our point of departure is that spontaneous stereotypy indeed has a cognitive anchor. We say so for the following reasons: First, the waxing and waning nature of stereotypy throughout a normal dark cycle, possibly points to outcome-feedback regulation being involved. To explain, it stands to reason that if stereotypy in the deer mouse was purely a manifestation typified by impulse control deficits or inadequate motor control, one would expect such behaviour to persist, irrespective of time course [80, 128]. Secondly, in a ‘three-animal-per-arena’ social paradigm, N¹ and H² mice appraise another differently in social contexts, meaning that N animals marginalise H animals, and vice versa, irrespective of the cohort structure used, e.g. NNH, HHN [145]. Hence, deer mice seem to perceive stereotypy in others and can distinguish their own behaviour from that of an animal of another cohort. To appraise, value and interpret what we see, require a significant degree of cognitive processing, a skill which is arguable showcased by deer mice in social contexts. That said, this is largely still speculative and continued studies are underway to investigate other potential cognitive underpinnings in deer mouse stereotypy. To this end, this work will also aim to address this question. Specifically, we will attempt to answer whether stereotypy is characterised by cognitive inflexibility, and we will do so by assessing N and H deer mice in a validated rodent test of cognition, i.e., the T-Maze [32, 33, 124].

2.7.3 Perspectives on the current investigation – A brief background on the T-maze assessment of cognitive flexibility

Cognitive tests for use in animal studies have been developed to examine different components of behaviours that are reminiscent of clinical neurological and neuropsychiatric conditions, e.g. OCD³ [58]. Here, the potential relationship between spontaneous, compulsive-like stereotypy in deer mice and potential cognitive deficits as reflected by naturalistic arm-choice alternation in a T-maze, will form the conceptual

¹ low-stereotypical

² high-stereotypical

³ obsessive-compulsive disorder

background of this project. T-maze assessment can be used in a number of ways to assess the cognitive ability of rodents [32, 33]. For example, it has been used as a tool to assess cognitive processes related to associative learning and spatial memory, habitual responses, stimulus satiation, and reactive inhibition [124]. Normally, during the first trial of a T-maze test, an animal is placed in the stem of a high-walled T-maze and allowed to habituate. Then, it may enter the maze and choose one of the perpendicular arms of the 'T'. This is repeated several times, after which an alternation score, i.e. the number of consecutive left-right or right-left choices out of the total choices, is calculated. Since rodents have a naturalistic tendency to explore a novel, but safe environment [71], it is proposed that lower alternation scores reflect cognitive aberrations like deficits in memory, persistently dominant motor routines, or habitual engagement. It is likely that the cognitive constructs scrutinised in deer mice, as in this work, will be more related to dominant motor routines and habitual engagement, rather than memory, since all trials (arm choices) made by each animal, will be concluded on a single day with a very brief inter-trial interval. This will be done to assess the naturalistic arm-choice behaviour of deer mice that have been prior selected for different levels of stereotypy. Hence, we aim to establish whether N¹ and H² animals would instinctively present with different instantaneous and non-trained alternating profiles.

2.8 Conclusion to Chapter 2

In this chapter, we briefly reviewed OCD³ against the background of its clinical epidemiology, manifestation, and psychobiology. We further explained some of the most prominent cognitive theories of OCD and how this may contribute in unique ways to compulsive symptom presentation. We then set out to explain how novel treatment targets, aimed at restoring potential deficits in cognitive function, e.g. SV2A⁴, may

¹ low-stereotypical

² high stereotypical

³ obsessive-compulsive disorder

⁴ synaptic vesicle glycoprotein 2A

provide a useful way forward for the treatment of conditions related to cognitive deficits. Last, we closed by briefly summarising the existing literature base on the deer mouse model of OCD. We explained why, in our view, the model suits itself well to the study of cognitive processes and their potential association with compulsive-like motor persistence, and how the model may be fruitful to investigate novel treatments aimed at restoring potentially aberrant cognitive processing.

2.9 References

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3 Journal Article

Spontaneous alternation in low- and high stereotypical deer mice and its response to escitalopram and levetiracetam

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3.1 Introduction

Obsessive-compulsive disorder (OCD)¹ is characterised by obsessions and compulsions [9]. Obsessions can be described as intrusive, unwanted, and often anxiogenic thoughts and urges, e.g. concerns about contamination, that are normally, but not always associated with rigid and repetitive neutralising behaviours (compulsions), e.g. washing routines [1, 12, 18]. Four main symptom phenotypes (excluding hoarding) of OCD are recognised, i.e. contamination/washing, symmetry/ordering, safety/checking or saving, and inappropriate thoughts relating to shameful ideas of a sexual, religious or violent nature; these are associated with covert mental routines, e.g. praying. OCD is generally described as a condition characterised by cortico-striatal hyposerotonergic signalling, since chronic, high-dose selective serotonin reuptake inhibitor (SSRI)² treatment is the mainstay of treatment [20, 53]. However, approximately a third of patients do not respond to first line pharmacotherapy with SSRIs [35], in which case psychotherapy, e.g. cognitive behavioural therapy (CBT)³ and exposure-response prevention (ERP)⁴, administered either alone or in combination with drug treatment, may be useful in up to 50 % of treatment-resistant patients [5, 7].

Interestingly, patients suffering from contamination/washing and symmetry/ordering OCD have been shown to respond relatively well to CBT, but less so to SSRI treatment [36, 37, 45, 50]. While these findings point to some degree of cognitive involvement in OCD, data pertaining to the general cognitive and executive functioning of OCD patients vs. healthy controls, are mostly inconclusive [2, 3, 19, 25, 41]. This can likely be ascribed to different patient inclusion criteria applied between the various investigations. Rather, regarded as a collective, an increasing body of evidence points to unique associations between different symptom phenotypes and specific cognitive alterations [3, 40], which deserve further elucidation to improve current treatment approaches. Indeed, if specific associations between certain cognitive domains and the expression of compulsive motor routines can be identified, it stands to reason that, non-serotonergic drugs that are able to improve cognitive function, may be of great

¹ obsessive-compulsive disorder

² selective serotonin reuptake inhibitor

³ cognitive behavioural therapy

⁴ exposure-response prevention

value in the treatment of some patients with OCD¹. To this end, levetiracetam (LEV)², clinically used as supplementary treatment for epilepsy, has shown some promise [34, 38]. Specifically, LEV resulted in improved set-shifting ability and attention [29, 30, 57]—two cognitive domains that are also affected in OCD [26, 42, 44] —in patients suffering from epilepsy, findings that were not attributable to its concomitant successful seizure control [23, 31, 32, 43, 57]. The influence of LEV on cognitive and behavioural processes, especially that of memory, has also been explored in animal studies of Alzheimer’s disease (AD)³ and epilepsy [16, 17]. Briefly, LEV modulates neurotransmitter release and signalling via its actions on synaptic vesicle glycoprotein 2A (SV2A)⁴, thereby influencing synaptic vesicle exocytosis. Instead of targeting any specific neurotransmitter system in isolation, it is possible that LEV may rather act to restore potential neurotransmitter imbalances [30, 57]; however, this remains to be established.

The use of animal models that varyingly present with naturalistic, repetitive behaviours could be useful to expand our understanding of the potential innate associations between such behaviours and cognitive processes. Here, we applied the deer mouse (*Peromyscus maniculatus bairdii*) model of naturalistic compulsive-like behavioural repetition [54] to establish whether such behaviour is associated with perturbations in cognitive flexibility as assessed by measuring the spontaneous alternation behaviour of mice in a T-maze [15]. We further sought to investigate whether stereotypy and cognitive flexibility would respond to the SSRI⁵, escitalopram (ESC)⁶, and/or LEV. When housed under standard laboratory conditions 35 – 45 % of male and female deer mice develop high-stereotypical (H)⁷ behaviour, and thus the deer mouse model may be well suited to investigate a research question of this nature.

¹ obsessive-compulsive disorder

² levetiracetam

³ Alzheimer’s disease

⁴ synaptic vesicle glycoprotein 2A

⁵ selective serotonin reuptake inhibitor

⁶ escitalopram

⁷ high-stereotypical

3.2 Materials and methods

3.2.1 Animals

Since only 35 – 45 % [13, 54, 55] of deer mice show H¹ behaviour, an initial pool of 124 mice of both sexes (aged 10 – 12 weeks at the onset of experimentation), were randomly sourced without litter or cage bias from the North-West University (NWU)² vivarium for initial stereotypical assessment. From these, 31 normal (low-stereotypical; N³) and 34 H animals were identified ($n = 65$ in total used for experimentation). Henceforth, animals were ear-tagged for identification purposes and group-housed [four same-sex mice per automatically climate-controlled cage; 35 cm (l) x 20 cm (w) x 13 cm (h); Techniplast® S.P.A., Varese, Italy]. Cages were maintained at 23°C, a relative humidity of 55 %, and on a normal 12-hour light/dark cycle (lights on and off at 06:00 and 18:00 respectively) [54]. Food and water (or drug solutions) were available *ad lib* throughout the course of the investigation. Cages were cleaned, and fresh corncob bedding and nestlet paper added, weekly. All aspects of this work were approved by the AnimCare Research Ethics Committee of the NWU (approval no. **NWU-00522-20-A5**).

