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The effects of parachloroamphetamine on monoamine levels and exploratory behaviour in Wistar female rats of high and low exploratory phenotypes

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Läbiv pealkiri: Wistar female behavior and monoamines after PCA denervation

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Paraklooramfetamiini mõjud emaste Wistar rottide monoamiinide tasemetele ja uudiskäitumisele eritasemelistes uudiskäitumise fenotüüpides

Kokkuvõte: Monoaminergiline tasakaaluhäire on seotud rohkete häiretega, kaasaarvatud depressiooniga. Loommudelid on olnud kriitilise tähtsusega depressiooni jaoks ravimite väljatöötamisel. Üheks sellistest on uudiskasti test, millega mõõdetakse ärevuslaadset käitumist loomades. Antud uuringu eesmärk oli välja selgitada paraklooramfetamiini tekitatud monoaminergilise denervatsiooni mõju emaste Wistar rottide uudiskäitumisele, ning samuti ka selle mõju monoamiinidele juttkehas ja hippokampuses. Tulemused näitavad statistiliselt olulisi vähenemisi juttkeha dopamiinis ja metaboliitides ~10-15% võrra ning hippokampuse serotoniinis ja selle metaboliidis ~29% võrra. Sellest hoolimata paraklooramfetamiin ei omanud olulist mõju uudiskäitumisele. Seda selgitatakse erinevustega Wistari sugudes, nii baastasemel kui ka paraklooramfetamiinijärgselt.

Võtmesõnad: depressioon, monoaminergiline tasakaaluhäire, paraklooramfetamiin, denervatsioon, juttkeha, hippokampus, uudiskäitumine, uudiskast, sooerinevused.

The effects of parachloroamphetamine on monoamine levels and exploratory behaviour in Wistar female rats of high and low exploratory phenotypes

Abstract: Monoaminergic imbalance has been implicated in numerous ailments, including depression. In studying cures for depression, animal models have provided a critical tool. One such model is the exploration box test, whereby anxiety-like behavior is measured. The purpose of this study was to delineate the effects of monoaminergic denervation with parachloroamphetamine on the female Wistar rat exploratory behavior as well as its effects monoamines in the striatum and hippocampus. The results show significant reduction of striatal dopamine and metabolites by about ~10-15%, and of hippocampal serotonin and its metabolite by about ~29%. However, parachloroamphetamine had no significant effect on exploratory behavior. Wistar sex differences are implicated, both at basal levels as well as in response to parachloroamphetamine.

Keywords: depression, monoaminergic imbalance, parachloroamphetamine, denervation, striatum, hippocampus, exploratory behavior, exploration box, sex differences.

1. Introduction:

Monoamines are a group of compounds that includes such neurotransmitters as serotonin and catecholamines (dopamine, adrenaline and noradrenaline), which perform a multitude of roles in various functions ranging from memory to gastrointestinal control. The monoaminergic system denotes such corresponding networks of neurons that use these monoamines. Imbalances in the monoaminergic system play a role in the etiology of a wide array of ailments, such as schizophrenia, Alzheimer's disease, Parkinson's disease, autism and others (Hornykiewicz, 1975; Sedvall, 1990; Kohls *et al.*, 2013; Yenkoyan, Fereshetyan, Matinyan, Chavushyan, Aghajanov, 2018). Depression has also been hypothesized to be a result of monoaminergic imbalances, as per the monoaminergic hypothesis of depression, in turn leaving subjects vulnerable to external stressors (Harro, Oreland, 2001; Elhwuegi, 2004; Harro, 2010; Hillhouse, Porter, 2015). The classic theory has been that the causal factor was a simple monoaminergic neurotransmitter deficit; however, while studies of monoamine depletion have successfully shown an exacerbation in depressed subjects, healthy subjects remained unaffected (Delgado, 2000; Harro, Oreland, 2001), implicating more systemic causes. This is corroborated by the delayed time it takes for antidepressants to have effect despite acute increases in neurotransmitters. The overall factor seems to be a finely tuned disbalance of monoamines instead, implicating both a lowered tonic release of monoamines, and an adaptively heightened as well as lowered postsynaptic sensitivity towards monoamines (*ibid.*).

The inherent complexity involved in trying to model depression also stems from the heterogeneity of monoamine contributions to the condition. There is considerable literature on the interplay between different monoamine types, such as the serotonergic system and the catecholaminergic system, which each produce a different effect upon depression. For example, the depletion of tryptophan – a serotonin (5HT) precursor – which reduces available 5HT, produces negative affect (sadness, hopelessness) in humans, whereas a catecholaminergic deficit produces mainly concentration difficulties and passivity (Homan, Neumeister, Nugent, Charney, Drevets, Hasler, 2015). Preliminary evidence has suggested that catecholaminergic antidepressants may be more effective in alleviating problems associated with a lack of positive affect (interest, energy, pleasure, alertness, etc.) than serotonergic antidepressants (Nutt *et al.*, 2007), indicative of distinct roles of these monoamines. This is critical for accurate prescription, for drugs aimed at serotonergic regulation (such as selective serotonin re–uptake inhibitors, SSRIs) will not have effect on the catecholaminergic system, which remains untreated in

patients to whose etiology it may have contributed. Such considerations must be accounted for, when trying to model depression.

Fortunately, animal models offer a viable path for modelling the various neurochemical conditions associated with depression, because the underlying mechanisms for stress-response and neurochemistry – such as anxiety, passivity, novelty seeking – are similar enough between humans and animals (Dellu, Piazza, Mayo, Le Moal, Simon, 1996; Harro, Oreland, 2001). Various tests have been developed for modelling anxiety/depressive phenotypes in animals, such as the elevated plus-maze test, forced swimming test, hole-board test, light/dark transition test, fourplate test, fear conditioning, etc. (Otter *et al.*, 1997). One of the main anxiety-related behavior modelling paradigms involves animal exploratory behavior.

Animal exploratory behavior is how the animal reacts to being presented with novel stimuli. It is regulated by the conflicting motivators of fear and curiosity – the resulting balance of which is critical for survival, for any such contact with a novelty presents both potential rewards as well as risks. Exploratory activity can be measured by various tests and animals can be divided into groups of persistently high and low exploratory activity (HE and LE, respectively). One of these tests is the open field test (OFT). Animal behavior in the OFT seems to reflect more than mere locomotor activity, but instead a wider temperament type of the animals, with LE belonging correlating with various assays of anxiety-like behavior – for example passivity in the forced swimming test (Valencia *et al.*, 2019), inhibition in the elevated plus-maze test (EPM) (Carola, D'Olimpio, Brunamonti, Mangia, Renzi, 2002; Mällo, 2008), decreased activity in the black-white box test (Mällo, 2008) and greater freezing in conditional fear conditioning (Ahn *et al.*, 2013) – suggesting that animals from the LE group exhibit more overall anxiety-like behaviors. An interpretation further corroborated by the fact that exploratory activity is modulated by anxiolytic treatment (Crawley, 1985; Costall, Jones, Kelly, Naylor, Tomkins, 1989). Similarly, rats selectively bred for high or low anxiety-like behavior by the EPM produced personality types that correlated with results in both OFT and forced swimming test (Liebsch, Montkowski, Holsboer, Landgraf, 1998). Overall, animals more active in one test are also generally more active in others and vice versa (Ho, Eichendorff, Schwarting, 2002; Mällo *et al.*, 2007; Lynn, Brown, 2009).

The exploration box testing is a type of emergence test, whereby the animal is introduced into a novel environment and the latency of its emergence from its initial box is measured, as well as various other exploratory activity indicators. It allows for repeated measuring and reveals stable personality types already on the second measuring, which remain

persistent even after 6 months (Mällo *et al.*, 2007). Additionally, the exploration box test successfully separates anxiolytic effects of psychoactive drugs from their stimulative effects, ie. emotional and motivational effects (Otter *et al.*, 1997). Finally, due to the complexity of animal behavior and the multitude of possible variables involved, the consistent use of one model in a laboratory after validation can alleviate such confounding factors (*ibid.*). Considering the aforementioned and the previous experience of our laboratory, this study will conduct measurements using the exploration box test.

