DISSERTATIONES PSYCHOLOGICAE UNIVERSITATIS TARTUENSIS
24

DISSERTATIONES PSYCHOLOGICAE UNIVERSITATIS TARTUENSIS
24

AET ALTTOA

Neurochemical regulation of rat exploratory behaviour: focus on dopaminergic and noradrenergic neurotransmission

TARTU UNIVERSITY
PRESS

Department of Psychology, University of Tartu, Estonia

Dissertation is accepted for the commencement of the degree of Doctor of Philosophy (in Psychology) on July 11, 2008 by the Doctoral Committee of the Department of Psychology, University of Tartu.

Supervisor: Professor Jaanus Harro, MD, PhD
University of Tartu, Estonia

Opponent: Professor Nicolas Singewald, PhD
University of Innsbruck, Austria

Commencement: September 17, 2008

ISSN 1024–3921
ISBN 978–9949–11–947–9 (trükis)
ISBN 978–9949–11–948–6 (PDF)

Autoriõigus Aet Alttoa, 2008

Tartu Ülikooli Kirjastus
www.tyk.ee
Tellimus nr 361

CONTENTS

LIST OF ORIGINAL PUBLICATIONS **7**

ABBREVIATIONS **8**

1. INTRODUCTION **9**
1.1. Exploratory behaviour 9
1.2. The neurobiology of exploratory behaviour 10
1.2.1. Dopaminergic neurotransmission in exploratory behaviour ... 10
1.2.2. Cholecystokinin-dopamine interaction 11
1.2.3. The locus coeruleus noradrenaline system and exploratory behaviour 12
1.2.4. Dopamine and noradrenaline in the behavioural effects of amphetamine 13
1.3. Individual differences in exploratory behaviour 13

2. AIMS OF THE PRESENT STUDIES **16**

3. MATERIALS AND METHODS **17**
3.1. Animals (Papers I – VI) 17
3.2. Drugs (Papers I – V) 17
3.3. Behavioural methods 17
3.3.1. Exploration box test and classification of animals as high or low explorers (Papers I – VI) 17
3.3.2. Other behavioural tests (Paper II) 19
3.4. Biochemical methods 21
3.4.1. In vivo microdialysis (Papers II and III) 21
3.4.2. Monoamine content measurements (Papers IV and V) 22
3.4.3. D2 receptor-stimulated [35S]GTPγS binding (Papers IV and V) 22
3.4.4. Measurement of the D2High receptors in the striatum (Paper III) 23
3.4.5. RNA preparation and gene expression profiling (Paper VI) .. 24
3.5. Statistical methods 24
3.5.1. Bioinformatics (Paper VI) 24
3.5.2. Data analysis 25

4. RESULTS AND DISCUSSION **27**
4.1. Role of CCK in amphetamine-stimulated exploratory behaviour and sensitization to amphetamine 27
4.1.1. Effect of CCK receptor blockade on amphetamine-stimulated locomotor activity (Paper I) 27

4.1.2. Effect of CCK receptor blockade on behavioural sensitization to amphetamine (Paper I) 29
4.2. Exploratory activity as a persistent trait: behavioural differences of rats with high and low exploratory activity 31
4.2.1. Persistent individual differences in exploratory activity in the exploration box test (Paper II) 31
4.2.2. Behavioural differences in high and low explorers in tests of anxiety and depression (Paper II) 33
4.3. Neurobiological differences in high and low explorers 37
4.3.1. Ex vivo monoamines and metabolites (Papers IV and V) 37
4.3.2. Extracellular dopamine in the striatum and the nucleus accumbens (Papers II and III) 38
4.3.3. Differences in the proportion of D2 receptors in the high affinity state in the striatum (Paper III) 39
4.3.4. Differences in genome-wide gene expression profiles (Paper VI) 40
4.4. Noradrenergic regulation of exploratory behaviour: behavioural and neurobiological effects of partial locus coeruleus denervation in high and low explorers 51
4.4.1. Behavioural effects of partial locus coeruleus denervation in high and low explorers (Papers IV and V) 51
4.4.2. Effect of partial locus coeruleus denervation on neurobiological measures (Papers IV and V) 54

5. GENERAL SUMMARY 58

ACKNOWLEDGEMENTS 59

WEB RESOURCES 60

REFERENCES 60

SUMMARY IN ESTONIAN 73

PUBLICATIONS 75

LIST OF ORIGINAL PUBLICATIONS

I **Alttoa, A.**, Harro, J. (2004) Effect of CCK_1 and CCK_2 receptor blockade on amphetamine-stimulated exploratory behavior and sensitization to amphetamine. *European Neuropsychopharmacology*, 14, 324–331.

II Mällo, T., **Alttoa, A.**, Kõiv, K., Tõnissaar, M., Eller, M., Harro, J. (2007) Rats with persistently low or high exploratory activity: behaviour in tests of anxiety and depression, and extracellular levels of dopamine. *Behavioural Brain Research*, 177, 269–281.

III **Alttoa, A.**, Seeman, P., Kõiv, K., Eller, M., Harro, J. Rats with persistently high exploratory activity have both higher extracellular dopamine levels and higher proportion of D_2^{High} receptors in the striatum. *Synapse* (in press).

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

V **Alttoa, A.**, Eller, M., Herm, L., Rinken, A., Harro, J. (2007) Amphetamine-induced locomotion, behavioral sensitization to amphetamine, and striatal D_2 receptor function in rats with high or low spontaneous exploratory activity: differences in the role of locus coeruleus. *Brain Research*, 1131, 138–148.

VI **Alttoa, A.**, Kõiv, K., Hinsley, T.A., Brass, A., Harro, J. Differential gene expression in rats with persistently high or low exploratory activity. (*in manuscript*).

The author of the present dissertation contributed to the individual publications presented herein as follows:

- for **Papers I, IV and V**, creating the experimental designs, carrying out all of the behavioural experiments and the data analysis, and writing the publications as the main author;
- for **Paper II**, performing some of the behavioural experiments and conducting the microdialysis studies, and participating in the preparation of the first draft;
- for **Paper III**, creating the experimental design, conducting the microdialysis experiment, performing all statistical analysis, and writing the publication;
- for **Paper VI**, performing some of the behavioural work, conducting the bioinformatical analysis of the microarray data and writing the manuscript.

ABBREVIATIONS

[^{35}S]GTPγS	[^{35}S]-guanosine-5'-(γ-thio)-triphosphate
5-HIAA	5-hydroxyindoleacetic acid
5-HT	5-hydroxytryptamine; serotonin
5-HTT	serotonin transporter
6-OHDA	6-hydroxydopamine
Atf2	gene for activating transcription factor 2
CB_1	cannabinoid receptor 1
CCK	cholecystokinin
Cnr1	gene for cannabinoid receptor 1 (brain)
Ctnnb1	gene for catenin (cadherin associated protein), beta 1
Cxxc4	gene for CXXC4 finger (Idax)
D_2	dopamine D_2 receptor
D_2^{High}	dopamine D_2 receptor in the high affinity state
DAVID	the Database for Annotation, Visualization and Integrated Discovery
DSP-4	N-(2-chloroethyl)-N-ethyl-2-bromobenzylamine
Fgf12	gene for fibroblast growth factor 12
GABA	γ-aminobutyric acid
Gabra1	gene for gamma-aminobutyric acid A receptor, subunit alpha 1
Gabra4	gene for gamma-aminobutyric acid A receptor, subunit alpha 4
Gabrg1	gene for gamma-aminobutyric acid A receptor, subunit gamma 1
Gabrg2	gene for gamma-aminobutyric acid A receptor, subunit gamma 2
GO	Gene Ontology
Grin2a	gene for glutamate receptor, ionotropic, N-methyl-D-aspartate 2A
Gria3	gene for glutamate receptor, ionotropic, AMPA3 (alpha 3)
GSK-3β	glycogen synthase kinase-3, beta
Gsk3b	gene for glycogen synthase kinase-3, beta
HE	high explorer
Htr1a	gene for 5-hydroxytryptamine (serotonin) receptor 1A
Htr2c	gene for 5-hydroxytryptamine (serotonin) receptor 2C
JNK	c-Jun N-terminal kinase
KEGG	Kyoto Encyclopedia of Genes and Genomes
LC	locus coeruleus
LE	low explorer
MAPK	mitogen-activated protein kinase
Mapk9	gene for mitogen-activated protein kinase 9
NMDA	N-methyl-D-aspartic acid
Rac1	gene for ras-related C3 botulinum toxin substrate 1
Senp2	gene for SUMO/sentrin specific protease 2 (Axam)
Tph1	gene for tryptophan hydroxylase 1
Tph2	gene for tryptophan hydroxylase 1
VTA	ventral tegmental area

1. INTRODUCTION

1.1. Exploratory behaviour

Rodents are attracted to novel objects and readily spend time exploring unfamiliar environments. Exploratory behaviour in rats is of a clear biological significance as it drives the animal to approach novel places and objects to find food, water, shelter, etc., thus greatly enhancing its chances for survival and reproduction (Crusio, 2001). Crusio and Abeelen (1986) have defined exploration as being "... evoked by novel stimuli ...", and consisting "... of behavioural acts and postures that permit the collection of information about new objects and unfamiliar parts of the environment".

Whenever an animal is exposed to a novel environment, a natural conflict occurs between its innate motivation to explore the unfamiliar and its initial tendency to avoid the unknown. Thus, the behavioural activity of an animal in a novel context is always a combination of its curiosity and fear (Harro, 1993, for review). Exploration tests have been widely used in behavioural pharmacology. The overlap in the neurobiological substrates of novelty- and drug-related behaviour (Bardo et al., 1996) makes exploration tasks useful in the research of drug addiction. Also, because of the component of neophobia in exploratory activity, novelty-based tests have been employed in studies of anxiety-like behaviours.

One of the most popular approaches to measure exploratory behaviour in rodents is the open-field test (Prut and Belzung, 2003). The open-field test is an example of a forced exploration test which involves placing the animal into a brightly lit unfamiliar arena from which there is no retreat. Consequently, it is difficult to determine whether the behaviours displayed in this inescapable apparatus are due to true exploratory drive or general locomotor activity. There is also evidence that forced exploration is stressful as corticosterone levels in rats increase in response to an exposure to an open field (Marin et al., 2007) but not to free-choice novelty (Misslin et al., 1982). Thus, a more valid measure of novelty-related behaviour would be obtained if an exploration test were used which would allow the animal to actively choose a novel environment over a familiar one.

In the studies described in this dissertation the exploration box test was used to measure exploratory behaviour in rats. Originally developed to assess the behavioural changes in novelty-related behaviour after the denervation of noradrenergic projections from the locus coeruleus (Harro et al., 1995), it contains elements of a free-choice exploration task. Specifically, the rat has an opportunity to both hide and stay close to the walls in a small dark chamber that resembles its home cage, or venture out of it into a novel open area that has unfamiliar objects placed in it (Otter et al., 1997).

1.2. The neurobiology of exploratory behaviour

1.2.1. Dopaminergic neurotransmission in exploratory behaviour

The dopamine neurons in the ventral midbrain are located mostly in the ventral tegmental area (VTA), which coincides with the A10 dopamine cell group, and in the substantia nigra (the A9 cell group) which is located laterally to the VTA (Dahlström and Fuxe, 1964). The dopaminergic neurons in the VTA send their axons to the limbic regions (including the nucleus accumbens) and cortical areas, giving rise to the mesocorticolimbic dopamine pathway, while the dopamine cells in the substantia nigra project mainly to the dorsal part of the striatum (caudate and putamen) in the nigrostriatal dopamine system.

The mesolimbic dopamine system has a central role in motivated and reward-related behaviours (Wise and Bozarth, 1985; Berridge and Robinson, 1998; Ikemoto and Panksepp, 1999); however, reward-related changes in dopaminergic activity have been shown in the striatum as well (Zald et al., 2004). The dopaminergic neurotransmission, particularly in the nucleus accumbens, is increased in response to natural rewards, such as feeding (Heffner et al., 1980; Westerink et al., 1994; Krügel et al., 2003; Richardson and Gratton, 2008), drinking (Young et al., 1992), and sexual interaction (Pfaus et al., 1990; Pfaus et al., 1995; Becker et al., 2001); and has been implicated in the mechanism of action of drugs with addictive properties (Di Chiara and Imperato, 1988; Cadoni and Di Chiara, 2007). Novel stimuli that initiate investigatory behaviour and orientation also activate the midbrain dopaminergic neurons (Bunzeck and Düzel, 2006) and increase extracellular dopamine levels in the brain (Rebec et al., 1997; De Leonibus et al., 2006). Thus, novelty and drugs of abuse activate, at least partly, the same neurobiological substrates, suggesting common mechanism underlying exploratory and drug-related behaviour (Bardo et al., 1996).

Enhanced dopaminergic neurotransmission in the nucleus accumbens and the striatum is crucial for the expression of the locomotor-stimulating effects of psychostimulants, e.g., amphetamine (Segal and Kuczenski, 1994, for review). Repeated administration of psychostimulants results in persistently increased locomotor activity in response to the drug (Vezina, 2004). It is not only the locomotor response that becomes sensitized after such treatments; the psychostimulant-induced dopamine release in the striatum and the nucleus accumbens is potentiated as well (Vezina, 1993; Paulson and Robinson, 1995; Vanderschuren et al., 1999). Recently, it has been shown that dopaminergic supersensitivity, either elicited by sensitization to psychostimulants or otherwise, is accompanied by the modifications in the affinity states of the striatal dopamine D_2 receptors (Seeman et al., 2005; Seeman et al., 2006). The D_2 receptors exist in high-affinity (D_2^{High}) and low-affinity (D_{2Low}) states, and the former has been proposed to be the physiologically functional state (George et

al., 1985). In psychostimulant-sensitized rats, the proportion of the high-affinity D_2 receptors in the striatum markedly increases, likely contributing to their heightened behavioural sensitivity to psychostimulants and dopamine agonists (Seeman et al., 2002; Seeman et al., 2007; Shuto et al., 2008; Simola et al., 2008; Briand et al., 2008).

1.2.2. Cholecystokinin-dopamine interaction

Cholecystokinin (CCK) is a gut–brain peptide, which acts via CCK_1 and CCK_2 receptor subtypes (Noble et al., 1999). CCK is involved in the regulation of feeding, pain perception, and learning and memory (Crawley and Corwin, 1994 and Moran and Schwartz, 1994), and possibly in the pathogenesis of anxiety and psychosis (see Bourin et al., 1996 and Harro, 2006, for review).

A subpopulation of the dopaminergic neurons in the ventral tegmental area projecting to the nucleus accumbens contains CCK as a cotransmitter (Hökfelt et al., 1980). It has been demonstrated that CCK modulates dopaminergic activity depending on the CCK receptor subtype involved. For example, CCK acting via CCK_1 receptors in the medial posterior nucleus accumbens potentiates dopaminergic activity while CCK acting on CCK_2 receptors in the anterior nucleus accumbens either has no effect or inhibits dopaminergic activity (Crawley, 1991 and Marshall et al., 1991). Similarly, behavioral studies have demonstrated that intracerebrally administered CCK potentiates dopamine-dependent behavior in a CCK_1 receptor-mediated manner while inhibits it via CCK_2 receptors (Crawley, 1991; Crawley, 1992; Crawley, 1994; Vaccarino and Rankin, 1989). Given the dopamine involvement in drug reward (Wise and Bozarth, 1985; Pierce and Kumaresan, 2006) as well as in psychotic disorders (Davis et al., 1991 for review), CCK may contribute to the pathogenesis of drug abuse or psychosis.

The selective CCK receptor antagonists have been shown to be ineffective in modulating baseline locomotor behavior, but they alter the behavioral changes induced by dopamine or dopamine agonists, e.g. the indirect agonist amphetamine (Phillips et al., 1993; Josselyn and Vaccarino, 1995; Tieppo et al., 2000). CCK receptors also seem to contribute to the development and expression of behavioral sensitization to amphetamine (DeSousa et al., 1999; Wunderlich et al., 2000; Wunderlich et al., 2004), a phenomenon known to occur after repeated exposure to psychostimulants (Vezina, 2004). Nevertheless, there are some inconsistencies in the studies describing the specific roles the CCK receptor subtypes may have in modulating the different behavioral effects of amphetamine, particularly when CCK receptor antagonists have been used to block the modulating role of endogenous CCK. In the rat, CCK_1 receptor blockade was reported not to influence amphetamine-induced hyperlocomotion (DeSousa et al., 1999) and stereotypy (Tieppo et al., 2000). Neither did a nonpeptide CCK_1 receptor antagonist affect amphetamine-induced disruption of

prepulse inhibition (Shilling and Feifel, 2002). Somewhat inconsistently, rats with the naturally-occurring CCK_1 receptor-deficiency were less sensitive to locomotor activity enhancing effects of amphetamine (Feifel et al., 2001), and devazepide, a CCK_1 receptor antagonist, reduced the locomotor stimulant effect of amphetamine in mice (Vasar et al., 1991). CCK_1 receptor blockade by devazepide treatment has been found to block the expression of amphetamine sensitization, but not the development of it (Wunderlich et al., 2000). Interestingly, devazepide has been shown to antagonise the acquisition of amphetamine-conditioned (Josselyn et al., 1996) as well as cocaine-conditioned activity (Josselyn et al., 1997), suggesting differential involvement of CCK_1 receptors in these behavioral phenomena. Regarding CCK_2 receptor blockade, the effect of an antagonist, L-365,260, on the development of amphetamine sensitization was found to be either reducing or potentiating depending upon dose (Wunderlich et al., 2000). In other studies, L-365,260 has been reported to enhance amphetamine-facilitated responding for conditioned rewards (Josselyn and Vaccarino, 1995) and to reduce amphetamine stereotypy (Tieppo et al., 2000).

1.2.3. The locus coeruleus noradrenaline system and exploratory behaviour

The *locus coeruleus* (LC) is a well-defined nucleus of noradrenaline-containing neurons, located in the brain stem next to the fourth ventricle (Berridge and Waterhouse, 2003). The LC neurons project to virtually the entire central nervous system; the noradrenergic innervation in the hippocampus and the neocortex originates exclusively from the LC. The spontaneous activity of the LC neurons in the rat varies with the state of arousal: it is highest during waking, decreased in slow-wave sleep and almost absent in paradoxical sleep (Aston-Jones et al., 1999). However, the tonic firing rate of the LC neurons is also moderately reduced during certain behaviours (e.g., grooming and feeding) when the animal is awake, but pays little attention to the environmental stimuli (Aston-Jones and Bloom, 1981a). Various sensory stimuli that also disrupt ongoing behaviour and evoke behavioural orienting responses typically induce large phasic activation of the LC neurons (Aston-Jones and Bloom, 1981b).

Aston-Jones and Bloom have proposed that the enhanced firing of the LC promotes the attention and orienting to salient environmental stimuli, novel stimuli in particular, and suppresses the central nervous system activity that is related to vegetative functions (Aston-Jones and Bloom, 1981b). Exposure to novelty increases extracellular noradrenaline levels in the prefrontal cortex (Feenstra et al., 2000), and intact noradrenergic neurotransmission is required for normal novelty-related behaviour (Harro et al., 1995). Near maximal denervation of the LC projections with a large dose (50 mg/kg) of the selective noradrenergic neurotoxin DSP-4 renders the animals neophobic and defective in coping with environmental changes (Archer, 1983; Harro et al., 1995), without affecting their spontaneous behaviour in familiar settings.

1.2.4. Dopamine and noradrenaline in the behavioural effects of amphetamine

The locus coeruleus noradrenergic system has been shown to regulate the activity of the ascending dopamine pathways. There is a connection of these monoamine systems at the level of the ventral tegmental area where noradrenaline locally either inhibits or excites the dopamine cell bodies (Grenhoff and Svensson, 1993; Grenhoff et al., 1995).

Lesioning the LC projections with DSP-4 decreases basal or stimulated dopamine release in the nucleus accumbens (Lategan et al., 1992 and Häidkind et al., 2002). However, in the case of a chronic noradrenaline deficiency, D_2 receptors upregulate in response to the reduced dopamine output and the animals become hyperreactive to amphetamine (Harro et al., 2000, Weinshenker et al., 2002, Haile et al., 2003, Harro et al., 2003). Behavioural studies investigating the effects of the LC lesions on amphetamine-induced changes in behaviour have however been inconclusive in determining the role of noradrenaline in psychostimulant effect of amphetamine (Ögren et al., 1983, Archer et al., 1986; Di Lullo and Martin-Iverson, 1991), possibly because of the contribution of the novelty in the experimental situation as large lesions of the LC projections increase neophobia (Harro et al., 1995).

The interaction between the LC noradrenergic and the mesotelencephalic dopaminergic systems also operates indirectly via the prefrontal cortex. Dopamine release in the prefrontal cortex is regulated by local noradrenergic nerve terminals (Gresch et al., 1995) and the electrical stimulation of the LC neurons increases both extracellular dopamine and noradrenaline in the prefrontal cortex (Devoto et al., 2005). Enhanced noradrenaline neurotransmission has been shown to contribute to the acute effects of amphetamine (Vanderschuren et al., 2003). Further, attenuation of the amphetamine-induced locomotion and dopamine release in the nucleus accumbens has been demonstrated after reduced noradrenergic neurotransmission in the prefrontal cortex (Darracq et al., 1998; Ventura et al., 2003). Activation of the prefrontal α_{1b}-adrenoceptors modulates the acute and sensitized effects of amphetamine, as an acute pharmacological blockade or a genetic knockout of these receptors results in an attenuation of the initial locomotor response to psychostimulants and psychostimulant sensitization (Darracq et al., 1998, Drouin et al., 2002; Weinshenker et al., 2002).

1.3. Individual differences in exploratory behaviour

Large and stable inter-individual differences physiologically exist in locomotor and exploratory behaviour, which can predict sensitivity to psychoactive drugs (see Kabbaj, 2006, for review). Animal models based on selective breeding or testing for these differences in novelty-related behaviour have often been used

in the research probing the neurobiological mechanisms underlying drug addiction and anxiety (Piazza et al., 1989; Landgraf and Wigger, 2002).

In humans, higher scores on the self-reported measures of sensation-seeking or novelty-seeking have been shown to be associated with the magnitude of the psychostimulant-induced dopamine release in the mesolimbic dopamine system and the propensity to use (and abuse) psychoactive drugs (Zuckerman, 1996; Leyton, 2007, for review). In a proposed animal model of novelty-seeking, rats are classified as high or low responders to novelty based on their locomotor response to an exposure to an inescapable unfamiliar apparatus (Piazza et al., 1989). Rats classified as high responders to novelty tend to be more sensitive to the stimulating and rewarding effects of acute amphetamine administration than low responders to novelty, but this seems to be contingent on the used selection procedures, the doses of amphetamine and the methods for evaluation of the drug effect (Piazza et al., 1989, Hooks et al., 1992, Exner and Clark, 1993, Gingras and Cools, 1997, Klebaur and Bardo, 1999; Klebaur et al., 2001; Dietz et al., 2005; Cain et al., 2008). For example, when an inescapable exploration test is used to assess the animals' reactivity to a novel environment, the high responders to novelty acquire amphetamine self-administration more readily (Piazza et al., 1989) and display higher locomotion to systemic amphetamine administration (0.5–1.0 mg/kg, i.p.) (Hooks et al., 1992). However, Erb and Parker (1994) did not find any correlation between activity in a novel chamber and amphetamine-induced place preference. In contrast, activity in a free-choice novelty test (playground maze) predicted greater amphetamine-conditioned place preference, but not higher locomotion to amphetamine or amphetamine self-administration in the high responders (Klebaur and Bardo, 1999; Klebaur et al., 2001; Cain et al., 2008).

The behavioural sensitization to repeated administration of amphetamine also differs in high versus low responders to novelty. The results of the studies investigating this issue are contrasting: Hooks et al. (1992) reported greater sensitization to amphetamine in high responders, whereas Piazza et al. (1989) demonstrated behavioural sensitization to amphetamine only in the low responders to novelty. These differences are possibly explained by the differential sensitivity of high and low responding animals to the contextual stimuli during drug administration, as the high responders developed behavioural sensitization to amphetamine only when the drug administration was paired with particular environmental cues and conversely, unconditioned sensitization occurred only in the low responders (Jodogne et al., 1994).

In addition to differences in the sensitivity to the rewarding and psychomotor effects of psychostimulants, high and low responders to novelty display distinct anxiety-like behaviours in tests related to affective disorders. Thus, as compared to high responders to novelty, low responders are more anxious in the elevated plus-maze and the light-dark box tests and exhibit a more passive coping style in the forced swimming test (Kabbaj et al., 2000). Similarly, rats selectively bred for their higher anxiety-related behaviour in the elevated plus-maze are

less active in the open-field test and use passive coping strategies on the first but not the second day of the forced swimming test (Liebsch et al., 1998). Thus, the differences in interacting with and reacting to novel environments in rats are actually predictive of activity levels across various testing conditions.

Differences in the activity of the dopaminergic system have been most commonly associated with the individual differences in the novelty-related traits. For example, high responders to novelty have higher *ex vivo* dopamine content in the striatum and the nucleus accumbens (Piazza et al., 1991; Thiel et al., 1999; Antoniou et al., 2008), higher *in vivo* basal and evoked dopamine release in the nucleus accumbens (Hooks et al., 1992), and increased firing rate of dopaminergic neurons in the VTA and the substantia nigra (Marinelli and White, 2000). Interestingly, the expression of D_2 receptor mRNA in the nucleus accumbens and the striatum has been shown to be lower in the rats with high locomotor response to novelty (Hooks et al., 1994).

As well as the differences in the dopaminergic neurotransmission, serotonin and noradrenaline have been implicated in determining the individual response to novelty. The locomotor response to novelty has been demonstrated to be negatively correlated to the measures of serotonergic activity in the prefrontal cortex (Piazza et al., 1989; Thiel et al., 1999; Antoniou et al., 2008), and highly correlated to the magnitude of the hippocampal noradrenaline release in stressful conditions (Rosario and Abercrombie, 1999).

In this dissertation, the exploration box test has been used to measure persistent baseline novelty-related behaviour in male Wistar rats (Otter et al., 1997). This emergence-type test typically yields a rather bimodal separation of rats into groups of high motivation to explore/low neophobia and low motivation to explore/high neophobia (HE and LE, respectively), with relatively few animals occupying the middle ground. The differences in the dopaminergic measures between the high and low explorers have been studied in the papers described in this dissertation. The HE- and LE-rats also differ in the properties of the serotonergic system in the prefrontal cortex and hippocampus (Mällo et al., 2008) that may underlie their anxiety-related behaviours. Specifically, although serotonin release in the prefrontal cortex and dentate gyrus in baseline conditions is similar in high and low exploring rats, the number of serotonin transporter binding sites is higher in the low explorers. Thus, after blocking the serotonin transporters in the prefrontal cortex by a local infusion of citalopram, the serotonin release in that brain region is significantly increased in the LE group (Mällo et al., 2008). Furthermore, the two groups have distinct cerebral metabolic activity in areas that are involved in defensive behaviours and cognitive processing of sensory stimuli (Matrov et al., 2007).

2. AIMS OF THE PRESENT STUDIES

The current dissertation aims at:

- studying the dopaminergic regulation of rat exploratory behaviour with the focus on the cholecystokinin-dopamine interactions in the behavioural effects of amphetamine (**Paper I**);
- investigating the existence and stability of individual differences in spontaneous exploratory activity in the exploration box, and characterizing the behaviour of the high and low exploring rats in other behavioural tests of anxiety and depression (**Paper II**);
- exploring the differences in the extracellular dopamine levels and the proportion of D_2 receptors in the functional high affinity state in the striatum of high and low explorers (**Papers II and III**);
- examining the effects of a partial denervation of the noradrenergic terminals originating from the *locus coeruleus* on the exploratory behaviour, amphetamine-stimulated activity and behavioural sensitization, and striatal D_2 receptor function in high and low explorers (**Papers IV and V**);
- analysing the genetic differences that might underlie the distinct behavioural profiles of the rats with persistently high or low exploratory activity using genome-wide microarrays to measure gene expression in these animals (**Paper VI**).

3. MATERIALS AND METHODS

3.1. Animals (Papers I–VI)

Male Wistar (**Papers I–VI**) or Sprague-Dawley (**Paper II**) rats from National Laboratory Animal Center, Kuopio, Finland (**Papers I and IV**) or Scanbur BK AB, Sweden (**Papers II, III, V and VI**), were used. The animals were housed in standard transparent polypropylene cages with free access to tap water and food pellets (diet R70, Lactamin, Sweden). The room temperature in the animal house was maintained at 21±2°C with a 12h:12h light-dark cycle (lights on at 08:00 h). Animals were housed in groups of three (Experiment 1, **Paper II**) or four (**Papers I–VI**) in the beginning of studies or were single-housed (Experiment 1, **Paper II**) 1–4 weeks after exploration box tests. All experiments were carried out 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.

3.2. Drugs (Papers I–V)

CCK_1 receptor antagonist devazepide and CCK_2 receptor antagonist L-365,260 (ML Laboratories/Panos Therapeutics, UK), administered in the dose of 10 μg/kg (**Paper I**), were suspended in a few drops of Tween-85®, diluted in distilled water, and were injected 30 min prior to behavioural testing. D-amphetamine sulphate, administered in a dose of 0.5 mg/kg (**Papers I–V**), was dissolved in distilled water and given as an intraperitoneal injection 15 min prior to behavioural testing. DSP-4 (N-(2-chloroethyl)-N-ethyl-2-bromobenzylamine; AstraZeneca, Södertälje, Sweden) (**Papers IV and V**) was administered in the dose of 10 mg/kg (expressed as for hydrochloride). Each dose was weighed separately, dissolved in distilled water and injected immediately. All drugs were administered intraperitoneally in a volume of 1 ml/kg. Control animals received a vehicle injection.

3.3. Behavioural methods

3.3.1. Exploration box test and classification of animals as high or low explorers (Papers I–VI)

Exploration box test

The exploration box test (**Papers I–VI**) was conducted as described previously (Harro et al., 1995; Otter et al., 1997). The exploration box was made of metal

and consisted of a 0.5 × 1 m open area with 40 cm side walls, and a 20 × 20 × 20 cm small compartment attached to one of the shorter sides of the open area. The open area was divided into eight equal squares and four objects, three novel and one familiar (a glass jar, a cardboard box, a wooden handle and a food pellet), were placed onto certain squares (which remained the same throughout the experiments). The floor of the small compartment was covered with wood shavings. The small compartment was directly linked to the open area through an opening. The exploration test began with placing the animal into the small compartment, which was covered with a lid for the time of test. The following behavioural parameters were registered: 1) latency (of entering the open area with all four paws), 2) the number of entries into the open area, 3) time spent exploring on the open area, 4) line crossings, 5) rearings and 6) the number of unfamiliar object investigations. For the measure of the sum of exploratory events, the scores of line crossings, rearings and object investigations were summed for each animal. A single test session lasted for 15 minutes and experiments were carried out under dim light conditions (3–7 lx in the open area).

In the amphetamine experiments (**Papers I, IV and V**) the same apparatus was used, but the passage between the open area compartment and the small compartment was closed; the animal was placed on the open area, and for 15 minutes line crossings, rearings and the number of object investigations were registered. The sum of these measures was also calculated.

Classification of animals as high or low explorers

The animals' behaviour in the exploration box can best be predicted by their behaviour on the second exposure to the exploration box (**Paper II**). Thus, on days 1 and 2, the rats were tested for their spontaneous exploratory activity in the exploration box. The distribution of rats' spontaneous exploration is rather bimodal – with most of the animals having very low or very high novelty-related activity, and only a few occupying the middle ground. For the behavioural experiments, the animals were classified as high or low explorers (HE or LE, respectively) based on the median split of the sum of exploratory events on the second exposure to the exploration box (**Papers II–V**). For the gene expression profiling, described in **Paper V**, to maximize the characteristic between-group differences and minimize the within-group variance due to the small number of microarrays used and pooling of the RNA samples, the twelve HE and twelve LE animals were selected from a larger preselected population. The LE group consisted of animals that never emerged from the small compartment while the HE animals were selected from the animals belonging to the upper quartile based on the sum of exploratory events on the second day of testing.

3.3.2. Other behavioural tests (Paper II)

Social interaction test
The test developed by File and Hyde (1978) was used as previously described (Tõnissaar et al., 2004). In this test, a pair of previously single-housed, weight-matched rats was placed into a novel, brightly lit chamber (30 cm × 30 cm × 60 cm) with floor covered with wood shavings. The total time spent in active social behaviour (allogrooming, sniffing the partner, crawling under and over, following) was recorded for 10 min. The mean social interaction time was calculated for each rat. In each test, the partners were new to each other. Animals were single-housed for 10 days prior to the first social interaction test, followed by two more social interaction tests with 10-day intervals.

Sucrose preference test
In Experiment 1, sucrose intake was measured in home cages of single-housed animals. In Experiments 2 and 3, the animals were placed into single cages immediately before the sucrose preference testing. Sucrose preference test was carried out with two bottles, one filled with 1% sucrose solution and the other with water. Sucrose and water consumption was measured for the period of 1 h by weighing pre-weighed bottles at the end of the test. Sucrose preference was measured by calculating the proportion of sucrose consumption out of total consumption of liquid. The test was carried out on two consecutive days. In Experiment 3, an 18-h food and water deprivation was assigned after a 24-h resting period, and a third sucrose preference test was carried out immediately after that.