3.2.2 Stereotypy assessment and selection of N and H animals

All of the initially included 124 mice underwent three separate 12-hour long stereotypy screening sessions during the dark cycle (spaced at least 48 hours apart). This approach with deer mice is necessary to establish a robust average baseline of stereotypical activity [54]. On each given assessment day, animals were moved from the housing environment to the behavioural screening room which is located on the same floor of the vivarium and maintained at the same conditions as the housing room. Each mouse was introduced to its own behavioural testing cage [21 cm (w) x 21 cm (l) x 35 cm (h); Accuscan® Inc., Columbus, Ohio, USA], constructed from clear, translucent Plexiglas®, and fitted with position-detecting infra-red beams. Corncob was provided in quantities enough to cover the floor of the test cages, but not so that it interrupted the scoring of behavioural data. Food and water (or drug solutions) were provided *ad lib* throughout the testing phase. Animals were introduced to the testing

¹ high-stereotypical

² North-West University

³ low-stereotypical

cages by 17:00 and habituated for at least an hour before assessment commenced at the onset of the dark cycle at 18:00. Test cages were cleaned after each screen. N¹ and H² animals (65 animals in total; 31 N; 34 H) were selected based on prior published criteria that account for both the intensity of and time spent expressing stereotypical behaviour during a 12-hour dark cycle [13] (**Table 3-1**; **Figure 3-1**). The 59 animals not selected for further investigation were euthanised according to standard vivarium protocol. After the completion of all rounds of stereotypical classification the selected animals were subjected to T-maze assessment. All screening rounds were conducted under dark conditions.

Table 3-1 Cut off criteria for N and H behavioural expression during a 30-minute bout across both stereotypes [54]; N: low-stereotypical; H: high-stereotypical.

Behavioural Topography	N	H
Pattern running	<150 cage revolutions	>200 cage revolutions
Vertical jumping	<500 vertical beam breaks	>2000 vertical beam breaks

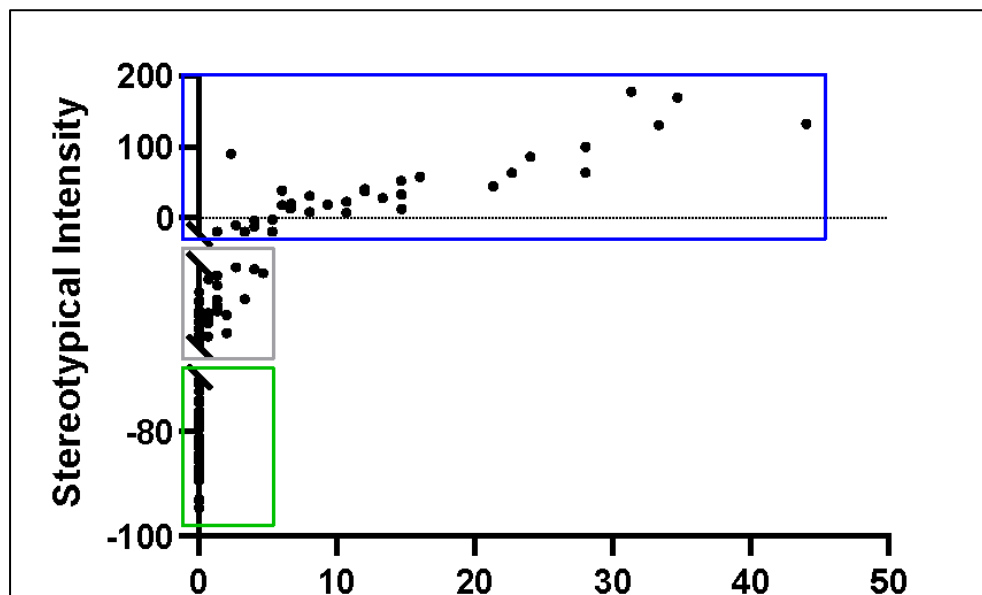


Figure 3-1 Graphical representation of animals selected for N and H behaviour N: low-stereotypical; H: high-stereotypical. H animals enclosed in blue. N animals enclosed in green. Animals not selected for investigation enclosed in grey. Stereotypical intensity is expressed as the % difference between the average of the 9 highest 30-minute stereotypical bouts over all three drug-naïve stereotypy assessments and the respective H criteria for cage revolutions or vertical jumping (Table 3-1). The % time spent expressing H was also calculated over all three drug-naïve stereotypy assessments. Animals could have expressed H behaviour in terms of running, jumping or both.

¹ low-stereotypical
² high-stereotypical

3.2.3 T-maze assessment

The T-maze apparatus used in this study was modified from the design of Gerlai [22] to accommodate the screening of hyperactive deer mice. Briefly, the T-maze consisted of a T-shaped maze constructed of opaque white Plexiglas®. Each of the respective arms (20 cm x 7.5 cm) could be closed off by a manually operated sliding door, while another door closed off a starting box (7.5 cm x 7.5 cm) at the base of the stem [49, 52] (**Figure 3-2**). Further, a removable arm divider was inserted during the first arm-choice trial to enforce a clear left-right decision [15]. As the current investigation aimed to assess the naturalistic alternation behaviour of mice separated according to the degree of motor stereotypy displayed, animals were not pre-trained according to a cue-reward paradigm or habituated to the maze before the assessment [58]. On days when T-Maze testing took place, mice were transferred to the testing room at 18:00 and left to habituate for one hour. Each animal was then individually placed in the start box of the T-maze and left for 30 seconds. The start box door was then raised for the first time which allowed animals to make their first entry into any one of the transverse arms of the maze [15]. Once the arm choice was made, the other arm was immediately closed off and animals were allowed to freely return to the start box on their own time where they resided once again for 30 seconds. The arm divider was now removed. Mice were allowed 14 more arm choices over a maximum period of 30 minutes, with a 30-second start box interval separating each choice [15]. Mice that failed to enter any of the transverse arms within 10 minutes after the onset of assessment, or mice that executed less than eight arm choices within 30 minutes, were excluded from further study and replaced by other animals [13]. Between assessments, animals were gently relocated to their respective home cages and moved back to the housing room, after which the T-maze was cleaned with 90 % ethanol (which was completely evaporated before the next assessment) to prevent any potential olfactory influence on the alternation behaviour of subsequently assessed mice [22]. All assessments were conducted under dim red light (40 lux). Since the first arm entry was regarded as a forced entry, the percentage alternation was calculated as the number of alternate arm choices out of a maximum of 14 arm entries (or the number of arm entries minus one—the first entry).

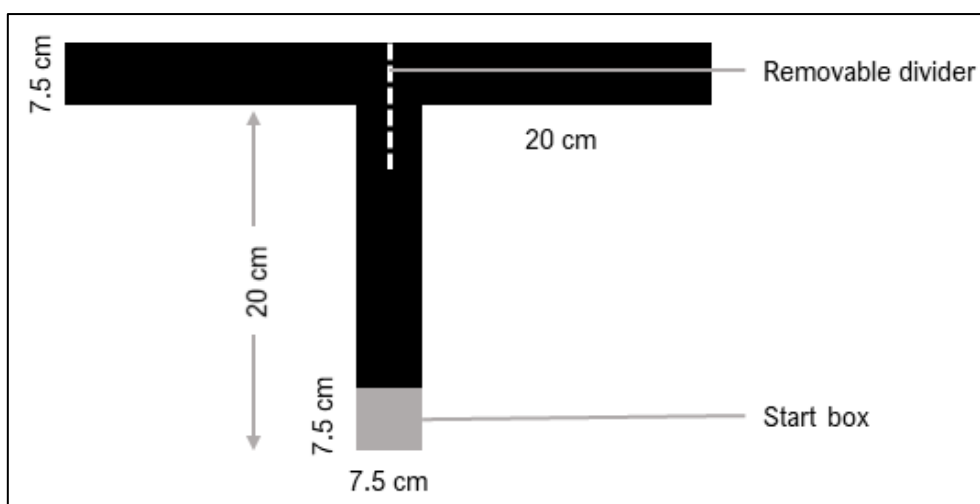


Figure 2-2 Schematic representation of the T-maze apparatus used.

3.2.4 Drugs and study termination

Following completion of the drug-naïve T-maze assessment, animals of each behavioural phenotype were randomly allocated to the different drug-exposure groups, i.e. normal water control, ESC¹ (50 mg/kg/day; [54]), and LEV² (75 mg/kg/day; [46]) (both obtained from BLD Pharm®, Shanghai, China). Final group numbers were as follows: N³ control & ESC: 11 each; N LEV: 9; H⁴ control and ESC: 11 each; H LEV: 12. Groups were as far as possible constituted by an equal number of male and female animals; the number of animals of the different sexes in each group, never differed by more than two. Both drugs were dissolved in the drinking water over 28 [54] days and the fluid intake were measured daily (supplementary data; Addendum A). On the second last night of water or drug exposure, all animals were once again assessed for alternation behaviour in the T-maze as described earlier, while a single post-drug exposure stereotypical assessment was performed on the last night of drug exposure. After this, animals were euthanised.