Expectedly, the differences in temperament of locomotor activity and anxiety-like behavior have been traced to differences in the brain chemistry of the animals. For example, rats with higher motor activity have a higher baseline extracellular dopamine (DA) and 5HT in striatum as well as a higher DA turnover in accumbens and striatum and a lower ratio in prefrontal cortex (Piazza *et al.*, 1991; Alttoa *et al.*, 2005). DA turnover is measured by the DOPAC/DA ratio, reflecting the metabolizing of DA into one of its main metabolites, DOPAC. High motor activity rats are also more responsive to cocaine and amphetamine administration, producing both, more locomotor activity and extracellular DA than in low motor activity rats (Hooks, Jones, Smith, Neill, Justice, 1991; Alttoa, Eller, Herm, Rinken, Harro, 2007). It is the more interesting when it is considered that cocaine and amphetamine attain their stimulative effect through monaminergic pathways, and consequently the lesioning of these attenuates it (Gawin, 1991; Antoniou, Kafetzopoulos, 1992; Pino, Awadallah, Norris, Torres, 2021). Taken together, the aforementioned characteristics allow to suggest an overall monoaminergic underperformance in low motor activity animals congruent with suggested causes of depression in humans (Harro, Oreland, 2001).

As mentioned, because depression is a result of the interaction between stress and monoaminergic imbalance, animal models have conformed to this by applying chronic and acute stress alone (Katz, Roth, Carroll, 1981) and in combination with neurotoxins (Harro *et al.*, 2001; Häidkind *et al.*, 2004), to create the necessary vulnerabilities in neurotransmitter systems similar to those in genetic predispositions. However, the risk has been that the wrong neurotoxin or too big a dosage will cause a complete destruction of the affected systems. Because depression does not entail such total destruction, a more limited usage of a neurochemically selective compound provides more relevant results (Kanarik, 2008). Such compound has been found in parachloroamphetamine (PCA): a neurotoxin, which thanks to its highly selective serotonergic nature, allows for systemic injection (Fuller, 1992). At dosages of 2mg/kg, PCA causes partial serotonergic denervation, attaining desired changes in

neurochemistry and behavior – increased anxiety and impulsivity – in rats both by PCA alone and in combination with stress (Harro, 2002; Häidkind *et al.*, 2004; Tõnissaar *et al.*, 2008).

The hippocampal brain region is critical in behavioral adaptability and learning, but also central in mood disorders, being one of the key loci for controlling anxiety (Engin, Treit, 2007; Brand, Möller, Harvey, 2015; Calhoon, Tye, 2015). The increase of 5HT levels in hippocampus is tied to a reduced anxiety-like behavior in rats (Tu *et al.*, 2014) and both, repeated and acute exposure to stressful stimulus has been shown to increase 5HT levels in hippocampus (Storey *et al.*, 2005; Mahar, Bambico, Mechawar, Nobrega, 2014; da Silva Rocha–Lopes, Machado, Suchecki, 2018), suggestive of the importance of 5HT levels in the hippocampus for stress response (Miyagishi *et al.*, 2020). Therefore, if PCA is to be used in animal models for depression and anxiety, especially with the intent of induced vulnerability to stress, it would first be beneficial to develop an understanding of its effects on monoamines and 5HT in particular in the hippocampus region. Thus, hippocampus will be one of the two brain regions observed in this study.

The striatum is involved in reward-learning, cognitive control and behavioral flexibility (Devan, Hong, McDonald, 2011) and only in the last 15 years has begun to be implicated in mood disorders (Price, Drevets, 2010; Lago, Davis, Grillon, Ernst, 2017). Abnormalities in the cortico–basal ganglia circuit have been widely associated with affective illness (Marchand, Yurgelun–Todd, 2010), however the exact nature of these has remained poorly understood. Striatum may offer an answer, being the primary input to this circuit (*ibid*.). Additionally, dopamine is the main neurotransmitter through which striatum may affect such conditions as depression and bipolar disorder (*ibid*.) and the release of dopamine in striatum is also how amphetamines have their effect (Drevets *et al.*, 2001; Heal, Smith, Gosden, Nutt, 2013) – striatum being one of the main brain regions affected by amphetamines (Antoniou, Kafetzopoulus, 1992). Finally, striatum has been proposed to play a role in neophobia and in general behavioral inhibition (Helfinstein, Fox, Pine, 2012). Consequently, the knowledge of PCA effects on striatal monoamines is pertinent to animal modelling for affective disorders in general and via exploratory behavior in specific, therefore striatum will be the second brain region to be observed in this study.

The aim of this study is to 1) delineate the effect of PCA on female non-naïve Wistar behavior in the exploration box test, 2) delineate the effect of PCA on female Wistar monoamine levels in striatum and hippocampus and 3) draw conclusions whether female Wistar rats exhibit characteristics different from previous observations in male counterparts.

2. Materials and methods:

2.1 Animals

Female Wistar rats (n = 31) were laboratory-bred locally. The animals were housed in standard transparent polypropylene cages under controlled light cycle (lights on from 08:00 to 20:00 h) and temperature (19–21 ◦C), with free access to tap water and food pellets (diet R70, Lactamin, Sweden). The first block of exploration testing (EB1, see 2.3) was conducted at animal age < 14–18 weeks.

2.2 Exploration box apparatus and procedure

The exploration box consists of a metal box that includes an open area 0.5m x 1m, divided into 8 equal sizes (see Appendix A). Inside the open area were placed three unfamiliar and one familiar object (a food pellet). The rat was inserted into the box via a small covered compartment attached to the shorter side of the box, after which the animal was free to either stay in the compartment or move into the open area. Observers took the following measurements: 1) latency of entering the open area, 2) total entries into the open area, 3) line crossings, 4) rearings, 5) exploration of the unfamiliar objects and 6) total time spent in the open area. A single test session lasted 15 min and the apparatus was cleaned after each session. The experiment was carried out under dim light conditions. A full description is provided by Mällo *et al.*, 2007.

2.3 General procedure

Two separate blocks of exploration box testing were conducted on the animals. The first, exploration box testing 1 (EB1, Fig. 1), to divide animals by phenotype into high exploratory (HE) and low exploratory (LE) groups on the basis of their median value of the sum of exploratory activity over 5 days of consecutive testing. After at least 4 weeks had passed from EB1, animals were administered either PCA or 0.9% NaCl. The second block of exploration box testing (EB2), occurred 1 week after administration, also on 5 days of consecutive testing. Animals were sacrificed and their brains dissected 1–2 weeks after day 5 of EB2.

2.4 Treatment

A single dose of PCA at 2mg/kg was injected intraperitoneally to animals at age 18–22 weeks. Control group received 0.9% NaCl as a vehicle injection.

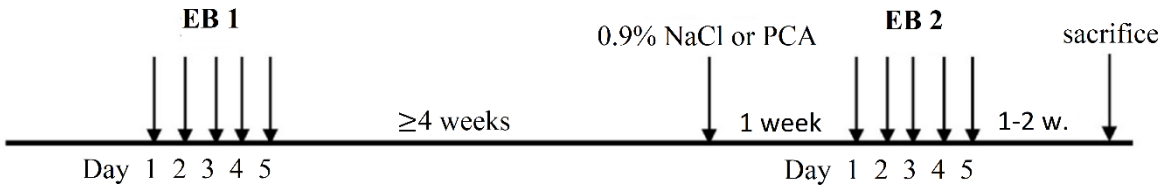


Fig. 1. Timeline of the experiment. EB1 and EB2 stand for exploration box testing blocks 1 and 2, respectively.

2.5 Behavioral activity scoring

A total of six parameters of animal behavior was measured: lines, rears, objects, entries, latency and time spent in field (see 2.2). Subsequently all measures were standardized according to the 0–1 scaling formula (Fig. 2A), so that the highest measurement in a given parameter over all 5 days of EB2 would equal 1, and the lowest 0. This was done to allow for inter-parameter comparisons across all days of EB2 on a common scale.

(A) $$x_{scaled} = \frac{x - x_{min}}{x_{max} - x_{min}}$$

(B) $$y = \sum_{k=1}^{4} \sqrt{(D_{(k+1)} - D_k)^2}$$

Fig. 2. Standardization formula (A) was used for 0–1 scaling, where x_{scaled} = standardized score, x = measurement to be standardized, x_{max} & x_{min} = highest and lowest measurements for the given parameter over all 5 days of EB2, respectively. Formula (B) was used for cumulative day-to-day change calculation, where y = total cumulative change, D = daily standardized group average score, k = number of day.

The overall activity score for each day per group was calculated by averaging all 6 of the parameter standardized scores for the given day for each animal in target group and then averaging the results.

