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EFFECTS OF PARACHLOROAMPHETAMINE ON LOCOMOTOR ACTIVITY AND SEROTONIN AND DOPAMINE LEVELS IN PREFRONTAL CORTEX AND SEPTUM IN RATS WITH DIFFERENT SOCIABILITY

research paper

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Running head: sociability, PCA and monoamines

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Effects of parachloroamphetamine on locomotor activity and serotonin and dopamine levels in prefrontal cortex and septum in rats with different sociability

Abstract

Social behaviour plays important role in many psychopathologies. Sociability has been shown to be a stable trait in animals and individual differences in sociability are related to 5-HT transmission in septum and prefrontal cortex. The aim of the current study was to investigate whether animals with different levels of sociability are differently affected by p-chloroamphetamine treatment. 5-HT, 5-HIAA and DA levels were measured in septum and PFC and locomotor activity was recorded. Although there was no difference in monoamine levels or locomotor activity between LS- and HS-animals after PCA treatment, our findings confirm previous research on the different neurodegenerative effect of PCA in different brain regions. PCA had stronger neurodegenerative effect in PFC as 5-HT and 5-HIAA levels there were lower than in septum.

Parakloramfetamiini mõju psühhomotoorsele aktiivsusele ja serotoniini ja dopamiini tasemetele septumis ja prefrontaalkorteksis erineva püsisotsiaalsusega loomadel

Kokkuvõte

Sotsiaalsel käitumisel on oluline roll paljudes psüühikahäiretes. Sotsiaalsus on loomades püsiv omadus ja seotud serotoniini transmissiooniga septumis ja prefrontaalkorteksis. Käesolevas uurimuse eesmärk oli uurida kas parakloramfetamiini mõju on erineva sotsiaalsusega loomadele erinev. Mõõtsime loomade liikumisaktiivsust ja DA, 5-HT ja 5-HIAA tasemeid prefrontaalkorteksis ja septumis. Kuigi monoamiinide tasemed ajus ja liikumisaktiivsus pärast PCA manustamist ei erinenud LS- ja HS-loomadel, kinnitavad käesoleva uurimuse tulemused varasemaid uuringuid PCA erinevast mõjust erinevatest ajupiirkondades. PCA neurodegeneratiivne mõju oli prefrontaalkorteksis suurem kui septumis, kuna 5-HT ja 5-HIAA tasemed olid prefrontaalkorteksis madalamad kui septumis.

1. Introduction

1.1 Sociability trait and psychopathology

Social behaviour has been evolutionally essential for the survival of the individual as well as the species and is the basis of one of the most generally accepted dimensions of personality – agreeableness. Difficulty engaging in or lack of social behaviour is often one of the symptoms of several psychopathologies for example autism spectrum disorders, schizophrenia, depression and social anxiety disorder (American Psychiatric Association, 2013).

Animal models are a reliable way to study the underlying mechanism of these disorders because the neurobiology that underlies affect is similar in all mammalian species (Panksepp, 1998). Individual differences in animal behaviour can reveal endophenotypes for the preclinical modeling of disorders listed above (Gould & Gottesman, 2006; Harro, 2010 & 2013) and studying biochemical and behavioural consequences of disturbed 5-HT regulation in animal models might reveal compensatory adaptations that could provide useful insight for developing new medication (Homberg et al., 2007).

Social interaction test was developed as an animal model for anxiety by File and Hyde (1978). Behaviour in social interaction test does not correlate well with behaviour in other animal models for anxiety – open field test, elevated plus maze (Ramos, Berton, Mormede & Chaouloff, 1997), so it was hypothesized that this model might have other underlying mechanisms in addition to anxiety, and could be used to study social behaviour and its neurobiology (Tõnissaar, Philips, Eller & Harro, 2004). Sociability in this test is defined as proneness to initiate and/or accept social contact with another animal on neutral territory (Tõnissaar et al., 2004). It has been shown that sociability is a persistent trait in rats (Tõnissaar et al., 2004) and even though sociability tends to decrease with age and weight gain, group differences remain significant (Tõnissaar et al., 2008).