Forced swimming test
The forced swimming test, first described by Porsolt et al. (1978) was carried out as described previously (Häidkind et al., 2004). Briefly, rats were placed into a vertical glass cylinder (diameter 22.5 cm and height 60 cm) containing about 35 cm water at 25°C. On the first day of experiment, the procedure lasted 15 min and the re-exposure 24 h later lasted 5 min. At the end of each session the rats were dried with laboratory tissues. The sessions were recorded with a video camera and the duration of immobility, swimming and struggling were measured by two independent experimentators. The measurements were based on the behavioural categories described by Armario et al. (1988). A rat was judged to be immobile when it remained floating in the water with all limbs motionless. The rat was judged to struggle whenever it made intense movements with all four limbs, with two front paws breaking the surface of water or touching the walls of the tank. The time spent in swimming was recorded when the rat was making active swimming motions, more than necessary to merely maintain its head above the water, e.g. moving around in the cylinder. The

average results of the two experimenters were used in the calculations. The inter-rater reliability between observers was high ($r = 0.88$).

Elevated plus-maze test

The method first described by Handley and Mithani (1984) and modified in our laboratory (Harro et al., 1990) was used. In brief, the plus-maze consisted of two open arms (50 cm × 10 cm) without any walls, two enclosed arms of the same size with 40 cm high side walls and end wall, and the central arena (10 cm × 10 cm) interconnecting the arms. The arms of the same type were opposite to each other. Both open arms were divided into three parts of equal size by lines which also separated the central arena from all arms. At the beginning of the experiment the rat was placed into a closed arm. The central arena and the open arms formed the 'open part' of the apparatus. An entry into the open arms was counted when the rat crossed the line between the central arena and an open arm with all four paws. The rat was considered to explore the open part of the apparatus when it had clearly crossed the line between an open arm and the central arena with its both forepaws. Behavioural measures taken during 4 min included (a) the latency entering the open part; (b) the number of line crossings; (c) time spent in the open part of the apparatus; (d) the number of approaches towards the central arena which were not completed (nose crossed the line but not both of the forepaws); (e) the number of open arm entries; (f) the total number of arm entries. From the two latter measures, the ratio open/total arm entries was calculated.

Fear conditioning test

Fear conditioning was studied in a standard shuttle box following the protocol described by Wallace and Rosen (2001). In short, rats were placed in the chamber for 3 min before a 1.5 mA, 1 s foot shock. Freezing, defined as a characteristic crouch position, was measured for 4 min immediately after the foot shock. Freezing was measured as a sample of freezing or not freezing every 10 s, for a total of 25 observations. A retention test of fear conditioning was conducted 24 h after the foot shock by placing the animals into the same chamber and recording freezing for 4 min as described above.

Activity in the novel cage

The cage used in the experiments was identical to the home cages of the animals, except for the absence of cage mates, food pellets and water. The new cage was located in the animal room on the same shelf as the animal's home cage. The floor was covered with wood shavings and the cage was covered with a standard metal grid cover. The animals was placed in the centre of the cage and observed for 10 min. In Experiment 4 (**Paper II**), the cage floor was divided into four squares and the following measures were counted: (a) transitions from one square to another, (b) rearings, (c) digging in wood shavings, (d) grooming, and (e) time of exploration. In Experiment 5 (**Paper II**), a novel object (a plastic

tube of 1 cm in diameter and 7 cm in length) was placed in one corner of the cage and the episodes and time of exploration of the object were measured. Every animal was tested in a separate new cage.

3.4. Biochemical methods

3.4.1. In vivo microdialysis (Papers II and III)

The animals were anaesthetized with chloral hydrate (350 mg/kg, i.p.) and mounted in a Kopf stereotactic frame. A self-made concentric Y-shaped microdialysis probe with 7 mm shaft length and 3 mm active tip was implanted into the left dorsal striatum according to the following coordinates: AP +0.7; ML +3.0; DV –7.0; and a probe with 8.0 mm shaft length and 2 mm active tip into nucleus accumbens according to the following coordinates: AP +1.3; ML +1.7; DV –8.0, according to Paxinos and Watson (1986). The dialysis membrane used was polyacrylonitrile/sodium methalyl sulphonate copolymer (Filtral 12; i.d.: 0.22 mm; o.d.: 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, the rats were placed in 21 cm × 36 cm × 18 cm individual cages in which they remained throughout the experiment. Rats were given about 24 h for recovery and microdialysis procedure was conducted in awake freely moving animals. The microdialysis probe was connected to a syringe pump (SP101, World Precision Instruments, Inc., Sarasota, FL, USA) and microfraction collector (CMA/142, CMA Microdialysis AB, Solna, Sweden) and 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; pH 7.20–7.22) at a constant rate of 1.5 µl/min. Connections to the infusion pump and microfraction collector were made with flexible FEP tubing (i.d. 0.12 mm, AgnTho's AB, Lidingö, Sweden). After connecting the animal to the microdialysis system, the perfusate was discarded during the first 60 min to allow stabilization. Then six baseline samples were collected, followed by administration of D-amphetamine (0.5 mg/kg, i.p.) in the beginning of the seventh sample, after which another nine samples were collected. The samples were collected with 15-min intervals into vials prefilled with 7.5 µl of 0.02 M acetic acid. Upon completion of the experiment the animals were deeply anaesthetized with chloral hydrate (350 mg/kg, i.p.) and decapitated; the brains were removed, immediately frozen in ice-cold acetone and kept at –80°C. The brains were sectioned on a cryostatic microtome (Microm GmbH, Walldorf, Germany) and probe placements were determined according to the atlas of Paxinos and Watson (1986). Only animals with the correct probe placements were included in the statistical analysis. The quantity of dopamine in the microdialysis samples was determined by high performance liquid chromatography with electrochemical detection. The chromatography system consisted of Hewlett Packard series 110 pump and autosampler, a

Hypersil BDS C-18 column (250 mm × 2 mm, 5 μm), an ESA 5011 analytical cell (working electrode potential +250 mV) and an ESA Coulochem II controller unit. The column temperature was 30°C. The mobile phase composition was 0.05 M sodium citrate buffer, pH 5.3, 0.02 mM EDTA, 3.1 mM sodium octylsuphonate, 9.5% acetonitrile.

3.4.2. Monoamine content measurements (Papers IV and V)

Monoamines and their metabolites were assayed by high performance liquid chromatography with electrochemical detection. The rat brain tissues were homogenized with Bandelin Sonopuls ultrasonic homogenizer (Bandelin Electronic, Berlin, Germany) in ice cold solution of 0.1 M perchloric acid (10–30 μl/mg) containing 5 mM sodium bisulfite and 0.4 mM EDTA to avoid oxidation. The homogenate was then centrifuged at 17,000 x *g* for 10 min at 4°C. Aliquots (10 μl) of the obtained supernatant were chromatographed on a Lichrospher 60 RP Select B column (250 mm × 3 mm; 5 μm). The separation was done in isocratic elution mode at column temperature of 30°C using the mobile phase containing 0.05 M sodium citrate buffer at pH 3.7; 0.02 mM EDTA; 1 mM KCl; 1 mM sodium octylsuphonate and 5.6% acetonitrile. The chromatography system consisted of a Hewlett Packard HP 1100 series isocratic pump, a thermostatted autosampler, a thermostatted column compartment and an HP 1049 electrochemical detector (Agilent, Waldbronn, Germany) with glassy carbon electrode. The measurements were done at an electrode potential of +0.6 V versus the Ag/AgCl reference electrode.

3.4.3. D_2 receptor-stimulated [^{35}S]GTPγS binding (Papers IV and V)

Membranes from the striatum and the nucleus accumbens were prepared as described previously (Lepiku et al., 1996). The tissues were homogenized in 100 vol (ww/v) of homogenization buffer (50 mM Tris-HCl, pH = 7.4) by Bandelin Sonopuls ultrasonic homogenizer (Bandelin Electronic, Berlin, Germany) (two passes, 10 s each). The membranes were collected by centrifugation at 40,000x*g* for 20 min at 4°C and were washed by homogenization and centrifugation two more times. The final pellet of striatal membranes were homogenized in 90 vol (ww/v) and accumbal membranes in 450 vol (ww/v) of the incubation buffer (20 mM K-HEPES, 7 mM $MgCl_2$, 100 mM NaCl, 1 mM EDTA, 1 mM DTT, pH = 7.4).

Binding of [^{35}S]-guanosine-5'-(γ-thio)-triphosphate ([^{35}S]GTPγS; Perkin Elmer Life Sciences, Boston, MA, USA) was carried out as described earlier (Rinken et al., 1999). In brief, the membranes (500 μg of striatal or 150 μg of nucleus accumbens membranes per tube) were incubated with 0.2 nM

[^{35}S]GTPγS and different concentrations of GDP (3 mM – 1 μM) and 1 mM dopamine or 1 mM butaclamol (all from Sigma-Aldrich Fine Chemicals, St. Louis, MO, USA) for 90 min at 30°C. The reaction was stopped by rapid filtration through GF/B glass-fiber filters (Whatman Int. Ltd., Madistone, UK) and the filters were washed three times with 5 ml of ice cold 20 mM phosphate buffer (pH = 7.4) containing 100 mM NaCl. The radioactivity content of the filters was counted in 5 ml of scintillation cocktail OptiPhase HiSafe®3 (Wallac Perkin Elmer Life Sciences, Cambridge, UK) by RackBeta 1219 liquid scintillation counter (Wallac Inc., Gaithersburg, MD, USA).

3.4.4. Measurement of the D_2^{High} receptors in the striatum (Paper III)

Eight HE and eight LE rats were sacrificed, their striata dissected and frozen at –70°C until used. The striata were homogenized in buffer (4 mg frozen tissue per ml buffer), using a teflon-glass homogenizer with the piston rotating at 500 rpm and 10 up and down strokes of the glass container. The buffer contained 50 mM Tris-HC1 (pH 7.4 at 20°), 1 mM EDTA, 5 mM KCl, 1.5 mM CaC12, 4 mM MgC12 and 120 mM NaCl.

[^{3}H]Domperidone was custom synthesized as [phenyl-^{3}H(N)]domperidone (42–68 Ci/mmol) by PerkinElmer Life Sciences Inc., Boston, MA, and used at a final concentration of 2 nM. Because the Kd of [^{3}H]domperidone is 0.47 nM (Seeman et al., 2003) at D_2 for rat striatum, the final concentration of 2 nM occupied 81% of the D_2 receptors, using the equation $f = C/(C + Kd)$, where f is the fraction of receptors occupied by [^{3}H]domperidone, and C is 2 nM.

The competition between dopamine and [^{3}H]domperidone for binding at the receptors was done as follows. Each incubation tube (12 × 75 mm, glass) received, in the following order, 0.5 ml buffer (containing dopamine at various concentrations, and with or without a final concentration of 10 μM S-sulpiride to define nonspecific binding to the dopamine D2 receptors), 0.25 ml [^{3}H]domperidone and 0.25 ml of tissue homogenate. The tubes, containing a total volume of 1 ml, were incubated for 2 h at room temperature (20°), after which the incubates were filtered, using a 12-well cell harvester (Titertek, Skatron, Lier, Norway) and buffer-presoaked glass fiber filter mats (Whatman GF/C). After filtering the incubate, the filter mat was rinsed with buffer for 15 s (7.5 ml buffer). The filters were pushed out and placed in scintillation polystyrene minivials (7 ml, 16 × 54 mm; Valley Container Inc., Bridgeport, Conn.). The minivials received 4 ml each of scintillant (Research Products International Corp., Mount Prospect, IL), and were monitored 6 h later for tritium in a Beckman LS5000TA scintillation spectrometer at 52% efficiency. The specific binding of [^{3}H]domperidone was defined as total binding minus that in the presence of 10 μM S-sulpiride.

The proportion of D_2^{High} receptors in the striata was measured by the competition of dopamine with [^{3}H]domperidone, as previously described (Seeman et al., 2003). Dopamine inhibited the binding of [^{3}H]domperidone in two phases. At low concentrations, corresponding to the high-affinity state of the dopamine D_2 receptor, D_2^{High}, dopamine inhibited the binding of [^{3}H]domperidone between 1 nM and 100 nM dopamine. A second phase of inhibition occurred above 100 nM dopamine. The demarcation between the two phases was sharp and unambiguous, clearly and readily permitting the measurement of the D_2^{High} component as a percent of the total amount of specific [^{3}H]domperidone binding, as defined by the presence of 10 μM S-sulpiride.

3.4.5. RNA preparation and gene expression profiling (Paper VI)

The frozen brain tissue samples were homogenized in 2 ml tubes containing 350 μl of lysis buffer RA1 (Macherey-Nagel GmbH & Co. KG, Germany) containing 3.5 μl β-mercaptoethanol. Isolation of total RNA from the lysate, including the 15 min DNase I treatment, was carried out using the Macherey-Nagel NucleoSpin RNA II kit (Macherey-Nagel GmbH & Co. KG, Germany) according to the manufacturer's protocol. The eluates were stored at –80°C.

RNA quality control and microarray hybridizations and processing were outsourced to ServiceXS (Leiden, The Netherlands). The quality of RNA in individual and pooled samples was checked with Agilent Bioanalyzer. For each microarray, three total RNA samples were pooled and for both groups (HE and LE) four hybridizations were performed per brain area. Thus, 24 Illumina RatRef-12 BeadChips (Illumina Inc., USA) were used in total.

3.5. Statistical methods

3.5.1. Bioinformatics (Paper VI)

3.5.1.1. Normalization of microarray data

The raw microarray data was processed using the beadarray (Dunning et al., 2007), preprocessCore (Bolstad et al., 2003) and puma (Rattray et al., 2006; PUMA) R packages of Bioconductor. In the beadarray package the backgroundCorrect method used was "minimum", whilst variables for the createbeadsummaryData method were as follows: log = TRUE, n=10. Data was normalised using the quantile normalisation algorithm from the preprocessCore package. Three functions from the puma package were used: pumaComb, pumaDE and write.reslts all with default settings.

Two filtering criteria were used to define statistically significant differentially expressed genes. Firstly, the expression fold change must be greater than (+ or –) 0.5. Secondly, the minimum pplr (Minpplr), which is simply the minimum of either the pplr statistic produced by puma or 1-pplr, was less than 0.001.

3.5.1.2. KEGG and GO analysis using DAVID 2007 Functional Annotation Tool

To assist in the functional interpretation of the microarray data, we used the online DAVID 2007 Functional Annotation Tool (http://david.abcc.ncifcrf.gov/home.jsp) to search the databases of Kyoto Encyclopedia of Genes and Genomes (KEGG) (http://www.genome.ad.jp/kegg/) and Gene Ontology (GO) (http://www.geneontology.org/). A major component of the KEGG database is the pathway module which consists of regularly updated manually curated graphical diagrams that represent the current knowledge of the molecular interactions involved in various cellular processes (Ogata et al., 1999). The GO database is helpful in assigning the differentially expressed genes to functional categories based on the three ontologies: biological process, molecular function and cellular component.

The gene identifiers (GID accessions) associated with each microarray feature were retrieved from the Illumina array datafiles and used in DAVID searches. All gene GID's on the Illumina array were used as the 'background' gene list in DAVID, whilst only the GID's for the statistically significant differentially expressed genes (filtered using the criteria above) were used as the 'gene list' in DAVID.

The depth of the GO Biological Process enrichment analysis was set at Level 3. To identify statistically significantly over-represented GO terms and KEGG pathways within the lists of differentially expressed genes, the group enrichment score (modified Fisher Exact p-value) (Huang et al., 2007) cutoff was set at 0.05. The results were ordered by the group enrichment score.

Additionally, the lists of differentially expressed genes were manually browsed to find the genes that encode neurotransmitter synthesizing enzymes, neurotransmitter receptor or receptor subunits.

3.5.2. Data analysis

The differences between the activity of the HE-and LE-rats in the selection experiment in **Paper IV** were analyzed using Mann-Whitney U test. All other behavioural data were analyzed using one-way (**Papers II and III**), two-way (**Papers I, II and IV**) or three-way (**Paper IV**) analysis of variance (ANOVA). Where appropriate, a repeated measures factor was added. Post hoc com-

parisons were done by Fisher's PLSD test. In correlative analyses, Pearson correlation coefficients were used (**Paper II**). All statistics were carried out using StatView 5.0 software (SAS Institute Inc., Cary, NC, USA). All binding data were analyzed by computer modelling, fitting it to appropriate formulas using non-linear least-squares regression, using GraphPad PRISM (GraphPad Software, San Diego, CA, USA) (**Papers IV and V**).

4. RESULTS AND DISCUSSION

4.1. Role of CCK in amphetamine-stimulated exploratory behaviour and sensitization to amphetamine

4.1.1. Effect of CCK receptor blockade on amphetamine-stimulated locomotor activity (Paper I)

Interactions between dopaminergic neurotransmission and CCK in the central nervous system may be important in the pathogenesis of psychotic disorders and substance abuse. In this study, it was demonstrated that while amphetamine stimulated exploratory behaviour in the exploration box during repeated testing, the CCK_1 receptor antagonist devazepide blocked this effect of amphetamine (Figure 1), but the CCK_2 receptor antagonist L-365,260 potentiated it (Figure 2). CCK receptor antagonists did not modulate exploratory behaviour when administered alone.

This is consistent with previous studies that have shown the involvement of both CCK receptor subtypes in the expression of behavioural responses to amphetamine and the inability of the CCK receptor antagonists alone to modify behaviour (Josselyn and Vaccarino, 1995 and Vasar et al., 1991). Consistently, Otsuka Long Evans Tokushima Fatty (OLETF) rats of the naturally-occurring CCK_1 receptor-deficient rat line have been demonstrated to be less sensitive to locomotor activity enhancing effects of amphetamine (Feifel et al., 2001) and they also exhibit less behavioural sensitization to cocaine (Beinfeld et al., 2001). CCK_2 receptor-deficient mice had an increased locomotor activity in response to amphetamine (Dauge et al., 2001 and Kõks et al., 2001) and also a pronounced behavioural sensitization to morphine (Dauge et al., 2001) compared to wild type mice.

Although amphetamine administration whether with or without L–365,260 coadministration stimulated animals' behaviour on all days of the 5-day experiment compared to controls, there was a significant difference in activity levels of amphetamine group and amphetamine/L–365,260 group animals on days 2–4 of the experiment. This time-dependency of L–365,260 effect on amphetamine's action in this study suggests that at least using the exploration test containing a free-choice element (animals can hide into the small chamber), single administration of the CCK_2 antagonist is not sufficient for the expression of the additive effect of amphetamine and CCK_2 receptor blockade. All groups exhibited the lowest levels of exploratory activity on the first day of the 5-day test. This appears to be related to the anxiogenic effect of the initial exposure to the testing environment in the exploration box (Harro et al., 1995). The present study suggests that CCK_2 receptor blockade induced potentiation of amphetamine-induced stimulation depends upon the level of anxiety.

Together these experiments demonstrate that the effects of amphetamine on exploratory behaviour are bidirectionally modulated by endogenous CCK, dependent upon receptor subtype. It has been suggested that the involvement of CCK in exploratory behaviour depends upon the time the animal has been able to explore the environment (Blacker et al., 1997).

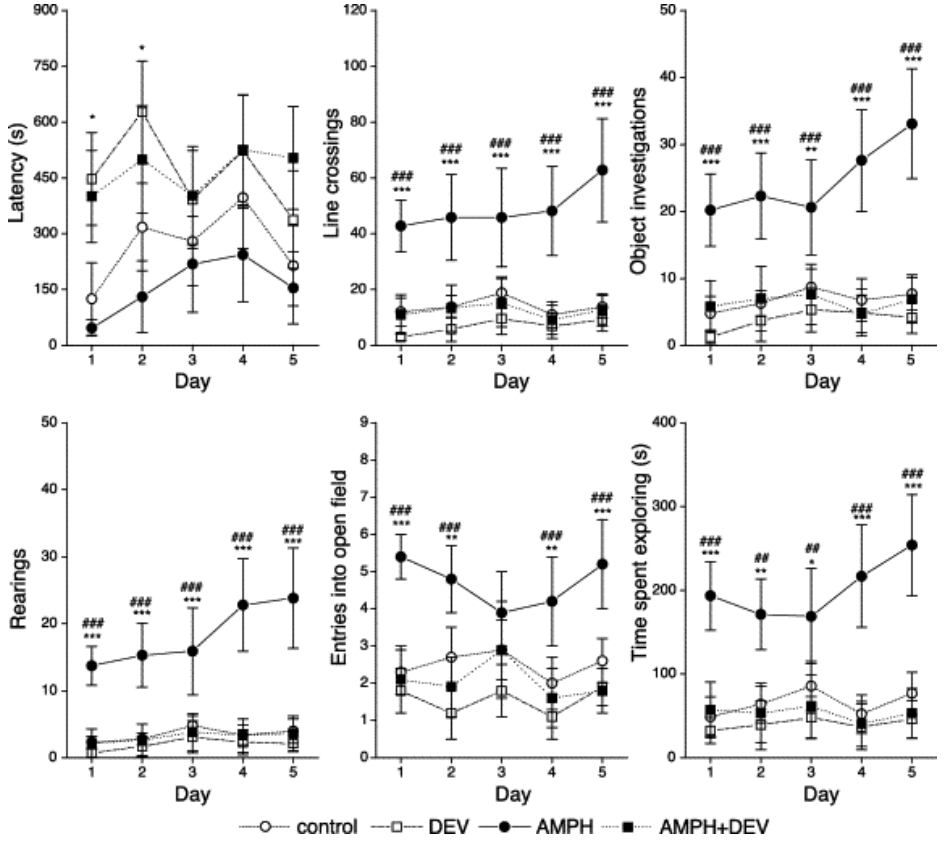

Figure 1. The effects of amphetamine (0.5 mg/kg, AMPH) and CCK_1 receptor antagonist devazepide (10 μg/kg, DEV) on rat exploratory behavior in the exploration box. *, $P<0.05$; **, $P<0.01$; ***, $P<0.001$ vs. control; #, $P<0.05$; ##, $P<0.01$; ###, $P<0.001$ vs. amphetamine + devazepide (AMPH + DEV).

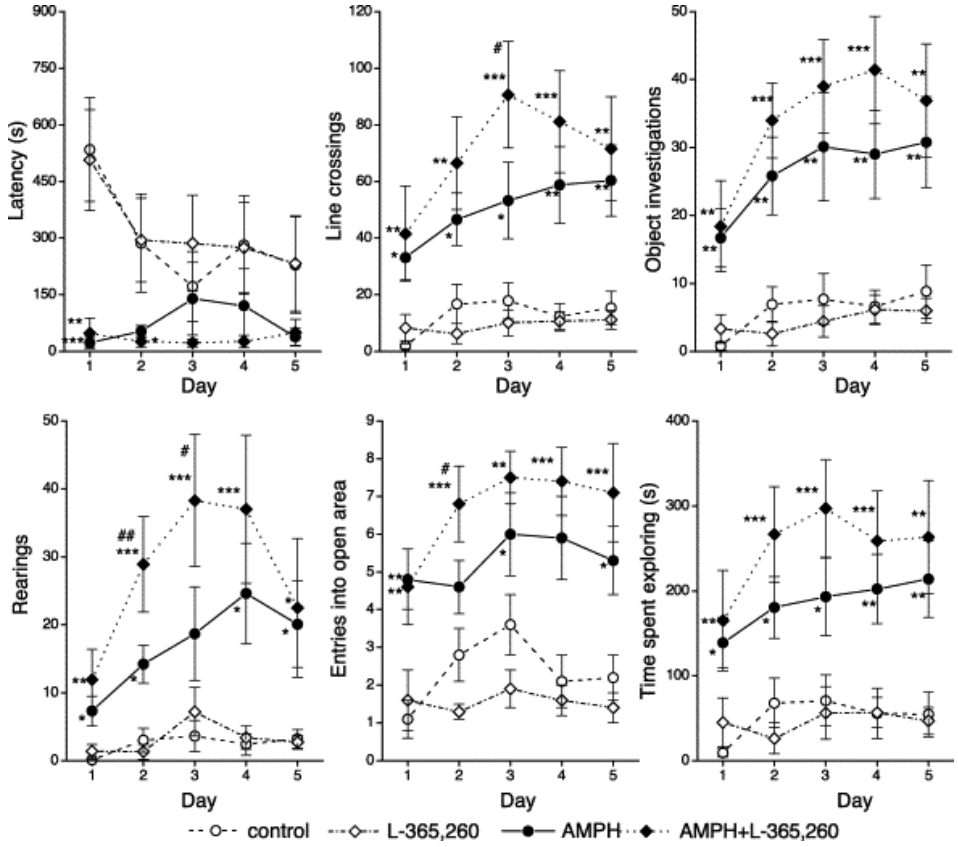

Figure 2. The effects of amphetamine (0.5 mg/kg, AMPH) and CCK_2 receptor antagonist L-365,260 (10 µg/kg) on rat exploratory behavior in the exploration box. *, $P<0.05$; **, $P<0.01$; ***, $P<0.001$ vs. control; #, $P<0.05$; ##, $P<0.01$ vs. amphetamine+L-365,260.

4.1.2. Effect of CCK receptor blockade on behavioural sensitization to amphetamine (Paper I)

CCK receptor blockade during repeated amphetamine administration altered the behavioural response to amphetamine administered after a withdrawal period. Devazepide pretreatment strongly tended to block (Figure 3) while L-365,260 pretreatment potentiated the development of behavioural sensitization (Figure 4).

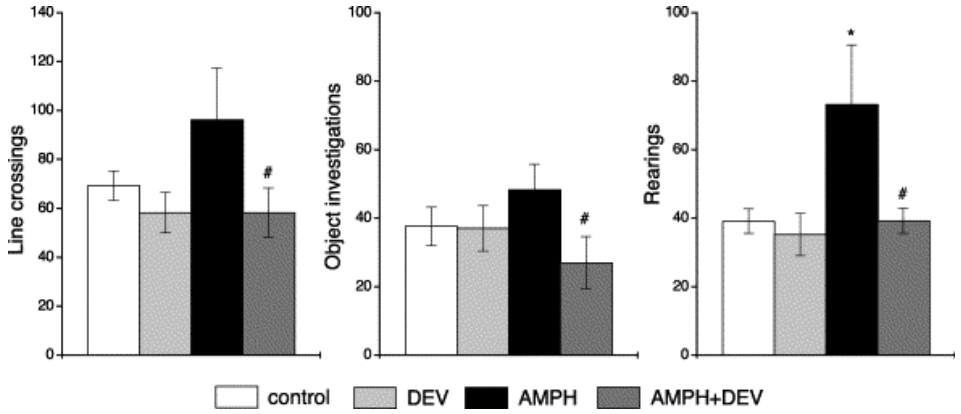

Figure 3. The effect of amphetamine challenge (0.5 mg/kg) on locomotor activity of rats pretreated with amphetamine (AMPH) and/or devazepide (DEV). *, $P<0.05$ vs. control; #, $P< 0.05$ vs. AMPH. Data on sum of the events are not shown.

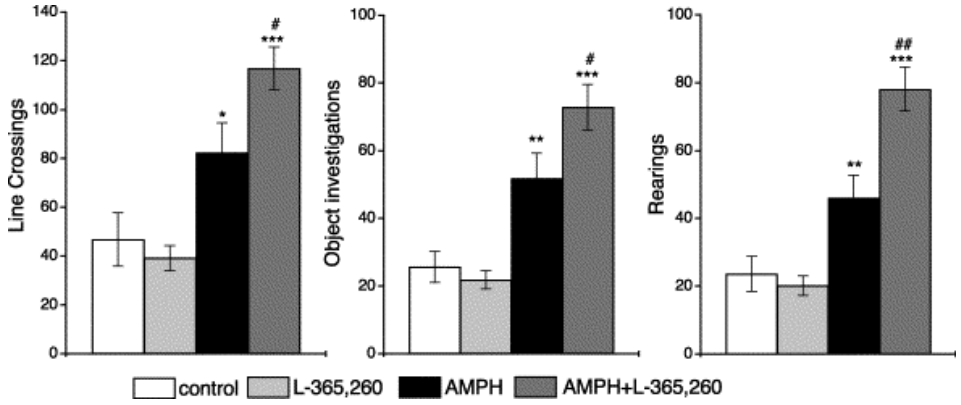

Figure 4. The effect of amphetamine challenge (0.5 mg/kg) on locomotor activity of rats pretreated with amphetamine (AMPH) and/or L–365,260. *, $P<0.05$; **, $P<0.01$, ***, $P<0.001$ vs. control; #, $P<0.05$; ##, $P<0.01$ vs. AMPH. Data on sum of the events are not shown.

Our results are somewhat different compared to DeSousa et al. (1999) and Wunderlich et al. (2000), who have suggested that endogenous CCK via CCK_1 receptors attenuates the expression (not studied in our present investigation) but not the development of behavioural sensitization. Regarding CCK_2 receptor blockade, Wunderlich et al. (2000) found similarly to us that a low dose (1 μg/kg in their study) potentiated the development of amphetamine sensitization; however, higher doses (including 10 μg/kg which was effective in our study) did not. In their study, a higher dose of amphetamine (1.5 mg/kg) was

used in the pretreatment phase and perhaps more importantly, the drug treatment, as emphasized by the authors themselves, was carried out in the home cages – the amphetamine pretreatment environment was distinct from the subsequent testing environment. Thus, to the extent the present data differ from the previous report, this may be due to the pairing of all amphetamine and CCK antagonist treatments with the environment for testing exploratory behaviour.

Thus, the results of this study reveal a significant differential involvement of endogenous CCK acting at CCK_1 and CCK_2 receptors in the expression of amphetamine-induced changes in exploratory behaviour and the development of sensitization to amphetamine. This involvement appears to depend upon the contingency between CCK receptor blockade and the environment where this occurs.

4.2. Exploratory activity as a persistent trait: behavioural differences of rats with high and low exploratory activity

4.2.1. Persistent individual differences in exploratory activity in the exploration box test (Paper II)

In this study, the persistence of the trait of novelty-related behaviour as expressed in exploratory activity in the exploration box test in male Wistar and Sprague-Dawley rats was investigated. It was found in both strains used that during repeated testing in the exploration box, the animals develop an individual level of activity in the apparatus (Figure 5). Their activity on the first exposure to the exploration box test is not consistently correlated with their activity observed in the future tests. However, already the behaviour on the second testing in the apparatus is reasonably predictive of the animal's subsequent performance, with the accuracy increasing with each testing. Thus, to minimize time cost and due to the acceptable correlation between the consequent behaviour and the activity on the second exposure to the exploration box test, this measure has been further used for the division of animals into high or low explorers (HE or LE, respectively).

When animals were classified on this basis into HE- and LE-rats, the difference between groups was still observed in further experiments, including a second block of tests carried out 1 month later in Experiment 1 and even as much as 6 months later in Experiment 4. In the latter, the levels of activity decreased in HE group, which was probably due to age- and weight-related decrease in exploratory drive, while the already low levels of LE group show little if any change. However, the difference between LE- and HE-rats was much more apparent in Wistar group, the patterns of exploratory activity over

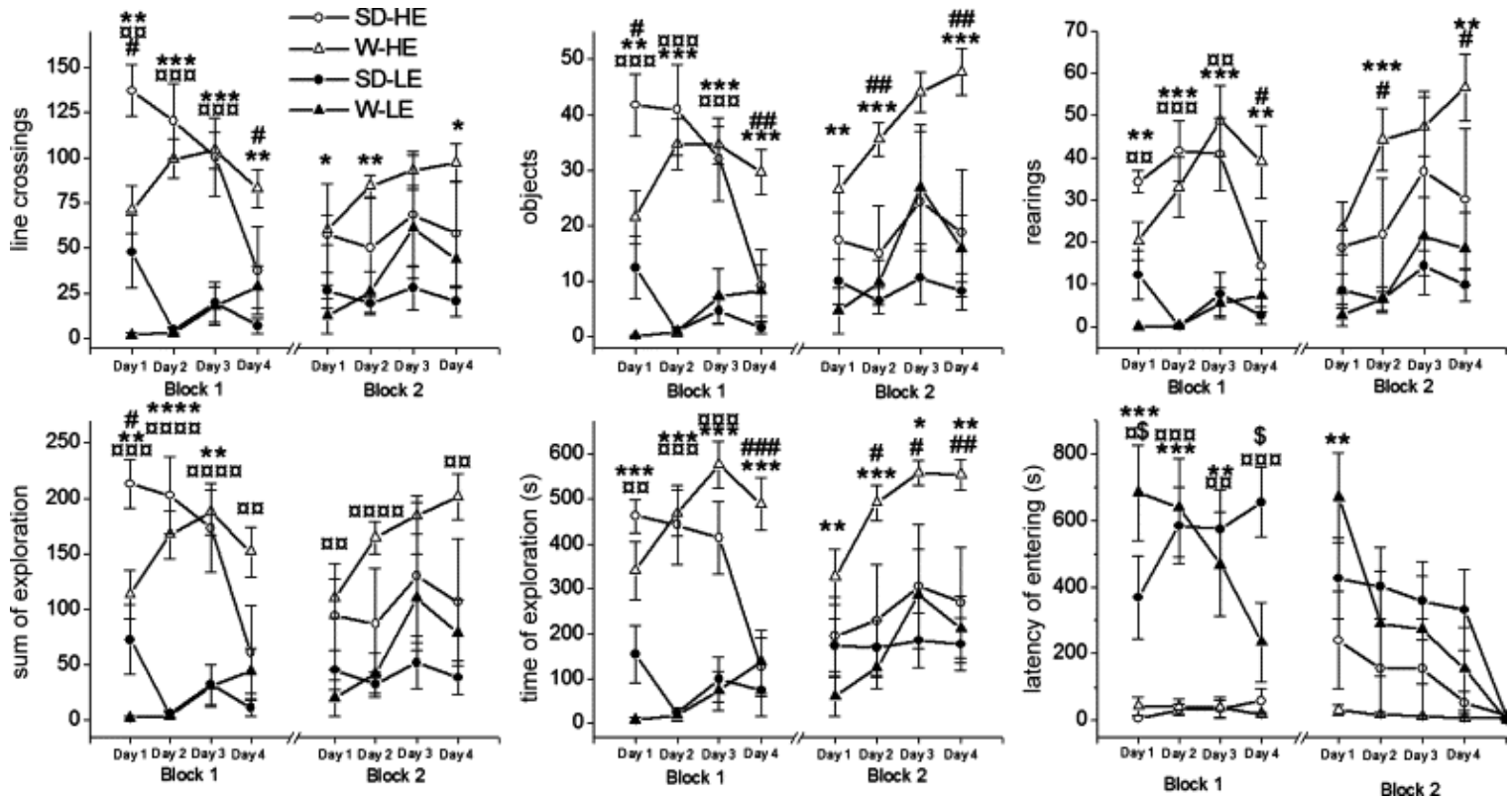

Figure 5. Exploratory behaviour during repeated testing in LE- and HE-rats of Wistar and Sprague–Dawley strains. n(W-LE) = 9; n(W-HE) = 9; n(SD-LE) = 9; n(SD-HE) = 9. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ LE vs. HE (Sprague–Dawley); $^{¤}p < 0.05$, $^{¤¤}p < 0.01$, $^{¤¤¤}p < 0.001$ LE vs. HE (Wistar); $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$ SD-HE vs. W-HE; $^{\$}p < 0.05$ SD-LE vs. W-LE. SD: Sprague–Dawley, W: Wistar, LE: low exploratory behaviour rats, HE: high exploratory behaviour rats. Data are presented as mean ± S.E.M.

repeated testing being quite different in the two strains in Experiment 1. In Wistar rats, the exploratory behaviour of the animals increased over the consecutive tests in the same block in both HE and LE animals during both test blocks, and the large difference between HE- and LE-rats was always maintained. In Sprague–Dawley rats, maximal activity was observed already during the first 15-min test in both HE and LE animals, with subsequent decline particularly in the HE-rats that eliminated the statistical significance between the groups by the fourth test. Thus, in an entirely novel environment, Wistar rats were more neophobic and possibly anxious as compared to Sprague–Dawley rats. Sprague–Dawley animals initially seem to exhibit very high levels of motivation to explore, which rapidly habituates with repeated testing. Therefore, assessing the behaviour in the exploration box using different rat strains enables to study different aspects of anxiety-related and motivated behaviours (like fear of novel environments and high or low motivation to explore novel areas).

