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KADRI KÕIV

Studies on monoaminergic regulation of inter-individual differences in exploratory behaviour and the activating and rewarding effects of psychostimulants in rats

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"But not only can we partially understand how it is that man is from various conflicting influences rendered capricious, but that the lower animals are, as we shall hereafter see, likewise capricious in their affections, aversions, and sense of beauty. There is also reason to suspect that they love novelty, for its own sake."

Charles Darwin

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LIST OF ORIGINAL PUBLICATIONS

I **Kõiv, K.**, Zobel, R., Raudkivi, K., Kivastik, T., Harro, J. The effect of denervation of the locus coeruleus projections with N-(2-chloroethyl)-N-ethyl-2-bromobenzylamine (DSP-4) on cocaine induced locomotion and place preference in rats. *Behavioural Brain Research (in press)*.

II 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 behaviour and dopamine D_2 receptor function in rats with high or low exploratory activity. *Neuroscience*, 132, 979–990.

III Alttoa, A., Seeman, P., **Kõiv, K.**, Eller, M. & Harro, J. (2009). Rats with persistently high exploratory activity have both higher extracellular dopamine levels and higher proportion of D_2^{High} receptors in the striatum. *Synapse*, 63, 443–446.

IV **Kõiv, K.** & Harro, J. Differences in 5-HT_{1A} receptor-mediated hypothermia in rats with low or high exploratory activity. *Behavioural Pharmacology (in press).*

V 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 Research*, 1194, 110–117.

In addition to the published papers, this thesis also contains some unpublished experiments, referred to as **Study I** – "Cocaine-conditioned place preference in rats with high or low exploratory activity" and **Study II** – "Extracellular striatal dopamine and serotonin levels in high and low exploring rats: the effect of acute imipramine on amphetamine-induced neurotransmitter release".

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

– for **Papers I**, **IV** and **Studies I** and **II**, creating the experimental designs, carrying out and scoring all the behavioural experiments and the data analysis, and writing the publications as the main author;
– for **Paper II**, performing the first part of the behavioural experiments (within-subjects design); contributed to data analysis;
– for **Paper III**, contributing to the experimental design, carrying out the behavioural experiments, contributing to statistical analysis of the data;
– and for **Paper V**, contributing to creating the experimental designs, conducting half of the microdialysis studies, and conducting some of the statistical analysis.

ABBREVIATIONS

[^{35}]GTPγS	[^{35}]-guanosine-5'-(γ-thio)-triphosphate
5-HIAA	5-hydroxyindoleacetic acid
5-HT_{1A}	5-hydroxytryptamine (serotonin) receptor 1A
5-HT	5-hydroxytryptamine; serotonin
5-HTT	5-hydroxytryptamine (serotonin) transporter
6-OHDA	6-hydroxydopamine
8-OH-DPAT	8-hydroxy-2-(dipropylamino)tetralin
ANOVA	analysis of variance
BDNF	brain-derived neurotrophic factor
cDNA	complementary desoxyribonucleinic acid
CPP	conditioned place preference
D_2	dopamine D_2 receptor
D_2^{High}	dopamine D_2 receptor in the high affinity state
DA	dopamine
DG	dentate gyrus
DOPAC	3,4-dihydroxyphenylacetic acid
DSP-4	N(2-chloroethyl)-N-ethyl-2-bromobenzylamine
GABA	γ-aminobutyric acid
GTP	guanosine-5'-triphosphate
HE	high explorer
HR	high responder (to novelty)
HPLC	high performance liquid chromatography
HVA	homovanillic acid
i.p.	intraperitoneal
LC	locus coeruleus
LE	low explorer
LR	low responder (to novelty)
mRNA	messenger ribonucleinic acid
NA	noradrenaline
NAT	noradrenaline transporter
NAC	nucleus accumbens
NGF	nerve growth factor
PCA	parachloroamphetamine
PFC	prefrontal cortex
SEM	standard error of the mean
SIH	stress-induced hyperthermia
TCA	tricyclic antidepressant
VTA	ventral tegmental area

INTRODUCTION

With facing the upcoming era of unipolar depressive disorder, predicted to become the leading cause of burden of disease by 2030 (Mathers & Loncar, 2006; Global burden of disease: 2004 update), there is a need for new personally adjusted interventions, taking into account both the genetic vulnerability and its interactions with the environmental factors of the affected subject. The present pharmacotherapies in practice generally utilize the same means of action – acutely boosting the monoaminergic neurotransmitter levels – as found, largely by chance, in the 1960s. To date, the initial monoamine hypothesis that considered depression to be the consequence of low levels of extracellular noradrenaline and serotonin (NA and 5-HT, respectively)(Bunney & Davis, 1965; Schildkraut, 1965) has been developed and refined, leading to the chemical/ molecular hypothesis of depression. Presently, it is acknowledged that complex alterations in the networks of monoaminergic (e.g., adaptive changes in monoamine receptors and their coupling to intracellular signal transduction) but also other systems, such as brain circuitries involving glutamate, neurotrophins and several neuropeptides, are related to the depressive-like phenotype and the delayed effect of antidepressants in ameliorating the depressive symptoms. Complementary to the chemical hypothesis, the network hypothesis proposes that problems in activity-dependent neuronal communication might underlie depression, and that antidepressants might work by improving information processing in the affected neural networks (Nestler et al., 2002; Castren, 2005; Drevets et al., 2008; Tanti & Belzung, 2010).

Mood and anxiety disorders, as defined by the Diagnostic and Statistic Manual IV-R (APA, 2000), show high comorbidity over lifetime and even greater longitudinal stability of comorbid anxiety and depression than either disorder alone; moreover, anxiety is more likely to predict the subsequent onset of depression than the other way around; thus, anxiety and depression may be manifestations of the same underlying diathesis, possibly with age-dependent expression, rather than comprising independent disorders (Merikangas et al., 2003). The 10^{th} revision of International Classification of Diseases (WHO, 1992) incorporates a specific category for mixed anxiety-depression based on concurrent expression of subthreshold-level anxiety and depression. Additionally, drug abuse and substance dependence have a complex bidirectional relationship with anxiety and affective disorders (Grant, 1995; Merikangas et al., 1998; Swendsen & Merikangas, 2000; Kessler et al., 2003; Volkow, 2004; Schuckit, 2006; Merikangas et al., 2008).

Depression is conceptualized as a multifaceted syndrome, involving a cognitive, affective and physiological component. Given the complexity and heterogeneity of anxiety and depression, there have been growing propositions for the diagnosis and treatment of these disorders to focus on individual behavioural, physiological or neurochemical endpoints, rather than the entire syndrome (Gottesman & Gould, 2003; Cryan & Holmes, 2005; Gould & Gottesman, 2006; Panksepp, 2006). Heightened negative emotionality, anxiety, loss of

interest, lack of reactivity and psychomotor change (retardation) are among the key components of the depressive phenotype (Hasler et al., 2004). Based on the assumption of all mammalian brains sharing a large number of basic emotional systems, rising from homologous subneocortical limbic networks (Panksepp, 1998, 2005, 2006), modelling of these features in animals (Gould & Gottesman, 2006) allows to elucidate the causal involvement of a given target by using more invasive techniques; and studying inter-individual differences in these animal models at behavioural, physiological and genetic levels are thought to help in revealing the substrates for vulnerability to depression and clarifying its pathogenetic mechanisms (Harro, 2010). Reduced motivation (to explore) and increased anxiety can be studied in laboratory animals in terms of exploratory behaviour, in which conflicting motivations – exploratory drive (curiosity) and fear evoked by novelty – are simultaneously contributing to behaviour (Montgomery, 1955).

Exploratory behaviour and its measurement

Neotic or novelty-related behaviour, usually called exploratory behaviour, is evoked by novel stimuli and consists of behavioural acts and postures that permit the collection of information about new objects and unfamiliar parts of the environment (Crusio & van Abeelen, 1986; Crusio, 2001); and novelty is considered to be a function of the degree of contrast between present perception and past experience (Corey, 1978). Daniel Berlyne (Berlyne, 1960) views exploratory behaviour as responses that alter the stimulus field, the principal function of exploratory responses being to afford access to environmental information that was not previously available. Exploratory behaviour comprises orienting responses (changes in posture, in the orientation of sense organs, or in the state of sense organs), locomotor exploration and investigatory responses, which effect changes in external objects. It is generally accepted that neotic behaviour consists of three phases: avoidance, approach and disinterest (Corey, 1978; Harro, 1993). Berlyne distinguished between extrinsic and intrinsic exploration. In extrinsic exploration, the stimuli are sought only as cues for the guidance of some succeeding response with an independent source of biological value or reinforcement, e.g., food. Intrinsic exploration, on the contrary, makes available stimuli that are sufficient in themselves, regardless of their immediate practical value. Thus, extrinsic exploration is essential for survival due to the enhanced possibilities it provides to find food, water, mating partner, shelter etc. but, simultaneously, such a behavioural activity will render an animal vulnerable to predation.

Among the attempts to give reasons for the motivation of intrinsic exploration have been the postulation of exploratory–investigatory drives (either primary or learned), appeals to concepts of optimal arousal, and explanations based on the energizing effects of fear (Hughes, 1997). However, responsiveness to novel stimuli can occur both in the form of approach (neophilia, neotic

approach) or avoidance (neophobia, neotic avoidance) (Berlyne, 1950; Montgomery & Monkman, 1955). Already William James recognised that "Curiosity and fear form a couple of antagonistic emotions liable to be awakened by the same outward thing, and manifestly both useful to their possessor" (James, 1950), paving the way to the dual-process theories of curiosity and fear, which assume that exploration and avoidance/withdrawal are conflicting forms of behaviour that are evoked by strangeness and novelty, and that the presence of both curiosity and fear results in an approach-avoidance conflict (Spielberger & Starr, 1994). Montgomery was among the first researchers to study the question empirically in rats, combining familiar living cage of the rat with possibility to explore novel mazes, and inferred that "Novel stimulation may evoke both the exploratory drive and the fear drive, thus generating approach-avoidance conflict behaviour" (Montgomery, 1955). Thus, exploratory behaviour of a rat is at any particular moment influenced by the conflicting motivations to explore the novel environment potentially dangerous by nature or stay within the secure and familiar surroundings.

Although studying exploratory behaviour *per se* has been relatively unpopular since mid 1960-s, measurements of exploration continue to feature in studies of the behavioural effects of a variety of pharmacological and neurophysiological manipulations (Hughes, 1997). The tests used for these purposes in rodents are diverse and routinely used to investigate anxiety-like behaviours (the inhibition of exploration by fear) and utilised for drug screening purposes (Harro, 1993). In addition, novelty-related tasks have also become valuable in addiction research, as it has been claimed that these behaviours may share some of a common neurobiological substrate (Bardo et al., 1996; Blanchard et al., 2009).

The means that are used to measure exploratory behaviour in the laboratory setting are diverse but can grossly be divided into 'forced' tests in which horizontal and vertical locomotor responses are assessed in animals placed into totally novel environments, and 'free' tests involving measurements of active choices/preference of differing degrees of novelty of either environments or novel objects in familiar environment. Probably the most widely used tests for characterization of the rat behaviour in a forced novel environment are the classical open field test and elevated plus-maze (reviewed in Harro, 1993). Nevertheless, because of the difficulty of distinguishing between extrinsic and intrinsic exploration with activity indices in the forced novel surrounding, tests of 'free' exploration are usually preferable (Hughes, 1997) since the essence of exploratory behaviour, as Sandra File has put it, "... is that the animal's own behaviour determines its exposure to the novel situation, i.e., the animal selects the stimuli to which it will attend, and the rate and duration of stimulus sampling" (File, 1985). The studies by Welker (Welker, 1957, 1959) revealed that the 'forced' and 'free' exploration tests may, indeed, measure different components of novelty-related behaviour as the activity of rats during forced sessions was greater than during free sessions, especially during the beginning of the session; there was an increase in activity over successive free sessions, but not over forced sessions, and when provided with an escape possibility from

the forced novel environment, it resulted in the animal's retreat as soon as the open doorway was encountered. Indeed, open field behaviour has been demonstrated to be influenced by factors of 'exploration' and 'emotional reactivity' (Whimbey & Denenberg, 1967). Large open areas in inescapable novel environment present an aversive stimulus to rodents and the behavioural 'response' measured in an open field is possibly more bound to animal's response to a stressful context (van den Buuse et al., 2001; Prut & Belzung, 2003) rather than appearance of its exploratory drive. Indeed, there is evidence that experiencing inescapable novelty is more stressful (Hennessy et al., 1979) than novelty that can be freely chosen (Misslin et al., 1982).

Another type of tests are a sort of 'emergence tests', containing elements of both forced and free exploration, where an animal is put into a novel situation (a novel environment is present and available for exploration) but has a choice whether to investigate it or stay in the more familiar surroundings/in a perceivably safer area (Montgomery, 1955; Fehrer, 1956; Welker, 1957; Glickman et al., 1964; Hughes, 1965; Valle, 1972; Paré et al., 2001). In our laboratory, we use the exploration box test, initially designed to assess the behavioural changes after the denervation of noradrenergic projections from locus coeruleus (Harro et al., 1995). During the exposure to the testing apparatus, the rat can stay either in the dark little chamber, which has home-cage-like features and provides a distinct home base in an unfamiliar environment, or explore a large dim open arena with novel objects in it (Otter et al., 1997). The test is designed to measure exploring both the new environment as a whole and the particular new items in it.

Dopamine systems underlying exploratory behaviour and psychostimulant activation and reward

Midbrain dopaminergic neurons constitute the major source of dopamine (DA) in the mammalian central nervous system (Dahlström & Fuxe, 1964). The mesencephalic dopaminergic system has been subdivided into several nominal systems. The nigrostriatal system, which mainly originates in the zona compacta of the substantia nigra (A9 region) and extends its fibers into the dorsal striatum (caudate-putamen), has an essential role in the control of voluntary motor movement and habit-forming (Ungerstedt, 1971; White, 1997). More medial to this pathway are the mesolimbic and mesocortical dopaminergic systems, which primarily arise from dopaminergic cells present in the ventral tegmental area (VTA, A10 region), considered to be crucial for motivational aspects of motor activity and have been implicated in reward and reinforcement mechanisms (Di Chiara & Imperato, 1988; Kalivas et al., 1993; Wise, 2004). The cells of the VTA project most prominently into the nucleus accumbens (NAC) and olfactory tubercle, but also innervate the septum, amygdala and hippocampus; cells in the medial VTA also project to the prefrontal, cingulate and perirhinal cortex (Swanson, 1982).

Dopamine receptors belong to the seven transmembrane domain G protein-coupled family of receptors which transduce signalling through guanosine-triphosphate (GTP) binding proteins and five receptor subtypes, designated D_1–D_5, have been isolated (Missale et al., 1998 for a review). D_1 receptor is the most widespread DA receptor, found in highest densities in the caudate putamen, NAC, and the olfactory tubercle. The distribution of D_2 is similar to that of D_1, being localized primarily to the mesencephalon (Emilien et al., 1999a). D_2-like receptors are of great interest as they are the primary targets of antipsychotic drugs and also appear to be of major importance in the treatment of Parkinson's disease, while both D_1 and D_2 receptors take part in addictive drug-related mechanisms (Missale et al., 1998; Emilien et al., 1999a; Emilien et al., 1999b; Seeman et al., 2006).