Net changes in behavior – the difference between day 1 and day 5 – were calculated by deducing from day 5 standardized score the day 1 standardized score.

Cumulative changes in behavior – the summed day-to-day absolute differences – were calculated by deducing from each days standardized score the standardized score of the previous day, and summing the absolute values (Fig. 2B). This was done both, on an individual basis as well as for group averages.

2.6 Measurement of monoamines and their metabolite levels

Monoamine levels were measured by high performance liquid chromatography (HPLC) using electrochemical detection: the rat brain tissue was first homogenized in ice cold solution by sonication, centrifuged, and the liquid was then pressurized and pumped through a column filled with a solid adsorbent. Due to differential interaction with the adsorbent, each component flows at a different rate, thereby allowing for separated measurement. A full description of the process is provided by Harro *et al.*, 2001.

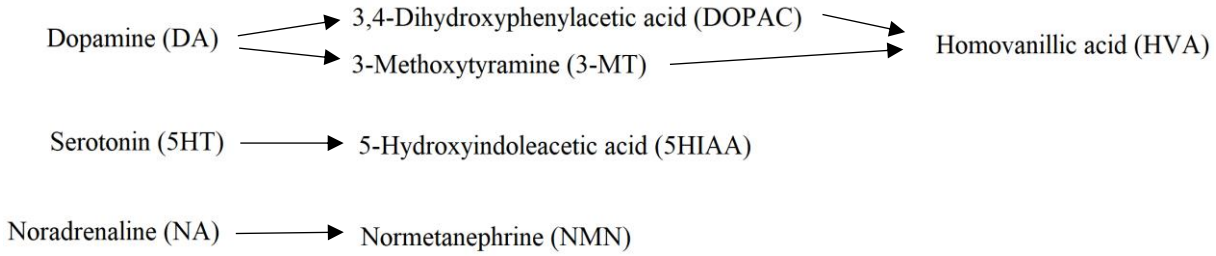


Fig. 3. Monoamines and their metabolic pathways.

Measurements were taken of the following molecules: DA and its metabolites DOPAC, NMN, 3MT, DA; 5HT and its metabolite 5HIAA; NA and its metabolite NMN (Fig. 3).

2.7 Data analysis

JASP 0.16.0.0 and Excel (Version 2203) were used to conduct ANOVA and correlations. Net as well as cumulative activity changes, monoamine turnover rates and 0-1 scaling were done in Excel.

The daily activity was calculated by averaging all the parameters (see 2.5) for the given day per animal. These scores were then summed for group and total averages.

Group differences in activity and monoamine levels were determined by a two-way ANOVA (Treatment*Phenotype). Subsequent pairwise comparisons were made by Tukey's post hoc or if equal variance was not met, by Games-Howell. Intra-group activity comparisons between different days of EB2 were analyzed by one-way ANOVA (Day).

Correlations between behavioral parameters and total activity on days 2-5; between molecules and behavioral parameters; between molecules and total activity, were conducted by Pearson's correlation coefficient. Correlations were conducted for the whole population as well as for each group separately. No adjustments for multiple correlations were made.

3. Results:

3.1. Activity in exploration box testing after treatment

The overall activity of all female Wistar rats remained stable during the entire 5 days of EB2 testing (Fig. 4). A slight peak was observed on day 3, followed by a slight non-significant decline until day 5. The differences amongst groups were the largest during day 1, after which a convergence of overall activity scores was observed, reflected in decreasing variance (Table 1).

A two-way ANOVA revealed a Phenotype main effect approaching significance on activity on day 1 ($F_{(1;27)} = 3.834$, $p = 0.061$), with HE rats exhibiting 0.58 mean activity as opposed to the 0.482 of LE rats. A Treatment+Phenotype interaction effect on activity on day 1 of EB2 also approached significance ($F_{(1, 27)} = 3.646$, $p = 0.067$), whereby PCA affected HE and LE animals differently (see 3.3).

Despite the fact that the saline HE and LE scores increasingly diverged over EB2 days, this did not approach statistical significance.

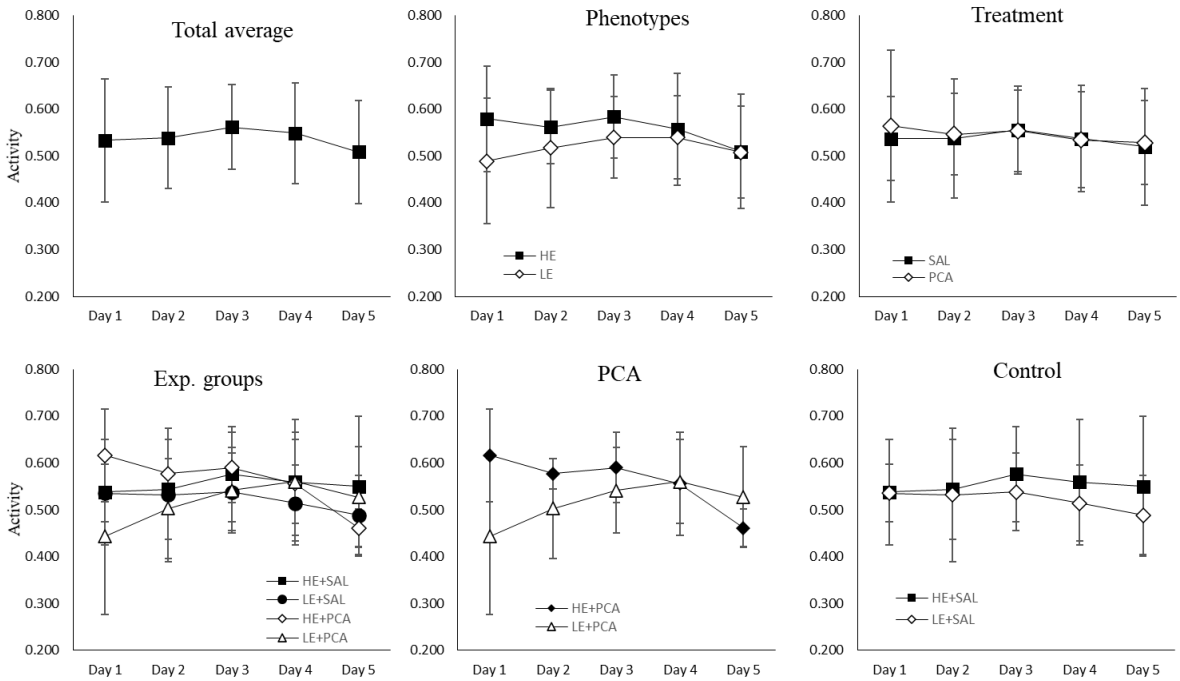


Fig. 4. Overall activity levels of exploration box testing block 2 (EB2), 0–1 scaled. Means±std. Top row shows total behavioral average, averages by phenotype and averages by treatment, respectively. Bottom row shows all experimental groups together, PCA groups separately, and control groups separately, respectively.

3.2. Behavioral parameters

Six of the parameters – linecrossings, rears, objects, entries, latency and time spent on field – correlated significantly with overall activity scores. The biggest component of overall activity, however, seems to be linecrossings, correlating with four of the other parameters. Linecrossings had the strongest correlation ($r_{(30)} = 0.89$, $p = < 0.001$) with overall activity levels as well (Table 2).

As expected, latency was the only parameter to correlate negatively with both overall activity scores ($r_{(30)} = – 0.471$, $p = 0.007$) and the other parameters separately. Latency also correlated the least with other parameters, only nearing significant correlation with linecrossings ($r_{(30)} = – 0.323$, $p = 0.076$).

3.3. PCA effect on behavior

A two-way ANOVA revealed a Treatment+Phenotype interaction effect on activity on day 1 that approached statistical significance ($F_{(1;27)} = 3.646$, $p = 0.067$). Tukey's post hoc revealed that the difference originated from between the HE+PCA and LE+PCA groups ($p = 0.045$). PCA had an excitatory effect on HE rats, increasing their mean activity to 0.616, highest amongst the groups, and an inhibitory effect on LE rats, decreasing their activity to 0.443, lowest amongst the groups – a significant difference between the two ($F_{(1, 14)} = 5.626$, $p = 0.033$). There was a gradual convergence between the two groups on the following days until day 4, after which their scores crossed on day 5 (Fig. 4).