4.2.2. Behavioural differences in high and low explorers in tests of anxiety and depression (Paper II)

The individual differences in exploratory activity between the HE- and LE-rats were also reflected in their behaviour in various other tests that have traditionally been used to measure different aspects of emotionality in studies on anxiolytic and antidepressant drugs.

There were no differences between HE and LE animals when tested for interaction with an unfamiliar object and activity in a novel cage similar to home cage. This suggests that the differences in the inherent baseline anxiety or motivation levels between HE and LE animals are specifically or preferentially revealed in the exploration box and possibly other more challenging conditions and not in environments that are perceived as more familiar and safe.

The elevated plus-maze is one of the most widely used test paradigms to investigate anxiety-related behaviour (Carobrez et al., 2005). The experiments described in **Paper II** show that LE-rats displayed higher anxiety levels in the elevated plus-maze behaviour (Figure 6). The decrease in the activity on the second trial (described by File, 1993), although occurring in both HE and LE animals, was more apparent in the LE-rats. Animals selectively bred for their high or low anxiety-related behaviour in the elevated plus-maze (HAB and LAB, respectively) also differ in other tests of anxiety; for example, the HAB rats were demonstrated to be less active in the black-white box and open field test (Liebsch et al., 1998). Also, when rats are preselected based on their locomotor reactivity in a forced exploration test, the rats with low levels of novelty-related locomotion (the low responders, LR) spend less time in the open arms and the middle square and more time in the closed arms of the elevated plus-maze as compared to the high responders (HR) (Kabbaj et al., 2000). Therefore, it is plausible that despite the differences between the less stressful free-choice (the

exploration box test and the elevated plus-maze) and the forced exploration paradigms (an inescapable open field), they also share a commonality in the measured construct.

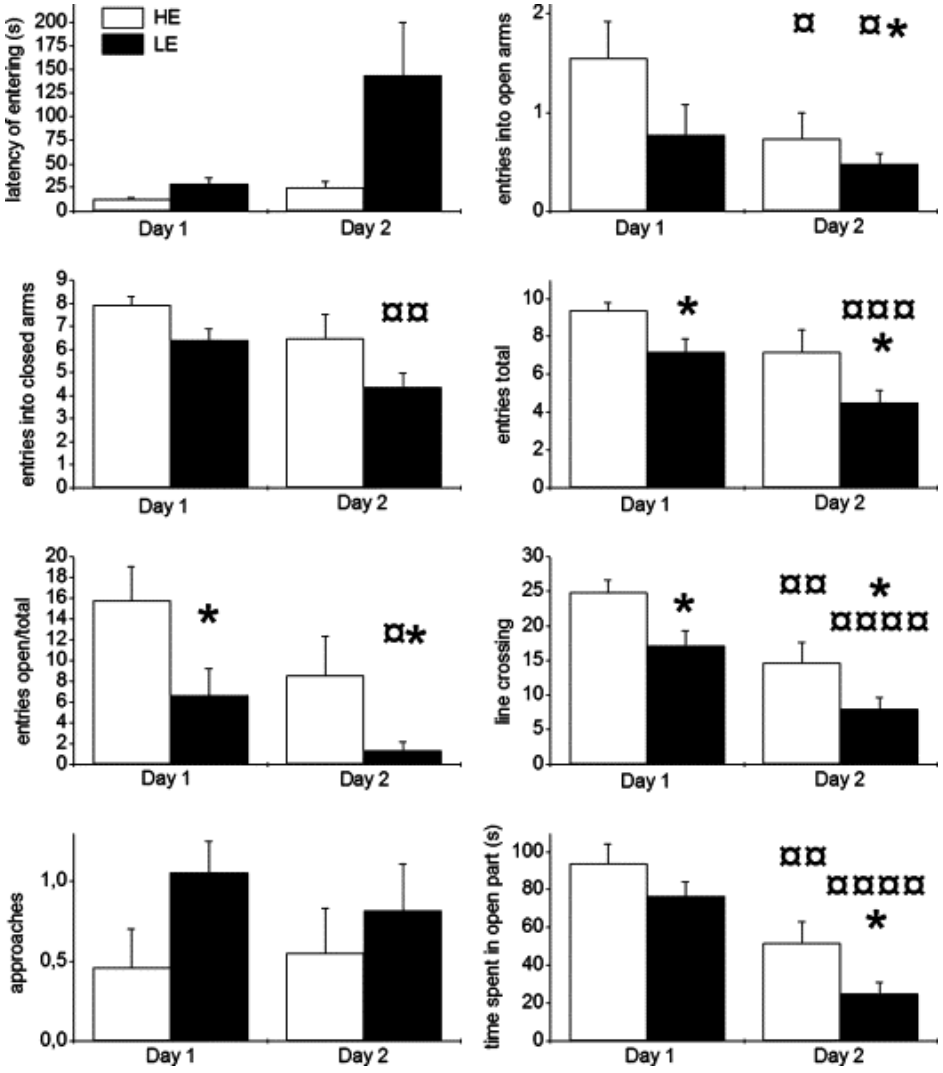

Figure 6. Plus-maze behaviour in LE- and HE-rats. n(LE) = 18; n(HE) = 14. $^{*}p < 0.05$ vs. HE; $^{¤}p < 0.05$, $^{¤¤}p < 0.01$, $^{¤¤¤¤}p < 0.0001$ vs. Day 1. LE: low exploratory behaviour rats, HE: high exploratory behaviour rats. Bars represent mean ± S.E.M.

Although social interaction time has often been shown to be negatively related with anxiety levels (File and Seth, 2003), the HE and LE animals did not differ in their social activity. It has previously been demonstrated that in male and female rats bred on the basis of their anxiety-related behaviour in the elevated plus-maze, the activity observed in the elevated plus-maze was consistently correlated with the time spent in active social interaction (Henniger et al., 2000). In our previous studies we have found that sociability – as measured by repeated social interaction tests – is a stable trait, with an animal's social behaviour in each single test highly correlated to its mean level of social activity (Tõnissaar et al., 2004). Interaction with both an unfamiliar environment and a novel partner can be motivating yet anxiety-provoking, but the present findings suggest that distinct mechanisms govern anxiety-related behaviours in those two different situations.

In the fear-conditioning paradigm, animals relate a formerly neutral environment or other cue to a stressful event and develop anxiety towards it. HE animals showed higher freezing levels on the first test day, suggesting that the initial fear response to acute stressors in HE-rats is higher (Figure 7).

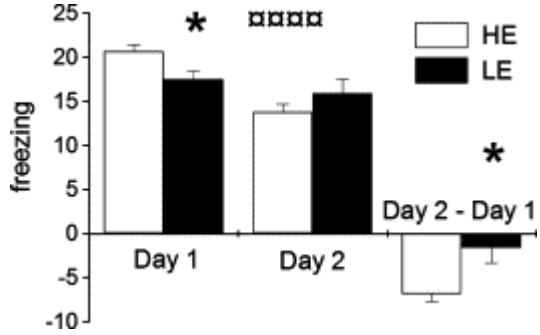

Figure 7. Freezing in fear conditioning test in LE- and HE-rats. n(LE) = 8; n(HE) = 8. $^{*}p < 0.05$ vs. HE; $^{¤}p < 0.05$ vs. Day 1. LE: low exploratory behaviour rats, HE: high exploratory behaviour rats. Bars represent mean ± S.E.M.

In a similar vein, White et al. (2007) demonstrated longer duration and shorter latency of the ultrasonic distress vocalizations emitted in response to loud startle-inducing auditory stimuli in high responders to novelty in an inescapable open field. Nevertheless, our results suggest that the extinction rate was faster in the HE group as the decrease in freezing between the two test days was greater in the HE animals, suggesting more active coping style. In contrast, the freezing levels between the two test days remained almost unchanged in the LE group, suggesting that they acquire a more persistent association between neutral and stressful stimuli. Borta et al. (2005) have found that rats selected for their low or high amount of time spent on the open arms of the elevated plus-maze also display differences in acute and conditioned responses in a fear conditioning

paradigm – namely, rats with low levels of time spent in the open arms of the plus-maze showing more freezing and emitting more distress calls.

The findings concerning the behaviour of the HE-and LE-rats in the forced swimming test are basically similar to those reported by Taghzouti et al. (1999) who demonstrated that novelty-related behaviour in a novel circular corridor is inversely correlated with the immobility time on the second day of the forced swimming test, with high responders to novelty being less immobile and swimming more. We found that HE animals show less immobility and more swimming on the second trial (Figure 8), which again suggests that these animals use more active coping strategies.

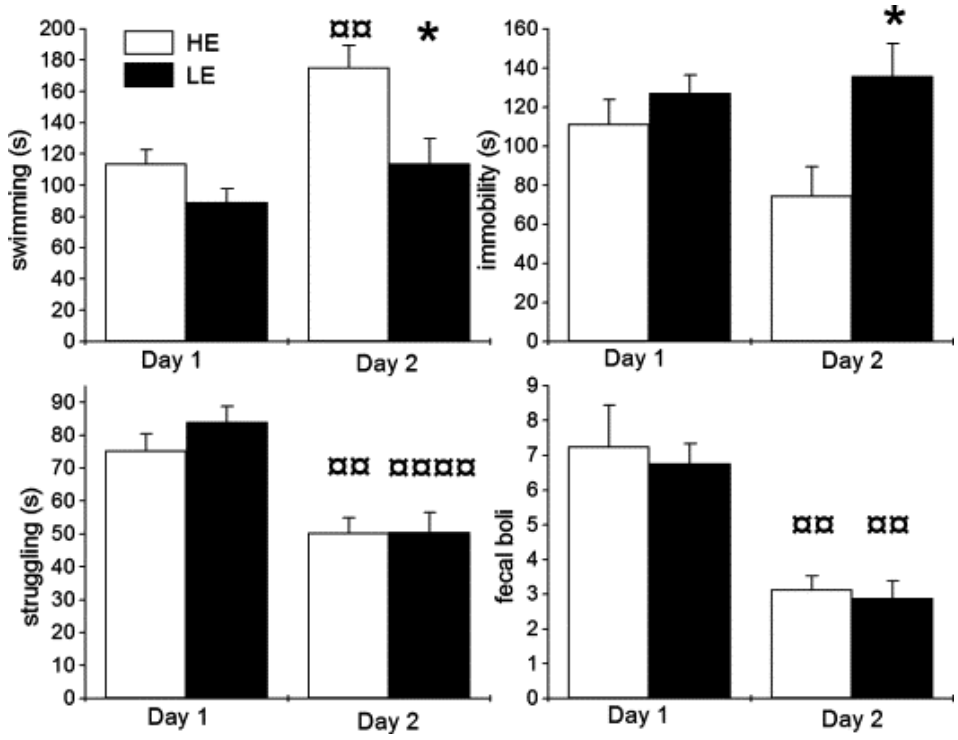

Figure 8. Forced swimming test in LE- and HE-rats. n(LE) = 18; n(HE) = 14. $^{*}p < 0.05$ vs. HE; $^{¤}p < 0.05$, $^{¤¤}p < 0.01$, $^{¤¤¤¤}p < 0.0001$ vs. Day 1. LE: low exploratory behaviour rats, HE: high exploratory behaviour rats. Bars represent mean ± S.E.M.

The sucrose preference test has been used as a measure of anhedonic tendencies in stress and depression models. In the present experiments, the LE-rats consumed more sucrose solution. This finding is somewhat surprising as decreased, not increased sucrose consumption in rats has generally been regarded as a behavioural sign of depression. In humans stress has been found to

affect food intake in a bidirectional way (Epel et al., 2004) and in the light of these findings both higher and lower sensitivity to the palatable properties of the sucrose solution could be interpreted as indicative of higher negative emotionality. One study from our group demonstrated that chronic variable stress resulted in a greater sucrose intake in rats with a partial denervation of the serotonergic neurotransmission (Harro et al., 2001). The differences in the sucrose intake between the HE- and LE-rats disappeared after an 18-hour food and water deprivation, as the increase in sucrose consumption after the fast was significantly greater in HE animals. This could indicate differences in reactivity to short-term stress like food deprivation between the HE and LE animals, or perhaps differences in attaining and maintaining individual energetic balance in these two groups.

4.3. Neurobiological differences in high and low explorers

4.3.1. Ex vivo monoamines and metabolites (Papers IV and V)

Regarding the tissue levels of monoamines, the HE- and LE-animals only differed in respect to the 5-HT and 5-HIAA content in the frontal cortex and striatum (**Paper IV**, which were higher in the HE-animals; and dopamine levels in the striatum, which were lower in the HE-rats (**Paper V**). This result is at variance with some studies (Piazza et al., 1991, Thiel et al., 1999, Antoniou et al., 2008), which reported higher dopamine levels in the nucleus accumbens and the striatum and/or lower 5-HT levels in the medial frontal cortex in high responders to novelty. One possible explanation for the discrepancy between the findings in the neurochemical differences in animals preselected for their spontaneous novelty-related behaviour from our laboratory and those of others is that the locomotor activity (Piazza et al., 1991) or rearing (Thiel et al., 1999) in an inescapable environment probably reflect aspects of coping with novelty that are distinct from the (true) exploratory behaviour measured by the emergence paradigm used in our study. While activity measured in the exploration box does involve novelty-induced locomotion, it also requires an entry into the open area, the incidence, latency and duration of which are strongly dependent on the individual level of curiosity and the tendency to avoid unfamiliar conditions. It has to be noted, however, that all the animals in the experiments described in **Papers IV and V** had received amphetamine at least once, so we cannot exclude the possibility of a confounding effect of amphetamine administration on the differences in the tissue monoamine levels between the HE and LE animals.

4.3.2. Extracellular dopamine in the striatum and the nucleus accumbens (Papers II and III)

Extracellular dopamine levels in dorsal striatum in baseline conditions as well as in response to amphetamine administration were significantly higher in HE animals than in LE animals, while no difference was detected in the nucleus accumbens (Figure 9).

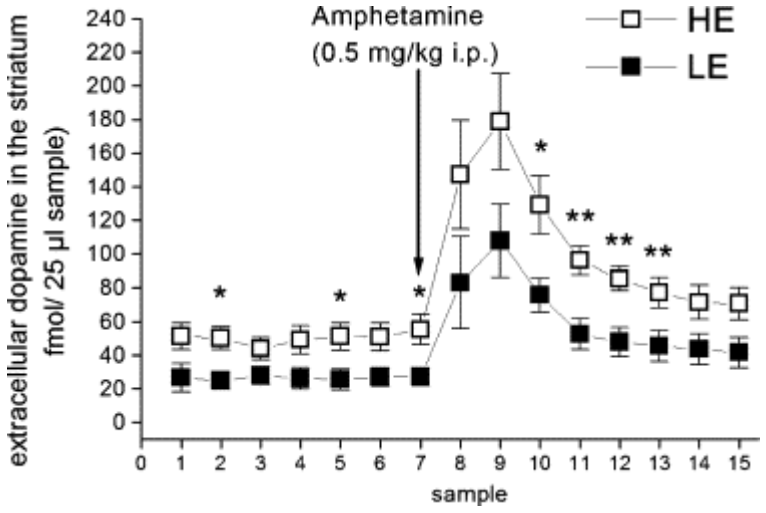

Figure 9. Dopamine release in the dorsal striatum in response to amphetamine administration in LE- and HE-rats. n(LE) = 6; n(HE) = 6. $^{*}p < 0.05$, $^{**}p < 0.01$ vs. LE. LE: low exploratory behaviour rats, HE: high exploratory behaviour rats, i.p.: intraperitoneal. Data are presented as mean ± S.E.M.

As will be shown in **Papers IV and V**, acute administration of a low dose of amphetamine induces a similar proportional increase in locomotor activity in HE- and LE-rats, and the activity levels of the HE-rats remain significantly higher as compared to the LE animals. Previously, higher basal dopamine release in the nucleus accumbens has been reported in rats with higher locomotor response to novelty that are also more sensitive to psychostimulants (Hooks et al., 1992). Also, when animals are preselected or bred for individual differences in neotic behaviour, higher levels of exploratory activity are associated with augmented novelty- and psychostimulant-induced increase in the extracellular dopamine in the nucleus accumbens (Hooks et al., 1992; Saigusa et al., 1999; Giorgi et al., 2007). Marinelli and White (2000) have shown that the high responders have higher firing rate and bursting activity of the the dopamine neurons in the ventral tegmental area and the substantia nigra than the low responders, which could explain the differences in the extracellular dopamine levels observed in these animals. It is therefore possible that absolute

differences in the activity of midbrain dopaminergic circuits are at least partly responsible for the differences in novelty-related behaviours in those two groups.

4.3.3. Differences in the proportion of D_2 receptors in the high affinity state in the striatum (Paper III)

It is not known whether an increase in both dopamine release and the modifications in the affinity states of the striatal D_2 receptors that accompany the behavioural sensitization to psychostimulants (Seeman et al., 2002; Seeman et al., 2007; Shuto et al., 2008; Simola et al., 2008; Briand et al., 2008) also form the physiological basis of the inter-individual differences that exist in locomotor and exploratory behaviour. The D_2 receptors exist in high-affinity (D_2^{High}) and low-affinity (D_{2Low}) states; the former has been proposed to be the functional state of the receptors (George et al., 1985). Thus, we examined whether high spontaneous exploratory activity in the HE-rats is associated with both higher dopamine output as well as a larger number of D_2^{High} in the striatum.

Indeed, rats with persistently high exploratory activity have elevated number of D_2 receptors in the functional high affinity state in the striatum (Figure 10), which coincides with higher extracellular dopamine levels in these animals (**Papers II and III**).

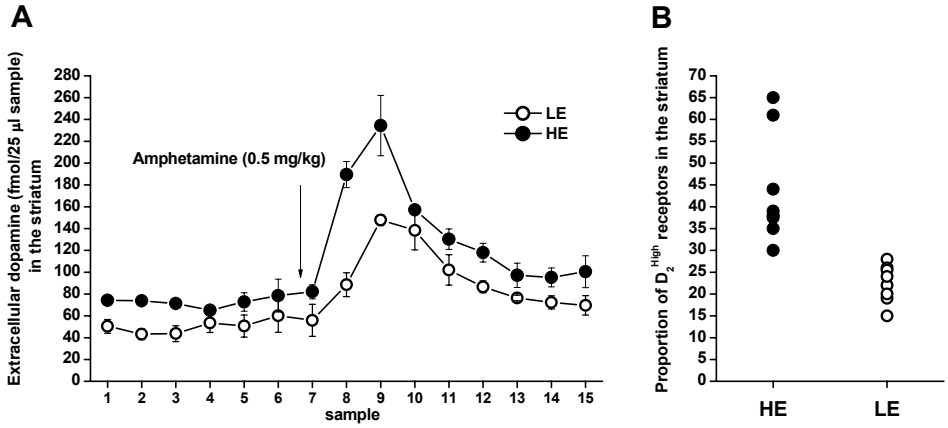

Figure 10. Extracellular dopamine in the striatum (A) and proportion of D_2^{High} receptors (B) in the striatum of rats with high or low exploratory activity. HE – high explorers, LE – low explorers. Data expressed as means ± S.E.M.

Interestingly, the magnitude of difference in the number of D_2^{High} receptors in the HE- and LE-rats is very similar to that observed in recent studies comparing psychostimulant-sensitized and control rats. For example, from control levels of about 20%, an approximately twofold increase (to 40–45%) in the proportion of striatal D_2^{High} receptors in rats has been demonstrated after psychomotor-sensitizing regimens of amphetamine (Seeman et al., 2002; Seeman et al., 2007), methamphetamine (Shuto et al., 2008), caffeine (Simola et al., 2008), and cocaine (Briand et al., 2008). In terms of the proportion of D_2 receptors in the high-affinity state, the HE-rats thus appear as naturally-occuring 'sensitized' animals. Our microdialysis data (**Papers II and III**) suggest that in a novel environment the novelty-induced dopamine release (see Horvitz, 2000) should be expected to be larger in the high exploring rats. Thus it could be speculated that such more extensive release of dopamine acting on a higher number of the D_2^{High} receptors in the striatum provides the physiological substrate for the increased behavioural activation observed in the rats with high exploratory activity.

4.3.4. Differences in genome-wide gene expression profiles (Paper VI)

In the present study, between the HE- and LE-rats, 816 transcripts in the raphe, 809 in the hippocampus, and 602 in the frontal cortex were significantly differentially expressed (pplr < 0.001) (see Table 1 for the numbers of up- and downregulated genes in LE- vs. HE-rats in each brain region). Moreover, there was considerable overlap in the expression profiles between the studied brain areas, as 390 transcripts were changed in the same direction and in common in the raphe, the hippocampus, and the frontal cortex. Of these, 264 genes were downregulated and 156 upregulated in the LE-rats.

Table 1. Significantly differentially expressed transcripts in the raphe, hippocampus and the frontal cortex between rats with high vs. low exploratory activity (at pplr < 0.001).

	Raphe	Hippocampus	Frontal cortex
Upregulated	555	459	322
Downregulated	261	350	280
Total number of differentially expressed transcripts*	816	809	602

* 390 transcripts were differentially expressed in the same direction in all three brain areas

4.3.3.1. Differential expression of genes coding for neurotransmitter-synthesizing enzymes, neurotransmitter receptors or receptor subunits

The expression of several genes coding for neurotransmitter receptors or receptor subunits that were expected to be biologically relevant to modulating affective symptomatology were significantly differetially expressed in the LE vs. HE animals (Table 2).

Table 2. Differentially expressed transcripts encoding neurotransmitter synthesizing enzymes, receptors or receptor subunits in the brains of rats with low vs. high exploratory activity (pplr < 0.001).

Genbank accession number	Gene symbol	Gene title	Log ratio		
			Raphe	Hippo-campus	Frontal cortex
NM_012784.3	*Cnr1*	Cannabinoid receptor 1 (brain)		0.76	0.63
NM_183326.1	*Gabra1*	Gamma-aminobutyric acid A receptor, alpha 1	–1.21	–0.77	–0.88
NM_080587.1	*Gabra4*	Gamma-aminobutyric acid A receptor, alpha 4			–0.54
NM_080586.1	*Gabrg1*	Gamma-aminobutyric acid A receptor, gamma 1	–1.30	–0.93	–0.72
NM_183327.1	*Gabrg2*	Gamma-aminobutyric acid A receptor, gamma 2	–1.21	–0.78	–0.79
NM_053296.1	*Glrb*	Glycine receptor, beta subunit	–1.20	–0.74	–0.68
NM_032990.1	*Gria3*	Glutamate receptor, ionotropic, AMPA3			0.65
XM_579337.1	*Grin2a*	Glutamate receptor, ionotropic, N-methyl D-aspartate 2A.		1.24	0.99
NM_012585.1	*Htr1a*	5-hydroxytryptamine (serotonin) receptor 1A		–0.65	
NM_012765.2	*Htr2c*	5-hydroxytryptamine (serotonin) receptor 2C	–0.82		
NM_173839.2	*Tph2*	Tryptophan hydroxylase 2	–0.55		

There is evidence from basic as well as clinical studies that suggest that dysregulation of serotonin neurotransmission is associated with depression (Deakin, 1996), as well as modulation of anxiety levels in animal models of these disorders (Griebel, 1995). Mällo et al. (2008) have recently reported region-specific differences in serotonergic function in the HE- and LE-rats. In the present study, we found that three genes involved in serotonin neurotransmission were downregulated in the LE-rats. In the raphe, Tph2 (tryptophan hydroxylase

2) and Htr2c (serotonin receptor 2C) were downregulated in the LE-rats. Of the two isoforms of the serotonin-synthesizing enzymes, Tph1 (tryptophan hydroxylase 1) and Tph2 (tryptophan hydroxylase 2), the latter is exclusively expressed in the brain and has a fundamental role in brain serotonin synthesis (Zhang et al., 2004). A functional single nucleotide polymorphism in the human Tph2 gene that results in a marked loss of function in serotonin production has been identified in patients with unipolar major depression (Zhang et al., 2005). Knockin mice that express the mutant form of the Tph2 gene have significantly reduced brain serotonin synthesis and are characterized by anxiety-like behaviours in the dark-light emergence test and tail suspension test (Beaulieu et al., 2008). Consistently with the idea of a reduced serotonergic neurotransmission posing an increased risk for developing mood disorders, the *Tph2* gene was downregulated in the raphe of the low exploring animals displaying the 'depressed' phenotype.

In general, the activation of 5-HT_{2C} receptors is thought to be anxiogenic, as 5-HT_{2C} agonists promote anxiety in animals (Menard and Treit, 1999), and the 5-HT_{2C}-knockout mice have a rather anxiolytic behavioural phenotype (Heisler et al., 2007). Thus, our finding of lower *Htr2c* expression in the raphe of LE animals which also have higher anxiety levels is in apparent discordance with the previous results. The 5-HT_{2C} receptor has a widespread expression in the CNS that is most abundant in serotonergic projection areas, consistent with its presumed postsynaptic localization, but has also been detected in the raphe nuclei prompting suggestions of its presynaptic localization on serotonergic neurons (Wright et al., 1995; Clemett et al., 2000). However, 5-HT_{2C} receptors do not suppress the firing of the raphe serotonergic neurons directly, but via activation of local GABA-ergic interneurons (Liu et al., 2000), which have been shown to express 5-HT_{2C} (Serrats et al., 2005; Boothman et al., 2006). In addition to lower *Htr2c* expression in the raphe, the genes encoding GABA-A receptor α_1, γ_1, and γ_2 subunits (*Gabra1*, *Gabrg1*, and *Gabrg2*, respectively) were downregulated in the same brain area of the LE animals. Therefore, the reduction of the expression of genes encoding 5-HT_{2C} receptors and several $GABA_A$ receptor subunits in the raphe of the more anxious LE-rats might contribute to an altered local interplay between serotonin and GABA, which may underlie the behavioural phenotype of this group. Alternatively, the reduced *Htr2c* expression in the raphe of the LE-rats might reflect a possible negative feedback mechanism to an increased number of 5-HT_{2C} receptors, as in behaviourally anxious 5-HTT-knockout mice lacking the serotonin transporter reduced levels of 5-HT_{2C} mRNA, but increased or unchanged density of 5-HT_{2C} receptors were found in specific brain regions (Li et al., 2003). There also remains a possibility that the anxiety-mediating role of the 5-HT_{2C} receptors is different depending on the localization in either the cell body region or the projection areas.

In the hippocampus, the expression of *Htr1a* (serotonin receptor 1A) was lower in the low exploring group of animals. Using drug challenges and *in vivo*

imaging, dysregulation of the 5-HT_{1A} receptor has been shown to be implicated in human affective and panic disorders (see Lesch and Gutknecht, 2004 and references therein). Moreover, Lesch and Gutknecht (2004) have suggested that because low 5-HT_{1A} function in major depressive disorder patients does not seem to be reversed by antidepressant treatment, this might reflect an inherent vulnerability mechanism to the disorder. Additionally, knockout mice lacking 5-HT_{1A} receptor display enhanced avoidance of novel situations, and decreased exploratory behaviour and increased fear of aversive environments (Ramboz et al., 1998; Parks et al., 1998). Interestingly, these behavioural effects of *Htr1a* inactivation were not only demonstrated in homozygous mutant mice but also in heterozygous mice that had about 60% reduction in the number of 5-HT_{1A} binding sites compared to wild-type levels (Ramboz et al., 1998). This supports the possibility that the downregulation of 5-HT_{1A} receptors in the brain may present a risk factor for mood disorders (Toth, 2003).

Several lines of evidence have indicated that decreased tone of the brain γ-aminobutyric acid (GABA) system might be implicated in anxiety and mood disorders (Shiah and Yatham, 1998; Kalueff and Nutt, 2007, for review). In line with multiple studies that have shown downregulation of GABA receptor genes in animal models of anxiety and depression (see Kalueff and Nutt, 2007), the expression of genes encoding GABA-A receptor subunits (*Gabra1*, *Gabra4*, *Gabrg1*, *Gabrg2*) was reduced in the low exploring rats. In addition to reduced GABA function, abnormal glutamatergic neurotransmission, including changes in glutamate receptors, have been reported in mood disorder patients (Sanacora et al., 2008). In the present study, we found an upregulation of *Grin2a* (NR2A subunit of the NMDA receptor) in the frontal cortex and hippocampus, and *Gria3* in the frontal cortex of low exploring animals. An insight into the behavioural functions of the NR2A comes from the findings of reduced anxiety-like and anti-depressive behaviour in mice with genetic inactivation of the *Grin2a* gene (Boyce-Rustay and Holmes, 2006). Thus, increased expression of *Grin2a* would be expected to have a diametrically opposite effect on anxiety- and depression-related behaviours.

Interestingly, CB_1 cannabinoid receptor gene (*Cnr1*) was upregulated in the frontal cortex and hippocampus of the rats with higher neophobia and lower motivation to explore. In mice, CB_1 cannabinoid receptors are known to mediate attenuated anxiety-like responses (Haller et al., 2004a) and CB_1-knockout mice context-dependently display higher stress responsiveness and altered coping strategies (Haller et al., 2004b). In contrast to mice, in several rat strains both CB_1 agonists and antagonists have a pro-anxiety profile; and in Wistar rats, specifically, it was found that CB_1 agonist WIN-55,212 administration induced anxiogenesis which was further augmented with the CB_1 antagonist AM-251 co-administration (Haller et al., 2007). Haller et al. (2007) have hypothesized that in mice the CB_1 activation which leads to reduced anxiety occurs mainly via modulation of GABA-ergic neurotransmission, whereas in rats the effects of CB_1 receptor stimulation are exerted mostly by a glutamate-mediated mecha-

nism. In line of this reasoning, the increase in the *Cnr1* expression together with decreases in GABA and increases in the expression of glutamate receptor genes in the LE-rats might underlie their persistently anxious phenotype as well as passive coping strategies displayed in behavioural tests of affective states (Mällo et al., 2007). The upregulation of *Cnr1* in the LE-rats might also result in a shift in the functional interaction between CB_1 and GABA-B receptors that has been proposed to finetune the balance of endocannabinoid and GABA-ergic signalling in the hippocampus (Cinar et al., 2008). At this point we have no information about the brain levels of endocannabinoids in the HE- and LE-rats, but we cannot rule out the possibility that the increased expression of *Cnr1* in the LE animals indeed reflects an adaptive response to low endocannabinoid levels in the brain.

4.3.3.2. KEGG pathway analysis

To find significantly over-represented pathways in our lists of differently regulated genes, we searched the curated pathway database of the Kyoto Encyclopedia of Genes and Genomes (KEGG) using the DAVID 2007 Functional Annotation Tool. The KEGG pathway database is a collection of manually drawn graphical diagrams that represent the current knowledge of molecular interactions involved in various cellular processes (http://www.genome.ad.jp/kegg/). In the raphe, the most significantly over-represented pathway was found to be the focal adhesion pathway, followed by axon guidance, B cell receptor signalling, leukocyte transendothelial migration, regulation of actin cytoskeleton, and Wnt signalling pathways (Table 3). In the hippocampus, the Wnt signalling pathway was the most significantly over-represented pathway, followed by MAPK signalling, axon guidance, regulation of actin cytoskeleton, focal adhesion, long-term depression, and long-term potentiation pathways. In the frontal cortex, the most significantly enriched pathway was the Wnt signalling pathway, followed by long-term depression, focal adhesion, axon guidance, and MAPK signalling pathway. Thus, the over-represented pathways were similar in the three brain regions.

