University of Tartu
Faculty of Social Sciences
Department of Psychology

Margus Kanarik

Search for brain regions involved in social behaviour and stress response: mapping of oxidative metabolism

Master's dissertation

Supervisor: prof. Jaanus Harro

Running head: Brain regions mediating sociability and stress response

Tartu 2007

Abstract

The aim of the study was to identify the brain regions mediating social behaviour, and response to chronic stress combined with partial serotonergic denervation. Regional oxidative energy metabolism was used to indicate of long-term neural function. Oxidative activity differed between rats, grouped according to their sociability level, in several brain regions: dorsomedial caudate putamen, median preoptic nucleus, supraoptic hypothalamus, paraventricular thalamus, ventral tegmental area, olfactory bulb, and median raphe. In the dorsomedial caudate putamen extracellular dopamine levels increased in response to a contact with a conspecific. Baseline, as well as parachloroamphetamine induced, extracellular levels of serotonin were higher in highly social animals. Chronic variable stress and serotonergic denervation had an interactive impact on oxidative activity in medial preoptic area, suprachiasmatic hypothalamus, hippocampal CA3, anteroventral thalamus, medial and cortical amygdala, and olfactory bulb. Conclusively, both animal models can be characterized by a specific pattern of neural long-term activity.

Keywords: Chronic variable stress, parachloroamphetamine, serotonin, dopamine, oxidative energy metabolism, cytochrome oxidase histochemistry, depression, animal models, sociability trait, social interaction, microdialysis

Lühikokkuvõte

Stressivastuse ja sotsiaalse käitumisega seotud ajupiirkondade tuvastamine: oksüdatiivse metabolismi kaardistamine

Uuringu eesmärgiks oli tuvastada ajupiirkonnad, mis vahendvad sotsiaalset käitumist, ning stressivastust serotoniinisüsteemi osalise denervatsiooni korral. Pikaajalise närvitegevuse indikaatorina kasutati regionaalset oksüdatiivset energiametabolismi. Oksüdatiivne aktiivsus erines sotsiaalsuse alusel grupeeritud rottidel mitmes ajupiirkonnas: dorsomediaalses caudate putamenis, mediaanses preoptilises tuumas, supraoptilises hüpotaalamuses, paraventrikulaarses taalamuses, ventraalses tegmentumis, haistesibulas ja mediaanses raphe-tuumas. Rakuvälise dopamiini tase tõusis dorsomediaalses caudate putamenis liigikaaslase juuresolekul. Nii baastasemel kui ka parakloramfetamiiniga stimuleeritult oli rakuvalise serotoniini tase sotsiaalsematel loomadel kõrgem. Krooniline muutlik stress ja serotonergiline denervatsioon avaldasid oksüdatiivsele aktiivsusele mitmes ajupiirkonnas koosmõju: mediaalses preoptilises alas, suprakiasmaatilises hüpotaalamuses, hippokamuse CA3 alas, anteroventraalses taalamuses, mediaalses ja kortikaalses amügdalas ja haistesibulas. Kokkuvõtteks - mõlemat loommudelit saab kirjeldada spetsiifilise pikaajalise neuronaalse aktivatsiooni mustriga.

Märksõnad: krooniline muutlik stress, parakloramfetamiin, serotoniin, dopamiin, oksüdatiivne energeetiline metabolism, tsütokroom oksüdaasi histokeemia, depressioon, loommmudelid, püsisotsiaalsus, sotsiaalne interaktsioon, mikrodialüüs

1. Introduction

This research focuses on the neuroanatomical and to a lesser extent on the neurochemical substrate of two central aspects of everyday life – sociability and chronic stress. For humans and probably for other animals, social environment is an important factor in well-being. Disturbances in this domain are associated with several psychopathological conditions, and good social relations can have a positive impact on (mental) health. Recognizing the link between certain psychopathology and core personality dimensions, understanding of the neural substrate of sociability has potentially a great practical value (Whittle, Allen, Lubman, & Yucel, 2006). Another unavoidable component of everyday life, chronic stress, can have harmful outcomes on mental health. Both social behaviour and stress response are at least to some extent dependent on serotonergic neural transmission. Furthermore, our group has recently demonstrated the interplay between sociability and chronic stress: single-housed rats with high sociability were more vulnerable to chronic stress induced anhedonia than animals with low sociability (Tõnissaar et al., 2007).

1.1. Social behaviour in human and animal research

Sociability is a stable characteristic of an animal that is expressed in behaviour as proneness to initiate or accept social contact with a conspecific in neutral circumstances. Most attempts to classify human personality have taken social behaviour into account. The Five-Factor Model encompasses social aspects of personality in Extraversion and Agreeableness dimensions (Costa & McRae, 1992), in Eysenck's Personality Questionnaire social facets are part of the Extraversion/Introversion and Psychotism/Socialization dimensions (Eysenck & Eysenck, 1975). The idea of evolutionary continuity suggests that these traits inherent in humans must be preceded by some related or similar phenomena in other animals (Gosling, Kwan, & John, 2003). Sociability has been identified as an independent component of *Macaca mulatta* and spotted hyena personality structure (Capitanio & Widaman, 2005; Gosling, 1998). Specific social behaviour is also an integral characteristic of several species from various classes eg. lizards, fish, birds and several rodents (Cote & Clobert, 2007; Gosling & John, 1999; Groothuis & Carere, 2005; Insel, 2003; Moy et al., 2004; Moy et al., 2007; Tõnissaar, Philips, Eller, & Harro, 2004). Sociability as a trait can be viewed as an adaptive tool that has evolved in animals living in organized groups

(Gosling & John, 1999), but some aspects of social behaviour have to be part of the personality structure of solitary animals, as contact with other animals is unavoidable.

No attempt to fully identify rat personality dimensions has been made, but there has been much research on several characteristics of underlying dispositions for social behaviour eg. abnormal aggressiveness, social recognition, pair bonding, maternal behaviour etc. (Bielsky & Young, 2004; Haller & Kruk, 2006; Insel, 2003). These specific measures are difficult to merge into a one coherent picture and determine what about them is universal to sociability and what is specific to a given model. In the present investigation we have utilized a simple test to measure an animal's general sociability. The social interaction (SI) test (File & Hyde, 1978; File & Seth, 2003) measures time spent in active social contact by two rats on a neutral arena. Two major factors probably contribute to rodent behaviour in the SI test – sociability and anxiety. The test was developed to measure anxiolytic and anxiogenic drug effects on behaviour, but besides assaying experimentally elicited changes in anxiety, the test is frequently used to show baseline differences between animals in anxiety. However, factor analytical studies indicate that the SI test scores do not load in the same factor with results from plus-maze and open field tests or black/white box (Ramos, Berton, Mormede, & Chaouloff, 1997). Besides anxiety there should be an ethological driving force that makes an animal interested in being involved in social contacts with a conspecific, and that would be revealed to us by the degree of sociability trait of the tested animal. Anxiety and sociability trait are further dissociated by data indicating an increase in social interaction and at the same time also an increase in anxiety related behaviour in the emergence test and plus-maze in response to acute methylenedioxymethamphetamine (MDMA) treatment (Morley, Arnold, & McGregor, 2005; Morley & McGregor, 2000). It can be assumed that sociability in rats is multifactorial – besides the desire to initiate social contact or to accept it the animals must be capable of affiliative, adequately agonistic and non-aggressive interaction and proper perception of social cues (Haller & Kruk, 2006).

While the expression of social behaviour in rats has proven to be individually stable over time, it is sensitive to social environment as moderate correlations between partners time spent in social interaction in the SI test are shown. Correlations between two separate tests are therefore relatively low, but the correlations between one test with the three others are high, meaning that in longer perspective sociability is a stable trait (Tõnissaar et al., 2004). The inherent nature of sociability is indicated by research done with inbred mice – certain strains seem to have more interest in social contacts and social novelty (Moy et al., 2004; Moy et al., 2007).

1.2. Impact of social behaviour on an individual's well-being

Depending on their nature, social contacts can have adverse or beneficial effects on an individual's well-being eg. mice with experimental stroke recovered better if they were in contact with another animal (Craft et al., 2005) and social defeat is a widely used method of inducing stress in rodents (Rygula et al., 2005). Besides the effect of social contact, inherent sociability also affects well-being – in humans greater sociability prevents an individual from being infected with common cold (Cohen, Doyle, Turner, Alper, & Skoner, 2003). In monkeys, the dominant males, who fight for their position in the group, whose behaviour is more agonistic than that of the more submissive group members, and who are frequently aggressively challenged by other dominant males, have a heightened risk for atherosclerosis (McEwen & Seeman, 2003).

Deficits in social behaviour are associated with a number of psychopathological conditions. In schizophrenic patients one of the negative symptoms is of social withdrawal (American Psychiatric Association [APA], 1994) and in animal models of schizophrenia social interaction is compromised (Torres, Meeder, Hallas, Gross, & Horowitz, 2005). The core of autism spectrum disorders is the inability to operate in social context (Happe, Ronald, & Plomin, 2006), and the rodent models of the disorder display deficits in social behaviour and information processing (Hammock & Young, 2006). Decreased interest in social interactions is one of the symptoms of depression, and avoidance of social contact is the core symptom of social anxiety disorder (APA, 1994).

1.3 Neurobiological substrate of social behaviour

The neurobiology of sociability trait involves the monoaminergic systems of the brain. Drugs that promote social behaviour, eg. MDMA and anti-depressants, operate partially through serotonergic mechanisms. MDMA, a drug that induces a massive release of serotonin and dopamine, increases time spent in active social contact in rats (Green, Mechan, Elliott, O'Shea, & Colado, 2003; Morley et al., 2005; Morley & McGregor, 2000). Several selective serotonin reuptake inhibitors are shown to modulate social behaviour if administered either acutely or chronically in normal humans (Harmer et al., 2003; Knutson et al., 1998). In macaques, levels of 5-hydroxyindole acetic acid in cerebrospinal fluid correlated positively with several measures of sociability (Mehlman et al., 1995) and in a rat sociability trait is negatively related to forebrain serotonin metabolism (Tõnissaar et al., 2004). Besides serotonin, another monoamine, dopamine is

involved in sociability. The role of dopamine in social behaviour lies in motivational and rewarding processes (Depue & Collins, 1999; Depue & Morrone-Strupinsky, 2005). Special forms of rodent social behaviour have been shown to include reward - play in juvenile rats has rewarding properties (Calcagnetti & Schechter, 1992; Normansell & Panksepp, 1990) and pair bonding in voles is dependent on dopaminergic reward mechanisms (Insel, 2003).

1.4. Chronic stress in humans and animals

Depression is a disorder arising from strong negative environmental stressors (Brown, 1998; Pine, Cohen, Johnson, & Brook, 2002; van Praag, 2005) and involves dysfunction in the monoamine systems (Chaouloff, 2000; Harro & Oreland, 2001). Stress-induced pattern of changes in hormonal and neuropeptide feedback systems is an important tool to adapt to changes in environment, but prolonged periods of stress can often have counter-adaptive effects on the behaviour (Akil, 2005; Korte, Koolhaas, Wingfield, & McEwen, 2005; Leonard, 2005). In an attempt to mimic excessive human day-to-day stress, several animal models have been developed. Methods using multiple stressors - chronic variable stress or chronic mild stress - rely on the ability of a sequence of relatively mild stressors to produce behavioural changes that are reversible by antidepressant treatment (Katz, 1982; Willner, 2005).

1.5. Neurobiological substrate of stress response

Many key proteins of the serotonergic system in different brain regions have been implicated in depression both in animal models and in human disorder. A few diverse examples of implications of serotonin in depression are changes in serotonin and its metabolite levels/turnover or release following chronic stress in different brain regions in animals (Bekris, Antoniou, Daskas, & Papadopoulou-Daifoti, 2005; Gamaro, Manoli, Torres, Silveira, & Dalmaz, 2003; Mangiavacchi et al., 2001); changes in serotonin receptor sensitivity in response to chronic stress (Leonard, 2005); therapeutic effect of antidepressant treatment by a cascade of events starting with increasing the amount of serotonin in the synapse and resulting in altered (auto)receptor sensitivity (Elhwuegi, 2004; Pineyro & Blier, 1999); depressogenic properties of acute dietary tryptophan (serotonin precursor) depletion (Bell, Hood, & Nutt, 2005); depressogenic effect of monoamine depletion by reserpine as an rodent model of depression and in people taking reserpine as hypertension treatment (O'Neil & Moore, 2003); more frequent occurrence of less functional

allelic variations of tryptophan hydroxylase 1 and 2 (enzymes synthesizing serotonin) genes among people with major depression and neurotic/anxious personality (Nash et al., 2005; Zhang et al., 2005). Conclusively, it is clear that the serotonergic system is malfunctioning in affective disorders and in animal models of depression, but there can be multiple simultaneous molecular mechanisms in a number of brain circuits that contribute to the depressive state.