1.2 Serotonin and social behaviour

Neurobiology behind sociability trait points to serotonin system. Serotonin is one of the oldest neurotransmitters that plays role in early brain development (Di Pino, Moessner, Lesch, Lauder & Persico, 2004), is widely distributed in the brain and regulates the activity and interaction of many other neurotransmitter systems (Lesch, 2007). 5-HT plays role in modulating emotional behaviour – anxiety, impulsivity, stress responses, aggression, motivation, cognition etc. Thus disturbed 5-HT regulation contributes to several disorders: affective disorders, drug addiction, schizophrenia, eating disorders, impulse control disorders etc (Murphy, Lerner, Rudnick & Lesch, 2004). Increased sensitivity to natural and experimental alterations of the serotonergic system known as serotonergic

vulnerability is one possible explanation why when for example exposed to stress, some people become depressed while others do not (Jans, Ridel, Markus & Blokland, 2007). The way people cope with stress is, among other things, affected by personality (Kendler, Karkowski & Prescott, 1999). Personality is partially determined by genetics, for example, 5-HTTLPR s-allele carriers compared to l/l carriers exhibit more likely anxiety-related personality traits such as neuroticism and harm avoidance (Lesch et al., 1996). Therefore certain personality traits might reflect serotonergic vulnerability.

5-HT partial depletion can be used as an animal model to study disturbed 5-HT regulation which has been shown to have similar long-lasting behavioural effects as chronic variable stress (CVS) - reduced immobility in the forced swimming test and anxiogenic effect in the social interaction test (Harro, Tõnissaar, Eller, Kask & Oreland, 2001). Tõnissaar et al., (2008) showed that stress affects animal behaviour and neurochemistry differently based on their sociability level – high sociability (HS) animals in response to stress developed anhedonia which is measured as a decrease in sucrose intake and low sociability (LS) animals showed increased swimming activity in forced swimming test. 5-HT levels decreased and DA levels increased in response to stress only in LS-animals thus persistently high social activity is associated with increased sensitivity to stress (Tõnissaar et al., 2008).

A study by Tõnissaar et al. (2004) found that sociability trait was negatively associated with 5-hydroxyindoleacetic acid (5-HIAA) levels in the frontal cortex. In single social interaction test though, social interaction might be dependent on 5-HT metabolism in septum because the negative correlation between social interaction time and 5-HT metabolism in septum was found after just one interaction test but not after four (Tõnissaar et al., 2004). Harro et al (2006) showed that the basal 5-HT levels in the PFC were significantly higher in LS animals and when treated with PCA, 5-HT release into synaptic cleft was similar in LS- and HS-animals and absolute levels of extracellular 5-HT in the PFC remained significantly higher in HS group. It was hypothesized that high 5-HT release to synaptic cleft desensitizes postsynaptic 5-HT receptors in PFC in LS-animals (Harro et al., 2006). Extracellular level of 5-HT is mostly regulated by the 5-HT transporter (SERT) which takes 5-HT from extracellular space back into the presynaptic neuron where it is broken down or stored for the future release (Blakely et al., 1991; Murphy et al., 1998). SERT knock-out animals display increased levels of anxiety and depression-like behaviours regardless of the test used (Olivier et al., 2008).

1.3 5-HT depletion

As mentioned above one way to study disturbed 5-HT regulation is 5-HT partial depletion which can be achieved by administration of 5-HT selective drugs such as 3,4-methylenedioxymethamphetamine (MDMA) or p-chloroamphetamine (PCA). MDMA is a well known and widely used illegal psychoactive drug that differs from other substituted amphetamines by having both stimulant and mild psychedelic effects. After consuming MDMA humans can experience connectedness, sense of well being, altered perception of time, mild hallucinations, increased self-confidence, reduced anxiety etc (Davison & Parrott, 1997). PCA is qualitatively similar but much more neurotoxic than MDMA. To achieve neurodegeneration in 5-HT neurons repeated administration of MDMA is required while PCA produces the effect after a single low dose.