The dopaminergic neurotransmission in the VTA-NAC axis is increased in response to both natural rewards such as food and sex, and brain stimulation reward (Joseph et al., 2003); VTA DA neurons respond to the presentation of salient and arousing environmental stimuli (Horvitz, 2000), and also have been implicated in the mechanism of action of drugs with addictive properties (Di Chiara & Imperato, 1988). Regarding recent theories, DA does not signal reward *per se* but rather mediates a learning signal that allows the system to predict better when rewards are likely to occur and thereby contribute to the optimization of reward-seeking behaviours (Montague et al., 1996; Montague et al., 2004). The mesolimbic dopaminergic pathway is also found to be involved in neotic behaviour. An increased transmission of NAC DA, particularly in the shell, is observed during the initial exploration of novel stimuli (Rebec et al., 1997a; Rebec et al., 1997b; De Leonibus et al., 2006). Lesions of forebrain DA systems produce a deficit in novelty-elicited behaviours as 6-hydroxydopamine (6-OHDA) injections along the medial forebrain bundle in the anterolateral hypothalamus bring about deficits in locomotor and novel object directed exploration (Fink & Smith, 1979, 1980b, a) and DA depletion in NAC with a local 6-OHDA lesion blocks novelty preference (Pierce et al., 1990). Moreover, intra-NAC injections of DA antagonists may abolish novelty-induced hyperactivity, while having no effect on general activity in familiar surroundings (Hooks & Kalivas, 1995). Recently, it has been shown that novel stimuli also activate the midbrain dopaminergic neurons in humans (Bunzeck & Düzel, 2006).

In addition to the facts that when given free access to both familiar and novel environment, rats will usually prefer the novel one (Hughes, 1965; Bardo et al., 1989) and the opportunity to discover complex novel places may function as an effective reinforcing agent in learning tasks (Montgomery, 1954), novelty and access to novel stimuli has also been shown to be rewarding using place preference conditioning (Besheer et al., 1999; Bevins & Bardo, 1999; Bevins et al., 2002). Conditioned place preference (CPP) is a popular experimental protocol used to study the rewarding properties of various stimuli (usually drug reward) in laboratory animals (Tzschentke, 1998; Bardo & Bevins, 2000 for a review), that appears to reflect a preference for a context due to a previous contiguous association between an unconditioned stimulus (e.g., a novel object,

drug administration) and the context (conditioned stimulus). DA-dependent mechanisms in preference for novelty and novel object conditioned place preference are involved as DA antagonists are able to block these phenomena (Bardo et al., 1989; Besheer et al., 1999).

Like access to novel stimuli, psychostimulants cocaine and amphetamine produce an increase in preference for the environment in which they are administered (Spyraki et al., 1982a, b; Mackey & van der Kooy, 1985; Morency & Beninger, 1986; Bardo et al., 1995). Amphetamine and cocaine evoke locomotor activity by enhancing monoamine signalling via interfering with presynaptic NA, DA and 5-HT transporter function and execute this by different mechanisms. Cocaine binds to the monoamine transporters, inhibiting reuptake of monoamines into the presynaptic nerve terminal, thereby prolonging their extracellular effects in the synapse and extrasynaptic space. Amphetamine blocks the aforementioned transporters and inhibits monoamine uptake, but concomitantly also is transported into the presynaptic terminal where it releases NA, DA and 5-HT from intracellular vesicles into the neuronal cytoplasm by interacting with vesicular monoamine transporter-2 (Fleckenstein et al., 2000; Partilla et al., 2006). This, in turn, results in a large build-up of monoamines in the cytosol, thereby reversing the direction of transporter to release monoamines into the extracellular space rather than facilitating their removal. Cocaine and amphetamine also differ in their relative affinity for monoamine transporters: while cocaine has approximately equal affinity for NA, DA and 5-HT transporters, amphetamine has relatively lower affinity for 5-HT transporter compared to that of DA and NA (Rothman et al., 2001; Rothman & Baumann, 2003; Han & Gu, 2006).

While multiple neurotransmitter systems (e.g., glutamatergic, GABA-ergic, opioid, etc.) are thought to modulate or interact with DA systems in the brain, the mesolimbic DA system is generally thought to be a critical component of the neural circuitry mediating psychomotor stimulant reward (reviewed in Koob & Nestler, 1997; Wise, 2004). Activation of mesoaccumbens dopamine transmission by psychostimulants is thought to be crucial in the acute motor stimulant effect of these drugs. Psychostimulants increase the extracellular concentration of DA in both NAC and striatum whereby amphetamine-induced stereotypy is correlated with DA released in striatum and locomotion with DA released in NAC (Sharp et al., 1987). Intracerebral self-administration studies support the notion that the NAC shell is among the primary sites of action for the psychostimulant effect (see McBride et al., 1999 for a review). However, cocaine is also self-administered into the medial prefrontal cortex and the olfactory tubercle (Goeders & Smith, 1983; Ikemoto, 2003). Dopamine is also thought to act in the dorsal striatum, amygdala, frontal cortex and other terminal sites to reward immediate behaviour and to establish conditioned motivational cues that will guide and motivate future behaviour (Wise, 2004).

Whereas the role of DA-ergic system in amphetamine-conditioned place preference is well acknowledged (Tzschentke, 1998), the extent to which mesolimbic dopamine system regulates the rewarding effects of cocaine as

measured by CPP remains somewhat unclear. 6-OHDA lesions of DA terminals within the NAC do not block cocaine place conditioning (Spyraki et al., 1982a) and microinjection of cocaine directly into the NAC fails to produce place preference (Hemby et al., 1992). However, this latter effect appears to be subregion-specific, as more recent investigations indicate that infusions of cocaine into the accumbens shell, but not core, produce CPP (Liao et al., 2000). Cocaine CPP seems to be more D_1 receptor mediated, as while D_2-like antagonists generally fail to influence place preference induced by cocaine, D_1-like antagonists administered systemically or intra-accumbally block cocaine elicited CPP (reviewed in Anderson & Pierce, 2005).

With repeated psychostimulant treatments, the magnitude of behavioural response, elicited by a psychostimulant after a withdrawal period, typically increases due to the sensitization of the dopaminergic system (see Vanderschuren & Kalivas, 2000, for a review). This phenomenon referred to as behavioural sensitization is considered an important contributor to the addictive potency of psychostimulants (Robinson & Berridge, 2001) and sensitization may also figure importantly in the reinstatement of drug taking in individuals that have been drug-free for some time (Vezina et al., 2002). The DA cell bodies of the VTA are critical in the induction of sensitization, whereas the DA terminals in the NAC are implicated in the expression of sensitization (Kalivas & Stewart, 1991; Vanderschuren & Kalivas, 2000). However, the induction and expression of behavioural sensitization to repeated psychostimulant treatment is also modulated by non-pharmacological factors, including environmental and contextual factors associated with drug administration (Badiani et al., 1995; Robinson & Berridge, 2001). Robinson with colleagues conclude that psychomotor sensitization is not an inevitable consequence of exposure to psychostimulant drugs, but is the result of interactions amongst the pharmacological actions of drugs and the circumstances surrounding drug administration (Robinson et al., 1998; Robinson & Berridge, 2001).

Recently, it has been shown that behavioural dopamine supersensitivity in rats is accompanied by a major elevation in brain striatal dopamine D_2^{High} receptors, which are dopamine D_2 receptors in the high-affinity state for dopamine (Seeman et al., 2005; Seeman et al., 2006), also considered to be the physiologically functional state of the receptor (George et al., 1985). Amphetamine sensitization markedly elevates the proportion of D_2 receptors in the state of high affinity for dopamine (Seeman et al., 2002; Seeman et al., 2005; Seeman, 2009) and cocaine self-administration experience produces an increase in the proportion of D_2^{High} receptors in the striatum with no change in the total number of D_2 receptors (Briand et al., 2008).

Noradrenergic locus coeruleus system in exploratory behaviour and psychostimulant activation and reward

Most of the NA-ergic innervation of the forebrain, including all NA-ergic innervation in the cerebral cortex and hippocampus, originates from the locus coeruleus (LC) A6 cell group, located near the wall of the fourth ventricle at the level of pons (reviewed in Moore & Bloom, 1979; Foote et al., 1983). Firing of LC neurons varies with state of wakefulness and vigilance. During quiet wakefulness, LC neurons fire at a regular slow rate (~1.5 Hz), they fire at a diminished rate during drowsiness and slow-wave sleep and are completely silent during rapid eye movement sleep (Aston-Jones & Bloom, 1981a). Consistent fluctuations in LC activity have been observed for certain waking behaviours: while discharge decreased during low vigilance behavioural states such grooming and consumption of sweet water, short bursts of impulses accompanied orienting responses to spontaneous or sensory-evoked interruptions of such ongoing behaviours. Neurons in the LC exhibit robust phasic activity in response to even mild sensory stimuli in the environment (Aston-Jones & Bloom, 1981b; Foote et al., 1983). NA release at forebrain sites is primarily determined by levels of activity in LC neurons. LC stimulation or iontophoretically applied NA generally depresses the spontaneous activity in many regions in the brain (Foote et al., 1983), while sparing the evoked response to sensory stimulation in different pathways, thus increasing the signal-to-noise ratio and fine-tuning the perceptual acuity (reviewed in Sara, 2009). LC neurons fire in phasic bursts when the rat encounters a novel object in a familiar hole-board, and this response habituates rapidly after a single exposure to the object (Vankov et al., 1995). The object exploring in the latter task is enhanced by drugs which increase the release of noradrenaline (e.g., α_2-adrenoceptor antagonist idazoxan) and decreased by drugs which block noradrenergic transmission (α_2 agonist clonidine, β antagonist propranolol) (Sara et al., 1995). Studies using lesions of LC or its projection pathways have shown that the behavioural impairments are quite subtle and appear mostly in demanding tasks such as in the context of novelty. Intact NA-ergic projections ascending from the LC have been found to be important for normal exploration in rats, as increased neophobia was observed after massive destruction of these projections (Harro et al., 1995).

In addition to the well-acknowledged participation of DA in the motor activating and reinforcing effects of psychostimulants, accumulating body of evidence using different experimental strategies is suggesting a substantial role for NA in modulating psychostimulant-induced behaviours such as locomotor activity, sensitization and reward (Weinshenker & Schroeder, 2007; Tassin, 2008). However, it is not clear which factors determine the exact role of NA in regulation of behaviour and in drug effects, as the findings do not yield a simple picture. For example, prazosin, an α_1-adrenoceptor antagonist, has generally been found to substantially suppress amphetamine-induced locomotion (Snoddy & Tessel, 1985; Dickinson et al., 1988; Blanc et al., 1994; Darracq et al., 1998; Drouin et al., 2002a; Drouin et al., 2002b; Vanderschuren et al., 2003); how-

ever, the effect of α_1-adrenoceptor blockade on cocaine-induced hyperlocomotion has been reported in several (Snoddy & Tessel, 1985; Berthold et al., 1992; Drouin et al., 2002a; Drouin et al., 2002b; Wellman et al., 2002) but not all (Thiebot et al., 1981; Filip et al., 2001; Vanderschuren et al., 2003) studies. Genetic modifications with impact on NA function have confirmed the critical role for NA in psychostimulant-induced locomotor activating effects. α_1-Adrenoceptor knockout (KO) mice display significant reductions in both amphetamine and cocaine-induced locomotor responses and sensitization (Drouin et al., 2002b; Auclair et al., 2004). In contrast, mice lacking NA transporters (NAT) that therefore display elevated extracellular levels and prolonged clearance of NA are hypersensitive to the locomotor stimulation by amphetamine and cocaine (Xu et al., 2000). Interestingly, DA β-hydroxylase KO mice that lack NA altogether are also hypersensitive to amphetamine and cocaine, possibly due to compensatory changes in the DA system (Weinshenker et al., 2002; Seeman et al., 2005; Schank et al., 2006).

The LC NA-ergic system is involved in regulating the activity of the ascending DA pathways at the level of the VTA, where NA-ergic neurons modulate the DA cell firing pattern via postsynaptic α_1-adrenoreceptors (Grenhoff et al., 1993; Grenhoff & Svensson, 1993), and the two systems also interact indirectly via the prefrontal cortex (PFC), as compromised NA-ergic neurotransmission in the prefrontal cortex leads to attenuation of DA release in the nucleus accumbens and amphetamine and cocaine-induced locomotion (Darracq et al., 1998; Ventura et al., 2003; Ventura et al., 2007). NA may also modulate striatal DA transmission indirectly by stimulating α_1-adrenoceptors on glutamatergic terminals, which facilitates glutamate release and potentiates DA transmission in the VTA (Rommelfanger et al., 2009). Electrical stimulation of the LC neurons increases both extracellular DA and NA in the PFC (Devoto et al., 2005b, a) and DA release in the PFC may also be regulated by local NA-ergic nerve terminals (Gresch et al., 1995). Dysregulation of LC NA-ergic neurotransmission has been suggested to be a major trigger in the pathogenesis of depression (Harro & Oreland, 2001).

The neurotoxin DSP-4 (N-(2-chloroethyl)-N-ethyl-2-bromobenzylamine) has a selective effect on NA-axonal terminal fields originating from the LC (Jonsson et al., 1981; Fritschy & Grzanna, 1989; Grzanna et al., 1989), with NA uptake carrier sites being the primary site of action (Ross, 1976), and hence it is a valuable tool in studies of functional actions of LC-derived NA in the brain. LC lesions have been shown to either increase or decrease amphetamine-induced hyperlocomotion (Ögren et al., 1983; Archer et al., 1986; Mohammed et al., 1986; Harro et al., 2000), the effect possibly being dependent upon the familiarity of the testing context (Harro et al., 1995; Harro et al., 2000).

Serotonergic system in stress-induced hyperthermia, exploratory behaviour and effects of psychostimulants

The central serotonergic system modulates the stress response, has been implicated in both the etiology of depression and anxiety disorders, and serves as a target for treatment in both conditions (Morilak & Frazer, 2004; Lanfumey et al., 2008). The pioneering mappings of serotonin system were done by Dahlström and Fuxe (1964) who described nine groups of serotonergic neurons and named these as B1-B9 (Dahlström & Fuxe, 1964). 5-HT-ergic neurons are located in the brainstem raphe nuclei and innervate multiple cortical brain regions to regulate a wide repertoire of behaviours, as well as cognition and mood. There are seven families of 5-HT receptors, 5-$HT_{1–7}$, and 15 genes encoding 5-HT receptors have been identified in the mammalian brain, most of them being G protein-coupled metabotropic receptors (reviewed in Bockaert et al., 2006). Particular attention has been paid to 5-HT_{1A} receptors, as these function as postsynaptic receptors in the forebrain and presynaptic autoreceptors on the 5-HT neurons' somas and dendrites (reviewed in Barnes & Sharp, 1999; Sharp et al., 2007). The density of 5-HT_{1A} binding sites is high in limbic brain areas, notably hippocampus and cortical areas (Barnes & Sharp, 1999). Genetic elimination of the 5-HT_{1A} receptor produces anxiety-type behaviour in adult mice (Heisler et al., 1998; Parks et al., 1998; Ramboz et al., 1998), and, during development, signalling through this receptor acts to establish normal behaviour with regard to anxiety in the adult (Gross et al., 2002). The 5-HT_{1A} receptor function also seems to be lower in emotionally less stable animals (Harro, 2010).

Converging evidence implicates the 5-HT_{1A} receptor dysfunction in the pathophysiology of anxiety and depression (Lesch & Gutknecht, 2004; Akimova et al., 2009). Depressed patients show blunted hypothermic and adrenocorticotropin/cortisol release relative to controls in response to a 5-HT_{1A} receptor challenge with ipsapirone (Lesch et al., 1990a; Lesch et al., 1990b) and lowered 5-HT_{1A} receptor binding potential in various brain regions, including cortex, hippocampus and raphe nuclei, as measured by positron emission tomography (Drevets et al., 1999; Sargent et al., 2000; Drevets et al., 2007). Furthermore, since also unmedicated clinically recovered patients have lower 5-HT_{1A}-receptor binding potential in cortical areas, this reduction was proposed as a trait variable associated with vulnerability to depression (Bhagwagar et al., 2004).

5-HT_{1A} receptors are, amongst others, also involved in modulation of body temperature (Lin et al., 1998; Cryan et al., 1999) and stress-induced hyperthermia (SIH), a short-lasting increase in body temperature in response to confronting a stressor (e.g., handling or a novel environment) (Singer et al., 1986; Van Der Heyden et al., 1997; Bouwknecht et al., 2007). The SIH paradigm utilizes the transient thermogenic effect mediated by the autonomic nervous system as a quantitative readout parameter for stress and this method may prove to be useful for screening novel anti-anxiety medications (Vinkers et al., 2008), as both classical benzodiazepines and 5-HT_{1A} receptor agonists consistently

reduce the SIH response and cause hypothermia (Hjorth, 1985; Cryan et al., 1999; Olivier et al., 2003).