1.6. The effect of parachloroamphetamine on brain monoamine system

One possible approach to elicit long-lasting serotonergic dysfunction is to apply neurochemically specific neurotoxins. Parachloroamphetamine (PCA) and other substituted amphetamines are potent neurotoxins with a multiphase, time dependent impact on neuronal functioning. The acute effect of PCA is a massive, dose dependent release of serotonin and dopamine, resulting in a transient depletion followed within days by the onset of degeneration of axon terminals, especially in neocortex, striatum and thalamus, leaving the preterminal axons and cell bodies intact (Mamounas & Molliver, 1988; Wilson, Mamounas, Fasman, Axt, & Molliver, 1993). The abovementioned studies use destruction of the serotonergic system as a tool to study morphology and morphological changes, but such a near-total lesion is of limited value from behavioural viewpoint. It has been suggested that partial lesions with small toxin doses can be utilized to add ecological validity to animal experiments (Datla & Curzon, 1996). Our group has previously shown that small doses of PCA (2 mg/kg) that cause a restricted reduction of serotonin in frontal cortex, cerebral cortex, hippocampus, hypothalamus and cerebellum can induce behavioural alterations bearing similarities to the negative impact of chronic stress – increased anxiety and impulsivity (Häidkind et al., 2004; Harro, 2002; Harro & Oreland, 2001; Harro, Tõnissaar, Eller, Kask, & Oreland, 2001).

1.7. Cytochrome oxidase activity – indicator of long-term neuronal metabolism

A possibility to monitor long term changes in brain in response to certain stimulation or to determine the neural substrate of certain inherent characteristics – traits, is to assess oxidative phosphorylation. Cytochrome c oxidase (CO, EC 1.9.3.1), an inner mitochondrial membrane protein, is the terminal complex of electron transport chain that catalyses the oxidation of the mobile electron carrier cytochrome c and reduction of oxygen to water (Brunori, Giuffre, & Sarti, 2005). In the processes of electron transport a proton gradient is generated, which is used to drive

the oxidative phosphorylation of ADP to ATP by F1/F0 ATPase (Oster, Wang, & Grabe, 2000). The activity of neurons is almost entirely dependent on oxidative metabolism and changes in CO expression in mitochondria can be used as a marker of long term metabolic activity. Strong input leading to high frequency of charging is associated with elevated Na^+/K^+ ATPase activity, which is proposed to be the main energy-consumer in neurons (Wong-Riley, Nie, Hevner, & Liu, 1998). CO histochemistry is a method that combines good anatomical resolution with a functional outcome – long term metabolic activity of a given brain region.

1.8. Stress diathesis model

It has been suggested by epidemiological research that the genetic makeup of an individual strongly interacts with stressful life situations (Kendler et al., 1995). Results demonstrating that lifetime stress interacts with the serotonin transporter promoter region polymorphism in causing depression have confirmed the notion of genetic susceptibility on molecular level (Caspi et al., 2003; Kendler, Kuhn, Vittum, Prescott, & Riley, 2005; Mandelli et al., 2006). The natural variability of the capacity of serotonin system causes chronic stress to induce a mild to severe depression-like state in a number of rats, but some animals remain unaffected. Partial denervation of the serotonergic system with PCA allows us to render the animals more susceptible to environmental stressors and to control for the vulnerability/protective properties of a certain neurotransmitter system.

1.9. Aims

The aims of these studies were: 1) to investigate the impact of partial serotonergic denervation with parachloroamphetamine and chronic variable stress (CVS) on rat brain regional long-term oxidative metabolism, to reveal which brain regions are most affected by these factors; 2) to locate brain regions where neuronal oxidative activity differs between animals with different levels of sociability trait, 3) to determine the extracellular monoamine levels, in a region selected on the basis of CO histochemistry (dorsomedial caudate putamen), in rats with different levels of sociability in response to social stimulation and acute parachloroamphetamine treatment.

2. Materials and methods

2.1. Animals

Male Wistar rats (from Scanbur BK AB, Sweden) were housed in standard polypropylene cages in a light controlled room (12-h light/dark cycle; lights on at 8:30 a.m.) maintained at 22°C. Food and water were available *ad libitum*. All experiments were in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC) and approved by the Ethics Committee of the University of Tartu. All efforts were made to minimize the number of animals used and their suffering.

In the PCA/Stress histochemistry experiment, animals (n=29, weighing 260-332 g at the beginning of the experiment) were housed four per cage and were submitted to CVS at 3 months of age.

In the sociability trait histochemistry experiment, animals (n=28, weighing 370-435 g at the first behavioural test) were single-housed ten days before behavioural testing at the age of two months and sacrificed at three month of age.

In the sociability trait microdialysis experiment, animals (n=28, weighing 343-485 g at the first behavioural test) were single-housed fifteen days before behavioural testing at the age of two months and one week and sacrificed in the course of four months.

2.2. Social interaction test

Rats were single-housed ten or fifteen days before the experiment, the three test sessions were separated by a ten-day period. An assay developed to measure changes in anxiety levels, the social interaction test (File & Hyde, 1978) was used to measure individual differences in sociability. Definition of sociability trait was based on the mean time in three SI tests spent in non-aggressive active social contact (allogrooming, sniffing the partner, crawling under and over, following) during a 10 minute session with a weight-matched strange rat on a 30x30 cm well lit arena. Aggressive encounters were not counted, for their extremely low occurrence. On the basis of the mean SI score animals were divided into groups with high, medium and low sociability trait (HS, MS, LS). In the sociability trait histochemistry experiment 8 rats with the lowest, 7 rats with the highest and 8 rats with medium levels of sociability were chosen to be analysed. In the sociability trait microdialysis experiment 28 animals were divided in two on the basis of SI score,

one animal was excluded because of wrong probe placement. In the PCA/Stress histochemistry experiment, in case of the dorsal part of the anterior olfactory bulb, one animal with the oxidative activity value exceeding the mean by two standard deviations was excluded from analysis.

2.3. General procedure of the sociability trait microdialysis experiment and social stimulation

The aim of the microdialysis study was to assess extracellular monoamine levels in three conditions: baseline, socially stimulated and PCA induced. Two weight-matched and preselected animals with intracerebral probes implanted were placed in a cage and connected to the syringe and a pump. The test cage was divided into two compartments (each 24x36 cm) by a grid that at the beginning of the experiment was covered – the animals were not in direct contact with each other. Before collecting the first sample, the tubing was washed through for an hour. The next seven samples were collected to acquire stable baseline levels of the monoamines. After the seventh sample the covers were removed from the grid separating the animals, allowing them to be in social contact. By the seventeenth sample the monoamine levels had stabilized again and the animals were treated with PCA to induce a release of serotonin and dopamine. The experiment was conducted at low illumination to promote social interaction.

2.4. PCA administration

Administration of the neurotoxin in the PCA/Stress histochemistry experiment was carried out one week before the CVS regimen. PCA (Sigma) in the dose of 2 mg/kg (expressed as for hydrochloride) was dissolved in distilled water and injected in a volume of 1 ml/kg intraperitoneally. Control animals received a vehicle injection. In the sociability trait microdialysis experiment PCA in the same concentration and via the same route was injected to animals connected to the microdialysis system.

2.5. CO histochemistry and image analysis

Unanesthesised rats were decapitated, brains removed and immediately frozen on dry ice. Brains were stored at -80°C until coronally sectioned (thickness 40 μm) in a cryostat microtome at -20°C, slides with sectioned tissue were kept refrigerated at -80°C until stained. The staining

procedure used is based on the protocol described by Gonzalez-Lima & Cada, 1998 with minor modifications. The 0.1 M Na_2HPO_4/NaH_2PO_4 buffer solution adjusted to pH of 7.4 was used. Automatic agitation was used with all the steps in the protocol. First the refrigerated sections were fixed for 5 min in 0.125% glutaraldehyde (v/v) solution in cold buffer (4°C). Next the specimens were washed with 4 changes (5 min each) of 10% sucrose in the buffer solution at room temperature. To enhance staining intensity, the sections were pre-incubated, for 10 min with 0.0275% cobalt chloride (w/v) and 0.5% dimethyl sulfoxide (DMSO, v/v) in 0.05 M Tris buffer with 10% sucrose (w/v) adjusted to pH to 7.4. with approximately 0.1% HCl (v/v). The metal ions included in the previous step were removed by a 5 min wash with the buffer solution. Thereafter the sections were stained for one hour at room temperature in an incubation solution consisting of 0.05% DAB (3,3'-diaminobenzidine tetrahydrochloride, AppliChem), 0.0075% cytochrome c (Sigma, prepared using TCA), 5% sucrose, 0.002% catalase (Sigma) and 0.25% DMSO (v/v) in sodium phosphate buffer. To avoid non-specific auto-oxidation the reaction was conducted in dark. Finally, the reaction was stopped by introducing the slides for 30 min to 3.5% formalin (v/v) and 10% sucrose in phosphate buffer. The sections were dehydrated in ethanol, cleared in xylene and coverslipped. Regions of interest (ROIs) to be compared in data analysis were stained in the same incubation medium.

Stained and coverslipped sections were digitized and saved in a non-compressed format. Image analysis was conducted using the Image J 1.34s freeware on the blue channel (resulting from a RGB split) of the background subtracted image. Eighty nine ROIs were detected from the stained images with the help of rat brain atlas Paxinos & Watson, 1986. Grayscale values were transformed to optical density values (OD) with the help of Kodak grayscale tablet with known grayscale and OD values. OD of any given ROI was sampled from three consecutive slices in one brain and averaged. The OD value was sampled randomly from right or left hemisphere of different animals, on the three consecutive slices of the brain the same hemisphere was sampled. ROIs were selected with a freehand selection tool covering the whole brain region, leaving out defective areas.

In the CO histochemistry experiment of sociability trait, internal standardization was used to calculate the activity of the enzyme. Activity of cytochrome oxidase in brain tissue homogenate of four adult male Wistar rats was assayed spectrophotometrically. Forty μm slices of the frozen homogenate were included in all incubation baths, and the OD measured on the standard was assigned the value previously determined spectrophotometrically yielding the results as μmole of substrate oxidised per minute and per gram of brain tissue.

2.6. Surgery and microdialysis

The preselected animals were anaesthetized with chloral hydrate (350 mg/kg, IP) and mounted in a Kopf stereotactic frame. Self-made concentric Y-shaped microdialysis probe with 7 mm shaft length and 2 mm active tip was implanted in the dorsomedial caudate putamen according to the following coordinates relative to bregma: AP: +0.2, ML: +3.0, DV: -5.34, angle 11.3° (according to Paxinos and Watson, 1986). The dialysis membrane used was polyacrylonitrile/sodium methalyl sulphonate copolymer (Filtral 12; inner diameter: 0.22 mm; outer diameter: 0.31 mm; AN 69, Hospal, Bologna, Italy). Two stainless steel screws and dental cement was used to fix the probe to the scull. After the surgery, rats were placed in individual cages (21 × 36 × 18 cm high) in which they remained throughout the experiment.

Microdialysis was conducted in awake, freely moving rats starting approximately 20 hours after the surgery in the course of 45 days. The probes were perfused with Ringer solution (147 mM NaCl, 4 mM KCl, 1.2 mM $CaCl_2$, 1.0 mM $MgCl_2$, 1.0 mM Na_2HPO_4, 0.2 mM NaH_2PO_4; pH 7.20-7.22) at a constant flow rate of 1.5 µl/min using syringe microdialysis pump (World Precision Instruments, USA). Connections to the infusion pump and microfraction collector (CMA/142, Sweden) were made with flexible FEB-tubing (ID 0.12 mm, AgnTho's AB, Sweden). After a stabilization period of approximately 1 h, 36 of 15 min samples were collected. Immediately after the collection of the 7 th sample a social stimulus was introduced and after the 17th sample animals received an IP injection of PCA (2 mg/kg). After the completion of the experiment, the animals were deeply anaesthetized with chloral hydrate (350 mg/kg, IP) and decapitated; the brains were removed, immediately frozen and kept at -80°C. The brains were sectioned in a cryostatic microtome; the probe placements were determined according to the atlas by Paxinos and Watson (1986) and data of animals with probe placements outside the appropriate area were excluded from the analysis.