Table 1
Descriptive statistics of 0-1 scaled behavioral activity in exploration box testing block 2 (EB2)

	Day 1	Day 2	Day 3	Day 4	Day 5
N	31	31	30	24	23
Mean	0.706	0.72	0.71	0.741	0.62
SD	0.168	0.136	0.12	0.147	0.133
Variance	0.028	0.018	0.015	0.022	0.018

PCA served to increase the activity change during EB2: a significant interaction of Treatment+Phenotype was revealed on the difference between activity scores on days 1 and 5 ($F_{(1, 19)} = 9.02$, $p = 0.007$). Post hoc revealed that the difference came from between the HE+PCA and LE+PCA groups (pTukey = 0.033). The HE+PCA group exhibited a decline in activity between days 1 and 5, falling from 0.616 to 0.461, a net change of –0.155 points. The LE+PCA on the other hand, exhibited an increase from 0.443 to 0.523, a net change of 0.084.

The HE+SAL and LE+SAL both experienced equally lower changes in activity between days 1–5, with a net difference of 0.064 and 0.059 points, respectively.

A one-way ANOVA revealed significant Treatment main effect on the group average cumulative changes ($F_{(1, 2)}$ = 45.9, p = 0.021). The average cumulative change was 0.181 for HE+PCA, and 0.15097 for LE+PCA and markedly lower at 0.064 and 0.059 for HE+SAL and LE+SAL, respectively. However, a two-way ANOVA with Treatment+Phenotype factors and individual rat cumulative day-to-day changes revealed no significant differences. This incongruency stems from the fact that in some of the groups rat score day-to-day changes balanced each other out, which was not reflected in group averages.

Table 2
Correlations between behavioral parameters and overall activity score in exploration box testing block 2 (EB2), all summed averages over days 2–5.

Variable	Lines	Rears	Objects	Entries	Latency	Time
Lines	—					
Rears	0.499 **	—				
Objects	0.443 *	0.536 **	—			
Entries	0.491 **	– 0.164	– 0.051	—		
Latency	– 0.323	– 0.011	– 0.054	– 0.251	—	
Time	0.451 *	0.773 ***	0.676 ***	– 0.301	0.148	—
Activity	0.890 ***	0.664 ***	0.663 ***	0.406 *	– 0.471 **	0.581 ***

* p < .05, ** p < .01, *** p < .001

3.4. Neurochemistry and behavioral correlates in striatum

Two-way ANOVA yielded several Treatment main effects: PCA reduced the levels of DA by ~12% ($F_{(1, 26)}$ = 8.558, p = 0.007), DOPAC by ~15% ($F_{(1, 27)}$ = 11.296, p = 0.002) and 5HIAA by ~11% ($F_{(1, 27)}$ = 5.684, p = 0.024), with 5HT nearing significance at a reduction of ~10% ($F_{(1, 27)}$ = 3.565, p = 0.07) (Table 3). Although a non–significant difference, DA fell by ~9% in HE animals and by ~16% in LE animals.

A significant Treatment+Phenotype interaction effect on striatal DA turnover was observed, whether measured by (DOPAC+HVA)/DA ratio ($F_{(1, 26)}$ = 4.386, p = 0.046) or the (DOPAC+HVA+3MT)/DA ratio ($F_{(1, 26)}$ = 5.663, p = 0.025). Either way, Games-Howell post hoc revealed no significant differences. Taking that into account, PCA decreased DA turnover in HE rats and increased it in LE rats (Fig. 1). One-way ANOVA revealed a Treatment effect in the HE animals on DA turnover ($F_{(1, 13)}$ = 4.654, p = 0.05). The turnover ratio was 0.164 in the HE+PCA group and 0.194 in the HE+SAL group (Table 3).

Of the striatal molecules, DOPAC had the most extensive correlations with animal behavior, correlating negatively with the overall activity score summed across days 2 to 5 ($r_{(30)}$

= – 0.438, p = 0.014) and with activity on day 5 separately ($r_{(30)}$ = – 0.426, p = 0.042). DOPAC also yielded the most significant correlations with specific behavioral parameters summed over days 2 to 5: linecrossings ($r_{(30)}$ = – 0.391, p = 0.03), rears ($r_{(30)}$ = – 0.375, p = 0.038), objects ($r_{(30)}$ = – 0.355, p = 0.05) and time spent on field ($r_{(30)}$ = – 0.364, p = 0.044). The only other striatal molecular–behavioral correlation was between 3MT and activity on day 4 ($r_{(30)}$ = – 0.482, p = 0.017).

Four of the six significant correlations between DOPAC and behavior originated from the LE+PCA group: linecrossings, rears, objects, time. The other two originated from the HE+SAL group: linecrossings and latency. All correlations with DOPAC were negative, except for latency.

The saline groups exhibited no significant differences by phenotype in the striatum.

3.5. Neurochemistry and behavioral correlates in hippocampus

PCA denervation significantly reduced the levels of 5HT ($F_{(1, 25)}$ = 56.966, $p < 0.001$) and 5HIAA ($F_{(1, 26)}$ = 31.709, $p < 0.001$) in the hippocampus, resulting in a ~29% decrease of each (Table 3). The amount of reduction did not differ beyond a few percentage points between HE and LE groups.

PCA did not affect any of the turnover rates in hippocampus. None of the present molecules in hippocampus yielded any significant correlations with behavior. No significant basal differences between HE and LE groups in the control condition were observed in hippocampus.

4. Discussion:

4.1. Overall behavioral activity

Male Wistar rats have been shown to exhibit more neophobia during the first days of exploration box testing, followed by a tendency of increased exploration during repeated testing (Mällo *et al.*, 2007). However, the results of this study on females were otherwise: female Wistar rat activity remained relatively stable throughout the 5 days of exploratory box testing, exhibiting only a slight non-significant decline (Fig. 3). Previously a decline in the Wistar strain has only been observed in male HE rats over a period of 6 months, suggested to be an age and weight–related decline (Mällo, 2008). LE males had remained unaffected by this – their activity being close to minimal already. However, in this study neither female HE nor LE exhibited a rise, and this could not have been related to aging or weight gain. Instead, this may be indicative of a sex difference, whereby female activity remains mostly stable during testing over consecutive days, as opposed to the male increase.

Table 3
Monoamine and metabolite levels (pmol/mg tissue) and turnover ratios in striatum and hippocampus.

	HE+SAL	LE+SAL	HE+PCA		LE+PCA	
Striatum						
5HT	5.21 ± 0.16	5.27 ± 0.30	4.84 ± 0.27	-7.15%	4.61 ± 0.24	-12.60%
DA	81.64 ± 2.77	83.87 ± 4.43	74.61 ± 3.41	-8.61%	70.40 ± 2.18	-16.06% *
NA	6.17 ± 0.36	6.25 ± 0.49	5.91 ± 0.37	-4.19%	5.97 ± 0.35	-4.42%
5HIAA	3.59 ± 0.13	3.52 ± 0.14	3.26 ± 0.19	-9.22%	3.08 ± 0.13	-12.63% *
DOPAC	7.05 ± 0.36	6.73 ± 0.31	6.05 ± 0.30	-14.08%	5.58 ± 0.21	-17.10% *
3MT	3.33 ± 0.10	3.16 ± 0.18	3.01 ± 0.17	-9.61%	3.00 ± 0.07	-5.11%
HVA	5.38 ± 0.67	5.09 ± 0.27	4.25 ± 0.15	-21.00%	4.55 ± 0.55	-10.47%
NMN	3.02 ± 0.16	3.10 ± 0.25	2.88 ± 0.07	-4.50%	2.95 ± 0.13	-5.04%
(HVA+DOPAC)/DA	0.15 ± 0.01	0.12 ± 0.01	0.12 ± 0.01	-19.26%	0.14 ± 0.01	10.23%
(HVA+3MT+DOPAC)/DA	0.19 ± 0.01	0.16 ± 0.01	0.16 ± 0.01	-15.39%	0.18 ± 0.01	10.67%
5HIAA/5HT	0.69 ± 0.03	0.68 ± 0.03	0.69 ± 0.07	0.50%	0.67 ± 0.02	-0.98%
NMN/NA	0.50 ± 0.03	0.50 ± 0.03	0.50 ± 0.03	0.50%	0.50 ± 0.03	-0.05%
Hippocampus						
5HT	3.88 ± 0.14	3.86 ± 0.19	2.80 ± 0.13	-27.82% ***	2.72 ± 0.05	-29.46% ***
DA	0.23 ± 0.02	0.25 ± 0.04	0.24 ± 0.02	8.15%	0.24 ± 0.02	-2.54%
NA	1.76 ± 0.14	1.56 ± 0.09	1.61 ± 0.10	-8.88%	1.74 ± 0.08	11.36%
5HIAA	2.35 ± 0.12	2.55 ± 0.15	1.73 ± 0.07	-26.64% ***	1.79 ± 0.09	-29.72% **
3MT	0.42 ± 0.05	0.40 ± 0.04	0.44 ± 0.04	3.11%	0.49 ± 0.03	22.12%
5HIAA/5HT	0.61 ± 0.03	0.64 ± 0.03	0.62 ± 0.02	2.06%	0.66 ± 0.04	3.66%
3MT/DA	1.83 ± 0.20	1.92 ± 0.28	1.79 ± 0.19	-2.54%	2.15 ± 0.24	12.05%

Mean±SEM. Percentages denote difference from corresponding saline group.
* $p < .05$, ** $p < .01$, *** $p < .001$; all compared to their corresponding saline groups.