Most drugs used to treat mood disorders via modulation of 5-HT system in humans have been found to modulate rodent behaviour in tests used in anxiety and depression research in a receptor subtype-specific manner (reviewed in Millan, 2003). Serotonin depletion has been associated with decreases in locomotor activity in a novel environment (Lipska et al., 1992; Dringenberg et al., 1995) and decreases in amphetamine-induced locomotion (Lipska et al., 1992). Recently it was demonstrated that while 5-HT_{1A} receptor agonist 8-OH-DPAT in small, autoreceptor preferring doses produced a dose-dependent inhibition of exploration of a novel object, treatment with WAY-100635, a selective antagonist of 5-HT_{1A} receptor, increased the investigatory response (Carey et al., 2008). The same group of authors have also shown that 5-HT_{1A} pharmacological agonism/antagonism at the 5-HT_{1A} receptor site with doses known to involve the postsynaptic component, can significantly modify the locomotor stimulant effects of cocaine. Overall, antagonism of the 5-HT_{1A} receptor site using WAY-100635 attenuated whereas the 5-HT_{1A} receptor agonist enhanced the locomotor stimulant effects of cocaine (Carey et al., 2000; Carey et al., 2002).

Inter-individual differences in novelty-related behaviour of rats and its implications for psychostimulant addiction and depression research

In the last two decades, behavioural neuroscience has benefited from research using inter-individual differences between conspecifics as a tool to analyse the underlying neural, biochemical and genetic differences relevant to the behavioural phenotype. This 'phenotype modelling' approach has helped to explain data variability in neurobiological and physiological measurements and thus serves as a unique path for psychobiological research, being amongst the important research strategies in anxiety, depression and addiction research.

Probably the most intensely studied dimensions of inter-individual differences of behaviour in animals relate to activity, exploratory behaviour and anxiety (Gosling, 2001; Pawlak et al., 2008). In order to reveal the underlying neurobiological regulation, these stable differences in the behaviour of laboratory animals have been used to model human traits and conditions such as sensation seeking, drug addiction, anxiety and depression. After the detection of a marked and relevant variability in behaviour, some models use a selection strategy based on spontaneous behaviour in a given population, and others engage in a breeding strategy, producing genetically diverging lines of animals that reveal a differing behavioural phenotype (Harro, 2010).

Although there had been scarce attempts to study inter-individual differences in exploration activity in the context of individual vulnerability to intracranial self-stimulation (Phillips & Valle, 1973), the field that made use of individual differences to study the vulnerability to reinforcing effects of drugs gained

momentum in 1990s based on the studies of Pier Vincenzo Piazza and colleagues, who discovered that differences in 'response' to novel environment, as measured by locomotion activity, would predict the subsequent self-administration of psychostimulants (Deminiere et al., 1989; Piazza et al., 1989). Since then, the 'high responder'/'low responder' (HR/LR, respectively) distinction has been proposed to model human trait of sensation seeking and vulnerability to drug addiction (Kabbaj, 2006; Blanchard et al., 2009). Originally, the Sprague-Dawley rats were classified based on median split of their two-hour locomotor activity in a novel inescapable photocell-equipped circular corridor (Piazza et al., 1989; Piazza et al., 1991). HR-rats were found to have more pronounced locomotion in the novel test apparatus as well as in response to amphetamine administration. In addition, the HR-rats were found to more readily acquire amphetamine intravenuous self-administration. Thereafter, the terms HR and LR have been used quite loosely by several research groups, denoting activity in an inescapable novel environment, either it is a small novel photocell-equipped plexiglas cage or a larger open field (Hooks et al., 1991b; Hooks et al., 1992; Hooks et al., 1994a; Hooks et al., 1994b; Chefer et al., 2003; Verheij et al., 2008; Verheij et al., 2009). More recently bred lines of HR/LR have also been introduced (Stead et al., 2006). HR-rats will also prefer novel and varied environments when given a free choice between novel and familiar (Dellu et al., 1993; Piazza & Le Moal, 1997), and display lower levels of anxiety in respective tests (Dellu et al., 1996; Kabbaj et al., 2000; Kabbaj, 2004; Verheij et al., 2009).

It has been argued that HR-rats' hyperactivity is associated exclusively with novelty and is not evident in familiar environment (Dellu et al., 1996; Saigusa et al., 1999). The rate of novelty-induced locomotion is shown to predict subsequent behavioural responses to psychostimulants: HR-rats exhibit higher rates of amphetamine- and cocaine-induced locomotor activity, develop stronger behavioural sensitization to repeated drug treatment and self-administer these drugs at lower doses than LR-rats do (Piazza et al., 1989; Hooks et al., 1991a). HR-rats also exhibit greater baseline and cocaine-induced elevations in extracellular concentrations of DA in NAC (Hooks et al., 1991b; Hooks et al., 1992) and have lower density of dopaminergic D_2 receptors in NAC and striatum (Hooks et al., 1994b) than LR-rats. HR-rats exhibit a prolonged corticosterone response after exposure to mild stress of a novel environment and exhibit greater stress-induced elevations of mesolimbic DA neurotransmission relative to their LR counterparts (Piazza et al., 1989; Dellu et al., 1996). Another modification of HR/LR model is based on the vertical activity (rearings) in a novel open field, which yields high/low rearing activity rats (HRA/LRA-rats). (Thiel et al., 1998; Thiel et al., 1999; Pawlak & Schwarting, 2002). Thiel with colleagues have reported higher levels of DA in ventral striatum and lower serotonin concentration in the medial prefrontal cortex in HRA-rats (Thiel et al., 1999). Taken together, these data indicate that individual differences in locomotor response to novelty implicate a role of DA in these effects.

Encountering the HR-LR distinction, however, one has to bear in mind that the inescapable novel environment presents an aversive stimulus to rodents and that the behavioural 'response' measured in such a context is possibly more bound to animal's response to a stressful context (Prut & Belzung, 2003; Roy et al., 2009) rather than appearance of its 'true' exploratory drive. In accordance with that notion, exposure to open field yields a significant increase in plasma corticosterone in rats (Hennessy et al., 1979; Wrona et al., 2004).

As already mentioned, another domain that has benefited from the inter-individual differences approach is anxiety and depression research. These models have been recently overviewed elsewhere (Pawlak et al., 2008; Harro, 2010). With regard to the present thesis, the following models are relevant and will be discussed: 1) Roman high avoidance/low avoidance rats (RHA/RLA-rats), bred based on learning speed of acquiring avoidance learning in shuttle-box, with RLA-rats displaying higher anxiety (Broadhurst & Bignami, 1965; Escorihuela et al., 1999; Giorgi et al., 2003; Giorgi et al., 2007; Fattore et al., 2009); 2) high/low anxiety-related behaviour rats (HAB/LAB-rats), bred based on behaviour in the elevated plus-maze test, with anxiety-related behaviour being higher in HAB-rats (Keck et al., 2005; Landgraf et al., 2007); 3) Flinders sensitive/resistant line (FSL/FRL-rats), originally bred for increased responses to an anticholinesterase agent, with FSL-rats displaying a depressive phenotype (Angelucci et al., 2000; Overstreet et al., 2005; Bjørnebekk et al., 2007). In addition, as there are inter-strain differences in rats with regard to anxiety- and depression-related behaviours, the relevant comparisons will be drawn.

In the present dissertation, the exploration box test (Harro et al., 1995; Otter et al., 1997) has been used to evaluate novelty-related behaviour in rats. This technique yields a rather bimodal distribution based on the sum of exploratory activity, and thus separates the animals tested into clusters with persistently high neophobia/low motivation and low neophobia/high motivation (low exploring and high exploring rats, LE- and HE-rats, respectively). The LE/HE-rats display their characteristic behavioural profile consistently over time. As compared to the HE-rats, the behaviourally 'depressive' LE-rats are more anxious in the plus-maze and light-dark box, display more passive coping strategies in the forced swimming test and acquire a more persistent association between stressful and neutral stimuli (Mällo et al., 2007 and unpublished data). In addition, the LE- and HE-rats have distinct cerebral metabolic activity in areas that are involved in defensive behaviours and cognitive processing of sensory stimuli (Matrov et al., 2007), and also have different gene expression profiles, e.g., involving several serotonergic, glutamatergic and GABA-ergic genes in brain regions previously implicated in mood disorders (Alttoa et al., 2010).

AIMS OF THE THESIS

The present dissertation consists of four main themes:

- studies on the significance of noradrenergic input from locus coeruleus in the behavioural effects of psychostimulants and exploratory behaviour (**Paper I** and **II**);
- studies on the low and high exploratory trait in behavioural responsiveness to psychostimulants (**Paper II** and **Study I**);
- studies on the role of dopaminergic neurotransmission in the inter-individual differences in exploratory behaviour (**Paper II**, **III**, **Study II**);
- and studies on the role of serotonergic neurotransmission in the inter-individual differences in exploratory behaviour (**Paper IV** and **V**, **Study II**).

Specifically, the objectives of the experiments were as follows:

- to clarify whether and to what extent the locomotor activity and conditioned place preference induced with cocaine are dependent upon integrity of the noradrenergic locus coeruleus system (**Paper I**);
- to determine whether cocaine-induced place preference depends on the rats' spontaneous exploratory activity levels as measured by the exploration box test (**Study I**);
- to examine the effects of a partial denervation of the noradrenergic terminals originating from the locus coeruleus on the exploratory behaviour, amphetamine-stimulated activity, and striatal D_2 receptor function in rats with low versus high spontaneous exploratory activity (**Paper II**);
- to compare basal and amphetamine-stimulated extracellular dopamine levels and the proportion of D_2 receptors in the functional high affinity state in the striatum of rats with low or high exploratory activity (**Paper III**);
- to learn whether acute administration of an antidepressant would modify basal and amphetamine-stimulated extracellular striatal dopamine and serotonin differently in low and high exploring rats (**Study II**);
- to investigate whether low and high exploring rats differ with regard to stress-induced hyperthermia response and in sensitivity to 5-HT_{1A} receptor challenge (**Paper IV**);
- to study whether low and high exploring rats would differ in regulation of extracellular serotonin levels and levels of neurotrophin mRNA in prefrontal cortex and hippocampus (**Paper V**).

METHODS

Animals

Male Wistar rats used in the experiments originated from Scanbur BK AB, Sweden, except for **Paper I** and **II**. The animals used for experiments in **Paper I** were acquired either from Scanbur BK AB, Sweden (Experiment 1) or from Harlan Laboratories, the Netherlands (Experiment 2). In **Paper II**, rats from the National Laboratory Animal Centre, Finland were used. All animals arrived to our animal facilities at the age of 3 weeks. The behavioural experiments started when the rats were at least 2 months old. The rats were housed in standard transparent polypropylene cages under controlled 12-h light/dark cycle [lights on at 07:00 (**Paper II**), 8:00 (**Paper V**) or 8:30 (**Papers I**, **III**, **IV**, **Studies I**, **II**)] in groups of four in a colony room maintained at 20±2ºC. The rats in **Paper IV** were singly housed in standard cages after behavioural preselection in the exploration box test. Food (diet R70, Lactamin, Sweden) and autoclaved water were available *ad libitum*. All behavioural experiments were carried out during the light cycle in an isolated experimental room. 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.

Drugs

D-amphetamine sulphate (Sigma, St. Louis, MO, USA), administered in the dose of 0.5 mg/kg (**Papers II**, **III**, **Study II**), was dissolved in distilled water and given as an intraperitoneal (i.p.) injection 15 minutes before behavioural testing (**Paper II**), or 90 minutes after the start of collecting the microdialysis samples (**Paper III**), or 120 minutes after acute imipramine injection in the microdialysis experiment (**Study II**). Imipramine (Sigma, St. Louis, MO, USA) was dissolved in distilled water and administered i.p. in the dose of 10 mg/kg acutely 75 minutes after the start of the collection of microdialysis samples (**Study II**). Cocaine hydrochloride (Synopharm GmbH & Co. KG, Germany) was dissolved in distilled water (**Paper I**) or saline (0.9% NaCl, **Study I**) and administered in the dose of 15 mg/kg i.p. immediately before CPP trainings (**Paper I, Study I**). Citalopram hydrobromide (1 μM; H. Lundbeck A/S, Copenhagen, Denmark) diluted in Ringer solution was administered locally via retrograde dialysis and DL-p-chloroamphetamine (PCA; Sigma, St. Louis, MO, USA) was administered i.p. in the dose of 2 mg/kg (**Paper V**). (±)8-OH-DPAT hydrobromide (Sigma, St. Louis, MO, USA) was dissolved in 0.9% saline and administered in the dose of 0.1 or 0.3 mg/kg i.p. immediately after baseline T_0 rectal temperature measurements (**Paper IV**). DSP-4 (N-(2-chloroethyl)-N-ethyl-2-bromobenzylamine; AstraZeneca, Södertälje, Sweden) was administered in the dose of 10 mg/kg (**Paper II**) or 10 and 50 mg/kg (**Paper I**). Each

dose of DSP-4 was weighed separately, dissolved in distilled water and injected immediately. Doses are expressed as for hydrochloride. All drugs administered intraperitoneally were delivered in the volume of 1 ml/kg, except for cocaine HCl and DSP-4, which were injected in the volume of 2 ml/kg. Control animals received a vehicle injection.

Behavioural and physiological methods

Exploration box test and classification of rats as low or high explorers (Papers II–V, Study I–II)

Exploration box test

The exploration box (Harro et al., 1995; Otter et al., 1997) was made of metal and consisted of a 50 x 100 cm open area (height of side walls 40 cm) with a 20 × 20 × 20 cm small compartment attached outside 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, respectively) were situated in certain places, which remained the same throughout the experiment. The small compartment, with its floor covered with wood shavings, was directly linked to the open area through an opening (size 20 × 20 cm), allowing free alternation between the open arena and small compartment for the 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 test. The following behavioural parameters were registered by an observer: 1) latency of entering the 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 investigations of the three unfamiliar objects. 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 was obtained. A single test session lasted 15 minutes and was carried out under dim light conditions (3–7 lux in the open area). The apparatus was cleaned with dampened laboratory tissue after each animal.

In the experiments measuring the effect of amphetamine (**Paper II**), the same apparatus was used, but the passage between the open area and the small compartment was closed, the animal was placed on a certain square of the open area and for 15 min 1) line crossings, 2) rearings and 3) the number of object investigations on the open arena were registered. Additionally, the sum of these measures was calculated.

Classification of rats as low or high explorers

When tested repeatedly in the exploration box test, the activity of rats on the first testing session does not always correlate highly with the following tests, but already the second exposure gives a reliable prediction of the activity levels

in subsequent tests (Mällo et al., 2007). Therefore in the present experiments, the rats were tested in the exploration box on two subsequent days and the sum of exploratory activity during the second testing was used for classification of rats as a low or high explorer. In **Papers II**, **III**, **V** and **Study I**, the rats were divided into low exploratory and high exploratory activity groups on the basis of the median value of the sum of exploratory events during the second testing session. In order to maximize the characteristic between-group differences and minimize the within-group variance, the LE and HE-rats used in **Study II**, **Paper IV** and **Paper III** (the rats used for D_2^{High} receptor measurements), were drawn from a larger preselected population of rats, belonging into the lower or upper quartile of the initial distribution of exploratory activity, respectively.

Place preference apparatus and place preference conditioning (Paper I and Study I)

Apparatus for conditioning of place preference

Cocaine-conditioned place preference (**Paper I** and **Study I**), cocaine-induced locomotor activity and possible sensitization to cocaine (**Paper I**) were studied in an apparatus consisting of four modules of two rectangular compartments (30 × 30 cm, 40 cm high walls), one with white and the other with dark grey walls and floor, in which the light intensity was 105-122 and 51-57 lux, respectively. The floor of each compartment was divided into four squares of equal size. The compartments were separated by a guillotine door and covered with a transparent Plexiglas ceiling. The experimenter left the room immediately after the beginning of each set of observations and behaviour of animals was recorded by an overhead video camera.