3.2.5 Statistical analysis

Statistical analyses were performed with GraphPad® Prism® version 9. Linear regression was applied to explore the effect of stereotypical intensity on spontaneous alternation behaviour, both before and after drug-exposure. Kruskal-Wallis Analysis

¹ escitalopram

² levetiracetam

³ low-stereotypical

⁴ high-stereotypical

of Variance (ANOVA)¹, followed by Dunn's multiple comparisons tests were applied to analyse the effect of drug exposure on the highest and average stereotypy scores, as well as the time spent engaging in H² activity, of H animals (expressed as the percentage change from the drug-naïve—or baseline—values). Drug exposure was set as the independent variable. The same analysis was used to analyse the effect of drug exposure on T-maze alternation. Time spent on *no*-stereotypical activity (defined as a 30-minute bout during which less than ten stereotypical movements were recorded) by N³ and H animals was analysed by means of ordinary two-way ANOVA. Here, stereotypical phenotype and drug exposure were set as independent variables. Where main effects were significant, pairwise comparisons of applicable data sets were carried out by means of Welch *t* tests. Statistical significance was set at $p < 0.05$ for all analyses.

3.3 Results

3.3.1 Behavioural categorisation

Spearman's rank-order correlation revealed a significant positive association between the degree of stereotypical intensity and the time spent engaging in H behaviour [$r_s(122) = 0.897$, $p < 0.0001$; **Figure 3-1**]. Thus, from the 124 initially screened animals, 31 N (enclosed in green), and 34 H (enclosed in blue) mice were selected for further investigation. Animals that expressed moderate levels of stereotypy, were excluded from further investigation (enclosed in grey).

3.3.2 Relationship between stereotypical intensity and spontaneous alternation in the T-maze

Figure 3-3 represents the baseline (pre-drug exposure) stereotypical intensity and alternation scores of all 65 animals selected for behavioural investigation. Based on linear regression analysis, we show that stereotypical intensity significantly predicts spontaneous alternation scores in deer mice [$F(1,61) = 4.48$, $p = 0.038$; $R^2 = 0.068$;

¹ Analysis of Variance

² high-stereotypical

³ low-stereotypical

Regression Equation: stereotypical intensity (%) = -1.345 alternation score (%) + 50.73].

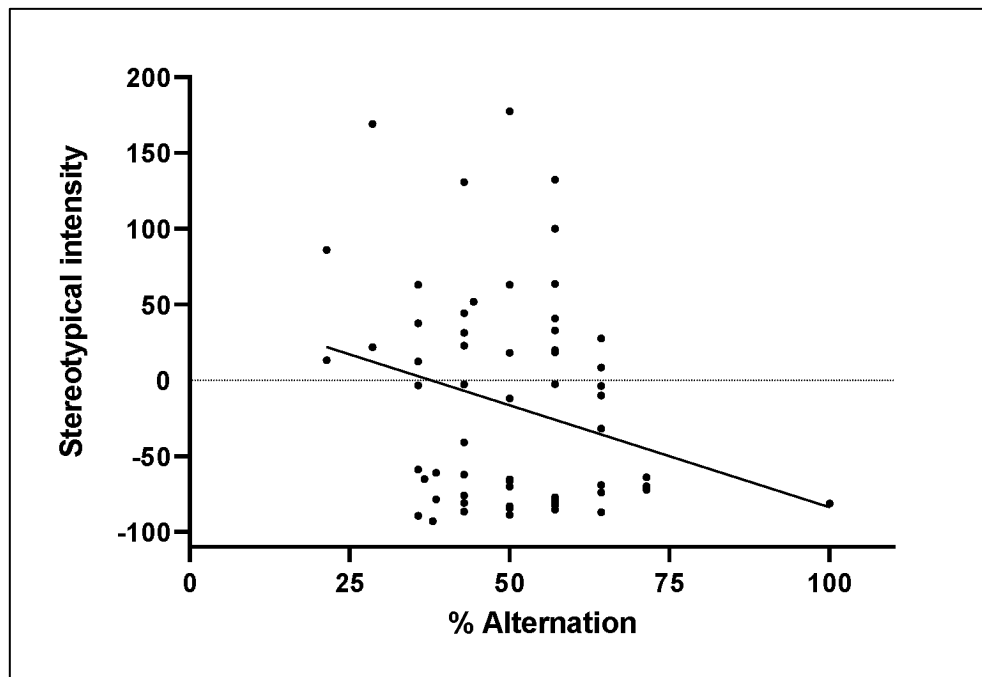


Figure 3-3 Stereotypical intensity vs. spontaneous alternation behaviour in deer mice

3.3.3 Response of stereotypical expression to pharmacological intervention

Figure 3-4 A – C represents the percentage change in the highest (**3-4A**) and average whole night (**3-4B**) stereotypy scores and the time spent expressing H¹ behaviour (**3-4C**), i.e. the number of 30-minute bouts during a dark cycle during which H animals engaged in H behaviour. **Figure 3-4D** represents the percentage change in the time spent engaging in *no*-stereotypical behaviour, i.e. the number of 30-minute dark cycle bouts during which animals expressed less than 10 stereotypical movements. In all cases, the % change reflects the change shown by each animal in the respective scores from the drug-naïve (baseline; pre-drug exposure) to the post-drug exposure values. One H animal in each of the ESC² and LEV³-exposed groups were excluded from statistical analysis, since their post-exposure behaviour reflected scores that were determined to be significantly deviating from the larger cohorts in at least one of the measures reported (as determined by Grubb's test; [24]).

¹ high-stereotypical

² escitalopram

³ levetiracetam

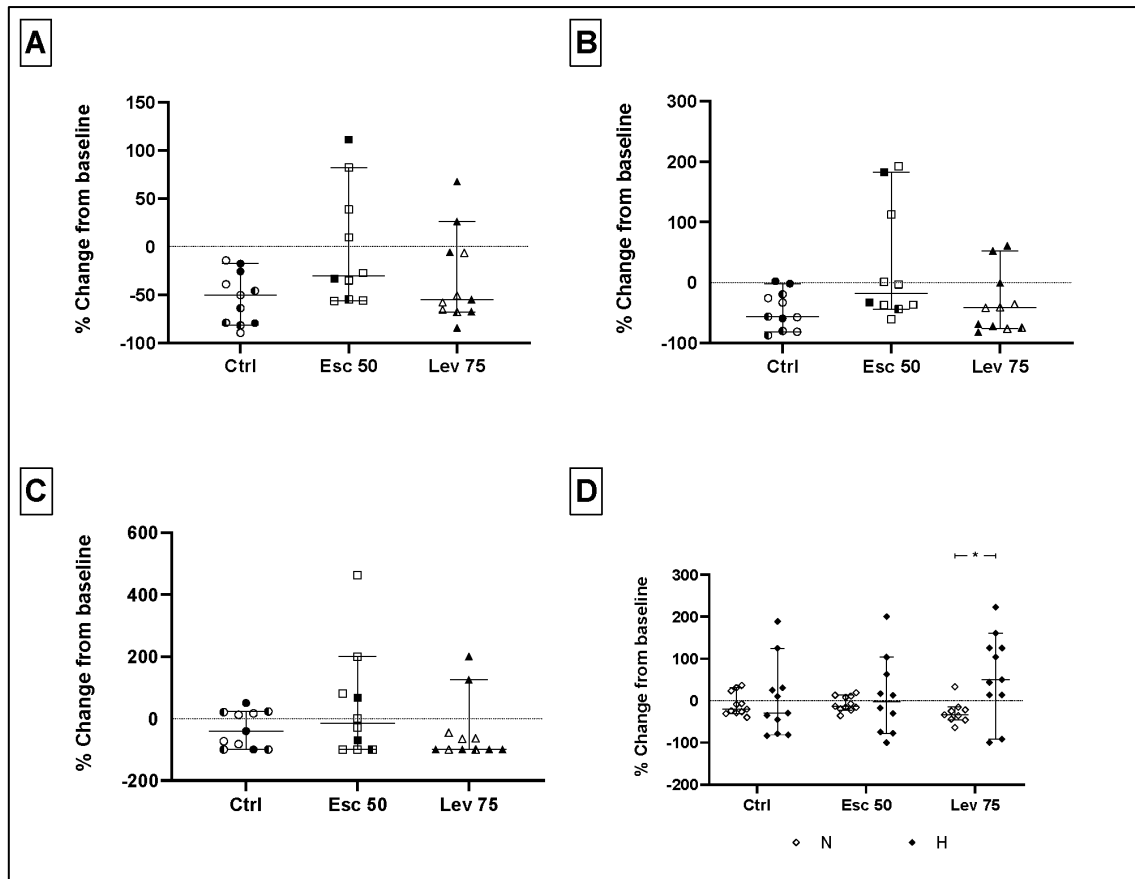


Figure 3-4 Effect of drug exposure on the highest (A) and average whole-night (B) stereotypy scores generated, and the time spent engaging in H activity by H animals. Data represent the % change noted in the post-drug exposure scores from the drug-naïve (pre-drug exposure; baseline) scores of the same animals.(C) (D): Data represent the % change noted from the drug-naïve values in the post-drug exposure amount of time that animals spent on no stereotypical activity. Calculations were based on the highest 9 baseline and 3 post-drug-exposure 30-minute bouts generated (A), and the averages of the 72 bouts generated over all three pre-drug exposure screens and the 24 bouts generated in the single post-drug exposure screen (B, C & D). A – C: Analysed by means of Kruskal-Wallis ANOVA followed by Dunn's multiple comparisons test. D: ordinary two-way ANOVA followed by Welch pairwise t-tests where applicable; * $p = 0.016$. Open symbols: animals expressing stereotypical running behaviour; Closed symbols: animals expressing stereotypical jumping behaviour; Dual symbols: animals expressing both stereotypical behaviours.