When it comes to sex differences in exploratory behavior, the literature is relatively lacking. Numerous species exhibit dispersal patterns such that males travel further from their

birthplace while females remain closer. Thus, a positive relationoship had been hypothesized between male dispersal patterns and higher exploratory activity, which has indeed been confirmed in birds (Dingemanse, Both, Noordwjik, Rutten, Drent, 2003). In rats, however, the relationship has not been demonstrated (Lynn, Brown, 2009). The general observation has been instead that male rats exhibit less exploratory activity (Blizard, Lippman, Chen, 1975) despite higher spatial ability (Tropp, Markus, 2001), with the differences becoming evident in the peripubeal age (Masur, Schutz, Boerngen, 1980). An alternative explanation has been that although rat males do disperse further, exploratory activity is higher in females precisely because they remain in the same environment and hence have a higher motivation to acquaint themselves with their immediate surroundings (Lynn, Brown, 2009). This may imply a different set of motivations to explore in females than in males, possibly rendering conventional phenotype interpretations inadequate for extrapolation between the sexes.

If the aforementioned is true, one would expect Wistar females to be higher in exploratory activity than males. Although the current study did not include males for comparison, an approximate estimation could be derived from Mällo *et al.*, 2007, where the average Wistar male sum of exploratory events ranged between 4 – 165, with LE animals scoring between 5 – 25 and HE animals between 50 – 150 over 4 days of exploration box testing. In the current study, the first 4 days of EB2 yielded a sum of exploratory events range between 172 – 285, with HE ranging between 187 – 285 and LE between 172 – 236. These are purely tentative figures, but could imply a lower variability amongst Wistar female than male rats in addition to higher general activity in the females. This may be due to a difference in distribution – in our laboratory, males have exhibited a bimodal distribution, while females a normal distribution (unpublished). A direct comparison of the sexes is necessary.

Congruent with lower female variability is the markedly smaller difference between Wistar female HE and LE phenotypes amongst the exploratory box non-naïve animals during EB2, in comparison with their male counterparts in previous studies (Mällo *et al.*, 2007; Mällo, 2008). Though the saline group activity scores did begin to diverge on day 2 of EB2, corroborating previous observations on the emergence of phenotypes on that day (*ibid.*), the differences did not become significant even by day 5. It is unlikely that habituation from EB1 *per se* was the cause by lowering HE motivation to explore – unless habituation is accelerated in females, in which case this would constitute a sex difference – for Mällo *et al.*, 2007 had demonstrated the stability of exploratory phenotypes in non-naïve males up to at least 6 months. It may also be that exploratory phenotypes are less stable in females. The male variability

hypothesis could also be recalled (Reinhold, Engqvist, 2013), though with limitations (Zajitschek *et al.*, 2020). All of these questions necessitate further research.

4.2. PCA and behavior

PCA induced a significant difference in animal behavior only on day 1 of EB2, when it had an excitatory effect on HE rats and an inhibitory effect on LE rats. That the differences diminished on the following days, as the scores converged, might suggest that PCA afflicted the ability of LE animals to adapt to a new environment, ameliorated by repeated exposure. Had the differences persisted, a different kind of change might have been implicated in LE animals, such as in temperament or motivation to explore.

The excitatory effect on HE rats seems to be more complex. Because PCA reduced the behavioral stability of HE animals, this might suggest that instead of an anxiolytic effect, PCA might have served to increase impulsivity in high explorers. Previous studies have observed that PCA at 2mg/kg decreases immobility in the forced swimming test, and suggested that this may signify increased impulsivity (Harro *et al.*, 2001, Häidkind *et al*., 2004). Similarly, the application of chronic variable stress in combination with PCA administration has been shown to increase sucrose consumption, which had been connected to carbohydrate cravings in depression and increased vulnerability to stress (Harro *et al.*, 2001; Kõiv *et al.*, 2019) – an effect not observed under chronic variable stress condition alone. These imply difficulties of adaptation produced by PCA. In HE animals under exploration box testing, this might find expression in transiently increased activity. Again, had the changes been more stable, a change in temperament might have been implicated instead, with a possible anxiolytic effect. Further clarification could be provided by Female Wistar anxiety assays after PCA denervation.

The difficulty with this explanation is the need to explain why PCA did not increase impulsivity in LE animals. This may suggest different coping mechanisms and corresponding neural differences in high and low explorers.

4.3. Monoamines and behavioral correlates in striatum

PCA at 2mg/kg had the effect of significantly lowering the levels of DOPAC, 5HIAA and DA by ~10–15% 1 week after administration, with 5HT nearing significance. Although the effect of PCA on striatal DA has been observed before in males (Schwartz, Weizman, Rehavi, 2006), in our laboratory PCA had previously failed to affect the male striatal DA at dosages of 2mg/kg, 4mg/kg or even 6mg/kg (Häidkind *et al.*, 2004). The current research, conducted on

females, proved first such confirmation of PCA effect on striatal DA in our laboratory. Additionally, in males there has been a significant difference in striatal DA both at basal levels as well as in response to PCA or amphetamine at 0.5mg/kg (Mällo *et al.*, 2007; Alttoa *et al.*, 2007; Alttoa, Seeman, Kõiv, Eller, Harro, 2009). Currently in females, no such differences were found between HE and LE animals, neither in response to PCA nor basal levels – the saline groups. Because the lack of these HE/LE differences cannot be explained purely by different dosages, this must be considered as a possible sex difference, whereby female Wistar striatal DA does not differ between HE and LE animals.

Significant interaction occurred between PCA administration and phenotype, whereby PCA decreased striatal DA turnover in HE rats while increasing it in LE rats. The decrease was extensive enough that the HE+PCA group came to resemble the LE+SAL group in DA turnover. As before with DA levels, this effect of PCA on DA turnover had not been observed in males, neither at current dosages of 2mg/kg, nor at 4mg/kg or 6mg/kg (Häidkind *et al.*, 2004). Again, this may point towards sex differences in the Wistar rat strain.

The prevalence of DOPAC in the striatum had a significant inhibitory effect on several of the measures of behavioral activity – overall activity scores averaged across days 2 to 5, day 5 separately and the parameters of linecrossings, rears, objects and time spent on field, all averaged across days 2 to 5. This extensive correlation with behavior was not mirrored neither by DA levels nor by any of the measures for DA turnover rates – DOPAC/DA, (DOPAC+HVA)/DA, (DOPAC+HVA+3MT)/DA – which may be explained by a combination of four factors: a) the preferential metabolism of DA into DOPAC as opposed to 3MT, due to the enzymatic characteristics of the striatum (Karoum, Chrapusta, Egan, 1994), b) with HVA being the end product of DOPAC and 3MT, and thereby one additional step removed from being a representative indicator of DA turnover, b) by a decrease of DA release, rendering the various turnover ratios constant due to simultaneous decreases in both DA and metabolites, thereby disconnecting them from DA release and presumably, its correlation with behavior and c) the PCA-reduced amounts of DA released possibly being metabolized too rapidly to retain any correlation between its total released amount and amount upon the time of measurement. These may explain why DOPAC was the only molecule in the striatum to have such extensive correlations with behavioral measurements.