2.7. Measurement of dopamine and serotonin in the microdialysates

The quantity of dopamine and serotonin in the samples was determined online by using HPLC with electrochemical detection. The samples were collected directly into a 50 µl loop of the electrically actuated injector (Cheminert C2V, Vici AG International, Switzerland) for 15 min and then injected automatically onto the column. The chromatography system consisted of a Shimadzu

LC-10AD series solvent delivery pump, a Luna C18(2) 5 µm column (150 x 2 mm) kept at 30ºC and Decade II digital electrochemical amperometric detector (Antec Leyden BV, the Netherlands) with electochemical flow cell VT-03 (2 mm GC WE, ISAAC reference electrode, Antec Leyden BV, the Netherlands). The mobile phase consisted of 0.05 M sodium citrate buffered to pH 5.3, 2 mM KCl, 0.02 mM EDTA, 3.5 mM sodium octanesulphonate and 14% acetonitrile. The mobile phase was filtered through a 0.22 µm pore size filter (type GV, Millipore, USA) and was pumped through the column at a rate of 0.2 ml/min. dopamine and serotonin eluted from the column (retention time 4.8-5 min and 8.9-9.2) was measured with a glassy carbon working electrode maintained at a potential of +0.4 V versus Ag/AgCl reference electrode. Data were acquired using a Shimadzu LC Solution system. Concentrations of monoamines were estimated by comparing peak heights from the microdialysates with those of external standards of known dopamine and serotonin concentration (Fluka, Germany). The limit of detection (three times baseline noise) was approximately 1 fmol/22.5 µl sample.

2.8. Chronic variable stress

Rats belonging to the stress group were submitted to the CVS procedure as previously described (Harro et al., 2001) in a separate room during 21 days. Various stressors of different duration were applied one by one every day, each one thrice altogether. The stressors, in the order of presentation, included: 1) movement restriction in a small cage (11×16×7 cm for 2 h), 2) cage tilt at 45° (for 24 h), 3) tail pinch with a clothes-pin placed 1 cm distal from the base of tail (5 min), 4) cold (4°) water and wet bedding (initially, 400 ml of water was poured on a rat, and the sawdust bedding was kept wet for the following 22 h), 5) forced swimming (5 min at room temperature), 6) strong illumination (900 lx) during the predicted dark phase (for 12 h) and 7) stroboscopic light (for 14 h, 10 Hz).

2.9 Data analysis

The obtained OD values were transformed to standard scores (T scores) for the PCA/Stress histochemistry experiment. All brain areas were treated as independent and a 2 x 2 (stress/no stress, PCA/vehicle) and 3 group (HS, MS and LS) ANOVA with Fisher LSD post hoc test used for group comparisons. In case of the microdialysis study groups were compared with t-test for

dependent and independent groups. Behavioural data was analysed with ANOVA and Fisher LSD test. Pearson correlations analysis was used. Normality of the distribution was assessed with the Shapiro-Wilk's test.

3. Results

3.1 Social interaction

The mean score of time spent in social interaction was distributed normally in both, the sociability trait histochemistry and microdialysis experiment (W=0.970 and 0.971 respectively, p - non-significant). The mean scores of SI tests in the sociability trait histochemistry experiment were 46±5.7, 87±3.0 and 113±5.3 (M±SEM, sec.) for Low, Medium and High Sociability animals respectively. Mean score of LS rats differed significantly from MS animals and mean score of MS rats differed significantly from HS animals ($F(1, 28)=50.1$, $p<0.00005$, $p<0.000005$ and $p<0.001$ respectively; LSD post hoc test). The mean scores of the SI test in microdialysis experiment were 40±2.8 and 73±2.6 (M±SEM, sec) for Low and High Sociability animals respectively, the difference was statistically significant ($F(1, 27)=77,5$, $p<0,00001$).

3.2 Sociability trait histochemistry experiment

Significant differences between LS and HS animals appeared in dorsomedial caudate putamen ($F(2, 23)=3.8$, $p<0.05$), where LS animals had higher CO activity than HS animals.

In median preoptic nucleus ($F(2, 22)=8.1$, $p<0.005$), posterior paraventricular thalamic nucleus ($F(2, 17)=4.7$, $p<0.05$) and median raphe ($F(2, 21)=10.7$, $p<0.001$) the relationship between sociability and oxidative metabolism was nonlinear: MS-rats had higher CO activity than LS- and HS-animals. In supraoptic nucleus the results were complex – in the anterior part there was a linear relationship ($F(2, 19)=3.6$, $p<0.05$), with HS-rats having higher CO activity than LS-animals, but in the posterior part the results were non-linear ($F(2, 23)=8,3$, $p<0.005$), with MS-rats having lower CO activity than LS-and HS-animals (Figure 1).

If only five of the most extreme and five of the average animals were analysed, despite the reduction in statistical power, a strong difference in CO activity appeared in ventral tegmental area ($F(2, 15)=10.5$, $p<0.005$), where LS-animals had lower CO activity from MS- and HS-animals ($p<0.005$, LSD post hoc test).

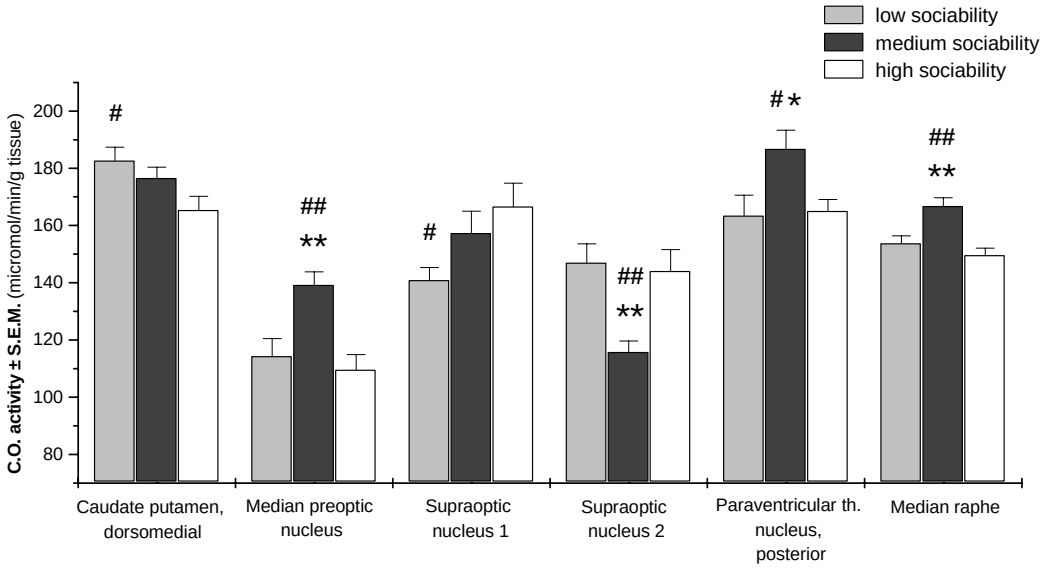

Figure 1. Cytochrome oxidase activity (μmol/min/g) in brain regions of rats with low, medium and high sociability. # vs high, p<0,05; ## vs high, p<0,01; * vs low, p<0,05; ** vs low, p<0,01 (LSD post hoc test).

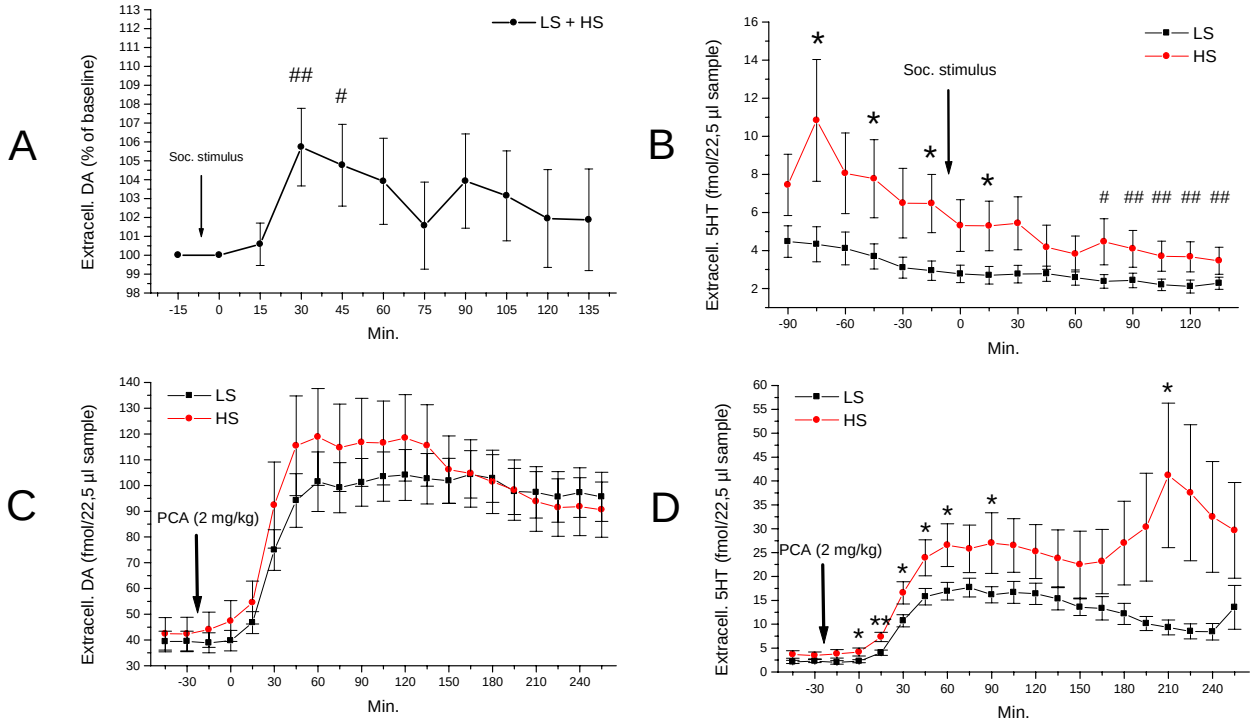

Figure 2. A – extracellular dopamine in CPDM in response to social stimulation; **B** - extracellular serotonin in CPDM in response to social stimulation (difference from baseline shown for HS-rats); **C -** extracellular dopamine in CPDM in response to PCA; **D** - extracellular serotonin in CPDM in response to PCA; * LS vs HS, p<0.05; ** LS vs HS, p<0.01; # vs baseline, p<0.05; ## vs baseline, p<0,01; means±S.E.M; T-test for dependent and independent samples.

Time spent in social interaction correlated with oxidative activity in ventral pallidum (r=-0.42, $p<0.05$) and dorsomedial caudate putamen (r=-4.8, $p<0.05$). A correlation of borderline significance between mean SI score and metabolic activity appeared in the lateral part of the anterior olfactory bulb (AOL) (r=-0.4, p=0.06).

3.3 Sociability trait microdialysis experiment

Since no curvilinear relations between sociability scores and monoamine release were found, the data is presented as two groups, HS- and LS-rats. dopamine release slightly, but significantly, increased in response to social stimulation in + 30 and +45 minute samples irrespective of the animal's sociability (baseline is the average of -15 and 0 minute samples, Figure 2A). Serotonin levels decreased irrespective of social stimulation in both groups, but the decrease was more pronounced in HS-rats (Figure 2B). Compared with 0 minute sample, in +75, +90, +105, +120 and +135 minute samples serotonin levels were significantly lower ($p<0.05$) in HS-rats. In LS-rats +75, +105 and +120 minute samples contained less serotonin than 0 minute sample ($p<0.05$). At -75, -45, -25 and +15 minute samples, HS animals had significantly higher serotonin levels than LS-animals, but from +30 minute sample on serotonin levels did not differ between HS- and LS-animals. PCA treatment induced a massive increase in both dopamine and serotonin release (Figure 2C, 2D), but only serotonin levels depended on the animals sociability – in HS-rats, compared to LS-animals, more serotonin was released in response to PCA. PCA induced serotonin release (mean of samples +15 to +255 minutes) correlated positively with the mean time spent in social interaction in three SI tests (r=0.4, $p<0.05$). The rise in extracellular serotonin during the ninth hour of the experiment depended entirely on the data of 3 animals only.

3.4. PCA/Stress histochemistry experiment

Chronic variable stress had a significant main effect on the weight of adrenal glands (left and right averaged, $F(1, 29)=10.7$, $p<0.005$), stressed animals having heavier adrenals than controls. An interaction between CVS and PCA was also found in adrenal glands ($F(1, 28)=5.0$, $p<0.05$), where stress blocked the weight loss evoked by PCA (Figure 3).

PCA had a significant main effect on CO activity in hippocampal CA3 region ($F(1, 28)=5.1$, $p<0.05$), anteroventral thalamus ($F(1, 26)=6.8$, $p<0.05$), substantia nigra ($F(1, 27)=5.3$, $p<0.05$) ventral tegmental area ($F(1, 27)=4.6$, $p<0.05$), granular retrosplenial cortex ($F(1, 27)=4.5$, $p<0.05$)

and cerebellar vermis ($F(1, 27)=6.8$, $p<0.05$). In all instances PCA caused an increase in oxidative activity.