Of specific interest in the context of the present study was the finding that the Wnt signalling pathway was the most enriched pathway in the hippocampus and the frontal cortex, while significantly over-represented also in the raphe. The Wnt proteins are secreted cysteine-rich glycoproteins that are critically involved in cell development, proliferation, and apoptosis (Logan and Nusse, 2004; Li et al., 2005). The Wnt proteins bind to the Frizzled family receptors and activate either canonical or non-canonical Wnt-Frizzled pathways. The canonical Wnt signalling controls gene transcription through preventing the degradation of the transcriptional regulator, β-catenin by displacing the key enzyme of this

Table 3. Significantly enriched KEGG pathways among the differentially expressed genes in the brains of rats with low vs. high exploratory activity.

Gene symbol	Gene title	Log ratio		
		Raphe	Hippo-campus	Frontal cortex
RNO04310: Wnt signalling pathway				
Camk2a	Calcium/calmodulin-dependent protein kinase II alpha subunit			–0.61
Camk2b	Calcium/calmodulin-dependent protein kinase II beta subunit		1.26	
Ctnnb1	Catenin (cadherin associated protein), beta 1, 88kda	–0.88	–0.91	–0.66
Cxxc4	CXXC finger 4	1.37	1.23	1.24
Gsk3b	Glycogen synthase kinase 3 beta	1.34	1.63	1.38
Mapk9	Mitogen-activated protein kinase 9	–1.05	–0.75	–0.68
Nfatc3_ predicted	Nuclear factor of activated t-cells, cytoplasmic, calcineurin-dependent 3 (predicted)	–0.51		
Ppp2r2c	Protein phosphatase 2 (formerly 2a), regulatory subunit b (pr 52), gamma isoform		–0.61	–0.63
Ppp3cb	Protein phosphatase 3, catalytic subunit, beta isoform	–0.76	–0.57	–0.58
Rac1	Ras-related c3 botulinum toxin substrate 1 (rho family, small gtp binding protein rac1)	–0.78	–0.80	–0.69
Rock1	Rho-associated coiled-coil forming kinase 1	–0.59		
Senp2	Sumo/sentrin specific protease 2	–0.57		
Wnt4	Wingless-related mmtv integration site 4		0.56	

Table 3. (Continuation)

Gene symbol	Gene title	Log ratio		
		Raphe	Hippo-campus	Frontal cortex
RNO04360: Axon guidance				
Gsk3b	Glycogen synthase kinase 3 beta	1.34	1.63	1.38
Gnai1	Guanine nucleotide binding protein, alpha inhibiting 1	–1.81	–1.18	–1.11
Kras2	Kirsten rat sarcoma viral oncogene homologue 2 (active)		0.76	0.80
Nras	Neuroblastoma ras oncogene	–0.54		
Nfatc3_ predicted	Nuclear factor of activated t-cells, cytoplasmic, calcineurin-dependent 3 (predicted)	–0.51		
Pak2	p21 (cdkn1a)-activated kinase 2	–0.66	–0.54	
Pak3	p21 (cdkn1a)-activated kinase 3	1.01	1.28	1.00
Ppp3cb	Protein phosphatase 3, catalytic subunit, beta isoform	–0.76	–0.57	–0.58
Rasa1	Ras p21 protein activator 1			0.51
Rac1	Ras-related c3 botulinum toxin substrate 1	–0.78	–0.80	–0.69
Rock1	Rho-associated coiled-coil forming kinase 1	–0.59		
Sema6d_ predicted	Sema domain, transmembrane domain (tm), and cytoplasmic domain, (semaphorin) 6d (predicted)	–0.53		
Slit1	Slit homolog 1 (drosophila)	0.62	0.66	
Unc5a	Unc-5 homolog a (c. elegans)		–0.68	–0.53
RNO04510: Focal adhesion				
Actb	Actin, beta	–1.15	–1.12	–0.95
Ctnnb1	Catenin (cadherin associated protein), beta 1, 88kda	–0.88	–0.91	–0.66
Cav	Caveolin	–0.91	–0.6	–0.73
Cav2	Caveolin 2	–0.56		
Fn1	Fibronectin 1	0.57		
Gsk3b	Glycogen synthase kinase 3 beta	1.34	1.63	1.38
Itga1	Integrin alpha 1		0.98	
Kras2	Kirsten rat sarcoma viral oncogene homologue 2 (active)		0.76	0.80
Mapk9	Mitogen-activated protein kinase 9	–1.05	–0.75	–0.68
Nras	Neuroblastoma ras oncogene	–0.54		

Table 3. (Continuation)

Gene symbol	Gene title	Log ratio		
		Raphe	Hippo-campus	Frontal cortex
RNO04510: Focal adhesion (continued)				
Pak2	p21 (cdkn1a)-activated kinase 2	–0.66	–0.54	
Pak3	p21 (cdkn1a)-activated kinase 3	1.01	1.28	1.00
Pik3r3	Phosphatidylinositol 3 kinase, regulatory subunit, polypeptide 3	–0.99	–0.55	–0.58
Pik3cb	Phosphatidylinositol 3-kinase, catalytic, beta polypeptide	–0.52		
Rac1	Ras-related c3 botulinum toxin substrate 1	–0.78	–0.80	–0.69
Rock1	Rho-associated coiled-coil forming kinase 1	–0.59		
Tnr	Tenascin r	1.61	1.98	1.96
Vegfa	Vascular endothelial growth factor a			–0.53
RNO04010: MAPK signalling pathway				
Atf2	Activating transcription factor 2		–0.79	–0.62
Acvr1c	Activin receptor-like kinase 7		1.37	1.54
Cacng3	Calcium channel, voltage-dependent, gamma subunit 3		–0.79	–0.64
Cacng8	Calcium channel, voltage-dependent, gamma subunit 8		–0.57	
Fgf12	Fibroblast growth factor 12		–1.32	–1.23
Gna12	Guanine nucleotide binding protein, alpha 12		–0.54	
Kras2	Kirsten rat sarcoma viral oncogene homologue 2 (active)		0.76	0.80
Max	Max protein		–0.68	–0.61
Mapk9	Mitogen-activated protein kinase 9		–0.75	–0.68
Ntrk1	Neurotrophic tyrosine kinase, receptor, type 1		0.67	
Pak2	p21 (cdkn1a)-activated kinase 2		–0.54	
Ppp3cb	Protein phosphatase 3, catalytic subunit, beta isoform		–0.57	–0.58
Rasgrp1	Ras guanyl releasing protein 1		–0.98	–0.77
Rasa1	Ras p21 protein activator 1			0.51
Rac1	Ras-related c3 botulinum toxin substrate 1		–0.80	–0.69

Table 3. (Continuation)

Gene symbol	Gene title	Log ratio		
		Raphe	Hippo-campus	Frontal cortex
RNO04810: Regulation of actin cytoskeleton				
Actb	Actin, beta	1.15	1.12	
Fgf12	Fibroblast growth factor 12	1.42	1.32	
Fn1	Fibronectin 1	–0.57		
Gna12	Guanine nucleotide binding protein, alpha 12		0.54	
Itga1	Integrin alpha 1		–0.98	
Kras2	Kirsten rat sarcoma viral oncogene homologue 2 (active)		–0.76	
Msn	Moesin	0.64	0.55	
Nras	Neuroblastoma ras oncogene	0.54		
Pak2	p21 (cdkn1a)-activated kinase 2	0.66	0.54	
Pak3	p21 (cdkn1a)-activated kinase 3	–1.01	–1.28	
Pfn1	Profilin 1	0.51	0.78	
Pik3cb	Phosphatidylinositol 3-kinase, catalytic, beta polypeptide	0.52		
Pik3r3	Phosphatidylinositol 3 kinase, regulatory subunit, polypeptide 3	0.99	0.55	
Rac1	Ras-related c3 botulinum toxin substrate 1 (rho family, small gtp binding protein rac1)	0.78	0.80	
Rock1	Rho-associated coiled-coil forming kinase 1	0.59		
Slc9a1	Solute carrier family 9, member 1	–0.54	–0.54	
RNO04730: Long-term depression				
Gna12	Guanine nucleotide binding protein, alpha 12		0.54	
Gnai1	Guanine nucleotide binding protein, alpha inhibiting 1		1.18	1.11
Gnao	Guanine nucleotide binding protein, alpha O		–1.24	–1.09
Gnaq	Guanine nucleotide binding protein, alpha Q polypeptide		0.93	0.65
Gria3	Glutamate receptor, ionotropic, AMPA3 (alpha 3)			–0.65
Kras2	Kirsten rat sarcoma viral oncogene homologue 2 (active)		–0.76	
Ppp2r2c	Protein phosphatase 2, regulatory subunit B, gamma isoform		0.61	0.63
Prkg2	Protein kinase, cGMP-dependent, type II		–0.57	–0.89

Table 3. (Continuation)

Gene symbol	Gene title	Log ratio		
		Raphe	Hippo-campus	Frontal cortex
RNO04720: Long-term potentiation				
Camk2b	Calcium/calmodulin-dependent protein kinase II beta subunit		–1.26	
Gnaq	Guanine nucleotide binding protein, alpha Q polypeptide		0.93	
Grin2a	Glutamate receptor, ionotropic, N-methyl-D-aspartate 2A		–1.24	
Kras2	Kirsten rat sarcoma viral oncogene homologue 2 (active)		–0.76	
Ppp3cb	Protein phosphatase 3, catalytic subunit, beta isoform		0.57	
Rapgef3	cAMP-regulated guanine nucleotide exchange factor 1		–0.57	

signalling pathway, glycogen synthase kinase-3 (GSK-3), from the multi-protein complex that in the absence of Wnt signalling functions to keep the levels of cytoplasmic β-catenin low. As a result of the activation of the Wnt signalling pathway, β-catenin accumulates in the cytoplasm and is transported into the nucleus where it activates the transcription of its target genes. Ubiquitously expressed in mammal tissues, GSK-3 has a crucial role in cell division, differentiation, proliferation and adhesion, and its dysfunction plays a role in the pathogenesis of cancer and diabetes (Frame and Cohen, 2001). The Wnt signalling pathway has recently been extensively studied in relation to several psychiatric, especially mood disorders. The mood-stabilizer lithium directly inhibits GSK-3 activity and increases Wnt- and β-catenin-mediated gene expression in rats (Gould et al., 2004; Gould et al., 2007a). Also, several serotonergic drugs have the ability to inhibit GSK-3 in the brain (Li et al., 2004; Li et al., 2007; Beaulieu, 2007). Moreover, while β-catenin overexpression mimicked the behavioural effects of lithium administration in mice (Gould et al., 2007b), forebrain-specific conditional inactivation of the β-catenin gene induced depression-like behaviour in tail suspension test in mice (Gould et al., 2008). In our experiment, the LE-rats had significantly higher *Gsk3b* (glycogen synthase kinase 3, beta isoform) expression and the expression of *Ctnnb1* (β-catenin) was downregulated across all brain regions in these animals.

Further, we found significant upregulation in the LE animals of *Cxxc4* (CXXC finger 4) gene encoding a protein, Idax, that functions as a negative regulator of the Wnt pathway (Hino et al., 2001). These results suggest less active Wnt signalling in LE-rats. In a recent study, it was shown that activation of GSK-3β accompanied a marked reduction in the brain serotonin levels and behavioural abnormalities in mice that express a mutant *Tph2* gene (Beaulieu et

al., 2008). The aberrant emotional behaviours were reversed by pharmacological or genetic inhibition of GSK-3β activity, suggesting an important role for GSK-3β signalling in psychiatric problems related to serotonin deficiency (Beaulieu et al., 2008).

However, we also found that *Senp2,* encoding for a SUMO-specific protease Axam (Axin-associating molecule, Kadoya et al., 2002), another inhibitor of Wnt signalling, was downregulated only in the raphe, but not the hippocampus or the frontal cortex of the LE-rats. At present, the relevance of this finding remains obscure.

Several genes involved in the mitogen-activated protein kinase (MAPK) signalling were differentially expressed in the LE- v. HE-rats, including *Fgf12*, *Rac1*, *Mapk9*, and *Atf2*, which were downregulated in the LE animals. The mitogen-activated protein kinase (MAPKs) cascades regulate diverse psysiological processes within the cell (from cell growth and proliferation to synaptic function and gene transcription) in response to a multitude of extracellular and intracellular stimuli (Sweatt, 2001; Raman et al., 2007). *Mapk9* encodes one of the c-Jun N-terminal kinases (JNKs), JNK2, which belongs to a subgroup MAPKs that have an important role in the regulation of the transcriptional activity of c-Jun, modulating gene transcription after neuronal stress (Herdegen et al., 1999). The reduced expression of *Mapk9*, as well as its upstream activators *Fgf12* and *Rac1*, and the downstream target activating transcription factor 2, *Atf2*, may indicate less active JNK signalling in the LE-rats.

GSK-3 and JNK signalling have been implicated in the modulation of synaptic plasticity and connectivity (Ciani and Salinas, 2005; Peineau et al., 2008; Chen et al., 2005). The reduced activity of Wnt and JNK2 pathways in the LE animals and the fact that the axon guidance, focal adhesion and the two main forms of synaptic plasticity in the brain, the long-term potentiation and long-term depression pathways were also significantly over-represented among the differentially expressed transcripts suggests that the major differences between the LE- and HE-rats lie in the remodelling of the neurons and the synapses. This is further supported by the search in the Gene Ontology (GO) database containing gene annotations where several GO terms relevant to the development, morphogenesis, differentiation and proliferation of neurons were found to be enriched.

4.4. Noradrenergic regulation of exploratory behaviour: behavioural and neurobiological effects of partial locus coeruleus denervation in high and low explorers

4.4.1. Behavioural effects of partial locus coeruleus denervation in high and low explorers (Papers IV and V)

4.4.1.1. Exploratory behaviour (Paper IV)

Our previous studies have consistently shown that after a large lesion of the LC projections by a standard dose (50 mg/kg) of DSP-4 all rats avoid entering to the open area of the apparatus during the first testing, and that this is strongly based on an increase in neophobia (Harro et al., 1995; and unpublished data). Several other studies that have also shown that DSP-4 pretreatment impairs adaptation with a novel environment are consistent with these observations (Delini-Stula et al., 1984 and Berridge and Dunn, 1990). In **Paper IV**, the administration of a low dose (10 mg/kg) of the selective neurotoxin DSP-4 which produced depletion of noradrenaline to an expected extent equally in both LE- and HE-rats had no effect on the exploratory activity of HE-rats, suggesting that the remaining noradrenergic terminals are sufficient to maintain their characteristic exploratory behaviour (Figure 11).

LE-rats display negligible exploration of the open area during the first testing, but after being accustomed to the environment, their activity increases, albeit not to the level of the HE-rats. In the present study, partial LC denervation completely prevented the increase of exploratory activity typically observed in the LE-rats. This suggests that either the LE-rats are more sensitive to lesions of the LC, or the increase in exploration of environments that occurs by a reduction of neophobia is strongly dependent on the LC neurotransmission whereas maintaining typical high exploratory activity as it occurs in the HE-rats is not.

4.4.1.2. Amphetamine-stimulated behaviour (Papers IV and V)

After acute administration of amphetamine (0.5 mg/kg) the proportional increase in amphetamine-stimulated locomotor activity was similar in HE- and LE-rats, the activity levels of LE-rats remaining significantly lower as compared to HE-rats (Figure 12) (**Papers IV and V**). However, the difference in the locomotor behaviour between amphetamine-treated and control LE-rats disappeared with repeated testing (**Paper V**), due to increased activity of the control LE-rats over repeated test sessions. In HE-rats the amphetamine-stimulated activity also seemed to habituate by the final days of the 5-day test

(**Paper V**). At a low dose, DSP-4 (10 mg/kg) did not produce changes in the baseline locomotor activity in either HE or LE groups (**Paper V**) and did not modify the effect of amphetamine on the behaviour of LE-rats, but it completely abolished the stimulating effect of amphetamine in HE-rats (**Paper IV**) even with repeated testing (**Paper V**).

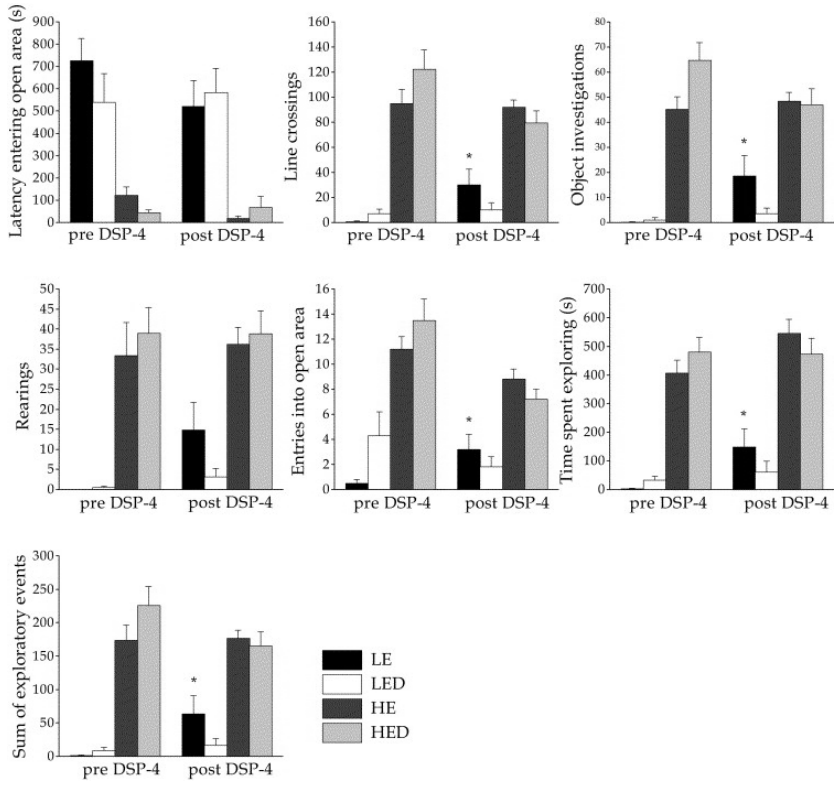

Figure 11. Exploratory behaviour in the exploration box before (pre-DSP-4) and after (post-DSP-4) DSP-4 (10 mg/kg) treatment. Data expressed as means±S.E.M. * $P<0.05$ vs. activity in pre DSP-4 test. (HED, high explorers which received DSP-4 treatment; LED, low explorers which received DSP-4 treatment).

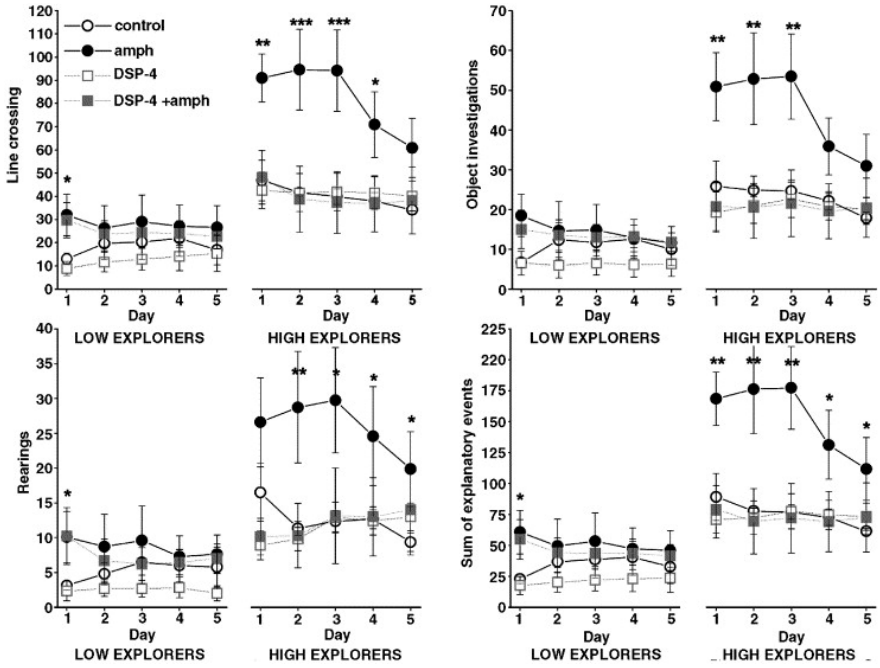

Figure 12. Locomotor activity on open area after amphetamine (0.5 mg/kg) on 5 consecutive days. ***$p < 0.001$; **$p < 0.01$; *$p < 0.05$ versus respective control group.

This implies that the expression of behavioural responses to amphetamine is dependent on an intact LC noradrenergic system in HE-, but not in LE-rats. Additionally, it was found to be a persistent phenomenon as DSP-4-pretreated HE-rats did not express the effect of amphetamine when tested five times on consecutive days.

4.4.1.3. Behavioural sensitization to amphetamine

After a withdrawal period, only the amphetamine-treated LE-rats with intact LC projections displayed a robust behavioural sensitization to a challenge dose of amphetamine (Figure 13). In contrast, a behavioural desensitization, rather than a sensitization, was evident in the amphetamine-treated HE-rats after this low dose of amphetamine. Although not directly measured in this experiment, we did not observe an increase in any of the stereotypical responses to amphetamine that could account for the decreased locomotor activity after amphetamine in these rats. The behavioural sensitization to amphetamine challenge is usually thought to be linked to augmented dopaminergic transmission in the terminal regions of the midbrain dopamine system, especially the nucleus accumbens and the dorsal striatum (Pierce and Kalivas, 1997, for review). In the present

experiment, repeated amphetamine treatment produced a significant increase in the striatal dopamine content in the amphetamine-treated LE-rats which also developed behavioural sensitization. Partial LC denervation prevented both behavioural sensitization to amphetamine and the striatal dopamine increase, which might thus be causally related to each other.

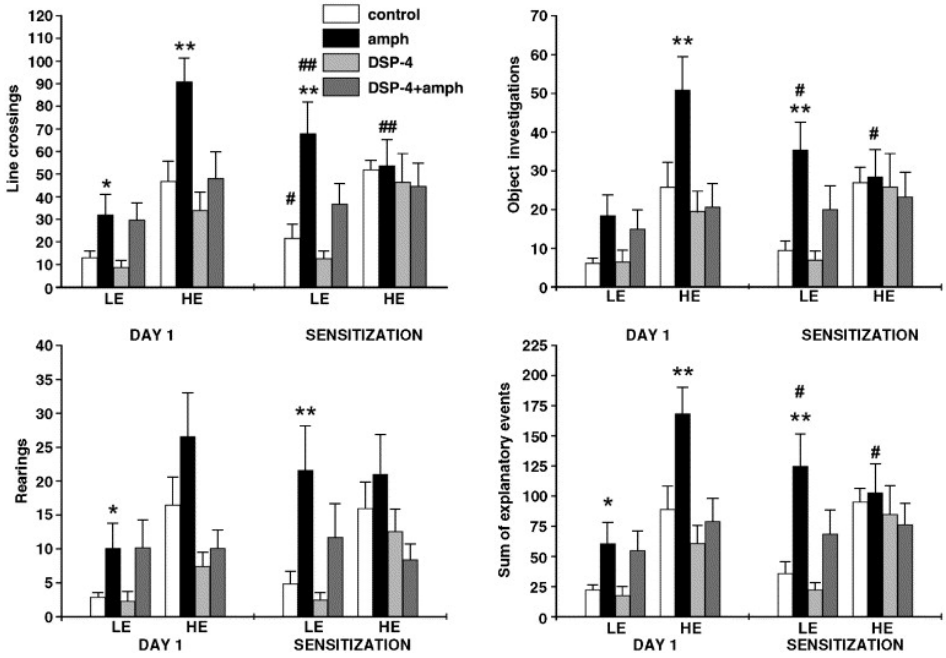

Figure 13. Behavioural sensitization to repeated amphetamine administration. LE – low explorers; HE – high explorers. $^{**}p < 0.01$; $^{*}p < 0.05$ versus respective control group; $^{\#\#}p < 0.01$; $^{\#}p < 0.05$ versus activity on Day 1.

4.4.2. Effect of partial locus coeruleus denervation on neurobiological measures (Papers IV and V)

4.4.2.1. Ex vivo monoamines

The administration of a low dose (10 mg/kg) of the noradrenergic neurotoxin DSP-4 produced a decrease in noradrenaline levels, comparable in the HE- and LE-rats, about 20–30% in the frontal cortex and about 15–20% in hippocampus, which are the brain areas receiving noradrenergic input only from the LC and thus reflecting the degree of denervation (**Papers IV and V**). Thus, at the dose used in these studies, DSP-4 effectively produces a partial noradrenergic denervation of frontal and hippocampal regions, as shown previously by measuring noradrenaline uptake (Harro et al., 1992). It remains unclear how the rate

of noradrenergic neurotransmission is altered after such a treatment because of the possible pre- and postsynaptic compensatory responses as is the case in all studies employing neurotoxins, but convergent data obtained in several studies suggest that the net basal noradrenergic input from LC is weakened in such conditions, perhaps most clearly shown by developing postsynaptic super-sensitivities (e.g. Pycock et al., 1975; see also Harro and Oreland, 2001 for a review). Indeed, even when baseline extracellular noradrenaline levels can be maintained in an acute experiment after extensive lesion produced by DSP-4 (50 mg/kg; Kask et al 1997 and Hughes and Stanford 1998), the possibility to increase noradrenaline release by inhibiting autoreceptor function is much smaller after DSP-4-induced lesion (Kask et al., 1997), which may suggest that over the course of time after administration of the neurotoxin, neurotransmitter levels are lower.

Although DSP-4 is selective for the noradrenergic nerve terminals originating from the locus coeruleus (Ross, 1976), this denervation seems to indirectly modify other monoaminergic systems as well. Even a partial denervation of the LC by 10 mg/kg DSP-4 was found to reduce dopamine release potential in the nucleus accumbens (Häidkind et al., 2002) and increase the number of dopamine D_2 receptor binding sites in the striatum (Harro et al., 2003). Tissue levels of dopamine and metabolites have not been found affected in animals not preselected. However, in the present investigations (**Papers IV and V**), pretreatment with a low dose of DSP-4 reduced the levels of dopamine and its metabolites in the frontal cortex, nucleus accumbens and the striatum, but only in the LE-rats. Whether this effect is causally related to the absence of novelty-related adaptation observed in these animals (**Paper IV**) after the partial denervation of the LC remains to be investigated. Additionally, levels of the serotonin (5-HT) metabolite, 5-HIAA were also reduced in the striatum and the nucleus accumbens selectively in the DSP-4-pretreated LE animals (**Papers IV and V**). Whether this could reflect a reduction in baseline 5-HT release should be examined by *in vivo* microdialysis. The mechanism by which this probably indirect and limited to LE-rats alteration in 5-HT systems is elicited is currently unknown, and determination of the causality of this change to behavioural deficits requires further investigation.

It is not known at present by which mechanism the measures of dopaminergic neurotransmission are changed by DSP-4 treatment. Possibly an intact LC input is necessary for adequate function of transcription factors in dopaminergic neurons. The findings that LC denervation by DSP-4 enhance the toxicity of drugs that lesion dopamin- or serotonergic nerve terminals (Fornai et al., 1996 and Ferrucci et al., 2002) and that MPTP toxicity is reduced in noradrenaline transporter knockout mice (Rommelfanger et al., 2004) allow for a speculation that intact noradrenergic activity protects from the damage from environmental toxins.

Interestingly, all the reductions in the dopamine measures in the nucleus accumbens after DSP-4 were normalized by repeated amphetamine treatment

(**Paper V**). It has been shown that a prelesion sensitizing regimen of amphetamine can prevent the effect of a nigrostriatal damage by 6-OHDA lesion on the behaviour and basal dopamine release (Moroz et al., 2004), but whether a postlesion repeated amphetamine treatment could be protective against DSP-4-induced changes in the dopamine system of the LE-rats remains to be investigated. Nevertheless, the group differences found clearly suggest differential regulation of monoamine systems in the LE- and HE-rats.

4.4.2.2. Striatal D_2 receptor function

The ability of dopamine to activate the receptor-coupled G proteins, measured by the increase of [^{35}S]GTPγS binding in the striatum, was found to be increased in the LE-rats and decreased in the HE-rats after the neurotoxin treatment in **Paper IV**. We hypothesized that the DSP-4-induced absence of the psychostimulant effect of amphetamine in the HE-rats is causally related to their reduced sensitivity of D_2 receptor-coupled G proteins in the striatum. In contrast, in neurotoxin-treated LE animals, locomotor activity after amphetamine remained similar to pre-toxin levels, possibly because the effects of the noradrenergic denervation were compensated for by the increase in D_2 receptor sensitivity.

However, in **Paper V** we demonstrated that the decrease in the sensitivity of striatal D_2 receptors occurred after repeated amphetamine treatment selectively in HE-rats, regardless of the integrity of the LC projections (Figure 14). Thus, we suggest that amphetamine, administered repeatedly, modulates the sensitivity of the striatal dopamine D_2 receptors depending on the level of baseline novelty-related behavior in rats. This reduction in the sensitivity of striatal D_2 receptors could explain the lack of behavioral sensitization to amphetamine in HE-rats (**Paper V**), but not the absence of the acute psychostimulant effect of amphetamine in DSP-4-treated HE-rats (**Papers IV and V**). It is possible that the results of the previous study were confounded by amphetamine administration – because the study design was such that all animals included in biochemical measurements received amphetamine twice – providing an explanation to this apparent inconsistency. In any case, it seems that the mechanism by which DSP-4 blocks the amphetamine effects in HE-rats is independent of the sensitivity of D_2 receptors and is probably related to the noradrenaline depletion in the frontal cortex.

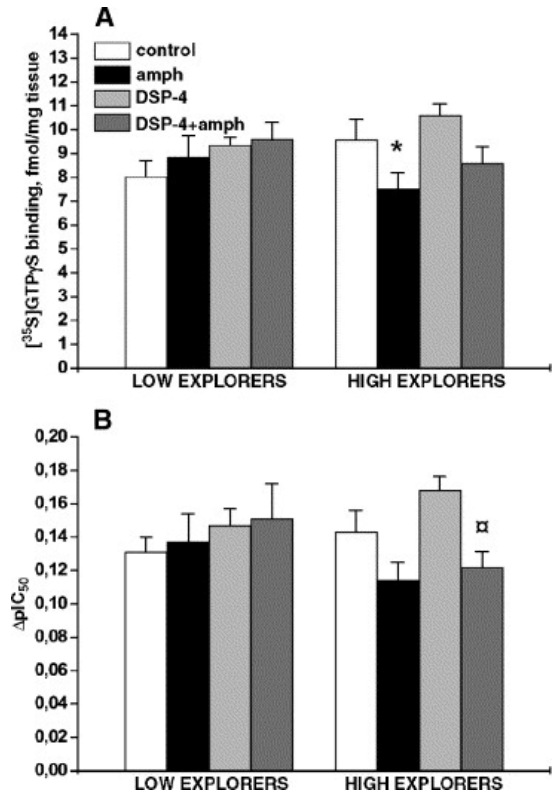

Figure 14. Dopamine-dependent activation of [^{35}S]GTPγS binding in the presence of 0.1 mM GDP (A) and the dopamine dependent change in GDP affinity (B) in rat striatal membranes. Data expressed as means ± SEM. *$p < 0.05$ versus HE control; ¤ $p < 0.05$ versus HE DSP-4 group.

Differences in D_2 receptor sensitivity between LE- and HE-rats were observed in striatum but not nucleus accumbens (**Papers IV**). The reasons for this remain to be clarified. It has been demonstrated that regulation of transcriptional activity by dopaminergic agonists differs greatly between these dopaminergic brain regions (McCoy et al., 2004). HE-rats have significantly higher baseline levels of dopamine release in striatum but no differences can be observed in the nucleus accumbens (**Papers II and III**), suggesting that the traitwise differences between HE- and LE-rats may in part be based on dopaminergic neurotransmission in the striatum.

5. GENERAL SUMMARY

The present studies confirmed the notion that both dopaminergic and noradrenergic neurotransmission are involved in the regulation of exploratory activity in rats.

The behavioural effects of the dopamine agonist amphetamine are modulated by endogenous cholecystokinin (CCK) released during exploration and acting on both receptor subtypes (CCK_1 and CCK_2). Inter-individual differences that exist in the novelty-related behaviour persist over time. These individual differences are at least partially determined by the extracellular dopamine levels and the properties of dopamine D_2 receptors in the striatum, as rats with high exploratory activity have higher extracellular basal and amphetamine-induced dopamine levels and larger proportion of the functional high affinity D_2 receptors in the striatum. The level of spontaneous exploratory behaviour also predicts the animals' behaviour in other tests of anxiety and depression, and determines whether they develop behavioural sensitization to amphetamine or not. The ability of repeated amphetamine administration to reduce the sensitivity of the striatal D_2 receptors was evident selectively in the rats with high exploratory activity. Partial denervation of the locus coeruleus projections has different effects on the dopaminergic system in animals which differ in their trait of novelty-related behaviour. Also, the locus coeruleus noradrenergic neurotransmission is differentially involved in dopaminergic mechanisms that mediate novelty-related vs. amphetamine-induced behaviour.

It seems that the behavioural activity in response to an exposure to a novel environment has a strong genetic foundation, as revealed by the differences between the high and low exploring rats in the expression profiles of several genes involved neurotransmission and intracellular signalling pathways mediating synaptic plasticity.

ACKNOWLEDGEMENTS

The studies described in this dissertation were supported by grants from the Estonian Science Foundation (Nos. 4531 and 6492), the Estonian Ministry of Education and Science (Nos. 0814, 0182643 and 0182734), the European Commission Framework 5 Project RECEPTOR HETERODIMER (QLG3-CT-2001-01056), the European Commission Framework 6 Integrated Project NEWMOOD (LSHMCT-2004-503474), the Ontario Mental Health Foundation, the Canadian Institutes for Health Research, the Dr. Karolina Jus estate, the Medland family, the O'Rorke family, the Rockert family, the Essel Foundation, Constance E. Lieber and Stephen Lieber.