Four of the total six DOPAC correlations with behavioral activity parameters averaged across days 2 to 5 originated from the LE+PCA group, correlating negatively with linecrossings, rears, objects and time spent on field. The remaining two correlations originated

from the HE+SAL group – a negative correlation with linecrossings and a positive correlation with latency. The negative correlations in the LE+PCA group are congruent by that group having the second-most highest activity on day 5, as well as the lowest levels of DOPAC, with an average of 5.582 (Table 3). However, that two of the six correlations with DOPAC would originate from the LE+PCA group is incongruent with the fact that the same group also had the highest activity on day 5 and simultaneously the highest average levels of DOPAC, at 7.046. It is also puzzling as to why the group with the lowest activity on day 5, HE+PCA, would have the second lowest average levels of DOPAC, at 6.054. It would seem that the inhibitive effect of striatal DOPAC played a more decisive role in determining behavioral outcomes in the LE+PCA group than in the LE+SAL and HE+PCA groups. The author currently cannot offer an explanation.

4.4. Monoamines and behavioral correlates in hippocampus

PCA at 2mg/kg served to significantly lower the levels of 5HT and 5HIAA 1 week after administration by about 29% each. These findings are in agreement with previous observations, where PCA served to reduce 5HT and 5HIAA levels by ~20–30% at 2mg/kg, and more at higher doses (Garcia–Osta, Frechilla, Del Rio, 2000; Harro, Tõnissaar, Eller, Kask, Oreland, 2001; Häidkind *et al.*, 2004). Of the correlates between hippocampal molecules and behavior, only one was significant: between 5HT and latency. However, considering the multiplicity of correlations made without adjustments, and that all of the other correlations between 5HT and behavioral measures were non-significant and very weak (Pearson's $r<0.19$), this is likely a chance find.

4.5. Limitations

One of the main difficulties of this study has been the small group sizes, exacerbated by the removal of the cases where animals exhibited boxbiting – when the rat is distracted by becoming engrossed in biting either objects or the exploration box itself – which became more common as days progressed. For example, on day 5 of EB2, 8 of the total 31 animal activity scores were disregarded due to boxbiting (Table 1), more than a quarter of total. The removal of boxbiting is necessary, because such behavior is ambivalent in terms of the measurements of exploration box testing – it is neither exploratory nor inhibited in nature. Additionally, the removal of outliers, both in behavioral scores and monoamine levels, served to compound the issue. This must be taken into account when interpreting the current study, as the chance of false negatives increases as sample size diminishes (Vadillo, Konstantinidis, Shanks, 2016).

5. Conclusion

Female Wistar behavior remained stable throughout the 5 days of exploration box testing. There were no significant differences in exploration box non-naïve female activity in EB2 between high and low explorer phenotypes. This may suggest a decreased phenotypic stability or an accelerated habituation in females. PCA also failed to affect significant changes in female behavior, only transiently on day 1 having a slight inhibitory effect on LE animals and a stimulating effect on HE animals – which may have been impulsivity instead of an anxiolytic effect. However, PCA caused significant monoaminergic denervation in striatum and hippocampus: decreasing striatal DA, DOPAC, 5HIAA and near-significantly 5HT, all by about ~10-15%, and hippocampal 5HT and 5HIAA by about ~29%. Additionally, PCA significantly decreased striatal DA turnover in HE animals and increased it in LE animals.

From these observations, clear sex differences emerged with previous studies – Wistar males have displayed more pronounced behavioral phenotypic differences between high and low explorers, which have remained significant for prolonged periods of time. PCA has also affected male phenotypic behavior significantly differently. Finally, male phenotypes have exhibited significant monoaminergic differences, both at basal levels as well as in response to PCA. None of these were observed in females in the current study.

References:

Ahn, S.H., Jang, E.H., Choi, J.H., Lee, H.R., Bakes, J., Kong, Y.Y., Kaang, B.K. (2013). Basal anxiety during an open field test is correlated with individual differences in contextually conditioned fear in mice. *Animal Cells and Systems, 17*(3), pp 154–159.

Altoa, A., Kõiv, K., Eller, M., Uustare, A., Rinken, A., Harro, J. (2005). Effects of low dose *N*–(2–chloroethyl)–*N*–ethyl–2–bromobenzylamine administration on exploratory and amphetamine–induced behavior and dopamine D_2 receptor function in rats with high or low exploratory activity. *Neuroscience, 132*(4), pp 979–990.

Alttoa, A., Kõiv, K., Eller, M., Uustare, A., Rinken, A., & Harro, J. (2005). Effects of low dose N–(2–chloroethyl)–N–ethyl–2–bromobenzylamine administration on exploratory and amphetamine–induced behavior and dopamine D2 receptor function in rats with high or low exploratory activity. *Neuroscience, 132*(4), pp 979–990.

Alttoa, A., Eller, M., Herm, L., Rinken, A., & Harro, J. (2007). Amphetamine-induced locomotion, behavioral sensitization to amphetamine, and striatal D2 receptor function in rats with high or low spontaneous exploratory activity: differences in the role of locus coeruleus. *Brain research, 1131*(1), pp 138–148.

Alttoa, A., Seeman, P., Kõiv, K., Eller, M., Harro, J (2009). Rats with persistently high exploratory activity have both higher extracellular dopamine levels and higher proportion of D(2) (High) receptors in the striatum. *Synapse, 63*(5), pp 443–446.

Antoniou, K., & Kafetzopoulos, E. (1992). Behavioral effects of amphetamine and apomorphine after striatal lesions in the rat. *Pharmacology, biochemistry, and behavior, 43*(3), 705–722.

Blizard, D. A., Lippman, H. R., & Chen, J. J. (1975). Sex differences in open–field behavior in the rat: the inductive and activational role of gonadal hormones. *Physiology & behavior, 14*(5), pp 601–608.

Brand SJ, Moller M, Harvey BH. A Review of Biomarkers in Mood and Psychotic Disorders: A Dissection of Clinical vs. Preclinical Correlates (2015). *Curr Neuropharmacol, 13*(3), pp 324–368.

Calhoon, G., Tye, K. (2015). Resolving the neural circuits of anxiety. *Nat Neurosci, 18*, pp 1394–1404.

Carola, V., D'Olimpio, F., Brunamonti, E., Mangia, F., & Renzi, P. (2002). Evaluation of the elevated plus-maze and open–field tests for the assessment of anxiety–related behaviour in inbred mice. *Behavioural brain research, 134*(1–2), pp 49–57.

Costall, B., Jones, B.J., Kelly, M.E., Naylor, R.J, Tomkins, D.M. (1989) Exploration of mice in a black and white test box: Validation as a model of anxiety. *Pharmacology Biochemistry and Behavior* 32(3), pp 777–785.

Crawley, J.N. (1985) Exploratory behavior models of anxiety in mice. *Neuroscience & Biobehavioral Reviews, 9*(1), pp 37–44.

Drevets, W. C., Gautier, C., Price, J. C., Kupfer, D. J., Kinahan, P. E., Grace, A. A., Price, J. L., & Mathis, C. A. (2001). Amphetamine-induced dopamine release in human ventral striatum correlates with euphoria. *Biological psychiatry, 49*(2), pp 81–96.

Elverfors, A., & Nissbrandt, H. (1992). Effects of d–amphetamine on dopaminergic neurotransmission; a comparison between the substantia nigra and the striatum. *Neuropharmacology, 31*(7), pp 661–670.

Homan, P., Neumeister, A., Nugent, A. C., Charney, D. S., Drevets, W. C., & Hasler, G. (2015). 5HT versus catecholamine deficiency: behavioral and neural effects of experimental depletion in remitted depression. *Translational psychiatry*, *5*(3), e532.

Nutt, D., Demyttenaere, K., Janka, Z., Aarre, T., Bourin, M., Canonico, P. L., Carrasco, J. L., & Stahl, S. (2007). The other face of depression, reduced positive affect: the role of catecholamines in causation and cure. *Journal of psychopharmacology (Oxford, England), 21*(5), pp 461–471.

Karoum, F., Chrapusta, S. J., & Egan, M. F. (1994). 3–Methoxytyramine is the major metabolite of released dopamine in the rat frontal cortex: reassessment of the effects of antipsychotics on the dynamics of dopamine release and metabolism in the frontal cortex, nucleus accumbens, and striatum by a simple two pool model. *Journal of neurochemistry*, *63*(3), pp 972–979.