5-HT selective neurotoxins act in two phases. In first few hours after the drug administration 5-HT is rapidly released, in next 24 hours the levels of 5-HT return to normal and in next few days 5-HT levels in brain tissue decrease due to neurodegeneration (Sanders-Bush & Steranka, 1978). PCA causes decrease in 5-HT and HIAA levels, density of 5-HT uptake sites, decreases in SERT expression, increased 5-HT axon calibre, huge swollen varicosities, fragmentation and dilated proximal axon stumps (Molliver et al., 1990) and this damage has been shown to persist for several months (Sanders-Bush, 1975). 2 mg/kg dosage of PCA causes approximately 20% reduction in 5-HT and 5-HIAA levels in the frontal cortex, hippocampus, and septum (Harro et al., 2001; Häidkind et al., 2004). Neurotoxic effect of PCA is time-dependent depending on the region, for example in septum the depleting effect of PCA occurred 2 weeks after the administration not right away (Häidkind et al., 2004) and this can be explained by the beaded nerve fibres in the septum that are more resistant to PCA than fine nerve fibres that innervate frontal cortex (Mamounas et al., 1991; Harro et al., 2001; Molliver et al., 1990).

Studies, where PCA was administered directly to 5-HT axons, showed no neurotoxic effect and 5-HT depletion by pretreatment with citalopram or p-chlorophenylalanin and reserpine protect against neurotoxic effects of PCA – that suggests that release of endogenous 5-HT is necessary for the neurotoxic effect (Berger, 1989).

5-HT selective neurotoxins enter 5-HT neurons through 5-HT uptake transporter and compete with endogenous 5-HT for reuptake and induce the release of 5-HT to the synaptic cleft (Berger, Gu & Azmitia, 1992).

Although these neurotoxins mainly affect 5-HT system without affecting catecholamines too much (Massari, Tibazi & Jacobowitz, 1978; Leonard, 1976) they nevertheless influences the release of dopamine and levels of its metabolites possibly due to the initial release of 5-HT (Schmidt, Wu & Lovenberg, 1986; Schmidt, 1987; Johnson, Huang & Nichols, 1991). The presynaptic release of DA in nucleus accumbens is related to the locomotor activity increasing effect of PCA and MDMA, this effect can be reduced by damage to DA neurons in nucleus accumbens (Gold, Hubner & Koob, 1989) or by 5-HT1A and oxytocin receptor antagonists (Kuteykin-Teplyakov & Maldonado, 2014). PCA and MDMA induced increase in locomotor activity is significantly different from amphetamine caused increase and most likely works partially through serotonin system (Frederick & Paule, 1997).

1.4 Aim of the study

The aim of the current study is to determine whether PCA administration has a different effect on monoamine levels in PFC and septum depending on the sociability level of the animal and does this have an effect on locomotor activity. The baseline serotonin levels are different in HS- and LS-animals (Tõnissaar et al., 2004) and their extracellular serotonin levels remained different after PCA treatment (Harro et al., 2006) and knowing that neurotoxic effect depends on the extracellular 5-HT level (Berger, 1989), we expect to see different neurodegenerative effect depending on the sociability of the animal. Since locomotor activity is regulated by both 5-HT and DA system (Gold, Hubner & Koob, 1989) we expect to find individual differences in locomotor activity as well. And in general gain some more insight into monoamine systems that underlie our sociability.

Hypothesis 1: Monoamine levels in septum and PFC after administration of PCA are lower in HS-animals than in LS-animals.

Hypothesis 2: Horizontal distance travelled in locomotor activity test is longer in HS-animals immediately after administration of PCA.

2. Method

2.1 Animals

Male Wistar rats (N=40) were group housed in standard cages in light and temperature controlled room with 12 hours light-dark cycle (lights on 7:00, lights out 19:00) and the temperature maintained at 22º C. Animals had access to food and water ad lib.

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Animals were two months old and weighed 267 – 350 g at the beginning of experiments. Animals were handled several times before the beginning of the experiments.

All experiments were in accordance with the EU Directive 2010/63/EU for animal experiments and approved by the Animal Experimentation Committee at the Estonian Ministry of Rural Affairs (license # 99).

2.2 General procedure

Animals were single-housed 9-10 days before the first social interaction test (SI). SI test was carried out 4 times, approximately 7 days between each test. Social interaction was measured for 10 minutes. After the last interaction test animals were divided into high sociability and low sociability groups based on the mean interaction time over 4 tests.