There was a CVS main effect of borderline significance only in hippocampal CA3 ($F(1, 28)=4.0$, $p=0.057$), with stress increasing oxidative activity. However, interaction between the serotonergic lesioning and chronic stress was the most prevalent finding in this study (Figure 4). Regions of interaction include: medial preoptic area ($F(1, 24)=4.9$, $p<0.05$), cortical amygdala ($F(1, 29)=9.9$, $p<0.005$) and medial amygdala ($F(1, 26)=6.3$, $p<0.05$), where PCA blocked the stress induced increase in oxidative activity; suprachiasmatic hypothalamus ($F(1, 26)=17.4$, $p<0.0005$), ventrolateral division of laterodorsal thalamus ($F(1, 28)=4.5$, $p<0.05$), anteroventral thalamus, with borderline significance ($F(1, 26)=4.1$, $p=0.054$) and cortical amygdala, where CVS reduced the oxidative activity induced by PCA treatment; dorsal division of the anterior olfactory bulb ($F(1, 28)=5.6$, $p<0.05$), where stress blocked the decrease in oxidative activity evoked by PCA. In anterior paraventricular nucleus ($F(1, 27)=5.7$, $p<0.05$) stress and PCA combined yielded in a reduction of metabolic activity compared to stress group.

Dorsal raphe nucleus was the only region with a large synergistic increase in oxidative activity in response to CVS and PCA combined (control 48.2 vs PCA/stress 56.5), but this effect was only seen at arithmetical level, the results of ANOVA were non-significant. Ventral tegmental area and retrosplenial cortex showed no significant post hoc comparisons, in case of cerebellar vermis the differences appeared only between the PCA treated and chronically stressed animals (data not presented).

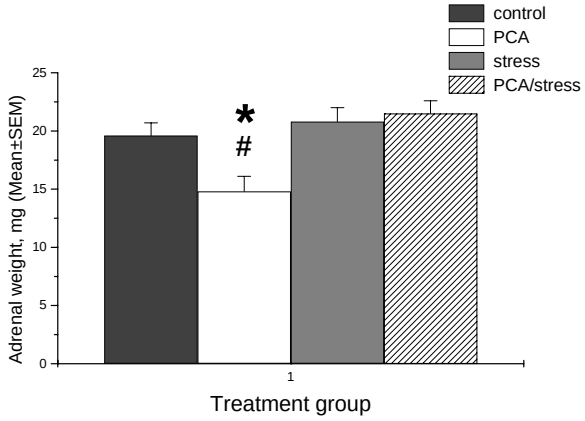

Figure 3. Weight of the adrenal glands (mg, M±SEM, left and right averaged) of chronic variable stress and parachloroamphetamine (PCA) treated rats. # vs control, p<0,05; * vs PCA/stress, p<0,001. Fisher LSD post hoc test.

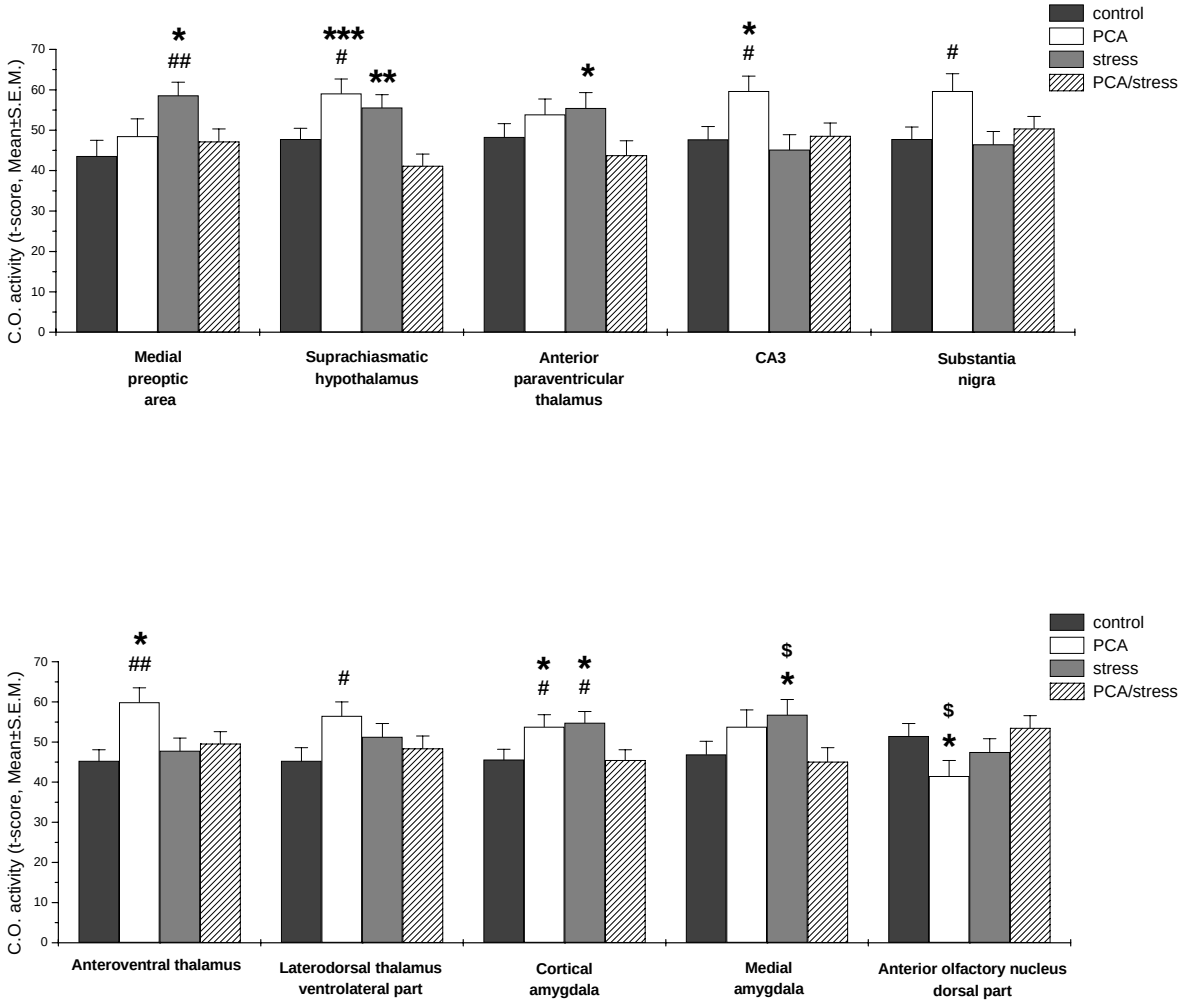

Figure 4. O.D. values (T-score, M±S.E.M.) of cytochrome oxidase stained brain slices of chronic variable stress and parachloroamphetamine (PCA) treated rats. # vs control, p<0,05; ## vs control, p<0,01; $ vs control, p=0,06; * vs PCA/stress, p<0,05; ** vs PCA/stress, p<0,01; *** vs PCA/stress, p<0,001. Fisher LSD post hoc test.

4. Discussion

Using neuronal oxidative metabolism as an index of the long-term activity of the brain in animals preselected on the basis of social interaction test, an attempt was made to describe brain regions underlying the trait of sociability. Several brain regions emerged, where animals with high, medium and low sociability levels differed in oxidative metabolism. Besides these basic differences we investigated whether HS- and LS-rats had baseline differences in monoamine release. The possibility that stimulation could alter monoamine release, and that this alteration is dependant on the level of sociability of the animal, was also tested. In addition we tried to improve the understanding of depression-related regional metabolic changes in the brain by using a stress-diathesis approach, combining the vulnerability caused by serotonin deficiency with chronic variable stress. In this model PCA treated animals should be at increased risk to environmental stress (Harro & Oreland, 2001), although there might also exist individual variations in susceptibility to the neurotoxicity of PCA (Mamounas & Molliver, 1988).

4.1. Brain regions involved in sociability trait

One of the main findings in this study was that in several brain areas – median preoptic nucleus, posterior paraventricular thalamus, median raphe and possibly supraoptic nucleus, the relationship of sociability trait to oxidative activity was curvilinear. U-shaped relations between behaviour and its neurobiological substrate have been described before. In case of the serotonergic system, which is also assumed to underlie sociability, it has been shown that persons with high or low levels of serotonergic capacity, as measured by platelet MAO activity, are at altered risk to psychopathology, as compared to individuals with medium serotonergic capacity (Schalling, Asberg, Edman, & Oreland, 1987). Furthermore, individuals with both high and low serotonergic capacities have an increased tendency of a specific behaviour - becoming a smoker (Harro, Fischer, Vansteelandt, & Harro, 2004). Smoking itself can be associated with extraversion (Munafo, Zetteler, & Clark, 2007), a personality dimension closely related to sociability. A sufficient explanation of the relation of non-linear oxidative metabolic activity and neurotransmitter release in these specific brain regions is yet to emerge as well as the functional relation of higher or lower metabolic activity to neurotransmitter release.

Posterior paraventricular thalamus, a region where MS-animals had higher oxidative activity than HS- and LS-rats, is one of the midline thalamic nuclei that have been associated with several

behaviours which can be related to social activity. Posterior paraventricular thalamus has connections with all major neurotransmitter systems: a dense dopaminergic input from ventral tegmental area, serotonergic input from dorsal raphe and noradrenergic input from locus coruleus. The region gives efferents to some cortical areas, nucleus accumbens, especially the shell, the adjacent caudate putamen, amygdala, and several hypothalamic areas eg. median preoptic nucleus (Van der Werf, Witter, & Groenewegen, 2002). One of the presumed functions of the paraventricular thalamus is assumed to be general arousal and incentive behaviour (Van der Werf et al., 2002). The anatomical connections of paraventricular thalamus mentioned above and the fact that it receives input of orexin (Kirouac, Parsons, & Li, 2005), a neuropeptide involved in several behaviours eg. food intake, vigilance and drug-seeking behaviour (Lawrence, Cowen, Yang, Chen, & Oldfield, 2006) have led several authors to a thought that this thalamic nucleus may be related to motivational/reward-mechanisms. The reward related aspects in social behaviour will be discussed below. It could be assumed that being in active social contact with another animal would require a higher "readiness" or general arousal to notice subtle social cues and act upon them. Of great interest is the fact that another brain region involved in vigilance, a small nucleus in the preoptic area, median preoptic nucleus, exhibits a CO activity profile similar to posterior paraventricular thalamus (Suntsova et al., 2007). Paraventricular thalamus is also implicated in some symptoms of autism – a disorder of social cognition, as implied by loss of nicotinergic acetylcholine receptor in the region in autopsy samples of autistic adults (Ray et al., 2005).

Another region that distinguished animals of different sociability levels was the supraoptic nucleus. The results appear complex, since CO activity from the anterior part of the nucleus yielded linear, but from the posterior part curvilinear relations with sociability, the statistical significance being greater in the latter case. While it is possible that there might be functional differences in different parts of the nucleus, the chance of an artefact must be kept in mind. Supraoptic nucleus is a small region consisting mainly of magnocellular oxytocinergic and vasopressinergic neurones. These neurones terminate in the posterior pituitary and give collaterals to amygdala and septum (Kiss & Mikkelsen, 2005). The pro-social compound MDMA increases social interaction in rats and at the same time activates immediate early genes in supraoptic nucleus. This is accompanied by the release of oxytocin in circulation and an elevation in plasma levels of oxytocin (Thompson, Callaghan, Hunt, Cornish, & McGregor, 2007). There is evidence, that central oxytocin release is one of the key features in several social/affiliative behaviours – sexual, maternal and aggressive behaviour, pair bonding and social memory (Insel, 2003;

Neumann, 2007). It has recently been suggested that some aspects of selective serotonin reuptake inhibitors might be mediated through oxytocinergic neurons in supraoptic and paraventicular hypothalamus, since the distribution of oxytocin-labelling and serotonin transporter-labelling coincides (Emiliano, Cruz, Pannoni, & Fudge, 2006). It is difficult to specify the role of peptide release from supraoptic nucleus in sociability trait, because in most of the behavioural studies it is not dissociated from the function of paraventricular hypothalamus – another peptidergic nucleus innervating the pituitary and several central brain regions (Kiss & Mikkelsen, 2005) and from some other oxytocin and vasopressin containing regions (Raggenbass, 2001). There is however some direct evidence of the involvement of supraoptic nucleus in social cognition – osmotic induction of vasopressin release in that region is accompanied by increased vasopressin release in septum with the behavioural result of improved social memory (Engelmann, Ludwig, & Landgraf, 1994).