Procedure for place preference conditioning

CPP procedure was conducted as described previously (Kivastik et al., 1996). Animals were randomly assigned either to cocaine or vehicle group and handled in the experiment room for 2 minutes on 3 consecutive days prior to the introduction to the CPP apparatus. For the next two days, rats were placed into the white part of the CPP apparatus and given free access to both compartments for 15 min. The latter session was recorded and served as a baseline for place preference conditioning. Next, during eight daily conditioning sessions (Days 1-8), rats were injected once every other day with either cocaine (cocaine group) or vehicle (control group), and then immediately confined into the nonpreferred (white) compartment for 30 min. On alternate days, all animals were injected with vehicle, and confined into the other (gray) compartment. On Day 9, no injections were given; rats were initially placed into the nonpreferred chamber and had then free access to both compartments for 15 min, their activity being recorded. The following measures from the recordings were monitored: 1) number of entries into the white compartment, 2) time spent in the white compartment, 3) line crossings and 4) rearings in the white compartment. The CPP

score was determined by the difference between the amounts of time spent in the cocaine-paired compartment before and after the conditioning sessions.

After a week, the second 8-day conditioning session and the free-choice test was repeated as described (**Study I**). To estimate the extinction of CPP, two weeks after that the rats were given a third access to both compartments for 15 min. The following measures in the white compartment were monitored from the videotape: 1) number of entries into the white compartment, 2) time spent in the white compartment, 3) line crossings and 4) rearings, the latter two were summed together as an index of 5) locomotor activity.

In order to estimate the effect of denervation of locus coeruleus (LC) on acute locomotion stimulating effect of cocaine and possible revelation of sensitization to cocaine, each animal's first and last training in the white (11–12th and 17–18th day after the DSP-4 administration) compartment were also recorded and for the first 15 minutes 1) rearings and 2) line crossings were scored (Experiment 1 of **Paper I**). The Experiment 2 of **Paper I** was an exact replication of the acute phase of Experiment 1. To replicate the effect of denervation of LC on acute locomotion stimulating effect of cocaine, on the 11th day after the neurotoxin treatment, rats were either injected with cocaine (cocaine group) or vehicle (control group), restricted into the white compartment and for 15 minutes, their locomotor activity was recorded and subsequently scored.

Stress-induced hyperthermia procedure (Paper IV)

In Experiment 1, measuring the rectal temperature and a clean novel empty cage (identical to the home cage of the rat but without saw-dust and void of food and water) served as stressors for stress-induced hyperthermia (SIH). Rectal temperature was measured at baseline (T_0) immediately before the rat was allocated into the new cage, and after 15 minutes (T_{15}) (Vinkers et al., 2008). Rats were lightly manually restrained and a lubricated flexible temperature probe (YSI, 400 series) was gently inserted 5 cm into the rectum and the readings from the temperature control unit (HB 101, Panlab, Harvard Apparatus) were taken after stabilization period of 1 minute. Three SIH tests with weekly intervals were undertaken and the average SIH response (T_{15}-T_0) over the three testing times was calculated for each rat.

In Experiment 2, in order to test the 5-HT_{1A} receptor sensitivity, a within-subject design was employed, each animal being treated with each dose of 5-HT_{1A} selective agonist 8-OH-DPAT according to a Latin Square design with weekly intervals. Measurement of rectal temperature and injection served as stressors for SIH. Rectal temperature was measured immediately before (T_0) and subsequently at 30 (T_{30}) and 60 minutes (T_{60}) after the 8-OH-DPAT injection. Rats were returned to their home-cages right after the injection. SIH response for the two time-points was calculated as $\Delta T_{30}=(T_{30} - T_0)$ and $\Delta T_{60}=(T_{60} - T_0)$.

Biochemical methods

Monoamine content measurements from tissue samples (Papers I and II)

Monoamines and their metabolites in rat brain tissue were assayed by high performance liquid chromatography (HPLC) with electrochemical detection. The 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 (**Paper II**) or 50 µl/mg (**Paper I**)] containing 5 mM sodium bisulphite and 0.4 mM EDTA to avoid oxidation. The homogenate was then centrifuged at 17,000×g for 10 min at 4°C. Aliquots (10 µl) of the obtained supernatant were chromatographed on a Lichrospher 60 RP Select B column (250×3 mm; 5 µm) (**Paper II**) or on Luna C18(2) column (150×2 mm; 5 µm) (**Paper I**). 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 octylsulphonate and 5.6 % (**Paper II**) or 7.5% acetonitrile (**Paper I**). 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.

In vivo microdialysis (Paper III, V and Study II)

Procedure

The animals preselected for their behaviour in the exploration box test were anaesthetized with chloral hydrate (350 mg/kg, IP) and mounted in a Kopf stereotactic frame. Self-made concentric Y-shaped microdialysis probes were implanted according to the rat brain atlas by Paxinos and Watson (1986). The dialysis membrane used was polyacrylonitrile/sodium methalyl sulphonate copolymer (Filtral 12; inner diameter: 0.22 mm; outer diameter: 0.31 mm; AN 69, Hospal, Bologna, Italy). Two stainless steel screws and dental cement was used to fix the probe to the scull. After the surgery, rats were placed in individual cages (21 × 36 × 18 cm high) in which they remained throughout the experiment.

Microdialysis was conducted in awake, freely moving rats starting approximately 20 hours after the surgery. The probes were perfused with Ringer solution (147 mM NaCl, 4 mM KCl, 1.2 mM $CaCl_2$, 1.0 mM $MgCl_2$, 1.0 mM Na_2HPO_4, 0.2 mM NaH_2PO_4; pH 7.20–7.22) at a constant flow rate of 1.5 µl/min using syringe microdialysis pump (World Precision Instruments, USA). Connections to the infusion pump and microfraction collector (CMA/142, Sweden) or collection loop were made with flexible FEB-tubing (ID

0.12 mm, AgnTho's AB, Sweden). After a stabilization period of approximately 1 h, samples were collected every 15 min. After the completion of the experiment, the animals were deeply anaesthetized with chloral hydrate (350 mg/kg, IP) and decapitated; the brains were removed, immediately frozen and kept at -80°C. The brains were sectioned in a cryostatic microtome (Microm GmbH, Germany); the probe placements were determined according to the atlas by Paxinos and Watson (1986) and data of animals with probe placements outside the appropriate area were excluded from the analysis.

In **Paper V**, a microdialysis probe with 5 mm shaft length and active membrane on the whole length was implanted into the medial prefrontal cortex (PFC) according to the following coordinates relative to bregma: AP: +3.3; ML: +0.8; DV: –5.0; and a probe with 4 mm shaft length and 1 mm active tip was implanted into dentate gyrus (DG) with the following coordinates: AP –4.3; ML: +2.2; DV: –3.8. The samples were collected directly into a 50 µl loop of the electrically actuated injector (Cheminert C2V, Vici AG International, Switzerland) and injected automatically into the column in order to determine the quantity of 5-HT in the samples 'online' by using HPLC with electrochemical detection. An intraperitoneal injection of parachloroamphetamine (PCA) (2 mg/kg) was given immediately after collection of the 7th sample. Local administration of citalopram (1 µM) by reverse dialysis, carried out on separate animals, started at the beginning of collection of the 7th sample and lasted for 2.5 h.

In **Paper III** and **Study II**, a probe with 7 mm shaft length and 3 mm active tip was implanted in the dorsal striatum according to the following coordinates: AP: +0.7; ML: +3.0; DV: –7.0. In **Study II**, immediately after the collection of the 5th sample, animals received an i.p. injection of either imipramine (10 mg/kg) or vehicle (controls). D-amphetamine (0.5 mg/kg i.p.) was administered immediately after the collection of the 13th sample (two hours after the imipramine/saline treatment) and samples were collected directly into a 50 µl loop and injected automatically into the column. In **Paper III**, D-amphetamine (0.5 mg/kg i.p.) was administered immediately after the collection of the 6th sample. The samples were collected in 15-min periods into vials prefilled with 7.5 µl of 0.02 M acetic acid.

Measurement of dopamine and serotonin in microdialysates

The quantity of DA and 5-HT in the samples was determined using HPLC with electrochemical detection. In **Paper V** and **Study II**, the samples were collected directly into a 50 µl loop of the electrically actuated injector (Cheminert C2V, Vici AG International, Switzerland) for 15 min and then injected automatically into the column. The chromatography system consisted of a Shimadzu LC-10AD series solvent delivery pump, a Luna C18(2) 5 µm column (150 x 2 mm) kept at 30ºC and Decade II digital electrochemical amperometric detector (Antec Leyden BV, the Netherlands) with electrochemical flow cell VT-03 (2 mm GC WE, ISAAC reference electrode, Antec Leyden BV, the Netherlands). The mobile phase consisted of 0.05 M sodium citrate buffered to pH 5.3,

2 mM KCl, 0.02 mM EDTA, 3.5 mM sodium octanesulphonate and 14% acetonitrile (**Study II**) or 0.05 M sodium citrate buffered to pH 5.3, 2 mM KCl, 0.02 mM EDTA, 4.9 mM sodium octanesulphonate and 18.5% acetonitrile (**Paper V**). The mobile phase was filtered through a 0.22μm pore size filter (type GV, Millipore, USA) and was pumped through the column at a rate of 0.2 ml/min. 5-HT and DA eluted from the column were measured with a glassy carbon working electrode maintained at a potential of +0.4 V versus Ag/AgCl reference electrode. Data were acquired using a Shimadzu LC Solution system. Concentrations of DA and 5-HT were estimated by comparing peak heights from the microdialysates with those of external standards of known concentration of DA and 5-HT (Fluka, Germany).

In **Paper III,** the chromatography system consisted of Hewlett Packard series 1100 pump and autosampler, a Hypersil BDS C-18 column (250×2 mm, 5 μm), an ESA 5011 analytical cell (working electrode potential +250 mV) and an ESA Coulochem II controller unit. The column temperature was 30ºC. 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.

D_2 receptor-stimulated [^{35}]GTPγS binding (Paper II)

Membranes from striatum and nucleus accumbens were prepared as described previously (Lepiku et al., 1996). Tissue samples were homogenized in 100 vol (ww/v) of homogenization buffer (50 mM Tris-HCl, pH=7.4) by Bandelin Sonopuls sonificator (two passes, 10 s each). The membranes were collected by centrifugation at 40,000×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 nucleus accumbens membranes in 450 vol (ww/v) of the incubation buffer A (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 accumbal 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 10 μM 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 LS 1800 scintillation counter (Beckman Coulter Inc, Fullerton, CA, USA). Raw data were analysed by means of a non-linear least squares fittings using GraphPad PRISM™ (GraphPad Software, San Diego, CA, USA) software.

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

The striata of LE- and HE-rats 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-HCl (pH 7.4 at 20°C), 1 mM EDTA, 5 mM KCl, 1.5 mM $CaCl_2$, 4 mM $MgCl_2$ and 120 mM NaCl.

[^{3}H]Domperidone was custom synthesized as [phenyl-^{3}H(N)]domperidone (42–68 Ci/ mmol) by PerkinElmer Life Sciences (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, based on 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 D_2 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, CT). 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.

[N-methyl-^{3}H]Citalopram binding to serotonin transporter (Paper V)

The PFC samples were collected from the right hemisphere (contralateral to the microdialysis site) of the frozen brains of the animals that had undergone the microdialysis experiment immediately before the probe localization determination, while the hippocampus samples were collected from the right hemisphere of naïve animals that were decapitated and brains immediately dissected on ice. The cortical tissues were homogenized in 5 ml of homogenization buffer (50 mM Tris-HCl, pH=7.4) by Bandelin Sonopuls sonicator (three passes, 10 s each). The membrane fragments were collected by centrifugation at 30, 000 × g for 20 min at 4˚C and washed by homogenization and centrifugation for two more times. The final pellet was resuspended in 100 vol (ww/v) of the incubation buffer (50 mM Tris-HCl, 120 mM NaCl, 5 mM KCl, pH=7.4). Binding of [N-methyl-^{3}H]Citalopram (75 Ci/mmol, Amersham Biosciences) was carried out by incubating membranes (1 mg of tissue per tube) in incubation buffer with different concentrations of radioligand (0.3–3.5 nM) for 60 min at 25˚C. Non-specific binding was determined in the presence of 1 μM nonradioactive fluoxetine. The reaction was stopped by rapid filtration through GF/B glass-fiber filters (Whatman Int. Ltd., presoaked with 0.3% polyethyleneimine before filtration) and the filters were washed three times with ice-cold incubation buffer. The filters were kept in 4 ml of scintillation coctail (OptiPhase HiSafe3, Wallac Perkin Elmer Life Sciences, Cambridge, UK) overnight and counted using a RackBeta 1219 liquid scintillation counter (Wallac Inc., Gaithersburg, MD, USA). Raw data were analysed by means of a non-linear least squares fittings using GraphPad PRISM™ (GraphPad Software, San Diego, CA, USA) software.

RNA isolation, cDNA synthesis and quantitative real-time PCR (Paper V)

Naïve LE- and HE-rats, not used in any experiments but for the selection, were decapitated and brains immediately dissected on ice. The dissected tissue samples were prepared for total RNA isolation and cDNA synthesis as described previously (Pruunsild et al., 2007). Levels of total BDNF and NGF mRNA were quantified with qPCR Core kit for SYBR® Green I No ROX (RT-SN10–05NR, Eurogentec, Belgium). All reactions were performed on Light-Cycler 2.0 thermocycler (Roche) using the following temperature cycling conditions: 10 min at 95°C (initial denaturation step), then 45 cycles of 5 s at 95°C, 10 s at 55°C and 10 s at 72°C. All PCR reactions were performed in triplicate and normalized to beta-actin (ACTB) mRNA levels. The following primers were used: BDNF_cod_s GGCCCAACGAAGAAAACCAT, BDNF_cod_as AGCATCACCCGGGAAGTGT, NGF_s TTGCCAAGGACG CAGCTTTCTA, NGF as CAACATGGACATTACGCTATGCA, ACTB_s ATGGAATCCTGTGGCATCCAT and ACTB_as CCACCAGACAGCACT GTGTTG. The Q-PCR data were expressed as BDNF or NGF mRNA levels relative to the reference ß-actin mRNA levels, with the expression level of 1.0 for a randomly selected sample.

Statistical analysis

In **Paper I**, data from the behavioural tests and HPLC measurements were analysed with two-factor analysis of variance (ANOVA), the Toxin (0 vs. 10 vs. 50 mg/kg of DSP-4) and Treatment (Vehicle vs. Cocaine) as independent variables. Where appropriate, a repeated measures factor (Day) was added. In **Paper II**, a two-factor ANOVA with Exploration (LE vs. HE) and Toxin (Vehicle vs. DSP-4) was employed, a repeated measure (pre- vs. post-DSP-4) or a third factor Treatment (Vehicle vs. Amphetamine) added when appropriate. In **Paper III**, data was analysed with a one-way ANOVA Exploration (LE vs. HE) with repeated measures variable (Sample) added in microdialysis experiment. In **Paper IV**, temperature data were analyzed using General Linear Model repeated measures procedure with Exploration (HE vs. LE) as an independent factor, and Time (30 vs. 60 min) and Dose (0, 0.1 and 0.3 mg/kg of 8-OH-DPAT) as within-subjects repeated measures factors. **In Paper V**, data were analyzed with a one-factor ANOVA, with a repeated measure (Sample) added for microdialysis data. In **Study I**, a two-factor ANOVA with Exploration (LE vs. HE) and Treatment (Cocaine vs. Vehicle) as independent variables was used and when appropriate, a repeated measures factor (Day) was added. In **Study II**, a two-factor ANOVA Exploration (LE vs. HE) and Treatment (Imipramine vs. Vehicle) with a repeated measures factor (Sample) was used.

Group differences after significant ANOVAs were measured by *post hoc* Fisher's PLSD test. For correlative analysis, Spearman's rank correlation coefficient was used. Statistical analysis was performed with Statview 5.0 (SAS Institute Inc., Cary, NC, USA) and Statistica 8.0 (Statsoft, Inc., Tulsa, OK, USA).