Animals were habituated with phenotyper cages 3 times for 10 minutes before the first locomotor activity test. HS and LS groups were further divided into PCA and saline treatment groups.

Animals were administered PCA or saline and immediately put into phenotyper cages. Locomotor activity was recorded twice, 7 days between the tests, for 60 minutes each time. Animals were decapitated 24 hours after the last locomotor activity test. Brain tissue was collected and stored until further analysis.

2.3 Social interaction test

The social interaction test developed by File and Hyde (1978) was used as described earlier (Harro et al, 2001). Animals were single-housed 9-10 days before the first SI test. Social interaction was measured on 4 separate occasions 7-10 days between each test. In dimly lit room two unfamiliar, weight-matched (weight difference no more than 10%) rats were placed in a transparent box (30 cm × 30 cm × 60 cm) with the floor covered with wood shavings for 10 minutes. The total time spent in active social behaviour (allogrooming, sniffing the partner, crawling under and over, following) was recorded by two or three observers. Based on the mean SI score over four interaction tests, animals were divided into LS and HS groups.

The two groups were assigned to the following treatment groups

1) Saline – rats were injected saline (0.9% sodium chloride) 0.05 ml/10g intraperitoneally before the first locomotor activity recording

2) PCA– rats were injected p-choloroamphetamine 2 mg/kg intraperitoneally before the first locomotor activity recording

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2.4 Locomotor activity recording

Horizontal locomotor activity was recorded for 60 minutes in 10 separate PhenoTyper 4500 cages (45x45x55 cm transparent cage) in a separate room. Horizontal distance travelled was recorded with the PhenoTyper top unit containing infrared sensitive camera with three arrays of infrared LED lights. This process was repeated 7 days later.

Animals were anaesthetized with CO2 and decapitated 24 hours after the second locomotor activity test

2.5 Brain dissection

Dissection of the tissues (nucleus accumbens, prefrontal cortex, hippocampus, raphe, locus coeruleus, dorsal striatum, and septum) was performed by two experienced researchers with the rat brain atlas of Paxinos and Watson (1998) as a guide. The tissues were immediately frozen in liquid nitrogen and stored at -80°C until analysis of the tissues.

PFC and septum were chosen for monoamine analysis because these brain regions have high concentrations of 5-HT, 5-HIAA and 5-HT uptake sites and previous studies (Tõnissaar et al 2004, 2008) found changes in these areas.

2.6 Monoamine concentration measurements with high-performance liquid chromatography

Monoamines and their metabolites were assayed by HPLC with electrochemical (amperometric) detection as described previously (Alttoa et al., 2005) with some minor modifications**.** The rat brain tissues were homogenized with an ultrasonic homogenizer (Bandelin Sonopuls, Bandelin Electronic, Berlin, Germany) in an ice-cold solution of 0.1 M perchloric acid (30 (for septum) or 50 µl/mg (for PFC)) containing 5 mM of sodium bisulphite and 0.4 mM EDTA to avoid oxidation. The homogenate was then centrifuged at 14.000 rpm for 10 min at 4°C. Aliquots (10 µl) of the obtained supernatant were chromatographed on a Luna C18(2) column (150 x 2 mm, 5 µm). The separation was done in isocratic elution mode at column temperature of 30°C using the mobile phase containing 0.05 M sodium citrate buffer at pH 3.7, 0.02 mM EDTA, 1 mM KCl, 1 mM sodium octanesulfonate and 7.5% acetonitrile. The chromatography system consisted of an isocratic pump (Hewlett Packard HP1100), a temperature-regulated autosampler, a temperature-regulated column compartment and an HP 1049 electrochemical detector (Agilent, Waldbronn, Germany) with glassy carbon electrode. The measurements were done at an electrode potential of +0.7 V versus the Ag/AgCl reference electrode. The limits of detection at signal-to-noise ratio (S/N)=3 were as follows: 0.08 pmol/mg tissue for DA; 0.10 pmol/mg tissue for HVA; 0.05 pmol/mg tissue for DOPAC; 0.1 pmol/mg tissue for 3MT; 0.03 pmol/mg tissue for NMN; 0.08 pmol/mg tissue for 5-HT; 0.04 pmol/mg tissue for 5-HIAA.