For most of the lower mammals, including rodents, olfaction is the primary means of communication in territorial, sexual and maternal behaviour as well as in social memory formation (Brennan & Kendrick, 2006). Although there is evidence that in animals with deficits in social cognition there is a similar activation in olfactory bulbs as in normal animals and their failure comes from higher centres – cortical amygdala (Ferguson, Aldag, Insel, & Young, 2001), we found a correlation of borderline significance between metabolic activity of one of the subregions of the main olfactory bulb – lateral pars of anterior olfactory bulb, and sociability, suggesting that basic differences may occur in more primary levels of perceptual processing. Interestingly this correlation appeared to be negative – the more socially active the animal, the less metabolically active the region.

Median raphe along with the dorsal raphe is the main source of the ascending serotonergic forebrain innervation. Although dorsal and median raphe innervate non-overlapping and complementary areas of the forebrain (Vertes, Fortin, & Crane, 1999) the two nuclei have strong reciprocal connections (Tischler & Morin, 2003). There is evidence that lesions of both dorsal and median raphe induce a reduction in social interaction but leave novelty suppressed drinking, a measure of anxiety related behaviour, intact (File & Deakin, 1980). To dissociate the function of the nuclei, rats with either median or dorsal raphe lesions were behaviourally tested. It was found that in reduced social interaction the role of dorsal raphe is probably primary, defective median raphe resulted in elevated defensive aggressiveness in a resident intruder test (File, Hyde, & Macleod, 1979). There is evidence that both median and dorsal raphe are involved in the regulation of aggressive behaviour, both defensive and predatory, suggesting a more universal role

of serotonin in inhibition of agonistic behaviour (Albert & Walsh, 1982). In hamsters, serotonergic inhibitory control of anterior hypothalamic peptide-mediated aggression, originating from both dorsal and median raphe, has been shown (Ferris, Stolberg, & Delville, 1999). As lesions of median raphe can promote agonistic behaviour, it is plausible that heightened activity of this region could imply better control of agonistic behaviour. Since MS-rats had the highest level of oxidative metabolism in median raphe, it can be speculated that both HS- and LS-animals might display more agonistic behaviour. As overt aggression in the SI tests conducted was observed too rarely to quantify, the agonistic behaviours are probably expressed in more subtle ways that should be established in future research. Serotonergic innervation arising from median raphe has been implicated in general control of arousal – induction of GABAergic inhibition in median raphe by muscimol results in hyperactivity (Wirtshafter, 2001). The curvilinear relationship between sociability and CO activity in median raphe suggests that MS-rats have, besides a better control of agonistic behaviour, a better control of behaviour overall. As mentioned above, some of the effects of serotonin on social behaviour and affiliative interaction might be mediated, at least to some extent, through peptidergic mechanisms (Emiliano et al., 2006; Thompson et al., 2007).

In two regions of the brain that have been implicated in motivational/reward related behaviour, CO activity either differed in animals of high, low or medium sociability trait or correlated with sociability. These areas were dorsomedial caudate putamen and ventral tegmental area. In the dorsomedial pars of caudate putamen lower sociability was related to higher neuronal oxidative metabolism. Although most of the research on reward related and motivational behaviour is engaged with the ventral part of the caudate putamen and the adjacent nucleus accumbens, the dorsal part is also regarded as an essential region in this circuit (Delgado, Locke, Stenger, & Fiez, 2003; Hyman, 2005). Both the dorsomedial caudate putamen and nucleus accumbens/ventral caudate putamen are activated in response to morphine treatment (D'Souza, Harlan, & Garcia, 1999), but since the connections of the two regions are only partially overlapping, somewhat different roles in incentive behaviour can be assumed (Fasano & Brambilla, 2002). When rats are treated with a pro-social drug MDMA, there is an induction of early genes in the medial part of the caudate putamen (Stephenson, Hunt, Topple, & McGregor, 1999), which could possibly imply involvement in both – drug related reward and social behaviour. Juvenile play, a behaviour that has been shown to have rewarding potential (Calcagnetti & Schechter, 1992; Normansell & Panksepp, 1990), can induce immediate early gene activation in the dorsal caudate putamen (Gordon, Kollack-Walker, Akil, & Panksepp, 2002).

Ventral tegmental area, a region that in this study was metabolically more active in highly social rats, is a dopaminergic nucleus giving efferents to nucleus accumbens and caudate putamen through the mesolimbic pathway and is central in the reinforcing mechanisms (McBride, Murphy, & Ikemoto, 1999; Wise, 2005). The role of ventral tegmental area has been exemplified in affiliative social behaviour – it is an essential part of the circuits underlying pair bond formation in voles – activation of this nucleus by glutamate or GABA antagonists resulted in partner preference (Curtis & Wang, 2005). Despite the fact, that most of the research on motivational aspects of social behaviour have been done on specific models – eg. juvenile or sexual interactions - it could be hypothesized that rats, or at least rats with high sociability, find social interaction rewarding. In humans the personality trait extraversion is presumed to have a dopaminergic motivational/rewarding neuronal substrate (Depue & Collins, 1999; Depue & Morrone-Strupinsky, 2005).

Ventral pallidum, where CO activity correlated negatively with social interaction time, is another region involved in pair bonding – when the vasopressin 1a receptors in that area were blocked by an antagonist, partner preference was not formed (Lim & Young, 2004).

42.2. Monoamine release in dorsomedial caudate putamen

In the microdialysis experiment the basal levels of neither dopamine nor serotonin differed between animals of high and low sociability in dorsomedial caudate putamen. The exposition of a social stimulus, another rat, to an animal, induced a small transient elevation in extracellular dopamine levels. It has been shown that presenting a conspecific of opposite sex to a rat induces increases in the number of dopamine concentration transients, indicative of burst firing of dopaminergic neurones, as measured by electrodes in dorsal caudate putamen (Robinson, Heien, & Wightman, 2002) . This effect is subject to rapid habituation, as the second stimulation induced less bursts. Our data also indicate that the raise in dopamine in response to social stimulation disappears within an hour. It should be taken into account that microdialysis and the voltammetry described above measure different aspects of dopamine transmission – tonic and phasic, possibly with different functions.

Extracellular serotonin levels in dorsomedial caudate putamen were in decline for the first two hours of the experiment, the decrease being more pronounced in HS animals. There is a reason to suspect processes of habituation or adaptation to environment, since the experiment was not carried out in the animal's home cage, but they were transferred there about one hour before

the first sample was taken – a time sufficient enough for dopamine levels to stabilize. There is evidence that serotonin is involved in habituation responses. Serotonin metabolism, as measured by serotonin concentration, catabolic enzyme activity and product accumulation is affected by repeated presentation of a stimulus: in the striatum serotonin concentration remained unchanged, whereas metabolite concentrations increased; serotonin levels decreased in amygdala and midbrain (Molodtsova, 2005). Although implying to the role of serotonin in habituation, there are major methodological differences between our and the aforementioned study. In our study the animals spent a longer period of time in one trial in the new environment, as opposed to several times for a short period, and we measured serotonin release as opposed to serotonin content in tissue.

In case of the higher extracellular levels of serotonin in HS-rats two explanations are possible. Firstly, there might be inherent differences in serotonin release, breakdown and transport between HS- and LS-animals. In that case the disappearance of differences remains a problem. Secondly, there might be differences between HS- and LS-rats in responsiveness to environmental stimulation. As the rats have already been in the test-cage an hour before the first sample was taken, their serotonin system has reacted differentially, revealing group differences, in the course of the following two hours habituation takes place. Unfortunately we do not no the basal serotonin levels from before the environmental change. The usual practice in our laboratory is to measure baseline and induced levels of serotonin in an animal's home cage, and in these circumstances problems with serotonin baselines have not been observed (unpublished).

In accordance with a previous study on ventral tegmental area, it was found that PCA induced serotonin release potential in dorsomedial caudate putamen was greater in HS-rats (Tõnissaar, Alttoa, Eller & Harro, 2007). In both occasions baseline differences were absent. It can be suspected that in individuals with highly social personality, serotonin releasing agents eg. an illicit drug MDMA can have more pronounced effects. Since the release of serotonin by PCA is dependent on serotonin transporter (Rudnick & Wall, 1992), differences in transporter function between animals of different sociability can be hypothesized.

In three animals a rise in serotonin levels appeared during the ninth hour of the experiment. As the experiment lasted for nine hours, it usually ended about eight o'clock PM, the dark phase in our animal house begins at half past eight. Although the light conditions in the lab did not change, it is possible that serotonin release increases during transition time from light to dark phase. There is no evidence for CP, but in the suprachiasmatic nucleus levels of tryptophan hydroxylase, the rate limiting enzyme in serotonin synthesis, are highest just before the onset of the dark phase and serotonin levels at the beginning of the dark phase (Barassin et al., 2002).

Our group has previously shown that animals with high sociability tend to be more reactive to chronic stress than low sociability animals, as shown by reduced sucrose intake (Tõnissaar et al, 2007). Some of the regions involved in sociability that emerged in this study can theoretically be related to possible vulnerability to chronic stress. Posterior paraventricular thalamus lesions inhibit an animal's ability to reduce hormonal reactiveness in the face of chronic stress (Bhatnagar, Huber, Nowak, & Trotter, 2002; Jaferi & Bhatnagar, 2006). In stressful situations the activation of the HPA axis is accompanied by peptide release from the supraoptic nucleus, the function of which is presumed to be behavioural coping with the situation (Neumann, 2002).

4.3. Brain regions involved in chronic variable stress and serotonergic denervation

Partial serotonergic denervation by PCA significantly increased oxidative activity in a number of brain areas, such as suprachiasmatic nucleus, CA3, substantia nigra, anteroventral and laterodorsal ventrolateral thalamus and cortical amygdala. It should also be mentioned that in many other brain areas PCA treatment tended to increase oxidative metabolism, and it can not be excluded that the small number of observations led to Type 2 error in some cases. Only in the dorsal part of anterior olfactory bulb the effect of partial serotonergic lesion was to decrease CO activity. Altogether it appears that the long-term effect of PCA is to release the serotonergic target areas from inhibition, as revealed by increased oxidative activity in several brain regions. In several parts of the rodent thalamus administration of serotonin or its agonists have been found to cause inhibition of target neurons firing rate and dorsal raphe lesions resulted in enhanced excitation in target areas (Blasiak, Siejka, Raison, Pevet, & Lewandowski, 2006; Grasso, Li Volsi, Licata, Ciranna, & Santangelo, 2006). The excitation/inhibition evoked by serotonin seems, however, to be dependent on serotonin receptor types predominantly expressed in a given region. (Di Mauro et al., 2003; Panksepp, 1998; Stanford, Kantaria, Chahal, Loucif, & Wilson, 2005).

In all brain regions where chronic stress had an impact on its own, the oxidative activity was increased. All these regions (medial preoptic area, cortical and medial amygdala) have been implicated in the regulation of hypothalamic-pituitary-adrenal (HPA) axis through paraventricular hypothalamus. It has been shown that medial amygdala (Dayas, Buller, & Day, 1999; Trneckova, Armario, Hynie, Sida, & Klenerova, 2006), some subdivisions of the cortical amygdala and the medial preoptic area (Herman & Cullinan, 1997; Herman et al., 2003) are all part of the acute stress response/modulation system. From our data it could be concluded that although acute and

chronic stress vary in several neurophysiological aspects, some regions controlling the HPA axis seem to be universally active in both circumstances.

In some cases it appeared that in animals with serotonergic dysfunction the regional energy metabolism was not reactive to stress. In medial preoptic area, cortical and medial amygdala PCA treatment inhibited the potential increase in oxidative metabolism induced by stress. Repeated social defeat induces a c-fos expression in both median and dorsal raphe nuclei (Chung, Martinez, & Herbert, 1999) and there is ample evidence of the involvement of dorsal raphe in chronic stress (Leonard, 2005). As reaction to stress is adaptive in essence, it can be suggested that the activation of serotonergic nuclei can be of benefit to the animal (Panksepp, 1998). If the serotonergic system is defective, these adaptive reactions can be compromised, leaving the animal more vulnerable to stress. There is evidence that rats with defective serotonin system have altered adaptive behavioural responses – animals pretreated with the neurotoxin 5,7-dihydroxytryptamine did not display the typical freezing behaviour in a social defeat situation (Chung et al., 1999). The reactivity of medial amygdala to chronic stress in an interactive manner with the condition of the serotonergic system has been previously shown using social defeat paradigm, where c-fos expression in that region was more pronounced in stressed animals with serotonergic lesions (Chung et al., 1999). On the other hand, there were several brain regions where stress had an impact only on PCA treated animals, decreasing the oxidative activity to control levels. These regions include suprachiasmatic hypothalamus, anteroventral thalamus, hippocampal CA3 and cortical amygdala. Though stress alone had no effect on oxidative activity of these regions, it did block the increase in metabolic activity in serotonin deficient rats. As we proposed, serotonergic lesions could release the target areas from inhibition, resulting in elevated metabolic activity. In line with this suggestion, the activation of dorsal raphe by CRF during stress could increase its output and decrease metabolic activity in target areas. There is evidence that amphetamine toxicity on striatal dopamine system is reversed by acute stress (Carlson & Wagner, 2006; Miller & O'Callaghan, 1996), the assumed mechanism involves an increase in circulating stress hormone levels. These results are not directly comparable to the present study due to vast methodological differences, but hormonal alterations in PCA treated and stressed animals can be assumed, enabling to speculate about the role of these hormones on PCA/stress interaction.