Prof. Svante Ross (AstraZeneca, Sweden) generously donated DSP-4, and Prof. Leslie Iversen (ML Laboratories/Panos Therapeutics, UK) devazepide and L-365,260. We thank Dr. H.-C. Guan for excellent technical assistance.

I would like to acknowledge many people who in one way or another have contributed to the completion of this thesis. First and most importantly, I would like to express my deepest gratitude to my supervisor, Professor Jaanus Harro, for his excellent guidance, incredible generosity with patience and time, for sharing his ideas and challenging me to shape my own.

My sincere thanks go to Tanel, for accompanying me on this academic journey through doctoral studies and sharing the joys and agonies along the way. I would also like to extend my gratitude to Kadri, Marika, Denis, Margus, Karita, and others from the psychophysiology group – many of you have been directly involved in the studies that make up this thesis, but all of you have made this time enjoyable.

Completion of this dissertation would have been much more difficult had it not been for the concern and the unwavering support from my parents, my sisters and their families, my friends, and my parents-in-law – thank you all. Finally, I am immensely grateful to my nearest and dearest, Kristjan, Martin, Markus, and Emilia, for your love, patience and encouragement, and for keeping my life in proper perspective and balance.

WEB RESOURCES

The URLs for resources presented herein are as follows:

Bioconductor, http://www.bioconductor.org
DAVID, http://david.abcc.ncifcrf.gov
Gene Ontology (GO), http://www.geneontology.org
Kyoto Encyclopedia of Genes and Genomes (KEGG), http://www.genome.ad.jp/kegg
PUMA, http://www.bioinf.man.ac.uk/resources/puma

REFERENCES

Agid, O., Mamo, D., Ginovart, N., Vitcu, I., Wilson, A.A., Zipursky, R.B., Kapur, S. (2006) Striatal vs extrastriatal dopamine D2 receptors in antipsychotic response – a double-blind pet study in schizophrenia. *Neuropsychopharmacology*, 32, 1209–1215.

Antoniou, K., Papathanasiou, G., Papalexi, E., Hyphantis, T., Nomikos, G.G., Spyraki, C., Papadopoulou-Daifoti, Z. (2008) Individual responses to novelty are associated with differences in behavioral and neurochemical profiles. *Behav Brain Res*, 187, 462–472.

Archer, T. (1983) Signalled and unsignalled avoidance impairments following noradrenaline depletion with DSP4: An hypothesis incorporating an associative and a non-associative factor. *Scand J Psychol*, 24, 75–87.

Archer, T., Fredriksson, A., Jonsson, G., Lewander, T., Mohammed, A.K., Ross, S.B., Soderberg, U. (1986) Central noradrenaline depletion antagonizes aspects of d-amphetamine-induced hyperactivity in the rat. *Psychopharmacology (Berl)*, 88, 141–146.

Armario, A., Gavalda, A., Marti, O. (1988) Forced swimming test in rats: Effect of desipramine administration and the period of exposure to the test on struggling behavior, swimming, immobility and defecation rate. *Eur J Pharmacol*, 158, 207–212.

Aston-Jones, G., Bloom, F.E. (1981) Activity of norepinephrine-containing locus coeruleus neurons in behaving rats anticipates fluctuations in the sleep-waking cycle. *J Neurosci*, 1, 876–886.

Aston-Jones, G., Bloom, F.E. (1981) Norepinephrine-containing locus coeruleus neurons in behaving rats exhibit pronounced responses to non-noxious environmental stimuli. *J Neurosci*, 1, 887–900.

Aston-Jones, G., Rajkowski, J., Cohen, J. (1999) Role of locus coeruleus in attention and behavioral flexibility. *Biol Psychiatry*, 46, 1309–1320.

Bardo, M.T., Donohew, R.L., Harrington, N.G. (1996) Psychobiology of novelty seeking and drug seeking behavior. *Behav Brain Res*, 77, 23–43.

Beaulieu, J.M. (2007) Not only lithium: Regulation of glycogen synthase kinase-3 by antipsychotics and serotonergic drugs. *Int J Neuropsychopharmacol*, 10, 3–6.

Beaulieu, J.M., Zhang, X., Rodriguiz, R.M., Sotnikova, T.D., Cools, M.J., Wetsel, W.C., Gainetdinov, R.R., Caron, M.G. (2008) Role of GSK3 beta in behavioral abnormalities induced by serotonin deficiency. *Proc Natl Acad Sci U S A*, 105, 1333–1338.

Becker, J.B., Rudick, C.N., Jenkins, W.J. (2001) The role of dopamine in the nucleus accumbens and striatum during sexual behavior in the female rat. *J. Neurosci.*, 21, 3236–3241.

Beinfeld, M.C., Connolly, K., Pierce, R.C. (2001) OLETF (otsuka long-evans tokushima fatty) rats that lack the CCK 1 (A) receptor develop less behavioral sensitization to repeated cocaine treatment than wild type LETO (long evans tokushima otsuka) rats. *Peptides*, 22, 1285–1290.

Berridge, C.W., Dunn, A.J. (1990) DSP-4-induced depletion of brain norepinephrine produces opposite effects on exploratory behavior 3 and 14 days after treatment. *Psychopharmacology (Berl)*, 100, 504–508.

Berridge, C.W., Waterhouse, B.D. (2003) The locus coeruleus-noradrenergic system: Modulation of behavioral state and state-dependent cognitive processes. *Brain Res Brain Res Rev*, 42, 33–84.

Berridge, K.C., Robinson, T.E. (1998) What is the role of dopamine in reward: Hedonic impact, reward learning, or incentive salience? *Brain Res Brain Res Rev*, 28, 309–369.

Blacker, D., Broberger, C., Ogren, S.O., Hokfelt, T. (1997) Cholecystokinin b receptor antagonists enhance the locomotor response to the n-methyl-d-aspartate antagonists phencyclidine and dizocilpine maleate. *Neuroscience*, 76, 1057–1067.

Bolstad, B.M., Irizarry, R.A., Astrand, M., Speed, T.P. (2003) A comparison of normalization methods for high density oligonucleotide array data based on variance and bias. *Bioinformatics*, 19, 185–193.

Boothman, L., Raley, J., Denk, F., Hirani, E., Sharp, T. (2006) In vivo evidence that 5-HT_{2C} receptors inhibit 5-HT neuronal activity via a gabaergic mechanism. *Br J Pharmacol*, 149, 861–869.

Borta, A., Schwarting, R.K. (2005) Inhibitory avoidance, pain reactivity, and plus-maze behavior in wistar rats with high versus low rearing activity. *Physiol Behav*, 84, 387–396.

Bourin, M., Malinge, M., Vasar, E., Bradwejn, J. (1996) Two faces of cholecystokinin: Anxiety and schizophrenia. *Fundam Clin Pharmacol*, 10, 116–126.

Boyce-Rustay, J.M., Holmes, A. (2006) Genetic inactivation of the NMDA receptor NR2A subunit has anxiolytic- and antidepressant-like effects in mice. *Neuropsychopharmacology*, 31, 2405–2414.

Briand, L.A., Flagel, S.B., Seeman, P., Robinson, T.E. (2008) Cocaine self-administration produces a persistent increase in dopamine $D2^{High}$ receptors. *Eur Neuropsychopharmacol*, 18, 551–556.

Bunzeck, N., Düzel, E. (2006) Absolute coding of stimulus novelty in the human substantia nigra/VTA. *Neuron*, 51, 369–379.

Cadoni, C., Chiara, G.D. (2007) Differences in dopamine responsiveness to drugs of abuse in the nucleus accumbens shell and core of Lewis and Fischer 344 rats. *J Neurochem*, 103, 487–499.

Cain, M.E., Denehy, E.D., Bardo, M.T. (2008) Individual differences in amphetamine self-administration: The role of the central nucleus of the amygdala. *Neuropsychopharmacology*, 33, 1149–1161.

Carobrez, A.P., Bertoglio, L.J. (2005) Ethological and temporal analyses of anxiety-like behavior: The elevated plus-maze model 20 years on. *Neurosci Biobehav Rev*, 29, 1193–1205.

Chen, J.T., Lu, D.H., Chia, C.P., Ruan, D.Y., Sabapathy, K., Xiao, Z.C. (2005) Impaired long-term potentiation in c-Jun N-terminal kinase 2-deficient mice. *J Neurochem*, 93, 463–473.

Ciani, L., Salinas, P.C. (2005) Wnts in the vertebrate nervous system: From patterning to neuronal connectivity. *Nat Rev Neurosci*, 6, 351–362.

Cinar, R., Freund, T.F., Katona, I., Mackie, K., Szucs, M. (2008) Reciprocal inhibition of G-protein signaling is induced by CB_1 cannabinoid and $GABA_B$ receptor interactions in rat hippocampal membranes. *Neurochem Int*, 52, 1402–1409.

Clemett, D.A., Punhani, T., Duxon, M.S., Blackburn, T.P., Fone, K.C. (2000) Immunohistochemical localisation of the 5-HT2C receptor protein in the rat CNS. *Neuropharmacology*, 39, 123–132.

Crawley, J.N. (1991) Cholecystokinin-dopamine interactions. *Trends Pharmacol Sci*, 12, 232–236.

Crawley, J.N. (1992) Subtype-selective cholecystokinin receptor antagonists block cholecystokinin modulation of dopamine-mediated behaviors in the rat mesolimbic pathway. *J. Neurosci.*, 12, 3380–3391.

Crawley, J.N. (1994) Cholecystokinin modulates dopamine-mediated behaviors. Differential actions in medial posterior versus anterior nucleus accumbens. *Ann N Y Acad Sci*, 713, 138–142.

Crawley, J.N., Corwin, R.L. (1994) Biological actions of cholecystokinin. *Peptides*, 15, 731–755.

Crusio, W.E. (2001) Genetic dissection of mouse exploratory behaviour. *Behav Brain Res*, 125, 127–132.

Crusio, W.E., van Abeelen, J.H. (1986) The genetic architecture of behavioural responses to novelty in mice. *Heredity*, 56, 55–63.

Dahlström, A., Fuxe, K. (1964) Localization of monoamines in the lower brain stem. *Experientia*, 20, 398–399.

Darracq, L., Blanc, G., Glowinski, J., Tassin, J.P. (1998) Importance of the noradrenaline-dopamine coupling in the locomotor activating effects of d-amphetamine. *J Neurosci*, 18, 2729–2739.

Dauge, V., Beslot, F., Matsui, T., Roques, B.P. (2001) Mutant mice lacking the cholecystokinin2 receptor show a dopamine-dependent hyperactivity and a behavioral sensitization to morphine. *Neurosci Lett*, 306, 41–44.

Davis, K.L., Kahn, R.S., Ko, G., Davidson, M. (1991) Dopamine in schizophrenia: A review and reconceptualization. *Am J Psychiatry*, 148, 1474–1486.

De Leonibus, E., Verheij, M.M.M., Mele, A., Cools, A. (2006) Distinct kinds of novelty processing differentially increase extracellular dopamine in different brain regions. *European Journal of Neuroscience*, 23, 1332–1340.

Deakin, J.F.W. (1996) 5-HT, antidepressant drugs and the psychosocial origins of depression. *J Psychopharmacol*, 10, 31–38.

Delini-Stula, A., Mogilnicka, E., Hunn, C., Dooley, D.J. (1984) Novelty-oriented behavior in the rat after selective damage of locus coeruleus projections by dsp-4, a new noradrenergic neurotoxin. *Pharmacol Biochem Behav*, 20, 613–618.

DeSousa, N.J., Wunderlich, G.R., De Cabo, C., Vaccarino, F.J. (1999) The expression of behavioral sensitization to amphetamine: Role of cck(a) receptors. *Pharmacol Biochem Behav*, 62, 31–37.

Devoto, P., Flore, G., Saba, P., Fa, M., Gessa, G.L. (2005) Co-release of noradrenaline and dopamine in the cerebral cortex elicited by single train and repeated train stimulation of the locus coeruleus. *BMC Neurosci*, 6, 31.

Di Chiara, G., Imperato, A. (1988) Drugs abused by humans preferentially increase synaptic dopamine concentrations in the mesolimbic system of freely moving rats. *Proc Natl Acad Sci U S A*, 85, 5274–5278.

Di Lullo, S.L., Martin-Iverson, M.T. (1991) Presynaptic dopaminergic neurotransmission mediates amphetamine-induced unconditioned but not amphetamine-conditioned locomotion and defecation in the rat. *Brain Res*, 568, 45–54.

Dietz, D.M., Tapocik, J., Gaval-Cruz, M., Kabbaj, M. (2005) Dopamine transporter, but not tyrosine hydroxylase, may be implicated in determining individual differences in behavioral sensitization to amphetamine. *Physiol Behav*, 86, 347–355.

Drouin, C., Darracq, L., Trovero, F., Blanc, G., Glowinski, J., Cotecchia, S., Tassin, J.P. (2002) Alpha1b-adrenergic receptors control locomotor and rewarding effects of psychostimulants and opiates. *J Neurosci*, 22, 2873–2884.

Dunning, M.J., Smith, M.L., Ritchie, M.E., Tavare, S. (2007) beadarray: R classes and methods for illumina bead-based data. *Bioinformatics*, 23, 2183–2184.

Epel, E., Jimenez, S., Brownell, K., Stroud, L., Stoney, C., Niaura, R. (2004) Are stress eaters at risk for the metabolic syndrome? *Ann NY Acad Sci*, 1032, 208–210.

Erb, S.M., Parker, L.A. (1994) Individual differences in novelty-induced activity do not predict strength of amphetamine-induced place conditioning. *Pharmacol Biochem Behav*, 48, 581–586.

Exner, M., Clark, D. (1993) Behaviour in the novel environment predicts responsiveness to d-amphetamine in the rat: A multivariate approach. *Behav Pharmacol*, 4, 47–56.

Feenstra, M.G.P., Botterblom, M.H.A., Mastenbroek, S. (2000) Dopamine and noradrenaline efflux in the prefrontal cortex in the light and dark period: Effects of novelty and handling and comparison to the nucleus accumbens. *Neuroscience*, 100, 741–748.

Feifel, D., Priebe, K., Shilling, P.D. (2001) Startle and sensorimotor gating in rats lacking CCK-A receptors. *Neuropsychopharmacology*, 24, 663–670.

Ferrucci, M., Gesi, M., Lenzi, P., Soldani, P., Ruffoli, R., Pellegrini, A., Ruggieri, S., Paparelli, A., Fornai, F. (2002) Noradrenergic loss enhances MDMA toxicity and induces ubiquitin-positive striatal whorls. *Neurol Sci*, 23 Suppl 2, S75–76.

File, S.E., Hyde, J.R. (1978) Can social interaction be used to measure anxiety? *Br J Pharmacol*, 62, 19–24.

File, S.E., Seth, P. (2003) A review of 25 years of the social interaction test. *Eur J Pharmacol*, 463, 35–53.

Foley, P., Gerlach, M., Double, K.L., Riederer, P. (2004) Dopamine receptor agonists in the therapy of parkinson's disease. *J Neural Transm*, 111, 1375–1446.

Fornai, F., Torracca, M.T., Bassi, L., D'Errigo, D.A., Scalori, V., Corsini, G.U. (1996) Norepinephrine loss selectively enhances chronic nigrostriatal dopamine depletion in mice and rats. *Brain Res*, 735, 349–353.

Frame, S., Cohen, P. (2001) GSK3 takes centre stage more than 20 years after its discovery. *Biochem J*, 359, 1–16.

George, S.R., Watanabe, M., Di Paolo, T., Falardeau, P., Labrie, F., Seeman, P. (1985) The functional state of the dopamine receptor in the anterior pituitary is in the high affinity form. *Endocrinology*, 117, 690–697.

Gingras, M.A., Cools, A.R. (1997) Different behavioral effects of daily or intermittent dexamphetamine administration in Nijmegen high and low responders. *Psychopharmacology (Berl)*, 132, 188–194.

Giorgi, O., Piras, G., Corda, M.G. (2007) The psychogenetically selected Roman high- and low-avoidance rat lines: A model to study the individual vulnerability to drug addiction. *Neurosci Biobehav Rev*, 31, 148–163.

Gould, T.D., Chen, G., Manji, H.K. (2004) In vivo evidence in the brain for lithium inhibition of glycogen synthase kinase-3. *Neuropsychopharmacology*, 29, 32–38.

Gould, T.D., Dow, E.R., O'Donnell, K.C., Chen, G., Manji, H.K. (2007) Targeting signal transduction pathways in the treatment of mood disorders: Recent insights into the relevance of the Wnt pathway. *CNS Neurol Disord Drug Targets*, 6, 193–204.

Gould, T.D., Einat, H., O'Donnell, K.C., Picchini, A.M., Schloesser, R.J., Manji, H.K. (2007) Beta-catenin overexpression in the mouse brain phenocopies lithium-sensitive behaviors. *Neuropsychopharmacology*, 32, 2173–2183.

Gould, T.D., O'Donnell, K.C., Picchini, A.M., Dow, E.R., Chen, G., Manji, H.K. (2008) Generation and behavioral characterization of beta-catenin forebrain-specific conditional knock-out mice. *Behav Brain Res*, 189, 117–125.

Grenhoff, J., North, R.A., Johnson, S.W. (1995) Alpha 1-adrenergic effects on dopamine neurons recorded intracellularly in the rat midbrain slice. *Eur J Neurosci*, 7, 1707–1713.

Grenhoff, J., Svensson, T.H. (1993) Prazosin modulates the firing pattern of dopamine neurons in rat ventral tegmental area. *Eur J Pharmacol*, 233, 79–84.

Gresch, P.J., Sved, A.F., Zigmond, M.J., Finlay, J.M. (1995) Local influence of endogenous norepinephrine on extracellular dopamine in rat medial prefrontal cortex. *J Neurochem*, 65, 111–116.

Griebel, G. (1995) 5-hydroxytryptamine-interacting drugs in animal models of anxiety disorders: More than 30 years of research. *Pharmacol Ther*, 65, 319–395.

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

Haile, C.N., During, M.J., Jatlow, P.I., Kosten, T.R., Kosten, T.A. (2003) Disulfiram facilitates the development and expression of locomotor sensitization to cocaine in rats. *Biol Psychiatry*, 54, 915–921.

Haller, J., Matyas, F., Soproni, K., Varga, B., Barsy, B., Nemeth, B., Mikics, E., Freund, T.F., Hajos, N. (2007) Correlated species differences in the effects of cannabinoid ligands on anxiety and on gabaergic and glutamatergic synaptic transmission. *Eur J Neurosci*, 25, 2445–2456.

Haller, J., Varga, B., Ledent, C., Barna, I., Freund, T.F. (2004) Context-dependent effects of CB1 cannabinoid gene disruption on anxiety-like and social behaviour in mice. *Eur J Neurosci*, 19, 1906–1912.

Haller, J., Varga, B., Ledent, C., Freund, T.F. (2004) CB1 cannabinoid receptors mediate anxiolytic effects: Convergent genetic and pharmacological evidence with CB1-specific agents. *Behav Pharmacol*, 15, 299–304.

Handley, S.L., Mithani, S. (1984) Effects of alpha-adrenoceptor agonists and antagonists in a maze-exploration model of 'fear'-motivated behaviour. *Naunyn Schmiedebergs Arch Pharmacol*, 327, 1–5.

Harro, J. (1993) Measurement of exploratory behaviour in rodents. In: Methods in neurosciences, vol. 14 (Conn, R.A., ed), pp 359–377 San Diego: Academic Press.

Harro, J. (2006) CCK and NPY as anti-anxiety treatment targets: Promises, pitfalls, and strategies. *Amino Acids*, 31, 215–230.

Harro, J., Jossan, S.S., Oreland, L. (1992) Changes in cholecystokinin receptor binding in rat brain after selective damage of locus coeruleus projections by DSP-4 treatment. *Naunyn Schmiedebergs Arch Pharmacol*, 346, 425–431.

Harro, J., Kiivet, R.A., Lang, A., Vasar, E. (1990) Rats with anxious or non-anxious type of exploratory behaviour differ in their brain CCK-8 and benzodiazepine receptor characteristics. *Behav Brain Res*, 39, 63–71.

Harro, J., Meriküla, A., Lepiku, M., Modiri, A.R., Rinken, A., Oreland, L. (2000) Lesioning of locus coeruleus projections by DSP-4 neurotoxin treatment: Effect on amphetamine-induced hyperlocomotion and dopamine D2 receptor binding in rats. *Pharmacol Toxicol*, 86, 197–202.

Harro, J., Tõnissaar, M., Eller, M., Kask, A., Oreland, L. (2001) Chronic variable stress and partial 5-HT denervation by parachloroamphetamine in the rat: effects on behaviour and monoamine neurochemistry. *Brain Res*, 899, 227–239.

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

Harro, J., Oreland, L., Vasar, E., Bradwejn, J. (1995) Impaired exploratory behaviour after DSP-4 treatment in rats: Implications for the increased anxiety after noradrenergic denervation. *Eur Neuropsychopharmacol*, 5, 447–455.

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

Heffner, T.G., Hartman, J.A., Seiden, L.S. (1980) Feeding increases dopamine metabolism in the rat brain. *Science*, 208, 1168–1170.

Heisler, L.K., Zhou, L., Bajwa, P., Hsu, J., Tecott, L.H. (2007) Serotonin 5-HT_{2C} receptors regulate anxiety-like behavior. *Genes Brain Behav*, 6, 491–496.

Henniger, M.S., Ohl, F., Holter, S.M., Weissenbacher, P., Toschi, N., Lorscher, P., Wigger, A., Spanagel, R., Landgraf, R. (2000) Unconditioned anxiety and social behaviour in two rat lines selectively bred for high and low anxiety-related behaviour. *Behav Brain Res*, 111, 153–163.

Herdegen, T., Mielke, K., Kallunki, T. (1999) Review : C-Jun and the c-Jun amino-terminal kinases: Bipotential components of the neuronal stress response. *Neuroscientist*, 5, 147–154.

Hino, S., Kishida, S., Michiue, T., Fukui, A., Sakamoto, I., Takada, S., Asashima, M., Kikuchi, A. (2001) Inhibition of the Wnt signaling pathway by Idax, a novel Dvl-binding protein. *Mol Cell Biol*, 21, 330–342.

Hooks, M.S., Colvin, A.C., Juncos, J.L., Justice, J.B., Jr. (1992) Individual differences in basal and cocaine-stimulated extracellular dopamine in the nucleus accumbens using quantitative microdialysis. *Brain Res*, 587, 306–312.

Hooks, M.S., Jones, G.H., Neill, D.B., Justice, J.B., Jr. (1992) Individual differences in amphetamine sensitization: Dose-dependent effects. *Pharmacol Biochem Behav*, 41, 203–210.

Hooks, M.S., Juncos, J.L., Justice, J.B., Jr., Meiergerd, S.M., Povlock, S.L., Schenk, J.O., Kalivas, P.W. (1994) Individual locomotor response to novelty predicts selective alterations in D1 and D2 receptors and mRNAs. *J Neurosci*, 14, 6144–6152.

Hooks, M.S., Kalivas, P.W. (1994) Involvement of dopamine and excitatory amino acid transmission in novelty-induced motor activity. *J Pharmacol Exp Ther*, 269, 976–988.

Horvitz, J.C. (2000) Mesolimbocortical and nigrostriatal dopamine responses to salient non-reward events. *Neuroscience*, 96, 651–656.

Huang, W., Sherman, B.T., Tan, Q., Collins, J.R., Alvord, W.G., Roayaei, J., Stephens, R., Baseler, M.W., Lane, H.C., Lempicki, R.A. (2007) The DAVID gene functional classification tool: A novel biological module-centric algorithm to functionally analyze large gene lists. *Genome Biol*, 8, R183.

Hughes, Z.A., Stanford, S.C. (1998) A partial noradrenergic lesion induced by DSP-4 increases extracellular noradrenaline concentration in rat frontal cortex: A microdialysis study in vivo. *Psychopharmacology (Berl)*, 136, 299–303.

Häidkind, R., Kivastik, T., Eller, M., Kolts, I., Oreland, L., Harro, J. (2002) Denervation of the locus coeruleus projections by treatment with the selective neurotoxin DSP-4 [N (2-chloroethyl)-n-ethyl-2-bromobenzylamine] reduces dopamine release potential in the nucleus accumbens shell in conscious rats. *Neurosci Lett*, 332, 79–82.

Hökfelt, T., Rehfeld, J.F., Skirboll, L., Ivemark, B., Goldstein, M., Markey, K. (1980) Evidence for coexistence of dopamine and CCK in meso-limbic neurones. *Nature*, 285, 476–478.

Ikemoto, S., Panksepp, J. (1999) The role of nucleus accumbens dopamine in motivated behavior: A unifying interpretation with special reference to reward-seeking. *Brain Res Brain Res Rev*, 31, 6–41.

Jodogne, C., Marinelli, M., Le Moal, M., Piazza, P.V. (1994) Animals predisposed to develop amphetamine self-administration show higher susceptibility to develop contextual conditioning of both amphetamine-induced hyperlocomotion and sensitization. *Brain Res*, 657, 236–244.

Josselyn, S.A., De Cristofaro, A., Vaccarino, F.J. (1997) Evidence for CCK_A receptor involvement in the acquisition of conditioned activity produced by cocaine in rats. *Brain Res*, 763, 93–102.

Josselyn, S.A., Franco, V.P., Vaccarino, F.J. (1996) Devazepide, a CCK_A receptor antagonist, impairs the acquisition of conditioned reward and conditioned activity. *Psychopharmacology (Berl)*, 123, 131–143.

Josselyn, S.A., Vaccarino, F.J. (1995) Interaction of CCK_B receptors with amphetamine in responding for conditioned rewards. *Peptides*, 16, 959–964.

Kabbaj, M. (2006) Individual differences in vulnerability to drug abuse: The high responders/low responders model. *CNS Neurol Disord Drug Targets*, 5, 513–520.

Kabbaj, M., Devine, D.P., Savage, V.R., Akil, H. (2000) Neurobiological correlates of individual differences in novelty-seeking behavior in the rat: Differential expression of stress-related molecules. *J. Neurosci.*, 20, 6983–6988.

Kadoya, T., Yamamoto, H., Suzuki, T., Yukita, A., Fukui, A., Michiue, T., Asahara, T., Tanaka, K., Asashima, M., Kikuchi, A. (2002) Desumoylation activity of Axam, a novel Axin-binding protein, is involved in downregulation of beta-catenin. *Mol Cell Biol*, 22, 3803–3819.

Kalueff, A.V., Nutt, D.J. (2007) Role of GABA in anxiety and depression. *Depress Anxiety*, 24, 495–517.

Kask, A., Harro, J., Tuomaine, P., Rago, L., Mannisto, P.T. (1997) Overflow of noradrenaline and dopamine in frontal cortex after [N-(2-chloroethyl)-n-ethyl-2-bromobenzylamine] (DSP-4) treatment: In vivo microdialysis study in anaesthetized rats. *Naunyn Schmiedebergs Arch Pharmacol*, 355, 267–272.

Klebaur, J.E., Bardo, M.T. (1999) Individual differences in novelty seeking on the playground maze predict amphetamine conditioned place preference. *Pharmacol Biochem Behav*, 63, 131–136.

Klebaur, J.E., Bevins, R.A., Segar, T.M., Bardo, M.T. (2001) Individual differences in behavioral responses to novelty and amphetamine self-administration in male and female rats. *Behav Pharmacol*, 12, 267–275.

Kõks, S., Volke, V., Veraksits, A., Runkorg, K., Sillat, T., Abramov, U., Bourin, M., Huotari, M., Männistö, P.T., Matsui, T., Vasar, E. (2001) Cholecystokinin2 receptor-deficient mice display altered function of brain dopaminergic system. *Psychopharmacology (Berl)*, 158, 198–204.

Krügel, U., Schraft, T., Kittner, H., Kiess, W., Illes, P. (2003) Basal and feeding-evoked dopamine release in the rat nucleus accumbens is depressed by leptin. *Eur J Pharmacol*, 482, 185–187.

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

Lategan, A.J., Marien, M.R., Colpaert, F.C. (1992) Suppression of nigrostriatal and mesolimbic dopamine release in vivo following noradrenaline depletion by DSP-4: A microdialysis study. *Life Sci*, 50, 995–999.

Lepiku, M., Rinken, A., Jarv, J., Fuxe, K. (1996) Kinetic evidence for isomerization of the dopamine receptor-raclopride complex. *Neurochem Int*, 28, 591–595.

Lesch, K.P., Gutknecht, L. (2004) Focus on the 5-HT1A receptor: Emerging role of a gene regulatory variant in psychopathology and pharmacogenetics. *Int J Neuropsychopharmacol*, 7, 381–385.

Leyton, M. (2007) Conditioned and sensitized responses to stimulant drugs in humans. *Prog Neuropsychopharmacol Biol Psych*, 31, 1601–1613.

Li, F., Chong, Z.Z., Maiese, K. (2005) Vital elements of the Wnt-Frizzled signaling pathway in the nervous system. *Curr Neurovasc Res*, 2, 331–340.

Li, Q., Wichems, C.H., Ma, L., Van de Kar, L.D., Garcia, F., Murphy, D.L. (2003) Brain region-specific alterations of 5-HT2A and 5-HT2C receptors in serotonin transporter knockout mice. *J Neurochem*, 84, 1256–1265.

Li, X., Rosborough, K.M., Friedman, A.B., Zhu, W., Roth, K.A. (2007) Regulation of mouse brain glycogen synthase kinase-3 by atypical antipsychotics. *Int J Neuropsychopharmacol*, 10, 7–19.

Li, X., Zhu, W., Roh, M.S., Friedman, A.B., Rosborough, K., Jope, R.S. (2004) In vivo regulation of glycogen synthase kinase-3beta (GSK-3beta) by serotonergic activity in mouse brain. *Neuropsychopharmacology*, 29, 1426–1431.

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

Liu, R., Jolas, T., Aghajanian, G. (2000) Serotonin 5-HT_2 receptors activate local GABA inhibitory inputs to serotonergic neurons of the dorsal raphe nucleus. *Brain Res*, 873, 34–45.

Logan, C.Y., Nusse, R. (2004) The Wnt signaling pathway in development and disease. *Annu Rev Cell Dev Biol*, 20, 781–810.

Marin, M.T., Cruz, F.C., Planeta, C.S. (2007) Chronic restraint or variable stresses differently affect the behavior, corticosterone secretion and body weight in rats. *Physiol Behav*, 90, 29–35.

Marinelli, M., White, F.J. (2000) Enhanced vulnerability to cocaine self-administration is associated with elevated impulse activity of midbrain dopamine neurons. *J Neurosci*, 20, 8876–8885.

Marshall, F.H., Barnes, S., Hughes, J., Woodruff, G.N., Hunter, J.C. (1991) Cholecystokinin modulates the release of dopamine from the anterior and posterior nucleus accumbens by two different mechanisms. *J Neurochem*, 56, 917–922.

Matrov, D., Kolts, I., Harro, J. (2007) Cerebral oxidative metabolism in rats with high and low exploratory activity. *Neurosci Lett*, 413, 154–158.

McCoy, M.T., Becker, K.G., Wood, W.H. III, Teichberg, D.R., Brown, R.W., Kostrzewa, R.M., Cadet, J.L. (2004) Differential quinpirole-induced transcriptional responses in the rat striatum and nucleus accumbens: evidence from microarray analysis. *Society of Neuroscience Astracts Program No. 123.9*

Menard, J., Treit, D. (1999) Effects of centrally administered anxiolytic compounds in animal models of anxiety. *Neurosci Biobehav Rev*, 23, 591–613.

Misslin, R., Herzog, F., Koch, B., Ropartz, P. (1982) Effects of isolation, handling and novelty on the pituitary – adrenal response in the mouse. *Psychoneuroendocrinology*, 7, 217–221.

Moran, T.H., Schwartz, G.J. (1994) Neurobiology of cholecystokinin. *Crit Rev Neurobiol*, 9, 1–28.

Moroz, I.A., Pecina, S., Schallert, T., Stewart, J. (2004) Sparing of behavior and basal extracellular dopamine after 6-hydroxydopamine lesions of the nigrostriatal pathway in rats exposed to a prelesion sensitizing regimen of amphetamine. *Exp Neurol*, 189, 78–93.

Mällo, T., Alttoa, A., Kõiv, K., Tõnissaar, M., Eller, M., Harro, J. (2007) Rats with persistently low or high exploratory activity: Behaviour in tests of anxiety and depression, and extracellular levels of dopamine. *Behav Brain Res*, 177, 269–281.

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

Noble, F., Wank, S.A., Crawley, J.N., Bradwejn, J., Seroogy, K.B., Hamon, M., Roques, B.P. (1999) International union of pharmacology. XXI. Structure, distribution, and functions of cholecystokinin receptors. *Pharmacol Rev*, 51, 745–781.

Ogata, H., Goto, S., Sato, K., Fujibuchi, W., Bono, H., Kanehisa, M. (1999) KEGG: Kyoto encyclopedia of genes and genomes. *Nucleic Acids Res*, 27, 29–34.

Otter, M.H., Matto, V., Sõukand, R., Skrebuhhova, T., Allikmets, L., Harro, J. (1997) Characterization of rat exploratory behavior using the exploration box test. *Methods Find Exp Clin Pharmacol*, 19, 683–691.

Parks, C.L., Robinson, P.S., Sibille, E., Shenk, T., Toth, M. (1998) Increased anxiety of mice lacking the serotonin1a receptor. *Proc Natl Acad Sci U S A*, 95, 10734–10739.