RESULTS AND DISCUSSION

Noradrenergic regulation of psychostimulant and rewarding effects of cocaine: behavioural and neurobiological effects of denervation of locus coeruleus (Paper I)

Tissue contents of monoamines and their metabolites

In Experiment 1, in order to confirm the efficacy of denervation of LC in response to DSP-4 administration, we measured the monoamine and their metabolite levels in brain regions that receive NA input exclusively from the LC, frontal cortex and hippocampus, which should reflect the degree of NA-ergic denervation. Twenty-three days after the administration of the toxin, the higher dose (50 mg/kg) of DSP-4 had reduced the tissue content of NA significantly in both areas to a comparable extent with previous reports (53% in frontal cortex and 74% in hippocampus) (Kask et al., 1997; Harro et al., 2003). However, the lower dose used, 10 mg/kg of DSP-4, had a statistically non-significant decreasing impact on the NA-levels (**Paper I Table 1**).

In Experiment 2, we measured NA content in the same brain regions earlier, 12–13 days after administration of the neurotoxin, and predictably found reduction in NA levels to be larger and dose-dependent: as compared to the controls, NA levels were decreased in frontal cortex and hippocampus in the 10 mg/kg-DSP-4 group by 32 and 28%, respectively, and the higher dose of 50 mg/kg had reduced NA levels by 74 and 85%, respectively (**Paper I Table 2**). It would be reasonable to assume that similar reduction in NA levels was also present at this time-point in Experiment 1 during the first training of CPP (11^{th}-12^{th} day after the DSP-4 treatment), when the locomotor activating effect was first studied, as this would also be in line with our previous results (Harro et al., 2003). In the latter study, the 10 mg/kg dose of DSP-4 reduced NA levels in the frontal cortex by 22% three days after the treatment, but one month later the NA levels were found to be completely recovered. Thus, the size of the effect of the smaller dose of DSP-4, as measured by NA levels, has been variable after the lag time for neurodegeneration, and not always consistently significant in all brain regions studied, but there have been functional consequences either with regard to neurochemical alterations in other monoamine systems or in behavioural measures.

Despite the specificity of the primary DSP-4 effect on the noradrenergic terminals (Ross, 1976; Fritschy & Grzanna, 1989; Fritschy et al., 1990), pre-treatment with this neurotoxin appears also to result in functional changes in other monoamine systems. In the experiments in **Paper I**, DSP-4 treatment also time-dependently modified DA and 5-HT levels. In Experiment 1, DA levels were upregulated in frontal cortex and there was a reduction of DA turnover that occurred after DSP-4 pre-treatment irrespective of the dose used. The trend observed in Experiment 2 was similar – DA levels were heightened, although not statistically significantly. Findings similar to our Experiment 1 concerning upregulation in

DA levels have been reported by others (Hallman et al., 1984; Fornai et al., 1996) who have used near-complete denervation, and commonly changes in DA measures have been reported two weeks and later after the DSP-4 treatment.

Changes in 5-HT levels also occurred: the 50 mg/kg dose of DSP-4 decreased 5-HIAA in FC both in Experiment 1 and 2. In Experiment 2, both doses lowered the turnover of 5-HT, as measured by 5-HIAA/5-HT ratio, in FC; and the dose of 50 mg/kg of DSP-4 lowered 5-HT in hippocampus but enhanced 5-HT turnover in this region. In several studies, 5-HT and/or 5-HIAA levels have been found changed after DSP-4 and there has been quite some discussion whether this is a direct or indirect effect and whether this bears relevance to the behavioural effects of DSP-4. We believe it is a secondary effect that has no direct established behavioural relevance. However, we cannot rule out the possibility that changes in 5-HT system are, to some extent, responsible for the behavioural effects seen after the DSP-4 treatment. In order to avoid the possible confound of altered 5-HT-systems, the selective uptake blocker of 5-HT before DSP-4 administration can be used, but, on the other hand, this would introduce global changes in the serotonergic tone. Another limitation of the present study is the lack of immunohistochemical or autoradiographic analysis of the size of lesions produced by DSP-4 administration, which would have permitted a detailed assessment of lesion effects on NA axons and direct correlations with cocaine-induced behaviour.

The effect of locus coeruleus denervation on the stimulant effect of cocaine on locomotor activity

The data from Experiment 1 indicated a large decrement in acute motor activating effect of cocaine in LC-denervated animals (**Paper I Fig. 1**). Already the smaller dose of DSP-4 was sufficient to reduce the number of cocaine-stimulated rearings and line crossings during the first training in the white compartment of the CPP apparatus, which was conducted 11–12 days after administration of DSP-4, and the effect was not distinguishable of that of the higher dose. During the last training in the cocaine-paired compartment, which took place on the 17–18th day after the neurotoxin administration, both doses of DSP-4 reduced cocaine-induced rearings, but only the higher denervation had an attenuating effect on line crossings. This supports the presumable recovery of NA levels in the 10mg/kg-group. Nonetheless, cocaine still had a significant stimulating effect in DSP-4 treated animals on line crossings, which indicates that LC-derived noradrenergic transmission greatly enhances but is not absolutely required to obtain locomotor responses to cocaine.

Because recovery of noradrenaline levels by the end of the Experiment 1 did not allow assessment of the efficacy of the neurotoxin, the effect of DSP-4 pretreatment on the acute psychomotor effect of cocaine was re-examined in an independent experiment (Experiment 2). The finding that extensive LC denervation reduces the locomotor activating effect of cocaine was confirmed (**Paper I Fig. 3**). However, the effect of the partial denervation was not significant in

this experiment. While this could have been because of either different source or age of the animals, lower acute locomotor response to cocaine, or different basal exploratory activity in the two populations of rats, it suggests that the smaller reductions in NA function have less robust impact on the motor activating effects of cocaine.

DSP-4 treatment has been shown to produce a behavioural deficit in challenging and changing (new) situations (Archer, 1983; Harro et al., 1995). We have previously seen that locomotor activity in largely NA-depleted animals in response to 1.5 mg/kg amphetamine depends upon the novelty of the testing environment: the denervation of LC caused a similar increase in locomotor activity in control and DSP-4-pretreated animals not familiar to the open field whereas when repeatedly (15 minutes on 5 consecutive days) habituated to the test apparatus, the effect of amphetamine on horizontal activity was significantly higher in the DSP-4-pretreated animals. The rats in **Paper I** were handled in the experimental room on three occasions and habituated to the CPP apparatus on two consecutive days before the conditioning sessions (Experiment 1) or measuring the effect of LC denervation on acute locomotor response to cocaine (Experiment 2). Thus, the confounding effect of neophobia should be minimized in these experiments. However, whereas DSP-4 did not produce a nonspecific locomotor deficit as the pre-treatment in Experiment 1, in Experiment 2, the locomotor activity of the 50 mg/kg-control group in the white compartment was also reduced and this could contribute to the locomotor attenuating effect of DSP-4 on cocaine seen in this group. A transient decrease in spontaneous activity after DSP-4 has also been reported before (Archer et al., 1983; Harro et al., 2000). As described earlier, an identical protocol for handling and introduction to the experimental room and CPP apparatus was used in both experiments, thus the reduction of activity in the 50 mg/kg-controls cannot be attributed to novelty. Nevertheless, in Experiment 1, the locomotor activity of the 50 mg/kg-control group was not decreased and these results from both experiments together allowed us to infer that the substantial denervation of LC brings about attenuation in the stimulant motor activating effect of cocaine.

The exact mechanism of this reduction in response to cocaine remains to be elucidated, but a simplistic explanation which does not take into account the possible compensatory mechanisms at receptor level would be as follows: the treatment with DSP-4 has been shown to reduce the tonic firing of LC neurons (Olpe et al., 1983); thus, when cocaine binds to the remaining NAT and blocks the uptake of NA, the accumulating extracellular NA levels at synapses are not enough to stimulate the postsynaptic neurons to the same extent as in controls. Alternatively and/or simultaneously, these reductions in cocaine-elicited motor activation could be mediated by changes in dopaminergic pathways. Impairment in LC projections is known to produce changes in the DA-systems, as even a partial denervation of the LC by 10 mg/kg DSP-4 can reduce DA release potential in the nucleus accumbens (Häidkind et al., 2002) and higher rates of LC denervation have been demonstrated to suppress DA release in NAC and striatum (Lategan et al., 1990, 1992), regions which are vital for locomotor

effects of psychostimulants. That NA-ergic control over DA is important for psychostimulant action has been demonstrated in studies of Ventura and colleagues who found prefrontal cortical NA depletion to impair both amphetamine and cocaine-induced accumbal DA release as well as CPP in mice (Ventura et al., 2003; Ventura et al., 2007).

The effect of locus coeruleus denervation on cocaine-conditioned place preference

In Experiment 1, we found clearly revealed cocaine-conditioned place preference only in rats with an intact projection from the LC. Cocaine CPP as measured by increment of time spent in the previously cocaine-paired chamber during drug-free conditions before and after cocaine-paired trainings, was entirely absent after the large lesion of LC projections by DSP-4 (50 mg/kg) (**Paper I Fig. 2**). The exact reason for this phenomenon, however, remains to be investigated: it is not clear whether the attenuation of CPP in LC-compromised animals is attributable to attenuation of rewarding properties of cocaine or impairment in contextual learning or both. Dorsal noradrenergic bundle (DNB) lesions have been shown to slow down the acquisition of conditional appetitive discrimination (Robbins, 1985) and experiments using DSP-4 have suggested impairments in acquisition of contextual learning in two-way avoidance paradigm (Ögren et al., 1980; Archer et al., 1982; Archer et al., 1984a; Archer et al., 1984b). Impaired learning of a spatial discrimination has also been observed following lesions of the DNB (Mason & Lin, 1980) or treatment with DSP-4 (Archer et al., 1983). On the contrary, enhancement of noradrenergic function has been demonstrated to improve the performance of rodents in attentional set-shifting tasks (Lapiz & Morilak, 2006; Lapiz et al., 2007). Nevertheless, taking also into account that the locomotor response to cocaine was attenuated in the 50 mg/kg DSP-4 group, it can be inferred that a crucial part in this effect is due to compromised NA-system. Prefrontal cortical NA depletion, which impairs both amphetamine and cocaine-induced accumbal DA release, also abolishes CPP in mice (Ventura et al., 2003; Ventura et al., 2007). Recent optogenetic studies have revealed that phasic activity in VTA dopaminergic cells is sufficient to mediate place preference conditioning (Tsai et al., 2009). Altogether these findings support the notion that psychostimulant reward depends on a well-integrated noradrenergic-dopaminergic coupling.

The modulation of brain noradrenergic systems offers one approach in the development of a pharmacotherapy for cocaine addiction. Indeed, disulfiram, which is an inhibitor of the NA biosynthetic enzyme dopamine β-hydroxylase, has demonstrated promising efficacy in the treatment of cocaine dependence in preliminary clinical trials (reviewed in Weinshenker & Schroeder, 2007; Sofuoglu & Sewell, 2009). Given the influence of LC NA-system on unconditioned and conditioned effects of cocaine found in **Paper I**, these findings have implications to the neurobiology of psychostimulant addiction.

Cocaine-conditioned place preference in low and high exploring rats (Study I)

Individual differences studied in relation to the CPP paradigm have yielded somewhat inconsistent results, possibly because of the differences in the preselection procedures used. Kosten and Miserendino (1998) and Gong et al. (1996) did not find any differences between the HR- and LR-rats using the place preference procedure (Gong et al., 1996; Kosten & Miserendino, 1998). The experiments of Robinet et al. (1998) and Klebaur & Bardo (1999), however, were able to demonstrate that when using a free-choice paradigm, animals which spent more time in the novel compartment during the test for novelty-induced place preference or engaged more in the exploration of a novel object in the playground maze also revealed a higher magnitude of amphetamine reward as measured by CPP (Robinet et al., 1998; Klebaur & Bardo, 1999). Thus, since the exploration box test involves both forced and free component of exploration, we investigated whether cocaine-induced place preference depends on the rats' spontaneous exploratory activity levels as measured by the exploration box test.

There were no differences in the baseline of time spent in the white part of the CPP apparatus between either HE- and LE-animals or in Cocaine vs. Saline groups (**Fig. 1**). However, the baseline activity of the HE animals was higher with regard to rearings and locomotor activity ($F(1,44)=4.26$, 4.27, $p<0.05$, respectively). There was a main effect of cocaine treatment over the repeated drug-free CPP tests on time spent in the white compartment and locomotor acitivity therein ($F(1,44)=10.9$, $p<0.01$; $F(1,44)=4.0$, $p=0.051$, respectively), with cocaine-trained animals also exhibiting a more pronounced locomotor activity over the testing times.

A two-factor repeated measures ANOVA revealed a significant effect of repeated testing on time spent, entries, rearings, line crossings and locomotor activity in the white compartment ($F(3,132)=26.3$, 25.8, 18.7, 30.7, 29.1, $p<0.0001$, respectively), all groups together being most active and spending more time in the white compartment after 8 conditionings. There also was a significant interaction of repeated testing and cocaine treatment on time ($F(3,132)=8.0$, $p<0.0001$), rearings ($F(3,132)=7.8$, $p<0.0001$), line crossings ($F(3,132)=3.6$, $p<0.05$) and locomotor activity ($F(3,132)=5.6$, $p<0.01$). Thus, cocaine conditioned place preference was revealed and the increase in locomotor activity was higher in cocaine-conditioned rats. *Post hoc* tests showed that while time in the white compartment remained significantly higher than baseline for both Cocaine groups throughout the three testing times after the conditionings, conditioned locomotor activity in the white part of the CPP apparatus differed between respective Cocaine and Saline groups only after eight CPP trainings, being higher in cocaine-trained groups.

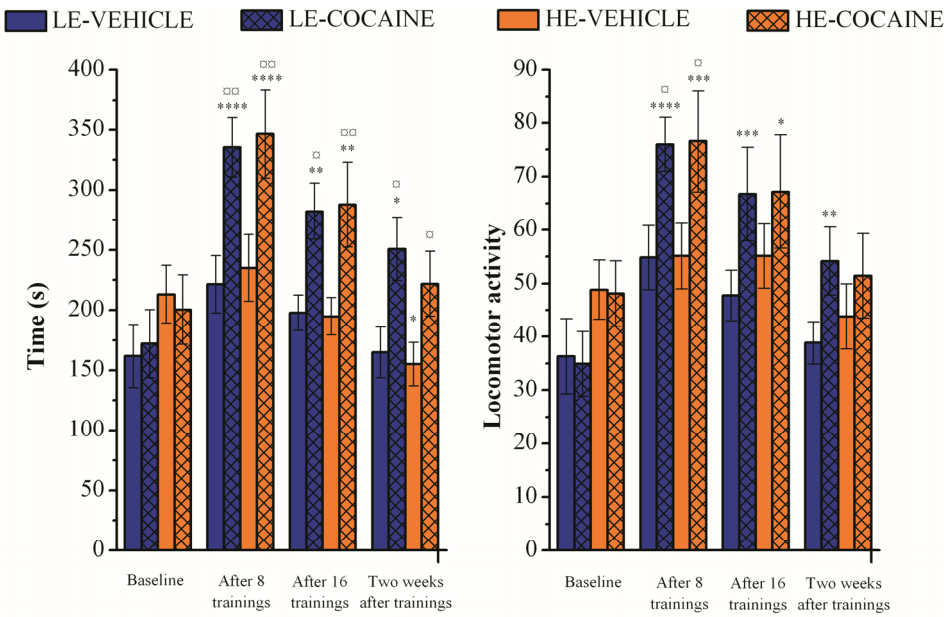

Figure 1. Time spent and locomotor activity (rearings + line crossings) in the white compartment of the conditioned place preference apparatus at baseline conditions, after 4 and 8 conditioning sessions with cocaine and two weeks after the conditioning series (data expressed as means±SEM; n=12 per group). ¤, ¤¤ – $p<0.05$, 0.01 vs. respective vehicle group; *, **, ***, **** – $p<0.05$, 0.01, 0.001, 0.0001 vs. respective group at baseline. LE – low explorers; HE – high explorers.