Although expression of H¹ behaviour seemed to reduce across all measures in all of the exposure groups (**3-4 A – C**), Kruskal-Wallis analyses failed to reveal significant differences in the median percentage change in baseline scores generated by H animals that were exposed to normal tap water (control; $n = 11$), ESC² ($n = 11$) and LEV³ ($n = 12$) [**3-4A**: $H(2) = 4.57$, $p = 0.102$; **3-4B**: $H(2) = 4.47$, $p = 0.107$; **3-4C**: $H(2) = 2.143$, $p = 0.342$]. However, although phenotype and drug exposure failed to interact significantly with respect to the time spent engaging in no-stereotypical activity

¹ high-stereotypical

² escitalopram

³ levetiracetam

[3-4D; $F(2,57) = 2.01, p = 0.143$], phenotype had a significant main effect on how N¹ and H² animals responded to drug intervention [$F(1,57) = 4.97, p = 0.03$]. A subsequent pairwise Welch's *t*-test revealed LEV³ to significantly increase the time spent in non-stereotypical activity by H, but not N animals [$t(2.8) = 11.78, p = 0.016, 95\text{CI: } 19.8 - 157.4$].

3.3.4 Response of spontaneous alternation in the T-maze to pharmacological intervention

Phenotype and drug exposure did not interact significantly with respect to the post-drug exposure spontaneous alternation behaviour of N and H animals [Figure 3-4; $F(2,57) = 1.19, p = 0.313$]. Further, neither drug exposure [$F(2,57) = 0.54, p = 0.59$] nor phenotype [$F(1,57) = 0.08, p = 0.776$], had a significant main effect on the alternation scores generated.

3.3.5 Influence of pharmacological drug exposure on the relationship between stereotypical intensity and spontaneous alternation in the T-maze

Both of the pharmacological drug exposures employed here, failed to result in linear predictions between the two parameters [ESC⁴: $F(1,19) = 0.63, p = 0.63$; **Regression Equation:** stereotypical intensity (%) = $0.7361 \text{ alternation score (\%)} - 51.45$; LEV: $F(1,18) = 0.14, p = 0.71$; **Regression Equation:** stereotypical intensity (%) = $-0.2223 \text{ alternation score (\%)} - 30.66$]. Although the linear relationship between the two parameters narrowly missed statistical significance in control-exposed animals, such relationship still trended towards the manner of prediction reported at baseline for all animals [$F(1,20) = 3.46, p = 0.078$; **Regression Equation:** stereotypical intensity (%) = $-1.34 \text{ alternation score (\%)} + 23.53$], i.e. that higher stereotypical intensity, predicted lower alternation scores.

3.4 Discussion

The main findings of this work are that 1) higher stereotypical intensity in deer mice significantly and negatively predicts spontaneous alternation scores in the T-maze, 2)

¹ low-stereotypical

² high-stereotypical

³ levetiracetam

⁴ escitalopram

LEV, but not control or ESC exposure, significantly increased the time spent engaging in non-stereotypical behaviour by H¹, compared to N² animals exposed to the same, and 3) that neither of the interventions employed here, affected spontaneous alternation scores in N and H deer mice.

Relationships between compulsive symptom expression in general and specific cognitive impairments, remain unclear. However, findings showing that some symptom phenotypes, e.g. contamination/washing, respond better to cognitive-behavioural interventions, point to a unique involvement of cognitive processes in some forms of OCD³. This is important, since it stands to reason that, novel non-serotonergic pharmacotherapies aimed at restoring said cognitive underpinnings of compulsivity, may be of benefit for some individuals suffering from the condition. Here, we show that stereotypical motor expression in deer mice, previously proposed as a model system of naturalistic compulsive-like behaviour [54], negatively predicts spontaneous alternation in a T-maze (**Figure 3-2**). Lower spontaneous alternation scores generated by rodents in a T-maze are believed to reflect a lower degree of exploratory behaviour which is often, but not without dispute [14, 15, 49], related to impaired cognitive flexibility. To this end, the T-Maze can be used in different ways, e.g. under untrained or trained circumstances, to assess cognitive dysfunction in animals, the choice of which is guided by the experimental question [8, 14, 15, 28, 47]. Since motor stereotypy devoid of direct cognitive involvement is a symptom of several neuropsychiatric disorders, e.g. autism spectrum disorders [4], Parkinson's disease [48], and Tourette's [6], it was important to establish whether such behaviours in deer mice are related to cognitive perturbations. Also, it was important for us not to train animals in terms of arm choice prior to the assessment, since our main aim was to establish if spontaneously H-expressing animals inherently present with an equally spontaneous change in alternation behaviour. Given that deer mouse stereotypy can be regarded as a mono-dimensional repetitive behaviour, i.e. not clouded by other forms of motor perturbations also, our data are congruent with that of clinical neurocognitive investigations which applied symptom-guided patient inclusion criteria [33]. It can be concluded with some degree of confidence that stereotypical jumping

¹ high-stereotypical

² low-stereotypical

³ obsessive-compulsive disorder

and pattern running in deer mice associate with cognitive involvement, the nature of which remains to be explored. This finding is useful, since it expands previous work in our laboratory that showed distinct social interaction profiles between animals of the same and different behavioural phenotypes, which at the time was interpreted as being indicative of outcome-feedback processing guided by cognitive mechanisms [56].

We also show here that neither ESC¹ nor LEV² influenced the expression of H³ behaviour, but that LEV, significantly increased the time that H animals engaged in no stereotypical activity compared to LEV-exposed N⁴ animals (**Figure 3-4**). Although the same trend was observed for both the control and ESC groups, the response of N animals in these groups undermined similar significant results. In whole, this finding is of paramount importance since it differs markedly from the results of our previous work with ESC [54], which showed that ESC significantly increased the time spent engaging in no-stereotypical activity. To provide an explanation, three noteworthy differences between this and our previous work need highlighting. First, in our earlier work, we employed single-housed cages throughout the eight-week duration of the study, as opposed to the social housing structure used here for four weeks. Such a lengthy study period was also needed, since the second difference between this and our earlier work was that each animal was its own drug-naïve control. Thus, animals were screened for stereotypy prior to the onset of ESC exposure, while screening continued after the onset of exposure. The last informing difference between this and our earlier work, pertains to the number of screens that animals underwent. Previously, each animal was observed on a weekly basis over the course of eight weeks to establish a clear drug-naïve stereotypy baseline, followed by a four-week assessment of sub-chronic and chronic drug response [54]. Here however, each animal was screened for stereotypy over three, relatively closely spaced, rounds before drug exposure and only once after four weeks of drug exposure. Thus, the lack of significant influences of ESC on the behaviour reported here, can likely be ascribed to the fact that the present experiment was on the one hand carried out in a less anxiogenic manner, i.e. devoid of social isolation, and on the other, with less

¹ escitalopram

² levetiracetam

³ high-stereotypical

⁴ low-stereotypical

opportunity for animals to habituate to the testing environment. In other words, it is likely that our earlier results with ESC could be ascribed to its well-known anxiolytic [11], rather than a purely anti-compulsive action. Here, we opted for socially enriched housing for two reasons. For one, we believed that stereotypy expressed by socially-reared deer mice would be stable and innate and that social housing onwards, would not have a significant impact on the behaviours displayed; we argued this, since animals are initially screened for stereotypy coming from socially housed environments. The second consideration was entirely based on ethical reasons. Nevertheless, against this background, the effect of LEV¹ on the time spent engaging in no stereotypical activity deserves emphasis. Deer mice are screened for stereotypical activity on a scale from low to high (**Figure 3-1**). Thus, N² animals mostly also express a handful of bouts of higher, though not H³, stereotypical activity, compared to the majority of bouts during which they show a lower level of behavioural expression. Therefore, compared to the behaviour of N animals, which did not show further normalising adaptation to LEV, H animals presented with significantly more bouts of normal rodent activity, i.e. bouts during which entirely *no* stereotypical activity, as opposed to merely a lower degree of stereotypy, was expressed. This effect cannot be ascribed to influences on general locomotor activity, since the behavioural expression of N animals remained mostly unaltered (**Figure 3-4 D**). Thus, within the current experimental framework, LEV indeed did engender a certain degree of control over the expression of H behaviour, which needs further elucidation.