Paulson, P.E., Robinson, T.E. (1995) Amphetamine-induced time-dependent sensitization of dopamine neurotransmission in the dorsal and ventral striatum: A microdialysis study in behaving rats. *Synapse*, 19, 56–65.

Peineau, S., Bradley, C., Taghibiglou, C., Doherty, A., Bortolotto, Z.A., Wang, Y.T., Collingridge, G.L. (2008) The role of GSK-3 in synaptic plasticity. *Br J Pharmacol*, 153 Suppl 1, S428–437.

Pfaus, J.G., Damsma, G., Nomikos, G.G., Wenkstern, D.G., Blaha, C.D., Phillips, A.G., Fibiger, H.C. (1990) Sexual behavior enhances central dopamine transmission in the male rat. *Brain Research*, 530, 345–348.

Pfaus, J.G., Damsma, G., Wenkstern, D., Fibiger, H.C. (1995) Sexual activity increases dopamine transmission in the nucleus accumbens and striatum of female rats. *Brain Research*, 693, 21–30.

Phillips, G.D., Le Noury, J., Wolterink, G., Donselaar-Wolterink, I., Robbins, T.W., Everitt, B.J. (1993) Cholecystokinin-dopamine interactions within the nucleus accumbens in the control over behaviour by conditioned reinforcement. *Behav Brain Res*, 55, 223–231.

Piazza, P.V., Deminiere, J.M., Le Moal, M., Simon, H. (1989) Factors that predict individual vulnerability to amphetamine self-administration. *Science*, 245, 1511–1513.

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

Pierce, R.C., Kalivas, P.W. (1997) A circuitry model of the expression of behavioral sensitization to amphetamine-like psychostimulants. *Brain Res Brain Res Rev*, 25, 192–216.

Pierce, R.C., Kumaresan, V. (2006) The mesolimbic dopamine system: The final common pathway for the reinforcing effect of drugs of abuse? *Neurosci Biobehav Rev*, 30, 215–238.

Porsolt, R.D., Anton, G., Blavet, N., Jalfre, M. (1978) Behavioural despair in rats: A new model sensitive to antidepressant treatments. *Eur J Pharmacol*, 47, 379–391.

Prut, L., Belzung, C. (2003) The open field as a paradigm to measure the effects of drugs on anxiety-like behaviors: A review. *Eur J Pharmacol*, 463, 3–33.

Pycock, C.J., Donaldson, I.M., Marsden, C.D. (1975) Circling behaviour produced by unilateral lesions in the region of the locus coeruleus in rats. *Brain Res*, 97, 317–329.

Raman, M., Chen, W., Cobb, M.H. (2007) Differential regulation and properties of MAPKs. *Oncogene*, 26, 3100–3112.

Ramboz, S., Oosting, R., Amara, D.A., Kung, H.F., Blier, P., Mendelsohn, M., Mann, J.J., Brunner, D., Hen, R. (1998) Serotonin receptor 1A knockout: An animal model of anxiety-related disorder. *Proc Natl Acad Sci U S A*, 95, 14476–14481.

Rattray, M., Liu, X., Sanguinetti, G., Milo, M., Lawrence, N.D. (2006) Propagating uncertainty in microarray data analysis. *Brief Bioinform*, 7, 37–47.

Rebec, G.V., Grabner, C.P., Johnson, M., Pierce, R.C., Bardo, M.T. (1997) Transient increases in catecholaminergic activity in medial prefrontal cortex and nucleus accumbens shell during novelty. *Neuroscience*, 76, 707–714.

Richardson, N.R., Gratton, A. (2008) Changes in nucleus accumbens dopamine transmission associated with fixed- and variable-time schedule-induced feeding. *Eur J Neurosci*, 27, 2714–2723.

Rinken, A., Finnman, U.B., Fuxe, K. (1999) Pharmacological characterization of dopamine-stimulated [^{35}S]-guanosine 5'(gamma-thiotriphosphate) ([^{35}S]GTPγS) binding in rat striatal membranes. *Biochem Pharmacol*, 57, 155–162.

Rommelfanger, K.S., Weinshenker, D., Miller, G.W. (2004) Reduced MPTP toxicity in noradrenaline transporter knockout mice. *J Neurochem*, 91, 1116–1124.

Rosario, L.A., Abercrombie, E.D. (1999) Individual differences in behavioral reactivity: Correlation with stress-induced norepinephrine efflux in the hippocampus of sprague-dawley rats. *Brain Res Bull*, 48, 595–602.

Ross, S.B. (1976) Long-term effects of N-2-chlorethyl-N-ethyl-2-bromobenzylamine hydrochloride on noradrenergic neurones in the rat brain and heart. *Br J Pharmacol*, 58, 521–527.

Saigusa, T., Tuinstra, T., Koshikawa, N., Cools, A.R. (1999) High and low responders to novelty: Effects of a catecholamine synthesis inhibitor on novelty-induced changes in behaviour and release of accumbal dopamine. *Neuroscience*, 88, 1153–1163.

Sanacora, G., Zarate, C.A., Krystal, J.H., Manji, H.K. (2008) Targeting the glutamatergic system to develop novel, improved therapeutics for mood disorders. *Nat Rev Drug Discov*, 7, 426–437.

Schank, J.R., Ventura, R., Puglisi-Allegra, S., Alcaro, A., Cole, C.D., Liles, L.C., Seeman, P., Weinshenker, D. (2006) Dopamine beta-hydroxylase knockout mice have alterations in dopamine signaling and are hypersensitive to cocaine. *Neuropsychopharmacology*, 31, 2221–2230.

Seeman, P., McCormick, P.N., Kapur, S. (2007) Increased dopamine $D2^{high}$ receptors in amphetamine-sensitized rats, measured by the agonist [^{3}H](+)PHNO. *Synapse*, 61, 263–267.

Seeman, P., Schwarz, J., Chen, J.F., Szechtman, H., Perreault, M., McKnight, G.S., Roder, J.C., Quirion, R., Boksa, P., Srivastava, L.K., Yanai, K., Weinshenker, D., Sumiyoshi, T. (2006) Psychosis pathways converge via $D2^{high}$ dopamine receptors. *Synapse*, 60, 319–346.

Seeman, P., Tallerico, T., Ko, F. (2003) Dopamine displaces [^{3}H]domperidone from high-affinity sites of the dopamine D2 receptor, but not [^{3}H]raclopride or [^{3}H]spiperone in isotonic medium: Implications for human positron emission tomography. *Synapse*, 49, 209–215.

Seeman, P., Tallerico, T., Ko, F., Tenn, C., Kapur, S. (2002) Amphetamine-sensitized animals show a marked increase in dopamine D2 high receptors occupied by endogenous dopamine, even in the absence of acute challenges. *Synapse*, 46, 235–239.

Seeman, P., Weinshenker, D., Quirion, R., Srivastava, L.K., Bhardwaj, S.K., Grandy, D.K., Premont, R.T., Sotnikova, T.D., Boksa, P., El-Ghundi, M., O'Dowd B, F., George, S.R., Perreault, M.L., Mannisto, P.T., Robinson, S., Palmiter, R.D., Tallerico, T. (2005) Dopamine supersensitivity correlates with $D2^{high}$ states, implying many paths to psychosis. *Proc Natl Acad Sci U S A*, 102, 3513–3518.

Segal, D.S., Kuczenski, R. (1994) Behavioral pharmacology of amphetamine. In: Amphetamine and its analogues: Psychopharmacology, toxicology and abuse(Cho, A.K.,Segal, D.S., eds), pp 115–150 San Diego: Academic Press, Inc.

Serrats, J., Mengod, G., Cortes, R. (2005) Expression of serotonin 5-HT2C receptors in GABAergic cells of the anterior raphe nuclei. *J Chem Neuroanat*, 29, 83–91.

Shiah, I.S., Yatham, L.N. (1998) GABA function in mood disorders: An update and critical review. *Life Sci*, 63, 1289–1303.

Shilling, P.D., Feifel, D. (2002) SR146131, a cholecystokinin-A receptor agonist, antagonizes prepulse inhibition deficits produced by dizocilpine and DOI. *Psychopharmacology (Berl)*, 164, 285–293.

Shuto, T., Seeman, P., Kuroiwa, M., Nishi, A. (2008) Repeated administration of a dopamine D1 receptor agonist reverses the increased proportions of striatal dopamine $D1^{high}$ and $D2^{high}$ receptors in methamphetamine-sensitized rats. *Eur J Neurosci*, 27, 2551–2557.

Simola, N., Morelli, M., Seeman, P. (2008) Increase of dopamine $D2^{high}$ receptors in the striatum of rats sensitized to caffeine motor effects. *Synapse*, 62, 394–397.

Sweatt, J.D. (2001) The neuronal MAP kinase cascade: A biochemical signal integration system subserving synaptic plasticity and memory. *J Neurochem*, 76, 1–10.

Zald, D.H., Boileau, I., El-Dearedy, W., Gunn, R., McGlone, F., Dichter, G.S., Dagher, A. (2004) Dopamine transmission in the human striatum during monetary reward tasks. *J Neurosci*, 24, 4105–4112.

Zhang, X., Beaulieu, J.M., Sotnikova, T.D., Gainetdinov, R.R., Caron, M.G. (2004) Tryptophan hydroxylase-2 controls brain serotonin synthesis. *Science*, 305, 217.

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

Zuckerman, M. (1996) The psychobiological model for impulsive unsocialized sensation seeking: A comparative approach. *Neuropsychobiology*, 34, 125–129.

Taghzouti, K., Lamarque, S., Kharouby, M., Simon, H. (1999) Interindividual differences in active and passive behaviors in the forced-swimming test: Implications for animal models of psychopathology. *Biol Psychiatry*, 45, 750–758.

Thiel, C.M., Muller, C.P., Huston, J.P., Schwarting, R.K. (1999) High versus low reactivity to a novel environment: Behavioural, pharmacological and neurochemical assessments. *Neuroscience*, 93, 243–251.

Tieppo, C.A., Ferreira, F.S., Sassatani, A.S., Felicio, L.F., Nasello, A.G. (2000) Opposite modulation of apomorphine- or amphetamine-induced stereotypy by antagonists of CCK receptors. *Eur J Pharmacol*, 387, 189–196.

Tõnissaar, M., Philips, M.A., Eller, M., Harro, J. (2004) Sociability trait and serotonin metabolism in the rat social interaction test. *Neurosci Lett*, 367, 309–312.

Toth, M. (2003) 5-HT1A receptor knockout mouse as a genetic model of anxiety. *Eur J Pharmacol*, 463, 177–184.

Vaccarino, F.J., Rankin, J. (1989) Nucleus accumbens cholecystokinin (CCK) can either attenuate or potentiate amphetamine-induced locomotor activity: Evidence for rostral-caudal differences in accumbens CCK function. *Behav Neurosci*, 103, 831–836.

Wallace, K.J., Rosen, J.B. (2001) Neurotoxic lesions of the lateral nucleus of the amygdala decrease conditioned fear but not unconditioned fear of a predator odor: Comparison with electrolytic lesions. *J Neurosci*, 21, 3619–3627.

Vanderschuren, L.J., Beemster, P., Schoffelmeer, A.N. (2003) On the role of noradrenaline in psychostimulant-induced psychomotor activity and sensitization. *Psychopharmacology (Berl)*, 169, 176–185.

Vanderschuren, L.J., Schmidt, E.D., De Vries, T.J., Van Moorsel, C.A., Tilders, F.J., Schoffelmeer, A.N. (1999) A single exposure to amphetamine is sufficient to induce long-term behavioral, neuroendocrine, and neurochemical sensitization in rats. *J Neurosci*, 19, 9579–9586.

Vasar, E., Harro, J., Lang, A., Põld, A., Soosaar, A. (1991) Differential involvement of CCK-A and CCK-B receptors in the regulation of locomotor activity in the mouse. *Psychopharmacology (Berl)*, 105, 393–399.

Weinshenker, D., Miller, N.S., Blizinsky, K., Laughlin, M.L., Palmiter, R.D. (2002) Mice with chronic norepinephrine deficiency resemble amphetamine-sensitized animals. *Proc Natl Acad Sci U S A*, 99, 13873–13877.

Ventura, R., Cabib, S., Alcaro, A., Orsini, C., Puglisi-Allegra, S. (2003) Norepinephrine in the prefrontal cortex is critical for amphetamine-induced reward and meso-accumbens dopamine release. *J Neurosci*, 23, 1879–1885.

Westerink, B.H.C., Teisman, A., De Vries, J.B. (1994) Increase in dopamine release from the nucleus accumbens in response to feeding: A model to study interactions between drugs and naturally activated dopaminergic neurons in the rat brain. *Naunyn-Schmiedeberg's Archives of Pharmacology*, 349, 230–235.

Vezina, P. (1993) Amphetamine injected into the ventral tegmental area sensitizes the nucleus accumbens dopaminergic response to systemic amphetamine: An in vivo microdialysis study in the rat. *Brain Research*, 605, 332–337.

Vezina, P. (2004) Sensitization of midbrain dopamine neuron reactivity and the self-administration of psychomotor stimulant drugs. *Neuroscience & Biobehavioral Reviews*, 27, 827–839.

Volkow, N.D., Fowler, J.S., Wang, G.J., Swanson, J.M., Telang, F. (2007) Dopamine in drug abuse and addiction: Results of imaging studies and treatment implications. *Arch Neurol*, 64, 1575–1579.

White, D.A., Kalinichev, M., Holtzman, S.G. (2007) Locomotor response to novelty as a predictor of reactivity to aversive stimuli in the rat. *Brain Res*, 1149, 141–148.

Wise, R.A., Bozarth, M.A. (1985) Brain mechanisms of drug reward and euphoria. *Psychiatr Med*, 3, 445–460.

Wright, D.E., Seroogy, K.B., Lundgren, K.H., Davis, B.M., Jennes, L. (1995) Comparative localization of serotonin$_{1A}$, $_{1C}$, and $_{2}$ receptor subtype mRNAs in rat brain. *J Comp Neurol*, 351, 357–373.

Wunderlich, G.R., DeSousa, N.J., Vaccarino, F.J. (2000) Cholecystokinin modulates both the development and the expression of behavioral sensitization to amphetamine in the rat. *Psychopharmacology (Berl)*, 151, 283–290.

Wunderlich, G.R., Rotzinger, S., Bush, D.E., DeSousa, N.J., Vaccarino, F.J. (2004) Cholecystokinin modulation of locomotor behavior in rats is sensitized by chronic amphetamine and chronic restraint stress exposure. *Brain Res*, 1001, 95–107.

Ögren, S.O., Archer, T., Johansson, C. (1983) Evidence for a selective brain nor-adrenergic involvement in the locomotor stimulant effects of amphetamine in the rat. *Neurosci Lett*, 43, 327–331.

Young, A.M.J., Joseph, M.H., Gray, J.A. (1992) Increased dopamine release in vivo in nucleus accumbens and caudate nucleus of the rat during drinking: A microdialysis study. *Neuroscience*, 48, 871–876.

SUMMARY IN ESTONIAN

ROTI UUDISTAVA KÄITUMISE NEUROKEEMILINE REGULATSIOON: DOPAMIINERGILISE JA NORADRENERGILISE NÄRVIÜLEKANDE ROLLIST

Uudistamine hõlmab igasuguseid käitumisi, mis aitavad keskkonna ning selles paiknevate objektide kohta informatsiooni koguda. Bioloogilises mõttes on uudistav käitumine kohastumuslik, sest võimaldab leida uusi toiduallikaid, vett, peidupaiku ning potentsiaalseid partnereid; kuid ka potentsiaalselt ohtlik, kuna aktiivselt ringi liikuv loom kujutab endast kiskjatele kergemat saaki. Seega määrab uudistamisaktiivsuse tundmatus keskkonnas alati uudishimu ning hirmu tasakaal.

Uudistamisel põhinevaid loomkatseid on käitumisfarmakoloogias laialdaselt kasutatud. Et vähemalt osaliselt langevad neurobioloogilised mehhanismid, mis uudsete stiimulitega kokkupuutudes aktiveeruvad, kokku nendega, mille kaudu vahenduvad sõltuvusttekitavate ainete toimed, on uudistamistestidele leitud rakendust uimastisõltuvuse uurimises. Uudistavas käitumises sisalduva hirmu aspekti tõttu on uudsusel põhinevatest katsetest kasu ka ärevuse- ja depressioonilaadsete seisundite uurimisel.

Üks traditsiooniliselt enimkasutatud uudsusel põhinevaid käitumisteste on nn. avarvälja test, milles loom asetatakse ristkülikukujulise põhiplaaniga eredalt valgustatud alale. Looma käitumise puhul selles sunduudistamise testis on aga raske eristada uudistamismotivatsiooni üldisest liikumisaktiivsusest. "Tõesema" pildi roti uudistamisaktiivsuse tasemest saab siis, kui kasutada selle mõõtmiseks katseseadet, kus loom võib vabalt valida, kas siseneda uudsesse keskkonda või mitte. Meie laboris välja töötatud uudiskast on just taoline vabal uudistamisel põhinev katsekast, mis koosneb suurest avatud väljast, millele on paigutatud mõned uudsed objektid ning mille ühel lühemal küljel asub väike kaanega suletav kamber, kuhu loomal on võimalik peituda.

Uudistamise neurokeemiline regulatsioon on keerukas, selles osalevad mitmesugused neurotransmitter- ja neuromodulaatorsüsteemid. Uudsed stiimulid võimendavad närviülekande aktiivsust nii keskajust alguse saavates dopamiiniradades kui ajutüve *locus coeruleus*'e (LC) ülenevas noradrenergilises juhtetees; nende virgatsainesüsteemide rollile uudistava käitumise regulatsioonis käesolev väitekiri keskendubki. Dopamiinergilise neurotransmissiooni aktiivsust moduleerib kahe retseptoritüübi kaudu endogeenne neuropeptiid koletsüstokiniin. Rottide uudistamisaktiivsuses on leitud olulisi individuaalseid erinevusi, mis on seotud loomade erineva tundlikkusega mitmesugustele farmakoloogilistele ning käitumuslikele manipulatsioonidele.

Et aju dopamiinergilise närviülekande ning koletsüstokiniini interaktsioon võib olla oluline psühhootiliste häirete ning uimastisõltuvuse patogeneesis, uuriti **Artiklis I**, kuidas endogeenne koletsüstokiniin (CCK) mõjutab amfetamiini toimet roti uudistamisaktiivsusele ning amfetamiinist-tingitud käitumus-

liku sensitisatsiooni kujunemisele. Selgus, et CCK_1 retseptori selektiivne antagonist devasepiid blokeeris täielikult ning CCK_2 retseptori antagonist L-365,260 võimendas amfetamiini uudistavat käitumist stimuleerivat efekti ja käitumusliku sensitisatsiooni kujunemist, ehkki kummalgi antagonistil iseseisvat uudistamisaktiivsust moduleerivat toimet ei ole; seega võib järeldada, et endogeenselt vabastatava CCK roll amfetamiini käitumuslike efektide väljendumises sõltub oluliselt sellest, millist tüüpi retseptorile ta mõjub.

Leiti, et uudistamisaktiivsuse alusel uudiskastis on võimalik jagada rotid kahte gruppi, mida iseloomustavad vastavalt kõrge uudistamismotivatsioon/madal ärevus ning madal uudistamismotivatsioon/kõrge ärevus. Taolised individuaalsed erinevused uudistamiskäitumises on väga püsivad ning ennustavad loomade käitumist teisteski ärevust ja depressiooni modelleerivates käitumiskatsetes (**Artikkel II**). Nimelt on kõrgema uudistamisaktiivsusega rotid ka teistes loomkatsetes aktiivsemad ning vähem ärevad. Palju- ja väheuudistavate loomade käitumuslike erinevustega kaasnevad olulised erinevused keskaju dopamiinisüsteemi aktiivsuses. Palju-uudistavatel rottidel on rakuvälise dopamiini tase nii baas- kui stimuleeritud tingimustes kõrgem, samuti on neil suurem funktsionaalsete, dopamiini suhtes kõrge afiinsusega D_2 retseptorite osakaal juttkehas (**Artiklid II ja III**). Käitumuslik sensitisatsioon amfetamiini korduvmanustamisele tekib ainult väheuudistavatel loomadel ning ainult palju-uudistavatel loomadel väheneb D_2 retseptorite tundlikkus (mõõdetuna dopamiinist-stimuleeritud [^{35}S]GTPγS seondumisena) amfetamiini korduva manustamise järel (**Artiklid IV ja V**).

Osalise *locus coeruleus*'e (LC) kahjustuse mõju uudistavale ja amfetamiinist-stimuleeritud käitumisele, käitumusliku sensitisatsiooni kujunemisele ning dopamiinisüsteemile oli palju- ja väheuudistavatel loomadel erinev (**Artiklid IV ja V**). Seega sõltub dopamiini poolt vahendatud käitumine oluliselt LC noradrenergilise süsteemi terviklikkusest ning dopamiini-noradrenaliini interaktsiooni moduleerivad individuaalsed erinevused uudistamisaktiivsuses.

Geenidekspressiooni profiilide erinevuste uurimine genoomiüleste mikrokiipide abil on üheks võimaluseks – käitumis- ja farmakoloogiliste eksperimentide kõrval – selgitada välja neurobioloogilisi mehhanisme, mis võiksid aluseks olla variatsioonidele spontaanses uudistamisaktiivsuses. **Artiklis VI** leiti, et võrreldes palju-uudistavate loomadega on väheuudistavatel rottidel üle- või alaekspresseeritud hulk geene, mis kodeerivad virgatsaine retseptoreid või nende alaühikuid ning osalevad mitmesugustes sünaptilist plastilisust reguleerivates rakusisestes signaaliradades.

PUBLICATIONS

I

Alttoa, A., Harro, J. (2004).
Effect of CCK_1 and CCK_2 receptor blockade
on amphetamine-stimulated exploratory behavior and sensitization
to amphetamine. *European Neuropsychopharmacology*, 14, 324–331.

II

Mällo, T., Alttoa, A., Kõiv, K., Tõnissaar, M., Eller, M., Harro, J. (2007).
Rats with persistently low or high exploratory activity:
behaviour in tests of anxiety and depression,
and extracellular levels of dopamine.
Behavioural Brain Research, 177, 269–281.

III

Alttoa, A., Seeman, P., Kõiv, K., Eller, M., Harro, J. Rats with persistently high exploratory activity have both higher extracellular dopamine levels and higher proportion of D_2^{High} receptors in the striatum. *Synapse* (in press).

RATS WITH PERSISTENTLY HIGH EXPLORATORY ACTIVITY HAVE BOTH HIGHER EXTRACELLULAR DOPAMINE LEVELS AND HIGHER PROPORTION OF D_2^{High} RECEPTORS IN THE STRIATUM

Aet Alttoa[1], Philip Seeman[2], Kadri Kõiv[1], Marika Eller[1], Jaanus Harro[1*]

[1] Department of Psychology, Centre of Behavioural and Health Sciences, University of Tartu, Tiigi 78, 50410 Tartu, Estonia
[2] Department of Pharmacology, University of Toronto, Toronto, Ontario, Canada M5S 1A8

[*] Corresponding author: Jaanus Harro

ABSTRACT

Increases in both striatal dopamine release and the proportion of the D_2 receptors in the high affinity state (D_2^{High}) accompany the behavioural sensitization to psychostimulants, but it is not known whether the physiological substrate of the inter-individual differences locomotor and exploratory behaviour is similar. Thus, we examined whether persistently high spontaneous exploratory activity is associated with extracellular dopamine as well as the proportion of D_2^{High} in the striatum. Extracellular dopamine levels were found to be significantly higher in rats with high exploratory activity (high explorers, HE) as compared to low explorers (LE) in baseline conditions as well as after administration of amphetamine (0,5 mg/kg, i.p.). Also, the HE animals had significantly higher proportion of striatal D_2^{High} receptors than the LE-rats (43.8 ± 4.4% and 22.5 ± 1.5%, respectively) . Thus, the present findings support the notion that concomitant higher extracellular dopamine levels and the proportion of D_2^{High} receptors in the striatum, whether naturally occurring and persistent or pharmacologically induced, are causally related to high behavioural activity.

Keywords: receptor binding, microdialysis, individual differences, locomotion, psychostimulant sensitization

Repeated administration of psychostimulants results in persistently increased locomotor activity in response to the drug (Vezina, 2004). It is not only the locomotor response that becomes sensitized after such treatments; the psychostimulant-induced dopamine release in the striatum and the nucleus accumbens

is potentiated as well (Vezina, 1993; Paulson and Robinson, 1995; Vanderschuren et al., 1999). Recently, it has been shown that dopaminergic supersensitivity, either elicited by sensitization to psychostimulants or otherwise, is accompanied by the modifications in the affinity states of the striatal D_2 receptors (Seeman et al., 2005; 2006). The D_2 receptors exist in high-affinity (D_2^{High}) and low-affinity (D_{2Low}) states, and the former has been proposed to be the physiologically functional state (George et al., 1985). In psychostimulant-sensitized rats, the proportion of the high-affinity D_2 receptors in the striatum markedly increases, likely contributing to their heightened behavioural sensitivity to psychostimulants and dopamine agonists (Seeman et al., 2002; Seeman et al., 2007; Shuto et al., 2008; Simola et al., 2008; Briand et al., 2008).

Large inter-individual differences physiologically exist in locomotor and exploratory behaviour, and these differences can predict sensitivity to psychoactive drugs (Kabbaj, 2006). It is not known whether the physiological basis of such individual differences similarly involves both higher dopamine output and a larger proportion of high affinity receptors. In our model of persistent differences in novelty-related behaviour (Alttoa et al., 2005; Mällo et al., 2007), extracellular dopamine levels in the striatum were found to be significantly higher in rats with high exploratory activity (high explorers, HE) as compared to low explorers (LE) (Mällo et al., 2007). The aim of the present study was to examine whether the spontaneous high behavioural activity in the HE-rats is associated with both elevated extracellular dopamine levels and a larger proportion of the D_2 receptors in the high affinity state in the striatum.

Twenty two male Wistar rats (6 in the microdialysis experiment and 16 in the binding experiment) from Scanbur BK AB, Sweden, weighing 293–332 g at the beginning of the experiments, were used. The animals were classified as high or low explorers on the basis of their spontaneous exploratory activity in the exploration box as described in Mällo et al. (2007). The animals were housed four per cage in standard polypropylene cages with free access to food (Lactamin R35, Sweden) and water. The temperature in the animal room was maintained at at 21 ± 2°C, and 12:12 h light-darkness cycle (lights on at 08:30 h) was applied.

In vivo microdialysis was carried out as previously described (Mällo et al., 2007). In brief, the animals (HE: n = 3; LE: n = 3) were anaesthetized with chloral hydrate (350 mg/kg, i.p.). A home-made concentric microdialysis probe with 7 mm shaft length and 3 mm active tip was implanted into the left dorsal striatum according to the following coordinates: AP: +0.7; ML: +3.0; DV: −7.0 according to Paxinos and Watson (1986). Rats were given about 24 h for recovery and microdialysis procedure was conducted in awake freely moving animals. Six baseline samples were collected in 15-min periods, followed by administration of d-amphetamine (0.5 mg/kg, i.p.) in the beginning of the seventh sample, after which another nine samples were collected. Upon completion of the experiment the animals were deeply anesthetized with chloral hydrate (350 mg/kg, i.p.) and decapitated. The probe placements were

determined according to the atlas of Paxinos and Watson (1986). The quantity of dopamine in the samples was determined by high-performance liquid chromatography with electrochemical detection.

For the measurement of D_2^{High} receptors in the striatum, eight HE and eight LE rats were sacrificed, their striata dissected and frozen at –70°C until used. The striata were homogenized in buffer (4 mg frozen tissue per ml buffer), using a teflon-glass homogenizer with the piston rotating at 500 rpm and 10 up and down strokes of the glass container. The buffer contained 50 mM Tris-HC1 (pH 7.4 at 20°), 1 mM EDTA, 5 mM KCl, 1.5 mM CaC12, 4 mM MgC12 and 120 mM NaCl. [^{3}H]Domperidone was custom synthesized as [phenyl-^{3}H(N)]domperidone (42–68 Ci/mmol) by PerkinElmer Life Sciences Inc., Boston, MA, and used at a final concentration of 2 nM. Because the Kd of [^{3}H]domperidone is 0.47 nM (Seeman et al., 2003) at D_2 for rat striatum, the final concentration of 2 nM occupied 81% of the D_2 receptors, using the equation f = C/(C + Kd), where f is the fraction of receptors occupied by [^{3}H]domperidone, and C is 2 nM.

The competition between dopamine and [^{3}H]domperidone for binding at the receptors was done as follows. Each incubation tube (12 × 75 mm, glass) received, in the following order, 0.5 ml buffer (containing dopamine at various concentrations, and with or without a final concentration of 10 μM S-sulpiride to define nonspecific binding to the dopamine D2 receptors), 0.25 ml [^{3}H]domperidone and 0.25 ml of tissue homogenate. The tubes, containing a total volume of 1 ml, were incubated for 2 h at room temperature (20°), after which the incubates were filtered, using a 12-well cell harvester (Titertek, Skatron, Lier, Norway) and buffer-presoaked glass fiber filter mats (Whatman GF/C). After filtering the incubate, the filter mat was rinsed with buffer for 15 s (7.5 ml buffer). The filters were pushed out and placed in scintillation polystyrene minivials (7 ml, 16 × 54 mm; Valley Container Inc., Bridgeport, Conn.). The minivials received 4 ml each of scintillant (Research Products International Corp., Mount Prospect, IL), and were monitored 6 h later for tritium in a Beckman LS5000TA scintillation spectrometer at 52% efficiency. The specific binding of [^{3}H]domperidone was defined as total binding minus that in the presence of 10 μM S-sulpiride.

The proportion of D_2^{High} receptors in the striata was measured by the competition of dopamine with [^{3}H]domperidone, as previously described (Seeman et al., 2003). Dopamine inhibited the binding of [^{3}H]domperidone in two phases. At low concentrations, corresponding to the high-affinity state of the dopamine D_2 receptor, D_2^{High}, dopamine inhibited the binding of [^{3}H]domperidone between 1 nM and 100 nM dopamine. A second phase of inhibition occurred above 100 nM dopamine. The demarcation between the two phases was sharp and unambiguous, clearly and readily permitting the measurement of the D_2^{High} component as a percent of the total amount of specific [^{3}H]domperidone binding, as defined by the presence of 10 μM S-sulpiride.

For the statistical analysis one-way ANOVA (Activity; HE vs LE) was used, with repeated measures factor added where appropriate. *Post hoc* comparisons were done with Fisher's LSD test.

In the microdialysis experiment, the basal and amphetamine-stimulated dopamine release was significantly higher in the striatum of HE animals compared to the LE animals [Activity effect ($F(1,14)=55.76$; $p<0.01$)] (Figure 1A). This reproduced our previous observation that HE-rats have increased extracellular dopamine levels in striatum, both in baseline conditions and after an acute administration of amphetamine (Mällo et al., 2007). Also, HE animals had more striatal D_2 receptors in the high-affinity state [Activity effect, $F(1,14)=20.47$; $p<0.001$)]. The proportion of D_2^{High} receptors was 22.5 ± 1.5% in the striata of LE-rats and 43.8 ± 4.4% in the striata of HE-rats, and there was no overlap in the data points between the two groups (Figure 1B).

The main finding of this study is that rats with persistently high exploratory activity have elevated number of D_2 receptors in the functional high affinity state in the striatum, which coincides with higher extracellular dopamine levels in these animals.

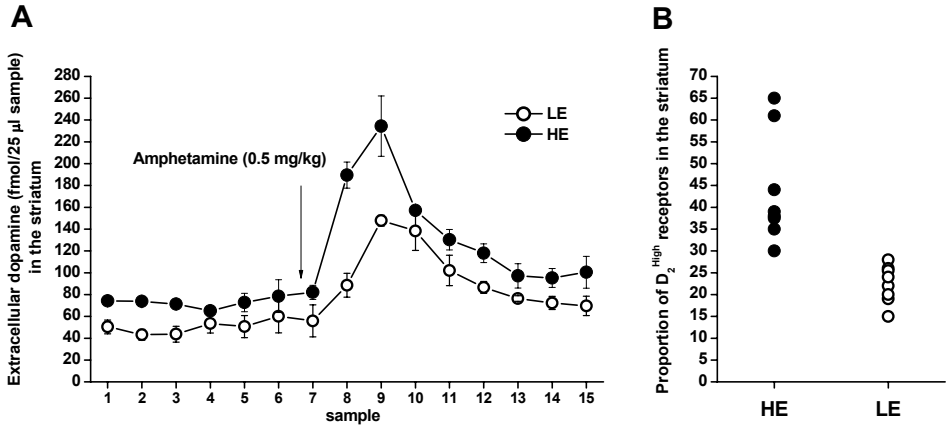

Figure 1. Extracellular dopamine (A) and proportion of D_2^{High} receptors (B) in the striatum of rats with high or low exploratory activity. HE – high explorers, LE – low explorers. Data expressed as means ± S.E.M.

Interestingly, the magnitude of difference in the number of D_2^{High} receptors in the HE- and LE-rats is very similar to that observed in recent studies comparing psychostimulant-sensitized and control rats. For example, from control levels of about 20%, an approximately twofold increase (to 40–45%) in the proportion of striatal D_2^{High} receptors in rats has been demonstrated after psychomotor-sensitizing regimens of amphetamine (Seeman et al., 2002; 2007), methamphetamine (Shuto et al., 2008), caffeine (Simola et al., 2008), and cocaine (Briand et al., 2008). In terms of the proportion of D_2 receptors in the high-affinity state, the

HE-rats thus appear as naturally-occuring 'sensitized' animals. Our microdialysis data suggest that in a novel environment the novelty-induced dopamine release (see Horvitz, 2000) should be expected to be larger in the high exploring rats. Thus it could be speculated that such more extensive release of dopamine acting on a higher number of the D_2^{High} receptors in the striatum provides the physiological substrate for the increased behavioural activation observed in the rats with high exploratory activity.