We found no differences in the magnitude of cocaine-conditioned place preference in LE- and HE-rats (interaction of Exploration×Treatment over repeated measuremants, NS). Interestingly, however, as compared to the baseline conditions, LE cocaine-trained animals exhibited pronounced locomotion even after two weeks after the end of the conditioning sessions whereas respective HE animals did not, showing a trend towards slower extinction of conditioned locomotor response in LE-rats. The finding that the conditioned locomotor activating effect of cocaine is more resistant in LE animals is worth of being studied further, because in an independent series of studies we have found that only the LE-rats developed a behavioural sensitization to a repeated low dose of amphetamine (0.5 mg/kg) administration (Alttoa et al., 2007), suggesting, at least in certain conditions, the LE-rats being more susceptible to repeated treatment effects with psychostimulants. On the other hand, the LE-rats have been shown to acquire a more persistent association between neutral and stressful stimuli in fear conditioning paradigm (Mällo et al., 2007), thus they may have deficits in relearning processes; therefore, the cognitive abilities of the LE/HE-rats should be tested. Nonetheless, studies on reinstatement of conditioned effects of psychostimulants would be of interest in the LE/HE model since NA-ergic system has been implicated in these processes (Wein-

shenker & Schroeder, 2007) and differential regulation of NA-ergic systems may contribute to the behavioural profile in those animals (**Paper II**, (Alttoa et al., 2007).

Previous experimental work has shown that individual differences in locomotor behaviour in an inescapable novel environment predict self-administration of amphetamine and cocaine, as the rats that show high activity in response to novel test situation reveal enhanced psychostimulant self-administration as compared to the low activity counterparts (Piazza et al., 1989; Pierre & Vezina, 1997, 1998; Marinelli & White, 2000; Piazza et al., 2000; Kabbaj et al., 2001; Klebaur et al., 2001; Ranaldi et al., 2001). The results of studies using conditioned place preference, another protocol used to estimate the rewarding effects of drugs and their abuse liability, seem to depend upon the specific pre-selection procedure used. While studies using the HR/LR distinction based on activity in a novel inescapable environment have shown that activity in a novel chamber does not predict the strength of a preference formed for an amphetamine-paired place (Erb & Parker, 1994; Robinet et al., 1998; Kabbaj, 2006), time spent in a novel area in a free-choice situation (Robinet et al., 1998) as well as novel object preference score in a previously familiarized playground-maze was found to predict the magnitude of amphetamine-induced CPP, with higher 'novelty seekers' found to be more susceptible to the rewarding effects of amphetamine (Klebaur & Bardo, 1999).

Our result that the LE- and HE-rats do not differ in cocaine-conditioned reward as measured by place preference protocol is in accordance with the studies which use an inescapable novel environment for the HR/LR selection procedure (Gong et al., 1996; Kosten & Miserendino, 1998). These CPP data suggest that the rewarding properties of cocaine may be similar in LE- and HE-rats. However, an alternative possibility would be that the stress of repeated injections may have equalized individual differences during the place preference conditionings. Indeed, it has been shown before that, e.g., social defeat stress equalises individual differences in cocaine self-administration in HR- and LR-rats (Kabbaj et al., 2001).

Exploration box test used in the present studies, which is an 'emergence test' by nature, contains both the elements of forced exploration and free-choice test and with regard to the property of not predicting the magnitude of CPP, its closer resemblance in this regard to the inescapable novel environment tasks might be proposed. Alternatively, it should be considered that previous studies having been able to demonstrate higher susceptibility of 'novelty seeking' rats to drug reward, the selection tests made use of investigation of either a relatively small novel area or a novel object, which may differ from tests placing higher requirements to inquisitive exploration. Furthermore, these studies used amphetamine as the drug reward, which may differ in respect with the dependency of its effect on exploratory behaviour. However, in order to further estimate the sensitivity of LE- and HE-rats to the reinforcing effects of psychostimulants, the self-administration paradigm could be the method of choice.

Neurobiological differences in low and high explorers in the NA system: behavioural and neurobiological effects of partial locus coeruleus denervation (Paper II)

Exploratory behaviour

Increased LC activity is observed in response to exposure to novel sensory stimuli (Foote et al., 1980; Aston-Jones & Bloom, 1981b), suggesting that NA participates in processing of the novel stimuli and familiarization with those. The selective neurotoxin DSP-4 primarily affects neuron terminals of NA-ergic cells arising from the locus coeruleus, producing substantial depletions of NA in cortex and hippocampus. Previous studies from our laboratory have repeatedly revealed that after a large lesion of the LC projections by a standard dose (50 mg/kg) of DSP-4, almost all rats avoid entering the open area of the exploration box apparatus during the first testing, and that this is strongly based on an increase in neophobia, since it can be reversed by a small dose of diazepam (Harro et al., 1995). Several other studies that have also reported DSP-4 pre-treatment to impair coping with a novel environment are consistent with these observations (Delini-Stula et al., 1984; Berridge & Dunn, 1990). In the experiments of Delini-Stula and colleagues, ten days after the pre-treatment, a high dose of DSP-4 decreased exploration-oriented locomotor responses in an unfamiliar environment and also resulted in longer latencies to approach a novel object in it. The effects of the DSP-4 treatment on novelty-behaviour appear to be time-dependent and/or species specific, as Berridge and Dunn demonstrated in mice that denervation using DSP-4 exerted opposite effects on exploratory behaviour observed 3 and 14 days following the pre-treatment.

Fourteen days after the administration of a low dose (10 mg/kg) of the selective neurotoxin DSP-4 which produced depletion of NA in a comparable level in both LE- and HE-rats, there was no effect of the toxin treatment on the exploratory activity of HE-rats, suggesting that the remaining NA-ergic terminals were sufficient to maintain their characteristic exploratory behaviour (**Paper II Fig. 2**). Wistar LE-rats display negligible exploration of the open area during the first testing but usually their activity increases from the third exposure to the exploration box, albeit not to the level of the HE-rats (Mällo et al., 2007). Partial LC denervation with the low dose of DSP-4 prevented the slight increase of exploratory activity typically observed in the LE-rats with repeated testing. We inferred 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 neurotransmission related to LC whereas maintaining typical high exploratory activity as it prevails in the HE-rats is not.

Amphetamine-stimulated behaviour

Data from HR/LR model indicate that the HR-rats display an enhanced locomotor response to psychostimulant administration (Piazza et al., 1989; Hooks et al., 1991b, a; Hooks et al., 1994a). After administration of a small dose of amphetamine (0.5 mg/kg), the locomotor activity in the open field remained higher in the HE-rats **(Paper II Fig. 3)**. The finding was repeated in an independent experiment which, by including control groups, allowed to infer that this dose of amphetamine stimulates locomotion in both LE- and HE-rats and to a rather similar extent in terms of proportional increase in activity with regard to baseline; the amphetamine-induced activity in the HE-rats remained significantly higher as compared with the LE-rats (**Paper II Fig. 4**). In a separate experiment, we also found similar proportional increase in locomotor response to cocaine (15 mg/kg), with HE-rats exhibiting a higher activity after cocaine administration in the open field (unpublished). However, in order to estimate the magnitude of 'pure' stimulating effects of psychostimulants on locomotor activity in HE and LE animals, prior to the test the rats should be let to habituate to the testing apparatus for several hours to exclude the confound of the immediate novelty response, and this remains to be carried out.

Both amphetamine studies revealed that only the HE-rats treated with DSP-4 displayed a marked decrease in all amphetamine-induced activity measures while in contrast, the effect of amphetamine was not modified in LE-rats (**Paper II Fig. 3 & 4**). In addition, a subsequent experiment by Alttoa and colleagues (2007) confirmed this finding using a 5-day repeated treatment schedule with the same dose of amphetamine, showing this to be a robust phenomenon (Alttoa et al., 2007). Thus, the accumulating evidence suggests that the expression of behavioural responses to amphetamine on an open field is dependent on an intact LC noradrenergic system in HE-, but not in LE-rats. Nevertheless, these results suggesting a different regulation of the NA-ergic neurochemical system in exploratory and amphetamine-activated behaviour in LE- and HE-rats should be addressed with direct acute pharmacological inhibition or stimulation of noradrenergic receptors, and tested in the same paradigm.

Concerning the behavioural results of the effect of partial denervation of LC on cocaine-induced locomotion in **Paper I**, which used rats not preselected for their exploratory activity, it could be that one of the reasons the pretreatment with 10 mg/kg dose of DSP-4 did not bring about the same reduction in the cocaine-induced locomotor activity in Experiment 2 as was seen in Experiment 1, was different level of exploratory activity and underlying sensitivity of brain mechanisms involved between the rat populations used in those two experiments. Indeed, the line crossings made in response to cocaine (15 mg/kg) in the control group was higher in Experiment 1 as compared to Experiment 2 in **Paper I**. As the HE-rats have higher locomotion after both amphetamine and cocaine (**Paper II** and unpublished) and the HE-rats' amphetamine-related behaviour is more dependent on an intact projection from LC, it could be speculated that there were more rats with high activity measures in Experiment

1 as compared to Experiment 2 in **Paper I**. Thus, the next logical step would be to investigate the role of different degrees of denervation of LC on un-conditioned and conditioned effects of cocaine in animals preselected for their exploratory activity.

Neurobiological measures

Tissue contents of monoamines and their metabolites

As compared to respective HE-rats, it was found that the control LE-rats had lower 5-HT content in frontal cortex and striatum (**Paper II Table 1**); however, this result was not replicated in a later experiment of ours (Alttoa et al., 2007). Some studies have reported higher dopamine levels in NAC and striatum and/or lower 5-HT levels in the frontal cortex in high responders to novelty (Piazza et al., 1991) (Hooks et al., 1991a; Thiel et al., 1999). One possible explanation for this discrepancy may be the different selection procedure used to classify the animals regarding their novelty-related behaviour. The basis for differentiation between high and low responders to novelty was rearing activity in an inescapable open field in Thiel et al. (1999), and locomotor response to a novel inescapable environment in Piazza et al. (1991) and Hooks et al. (1991), but the present study made use of an emergence paradigm, thus probably reflecting different aspects of coping with novelty. In addition, these tissue levels of monoamines need to be interpreted with precaution because due to the study design, the animals used for these measurements had received amphetamine twice during the behavioural testings. In a subsequent experiment we found that repeated amphetamine treatment may modify both dopamine and 5-HT levels in frontal cortex and striatum (Alttoa et al., 2007).

The neurotoxin DSP-4 administration in the dose of 10 mg/kg 19–21 days before the sacrifice of animals had caused a significant decrease in the NA content in FC and hippocampus in both LE- and HE-rats to a comparable extent. Since both brain areas receive NA-ergic input only from the LC, this result reflects a similar degree of denervation of NA-ergic terminals of LC in LE/HE-rats. However, the toxin also had a significant reducing effect on 5-HIAA in striatum and NAC of the LE animals only; and additionally the DA-system of LE animals was compromised after the neurotoxin treatment as DA and its metabolites HVA and DOPAC were significantly reduced in the NAC area, a finding replicated in an independent experiment (Alttoa et al., 2007). Whether these alterations in monoamine and their metabolite levels are causally related to difficulties with adaptation to environment in the LE phenotype rats, remains to be investigated in experiments combining, e.g., behavioural and *in vivo* microdialysis and electrophysiological measurements.

D_2 receptor-stimulated [^{35}]GTPγS binding

Even a partial LC denervation with DSP-4 has been shown to increase the number of D_2 receptor binding sites after a time lag in rats not preselected for

their exploratory activity (Harro et al., 2003). As the partial denervation of LC altered novelty-related and amphetamine-induced behaviour differently in LE- and HE-rats, we investigated whether the denervation of LC would be differentially reflected in functional D_2 receptor-mediated transmission as measured by DA-dependent [^{35}S]GTPγS binding. In **Paper II**, D_2 receptor function in striatum, but not in NAC, as determined by DA-stimulated increase of [^{35}S]GTPγS binding, was elevated in the LE-group which had received DSP-4 pre-treatment, but reduced in HE-rats pre-treated with DSP-4 (**Paper II Fig. 5**). Thus, we attributed this effect to partial denervation of LC and related the absence of behavioural amphetamine-stimulation in HE-rats to their reduced sensitivity of D_2-receptor-coupled G-proteins in striatum, and the unchanged amphetamine-induced behaviour in LE-rats to the increase of D_2 receptor sensitivity compensating for the NA-ergic denervation. However, since the receptor data was also, as tissue samples for monoamine assays, acquired from animals in the experiment using within-subjects design and all the animals had also received amphetamine injections (0.5 mg/kg) twice, this effect was interpreted with caution and re-examined in a following study also using control groups (Alttoa et al., 2007). Indeed, in the latter study it was demonstrated that the decrease in the sensitivity of D_2 receptor function at the level of D_2 receptor-G protein interaction occurred due to repeated amphetamine treatment selectively in HE-rats, regardless of the integrity of the LC projections.

Neurobiological differences in low and high explorers in the DA system (Paper III and Study II)

Extracellular DA release in striatum at baseline and amphetamine-stimulated conditions

Higher basal, novelty- and psychostimulant-evoked DA release has been reported in animals with higher novelty-related locomotor activity levels accompanied with elevated motor responses to psychostimulants (Hooks et al., 1991b; Hooks et al., 1992; Saigusa et al., 1999). Roman high avoidance rats, which display a more robust exploratory behaviour phenotype and less anxiety than their low avoidance counterparts (Escorihuela et al., 1999) (Fernandez-Teruel et al., 2002), also consistently differ in the functional properties of the dopaminergic system (reviewed in Giorgi et al., 2007). Given these results, we investigated DA release in the terminal areas of mesolimbic and mesostriatal dopaminergic pathways using *in vivo* microdialysis at basal as well as DA-stimulated circumstances.

Extracellular dopamine levels in dorsal striatum in baseline conditions as well as in response to amphetamine (0.5 mg/kg) administration were significantly higher in HE animals than in LE animals (**Paper III Fig. 1A**), while no difference had been previously detected in NAC (Mällo et al., 2007). However, when taken as a percentage from extracellular baseline DA, the magnitude of increase in DA release in response to amphetamine administration was similar

in HE- and LE-rats. We have demonstrated that acute administration of the same dose of amphetamine induces a similar proportional increase in locomotor activity in HE- and LE-rats; the activity levels of the HE-rats remaining significantly higher as compared to these of LE animals (**Paper II**). As differences in the basal and bursting impulse activity of DA neurons in VTA and substantia nigra have been shown to be implicated in the different phenotypes of HR and LR (Marinelli & White, 2000), differences in basal midbrain dopaminergic neuron firing could also be hypothesized to underlie, at least in part, the differences in extracellular DA levels found in the striatal complex between HE- and LE-rats.

Proportion of D_2 receptors in the high affinity state in the striatum

Dopamine D_2 receptors can exist in a state of high affinity for dopamine or in a state of low affinity for dopamine, with D_2^{High} being the functional high-affinity state of the D_2 receptor (George et al., 1985; Seeman et al., 2006). In order to reveal the D_2^{High} component and its variations in response to various treatments, a method of choice is to use the competition between dopamine and [^{3}H]domperidone (Seeman et al., 2003; Seeman et al., 2006).

The main finding of **Paper III** is that rats with persistently high exploratory activity have elevated number of D_2 receptors in the functional high affinity state for the agonist in the striatum, which coincides with higher extracellular dopamine levels in these animals (**Paper III Fig. 1B**). Further, in order to confirm the interdependency of exploratory activity and level of D_2^{High} receptors at the individual level, the sum of exploratory activity on the first and second day of the preselection procedure in the exploratory box test was correlated with the proportion of D_2 receptors in the high affinity state. We found strong positive correlations between these variables (Spearman's rank correlation coefficients $r=0.67$ $p<0.01$ and $r=0.78$ $p<0.001$ for Day 1 and Day 2, respectively).