Our last main finding pertains to the effect of drug exposure on spontaneous alternation and its association with stereotypical expression. We show here that neither of the interventions significantly influenced said constructs. Thus, the effect of LEV on the expression of normal rodent activity, i.e. increased time spent on no stereotypy (**Figure 3-4 D**), was not paralleled by an improvement in the alternation scores of the same animals. Given the aforementioned relationship between spontaneous stereotypy and alternation, this result is interesting as it points to an exclusive effect of LEV on motor output that does not necessarily associate with a change in cognitive function, as measured here. This finding is echoed in the post-

¹ levetiracetam

² low-stereotypical

³ high-stereotypical

drug exposure relationship between stereotypy and alternation behaviour. While higher stereotypy scores predicted lower alternation scores and vice versa at baseline, the opposite was not shown for ESC¹- or LEV-exposed animals after drug exposure, i.e. pointing to a dissonance in the association between behavioural inflexibility and high motor stereotypy after drug exposure. However, since the baseline negative relationship between stereotypy and alternation narrowly missed statistical significance in control-exposed animals (likely due to *n* equalling only 22 in this data set), the relationship between stereotypy and alternation remained largely stable over time; this was not the case for ESC²- or LEV³-exposed animals, albeit a significantly different relationship not being shown. Thus, in terms of applying LEV as a potential novel cognitive enhancer, our results will need future exploration. For example, it is possible that any potential effect on cognitive processes in deer mice that may relate to a change in stereotypical motor output, are masked by the methods followed here. It is possible that the therapeutic benefit of LEV on cognition can only be highlighted in a different behavioural paradigm, e.g. under trained, cue-reward contingency circumstances where it is possible to assess the reversal-learning abilities of rodents [27, 39]. The effects of other non-related compounds, i.e. the metabotropic glutamate 5 (mGlu5)⁴ receptor allosteric modulator, 3-cyano-N-(1,3 diphenyl-1H-hyrazol-5-yl) benzamide (CDPPB)⁵ [21], the cannabinoid receptor antagonist, SR141716A (rimonabant) [51] and others [10] on cognitive flexibility were shown in a learned T-Maze paradigm that required a significant degree of reversal learning. That said, our main aim was to establish whether H⁶ animals are inherently more rigid than N⁷ animals, which seems to hold true. To this extent, future research is needed to elucidate the nature of this association and how so-called cognitive enhancers may be used to manipulate such associations.

As is the case with most pre-clinical research, this study was not without shortcomings. First, it would have been useful to compare this data with a socially isolated group of animals since it is not possible to interrogate the effect of the current drug interventions

¹ escitalopram

² escitalopram

³ levetiracetam

⁴ metabotropic glutamate receptor 5

⁵ 3-cyano-N-(1,3 diphenyl-1H-hyrazol-5-yl) benzamide

⁶ high-stereotypical

⁷ low-stereotypical

against the background of our earlier findings with ESC. Secondly, although our methods were based on prior research [13], we now believe that increasing the number of arm-choices from 15, would have been useful. When applying 14 trials only (minus the first arm entry), a single arm-choice contributes 7.14% to the final alternation percentage (and even more if animals executed less than 15 arm choices), resulting in reduced granularity in the relationship between stereotypy and alternation score. This is to be expected, since such large increments in the alternation scores, do not allow for a more precise regression or correlational analysis. Finally, we believe that a paradigm based on prior training, using a separate group of animals would have been informative, especially since LEV¹ showed a promising effect in terms of exerting some degree of behavioural control over the expression of stereotypical behaviour. While our data suggest LEV to be ineffective in terms of altering cognitive processes, this conclusion cannot be drawn until further investigation.

¹ levetiracetam

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4 Summary and conclusion

4.1 Background and main findings

Patients with obsessive-compulsive disorder (OCD)¹ frequently present with perturbations in cognitive processing which are exemplified by, among others, an over-reliance on habit [7, 8, 10, 17] impaired task-switching ability and attentional bias [1, 14]. However, such findings remain inconclusive, including the relationship between compulsive motor routines and cognitive underpinnings, which requires further elaboration. In the present work we aimed to explore whether naturalistic high-stereotypical (H)² motor behaviour expressed by deer mice (*Peromyscus maniculatus bairdii*), which has previously been proposed as a rodent model of compulsive-like behavioural persistence [18], is associated with innate deficits in cognitive flexibility as reflected by reduced spontaneous and untrained alternation in a T-maze. We further sought to establish whether such an association—should it be shown—would respond to the first line pharmacotherapy for OCD, i.e. high-dose, chronic intervention with the selective serotonin reuptake inhibitor (SSRI)³, escitalopram (ESC)⁴. Last, we aimed to establish whether both motor stereotypy and its cognitive component—which is reduced alternation in a T-maze (if present)—would adapt to chronic intervention with a potentially novel cognitive enhancer, i.e. levetiracetam (LEV)⁵. The latter was an important focus of study, since it was theorised that motor compulsivity associated with deficits in cognitive flexibility, could benefit from drugs targeting said cognitive processes, rather than the well-studied serotonergic system, especially since 40 – 60 % of patients remain refractory to SSRI intervention [2, 5, 12].

The main findings of this work are that 1) higher stereotypical intensity in deer mice significantly and negatively predicts spontaneous alternation scores in the T-maze, 2) LEV, but not control or ESC exposure, significantly increases the time that H, but not low-stereotypical (N)⁶ animals spent engaging in no stereotypical behaviour, and 3)

¹ obsessive-compulsive disorder

² high-stereotypical

³ selective serotonin reuptake inhibitor

⁴ escitalopram

⁵ levetiracetam

⁶ low-stereotypical

neither of the interventions employed here, affect spontaneous alternation scores in N¹ and H² deer mice.

With respect to our first main finding, it is important to reiterate that symptom-non-specific relationships between compulsive symptom expression and cognitive impairments, remain unclear and thus, that continued research of this theme, is vital. Here, we showed that stereotypical motor expression in deer mice negatively predicted spontaneous alternation in a T-maze. This finding was striking, since motor stereotypy is a symptom of several neurological and psychiatric disorders devoid of direct cognitive involvement, e.g. autism spectrum disorders [3] and Parkinson's disease [15]. Since deer mouse stereotypy is associated to some degree with impaired cognitive flexibility as quantified by decreased spontaneous alternation scores in a T-maze, we argue that such behaviour is not simply an artefact of aberrant processes regulating motor control, but that deficits in cognitive ability, e.g. action-outcome-planning, may underlie the expression of such routines. While this notion remains to be explored in future studies, the validity of the deer mouse model is strengthened as a framework in which to investigate the psychobiology of naturalistic compulsive-like behaviours.

Secondly, we showed that LEV³ significantly increased the time that H animals engaged in no stereotypical activity compared to LEV-exposed N animals. In different terms, when compared to their baseline behaviour, LEV-exposed H animals engaged in *no* stereotypical rodent activity, i.e. engaging in more bouts of normal rodent activity, after drug exposure, than did LEV-exposed N animals or most animals of the control and ESC⁴ groups; this despite their continued engagement in H activity during other periods of the night. In fact, that neither ESC nor LEV influenced the expression of H behaviour per se, was striking, since such a lack of H adaptation to ESC differs markedly from the results of our previous work [18]. Potential explanations for this

¹ low-stereotypical

² high-stereotypical

³ levetiracetam

⁴ escitalopram

unexpected result are provided in Chapter 3, although future work is much needed to elucidate the exact reasons underlying our present data. Suffice to say that H¹ behaviour might arise from a complex interaction between the psychobiological architectures of anxiety- and compulsive-like processes, for which the appropriate controls, e.g. anxiolytic drug intervention and additional anxiety testing, were unfortunately not included here.

Last, irrespective of its beneficial effect on the expression of no stereotypical activity in H animals, this response did also not associate with a change in cognitive function, as measured in the T-maze. Further, neither control nor ESC² intervention influenced T-maze alternation. While this result could have been expected for ESC—if we argue that ESC targets hyperactive cortico-striatal motor circuits instead of cognitive (read executive) processes—the LEV³ result is somewhat confounding. Nevertheless, at this stage, we cannot conclude with certainty that LEV is devoid of a cognitive mechanism of action. For example, it is likely that restoration of such effects in deer mice by LEV are masked by the methods followed here and that a different paradigm, i.e. trained, cue-reward contingency T-maze alternation, might be more informing [11, 13]; indeed, such paradigms have proven useful to demonstrate the pro-cognitive effects of other novel and potentially useful cognition-enhancing drugs [4, 9, 16].

4.2 Study shortcomings and future work

As alluded to earlier, clinical research is inconclusive in terms of OCD⁴ patients demonstrating distinct deficits in cognitive performance [1, 10, 14]. Thus, for this work, we selected deer mice based on their presentation of stereotypical behaviour, i.e. a single behavioural phenotype, in an attempt to isolate a window onto the relationship between a specific compulsive-like phenotype and cognitive perturbations. Still, even

¹ high-stereotypical

² escitalopram

³ levetiracetam

⁴ obsessive-compulsive disorder

this approach was somewhat lacking, since our research highlighted some key and likely important methodological influences on our data.

First, the inclusion of a socially isolated group of animals, not only to align our present findings with earlier data from our lab, but also to investigate the potential contribution of isolation-induced anxiety on the data presented here, would have been useful. Further, although our methods are based on prior research [6], it is possible that an increased number of arm-choices, greater than the 15 allowed here, would have been useful. This is because a single arm-choice contributed 7.14 % to the final alternation score, preventing a detailed picture of the relationship between stereotypy and alternation scores to be sketched. Last, we believe that a trained paradigm in a separate group of animals would have been informative, especially since LEV¹ showed promising effects in terms of exerting some degree of behavioural control over the expression of H² behaviour. Given the association between stereotypy and spontaneous alternation, it is thus likely that LEV may in fact modulate cognitive processes also, a possibility that can only be investigated in a different cognitive assessment paradigm. Thus, while our data at this stage suggest LEV to be ineffective in terms of altering cognitive processes, this conclusion cannot be drawn until further investigation.