In conclusion, the present findings support the notion that concomitant higher extracellular dopamine levels and the proportion of D_2^{High} receptors in the striatum, whether naturally occurring and persistent or pharmacologically induced, are causally related to high behavioural activity.

ACKNOWLEDGEMENTS

We thank Dr. H.-C. Guan for excellent technical assistance. This work was supported by the Estonian Ministry of Education and Science project No 0182643, the EC Framework 6 Integrated Project NEWMOOD (LSHM-CT-2004-503474), the Ontario Mental Health Foundation, the Canadian Institutes for Health Research, the Dr. Karolina Jus estate, the Medland family, the O'Rorke family, the Rockert family, the Essel Foundation, Constance E. Lieber and Stephen Lieber.

REFERENCES

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

Briand LA, Flagel SB, Seeman P, Robinson TE. 2008. Cocaine self-administration produces a persistent increase in dopamine D2High receptors. Eur Neuropsychopharmacol 18:551–556.

George SR, Watanabe M, Di Paolo T, Falardeau P, Labrie F, Seeman P. 1985. The functional state of the dopamine receptor in the anterior pituitary is in the high affinity form. Endocrinology 117:690–697.

Horwitz JC. 2000. Mesolimbocortical and nigrostriatal dopamine responses to salient non-reward events. Neuroscience 96:651–656.

Kabbaj M. 2006. Individual difference in vulnerability to drug abuse: the high responders/low responders model. CNS Neurol Disord Drug Targets 5:513–520.

Mällo T, Alttoa A, Kõiv K, Tõnissaar M, Eller M, Harro J. 2007. Rats with persistently low or high exploratory activity: Behaviour in tests of anxiety and depression, and extracellular levels of dopamine. Behav Brain Res 177**:**269–281.

Paulson PE, Robinson TE. 1995. Amphetamine-induced time-dependent sensitization of dopamine neurotransmission in the dorsal and ventral striatum: a microdialysis study in behaving rats. Synapse 19:56–65.

Paxinos G, Watson C. 1986. The rat brain in stereotaxic coordinates. Second edition. New York: Academic Press.

Seeman P, Tallerico T, Ko F, Tenn C, Kapur S. 2002. Amphetamine-sensitized animals show a marked increase in dopamine $D2^{high}$ receptors occupied by endogenous dopamine, even in the absence of acute challenges. Synapse 46:235–239.

Seeman P, Tallerico T, and Ko F. 2003. Dopamine displaces [^{3}H]domperidone from high-affinity sites of the dopamine D2 receptor, but not [^{3}H]raclopride or [^{3}H]spiperone in isotonic medium: Implications for human positron emission tomography. Synapse 49:209–215.

Seeman P, Weinshenker D, Quirion R, Srivastava LK, Bhardwaj SK, Grandy DK, Premont RT, Sotnikova TD, Boksa P, El-Ghundi M, O'Dowd BF, George SR, Perreault ML, Männisto PT, Robinson S, Palmiter RD, Tallerico T. 2005. Dopamine supersensitivity correlates with $D2^{High}$ states, implying many paths to psychosis. Proc Natl Acad Sci U S A 102:3513–3518.

Seeman P, Schwartz J, Chen JF, Szechtman H, Perreault HM, McKnight GS, Roder JC, Quirion R, Boksa P, Srivastava LK, Yanai K, Weinshenker D, Sumiyoshi T. 2006. Psychosis pathways converge via $D2^{High}$ dopamine receptors. Synapse 60:319–346.

Seeman P, McCormick PN, Kapur S. 2007. Increased dopamine $D2^{High}$ receptors in amphetamine-sensitized rats, measured by the agonist [^{3}H](+)PHNO. Synapse 61:263–267.

Shuto T, Seeman P, Kuroiwa M, Nishi A. 2008. Repeated administration of a dopamine D1 receptor agonist reverses the increased proportions of striatal dopamine D1High and D2High receptors in methamphetamine-sensitized rats. Eur J Neurosci 27:2551–2557.

Simola N, Morelli M, Seeman P. 2008. Increase of dopamine $D2^{High}$ receptors in the striatum of rats sensitized to caffeine motor effects. Synapse 62:394–397.

Vanderschuren LJ, Schmidt ED, De Vries TJ, Van Moorsel TA, Tilders FJ, Schoffelmeer, AN. 1999. A single exposure to amphetamine is sufficient to induce long-term behavioral, neuroendocrine, and neurochemical sensitization in rats. J Neurosci 19:9579–9586.

Vezina P. 1993. Amphetamine injected into the ventral tegmental area sensitizes the nucleus accumbens dopaminergic response to systemic amphetamine: an in vivo microdialysis study in the rat. Brain Res 605:332–337.

Vezina P. 2004. Sensitization of midbrain dopamine neuron reactivity and the self-administration of psychomotor stimulant drugs. Neurosci Biobehav Rev 27:827–839.

IV

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

V

Alttoa, A., Eller, M., Herm, L., Rinken, A., Harro, J. (2007). Amphetamine-induced locomotion, behavioral sensitization to amphetamine, and striatal D_2 receptor function in rats with high or low spontaneous exploratory activity: differences in the role of locus coeruleus. *Brain Research*, 1131, 138–148.

VI

Alttoa, A., Kõiv, K., Hinsley, T.A., Brass, A., Harro, J.
Differential gene expression in rats with persistently
high or low exploratory activity.
(*in manuscript*).

DIFFERENTIAL GENE EXPRESSION IN RATS WITH PERSISTENTLY HIGH OR LOW EXPLORATORY ACTIVITY

Aet Alttoa[1], Kadri Kõiv[1], Timothy A. Hinsley[2], Andrew Brass[2], Jaanus Harro[1]

[1] Department of Psychology, Centre of Behavioural and Health Sciences, University of Tartu, Tiigi 78, 50410 Tartu, Estonia
[2] Department of Computer Science, University of Manchester, Manchester M13 9PL, United Kingdom

ABSTRACT

Behaviour in novel environments is a function of conflicting motivations to explore unfamiliar surroundings (curiosity) and avoid potential danger (neophobia). Affective disorders are often accompanied by changes in these motivational processes, thus, we have developed the exploration box test that allows separation of animals from the outbred Wistar rat line belonging into clusters of inherent and stable high anxiety/low motivation to explore and low anxiety/high motivation to explore (low explorers and high explorers, LE and HE, respectively). The present study made use of a genome-wide microarray analysis on the Illumina platform to identify differentially expressed genes in three brain regions, previously known to be involved in mood disorders (i.e., raphe, hippocampus and the frontal cortex), that may underlie the distinct behavioural phenotypes of HE- and LE-animals. Compared to the HE-rats, 816 up- or downregulated genes in the raphe, 809 in the hippocampus, and 602 in the frontal cortex of the LE-animals were found. Several genes encoding neurotransmitter synthesizing enzymes (*Tph2*), receptors (*Htr2c*, *Htr1a*, *Cnr1*), or receptor subunits (*Gabra1*, *Gabra4*, *Gabrg1*, *Gabrg2*, *Grin2a*, *Gria3*) were differentially expressed in the LE- and HE-rats. Using the DAVID 2007 Functional Annotation Tool, the analysis of KEGG pathway terms associated with these genes identified a significant overrepresentation of genes involved in the Wnt signalling pathway among the differentially expressed transcripts in all three brain regions. The *Gsk3b* gene coding for glycogen synthase kinase-3 was upregulated, while the *Ctnnb1* gene coding for beta-catenin was downregulated in the LE- vs. HE-rats. This suggests less active Wnt signalling in the LE-animals. In the hippocampus and the frontal cortex, but not in the raphe, a significant overrepresentation of genes involved in the MAPK signalling, long-term potentiation and long-term depression pathways was found. Thus, the behavioural differences between rats with persistently high or low exploratory activity may, at least partly, be related to differences in the synaptic plasticity in the projection areas of the raphe nuclei.

INTRODUCTION

Exploratory behaviour is evoked by novel stimuli and consists of behavioural acts and postures that allow obtaining information about the environment and novel objects therein, and as such, is crucial to survival as it increases the possibility to find various resources, e.g. food, water, shelter, etc. (Crucio and Abeelen, 1986). Animals' behaviour in unfamiliar contexts is always a function of their natural curiosity and fear of the unknown (Harro, 1993).

The baseline behavioural reactivity in rodents has been found to be extremely variable, and the differences in exploratory behaviour are accompanied by differences in monoaminergic neurotransmission and sensitivity to pharmacological manipulations (Piazza et al., 1989; Hooks et al., 1992; Rouge-Pont et al., 1998). In recent years, animals bred or preselected for individual variability are proving to be increasingly useful in modelling aspects of human psychopathology, particularly addiction and anxiety-related disorders (Piazza et al., 1989; Landgraf and Wigger, 2002; Kabbaj, 2006; White et al., 2007).

One of the ways to measure baseline novelty-related behaviour in male Wistar rats is the exploration box test (Otter et al., 1997). This emergence-type test typically yields a rather bimodal separation of rats into groups of high motivation to explore/low neophobia and low motivation to explore/high neophobia (HE and LE, respectively), with relatively few animals occupying the middle ground. These differences in spontaneous exploratory activity levels are stable over time, persist with repeated testing and predict activity in other behavioural tests (Mällo et al., 2007). The differences in the behaviour of HE- and LE-rats are due to novelty and not differences in their locomotor drive, as the two groups did not diverge in their locomotor response to a novel home cage. However, compared to the HE-animals, the LE-rats were less active and more anxious in the elevated plus-maze and displayed passive coping strategies in the forced swimming test. In fear conditioning test, the LE group acquired a more persistent association between neutral and stressful stimuli (Mällo et al., 2007). The LE-rats also develop behavioural sensitization to repeated amphetamine treatment more readily (Alttoa et al., 2007).

The distinct behavioural profiles of HE- and LE-rats are also reflected in the differences in their neurochemistry. We have shown previously that the HE animals have higher basal and stimulated extracellular dopamine levels in the striatum but not in the nucleus accumbens (Mällo et al., 2007). There are also differences between high and low exploring groups in the properties of the serotonergic system in the prefrontal cortex and hippocampus (Mällo et al., 2008) that may underlie their anxiety-related behaviours. Specifically, although serotonin release in the prefrontal cortex and dentate gyrus in baseline conditions is similar in HE- and LE-rats, the number of serotonin transporter binding sites is higher in the LE animals. Thus, after blocking the serotonin transporters in the prefrontal cortex by a local infusion of citalopram, the serotonin release in that brain region is significantly increased in the LE group (Mällo et al., 2008).

The drug-free, amphetamine-stimulated and amphetamine-sensitized behaviour of the HE- and LE-rats is differentially regulated by noradrenaline, as revealed after a partial noradrenergic denervation of locus coeruleus projections with a selective neurotoxin DSP-4 (Alttoa et al., 2005; Alttoa et al., 2007). Furthermore, the two groups have distinct cerebral metabolic activity in areas that are involved in defensive behaviours and cognitive processing of sensory stimuli (Matrov et al., 2007).

Taken together, the LE-rats demonstrate a behavioural profile that is characterized by low motivation to explore, high anxiety, high vulnerability to stress and cognitive rigidity – the core symptoms of depression –, and thus, resembles a 'depressed' phenotype. This notion is also, albeit to an extent, supported by the neurochemical and pharmacological findings in this group.

Although behavioural and pharmacological studies conducted in animals that differ in some persistent trait – e.g., anxiety-related behaviour –, are of undeniable value in probing the neurobiological mechanisms of the disorders they are designed to model, to investigate the genetic differences between the 'healthy' and the 'depressed', and identify novel mechanisms that contribute to or underlie the observed phenotype, genome-wide microarrays can be used. Thus, the aim of the current study was to identify the genes that contribute to the differences between HE- and LE-rats by examining the gene expression profiles in three brain regions implicated in the pathogenesis of depression and anxiety (the raphe, the hippocampus and the frontal cortex) using Illumina RatRef-12 BeadChips.

MATERIALS AND METHODS

Animals

Male Wistar rats (n = 24, weighing 275–472 g, Scanbur BK AB, Sweden) were housed four per cage in standard polypropylene cages in a light controlled room (12-h light/dark cycle; lights on at 7:00 a.m.) maintained at 22°C. Food and water were available *ad libitum*. All behavioural experiments were carried out between 13:00 and 19:00. 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.

Apparatus

The exploration box test was conducted as described previously (Harro et al., 1995 and Otter et al., 1997). The exploration box was made of metal and consisted of a 0.5 × 1 m open area (side walls 40 cm) with a 20 × 20 × 20 cm small compartment attached to one of the shorter sides of the open area. The open area was divided into eight squares of equal size. In the open area, four objects, three novel and one familiar (a glass jar, a cardboard box, a wooden

handle and a food pellet) were situated in certain places (which remained the same throughout the experiment). The small compartment had its floor covered with wood shavings and was directly linked to the open area through an opening (size 20 × 20 cm). The apparatus was cleaned with a dampened cloth after each animal. The exploration test was initiated by placing a rat into the small compartment, which was then covered with a lid for the time of the test. A single test session lasted 15 min and the following behavioural parameters were registered: (1) latency (of entering open area with all four paws), (2) number of entries into the open area, (3) time spent exploring on the open area, (4) line crossings, (5) rearings and (6) number of unfamiliar object investigations. To provide an index of exploration the scores of line crossing, rearing and object investigation were summed for each animal and thus (7) the sum of exploratory events obtained.

General procedure

All rats were tested on two consecutive days for their spontaneous exploratory activity in the exploration box. The animals were classified as high or low explorers (HE or LE, respectively) based on the sum of the exploratory events on the second exposure to the exploration box test. Only animals that did not emerge from the small chamber were categorized as low explorers, while rats belonging to the upper quartile of the number of exploratory events on the second exposure were classified as high explorers. Of these animals, 12 HE and 12 LE animals were selected for the microarray experiments. The rats were decapitated 10 days after the last behavioural experiment, their brains removed and the raphe nuclei, hippocampi and frontal cortex dissected on ice. The brain tissues were immediately frozen in liquid nitrogen and stored at –80 ºC until RNA extraction.

RNA preparation and expression profiling

The frozen brain tissue samples were homogenized in 2 ml tubes containing 350 µl of lysis buffer RA1 (Macherey-Nagel GmbH & Co. KG, Germany) containing 3.5 µl β-mercaptoethanol. Isolation of total RNA from the lysate, including the 15 min DNase I treatment, was carried out using the Macherey-Nagel NucleoSpin RNA II kit (Macherey-Nagel GmbH & Co. KG, Germany) according to the manufacturer's protocol. The eluates were stored at –80°C.

RNA quality control and microarray hybridizations and processing were outsourced to ServiceXS. The quality of RNA in individual and pooled samples was checked with Agilent Bioanalyzer. For each microarray, three total RNA samples were pooled and for both groups (HE and LE) four hybridizations were performed per brain area. Thus, 24 Illumina RatRef-12 BeadChips (Illumina Inc., USA) were used in total.

Data normalisation

The raw microarray data was processed using the beadarray (Dunning et al., 2007) preprocessCore (Bolstad et al., 2003) and puma (Rattray et al., 2006; PUMA) R packages of bioconductor (Bioconductor). In the beadarray package the backgroundCorrect method used was "minimum", whilst variables for the createbeadsummaryData method were as follows: log = TRUE, n=10. Data was normalised using the quantile normalisation algorithm from the preprocessCore package. Three functions from the puma package were used: pumaComb, pumaDE and write.reslts all with default settings.

Two filtering criteria were used to define statistically significant differentially expressed genes. Firstly, the expression fold change must be greater than (+ or –) 0.5. Secondly, the minimum pplr (Minpplr), which is simply the minimum of either the pplr statistic produced by puma or 1-pplr, was less than 0.001.

KEGG and GO analysis using DAVID 2007 Functional Annotation Tool

To assist in the functional interpretation of the microarray data, we used the online DAVID 2007 Functional Annotation Tool (http://david.abcc.ncifcrf.gov/home.jsp) to search the databases of Kyoto Encyclopedia of Genes and Genomes (KEGG) (http://www.genome.ad.jp/kegg/) and Gene Ontology (GO) (http://www.geneontology.org/). A major component of the KEGG database is the pathway module which consists of regularly updated manually curated graphical diagrams that represent the current knowledge of the molecular interactions involved in various cellular processes (Ogata et al., 1999). The GO database is helpful in assigning the differentially expressed genes to functional categories based on the three ontologies: biological process, molecular function and cellular component.

The gene identifiers (GID accessions) associated with each microarray feature were retrieved from the Illumina array datafiles and used in DAVID searches. All gene GID's on the Illumina array were used as the 'background' gene list in DAVID, whilst only the GID's for the statistically significant differentially expressed genes (filtered using the criteria above) were used as the 'gene list' in DAVID.

The depth of the GO Biological Process enrichment analysis in DAVID was set at Level 3. To identify statistically significantly over-represented GO terms and KEGG pathways within the lists of differentially expressed genes, the group enrichment score (modified Fisher Exact p-value) (Huang et al., 2007) cutoff was set at 0.05. The results were ordered by the group enrichment score.

Additionally, the lists of differentially expressed genes were manually browsed to find the genes that encode neurotransmitter synthesizing enzymes, neurotransmitter receptor or receptor subunits.

RESULTS

Individual differences in spontaneous exploratory activity

On the second exposure to the exploration box, which was the basis for the differentiation between high exploring (HE) and low exploring (LE) animals, none of the LE-rats emerged from the small chamber of the exploration box, while the mean number of exploratory events of the HE group was 216 (SEM 6.78). Thus, the HE-rats were significantly more active than the LE animals in their spontaneous novelty-related behaviour in the exploration box (Mann-Whitney *U* test, $p<0.00001$ for latency, line crossings, object investigations, rearings, entries into the open area, time spent exploring, and sum of exploratory events).

Gene expression analysis

In the present study, between the HE- and LE-rats, 816 transcripts in the raphe, 809 in the hippocampus, and 602 in the frontal cortex were significantly differentially expressed (see Table 1 for the numbers of up- and downregulated genes in LE- vs. HE-rats in each brain region). Moreover, there was considerable overlap in the expression profiles between the studied brain areas, as 390 transcripts were changed in the same direction and in common in the raphe, the hippocampus, and the frontal cortex. Of these, 264 genes were downregulated and 156 upregulated in the LE-rats.

The expression of several genes coding for neurotransmitter receptors or receptor subunits that were expected to be biologically relevant to modulating affective symptomatology were significantly altered in the LE vs. HE animals (Table 2). Specifically, in the raphe, *Gabra1*, *Gabrg1*, *Gabrg2*, *Glrb*, and *Htr2c* were downregulated in the LE-rats. Additionally, the tryptophan hydroxylase 2 (*Tph2*) gene was found to be downregulated in the LE animals. In the hippocampus, *Gabra1*, *Gabrg1*, *Gabrg2*, *Glrb*, and *Htr1a* were downregulated; *Grin2a* and *Cnr1* were upregulated in rats with low exploratory activity. In the frontal cortex, compared to the high explorers, LE-rats had lower expression of *Gabra1*, *Gabra4*, *Gabrg1*, *Gabrg2*, and *Glrb*; while the expression of *Gria3*, *Grin2a*, and *Cnr1* was higher in these animals.

To identify enriched biological themes, the DAVID Functional Annotation Tool was used to map the lists of differentially expressed transcripts onto the Biological Process terms according to the gene ontology of the GO Consortium. Several functional categories, including macromolecule metabolism, transport, cell organization and biogenesis, nervous system development, cellular localization, cellular morphogenesis, neuron differentiation, and ion homeostasis, were over-represented across all brain areas (Figure 1).

Table 1. Significantly differentially expressed transcripts in the raphe, hippocampus and the frontal cortex between rats with high vs. low exploratory activity (at pplr < 0.001).

	Raphe	Hippocampus	Frontal cortex
Upregulated	555	459	322
Downregulated	261	350	280
Total number of differentially expressed transcripts*	816	809	602

* 390 transcripts were differentially expressed in the same direction in all three brain areas

Table 2. Differentially expressed transcripts encoding neurotransmitter synthesizing enzymes, receptors or receptor subunits in the brains of rats with low vs. high exploratory activity (pplr < 0.001).

Genbank accession number	Gene symbol	Gene title	Log ratio		
			Raphe	Hippo-campus	Frontal cortex
NM_012784.3	*Cnr1*	Cannabinoid receptor 1 (brain)		0.76	0.63
NM_183326.1	*Gabra1*	Gamma-aminobutyric acid A receptor, alpha 1	–1.21	–0.77	–0.88
NM_080587.1	*Gabra4*	Gamma-aminobutyric acid A receptor, alpha 4			–0.54
NM_080586.1	*Gabrg1*	Gamma-aminobutyric acid A receptor, gamma 1	–1.30	–0.93	–0.72
NM_183327.1	*Gabrg2*	Gamma-aminobutyric acid A receptor, gamma 2	–1.21	–0.78	–0.79
NM_053296.1	*Glrb*	Glycine receptor, beta subunit	–1.20	–0.74	–0.68
NM_032990.1	*Gria3*	Glutamate receptor, ionotropic, AMPA3			0.65
XM_579337.1	*Grin2a*	Glutamate receptor, ionotropic, N-methyl D-aspartate 2A.		1.24	0.99
NM_012585.1	*Htr1a*	5-hydroxytryptamine (serotonin) receptor 1A		–0.65	
NM_012765.2	*Htr2c*	5-hydroxytryptamine (serotonin) receptor 2C	–0.82		
NM_173839.2	*Tph2*	Tryptophan hydroxylase 2	–0.55		

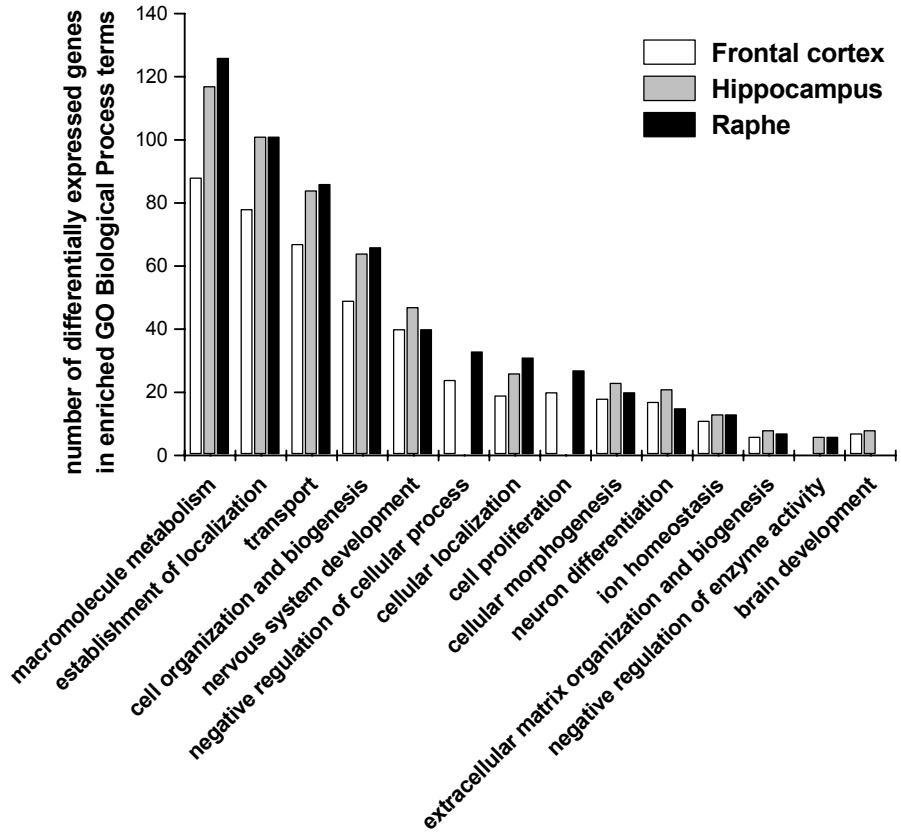

Figure 1. Significantly enriched Gene Ontology (GO) biological process terms in the lists of differentially expressed genes in the brains of the low vs high explorers.

To find significantly over-represented pathways in our lists of differently regulated genes, we searched the curated pathway database of the Kyoto Encyclopedia of Genes and Genomes (KEGG) using the DAVID 2007 Functional Annotation Tool. The KEGG pathway database is a collection of manually drawn graphical diagrams that represent the current knowledge of molecular interactions involved in various cellular processes (http://www.genome.ad.jp/kegg/). In the raphe, the most significantly over-represented pathway was found to be the focal adhesion pathway, followed by axon guidance, B cell receptor signalling, leukocyte transendothelial migration, regulation of actin cytoskeleton, and Wnt signalling pathways (Table 3). In the hippocampus, the Wnt signalling pathway was the most significantly over-represented pathway, followed by MAPK signalling, axon guidance, regulation of actin cytoskeleton, focal adhesion, long-term depression, and long-term potentiation pathways. In the frontal cortex, the most significantly enriched

pathway was the Wnt signalling pathway, followed by long-term depression, focal adhesion, axon guidance, and MAPK signalling pathway. Thus, the over-represented pathways were similar in the three brain regions.

Table 3. Significantly enriched KEGG pathways among the differentially expressed genes in the brains of rats with low vs. high exploratory activity.

KEGG pathway/ Gene symbol	Gene title	Log ratio		
		Raphe	Hippo-campus	Frontal cortex
RNO04310: Wnt signalling pathway				
Camk2a	Calcium/calmodulin-dependent protein kinase II alpha subunit			–0.61
Camk2b	Calcium/calmodulin-dependent protein kinase II beta subunit		1.26	
Ctnnb1	Catenin (cadherin associated protein), beta 1, 88kda	–0.88	–0.91	–0.66
Cxxc4	CXXC finger 4	1.37	1.23	1.24
Gsk3b	Glycogen synthase kinase 3 beta	1.34	1.63	1.38
Mapk9	Mitogen-activated protein kinase 9	–1.05	–0.75	–0.68
Nfatc3_ predicted	Nuclear factor of activated t-cells, cytoplasmic, calcineurin-dependent 3 (predicted)	–0.51		
Ppp2r2c	Protein phosphatase 2 (formerly 2a), regulatory subunit b (pr 52), gamma isoform		–0.61	–0.63
Ppp3cb	Protein phosphatase 3, catalytic subunit, beta isoform	–0.76	–0.57	–0.58
Rac1	Ras-related c3 botulinum toxin substrate 1 (rho family, small gtp binding protein rac1)	–0.78	–0.80	–0.69
Rock1	Rho-associated coiled-coil forming kinase 1	–0.59		
Senp2	Sumo/sentrin specific protease 2	–0.57		
Wnt4	Wingless-related mmtv integration site 4		0.56	

Table 3. (Continuation)

KEGG pathway/ Gene symbol	Gene title	Log ratio		
		Raphe	Hippo-campus	Frontal cortex
RNO04360: Axon guidance				
Gsk3b	Glycogen synthase kinase 3 beta	1.34	1.63	1.38
Gnai1	Guanine nucleotide binding protein, alpha inhibiting 1	–1.81	–1.18	–1.11
Kras2	Kirsten rat sarcoma viral oncogene homologue 2 (active)		0.76	0.80
Nras	Neuroblastoma ras oncogene	–0.54		
Nfatc3_ predicted	Nuclear factor of activated t-cells, cytoplasmic, calcineurin-dependent 3 (predicted)	–0.51		
Pak2	p21 (cdkn1a)-activated kinase 2	–0.66	–0.54	
Pak3	p21 (cdkn1a)-activated kinase 3	1.01	1.28	1.00
Ppp3cb	Protein phosphatase 3, catalytic subunit, beta isoform	–0.76	–0.57	–0.58
Rasa1	Ras p21 protein activator 1			0.51
Rac1	Ras-related c3 botulinum toxin substrate 1	–0.78	–0.80	–0.69
Rock1	Rho-associated coiled-coil forming kinase 1	–0.59		
Sema6d_ predicted	Sema domain, transmembrane domain (tm), and cytoplasmic domain, (semaphorin) 6d (predicted)	–0.53		
Slit1	Slit homolog 1 (drosophila)	0.62	0.66	
Unc5a	Unc-5 homolog a (c. elegans)		–0.68	–0.53
RNO04510: Focal adhesion				
Actb	Actin, beta	–1.15	–1.12	–0.95
Ctnnb1	Catenin (cadherin associated protein), beta 1, 88kda	–0.88	–0.91	–0.66
Cav	Caveolin	–0.91	–0.6	–0.73
Cav2	Caveolin 2	–0.56		
Fn1	Fibronectin 1	0.57		
Gsk3b	Glycogen synthase kinase 3 beta	1.34	1.63	1.38
Itga1	Integrin alpha 1		0.98	
Kras2	Kirsten rat sarcoma viral oncogene homologue 2 (active)		0.76	0.80
Mapk9	Mitogen-activated protein kinase 9	–1.05	–0.75	–0.68
Nras	Neuroblastoma ras oncogene	–0.54		

Table 3. (Continuation)

KEGG pathway/ Gene symbol	Gene title	Log ratio		
		Raphe	Hippo-campus	Frontal cortex
RNO04510: Focal adhesion (continued)				
Pak2	p21 (cdkn1a)-activated kinase 2	–0.66	–0.54	
Pak3	p21 (cdkn1a)-activated kinase 3	1.01	1.28	1.00
Pik3r3	Phosphatidylinositol 3 kinase, regulatory subunit, polypeptide 3	–0.99	–0.55	–0.58
Pik3cb	Phosphatidylinositol 3-kinase, catalytic, beta polypeptide	–0.52		
Rac1	Ras-related c3 botulinum toxin substrate 1	–0.78	–0.80	–0.69
Rock1	Rho-associated coiled-coil forming kinase 1	–0.59		
Tnr	Tenascin r	1.61	1.98	1.96
Vegfa	Vascular endothelial growth factor a			–0.53
RNO04010: MAPK signalling pathway				
Atf2	Activating transcription factor 2		–0.79	–0.62
Acvr1c	Activin receptor-like kinase 7		1.37	1.54
Cacng3	Calcium channel, voltage-dependent, gamma subunit 3		–0.79	–0.64
Cacng8	Calcium channel, voltage-dependent, gamma subunit 8		–0.57	
Fgf12	Fibroblast growth factor 12		–1.32	–1.23
Gna12	Guanine nucleotide binding protein, alpha 12		–0.54	
Kras2	Kirsten rat sarcoma viral oncogene homologue 2 (active)		0.76	0.80
Max	Max protein		–0.68	–0.61
Mapk9	Mitogen-activated protein kinase 9		–0.75	–0.68
Ntrk1	Neurotrophic tyrosine kinase, receptor, type 1		0.67	
Pak2	p21 (cdkn1a)-activated kinase 2		–0.54	
Ppp3cb	Protein phosphatase 3, catalytic subunit, beta isoform		–0.57	–0.58
Rasgrp1	Ras guanyl releasing protein 1		-0.98	–0.77
Rasa1	Ras p21 protein activator 1			0.51
Rac1	Ras-related c3 botulinum toxin substrate 1		–0.80	–0.69

Table 3. (Continuation)

KEGG pathway/ Gene symbol	Gene title	Log ratio		
		Raphe	Hippo-campus	Frontal cortex
RNO04810: Regulation of actin cytoskeleton				
Actb	Actin, beta	1.15	1.12	
Fgf12	Fibroblast growth factor 12	1.42	1.32	
Fn1	Fibronectin 1	–0.57		
Gna12	Guanine nucleotide binding protein, alpha 12		0.54	
Itga1	Integrin alpha 1		–0.98	
Kras2	Kirsten rat sarcoma viral oncogene homologue 2 (active)		–0.76	
Msn	Moesin	0.64	0.55	
Nras	Neuroblastoma ras oncogene	0.54		
Pak2	p21 (cdkn1a)-activated kinase 2	0.66	0.54	
Pak3	p21 (cdkn1a)-activated kinase 3	–1.01	–1.28	
Pfn1	Profilin 1	0.51	0.78	
Pik3cb	Phosphatidylinositol 3-kinase, catalytic, beta polypeptide	0.52		
Pik3r3	Phosphatidylinositol 3 kinase, regulatory subunit, polypeptide 3	0.99	0.55	
Rac1	Ras-related c3 botulinum toxin substrate 1 (rho family, small gtp binding protein rac1)	0.78	0.80	
Rock1	Rho-associated coiled-coil forming kinase 1	0.59		
Slc9a1	Solute carrier family 9, member 1	–0.54	–0.54	
RNO04730: Long-term depression				
Gna12	Guanine nucleotide binding protein, alpha 12		0.54	
Gnai1	Guanine nucleotide binding protein, alpha inhibiting 1		1.18	1.11
Gnao	Guanine nucleotide binding protein, alpha O		–1.24	–1.09
Gnaq	Guanine nucleotide binding protein, alpha Q polypeptide		0.93	0.65
Gria3	Glutamate receptor, ionotropic, AMPA3 (alpha 3)			–0.65
Kras2	Kirsten rat sarcoma viral oncogene homologue 2 (active)		–0.76	
Ppp2r2c	Protein phosphatase 2, regulatory subunit B, gamma isoform		0.61	0.63
Prkg2	Protein kinase, cGMP-dependent, type II		–0.57	–0.89

Table 3. (Continuation)

KEGG pathway/ Gene symbol	Gene title	Log ratio		
		Raphe	Hippo-campus	Frontal cortex
RNO04720: Long-term potentiation				
Camk2b	Calcium/calmodulin-dependent protein kinase II beta subunit		–1.26	
Gnaq	Guanine nucleotide binding protein, alpha Q polypeptide		0.93	
Grin2a	Glutamate receptor, ionotropic, N-methyl-D-aspartate 2A		–1.24	
Kras2	Kirsten rat sarcoma viral oncogene homologue 2 (active)		–0.76	
Ppp3cb	Protein phosphatase 3, catalytic subunit, beta isoform		0.57	
Rapgef3	cAMP-regulated guanine nucleotide exchange factor 1		–0.57	

Within the Wnt signalling pathway, two genes involved in negative regulation of canonical Wnt signalling (*Gsk3b*, glycogen synthase kinase 3 beta, and *Cxxc4*, CXXC finger 4, Idax) were upregulated in the low exploring animals while the *Ctnnb1* (beta-catenin) gene was downregulated in these rats. Several genes contributed to more than one KEGG pathway. For example, the *Gsk3b* gene is also involved in the axon guidance, focal adhesion, and MAPK signalling pathways, as are *Kras2* (Kirsten rat sarcoma viral oncogene homologue 2) and *Pak3* (p21 (cdkn1a)-activated kinase 3), both upregulated; and *Pak2* (p21 (cdkn1a)-activated kinase 2) and *Rac1* (Ras-related c3 botulinum toxin substrate 1), which were downregulated in the LE-rats. The expression of mitogen-activated protein kinase 9 gene (*Mapk9*), which is part of MAPK signalling and non-canonical Wnt signalling, and also plays a role in focal adhesion formation, is lower in the LE animals.