To our knowledge, this is the first time that such a two-fold difference in the proportion of D_2^{High} in a naturally occurring phenotype was demonstrated. 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 instance, an approximately twofold increase (from 20% to 40–45%) in the proportion of striatal D_2^{High} receptors in rats has been demonstrated after a variety of psychomotor-sensitizing regimens of amphetamine (Seeman et al., 2002; Seeman et al., 2007; Seeman, 2009), 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 a naturally occurring ''sensitized'' phenotype. Thus, it would be relevant to measure the D_2^{High} proportion also in HE- and LE-rats being treated with sensitizing psychostimulant regimens known to produce changes in the D_2^{High} proportion.

Our microdialysis data suggest that in a novel environment, the novelty-induced dopamine release (Saigusa et al., 1999; Horvitz, 2000) could also 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.

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, might be causally related to high behavioural activity. In addition, recent studies show that the upregulation of D_2^{High} proportion can be also interpreted in terms of dopamine D_2 receptor dimerization (Franco et al., 2010; Wang et al., 2010).

The effect of acute imipramine on amphetamine-stimulated DA and 5-HT release in the striatum

The monoamine hypothesis of depression has mainly focused on noradrenaline and serotonin as neural substrates of depression and antidepressant drug action. However, some symptoms of major depression, e.g., anhedonia, can be related to altered functioning of the mesolimbic systems (Nestler & Carlezon, 2006), and a role for the involvement of the dopamine system in the action of anti-depressants has also been suggested (Willner et al., 2005; Dunlop & Nemeroff, 2007; Nutt et al., 2007). A general finding is that antidepressants augment the behavioural response to DA receptor agonists. Nevertheless, there have been mixed results in the literature, since the effects have been shown to be brain region- and particular substance-dependent (reviewed in D'Aquila et al., 2000). For example, it has been demonstrated that chronic treatment with imipramine, a tricyclic antidepressant (TCA), but not fluoxetine, a 5-HT reuptake inhibitor, heightens extracellular DA levels at baseline and potentiates the effect of 0.5 mg/kg amphetamine treatment in NAC (Ichikawa et al., 1998). In our lab, we had had similar result with a two-week chronic imipramine (10 mg/kg) schedule, but the potentiated DA-release in striatum after this treatment was seen only in HE animals (unpublished data). Taking also into consideration the higher extracellular DA-levels in HE-rats in striatum (**Paper III**), but not nucleus accumbens (Mällo et al., 2007), we investigated the potency of imipramine to alter amphetamine-stimulated dopamine and serotonin release in striatum in our low and high exploratory rat model.

While no differences between LE and HE groups in baseline conditions (samples 3-5) in DA levels in striatum were observed in this experiment (**Figure 2**), preceding acute imipramine administration produced a more robust increase in the amphetamine-stimulated DA release in high exploring rats (interaction of Exploration, Treatment and Sample ($F(23,483)=2.1$, $p<0.01$). There also were no differences in the baseline of 5-HT levels in striatum in LE- and HE-rats, and this stayed unchanged after imipramine and amphetamine treatments (interaction of Exploration, Treatment and Sample ($F(23,414)=0.8$,

NS). Pre-administration of imipramine had an elevating effect on acute amphetamine treatment both on DA and 5-HT levels (effect of Treatment $F(1,21)=15.1$, $p<0.001$ and $F(1,18)=7.1$, $p<0.05$; interaction of Treatment× Sample $F(23,483)=12,4$, $p<0.0001$ and $F(23,414)=13.1$, $p<0.0001$ for extracellular DA and 5-HT, respectively).

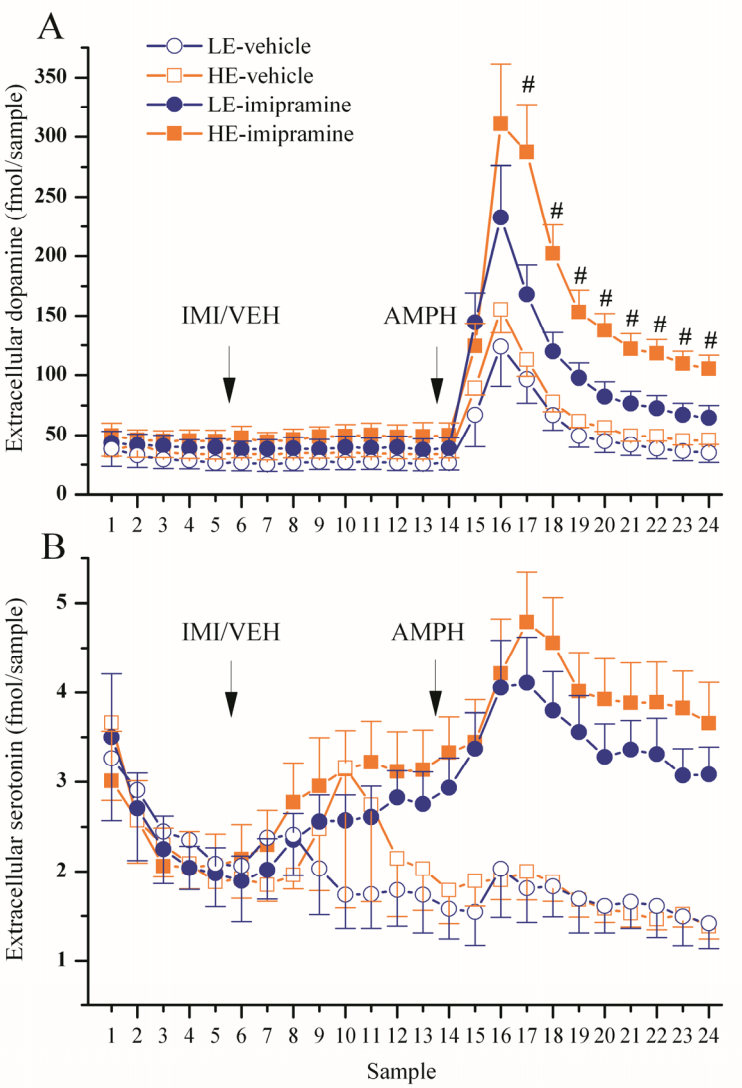

Figure 2. Extracellular dopamine (A) and serotonin (B) levels in the striatum of rats with low (LE) or high (HE) exploratory activity. Samples were collected every 15 min (22.5 µl per sample). Imipramine (10 mg/kg intraperitoneally) or vehicle (controls) was administered immediately after the collection of the 5th sample. D-amphetamine (0.5 mg/kg i.p.) was administered immediately after the collection of the 13th sample (two hours after the imipramine/vehicle treatment). Data expressed as means±SEM, N=4-7 per group. # - $p<0.05$ vs. LE-imipramine group.

In previous experiments, we have seen a difference in both baseline and after amphetamine (0.5 mg/kg) stimulation between LE- and HE-rats in striatum (**Paper III**). However, in this experiment, this observation was not statistically significant, although the direction, LE-rats to have lower levels of extracellular DA, was repeated. The present DA levels at baseline are lower than presented in **Paper III**. Methodological differences could account for the discrepant findings, the age of the animals used, and the level of exploratory activity itself. Specifically, in **Paper III**, the dialysates were collected into vials with 0.02M acetic acid in it and measured using a different chromatography system, and the animals used in the present paper were one month older at the time of the microdialysis experiments. It should also be noted that the sum of exploratory activity of HE-rats in **Paper III** microdialysis experiment were twice lower than in the present experiment. Thus, using the median split of the distribution of exploratory activity and using rats from the upper or lower quartile from a larger preselected population may yield somewhat different results. This, in turn, could mean that a comparison group of rats occupying the middle ground in the distribution of exploratory activity would be relevant in future studies.

Taken together, acute preceding imipramine treatment potentiated amphetamine-induced dopamine release more in HE-rats whereas no difference between LE- and HE-rats was seen in serotonin overflow. While the mechanism for this remains to be elucidated, this difference in sensitivity to imipramine may model the individual differences in response to antidepressant treatment. Nevertheless, this result needs to be elaborated by also using other antidepressants.

Neurobiological differences in low and high explorers in the 5-HT system (Papers IV and V)

Extracellular 5-HT and 5-HTT binding

The frontal cortex has widespread influences on multiple components of forebrain circuits regulating anxiety-related behaviour (reviewed in Millan, 2003). The hippocampal 5-HT-ergic system has been acknowledged to mediate anxiogenic responses (e.g., (File et al., 1987, 2000), and dysfunction in hippocampal region, especially in dentate gyrus (DG) has been ascribed an important role in stress reactions (Dremencov et al., 2003; Airan et al., 2007; Tanti & Belzung, 2010).

Previously, we had found LE-rats to have higher levels of metabolic activity in dorsal raphe in LE animals (Matrov et al., 2007), but lower levels of 5-HT in frontal cortex in tissue samples (**Paper II**). Thus, we addressed the question of putative differential serotonergic regulation in LE- and HE-rats by using *in vivo* microdialysis to monitor extracellular 5-HT levels in prefrontal cortex (PFC) and DG at baseline levels as well as in response to manipulations to the 5-HT-ergic system. The baseline levels of 5-HT in PFC and also in DG were similar

in LE- and HE-rats, and no differences were detected in PCA-induced depolarization-independent 5-HT release in these regions (**Paper V, Fig. 1**). However, after local infusion with citalopram, the increase in extracellular 5-HT output in LE group was more robust in the PFC (**Paper V, Fig. 3**). It is possible that the firing rate of the 5-HT-ergic projections to the PFC is higher in LE-rats, resulting in higher extracellular 5-HT levels after blockade of 5-HT transporter (5-HTT), while at baseline conditions the greater release of 5-HT is balanced by the increased re-uptake in the LE-rats that we found to have higher levels of 5-HTT in this region (**Paper V, Fig. 2**). It has been demonstrated that rats with a greater increase in extracellular levels of 5-HT caused by an inescapable stress were more likely to become helpless in two-way avoidance task than rats with less 5-HT increase in frontal cortex, whereas the basal 5-HT levels did not predict the behavioural outcome (Petty et al., 1994). Contrary to our findings, Giorgi with colleagues (2003) have reported higher levels of [^{3}H]-citalopram binding to 5-HT reuptake sites and greater increase in 5-HT output in the frontoparietal cortex elicited by either systemic or local application of a SSRI in Roman high avoidance rats (RHA), while the release of 5-HT was similar at baseline conditions with the low avoidance rats (RLA) (Giorgi et al., 2003). Although some reports have found RHA-rats to be more anxious in the elevated plus-maze and light/dark compartment test, most of the large number of behavioural studies have led to the conclusion that RHA-rats reveal less anxiety-related behaviour under stressful or conflict conditions as well as higher novelty-directed, exploratory behaviour than RLA-rats do (reviewed in Escorihuela et al., 1999; Pawlak et al., 2008). It thus appears that both higher and lower 5-HT-ergic activity in rostral parts of cerebral cortex can be found in animals revealing more anxiety-related behaviour.

In DG during citalopram perfusion, on the contrary, the LE-rats had lower increase in extracellular 5-HT levels, but this was not accompanied by lower binding capacities of 5-HTT in this area. Wistar-Kyoto strain of rats, which is more anxious and passive in the plus-maze test than spontaneously hypertensive rat (SHR) strain, had lower number of [^{3}H]citalopram binding sites in hippocampus, and their extracellular 5-HT levels tended to be lower to 1μM citalopram perfusion as compared to SHR in ventral hippocampus (Pollier et al., 2000). Animals bred for high anxiety-related behaviour (HAB) show blunted 5-HT release in the dorsal hippocampus, which is apparent in response to an emotional stressor but not under basal conditions. However, HAB rats have increased amount of 5-HTT binding sites and decreased 5-HT$_{1A}$ receptor mRNA in the hippocampus as compared with LAB rats (Keck et al., 2005). Inbred Lewis rats, also considered more anxious in plus-maze than SHRs, however, did not differ with regard to [^{3}H]citalopram binding in hippocampus while displaying a 3–4 fold higher baseline level of extracellular 5-HT in this region (Kulikov et al., 1997).

A possible mechanism for decreased 5-HT levels in DG region in LE-rats after local perfusion with citalopram could be the differential activity of 5-HT$_{1B}$ autoreceptors, which regulate the release of 5-HT by inhibitory feedback and

have been hypothesized to be supersensitive in depression and anxiety (Moret & Briley, 2000). Indeed, in learned helpless rats, 5-HT_{1B} receptors are up-regulated in the cortex, hippocampus and septum and down-regulated in the hypothalamus (Edwards et al., 1991), compared to control animals.

Altogether these results provide no simple picture with regard to serotonin/5-HTT levels and anxiety, as both excessively high and excessively low 5-HT loads are associated with anxiety-related behaviours, and also differences in regional serotonergic orchestration play a major role in the anxious phenotype (Harro, 2010).

Stress-induced hyperthermia and 5-HT_{1A} receptor function (Paper IV)

We found no differences in the magnitude of stress-induced hyperthermia in LE- and HE- rats, irrespective of stressor and measurement time (15 vs. 30 minutes after the acute stress). Nevertheless, when combining the data from both Experiment 1 and 2, it may be hypothesized that the SIH response habituates slower in the LE animals and/or the stressors used (novel cage vs. injection) are of differing intensity to LE- and HE-rats, with HE being more reactive to novelty-related conditions [average ΔT_{15} in Experiment 1: 1.3 vs. 1.5 ºC and ΔT_{30} in Experiment 2 (vehicle conditions): 1.3 vs. 1.0 ºC, in LE- and HE-rats, respectively]. These hypotheses are worth being studied experimentally; however, a telemetric system should be employed, as handling and rectal temperature measurements alone activate the autonomic stress response (Van Der Heyden et al., 1997; Vinkers et al., 2008).

Administration of 8-OH-DPAT (0.3 mg/kg) reduced SIH response in both LE and HE animals, but to a significantly higher extent in HE animals, bringing about hypothermia (**Paper IV Fig.1**). This finding was confirmed by correlation analysis at the individual level, showing lower sensitivity to 5-HT_{1A} agonist-elicited hypothermia in rats with lower exploration scores (**Paper IV Table 1**). Interestingly, correlation was stronger with behaviour in the exploration box during the first testing, when the role of anxiety in driving the performance is greater.

Lower 5-HT_{1A} receptor sensitivity to a higher agonist dose in the more behaviourally inhibited LE-rats, as measured by the lower antihyperthermic response to 8-OH-DPAT, is in good accordance with previous data from rodent and human studies showing defects in the regulation of 5-HT_{1A}-mediated neurotransmission accompanying anxiety- and depressive-like symptoms. 5-HT_{1A} knock-out (KO) mice consistently display a more anxious phenotype than the corresponding wild-types, but also less of depression-related behaviour as revealed by decreased behavioural despair in response to stress, and also lack hypothermic effects of 8-OH-DPAT (Heisler et al., 1998; Parks et al., 1998; Ramboz et al., 1998). 5-HT_{1A} KO mice also have been found to have an exaggerated SIH response to injection-stress and blunted hypothermic response to flesinoxan, another 5-HT_{1A} agonist (Pattij et al., 2002). LE-rats also resemble the more

anxious and depressive 5-HTT KO rats for which flesinoxan had no hypothermic effect as compared to 5-HTT$^{+/+}$ rats (Olivier et al., 2008) and also displayed a reduced hypothermic response to 8-OH-DPAT (Homberg et al., 2008). Similarly, 5-HTT KO mice, which display increased anxiety-like behaviour and inhibited exploratory activity relative to 5-HTT +/+ mice (Holmes et al., 2003a; Holmes et al., 2003b), also have decreased 5-HT$_{1A}$ receptor binding and function, as 5-HT$_{1A}$ receptor 8-OH-DPAT-mediated hypothermia was shown to be eliminated in 5-HTT null mutant mice (Li et al., 1999; Fox et al., 2008).