¹ levetiracetam

² high-stereotypical

4.3 Final summary

Table 4-1 Summary of final outcomes.

Study question	Outcome
1. Will N ¹ and H ² deer mice show distinct patterns of spontaneous alternation as assessed in a T-maze?	 By plotting the alternation scores of all N and H animals against their respective stereotypical intensities, we showed that stereotypical intensity indeed negatively predicted alternation scores. It can thus be concluded that N and H animals do in fact present with distinct patterns of spontaneous alternation in a T-maze.
2. Will escitalopram, a known anti-compulsive intervention which has been shown to reduce spontaneous stereotypy in deer mice, also improve any potential differences in spontaneous T-maze alternation behaviour as shown by N and H mice?	 Strikingly, our present results differ from our prior research. This can likely be ascribed to the research methodology followed here, which applied social housing structures and a four-week interval between stereotypy assessments which highlighted a potential influence of anxiety-related processes in our current and previous data. This finding will need considerable future investigation to clarify.
3. How will the effects of levetiracetam compare to that of escitalopram on both spontaneous stereotypy and alternation behaviour?	While neither intervention influenced the expression of H behaviour per se, LEV³, but not the other interventions, increased the time that H animals engaged in normal rodent activity, as evinced by the increase in the time that H mice expressed bouts during which no stereotypical activity at all, was shown. However, in light of the findings alluded to in (2) above, future work needs to establish the therapeutic value of such a response for deer mouse stereotypy as a model of compulsive-like behaviour.

¹ low-stereotypical

² high-stereotypical

³ levetiracetam



4. Would it be valuable to explore novel treatment strategies that are devoid of direct monoaminergic action for the treatment of compulsive-like traits that may be associated with cognitive impairment?

Yes. Although much remains to be established, this investigation provided sufficient proof of concept for future investigations exploring the potential benefits of drug molecules described as cognitive enhancers in the deer mouse model of compulsivity. Not only did we show an interaction between stereotypical intensity and cognitive flexibility, but we have also demonstrated that LEV¹ modulated the behavioural presentation of H² mice, albeit with respect to one measure only.

4.4 Conclusion

In this work we explored whether high motor stereotypy as observed in deer mice, is associated with cognitive rigidity as measured in a T-Maze, which was shown to be so. Although our findings regarding the effect of ESC³ on stereotypy is inconsistent with our previous work, the data presented here provided valuable insights into the potential interactions between anxiety- and compulsive-like processes that may act in concert to give rise to naturalistic stereotypy; this will need careful and well-conceptualised future work to clarify. Nevertheless, our data are promising in terms of showing that naturalistic stereotypy in deer mice is innately associated with perturbations in cognitive flexibility. This paves the way for future cognitive and anti-compulsive therapeutic research in this species.

¹ levetiracetam

² high-stereotypical

³ escitalopram

4.5 References

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Addendum A

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

Additional information pertaining to animal husbandry, housing, and care

- Prior to the onset of the study, all animals used in this investigation were bred and supplied by the North-West University (NWU)¹ vivarium, Potchefstroom, South Africa (SAVC² Reg no.: FR15/13458; SANAS³ GLP⁴ compliance no.: G0019; AAALAC⁵ accreditation file: 1717).
- At postnatal day 21, mice were weaned, group-housed and raised in cages of 4 - 6 same sex animals per cage [35 cm (l) x 20 cm (w) x 13 cm (h); Tecniplast® S.P.A., Varese, Italy], with a maximum of 6 days age difference between mice. Mice were housed as such until adulthood at 10 weeks.
- At age 10 weeks, animals were randomly selected, from different group housed cages, ear-tagged for identification purposes, and reallocated to new home cages in groups of four same sex animals per cage. They remained so for the duration of the study.
- At age 12 weeks, stereotypy assessment commenced. All animals were screened over three rounds. Eight animals could be screened per night, with a minimum period of 48 hours in between the different rounds of screening.
- Mouse pellets (LabChef®, Johannesburg, South Africa, SAVC Reg no.: 2012/141960/07) were autoclaved at 90°C for 15 minutes before use.

Daily routine during the behavioural investigation

- Throughout the course of this work, animal welfare was monitored between 08:00 and 10:00 each morning, and water or drug solution containing bottles were weighed to track fluid consumption. Fresh drug solutions, where applicable, were provided every day. Apart from the weekly cage maintenance routine (see below), animals were left undisturbed for the remainder of the day, except if experimentation commenced at night.

¹ North-West University

² South African Veterinary Council

³ South African National Accreditation System

⁴ Good Laboratory Practice

⁵ Association for Assessment and Accreditation of Laboratory Animal Care

- Further, as part of the normal caretaking routine, the following parameters were routinely recorded: 1) humidity, 2) temperatures of the housing and experimental rooms, and 3) food and water or drug availability.
- Once a week, cages were cleaned, and fresh corncob and paper towel provided. Cages were cleaned with F10® SCXD Veterinary Disinfectant Solution (Reg No.: G3073).
- When stereotypy assessment took place, mice were transferred from the housing room to the experimental room 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. Mice were 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 ample food and water throughout the night.
- On days of T-maze testing, mice were transferred to the testing room at 18:00 and left to habituate until 19:00. Mice were then individually placed in the start box of the T-maze and left for 30 seconds. Then, the start box door was raised for the first time (see Chapter 3 for details). Importantly, mice had to meet specific criteria to pass the test, i.e. they must have left the start box for the first time within the first 10 min of assessment, they must have made at least 8 transverse arm choices within a 30-min window, or a total of 15 arm choices.
- 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.

Additional information pertaining to drug administration

- Drugs: Escitalopram oxalate (ESC)¹ (50 mg/kg/day; [2]), levetiracetam (LEV)² (75 mg/kg/day; [1]) -, were orally administered by dissolving them in the drinking water. Control-treated animals received only tap water.

¹ escitalopram

² levetiracetam

- To establish baseline fluid consumption of four group housed mice, 40 ml of water was given to each cage daily for 7 days prior to the onset of behavioural testing. After allocation of animals to different stereotypical and drug exposure groups and after completion of the first baseline (pre-drug-exposure) behavioural assessment, all animals were exposed to said interventions for 28 days and the daily fluid intake measured (**Figure A-1**).
- Treatment involved the daily provision of 40 ml of the relevant drug solutions to the subjects in their respective group housing cages.
- Drug concentrations were based on the average fluid intake of deer mice (*Peromyscus maniculatus bairdii*), i.e. 0.25 ml/g/day [2]. Thus, ESC¹ was constituted by dissolving 25.4 mg of the drug salt in 100 ml of water, while 30 mg of LEV² was dissolved in 100 ml [1].
- On the second last day of water or drug exposure, all low-stereotypical (N)³ and high-stereotypical (H)⁴ animals were again assessed for alternation behaviour in the T-maze.
- On the last day of water or drug exposure, all N and H animals were reassessed for stereotypical behaviour, after which subjects were euthanised as per the ethically approved protocol of the Vivarium of the NWU⁵

¹ escitalopram

² levetiracetam

³ low-stereotypical

⁴ high-stereotypical

⁵ North-West University

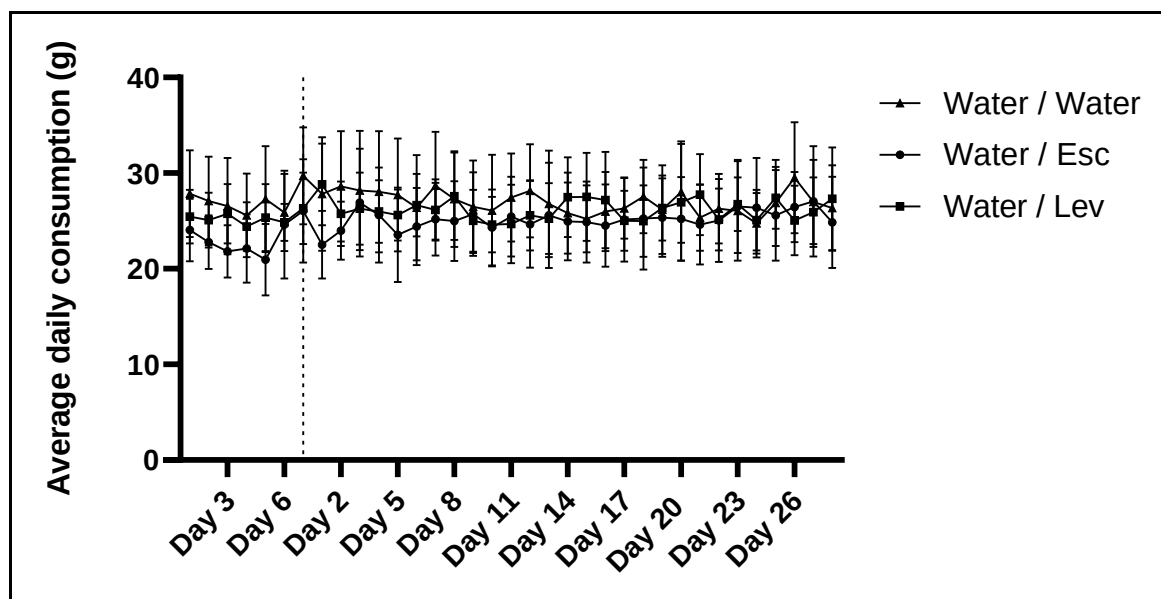


Figure A-1 The average daily fluid intake. Four mice per cage. Data is representative of the average daily fluid intake of all animals that received the respective solutions, irrespective of phenotype.