2.7 Statistical analysis

All data were analyzed using the IBM SPSS Statistics 21 software. Three-way mixed analysis of variance (ANOVA) and two-way ANOVA were conducted for group comparisons. Occasionally assumptions for ANOVA – homogeneity of variances, the absence of outliers and normality were violated but transforming data ruled out these violations. All analysis were run both with and without outliers and since results did not differ significantly the results are reported on original data. For association analysis, Pearson correlation was run. Data are mean ± SEM unless otherwise stated and significance set at $p < 0.05$.

3. Results

3.1 Individual differences in social interaction time.

After dividing HS- and LS-animals into two treatment groups analysis was run to determine there were no statistically significant differences in the mean interaction time between treatment groups for neither sociability group.

There was statistically significant main effect of sociability ($F(1, 36)= 43.17$, $p < .001$, partial $\eta 2 = .545$). Social interaction time was significantly higher in HS-animals (204.21 ± 6.64 s) than LS-animals (142.54 ± 6.64 s) with the mean difference of (61.67 ± 9.39 seconds, $p < .001$.) for both treatment groups (fig.1). The difference in social interaction time between treatment groups was not statistically significant ($p= .96$).

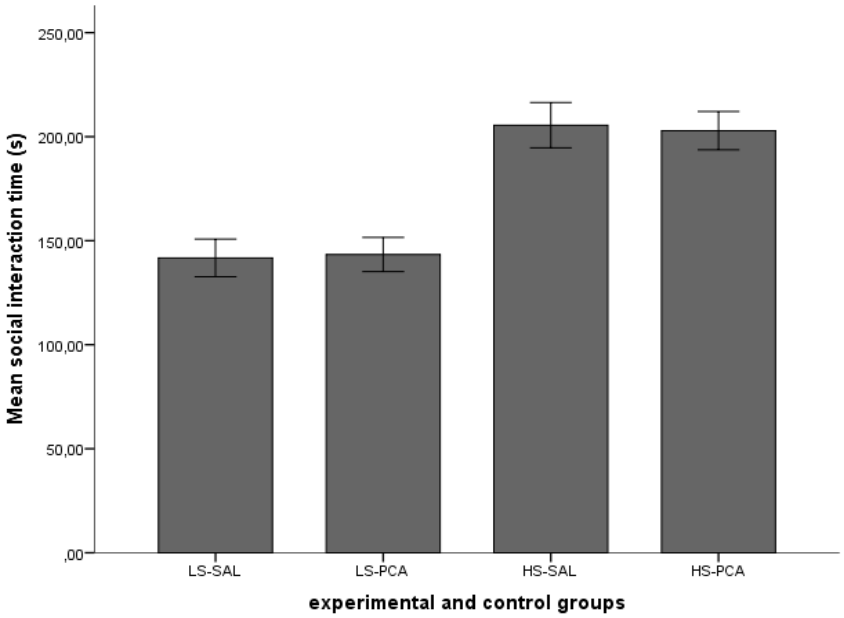

Figure 1. *Mean social interaction time in SI test. Error bars indicate SEM.*

3.2 Individual differences in locomotor activity

A three-way mixed ANOVA was run to understand the effects of sociability, treatment and time on horizontal distance travelled in locomotor activity test. There was no statistically significant three-way interaction between time, treatment and sociability ($F(1, 36) = .003$ $p = .95$. $\eta 2 < .001$). Difference in locomotor activity was not statistically significant in sociability or treatment group neither on the first ($F(1, 36) = .03$, $p = .87$) nor on the second ($F(1, 36) = .07$, $p = .80$) locomotor activity recording (Fig. 2).