Chronic stress regimens, which are thought to bring about/precipitate depressive states, have been shown to result in down-regulation of 5-HT$_{1A}$ gene expression and receptor binding in the hippocampus and PFC (Kieran et al.; Watanabe et al., 1993; Lopez et al., 1998). Bred HAB rats have decreased 5-HT$_{1A}$ receptor mRNA in the hippocampus as compared with LAB rats (Keck et al., 2005). Similarly, LE-rats, animals with naturally occurring low exploring behavioural phenotype, representing anxiety- and depression-like features, have lower 5-HT$_{1A}$ receptor gene expression in hippocampus (Alttoa et al., 2010) and also lower extracellular 5-HT levels in response to 5-HTT blockade with citalopram in hippocampus as compared to HE-rats (**Paper V)**. In the present study we have found that the LE-rats indeed have a lower 5-HT$_{1A}$ receptor function as revealed by agonist-induced hypothermia. It is mainly found in the literature that 5-HT$_{1A}$ receptor-induced hypothermia is mediated by postsynaptic 5-HT$_{1A}$ receptors in rats (e.g., Bill et al., 1991; Millan et al., 1993; Homberg et al., 2008). Nevertheless, in our LE/HE model, further autoradiographic and binding studies are needed to locate the brain areas which contribute to the observed difference in 5-HT$_{1A}$ receptor function.

Neurobiological differences in low and high explorers in the levels of expression of neurotrophic factors (Paper V)

Brain-derived neurotrophic factor (BDNF) and nerve growth factor (NGF) are proteins involved in neuronal survival and plasticity of neurons in the central nervous system (reviewed in, e.g., Castren et al., 2007; Andressoo & Saarma, 2008; Martinowich & Lu, 2008). Decreased expression of BDNF and other neurotrophic factors have been suggested to contribute to depression, as stress, which often precipitates depressive-like states, generally decreases BDNF content and antidepressant treatments are found to upregulate the BDNF levels in brain areas implicated in depression (Nibuya et al., 1995; Altar, 1999; Duman & Monteggia, 2006). With regard to neurotrophins, the hippocampus and frontal regions of cerebral cortex have received particular attention in animal research on depression and antidepressants (Dranovsky & Hen, 2006). Contrary to the suggested 'neurotrophin hypothesis of depression', we found that in the left prefrontal cortex of the more behaviourally depressed LE-rats, the relative levels of BDNF mRNA were higher as compared to HE-rats,

suggesting that BDNF gene expression is increased at the level of transcription or mRNA stability, whereas no differences were found in hippocampus (**Paper V Fig. 4**). NGF mRNA levels did not differ between LE- and HE-rats.

Although a great number of preclinical studies indeed observe decreased cerebral BDNF expression to be associated with a depressive phenotype, there is accumulating evidence that is not consistent with such stress- and treatment-related BDNF regulation. Recent studies using chronic stress in rats resulted in upregulation of BDNF mRNA or protein in hippocampus (Schulte-Herbrüggen et al., 2009; Larsen et al., 2010). BDNF was also found to be selectively increased following early maternal deprivation stress in female rhesus macaques which displayed behavioural passivity indicative of a depression-like state (Cirulli et al., 2009). Increased hippocampal BDNF protein levels were found in mice communally nested during early life showing increased anxiety- and depressive-related behaviour in adulthood (Branchi et al., 2006). Further, while BDNF protein levels were not modified in a mouse model of learned helplessness (Schulte-Herbrüggen et al., 2006), these were found to be significantly increased in hippocampus and frontal cortex in a standard depressive-related mouse model of olfactory bulbectomy (Hellweg et al., 2007). Additionally, in an earlier report describing altered levels of neurotrophins in Flinders Sensitive Line (FSL) rats, higher levels of BDNF and NGF were found in frontal and occipital cortex, and hypothalamus (Angelucci et al., 2000). Apparently, involvement of BDNF in depression-related phenotypes appears to be more complex than a down- or up-change of the total BDNF levels in the brain.

Higher levels of BDNF mRNA combined with the higher levels of [^{3}H]citalopram binding in LE-rats are in alignment with the findings that, inversely, in heterozygous BDNF KO mice, 5-HTT function is decreased (Daws et al., 2007; Guiard et al., 2008). Nevertheless, it is acknowledged that our results are not indicative of any direct association between BDNF and 5-HTT expression. A recent study in humans found that carriers of the genotype associated with enhanced BDNF function also displayed higher 5-HTT availability in male subjects in various brain areas (Henningsson et al., 2009). In line with this, intracerebrally infused BDNF has been demonstrated to increase 5-HTT function (Benmansour et al., 2008).

These results suggest that the link between BDNF in depression-like behaviours is more complex than originally proposed. The possibility has to be considered that the upregulation in BDNF levels in rodent models of anxiety and depression might indicate an attempt towards neurochemical adaptation, a view which would be consistent with the proposal of neurotrophic factors to act as necessary tools in the activity-dependent modulation of brain networks, with the function of these networks determining whether activity-dependent plasticity produces a depression-like or antidepressant behavioural response (Castren et al., 2007).

GENERAL SUMMARY

Dopaminergic neural circuits have been implicated in motivational and reward systems, while noradrenergic and serotonergic systems are associated with the regulation of organism's responses to novel and aversive stimuli. With regard to psychostimulant reward, we found support to the idea linking noradrenaline to critical aspects of dopamine signalling and drug addiction. According to our studies, unconditioned and conditioned effects of cocaine, at least to a certain extent, depend upon integrity of the noradrenergic projections from locus coeruleus, as near-complete denervation of these projections reduced the locomotor activating and place conditioning effect of cocaine.

Studies presented in this thesis applying phenotype-based approach to model depression-like features indicate that rats with different exploratory phenotype also differ with regard to regulation of underlying monoaminergic systems. There appear to be profound differences in the regulation of midbrain dopaminergic systems between LE- and HE-rats. HE-rats revealed increased striatal dopamine release both at baseline and amphetamine conditions, and the proportion of the D_2 receptors in the high affinity state for dopamine was higher in HE animals as well. Partial denervation of the noradrenergic projections from locus coeruleus had phenotype-dependent effects on the midbrain dopaminergic systems, e.g., affecting the dopamine and its metabolite levels in striatal areas. LE- and HE-rats differed with regard to noradrenergic regulation of novelty and amphetamine-induced behaviour, as partial locus coeruleus denervation prevented the habituation-related increase in exploratory behaviour over repeated testing in LE-rats, but did not affect exploration in HE-rats. In contrast, the amphetamine-induced locomotor activation was attenuated by partial locus coeruleus denervation in HE animals only. In addition, serotonergic neurotransmission (with possible influences from BDNF) in prefrontal cortex and hippocampus was found to be regionally differentially regulated in LE and HE phenotypes, as revealed by 5-HT transporter binding and extracellular levels of 5-HT in response to blocking the 5-HT uptake. As the LE-rats exhibited a lower 5-HT_{1A} receptor agonist elicited antihyperthermic effect, it suggests a less effective 5-HT_{1A} function in the LE-rats.

Taken together, the results of our studies implicate the midbrain DA-ergic and 5-HT neurotransmission in prefrontal cortex and hippocampus contributing to the inter-individual variations in novelty-related behavioural disposition. Altogether these results lend support to the notion for using LE/HE model for revealing (novel) endophenotypes of anxiety and depression.

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SUMMARY IN ESTONIAN

Indiviididevaheliste erinevuste monoamiinergiline regulatsioon rottide uudistavas käitumises ning psühhostimulaatorite käitumist aktiveerivad efektid

Depressioon ning sellega tihti kaasuvad ärevus- ja sõltuvushäired on aina enam haiguskoormust tekitavad patoloogiad, mille raviks on küll olemas teatud osa patsientide jaoks efektiivsed farmakoteraapiad, kuid mille tekke ning kasutusel olevate ravimite toimemehhanismide kohta puudub ammendav teooria. Siiani olulisim teooria depressiooni neurobioloogiliste aluste kohta püüab depressiivset sümptomatoloogiat seletada läbi monoamiinergiliste – noradrenaliini, serotoniini ja dopamiini – transmittersüsteemide vähenenud aktiivsuse. Hilisemalt on lisandunud ka mitmeid mitte-amiinergilisi mehhanisme ning närvivõrkude aktiivsust kaasavaid teooriaid. Üks viis, kuidas depressiooni kui mitmetahulist sündroomi uurida, on modelleerida selle erinevaid sümptomeid katseloomadel.

Uudistav käitumine hõlmab uudsete stiimulite poolt esilekutsutud ning neile suunatud käitumisi, mis võimaldavad koguda informatsiooni uute objektide ja seniavastamata alade kohta, eesmärgiga tagada efektiivne hakkamasaamine ümbritsevas keskkonnas. Uudistavat käitumist mõjutavad samaaegselt nii uudishimu kui ka hirm tundmatute objektide ees. Seetõttu põhineb suur hulk psühhofarmakoloogias kasutusel olevaid ärevuse mõõtmisega tegelevaid käitumisteste just uudistava käitumisega seotud lähenemise-vältimise konfliktsituatsioonil. Varasemalt on leitud, et vähemalt osaliselt on uudistamise ning erinevate sõltuvusttekitavate ainete, nagu näiteks psühhostimulaatorite, mõju neurobioloogilised alusmehhanismid kattuvad ning nii uudistava käitumise regulatsioonis kui ka sõltuvuskäitumises mängivad olulist rolli monoamiinergilised transmittersüsteemid.

Eelnevalt on näidatud, et dopamiinil on oluline roll psühhostimulaator kokaiini käitumist modifitseerivas toimes, aga kuivõrd osaleb selles noradrenergiline süsteem, oli suuresti määratlemata. Seetõttu uurisimegi (**Artikkel I**), kuidas mõjustab eesajus põhilise noradrenaliini allika *locus coeruleus*e (sinav tuum) närvilõpmete kahjustus kokaiini stimuleerivat ning käitumist tingivat toimet. Leidsime, et ulatuslik *locus coeruleus*est lähtuvate noradrenergiliste närvirakkude lõpmete kahjustus selektiivse närvimürgiga DSP-4 [N-(2-kloroetüül-N-etüül-2-bromobensüülamiin], mida kasutatakse *locus coeruleus*e düsfunktsionaalsuse modelleerimiseks katseloomadel, vähendas oluliselt kokaiini poolt stimuleeritud liikumisaktiivsust ega lasknud ilmneda kokaiiniga tingitud paigaeelistusel, mis kontrollrühmal selgelt väljendus. Seega leidsime, et kokaiini aktiveerivas ning sarrustavas toimes on oluline roll ka intaktsel noradrenergilisel närviülekandel.

Erinevatel käitumuslikel meetoditel on näidatud, et katseloomad erinevad oma käitumiselt uudses keskkonnas. Meie laboris on kasutusel vaba uudistamise aspekti hõlmav uudiskastitest, mis lubab rotil valida kodupuuri elemente

sisaldava väikese kambri ning suurema avarvälja vahel, millele on paigutatud ka mitu uudset objekti. Eelnevalt oleme kindlaks teinud, et sellisel meetodil määratud indiviididevahelised erinevused uudistamisaktiivsuses on ajas püsivad ning need stabiilsed käitumisjooned ennustavad ka rottide tegutsemist teistes ärevuse ning depressiooniuuringute kontekstis kasutatavates käitumistestides: madala uudistamisaktiivsusega rotid on ka teistes käitumistestides ärevamad ning passiivsema käitumisprofiiliga, mistõttu võiks nende käitumist pidada 'depressiivseks'. Sellised käitumiseripärad palju- ja väheuudistavate rottide vahel lubavad oletada ka varieeruvust neis käitumise aluseks olevais neurobioloogilistes substraatides. Järgnevad eksperimendid (**Artiklid II–V**) viidigi läbi kirjeldamaks nende kahe fenotüübi võimalikke neurokeemilisi erisusi ning tundlikkust psühhostimulaatorite käitumuslikele efektidele.

Isased Wistar rotid klassifitseeriti uudiskasti meetodil teise katsepäeva uudistamisaktiivsuse alusel palju- ja väheuudistavateks loomadeks. Leidsime, et vaba uudistamist kaasaval uudiskasti meetodil määratud individuaalsed erinevused uudistamisaktiivsuses ennustavad rottide aktiivsust reageerivuses amfetamiinile: vähe-uudistavad rotid olid ka amfetamiini manustamise järgselt proportsionaalselt vähem ringiliikuvad kui palju-uudistavad elukad. Küll aga avaldus kokaiiniga tingitud paigaeelistus vähe- ja palju-uudistavatel rottidel samas ulatuses. Leidsime, et palju- ja väheuudistavate rottide käitumuslike erinevustega kaasnevad olulised erinevused keskaju dopamiinisüsteemi aktiivsuses. Vähe-uudistavatel rottidel on väiksem dopamiini D_2 funktsionaalsete, dopamiini suhtes kõrge afiinsusega retseptorite osakaal juttkehas. Samuti oli väheuudistavate rottide juttkeha dopamiinisüsteem vähemtundlik tritsüklilise antidepressandi imipramiini amfetamiinipotentseerivale mõjule, mõõdetuna *in vivo* mikrodialüüsil.

*Locus coeruleus*e osaline denervatsioon ei mõjustanud uudistamisaktiivsust palju-uudistavatel loomadel, väheuudistavatel loomadel pärssis *locus coeruleus*e osaline kahjustus uudiskasti testsituatsiooniga kohandumist. *Locus coeruleus*e osaline kahjustus vähendas ainult palju-uudistavateks klassifitseeritud rottide amfetamiini manustamisest põhjustatud aktiivsust, kuid see-eest vähendas selektiivselt väheuudistavate rottide dopamiini ning selle metaboliitide sisaldust olulises dopamiinergiliste rakkude projektsioonisalas *nucleus accumbens*is. Järeldasime, et dopamiini poolt vahendatud käitumine sõltub oluliselt noradrenergilise süsteemi terviklikkusest ning dopamiini-noradrenaliini interaktsiooni moduleerivad indiviididevahelised erinevused uudistamisaktiivsuses.

Leidsime, et lisaks erinevustele dopamiinergilises ja noradrenergilises närviülekandes avalduvad vähe- ja palju-uudistate rottide vahel erinevused ka serotonergiliste süsteemide regulatsioonis. Väheuudistavatel rottidel oli väiksem funktsionaalne tundlikkus serotoniin 1A retseptori agonistile mõõdetuna läbi keha süvatemperatuuri muutuse. Mikrodialüüsil selgus, et parakloramfetamiini serotoniini vabastavas mõjus ei olnud vähe- ja palju-uudistavate gruppide vahel erinevusi, kuid serotoniini tagasihaarde blokeerimisel sõltusid rakuvälise serotoniini tasemed ajupiirkonniti erinevustest uudistamisaktiivsuses, haakudes piirkonniti ka närvikasvufaktori BDNF mRNA tasemetega.

Kokkuvõttes võib vähe- ja palju-uudistaval käitumuslikul fenotüübil põhinev loomkatsemudel osutuda kasulikuks ärevuse ja depressiooni-uuringutes patogeneetiliste mehhanismide ning võimalike uute farmakoloogiliste sihtmärkide tuvastamisel.

PUBLICATIONS

CURRICULUM VITAE

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Professional employment

2009–...	Research fellow in psychophysiology, Department of Psychology, University of Tartu
2006–2009	Extraordinary research fellow, Department of Psychology, University of Tartu
2005–2006	Laboratory assistant, Department of Psychology, University of Tartu
2003–2005	Coordinator of open university studies in psychology, Department of Psychology, University of Tartu

Scientific activity

Main research area:
- Animal models of affective disorders; exploratory behaviour and its neurochemical regulation in the rat, positive affect and addictive behaviour in the rat

Membership:
- Union of Estonian psychologists
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Publications:

Kõiv, K. & Harro, J. Differences in 5-HT_{1A} receptor-mediated hypothermia in rats with low or high exploratory activity. *Behavioural Pharmacology (in press)*.

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2005–2006	laborant, psühholoogia instituut, Tartu Ülikool
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Teadustegevus

Teadustöö põhisuunad:
- afektiivsete häirete loommudelid, roti uudistav käitumine ja selle neurobioloogiline regulatsioon, positiivne afektiivsus ja sõltuvuskäitumine rotil

Liikmelisus:
- Eesti Psühholoogide Liit
- Eesti Farmakoloogia Selts

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