Kohls, G., Schulte–Rüther, M., Nehrkorn, B., Müller, K., Fink, G.R., Kamp–Becker, I., Herpertz–Dahlmann, B., Schultz, R.T., Konrad, K. (2013). Reward system dysnfunction in autism spectrum disorders. *Social Cognitive and Affective Neuroscience, 8*(5), pp 565–572.

Kõiv, K., Vares, M., Kroon, C., Metelitsa, M., Tiitsaar, K., Laugus, K., Jaako, K., & Harro, J. (2019). Effect of chronic variable stress on sensitization to amphetamine in high and low sucrose-consuming rats. *Journal of psychopharmacology (Oxford, England), 33*(12), 1512–1523.

Delgado, P.L. (2000). Depression: The Case for a Monoamine Deficiency. *Journal of Clinical Psychiatry 61(suppl)*, pp 7–11.

Devan, B. D., Hong, N. S., & McDonald, R. J. (2011). Parallel associative processing in the dorsal striatum: segregation of stimulus–response and cognitive control subregions. *Neurobiology of learning and memory*, *96*(2), pp 95–120.

Elhwuegi, A.S. (2004). Central monoamines and their role in major depression. *Progress in neuro–Psychopharmacology and Biological Psychiatry, 28*(3): pp 435–451.

Engin, E., & Treit, D. (2007). The role of hippocampus in anxiety: intracerebral infusion studies. *Behavioural pharmacology*, *18*(5–6), pp 365–374.

Katz, R.J., Roth, K.A., Carroll, B.J. (1981). Acute and chronic stress effects on open field activity in the rat: Implications for a model of depression. *Neuroscience & Biobehavioral Reviews*, *5*(2), pp 247–251.

Landgraf, R. (2003). HAB/LAB rats: an animal model of extremes in trait anxiety and depression. *Clinical Neuroscience Research, 3*(4–5): pp 239–244.

Landgraf, R., Wigger, A. (2002). High vs low anxiety–related behavior rats: an animal model of extremes in trait anxiety. *Behavior Genetics, 32*(5): pp 301–314.

Liebsch G, Montkowski A, Holsboer F, Landgraf R. (1998) Behavioural profiles of two Wistar rat lines selectively bred for high or low anxiety–related behaviour. *Behav Brain Res 94*, pp 301–310.

Datla, K.P., Curzon, G. (1996). Effect of p–chlorophenylalanine at moderate dosage on 5–HT and 5–HIAA concentrations in brain regions of control and p–chloroamphetamine treated rats. *Neuropharmacology, 35*(3): pp 315–320.

Dellu, F., Piazza, P.V., Mayo, W., Le Moal, M., Simon, H. (1996). Novelty–Seeking in Rats–Biobehavioral Characteristics and Possible Relationship with the Sensation–Seeking Trait in Man. *Neuropsychobiology, 34*, pp 136–145.

Scholl, J.L., Renner, K.J., Forster, G.L., Tejani–Butt, S. (2010). Central monoamine levels differ between rat strains used in studies of depressive behavior. *Brain Research, 1355*, pp 41–51.

da Silva Rocha–Lopes, J., Machado, R. B., & Suchecki, D. (2018). Chronic REM Sleep Restriction in Juvenile Male Rats Induces Anxiety-like Behavior and Alters Monoamine Systems in the Amygdala and Hippocampus. *Molecular neurobiology, 55*(4), pp 2884–2896.

Tu, W., Cook, A., Scholl, J. L., Mears, M., Watt, M. J., Renner, K. J., & Forster, G. L. (2014). 5HT in the ventral hippocampus modulates anxiety-like behavior during amphetamine withdrawal. *Neuroscience, 281*, pp 35–43.

Gawin F. H. (1991). Cocaine addiction: psychology and neurophysiology. *Science (New York, N.Y.), 251*(5001), pp 1580–1586.

García–Osta, A., Frechilla, D., & Del Río, J. (2000). Effect of p–chloroamphetamine on 5–HT1A and 5–HT7 5HT receptor expression in rat brain. *Journal of neurochemistry, 74*(5), pp 1790–1797.

Gould, T.D., Dao, D.T., Kovacsics, C.E. (2009) The Open Field Test. *Mood and Anxiety Related Phenotypes in Mice. Neuromethods, vol 42*, pp 1–20.

Harro, J., Tõnissaar, M., Eller, M., Kask, A., & Oreland, L. (2001). Chronic variable stress and partial 5–HT denervation by parachloroamphetamine treatment in the rat: effects on behavior and monoamine neurochemistry. *Brain research*, 899(1–2), pp 227–239.

Harro, J., Oreland, L. (2001) Depression as a spreading adjustment disorder of monoaminergic neurons: a case for primary implication of the locus coeruleus. *Brain Research Reviews 38*(1–2), pp 79–128.

Harro, J. (2002). Long–term partial 5–HT depletion: interference of anxiety and impulsivity? *Psychopharmacology, 164*, 433–34.

Harro, J., Terasmaa, A., Eller, M., Rinken, A. (2003). Effect of denervation of the locus coeruleus projections by DSP–4 treatment on [^{3}H]–raclopride binding to dopamine D_2 receptors and D_2 receptor–G protein interaction in the rat striatum. *Brain Research 976*(2), pp 209–216.

Harro J. (2010). Inter-individual differences in neurobiology as vulnerability factors for affective disorders: implications for psychopharmacology. *Pharmacology & therapeutics*, *125*(3), 402–422.

Heal, D. J., Smith, S. L., Gosden, J., & Nutt, D. J. (2013). Amphetamine, past and present—a pharmacological and clinical perspective. *Journal of psychopharmacology (Oxford, England)*, *27*(6), pp 479–496.

Helfinstein, S. M., Fox, N. A., & Pine, D. S. (2012). Approach–withdrawal and the role of the striatum in the temperament of behavioral inhibition. *Developmental psychology*, *48*(3), pp 815–826.

Ho, Y.H., Eichendorff, J., Schwarting, R.K.W. (2002). Individual response profiles of male Wistar rats in animal models for anxiety and depression. *Behavioural Brain Research, 136*(1), pp 1–12.

Hooks, M.S., Jones, G.H., Smith, A.D., Neill, D.B., Justice, Jr J.B. (1991) Response to novelty predicts the locomotor and nucleus accumbens dopamine response to cocaine. *Synapse* 9. pp 121–128.

Häidkind, R., Eller, M., Kask, A., Harro, M., Rinken, A., Oreland, L., Harro, J. (2004). Increased behavioural activity of rats in forced swimming test after partial denervation of serotonergic system by parachloroamphetamine treatment. *Neurochemistry International, 45*, pp 721–32.

Borsini, F. (1995). Role of the serotonergic system in the forced swimming test. *Neuroscience & Biobehavioral Reviews*, *19*(3), pp 377–395.

Hornykiewicz, O. (1975). Brain monoamines and parkinsonism. *National Institute on Drug Abuse Research Monograph Series*,(3), pp 13–21.

Yenkoyan, K., Fereshetyan, K., Matinyan, S., Chavushyan, V., Aghajanov, M. (2018). The role of monoamines in the development of Alzheimer's disease and neuroprotective effect of a proline rich polypeptide. *Progress in Neuro–Psychopharmacology & Biological Psychiatry*, *30*(86), pp 76–82.

Schwartz, K., Weizman, A., & Rehavi, M. (2006). The effect of psychostimulants on [3H]dopamine uptake and release in rat brain synaptic vesicles. *Journal of neural transmission (Vienna, Austria : 1996)*, *113*(9), pp 1347–1352.

Sedvall, G. (1990). Monoamines and schizophrenia. *Acta Psychiatrica Scandinavica, 358*: pp 7–13.

Storey, J. D., Robertson, D. A., Beattie, J. E., Reid, I. C., Mitchell, S. N., & Balfour, D. J. (2006). Behavioural and neurochemical responses evoked by repeated exposure to an elevated open platform. *Behavioural brain research*, *166*(2), 220–229.

Hillhouse, T.M., Porter, J.H. (2015). A brief history of the development of antidepressant drugs: From monoamines to glutamate. *Experimental and Clinical Psychopharmacology, 23(1),* 1–21.

Kanarik, M., Matrov, M., Kõiv, K., Eller, M., Tõnissaar, M., Harro, J. (2008). Changes in regional long–term oxidative metabolism induced by partial serotonergic denervation and chronic variable stress in rat brain. *Neurochemistry International, 52*, pp 432–437.