DISCUSSION

In the present study, we report the gene expression data from three brain regions of rats with persistently high or low exploratory activity. The focus on the raphe nuclei, the hippocampus and the frontal cortex, comprising the ascending serotonergic pathway, was determined by their role in the pathogenesis and pathophysiology of anxiety and depression.

There is evidence from basic as well as clinical studies that suggest that dysregulation of serotonin neurotransmission is associated with depression (Deakin, 1996), as well as modulation of anxiety levels in animal models of

these disorders (Griebel, 1995). Mällo et al. (2008) have recently reported region-specific differences in serotonergic function in the HE- and LE-rats. In the present study, we found that three genes involved in serotonin neurotransmission were downregulated in the LE-rats. In the raphe, *Tph2* (tryptophan hydroxylase 2) and *Htr2c* (serotonin receptor 2C) were downregulated in the LE-rats. Of the two isoforms of the serotonin-synthesizing enzymes, *Tph1* (tryptophan hydroxylase 1) and *Tph2* (tryptophan hydroxylase 2), the latter is exclusively expressed in the brain and has a fundamental role in brain serotonin synthesis (Zhang et al., 2004). A functional single nucleotide polymorphism in the human *Tph2* gene that results in a marked loss of function in serotonin production has been identified in patients with unipolar major depression (Zhang et al., 2005). Knock-in mice that express the mutant form of the *Tph2* gene have significantly reduced brain serotonin synthesis and are characterized by anxiety-like behaviours in the dark-light emergence test and tail suspension test (Beaulieu et al., 2008). Consistently with the idea of a reduced serotonergic neurotransmission posing an increased risk for developing mood disorders, the *Tph2* gene was downregulated in the raphe of the low exploring animals displaying the 'depressed' phenotype.

In general, the activation of 5-HT_{2C} receptors is thought to be anxiogenic, as 5-HT_{2C} agonists promote anxiety in animals (Menard and Treit, 1999), and the 5-HT_{2C}-knockout mice have a rather anxiolytic behavioural phenotype (Heisler et al., 2007). Thus, our finding of lower *Htr2c* expression in the raphe of LE animals which also have higher anxiety levels is in apparent discordance with the previous results. The 5-HT_{2C} receptor has a widespread expression in the CNS that is most abundant in serotonergic projection areas, consistent with its presumed postsynaptic localization, but has also been detected in the raphe nuclei prompting suggestions of its presynaptic localization on serotonergic neurons (Wright et al., 1995; Clemett et al., 2000). However, 5-HT_{2C} receptors do not suppress the firing of the raphe serotonergic neurons directly, but via activation of local GABA-ergic interneurons (Liu et al., 2000), which have been shown to express 5-HT_{2C} (Serrats et al., 2005; Boothman et al., 2006). In addition to lower *Htr2c* expression in the raphe, the genes encoding GABA-A receptor α_1, γ_1, and γ_2 subunits (*Gabra1*, *Gabrg1*, and *Gabrg2*, respectively) were downregulated in the same brain area of the LE animals. Therefore, the reduction of the expression of genes encoding 5-HT_{2C} receptors and several $GABA_A$ receptor subunits in the raphe of the more anxious LE-rats might contribute to an altered local interplay between serotonin and GABA, which may underlie the behavioural phenotype of this group. Alternatively, the reduced *Htr2c* expression in the raphe of the LE-rats might reflect a possible negative feedback mechanism to an increased number of 5-HT_{2C} receptors, as in behaviourally anxious 5-HTT-knockout mice lacking the serotonin transporter reduced levels of 5-HT_{2C} mRNA, but increased or unchanged density of 5-HT_{2C} receptors were found in specific brain regions (Li et al., 2003). There also remains a possibility that the anxiety-mediating role of the 5-HT_{2C} receptors is

different depending on the localization in either the cell body region or the projection areas.

In the hippocampus, the expression of *Htr1a* (serotonin receptor 1A) was lower in the low exploring group of animals. Using drug challenges and *in vivo* imaging, dysregulation of the 5-HT_{1A} receptor has been shown to be implicated in human affective and panic disorders (see Lesch and Gutknecht, 2004 and references therein). Moreover, Lesch and Gutknecht (2004) have suggested that because low 5-HT_{1A} function in major depressive disorder patients does not seem to be reversed by antidepressant treatment, this might reflect an inherent vulnerability mechanism to the disorder. Additionally, knockout mice lacking 5-HT_{1A} receptor display enhanced avoidance of novel situations, and decreased exploratory behaviour and increased fear of aversive environments (Ramboz et al., 1998; Parks et al., 1998). Interestingly, these behavioural effects of *Htr1a* inactivation were not only demonstrated in homozygous mutant mice but also in heterozygous mice that had about 60% reduction in the number of 5-HT_{1A} binding sites compared to wild-type levels (Ramboz et al., 1998). This supports the possibility that the downregulation of 5-HT_{1A} receptors in the brain may present a risk factor for mood disorders (Toth, 2003).

Several lines of evidence have indicated that decreased tone of the brain γ-aminobutyric acid (GABA) system might be implicated in anxiety and mood disorders (Shiah and Yatham, 1998; Kalueff and Nutt, 2007, for review). In line with multiple studies that have shown downregulation of GABA receptor genes in animal models of anxiety and depression (see Kalueff and Nutt, 2007), the expression of genes encoding GABA-A receptor subunits (*Gabra1*, *Gabra4*, *Gabrg1*, *Gabrg2*) was reduced in the low exploring rats. In addition to reduced GABA function, abnormal glutamatergic neurotransmission, including changes in glutamate receptors, have been reported in mood disorder patients (Sanacora et al., 2008). In the present study, we found an upregulation of *Grin2a* (NR2A subunit of the NMDA receptor) in the frontal cortex and hippocampus, and *Gria3* in the frontal cortex of low exploring animals. An insight into the behavioural functions of the NR2A comes from the findings of reduced anxiety-like and anti-depressive behaviour in mice with genetic inactivation of the *Grin2a* gene (Boyce-Rustay and Holmes, 2006). Thus, increased expression of *Grin2a* would be expected to have a diametrically opposite effect on anxiety- and depression-related behaviours.

Interestingly, CB_1 cannabinoid receptor gene (*Cnr1*) was upregulated in the frontal cortex and hippocampus of the rats with higher neophobia and lower motivation to explore. In mice, CB_1 cannabinoid receptors are known to mediate attenuated anxiety-like responses (Haller et al., 2004a) and CB_1-knockout mice context-dependently display higher stress responsiveness and altered coping strategies (Haller et al., 2004b). In contrast to mice, in several rat strains both CB_1 agonists and antagonists have a pro-anxiety profile; and in Wistar rats, specifically, it was found that CB_1 agonist WIN-55,212 administration induced anxiogenesis which was further augmented with the CB_1

antagonist AM-251 co-administration (Haller et al., 2007). Haller et al. (2007) have hypothesized that in mice the CB_1 activation which leads to reduced anxiety occurs mainly via modulation of GABA-ergic neurotransmission, whereas in rats the effects of CB_1 receptor stimulation are exerted mostly by a glutamate-mediated mechanism. In line of this reasoning, the increase in the *Cnr1* expression together with decreases in GABA and increases in the expression of glutamate receptor genes in the LE-rats might underlie their persistently anxious phenotype as well as passive coping strategies displayed in behavioural tests of affective states (Mällo et al., 2007). The upregulation of *Cnr1* in the LE-rats might also result in a shift in the functional interaction between CB_1 and GABA-B receptors that has been proposed to finetune the balance of endocannabinoid and GABA-ergic signalling in the hippocampus (Cinar et al., 2008). Although at this point we have no information about the brain levels of endocannabinoids in the HE- and LE-rats, it is possible that the increased expression of *Cnr1* in the LE animals indeed reflects an adaptive response to low endocannabinoid levels in the brain.

The database of the Kyoto Encyclopedia of Genes and Genomes (KEGG) contains biochemical paths that describe the physiological processes in the cell. A search in that database can help identify the molecular pathways with over-representation among microarray data. The KEGG pathway analysis revealed a number of significantly enriched pathways among the differentially expressed genes. In particular, the Wnt signalling, focal adhesion, and axon guidance pathways were found to be over-represented across all three brain regions. Genes involved in the regulation of actin cytoskeleton were over-represented in the raphe and the hippocampus, and the MAPK signalling and the long-term depression pathways were significantly enriched in the hippocampus and the frontal cortex. Further, the long-term potentiation pathway was over-represented in the hippocampus. These pathways are highly interrelated, as is evident by the considerable overlap in the differently regulated genes contributing to each of the pathways.

Of specific interest in the context of the present study was the finding that the Wnt signalling pathway was the most enriched pathway in the hippocampus and the frontal cortex, while significantly over-represented also in the raphe. The Wnt proteins are secreted cysteine-rich glycoproteins that are critically involved in cell development, proliferation, and apoptosis (Logan and Nusse, 2004; Li et al., 2005). The Wnt proteins bind to the Frizzled family receptors and activate either canonical or non-canonical Wnt-Frizzled pathways. The canonical Wnt signalling controls gene transcription through preventing the degradation of the transcriptional regulator, β-catenin by displacing the key enzyme of this signalling pathway, glycogen synthase kinase-3 (GSK-3), from the multi-protein complex that in the absence of Wnt signalling functions to keep the levels of cytoplasmic β-catenin low. As a result of the activation of the Wnt signalling pathway, β-catenin accumulates in the cytoplasm and is transported into the nucleus where it activates the transcription of its target

genes. Ubiquitously expressed in mammal tissues, GSK-3 has a crucial role in cell division, differentiation, proliferation and adhesion, and its dysfunction plays a role in the pathogenesis of cancer and diabetes (Frame and Cohen, 2001). The Wnt signalling pathway has recently been extensively studied in relation to several psychiatric, especially mood disorders. The mood-stabilizer lithium directly inhibits GSK-3 activity and increases Wnt- and β-catenin-mediated gene expression in rats (Gould et al., 2003; Gould et al., 2007a). Also, several serotonergic drugs have the ability to inhibit GSK-3 in the brain (Li et al., 2004; Li et al., 2007; Beaulieu et al., 2007). Moreover, while β-catenin overexpression mimicked the behavioural effects of lithium administration in mice (Gould et al., 2007b), forebrain-specific conditional inactivation of the β-catenin gene induced depression-like behaviour in tail suspension test in mice (Gould et al., 2008). In our experiment, the LE-rats had significantly higher *Gsk3b* (glycogen synthase kinase 3, beta isoform) expression and the expression of *Ctnnb1* (β-catenin) was downregulated across all brain regions in these animals. Further, we found significant upregulation in the LE animals of *Cxxc4* (CXXC finger 4) gene encoding a protein, Idax, that functions as a negative regulator of the Wnt pathway (Hino et al., 2001). These results suggest less active Wnt signalling in LE-rats. In a recent study, it was shown that activation of GSK-3β accompanied a marked reduction in the brain serotonin levels and behavioural abnormalities in mice that express a mutant *Tph2* gene (Beaulieu et al., 2008). The aberrant emotional behaviours were reversed by pharmacological or genetic inhibition of GSK-3β activity, suggesting an important role for GSK-3β signalling in psychiatric problems related to serotonin deficiency (Beaulieu et al., 2008).

However, we also found that *Senp2,* encoding for Axam, another inhibitor of Wnt signalling, was downregulated only in the raphe, but not the hippocampus or the frontal cortex of the LE-rats. At present, the relevance of this finding remains obscure.

Several genes involved in the mitogen-activated protein kinase (MAPK) signalling were differentially expressed in the LE- v. HE-rats, including *Fgf12*, *Rac1*, *Mapk9*, and *Atf2*, which were downregulated in the LE animals. The mitogen-activated protein kinase (MAPKs) cascades regulate diverse psysiological processes within the cell (from cell growth and proliferation to synaptic function and gene transcription) in response to a multitude of extracellular and intracellular stimuli (Sweatt, 2001; Raman et al., 2007). *Mapk9* encodes one of the c-Jun N-terminal kinases (JNKs), JNK2, which belongs to a subgroup MAPKs that have an important role in the regulation of the transcriptional activity of c-Jun, modulating gene transcription after neuronal stress (Herdegen et al., 1999). The reduced expression of *Mapk9*, as well as its upstream activators *Fgf12* and *Rac1*, and the downstream target activating transcription factor 2, *Atf2*, may indicate less active JNK signalling in the LE-rats.

GSK-3 and JNK signalling have been implicated in the modulation of synaptic plasticity and connectivity (Ciani and Salinas, 2005; Peineau et al., 2008; Chen et al., 2005). The reduced activity of Wnt and JNK2 pathways in

the LE animals and the fact that the axon guidance, focal adhesion and the two main forms of synaptic plasticity in the brain, the long-term potentiation and long-term depression pathways were also significantly over-represented among the differentially expressed transcripts suggests that the major differences between the LE- and HE-rats lie in the remodelling of the neurons and the synapses. This is further supported by the search in the Gene Ontology (GO) database containing gene annotations where several GO terms relevant to the development, morphogenesis, differentiation and proliferation of neurons were found to be enriched.

ACKNOWLEDGEMENTS

This study was supported by the European Commission Framework 6 Integrated Project NEWMOOD (LSHMCT-2004-503474).

WEB RESOURCES

The URLs for resources presented herein are as follows:

Bioconductor, www.bioconductor.org
DAVID, http://david.abcc.ncifcrf.gov
Gene Ontology (GO), http://www.geneontology.org
Kyoto Encyclopedia of Genes and Genomes (KEGG), http://www.genome.ad.jp/kegg
PUMA, http://www.bioinf.man.ac.uk/resources/puma

REFERENCES

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

Alttoa, A., Eller, M., Herm, L., Rinken, A., Harro, J., 2007. Amphetamine-induced locomotion, behavioral sensitization to amphetamine, and striatal D_2 receptor function in rats with high or low spontaneous exploratory activity: differences in the role of locus coeruleus. Brain Res. 1131, 138–148.

Beaulieu, J.M., 2007. Not only lithium: Regulation of glycogen synthase kinase-3 by antipsychotics and serotonergic drugs. Int J Neuropsychopharmacol. 10, 3–6.

Beaulieu, J.M., Zhang, X., Rodriguiz, R.M., Sotnikova, T.D., Cools, M.J., Wetsel, W.C., Gainetdinov, R.R., Caron, M.G., 2008. Role of GSK3 beta in behavioral abnormalities induced by serotonin deficiency. Proc Natl Acad Sci U S A. 105, 1333–1338.

Bolstad, B.M., Irizarry, R. A., Astrand, M, and Speed, T.P., 2003. A Comparison of Normalization Methods for High Density Oligonucleotide Array Data Based on Bias and Variance. Bioinformatics 19, 185–193.

Boothman, L., Raley, J., Denk, F., Hirani, E., Sharp, T., 2006. In vivo evidence that 5-HT2C receptors inhibit 5-HT neuronal activity via a GABAergic mechanism. Br J Pharmacol. 149, 861–869.

Boyce-Rustay, J.M., Holmes, A., 2006. Genetic Inactivation of the NMDA Receptor NR2A Subunit has Anxiolytic- and Antidepressant-Like Effects in Mice. Neuropsychopharmacology. 31, 2405–2414.

Chen, J.-T., Lu, D.-H., Chia, C.-P., Ruan, D.-Y., Sabapathy, K., Xiao, Z.-C., 2005. Impaired long-term potentiation in c-Jun N-terminal kinase 2-deficient mice. Journal of Neurochemistry. 93, 463–473.

Ciani, L., Salinas, P.C., 2005. WNTS in the vertebrate nervous system: from patterning to neuronal connectivity. Nat Rev Neurosci. 6, 351–362.

Cinar, R., Freund, T.F., Katona, I., Mackie, K., Szucs, M., 2008. Reciprocal inhibition of G-protein signaling is induced by CB_1 cannabinoid and $GABA_B$ receptor interactions in rat hippocampal membranes. Neurochem Int. 52, 1402–1409.

Clemett, D.A., Punhani, T., S. Duxon, M., Blackburn, T.P., Fone, K.C.F., 2000. Immunohistochemical localisation of the 5-HT2C receptor protein in the rat CNS. Neuropharmacology. 39, 123–132.

Crusio, W.E., van Abeelen, J.H., 1986. The genetic architecture of behavioural responses to novelty in mice. Heredity. 56, 55–63.

Deakin, J.F.W., 1996. 5 -HT, antidepressant drugs and the psychosocial origins of depression. J Psychopharmacol. 10, 31–38.

Dunning, M.J., Smith, M.L., Ritchie, M.E., Tavare, S., 2007. beadarray: R classes and methods for Illumina bead-based data, Bioinformatics, 23, 2184–2185.

Frame, S., Cohen, P., 2001. GSK3 takes centre stage more than 20 years after its discovery. Biochem J. 359, 1–16.

Gould, T.D., Chen, G., Manji, H.K., 2003. In vivo evidence in the brain for lithium inhibition of glycogen synthase kinase-3. Neuropsychopharmacology. 29, 32–38.

Gould, T.D., Dow, E.R., O'Donnell, K.C., Chen, G., Manji, H.K., 2007a. Targeting signal transduction pathways in the treatment of mood disorders: recent insights into the relevance of the Wnt pathway. CNS Neurol Disord Drug Targets. 6, 193–204.

Gould, T.D., Einat, H., O'Donnell, K.C., Picchini, A.M., Schloesser, R.J., Manji, H.K., 2007b. [beta]-Catenin Overexpression in the Mouse Brain Phenocopies Lithium-Sensitive Behaviors. Neuropsychopharmacology. 32, 2173–2183.

Gould, T.D., O'Donnell, K.C., Picchini, A.M., Dow, E.R., Chen, G., Manji, H.K., 2008. Generation and behavioral characterization of [beta]-catenin forebrain-specific conditional knock-out mice. Behavioural Brain Research. 189, 117–125.

Griebel, G., 1995. 5-hydroxytryptamine-interacting drugs in animal models of anxiety disorders: More than 30 years of research. Pharmacol Ther. 65, 319–395.

Haller, J.a., Varga, B.a., Ledent, C.b., Freund, T.F.a., 2004a. CB1 cannabinoid receptors mediate anxiolytic effects: convergent genetic and pharmacological evidence with CB1-specific agents. Behavioural Pharmacology. 15, 299–304.

Haller, J., Varga, B., Ledent, C., Barna, I., Freund, T.F., Matyas, F., Soproni, K., Barsy, B., Nemeth, B., Mikics, E., Hajos, N., 2004b. Context-dependent effects of CB1 cannabinoid gene disruption on anxiety-like and social behaviour in mice. Eur J Neurosci. 19, 1906–1912.

Haller, J., Matyas, F., Soproni, K., Varga, B., Barsy, B., Nemeth, B., Mikics, E., Freund, T.F., Hajos, N., 2007. Correlated species differences in the effects of cannabinoid ligands on anxiety and on GABAergic and glutamatergic synaptic transmission. Eur J Neurosci. 25, 2445–2456.

Harro, J., 1993. Measurement of exploratory behaviour in rodents. In: Methods in neurosciences, vol. 14 (Conn, R.A., ed), pp 359–377. San Diego: Academic Press.

Harro, J., Oreland, L., Vasar, E., Bradwejn, J., 1995. Impaired exploratory behaviour after DSP-4 treatment in rats: Implications for the increased anxiety after noradrenergic denervation. Eur Neuropsychopharmacol. 5, 447–455.

Heisler, L.K., Zhou, L., Bajwa, P., Hsu, J., Tecott, L.H., 2007. Serotonin 5-HT2C receptors regulate anxiety-like behavior. Genes, Brain and Behavior. 6, 491–496.

Herdegen, T., Mielke, K., Kallunki, T., 1999. Review : c-Jun and the c-Jun amino-terminal kinases: bipotential components of the neuronal stress response. Neuroscientist. 5, 147–154.

Hino, S.-I., Kishida, S., Michiue, T., Fukui, A., Sakamoto, I., Takada, S., Asashima, M., Kikuchi, A., 2001. Inhibition of the Wnt signaling pathway by Idax, a novel Dvl-binding protein. Mol. Cell. Biol. 21, 330–342.

Hooks, M.S., Colvin, A.C., Juncos, J.L., Justice, J.B., Jr., 1992. Individual differences in basal and cocaine-stimulated extracellular dopamine in the nucleus accumbens using quantitative microdialysis. Brain Res. 587, 306–312.

Kabbaj, M., 2006. Individual differences in vulnerability to drug abuse: The high responders/low responders model. CNS Neurol Disord Drug Targets. 5, 513–520.

Kalueff, A.V., Nutt, D.J., 2007. Role of GABA in anxiety and depression. Depression and Anxiety. 24, 495–517.

Landgraf, R., Wigger, A., 2002. High vs low anxiety-related behavior rats: An animal model of extremes in trait anxiety. Behav Genetics. 32, 301–314.

Lesch, K.P., Gutknecht, L., 2004. Focus on the 5-HT1A receptor: Emerging role of a gene regulatory variant in psychopathology and pharmacogenetics. Int J Neuropsychopharmacol. 7, 381–385.

Li, Q., Wichems, C.H., Ma, L., Van de Kar, L.D., Garcia, F., Murphy, D.L., 2003. Brain region-specific alterations of 5-HT2A and 5-HT2C receptors in serotonin transporter knockout mice. Journal of Neurochemistry. 84, 1256–1265.

Li, X., Zhu, W., Roh, M.S., Friedman, A.B., Rosborough, K., Jope, R.S., 2004. In vivo regulation of glycogen synthase kinase-3beta (GSK-3beta) by serotonergic activity in mouse brain. Neuropsychopharmacology 29, 1426–1431.

Li, F., Chong, Z.Z., Maiese, K., 2005. Vital elements of the Wnt-Frizzled signaling pathway in the nervous system. Curr Neurovasc Res. 2, 331–40.

Li, X., Rosborough, K.M., Friedman, A.B., Zhu, W., Roth, K.A., 2007. Regulation of mouse brain glycogen synthase kinase-3 by atypical antipsychotics. Int J Neuropsychopharmacol. 10, 7–19.

Liu, R., Jolas, T., Aghajanian, G., 2000. Serotonin 5-HT2 receptors activate local GABA inhibitory inputs to serotonergic neurons of the dorsal raphe nucleus. Brain Research. 873, 34–45.

Mällo, T., Alttoa, A., Kõiv, K., Tõnissaar, M., Eller, M., Harro, J., 2007. Rats with persistently low or high exploratory activity: behaviour in tests of anxiety and depression, and extracellular levels of dopamine. Behavioural Brain Research. 177, 269–281.

Mällo, T., Kõiv, K., Koppel, I., Raudkivi, K., Uustare, A., Rinken, A., Timmusk, T., Harro, J., 2008. Regulation of extracellular serotonin levels and brain-derived

neurotrophic factor in rats with high and low exploratory activity. Brain Res. 1194, 110–117.
Matrov, D., Kolts, I., Harro, J., 2007. Cerebral oxidative metabolism in rats with high and low exploratory activity. Neurosci Lett. 413, 154–158.
Menard, J., Treit, D., 1999. Effects of centrally administered anxiolytic compounds in animal models of anxiety. Neuroscience & Biobehavioral Reviews. 23, 591–613.
Ogata, H., Goto, S., Sato, K., Fujibuchi, W., Bono, H., Kanehisa, M., 1999. KEGG: Kyoto encyclopedia of genes and genomes. Nucleic Acids Res. 27, 29–34.
Otter, M.H., Matto, V., Sõukand, R., Skrebuhhova, T., Allikmets, L., Harro, J., 1997. Characterization of rat exploratory behavior using the exploration box test. Methods Find Exp Clin Pharmacol. 19, 683–691.
Parks, C.L., Robinson, P.S., Sibille, E., Shenk, T., Toth, M., 1998. Increased anxiety of mice lacking the serotonin1A receptor. Proceedings of the National Academy of Sciences. 95, 10734–10739.
Peineau, S., Bradley, C., Taghibiglou, C., Doherty, A., Bortolotto, Z.A., Wang, Y.T., Collingridge, G.L., 2008. The role of GSK-3 in synaptic plasticity. Br J Pharmacol. 153, S428–437.
Piazza, P.V., Deminiere, J.M., Le Moal, M., Simon, H., 1989. Factors that predict individual vulnerability to amphetamine self-administration. Science. 245, 1511–1513.
Raman, M., Chen, W., Cobb, M.H., Differential regulation and properties of MAPKs. Oncogene. 26, 3100–3112.
Ramboz, S., Oosting, R., Amara, D.A., Kung, H.F., Blier, P., Mendelsohn, M., Mann, J.J., Brunner, D., Hen, R., 1998. Serotonin receptor 1A knockout: An animal model of anxiety-related disorder. Proceedings of the National Academy of Sciences. 95, 14476–14481.
Rattray, M., Liu, X., Sanguinetti, G., Milo, M. and Lawrence, N.D., 2006. Propagating uncertainty in Microarray data analysis. Briefings in Bioinfomatics, 7:37–47.
Rouge-Pont, F., Deroche, V., Le Moal, M., Piazza, P.V., 1998. Individual differences in stress-induced dopamine release in the nucleus accumbens are influenced by corticosterone. Eur J Neurosci. 10, 3903–3907.
Sanacora, G., Zarate, C.A., Krystal, J.H., Manji, H.K., 2008. Targeting the glutamatergic system to develop novel, improved therapeutics for mood disorders. Nat Rev Drug Discov. 7, 426–437.
Serrats, J., Mengod, G., Cortés, R., 2005. Expression of serotonin 5-HT2C receptors in GABAergic cells of the anterior raphe nuclei. Journal of Chemical Neuroanatomy. 29, 83–91.
Shiah, I.S., Yatham, L.N., 1998. GABA function in mood disorders: An update and critical review. Life Sciences. 63, 1289–1303.
Sweatt, J.D., 2001. The neuronal MAP kinase cascade: a biochemical signal integration system subserving synaptic plasticity and memory. Journal of Neurochemistry. 76, 1–10.
Toth, M., 2003. 5-HT1A receptor knockout mouse as a genetic model of anxiety. European Journal of Pharmacology. 463, 177–184.
White, D.A., Kalinichev, M., Holtzman, S.G., 2007. Locomotor response to novelty as a predictor of reactivity to aversive stimuli in the rat. Brain Res. 1149, 141–148.
Wright, D.E., Seroogy, K.B., Lundgren, K.H., Davis, B.M., Jennes, L., 1995. Comparative localization of serotonin$_{1A}$, $_{1C}$, and $_{2}$ receptor subtype mRNAs in rat brain. J Comp Neurol. 351, 357–373.

Zhang, X., Beaulieu, J.-M., Sotnikova, T.D., Gainetdinov, R.R., Caron, M.G., 2004. Tryptophan hydroxylase-2 controls brain serotonin synthesis. Science. 305, 217–.

Zhang, X., Gainetdinov, R.R., Beaulieu, J.-M., Sotnikova, T.D., Burch, L.H., Williams, R.B., Schwartz, D.A., Krishnan, K.R.R., Caron, M.G., 2005. Loss-of-function mutation in tryptophan hydroxylase-2 identified in unipolar major depression. Neuron. 45, 11–16.

CURRICULUM VITAE

Aet Alttoa

Date of Birth	September 16, 1974
Citizenship	Estonian
Address	Department of Psychology, University of Tartu, Tiigi 78, 50410 Tartu, Estonia
Telephone	+372 55 967 304
e-mail	aet.alttoa@ut.ee

Education

2004–2008	Doctoral studies, Dept of Psychology, University of Tartu
2002–2004	Master's studies (MSc), Dept of Psychology, University of Tartu
1997–2002	Bachelor's studies, Dept of Psychology, University of Tartu

Proffessional employment

2007 – ...	Research fellow, Department of Psychology, University of Tartu
2004–2007	Extraordinary research fellow, Department of Psychology, University of Tartu
2002–2004	Technician, Department of Psychology, University of Tartu

Scientific activity

Main research area:

Affective states and disorders: development of animal models and investigating the underlying psychobiological mechanisms

Membership:

Union of Estonian Psychologists
Estonian Society of Pharmacology

CURRICULUM VITAE

Aet Alttoa

Sünniaeg ja koht	16. september 1974, Tartu
Kodakondsus	Eesti
Aadress	Psühholoogia instituut, Tiigi 78, Tartu 50410, Eesti
Telefon	+372 55 967 304
e-mail	aet.alttoa@ut.ee

Haridus

2004–2008	Doktoriõpe, psühholoogia instituut, Tartu Ülikool
2002–2004	Magistriõpe, psühholoogia instituut (MSc), Tartu Ülikool
1997–2002	Bakalaureuseõpe, psühholoogia instituut (BSc), Tartu Ülikool

Teenistuskäik

2007– ...	psühhofüsioloogia teadur, psühholoogia instituut, Tartu Ülikool
2004–2007	erakorraline teadur, psühholoogia instituut, Tartu Ülikool
2002–2004	laborant, psühholoogia instituut, Tartu Ülikool

Teadustegevus

Teadustöö põhisuunad:

Afektiivsete seisundite ja häirete modelleerimine ning psühhobioloogiliste alusmehhanismide selgitamine rottidel

Liikmelisus:

Eesti Psühholoogide Liit
Eesti Farmakoloogia Selts

DISSERTATIONES PSYCHOLOGICAE UNIVERSITATIS TARTUENSIS

1. **Jüri Kruusvall.** Environmental and social influence on human activity. Tartu, 1994, 135 p.
2. **Dagmar Kutsar.** Transformation in Estonia as reflected in families: Insight into social stress and poverty. Tartu, 1995, 171 p.
3. **Aleksander Pulver.** Measurement of elementary movement vectors in human visual system. Tartu, 1995, 123 p.
4. **Ritva Fagerström.** The correlations between psychological factors and vision in aged cataract operation patients. Tartu, 1996, 145 p.
5. **Eve Kikas.** Conceptual development in school-aged children: The impact of teaching. Tartu, 1997, 112 p.
6. **Anu Realo.** Individualism and collectivism: An exploration of individual and cultural differences. Tartu, 1999, 164 p.
7. **Aaro Toomela.** Cultural-historical psychology: three levels of analysis. Tartu, 2000, 171 p.
8. **Anneli Kolk.** Cognitive development of children with non-progressive unilateral brain lesion. Tartu 2001, 95 p.
9. **Aune Valk.** Two facets of ethnic identity: pride and differentiation. Tartu, 2001, 153 p.
10. **Anu Aluoja.** Depression in the population: assessment, prevalence and relationships with socio-demographic factors and cognitive aspect of social adjustment. Tartu 2002, 73 p.
11. **Talvi Kallasmaa.** Personality traits and ways of coping: their characteristics and interrelations. Tartu 2002, 119 p.
12. **Luule Mizera.** Socialization in Estonian families: attitudes and behavior in comparative perspective. Tartu 2003, 117 p.
13. **Kairi Kreegipuu.** Availability and accessibility of information in perception of moving stimuli. Tartu 2004, 97 p.
14. **Riina Häidkind.** Monoaminergic mechanisms in mood-associated behaviours and neurochemistry in rats. Tartu 2004, 123 p.
15. **Evelin Kiive.** Studies on peripheral markers of central serotonergic activity and behaviour. Tartu, 2005, 113 p.
16. **Helle Pullmann.** The development of intelligence and personality traits among Estonian schoolchildren. Tartu, 2005, 112 p.
17. **Kenn Konstabel.** The structure and validity of self- and peer-reported personality traits. Tartu, 2006, 103 p.
18. **Toivo Aavik.** Lexical analysis of Estonian personal values vocabulary and relation to socially desirable responding and parenting practices. Tartu, 2006, 113 p.

19. **Margus Tõnissaar.** Stress and sociability: individual differences and their neurochemical substrate. Tartu, 2006, 161 p.
20. **Kaia Laidra.** Adolescent personality: Development, interrater agreement, and relation to academic achievement. Tartu, 2007, 117 p.
21. **Iiris Luiga.** Interaction of object perception and visual attentional selection processes. Tartu, 2007, 116 p.
22. **Marika Paaver.** Types of impulsivity, their behavioural expression, and association with the markers of vulnerability of serotonin system. Tartu, 2007, 140 p.
23. **Tanel Mällo.** Exploratory behaviour and 50-kHz ultrasonic vocalizations in rats: behavioural and neurochemical profiles of persistent inter-individual differences. Tartu, 2008, 216 p.