Examples of raw data exports from stereotypical assessments

W410		=SUM(U410:V410)																				
A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W
403	B3 145-19 Cag6	Cycle1	Phase 1	32	6:20:02 am	1800	1841.7	2616	2434	1373.05	184	426.83	184	35.23	84	182	145	87	480.22	4	11	1
404	B3 145-19 Cag6	Cycle1	Phase 1	1	2:50:02 pm	1800	3362.04	5933	2074	921.16	319	877.67	320	147.31	291	859	282	656	1068.09	9	30	3
405	B3 145-19 Cag6	Cycle1	Phase 1	2	3:20:02 pm	1800	1423.38	1924	1706	1505.5	122	294.63	121	38.64	75	218	149	350	373.48	4	12	3
406	B3 145-19 Cag6	Cycle1	Phase 1	3	3:50:02 pm	1800	36.37	149	128	1768.27	39	31.73	38	6.6	6	21	70	0	52.4	1	0	0
407	B3 145-19 Cag6	Cycle1	Phase 1	4	4:20:02 pm	1800	19.93	73	62	1784.2	10	15.8	9	3.59	7	11	16	0	22.64	0	0	0
408	B3 145-19 Cag6	Cycle1	Phase 1	5	4:50:02 pm	1800	189.07	451	403	1712	63	88	62	9.14	26	48	71	0	131.48	1	0	0
409	B3 145-19 Cag6	Cycle1	Phase 1	6	5:20:02 pm	1800	28.27	62	60	1791.55	21	8.45	20	0.09	1	2	28	0	12.67	0	0	0
410	B3 145-19 Cag6	Cycle1	Phase 1	7	5:50:02 pm	1800	9.93	46	43	1788.52	15	11.48	14	0.41	3	3	17	0	15.74	0	0	0
411	B3 145-19 Cag6	Cycle1	Phase 1	8	6:20:02 pm	1800	92.5	663	467	1665.42	122	134.58	121	48.31	59	196	158	0	189.09	0	0	0
412	B3 145-19 Cag6	Cycle1	Phase 1	9	6:50:02 pm	1800	1245.66	4017	3456	1129.11	253	670.38	253	113.49	229	561	254	477	791.55	9	7	0
413	B3 145-19 Cag6	Cycle1	Phase 1	10	7:20:02 pm	1800	693.94	2432	2099	1412.34	139	387.65	139	66.74	113	333	138	320	474.07	1	2	0
414	B3 145-19 Cag6	Cycle1	Phase 1	11	7:50:02 pm	1800	232.86	889	788	1659.97	108	140	108	24.64	38	101	128	94	212.88	0	0	0
415	B3 145-19 Cag6	Cycle1	Phase 1	12	8:20:02 pm	1800	103.76	468	375	1696.51	94	103.49	93	19.84	35	93	113	0	151.65	0	0	0
416	B3 145-19 Cag6	Cycle1	Phase 1	13	8:50:02 pm	1800	21.57	116	76	1775.76	26	24.24	25	7	14	40	44	0	32.43	0	0	0
417	B3 145-19 Cag6	Cycle1	Phase 1	14	9:20:02 pm	1800	37.77	188	160	1763.55	50	36.46	49	7.35	11	28	76	0	59.9	0	0	0
418	B3 145-19 Cag6	Cycle1	Phase 1	15	9:50:02 pm	1800	1031.29	3087	2765	1350.73	211	449.27	210	58.51	128	322	238	469	604.68	3	2	0
419	B3 145-19 Cag6	Cycle1	Phase 1	16	10:20:02 pm	1800	22.61	89	86	1778.43	16	21.57	15	0.41	3	3	29	0	23.84	0	0	0
420	B3 145-19 Cag6	Cycle1	Phase 1	17	10:50:02 pm	1800	32.31	207	154	1750.19	73	49.81	72	13.81	20	53	89	0	75.55	0	0	0
421	B3 145-19 Cag6	Cycle1	Phase 1	18	11:20:02 pm	1800	53.77	296	236	1734.9	61	65.1	60	13.65	17	60	112	0	107.64	1	0	0
422	B3 145-19 Cag6	Cycle1	Phase 1	19	11:50:02 pm	1800	862.34	2431	1935	1361.18	191	438.81	191	110.31	148	496	179	364	525.68	2	7	0
423	B3 145-19 Cag6	Cycle1	Phase 1	20	12:20:02 am	1800	1376.62	3102	2755	1338.51	181	461.43	181	69.23	120	347	195	677	579.03	7	8	1
424	B3 145-19 Cag6	Cycle1	Phase 1	21	12:50:02 am	1800	74.66	308	265	1719.98	61	80.02	60	10.92	19	43	87	0	111.51	0	0	0
425	B3 145-19 Cag6	Cycle1	Phase 1	22	1:20:02 am	1800	114.47	4085	3610	1227.82	253	572.84	253	90.05	160	475	255	1245	802.9	4	4	0
426	B3 145-19 Cag6	Cycle1	Phase 1	23	1:50:02 am	1800	2359.59	6580	5694	832.35	306	966.25	307	139.26	267	886	239	2672	1129.94	12	9	0
427	B3 145-19 Cag6	Cycle1	Phase 1	24	2:20:02 am	1800	1902.86	6400	5639	799.27	307	1000.2	308	112.22	264	761	263	4238	1096.57	8	10	0
428	B3 145-19 Cag6	Cycle1	Phase 1	25	2:50:02 am	1800	15.29	139	143	1751.45	110	48.55	109	9.5	23	50	114	0	61.9	0	0	0
429	B3 145-19 Cag6	Cycle1	Phase 1	26	3:00:02 am	1800	94.121	3310	2833	1247.43	312	552.16	312	83.54	150	477	267	936	677.45	1	1	0
430	B3 145-19 Cag6	Cycle1	Phase 1	27	3:50:02 am	1800	1645.83	3838	3489	1294.99	326	505.01	325	64.4	118	349	169	1657	671.53	12	8	2
431	B3 145-19 Cag6	Cycle1	Phase 1	28	4:20:02 am	1800	17.19	175	157	1756.46	58	43.99	57	3.58	9	18	75	0	71.44	0	0	0
432	B3 145-19 Cag6	Cycle1	Phase 1	29	4:50:02 am	1800	1389.35	2375	2147	1382.38	175	417.62	174	43.28	77	228	157	362	507.12	13	4	0
433	B3 145-19 Cag6	Cycle1	Phase 1	30	5:20:02 am	1800	52.64	273	205	1713.12	72	86.88	71	22.11	26	68	95	0	106.02	1	0	0
434	B3 145-19 Cag6	Cycle1	Phase 1	31	5:50:02 am	1800	16.94	80	73	1782.7	21	17.3	20	0.2	4	7	41	0	22.57	0	0	0
435	B3 145-19 Cag6	Cycle1	Phase 1	32	6:20:02 am	1800	18.94	178	156	1760.44	49	39.56	48	4.57	11	22	88	0	60.42	0	0	0
436	B3 145-19 Cag7	Cycle1	Phase 1	1	2:50:02 pm	1800	11608.1	10982	10217	589.02	191	1210.98	190	99.17	137	765	185	960	1303.86	34	113	14
437	B3 145-19 Cag7	Cycle1	Phase 1	2	3:20:02 pm	1800	8690.09	8541	7888	803.92	229	995.08	228	107.08	239	653	228	858	1306.68	39	93	13
438	B3 145-19 Cag7	Cycle1	Phase 1	3	3:50:02 pm	1800	1129.85	2027	1876	1569.8	77	230.2	76	27.01	73	151	200	114	431.43	12	4	0
439	B3 145-19 Cag7	Cycle1	Phase 1	4	4:20:02 pm	1800	544.65	970	918	1682.25	48	117.75	47	6.66	27	52	109	9	231.17	2	4	0
440	B3 145-19 Cag7	Cycle1	Phase 1	5	4:50:02 pm	1800	127.3	540	522	1755.05	31	44.95	30	4.6	9	18	93	0	166.98	2	4	0
441	B3 145-19 Cag7	Cycle1	Phase 1	6	5:20:02 pm	1800	4244.62	5170	4729	1170.84	126	629.74	125	91.33	157	441	179	380	739.86	16	41	0

Figure A-3 Excerpt from the collated vertical jumping data for each animal (one column represents one animal) after the three pre-drug exposure stereotypy screens (see Chapter 3 for detail), sorted from low (top) to high (bottom). Data of the 36 highest intervals out of 72 intervals (three nights times 24 30-minute bouts each) of 26 animals reflected. Values exceeding the cut-off criteria for vertical jumping, i.e. 2000 beam breaks per 30 minutes, are highlighted in red. This data was used to constitute Figure 3-1. For animal 'V', stereotypical intensity was calculated as follows:

(Average of the 9 highest bouts – 2000) / 2000 x 100. Time spent expressing stereotypy was calculated as follows:

10 bouts of H behaviour / total of 72 bouts x 100.