Chronic stress or depression can alter the daily rhythms in rodents, for example chronic mild stress exerts disturbances of the diurnal and circadian rhythms of the locomotor activity and circadian body temperature changes in the rats (Gorka, Moryl, & Papp, 1996; Ushijima, Morikawa, To, Higuchi, & Ohdo, 2006). The relationship of circadian activity rhythms

and chronic stress was further indirectly confirmed by this study. Stress had an impact on oxidative activity of suprachiasmatic nucleus, the internal pacemaker of the organism, but only in PCA pretreated animals. On the other hand, it is possible that instead non-photic stimulation (stressors and everyday handling) the photic stimuli (stroboscope and light during the habitual dark phase) have affected the function of suprachiasmatic nucleus (Mistlberger, 2006). There is also evidence that rodent brain lesions caused by PCA or another neurotoxin, MDMA, modulate circadian rhythm changes (Morin & Allen, 2006; Penev, Turek, & Zee, 1995) and that non-photic stimulation eg. sleep deprivation by handling, which can be regarded as a stressor to an animal, increases serotonin release in suprachiasmatic nucleus (Grossman, Mistlberger, Antle, Ehlen, & Glass, 2000). Our results suggest that from a long term metabolic perspective, stressors could have more marked effect on suprachiasmatic function in animals with serotonergic deficit.

Anteroventral thalamus, the region in our study where stress reversed the effect of PCA, but had no effect on its own, has usually been linked to allocentric spatial learning and memory processes (van Groen, Kadish, & Wyss, 2002). It is known that chronic stress has a strong impact on spatial memory formation in Y-maze and Morris water maze (Kleen, Sitomer, Killeen, & Conrad, 2006; Song, Che, Min-wei, Murakami, & Matsumoto, 2006). It could be hypothesized that the adverse impact of chronic stress on memory can partially be mediated by alterations in the anteroventral thalamus, at least among a specific subgroup of animals with inferior capacity of serotonergic system. The impact of PCA and stress on another region of the brain extensively associated with memory, hippocampal CA3, is identical to that of anteroventral thalamus – chronic stress having an impact only on the background of serotonergic dysfunction.

Chronic stress had an effect on dorsal division of the anterior olfactory bulb CO activity, though again only in animals with serotonergic dysfunction which had their metabolic activity reduced. Dorsal division of the anterior olfactory bulb is a part of the olfactory system with numerous connections to the limbic system, including the cortical amygdala (Song & Leonard, 2005). This system (olfactory bulbs and amygdaloid complex) has previously been described as a possible part of chronic stress neural substrate, showing long-lasting c-fos activation in response to chronic stressors (Matsuda et al., 1996).

Although chronic stress had a substantial main effect on adrenal weight, this was due to reversibility of PCA induced adrenal weight decrease by CVS and stress alone had no impact. Effect of CVS on adrenal glands has been found in some studies, including in one of our own, but it is not a consistent finding with this stress regime (Harro et al., 2001; Ulrich-Lai et al., 2006). The decrease in the weight of adrenal glands induced by PCA treatment can be interpreted in two

ways – by the impact of altered body temperature and/or by the changes inflicted on the HPA axis. It is known that PCA causes an acute rise of body temperature (Colado, Murray, & Green, 1993; Sugimoto, Ohkura, Inoue, & Yamada, 2001) and that acute elevations in ambient temperature can cause a reduction of adrenal mass and volume (Koko, Djordjeviae, Cvijiae, & Davidoviae, 2004). It is, however, unknown how long such reductions would last and whether the rise in body temperature induced by a small dose of PCA is sufficient to decrease the adrenals. The second explanation rests on the assumption that serotonergic denervation causes alterations in ACTH and androgen systems. Since ACTH acts a trophic factor for adrenal cells (Nussdorfer & Mazzochi, 1971), it can be hypothesized that PCA causes a reduction in ACTH through damage to serotonergic neurons involved in ACTH release. It is reported that serotonin is involved in ACTH release and regulation (Rittenhouse et al., 1994) and it has been shown that serotonergic neurotoxin MDMA causes a blunted ACTH release in response to fenfluramine stimulation (Poland et al., 1997). It can be also assumed that PCA treatment causes a change in the inhibitory role of serotonin on the amount of circulating testosterone (Hull, Muschamp, & Sato, 2004; Sodersten, Berge, & Hole, 1978) and elevated levels of testosterone in turn have been shown to reduce adrenal weight (Rifka, Cutler, Sauer, & Loriaux, 1978). In this case the reversibility of PCA induced decrease by chronic stress could be explained by an elevation in ACTH levels and reduction in testosterone levels in chronically stressed animals.

5. Conclusions

There are several brain regions that differentiate animals of high, medium or low sociability on the basis of long-term neuronal metabolism. In one of these regions, dorsomedial part of the caudate putamen, social stimulation increases extracellular dopamine irrespective of the animal's sociability. Rats with high sociability have higher baseline levels of serotonin, and PCA induced serotonin release is more pronounced in rats with high levels of sociability trait in dorsomedial caudate putamen. Partial serotonergic denervation with parachloroamphetamine and chronic variable stress both had independent effects on cytochrome oxidase activity, an indicator of long-term energy metabolism, in several rat brain structures. Both manipulations tended to increase energy metabolism. The main finding of this study is, however, that partial serotonergic denervation by parachloroampetamine and chronic variable stress had an interactive impact on energy metabolism, the combination of manipulations resulting in oxidative energy metabolism

comparable to control animals. The functional and behavioural significance of this interaction is yet to be established, but could be related to a reduction in the adaptive capacity of the brain.

Acknowledgements: This work was supported by the Estonian Ministry of Education and Science project 0182643 and the EU Framework 6 Integrated Project NEWMOOD (LSHM-CT-2004-503474).

6. References

Akil, H. (2005). Stressed and depressed. *Nature Medicine, 11*, 116-118.

Albert, D. J., & Walsh, M. L. (1982). The inhibitory modulation of agonistic behavior in the rat brain: A review. *Neuroscience & Biobehavioral Reviews, 6*, 125-143.

American Psychiatric Association. (1994). Diagnostic and statistical manual of mental disorders (4th ed.). Washington, DC: Author.

Barassin, S., Raison, S., Saboureau, M., Bienvenu, C., Maitre, M., Malan, A., et al. (2002). Circadian tryptophan hydroxylase levels and serotonin release in the suprachiasmatic nucleus of the rat. *European Journal of Neuroscience 15*, 833-840.

Bekris, S., Antoniou, K., Daskas, S., & Papadopoulou-Daifoti, Z. (2005). Behavioural and neurochemical effects induced by chronic mild stress applied to two different rat strains. *Behavioural Brain Research, 161*, 45-59.

Bell, C. J., Hood, S. D., & Nutt, D. J. (2005). Acute tryptophan depletion. Part II: Clinical effects and implications. *Australian and New Zeland Journal of Psychiatry, 39*, 565-574.

Bhatnagar, S., Huber, R., Nowak, N., & Trotter, P. (2002). Lesions of the posterior paraventricular thalamus block habituation of hypothalamic-pituitary-adrenal responses to repeated restraint. *Journal of Neuroendocrinology, 14*, 403-410.

Bielsky, I. F., & Young, L. J. (2004). Oxytocin, vasopressin, and social recognition in mammals. *Peptides, 25*, 1565-1574.

Blasiak, T., Siejka, S., Raison, S., Pevet, P., & Lewandowski, M. H. (2006). The serotonergic inhibition of slowly bursting cells in the intergeniculate leaflet of the rat. *European Journal of Neuroscience, 24*, 2769-2780.

Brennan, P. A., & Kendrick, K. M. (2006). Mammalian social odours: attraction and individual recognition. *Philosophical Transactions of the Royal Society B: Biological Sciences, 361*, 2061-2078.

Brown, G. W. (1998). Genetic and population perspectives on life events and depression. *Social Psychiatry and Psychiatric Epidemiology, 33*, 363-372.

Brunori, M., Giuffre, A., & Sarti, P. (2005). Cytochrome c oxidase, ligands and electrons. *Journal of Inorganic Biochemistry, 99*, 324-336.

Calcagnetti, D. J., & Schechter, M. D. (1992). Place conditioning reveals the rewarding aspect of social interaction in juvenile rats. *Physiology & Behavior, 51*, 667-672.

Capitanio, J. P., & Widaman, K. F. (2005). Confirmatory factor analysis of personality structure in adult male rhesus monkeys (*Macaca mulatta*). *American Journal of Primatology, 65*, 289-294.

Carlson, K. M., & Wagner, G. C. (2006). Shock stress protects mice against amphetamine-induced dopaminergic toxicity. *Brain Research, 1087*, 186-189.

Caspi, A., Sugden, K., Moffitt, T. E., Taylor, A., Craig, I. W., Harrington, H., et al. (2003). Influence of life stress on depression: moderation by a polymorphism in the 5-HTT gene. *Science, 301*, 386-389.

Chaouloff, F. (2000). Serotonin, stress and corticoids. *Journal of Psychopharmacology, 14*, 139-151.

Chung, K. K. K., Martinez, M., & Herbert, J. (1999). c-fos expression, behavioural, endocrine and autonomic responses to acute social stress in male rats after chronic restraint: modulation by serotonin. *Neuroscience, 95*, 453-463.

Cohen, S., Doyle, W. J., Turner, R., Alper, C. M., & Skoner, D. P. (2003). Sociability and susceptibility to the common cold. *Psychological Science, 14*, 389-395.

Colado, M. I., Murray, T. K., & Green, A. R. (1993). 5-HT loss in rat brain following 3,4-methylenedioxymethamphetamine (MDMA), p-chloroamphetamine and fenfluramine administration and effects of chlormethiazole and dizocilpine. *British Journal of Pharmacology., 108*, 583-589.

Costa, P. T., & McRae, R. M. (1992). *Revised NEO Personality Inventory (NEO-PI-R) and NEO Five Factor Inventory (NEO-FFI). Professional manual.* Odessa, Florida: Psychological Assessment Resources.

Cote, J., & Clobert, J. (2007). Social personalities influence natal dispersal in a lizard. *Proceedings of the Royal Society B: Biological Sciences, 274*, 383-390.

Craft, T. K. S., Glasper, E. R., McCullough, L., Zhang, N., Sugo, N., Otsuka, T., et al. (2005). Social interaction improves experimental stroke outcome. *Stroke, 36*, 2006-2011.

Curtis, J. T., & Wang, Z. (2005). Ventral tegmental area involvement in pair bonding in male prairie voles. *Physiology & Behavior, 86*, 338-346.

D'Souza, D. N., Harlan, R. E., & Garcia, M. M. (1999). Sexual dimorphism in the response to N-methyl--aspartate receptor antagonists and morphine on behavior and c-Fos induction in the rat brain. *Neuroscience, 93*, 1539-1547.

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*, 315-320.

Dayas, C. V., Buller, K. M., & Day, T. A. (1999). Neuroendocrine responses to an emotional stressor: evidence for involvement of the medial but not the central amygdala. *European Journal of Neuroscience, 11*, 2312-2322.

Delgado, M. R., Locke, H. M., Stenger, V. A., & Fiez, J. A. (2003). Dorsal striatum responses to reward and punishment: Effects of valence and magnitude manipulations. *Cognitive, Affective, & Behavioral Neuroscience, 3*, 27-38.

Depue, R. A., & Collins, P. F. (1999). Neurobiology of the structure of personality: dopamine, facilitation of incentive motivation, and extraversion. *Behavioural Brain Science, 22*, 491-517.

Depue, R. A., & Morrone-Strupinsky, J. V. (2005). A neurobehavioral model of affiliative bonding: implications for conceptualizing a human trait of affiliation. *Behavioural Brain Science, 28*, 313-350.