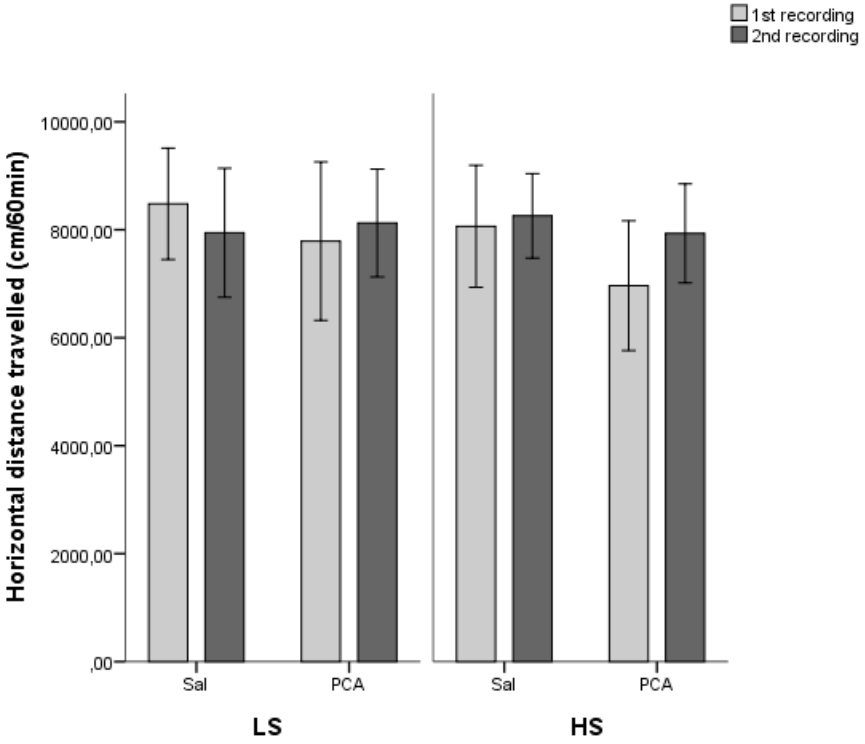

Figure 2. *Mean locomotor activity on first and second locomotor activity recording. Error bars indicate SEM.*

3.3 Changes in 5-HT and 5-HIAA levels in septum and PFC

The effects of PCA (2 mg/kg) on the levels of 5-HT, 5-HIAA and 5-HIAA/5-HT ratio in the PFC and septum are given in Table 1.

The interaction effect between treatment and sociability on 5-HT levels in septum was not statistically significant ($F(1, 35) = 2.07$, $p = .16$, partial $\eta 2 = .06$). 5-HT levels in septum were not statistically significantly different for HS and LS animals ($p > .05$). 5-HT levels in septum for PCA treated animals were lower than for saline treated animals with mean difference (.87± .42 pmol/mg, $p = .048$.) (Table

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1). 5-HIAA levels in septum were not statistically significantly different in neither sociability nor treatment group (Fig. 4).

The interaction effect between treatment and sociability on 5-HT levels in PFC was not statistically significant ($F(1, 36) = .32$, $p = .58$, partial $\eta 2 = .01$). 5-HT levels in PFC were not statistically significantly different for HS and LS animals ($p > .05$). 5-HT levels in PFC were lower in PCA treated animals than saline treated animals with mean difference of ($1.23 \pm .20$, $p < .001$) (Table 1). 5-HIAA levels in PFC were lower in PCA treated animals than saline treated animals with mean difference of ($.50 \pm .13$, $p < .001$) (Table 1).

Table 1. *Mean 5-HT and 5-HIAA levels (pmol/mg tissue) and 5-HT turnover in septum and PFC after PCA treatment*

	5-HT		5-HIAA		5-HIAA/5-HT	
	septum	PFC	septum	PFC	septum	PFC
Saline	4.75 ± 0.30	3.18 ± 0.17	3.38 ± 0.18	1.92 ± 0.11	0.75 ± 0.05	0.64 ± 0.05
PCA	3.86 ± 0.29*	1.94 ± 0.10**	2.96 ± 0.17	1.42 ± 0.06**	0.82 ± 0.05	0.74 ± 0.03