Lago, T., Davis, A., Grillon, C., & Ernst, M. (2017). Striatum on the anxiety map: Small detours into adolescence. *Brain research*, *1654*(Pt B), pp 177–184.

Lynn, D. A., & Brown, G. R. (2009). The ontogeny of exploratory behavior in male and female adolescent rats (Rattus norvegicus). *Developmental psychobiology*, *51*(6): pp 513–520.

Piazza P.V., Rouge–Pont F., Deminiere J.M., Kharoubi M., Le Moal M., Simon H. (1991) Dopaminergic activity is reduced in the prefrontal cortex and increased in the nucleus accumbens of rats predisposed to develop amphetamine self–administration. *Brain Res, 567*, pp 169–174.

Mahar, I., Bambico, F. R., Mechawar, N., & Nobrega, J. N. (2014). Stress, 5HT, and hippocampal neurogenesis in relation to depression and antidepressant effects. *Neuroscience and biobehavioral reviews*, *38*, 173–192.

Masur, J., Schutz, M. T., & Boerngen, R. (1980). Gender differences in open–field behavior as a function of age. *Developmental psychobiology*, *13*(2), pp 107–110.

Miyagishi, H., Tsuji, M., Miyagawa, K., Kurokawa, K., Mochida–Saito, A., Takahashi, K., Ishige, K., & Takeda, H. (2020). Characterization of 5–HT_{1A} receptor and transport protein KIF13A expression in the hippocampus of stress–adaptive and –maladaptive mice. *Neuroscience letters*, *733*, 135082.

Mällo, T., Alttoa, A., Kõiv, K., Tõnissaar, T., Eller, M., Harro, J. (2007). Rats with persistently low or high exploratory activity: Behaviour in tests of anxiety and depression, and extracellular levels of dopamine. *Behavioral Brain Research*, 177, pp 269–81.

Mällo, T. (2008) Exploratory behavior and 50–kHz ultrasonic vocalizations in rats: behavioral and neurochemical profiles of persistent inter-individual differences. *Dissertationes Psychologicae Universitas Tartuensis 23*.

Mällo, T., Kõiv, K., Koppel, I., Raudkivi, K., Uustare, A., Rinken, A., Timmusk, T., Harro, J. (2008). Regulation of extracellular 5HT levels and brain–derived neurotrophic factor in rats with high and low exploratory activity. *Brain Research, 1194*: pp 110–117.

Otter, M. H., Matto, V., Sõukand, R., Skrebuhhova, T., Allikmets, L., & Harro, J. (1997). Characterization of rat exploratory behavior using the exploration box test. *Methods and findings in experimental and clinical pharmacology*, *19*(10), pp 683–691.

Pino, J. A., Awadallah, N., Norris, A. M., & Torres, G. E. (2021). A Plate-Based Assay for the Measurement of Endogenous Monoamine Release in Acute Brain Slices. *Journal of visualized experiments : JoVE*, (174),

Porsolt, R.D., Bertin, A., Blavet, N., Deniel, M., Jalfre, M. (1979). Immobility, induced by forced swimming in rats: effects of agents which modify central catecholamine and 5HT activity. *European Journal of Pharmacology, 57*(2–3), pp 201–210.

Price, J. L., & Drevets, W. C. (2010). Neurocircuitry of mood disorders. *Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology*, *35*(1), pp 192–216.

Reinhold, K., & Engqvist, L. (2013). The variability is in the sex chromosomes. *Evolution; international journal of organic evolution*, *67*(12), pp 3662–3668.

Tropp, J., & Markus, E. J. (2001). Sex differences in the dynamics of cue utilization and exploratory behavior. *Behavioural brain research*, *119*(2), pp 143–154.

Tõnissaar, M., Mällo, T., Eller, M., Häidkind, R., Kõiv, K., Harro, J. (2008). Rat behavior after chronic variable stress and partial lesioning of 5–HT–ergic neurotransmission: effects of citalopram. *Progress in Neuro–Psychopharmacology & Biological Psychiatry, 32*(1), pp 164–177.

Vadillo, M. A., Konstantinidis, E., & Shanks, D. R. (2016). Underpowered samples, false negatives, and unconscious learning. *Psychonomic bulletin & review*, *23*(1), pp 87–102.

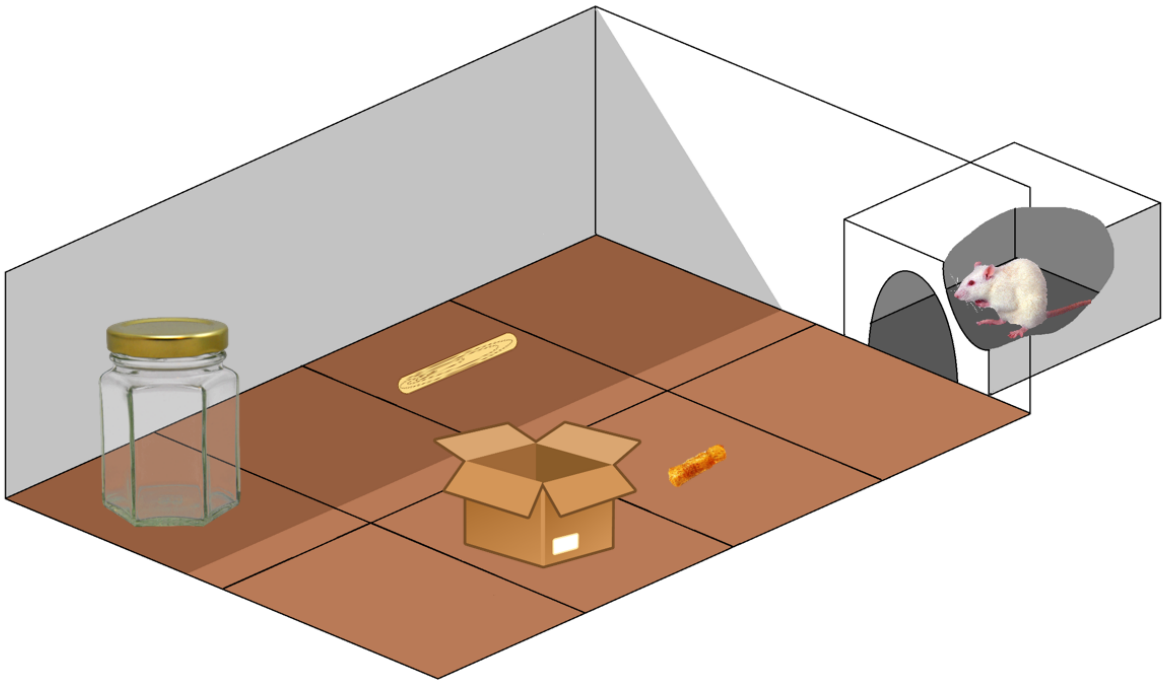
Valencia, S., Gonzales, E. L., Adil, K. J., Jeon, S. J., Kwon, K. J., Cho, K. S., & Shin, C. Y. (2019). Comparative Behavioral Correlation of High and Low–Performing Mice in the Forced Swim Test. *Biomolecules & therapeutics*, *27*(4), pp 349–356.

Fuller, R.W. (1992). Effects of p–chloroamphetamine on brain 5HT neurons. *Neurochemical Research, 17*(5), pp 449–56.

Zajitschek, S. R., Zajitschek, F., Bonduriansky, R., Brooks, R. C., Cornwell, W., Falster, D. S., Lagisz, M., Mason, J., Senior, A. M., Noble, D. W., & Nakagawa, S. (2020). Sexual dimorphism in trait variability and its eco–evolutionary and statistical implications. *eLife*, *9*, e63170.

Appendix A

The exploration box



The exploration box. The box consists of two areas: an open area of 0.5m x 1m, divided into 8 equal parts, delineated by lines as shown above; and an enclosed smaller box, by which the rat is attached into the open area and allowed to either leave or remain inside of. Four objects are dispersed in the open area in positions as shown here, with 3 of them being unfamiliar objects (a glass jar, a wooden handle, a cardboard box) and one familiar (a food pellet).

Käesolevaga kinnitan, et olen korrektselt viidanud kõigile oma töös kasutatud teiste autorite poolt loodud kirjalikele töödele, lausetele, mõtetele, ideedele või andmetele.

Olen nõus oma töö avaldamisega Tartu Ülikooli digitaalarhiivis DSpace.

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