Di Mauro, M., Fretto, G., Caldera, M., Li Volsi, G., Licata, F., Ciranna, L., et al. (2003). Noradrenaline and 5-hydroxytryptamine in cerebellar nuclei of the rat: functional effects on neuronal firing. *Neuroscience Letters, 347*, 101-105.

Elhwuegi, A. S. (2004). Central monoamines and their role in major depression. *Progress in Neuro-Psychopharmacology and Biological Psychiatry, 28*, 435-451.

Emiliano, A. B. F., Cruz, T., Pannoni, V., & Fudge, J. L. (2006). The interface of oxytocin-labeled cells and serotonin transporter-containing fibers in the primate hypothalamus: a substrate for SSRIs therapeutic effects? *Neuropsychopharmacology, 32*, 977-988.

Engelmann, M., Ludwig, M., & Landgraf, R. (1994). Simultaneous monitoring of intracerebral release and behavior: endogenous vasopressin improves social recognition. *Journal of Neuroendocrinology, 6*, 391-395.

Eysenck, H. J., & Eysenck, S. B. G. (1975). *The Eysenck Personality Questionnaire*. London: Hodder & Stoughton.

Fasano, S., & Brambilla, R. (2002). Cellular mechanisms of striatum-dependent behavioral plasticity and drug addiction. *Current Molecular Medicine, 2*, 649-665.

Ferguson, J., Aldag, J. M., Insel, T. R., & Young, L. J. (2001). Oxytocin in the medial amygdala is essential for social recognition in the mouse. *Journal of Neuroscience, 21*, 8278-8285.

Ferris, C. F., Stolberg, T., & Delville, Y. (1999). Serotonin regulation of aggressive behavior in male golden hamsters (Mesocricetus auratus). *Behavioural Neuroscience, 113*, 804-815.

File, S. E., & Deakin, J. F. (1980). Chemical lesions of both dorsal and median raphe nuclei and changes in social and aggressive behaviour in rats. *Pharmacology, Biochemistry and Behaviour, 12*, 855-859.

File, S. E., & Hyde, J. R. (1978). Can social interaction be used to measure anxiety? *British Journal of Pharmacology, 62*, 19-24.

File, S. E., Hyde, J. R. G., & Macleod, N. K. (1979). 5,7-Dihydroxytryptamine lesions of dorsal and median raphe nuclei and performance in the social interaction test of anxiety and in a home-cage aggression test. *Journal of Affective Disorders, 1*, 115-122.

File, S. E., & Seth, P. (2003). A review of 25 years of the social interaction test. *European Journal of Pharmacology, 463*, 35-53.

Gamaro, G. D., Manoli, L. P., Torres, I. L. S., Silveira, R., & Dalmaz, C. (2003). Effects of chronic variate stress on feeding behavior and on monoamine levels in different rat brain structures. *Neurochemistry International, 42*, 107-114.

Gonzalez-Lima, F., & Cada, A. (1998). Quantitative histochemistry of cytochrome oxidase activity. In F. E. Gonzalez-Lima (Ed.), *Cytochrome oxidase in neuronal metabolism and Alzheimer's disease* (pp. 54-90): New York: Plenum Press.

Gordon, N. S., Kollack-Walker, S., Akil, H., & Panksepp, J. (2002). Expression of c-fos gene activation during rough and tumble play in juvenile rats. *Brain Research Bulletin, 57*, 651-659.

Gorka, Z., Moryl, E., & Papp, M. (1996). Effect of chronic mild stress on circadian rhythms in the locomotor activity in rats. *Pharmacology Biochemistry and Behavior, 54*, 229-234.

Gosling, S. D. (1998). Personality dimensions in spotted hyenas *(Crocuta crocuta)*. *Journal of Comparative Psychology, 112*, 107-118.

Gosling, S. D., & John, O. P. (1999). Personalty dimensions in nonhuman animals: a cross-species reveiw. *Current Directions in Psychological Science, 8*, 69-75.

Gosling, S. D., Kwan, V. S. Y., & John, O. P. (2003). A dog's got personality: a cross-species comparative approach to personality judgements in dogs and humans. *Journal of Personality and Social Psychology, 85*, 1161-1169.

Grasso, C., Li Volsi, G., Licata, F., Ciranna, L., & Santangelo, F. (2006). Aminergic control of neuronal firing rate in thalamic motor nuclei of the rat. *Archives Italiennes de Biologie, 144*, 173-196.

Green, A., Mechan, A. O., Elliott, J. M., O'Shea, E., & Colado, M. I. (2003). The pharmacology and clinical pharmacology of 3,4-methylenedioxymethamphetamine (MDMA, "Ecstasy"). *Pharmacological Reviews, 55*, 463-508.

Groothuis, T. G. G., & Carere, C. (2005). Avian personalities: characterization and epigenesis. *Neuroscience & Biobehavioral Reviews, 29*, 137-150.

Grossman, G. H., Mistlberger, R. E., Antle, M. C., Ehlen, J. C., & Glass, J. D. (2000). Sleep deprivation stimulates serotonin release in the suprachiasmatic nucleus. *Neuroreport, 11*, 1929-1932.

Häidkind, R., Eller, M., Kask, A., Harro, M., Rinken, A., Oreland, L., et al. (2004). Increased behavioural activity of rats in forced swimming test after partial denervation of serotonergic system by parachloroamphetamine treatment. *Neurochemistry International, 45*, 721-732.

Haller, J., & Kruk, M. R. (2006). Normal and abnormal aggression: human disorders and novel laboratory models. *Neuroscience & Biobehavioral Reviews, 30*, 292-303.

Hammock, E. A. D., & Young, L. J. (2006). Oxytocin, vasopressin and pair bonding: implications for autism. *Philosophical Transactions of the Royal Society B: Biological Sciences, 361*, 2187-2198.

Happe, F., Ronald, A., & Plomin, R. (2006). Time to give up on a single explanation for autism. *Nature Neuroscience, 9*, 1218-1220.

Harmer, C. J., Bhagwagar, Z., Perrett, D. I., Vollm, B. A., Cowen, P. J., & Goodwin, G. M. (2003). Acute SSRI administration affects the processing of social cues in healthy volunteers. *Neuropsychopharmacology, 28*, 148-152.

Harro, J. (2002). Long-term partial 5-HT depletion: interference of anxiety and impulsivity? *Psychopharmacology (Berl). 164*, 433-434.

Harro, J., Fischer, K., Vansteelandt, S., & Harro, M. (2004). Both low and high activities of platelet monoamine oxidase increase the probability of becoming a smoker. *European Neuropsychopharmacology, 14*, 65-69.

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*, 79-128.

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*, 227-239.

Herman, J. P., & Cullinan, W. E. (1997). Neurocircuitry of stress: central control of the hypothalamo-pituitary-adrenocortical axis. *Trends in Neurosciences, 20*, 78-84.

Herman, J. P., Figueiredo, H., Mueller, N. K., Ulrich-Lai, Y., Ostrander, M. M., Choi, D. C., et al. (2003). Central mechanisms of stress integration: hierarchical circuitry controlling hypothalamo-pituitary-adrenocortical responsiveness. *Frontiers in Neuroendocrinology, 24*, 151-180.

Hull, E. M., Muschamp, J. W., & Sato, S. (2004). Dopamine and serotonin: influences on male sexual behavior. *Physiology & Behavior, 83*, 291-307.

Hyman, S. E. (2005). Addiction: A Disease of Learning and Memory. *American Journal of Psychiatry 162*, 1414-1422.

Insel, T. R. (2003). Is social attachment an addictive disorder? *Physiology & Behavior, 79*, 351-357.

Jaferi, A., & Bhatnagar, S. (2006). Corticosterone can act at the posterior paraventricular thalamus to inhibit hypothalamic-pituitary-adrenal activity in animals that habituate to repeated stress. *Endocrinology, 147*, 4917-4930.

Katz, R. J. (1982). Animal model of depression: pharmacological sensitivity of a hedonic deficit. *Pharmacology, Biochemistry and Behavior, 16*, 965-968.

Kendler, K. S., Kessler, R. C., Walters, E. E., MacLean, C., Neale, M. C., Heath, A. C., et al. (1995). Stressful life events, genetic liability, and onset of an episode of major depression in women. *American Journal of Psychiatry, 152*, 833-842.

Kendler, K. S., Kuhn, J. W., Vittum, J., Prescott, C. A., & Riley, B. (2005). The interaction of stressful life events and a serotonin transporter polymorphism in the prediction of episodes of major depression: a replication. *Archives of General Psychiatry, 62*, 529-535.

Kirouac, G. J., Parsons, M. P., & Li, S. (2005). Orexin (hypocretin) innervation of the paraventricular nucleus of the thalamus. *Brain Research, 1059*, 179-188.

Kiss, A., & Mikkelsen, J. D. (2005). Oxytocin - anatomy and functional assignments: a minireview. *Endocrine Regulations, 39*, 97-105.

Kleen, J. K., Sitomer, M. T., Killeen, P. R., & Conrad, C. D. (2006). Chronic stress impairs spatial memory and motivation for reward without disrupting motor ability and motivation to explore. *Behavioural Neuroscience, 120*, 842-851.

Knutson, B., Wolkowitz, O. M., Cole, S. W., Chan, T., Moore, E. A., Johnson, R. C., et al. (1998). Selective alteration of personality and social behavior by serotonergic intervention. *American Journal of Psychiatry, 155*, 373-379.

Koko, V., Djordjeviae, J., Cvijiae, G., & Davidoviae, V. (2004). Effect of acute heat stress on rat adrenal glands: a morphological and stereological study. *Journal of Experimental Biology, 207*, 4225-4230.

Korte, S. M., Koolhaas, J. M., Wingfield, J. C., & McEwen, B. S. (2005). The Darwinian concept of stress: benefits of allostasis and costs of allostatic load and the trade-offs in health and disease. *Neuroscience & Biobehavioral Reviews, 29*, 3-38.

Lawrence, A. J., Cowen, M. S., Yang, H.-J., Chen, F., & Oldfield, B. (2006). The orexin system regulates alcohol-seeking in rats. *British Journal of Pharmacology, 148*, 752-759.

Leonard, B. E. (2005). The HPA and immune axes in stress: The involvement of the serotonergic system. *European Psychiatry, 20*, S302-S306.

Lim, M. M., & Young, L. J. (2004). Vasopressin-dependent neural circuits underlying pair bond formation in the monogamous prairie vole. *Neuroscience, 125*, 35-45.

Mamounas, L. A., & Molliver, M. E. (1988). Evidence for dual serotonergic projections to neocortex: axons from the dorsal and median raphe nuclei are differentially vulnerable to the neurotoxin p-chloroampetamine (PCA). *Experimental Neurology, 102*, 23-36.

Mandelli, L., Serretti, A., Marino, E., Pirovano, A., Calati, R., & Colombo, C. (2006). Interaction between serotonin transporter gene, catechol-O-methyltransferase gene and stressful life events in mood disorders. *International Journal of Neuropsychopharmacology, 7*, 1-11.

Mangiavacchi, S., Masi, F., Scheggi, S., Leggio, B., De Montis, M. G., & Gambarana, C. (2001). Long-term behavioral and neurochemical effects of chronic stress exposure in rats. *Journal of Neurochemistry, 79*, 1113-1121.

Matsuda, S., Peng, H., Yoshimura, H., Wen, T.-C., Fukuda, T., & Sakanaka, M. (1996). Persistent c-fos expression in the brains of mice with chronic social stress. *Neuroscience Research, 26*, 157-170.

McBride, W. J., Murphy, J. M., & Ikemoto, S. (1999). Localization of brain reinforcement mechanisms: intracranial self-administration and intracranial place-conditioning studies. *Behavioural Brain Research, 101*, 129-152.

McEwen, B. S., & Seeman, T. (2003). Stress and affect: applicability of the concepts of allostasis and allostatic load. In R. J. Davidson, K. R. Scherer & H. H. Goldsmith (Eds.), *Handbook of Affective Sciences*. New York: Oxford University Press.

Mehlman, P. T., Higley, J. D., Faucher, I., Lilly, A. A., Taub, D. M., Vickers, J., et al. (1995). Correlation of CSF 5-HIAA concentration with sociality and the timing of emigration in free-ranging primates. *American Journal of Psychiatry, 152*, 907-913.

Miller, D. B., & O'Callaghan, J. P. (1996). Neurotoxicity of d-amphetamine in the C57BL/6J and CD-1 mouse. Interactions with stress and the adrenal system. *Annals of the New York Acadamy of Sciences, 801*, 148-167.

Mistlberger, R. E. (2006). Illuminating serotonergic gateways for strong resetting of the mammalian circadian clock. *American Journal of Physiology. Regulatory, Integrative and Comparative Physiology, 291*, R177-179.