* $p < 0.05$

** $p < 0.001$

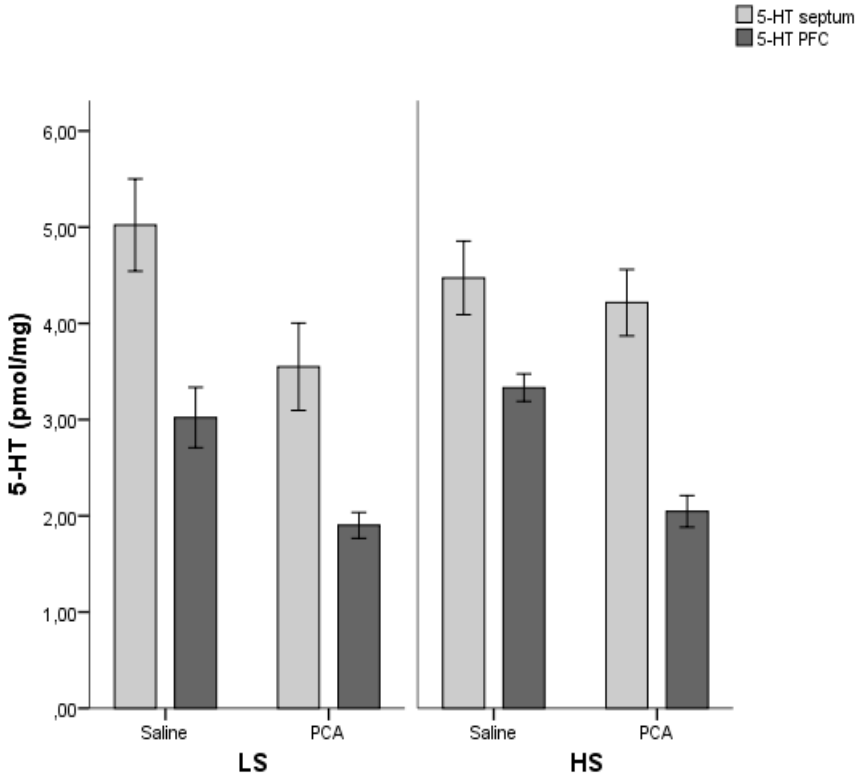

Figure 3. *Mean 5-HT levels in septum and PFC. Error bars indicate SEM.*

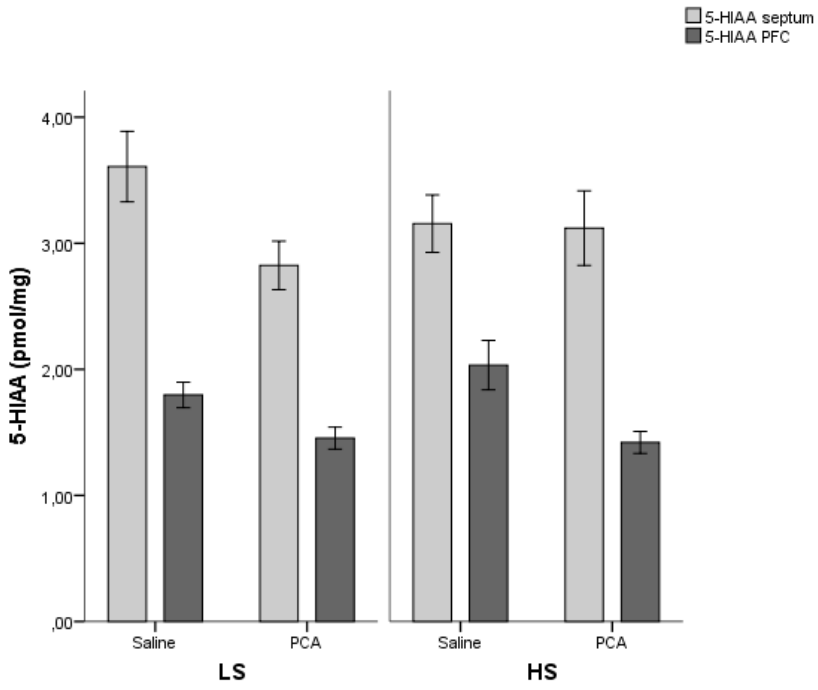

Figure 4. *Mean 5-HIAA levels in septum and PFC. Error bars indicate SEM.*

3.4 Changes in DA levels in septum and PFC

There were no statistically significant interaction effects or main effects of sociability or treatment on DA levels in septum or PFC ($p > .05$).