Schematic representation of the T-maze procedure

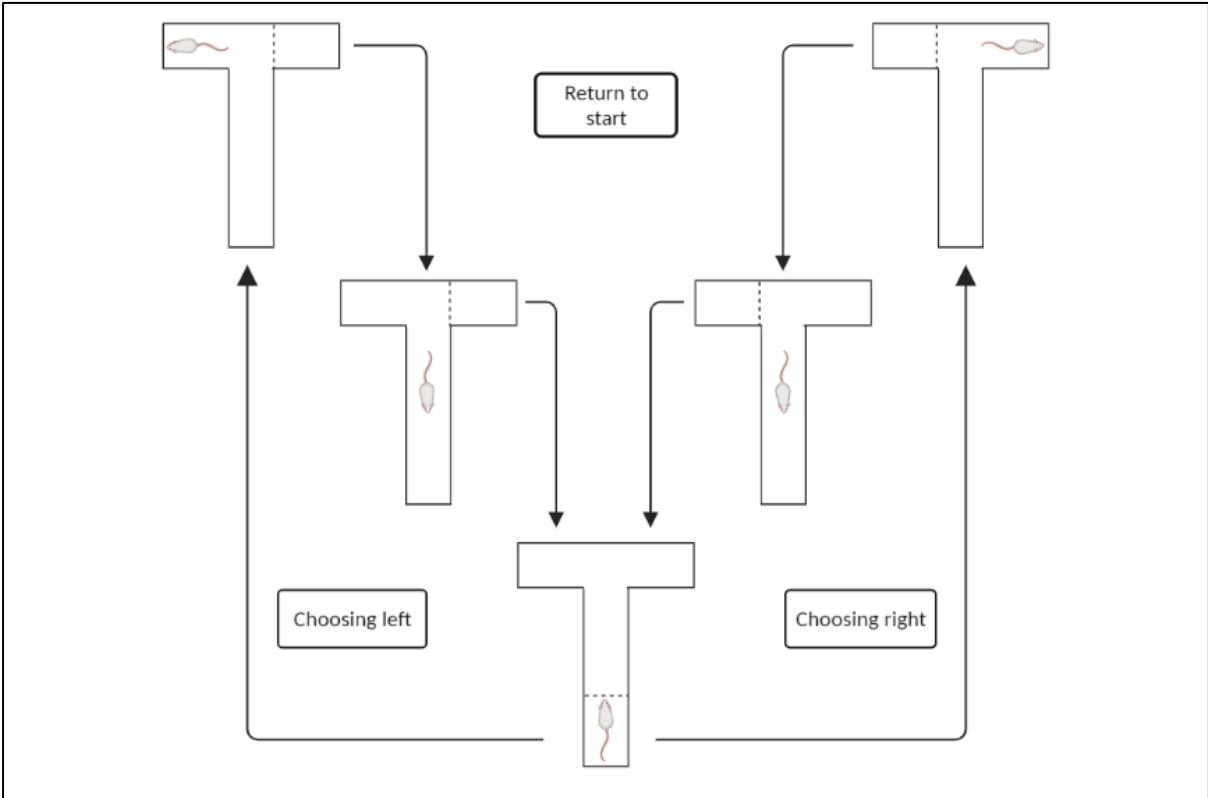


Figure A-4 Schematic procedural layout of T-maze assessment

NS Water	Pre Rx											
Mouse	25,1	25,4	27,4	29,1	29,3	30,3	32,1	37,1	37,3	37,4	38,4	40,1
Forced ch	L	L	L	R	R	L	R	L	L	L	R	L
	R	R	L	L	R	R	L	R	L	R	R	R
	R	L	R	R	R	L	R	L	R	L	L	L
	L	R	L	R	R	R	L	R	L	R	R	L
	R	R	R	L	L	R	L	L	R	R	R	L
	R	L	R	R	R	R	R	L	R	L	R	R
	R	R	R	R	R	R	R	R	R	R	L	L
	L	R	L	R	L	L	L	L	L	R	L	R
	L	L	L	L	R	R	R	R	L	R	R	R
	R	L	R	R	L	L	R	R	L	L	L	R
	L	R	R	L	R	R	L	L	R	R	R	L
	L	R	R	R	R	R	R	L	R	R	R	L
	L	L	L	L	L	R	L	R	L	R	L	R
	L	R	L	R	R	R	R	L	R	L	R	L
	R	R	L	L	R	L	L	R	R	R	R	L

Figure A-5 Example of the completed data output of 12 mice which completed the T-Maze assessment
For animal 25.1 the alternation score was calculated as follows:
 $7 \text{ left (L)-right (R) or R-L alternations made} / 14 \text{ arm choices made} \times 100$

Supplementary images pertaining to animal housing, husbandry, and care

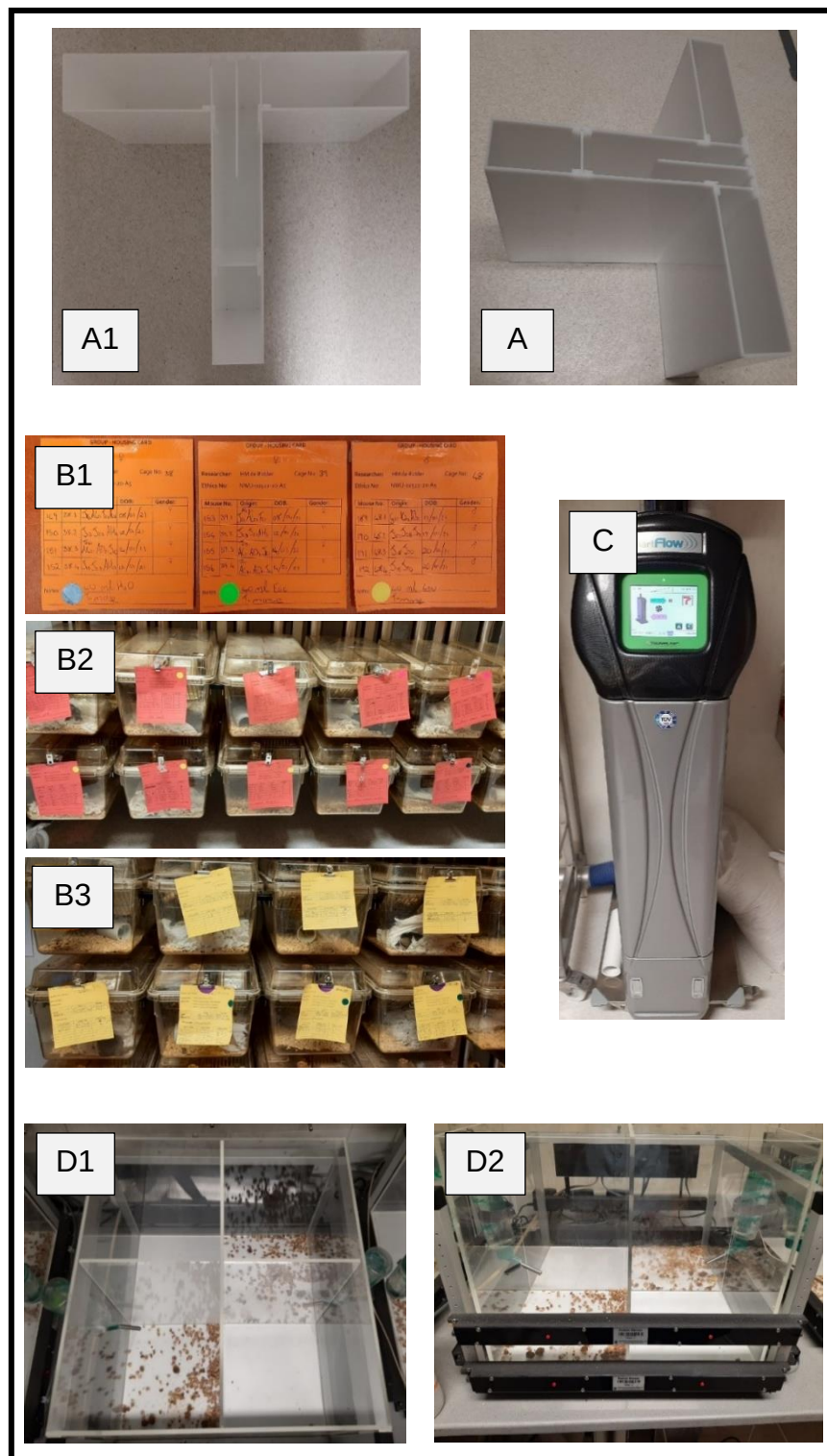


Figure A-6 (**A1-A2**): T-Maze; (**B1-3**): Deer mice housing system (cages: 35 cm (l) x 20 cm (w) x 13 (h) cm; Techniplast® S.P.A., Varese, Italy); (**B1**): Experimental cages housing cards, stickers indicated the treatment the cage received; blue: water control; green: escitalopram; yellow: levetiracetam; (**B2**): Weaned cages; (**B3**): Breeding cages; (**C**): Techniplast® Smart Flow for the monitoring of ventilation, humidity and temperature in the cages; (**D1-D2**): Accuscan®, illustrating lasers that capture vertical and horizontal stereotypical motor patterns.

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