Molodtsova, G. F. (2005). Changes in serotonin metabolism in the rat brain on presentation of a habituated stimulus. *Neuroscience and Behavioral Physiology, 35*, 561-566.

Morin, L. P., & Allen, C. N. (2006). The circadian visual system, 2005. *Brain Research Reviews, 51*, 1-60.

Morley, K. C., Arnold, J. C., & McGregor, I. S. (2005). Serotonin (1A) receptor involvement in acute 3,4-methylenedioxymethamphetamine (MDMA) facilitation of social interaction in the rat. *Progress in Neuro-Psychopharmacology and Biological Psychiatry, 29*, 648-657.

Morley, K. C., & McGregor, I. S. (2000). (+/-)-3,4-Methylenedioxymethamphetamine (MDMA, `Ecstasy') increases social interaction in rats. *European Journal of Pharmacology, 408*, 41-49.

Moy, S. S., Nadler, J. J., Perez, A., Barbaro, R. P., Johns, J. M., Magnuson, T. R., et al. (2004). Sociability and preference for social novelty in five inbred strains: an approach to assess autistic-like behavior in mice. *Genes, Brain and Behavior, 3*, 287-302.

Moy, S. S., Nadler, J. J., Young, N. B., Perez, A., Holloway, L. P., Barbaro, R. P., et al. (2007). Mouse behavioral tasks relevant to autism: Phenotypes of 10 inbred strains. *Behavioural Brain Research, 176*, 4-20.

Munafo, M. R., Zetteler, J. I., & Clark, T. G. (2007). Personality and smoking status: A meta-analysis. *Nicotine & Tobacco Research, 9*, 405-413.

Nash, M. W., Sugden, K., Huezo-Diaz, P., Williamson, R., Sterne, A., Purcell, S., et al. (2005). Association analysis of monoamine genes with measures of depression and anxiety in a

selected community sample of siblings. *American Journal of Medical Genetics. Part B, Neuropsychiatric Genetics, 135*, 33-37.

Neumann, I. D. (2002). Involvement of the brain oxytocin system in stress coping: interactions with the hypothalamo-pituitary-adrenal axis. *Progress in Brain Research, 139*, 147-162.

Neumann, I. D. (2007). Oxytocin: The neuropeptide of love reveals some of its secrets. *Cell Metabolism, 5*, 231-233.

Normansell, L., & Panksepp, J. (1990). Effects of morphine and naloxone on play-rewarded spatial discrimination in juvenile rats. *Developmental Psychobiology, 23*, 75-83.

Nussdorfer, G. G., & Mazzochi, G. (1971). Effect of ACTH on mitochondrial RNA synthesis of rat adrenocortical cells. *Zeitschrift fur Zellforschung und Mikroskopische Anatomie, 118*, 35-48.

O'Neil, M. F., & Moore, N. A. (2003). Animal models of depression: are there any? *Human Psychopharmacology: Clinical and Experimental, 18*, 239-254.

Oster, G., Wang, H., & Grabe, M. (2000). How Fo-ATPase generates rotary torque. *Philosophical Transactions of the Royal Society B: Biological Sciences, 355*, 523–528.

Panksepp, J. (1998). *Affective Neuroscience: The Foundations of Human and Animal Emotions*. New York: Oxford University Press.

Paxinos, G., & Watson, C. (1986). *The Rat Brain in Stereotaxic Coordinates.* San Diego: Academic Press.

Penev, P. D., Turek, F. W., & Zee, P. C. (1995). A serotonin neurotoxin attenuates the phase-shifting effects of triazolam on the circadian clock in hamsters. *Brain Research, 669*, 207-216.

Pine, D. S., Cohen, P., Johnson, J. G., & Brook, J. S. (2002). Adolescent life events as predictors of adult depression. *Journal of Affective Disorders, 68*, 49-57.

Pineyro, G., & Blier, P. (1999). Autoregulation of serotonin neurons: role in antidepressant drug action. *Pharmacological Reviews, 51*, 533-591.

Poland, R. E., Preetam, L., James, T. M., Jing-Ping, Z., Gary, L. B., Charles, S. G., et al. (1997). Abnormal ACTH and prolactin responses to fenfluramine in rats exposed to single and multiple doses of MDMA. *Psychopharmacology, 131*, 411-419.

Raggenbass, M. (2001). Vasopressin- and oxytocin-induced activity in the central nervous system: electrophysiological studies using in-vitro systems. *Progress in Neurobiology, 64*, 307-326.

Ramos, A., Berton, O., Mormede, P., & Chaouloff, F. (1997). A multiple-test study of anxiety-related behaviours in six inbred rat strains. *Behavioural Brain Research, 85*, 57-69.

Ray, M. A., Graham, A. J., Lee, M., Perry, R. H., Court, J. A., & Perry, E. K. (2005). Neuronal nicotinic acetylcholine receptor subunits in autism: an immunohistochemical investigation in the thalamus. *Neurobiology of Disease, 19*, 366-377.

Rifka, S. M., Cutler, G. B., Jr, Sauer, M. A., & Loriaux, D. L. (1978). Rat adrenal androgen receptor: a possible mediator of androgen-induced decreased in rat adrenal weight. *Endocrinology, 103*, 1103-1110.

Rittenhouse, P. A., Bakkum, E. A., Levy, A. D., Li, Q., Carnes, M., & van de Kar, L. D. (1994). Evidence that ACTH secretion is regulated by serotonin2A/2C (5-HT2A/2C) receptors. *Journal of Pharmacology and Experimental Therapeutics, 271*, 1647-1655.

Robinson, D. L., Heien, M. L., & Wightman, R. M. (2002). Frequency of dopamine concentration transients increases in dorsal and ventral striatum of male rats during introduction of conspecifics. *Journal of Neuroscience, 22*, 10477-10486.

Rudnick, G., & Wall, S. C. (1992). p-Chloroamphetamine induces serotonin release through serotonin transporters. *Biochemistry, 31*, 6710-6718.

Rygula, R., Abumaria, N., Flugge, G., Fuchs, E., Ruther, E., & Havemann-Reinecke, U. (2005). Anhedonia and motivational deficits in rats: Impact of chronic social stress. *Behavioural Brain Research, 162*, 127-134.

Schalling, D., Asberg, M., Edman, G., & Oreland, L. (1987). Markers for vulnerability to psychopathology: temperament traits associated with platelet MAO activity. *Acta Psychiatrica Scandinavica, 76*, 172-182.

Sodersten, P., Berge, O. G., & Hole, K. (1978). Effects of p-chloroamphetamine and 5,7-dihydroxytryptamine on the sexual behavior of gonadectomized male and female rats. *Pharmacology, Biochemistry, and Behaviour, 9*, 499-508.

Song, C., & Leonard, B. E. (2005). The olfactory bulbectomised rat as a model of depression. *Neuroscience & Biobehavioral Reviews, 29*, 627-647.

Song, L., Che, W., Min-wei, W., Murakami, Y., & Matsumoto, K. (2006). Impairment of the spatial learning and memory induced by learned helplessness and chronic mild stress. *Pharmacology Biochemistry and Behavior, 83*, 186-193.

Stanford, I. M., Kantaria, M. A., Chahal, H. S., Loucif, K. C., & Wilson, C. L. (2005). 5-Hydroxytryptamine induced excitation and inhibition in the subthalamic nucleus: Action at 5-HT2C, 5-HT4 and 5-HT1A receptors. *Neuropharmacology, 49*, 1228-1234.

Stephenson, C. P., Hunt, G. E., Topple, A. N., & McGregor, I. S. (1999). The distribution of 3,4-methylenedioxymethamphetamine "Ecstasy"-induced c-fos expression in rat brain. *Neuroscience, 92*, 1011-1023.

Sugimoto, Y., Ohkura, M., Inoue, K., & Yamada, J. (2001). Involvement of serotonergic and dopaminergic mechanisms in hyperthermia induced by a serotonin-releasing drug, p-chloroamphetamine in mice. *European Journal of Pharmacology, 430*, 265-268.

Suntsova, N., Guzman-Marin, R., Kumar, S., Alam, N., Szymusiak, R., & McGinty, D. (2007). The median preoptic nucleus reciprocally modulates activity of arousal-related and sleep-related neurons in the perifornical lateral hypothalamus. *Journal of Neuroscience, 27*, 1616-1630.

Zhang, X., Gainetdinov, R. R., Beaulieu, J. M., Sotnikova, T. D., Burch, L. H., Williams, R. B., et al. (2005). Loss-of-function mutation in tryptophan hydroxylase-2 identified in unipolar major depression. *Neuron, 45*, 11-16.

Thompson, M. R., Callaghan, P. D., Hunt, G. E., Cornish, J. L., & McGregor, I. S. (2007). A role for oxytocin and 5-HT(1A) receptors in the prosocial effects of 3,4 methylenedioxymethamphetamine ("ecstasy"). *Neuroscience, 146*, 509-514.

Tischler, R. C., & Morin, L. P. (2003). Reciprocal serotonergic connections between the hamster median and dorsal raphe nuclei. *Brain Research, 981*, 126-132.

Tõnissaar, M., Alttoa, A., Eller, M., & Harro, J. Extracellular levels of serotonin in prefrontal cortex and ventral tegmental area in rats with high and low sociability. (2007). Submitted for publication.

Tõnissaar, M., Herm, L., Eller, M., Kõiv, K., Rinken, A., & Harro, J. (2007). Rats with high or low sociability are differently affected by chronic variable stress. Submitted for publication.

Tõnissaar, M., Philips, M.-A., Eller, M., & Harro, J. (2004). Sociability trait and serotonin metabolism in the rat social interaction test. *Neuroscience Letters, 367*, 309-312.

Torres, G., Meeder, B. A., Hallas, B. H., Gross, K. W., & Horowitz, J. M. (2005). Preliminary evidence for reduced social interactions in Chakragati mutants modeling certain symptoms of schizophrenia. *Brain Research, 1046*, 180-186.

Trneckova, L., Armario, A., Hynie, S., Sida, P., & Klenerova, V. (2006). Differences in the brain expression of c-fos mRNA after restraint stress in Lewis compared to Sprague-Dawley rats. *Brain Research, 1077*, 7-15.

Ulrich-Lai, Y. M., Figueiredo, H. F., Ostrander, M. M., Choi, D. C., Engeland, W. C., & Herman, J. P. (2006). Chronic stress induces adrenal hyperplasia and hypertrophy in a subregion-

specific manner. *American Journal of Physiology, Endocrinology and Metabolism, 291*, E965-973.

Ushijima, K., Morikawa, T., To, H., Higuchi, S., & Ohdo, S. (2006). Chronobiological disturbances with hyperthermia and hypercortisolism induced by chronic mild stress in rats. *Behavioural Brain Research, 173*, 326-330.

Van der Werf, Y. D., Witter, M. P., & Groenewegen, H. J. (2002). The intralaminar and midline nuclei of the thalamus. Anatomical and functional evidence for participation in processes of arousal and awareness. *Brain Research Reviews, 39*, 107-140.

van Groen, T., Kadish, I., & Michael Wyss, J. (2002). Role of the anterodorsal and anteroventral nuclei of the thalamus in spatial memory in the rat. *Behavioural Brain Research, 132*, 19-28.

van Praag, H. M. (2005). Can stress cause depression? *World Journal of Biological Psychiatry, 6*, 5-22.

Vertes, R. P., Fortin, W. J., & Crane, A. M. (1999). Projections of the median raphe nucleus in the rat. *Journal of Comparative Neurology, 407*, 555-582.

Whittle, S., Allen, N. B., Lubman, D. I., & Yucel, M. (2006). The neurobiological basis of temperament: Towards a better understanding of psychopathology. *Neuroscience & Biobehavioral Reviews, 30*, 511-525.

Willner, P. (2005). Chronic mild stress (CMS) revisited: consistency and behavioural-neurobiological concordance in the effects of CMS. *Neuropsychobiology., 52*, 90-110.

Wilson, M. A., Mamounas, L. A., Fasman, K. H., Axt, K. J., & Molliver, M. E. (1993). Reactions of 5-HT neurons to drugs of abuse: neurotoxicity and plasticity. *NIDA Research Monograph, 136*, 155-178.

Wirtshafter, D. (2001). The control of ingestive behavior by the median raphe nucleus. *Appetite, 36*, 99-105.

Wise, R. A. (2005). Forebrain substrates of reward and motivation. *Journal of Comparative Neurology, 493*, 115-121.

Wong-Riley, M. T. T., Nie, F., Hevner, R. F., & Liu, S. (1998). Brain cytochrome oxidase: Functional significance and bigenomic regulation in the CNS. In F. Gonzalez-Lima (Ed.), *Cytochrome oxidase in neuronal metabolism and Alzheimer's disease* (pp. 1-53): New York: Plenum Press.