3.5 Relationship between locomotor activity and monoamine levels in septum and PFC

Correlations between locomotor activity and monoamines are given in Table 2. There was statistically significant positive correlation between locomotor activity during first locomotor activity recording and norepinephrine (NA) in PFC ($r(38) = .48$, $p = .03$) and normetanephrine (NMN) in PFC ($r(38) = .45$, $p = .05$) in saline treated animals. No significant correlations were found in PCA treatment group.

Table 2. *Pearson correlation for locomotor activity and monoamine levels*

	treatment			
	Saline		PCA	
	1st recording	2nd recording	1st recording	2nd recording
5-HT septum	,11	-,09	,02	,08
5-HIAA septum	-,18	-,36	-,11	-,22
5-HT PFC	,31	,32	,07	,06
5-HIAA PFC	,38	,12	-,04	-,17
NA septum	-,15	-,25	-,09	-,03
NMN septum	-,27	-,23	-,30	-,52
NA PFC	,48*	,15	,43	,29
NMN PFC	,45*	,11	,39	,33
DA septum	,31	,21	-,18	-,11
DA PFC	-,01	-,13	-,20	-,38

* $p < 0.05$

4. Discussion

Disturbed 5-HT regulation is related to several psychopathologies and partial 5-HT depletion can be used to study underlying neurobiological mechanisms of these disorders. Some personality traits that play role in the development of psychopathologies are regulated partially by the 5-HT system. One such personality trait is sociability. It is widely accepted that sociability is a rather persistent trait in humans and it has been shown that this is the case for animals too (Tõnissaar et al., 2004). Tõnissaar et al. (2004) found that animals with different sociability level had different 5-HIAA and 5-HT levels in septum and frontal cortex – LS-animals having higher levels of 5-HT in frontal cortex than HS-animals. The aim of the current study was to further investigate the relationship between 5-HT transmission and sociability by causing partial depletion with 5-HT selective neurotoxin PCA.

Knowing that PCA affects DA through the 5-HT system (Schmidt, Wu & Lovenberg, 1986; Schmidt 1987; Johnson, Huang & Nichols, 1991) we hypothesized that locomotor activity increases in response to PCA treatment and more so in HS-animals.

In this study, we showed that neurodegeneration measured as a decrease in 5-HT and 5-HIAA levels in brain tissue occurred in both septum and PFC. PCA caused 39% reduction in 5-HT levels and 26% reduction in 5-HIAA levels in PFC. In septum PCA caused 19% reduction in 5-HT and 12% reduction in 5-HIAA levels. These findings are similar to previous findings by Häindkind et al. (2004) and Harro et al. (2001). Häidkind et al. (2004) showed that neurodegeneration in septum was time-dependent and reached the level of 20% two weeks after the administration of PCA. The reason, why neurodegeneration takes longer to appear in septum, might be due to the fact that serotonergic neurons

from dorsal raphe nucleus to septum are different than serotonergic neurons from median raphe nucleus to PFC. Septum has more beaded nerve fibres than PFC and these have been shown to be more resistant to the neurodegenerative effect of PCA (Mamounas et al., 1991).

No effect of sociability group on 5-HT transmission was found which is not in accordance with previous studies that have found that extracellular and tissue levels of 5-HT and 5-HIAA are different in LS- and HS-animals (Tõnissaar et al., 2004 & 2008; Harro et al., 2001 & 2006). Failure to detect that difference could be because of the small sample size and the fact that we had no medium sociability group that would have made mean social interaction time between HS- and LS-animals larger.

No effect of PCA or sociability level on locomotor activity was found. Neither did DA and 5-HT levels correlate with the locomotor activity. It did, however, correlate with NA and NMN levels. We found a moderate correlation between locomotor activity during first recording and NA and NMN levels in PFC in saline treated animals. Although locomotor activity is associated more with DA regulation and partially with serotonin since it has an effect on DA, it has been suggested that locomotor actvity induced by PCA is associated with changes in NA metabolism in the brain (Strada, Sanders-Bush & Sulser, 1970), but that does not explain our finding since it occurred in saline treated animals.

In conclusion, this study confirmed some previous findings. In addition, some interesting not anticipated findings, like the correlations mentioned above or no statistically significant difference in locomotor activity or monoamine levels between HS- and LS-animals, were found. Thus this topic might be worth studying further.

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