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THE NEUROCHEMICAL REGULATION OF RAT EXPLORATORY BEHAVIOUR: FOCUS ON DOPAMINERGIC AND NORADRENERGIC NEUROTRANSMISSION

Master's thesis

Running head: Dopamine and noradrenaline in exploratory behaviour
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Tartu 2004

ABSTRACT

Exploratory and amphetamine-stimulated behaviour in rats was studied in four experiments. Amphetamine (0.5 mg/kg) increased exploratory activity in the exploration box on five consecutive testing days, while cholecystokinin (CCK)-1 receptor antagonist devazepide (10 μg/kg) blocked and CCK_2 receptor antagonist L-365,260 (10 μg/kg) enhanced amphetamine-induced stimulation of activity. Devazepide coadministration prevented the development of sensitization to amphetamine, while coadministration of L-365,260 with amphetamine potentiated the locomotor effect of a challenge dose of amphetamine. Thus endogenous CCK, released during exploratory activity, shapes behavioural responses to amphetamine by acting on both receptor subtypes, and modulates the development of sensitization to amphetamine. When animals were preselected for their spontaneous exploratory activity, we found that these individual differences were stable and able to predict subsequent amphetamine-stimulated behaviour. Only the low explorers (the LEs) developed behavioural sensitization to repeated amphetamine treatment, suggestive of a differential vulnerability to addictive drugs in the LEs and the high explorers (the HEs). The HEs and LEs did not differ in their local depolarization- or drug-induced dopamine release in the nucleus accumbens. Locus coeruleus (LC) noradrenergic system regulates dopamine release in the VTA via both α_1- and α_2-receptors. Partial LC denervation with the selective neurotoxin DSP-4 (10 mg/kg) prevented the increase in exploratory activity over two test sessions in LE animals, but had no effect in HE rats. Amphetamine induced locomotor activity was attenuated by DSP-4 pretreatment only in HE animals. These results suggest differential involvement of the LC noradrenergic transmission in novelty- and amphetamine-induced behaviour in animals with different levels of exploratory activity. DSP-4 treatment also decreased the content of dopamine and its metabolites in the nucleus accumbens, but only in LE animals. The D_2 receptor function in the striatum was differentially affected by partial LC denervation – decreased in the HEs and increased in the LEs.

Keywords: exploratory behaviour, amphetamine, dopamine, nucleus accumbens, striatum, D_2 receptors, cholecystokinin (CCK), noradrenaline, locus coeruleus (LC), DSP-4, microdialysis.

KOKKUVÕTE

Neljas eksperimendis uuriti rottide uudistavat ja amfetamiinist stimuleeritud käitumist. Amfetamiin (0.5 mg/kg) stimuleeris uudistavat käitumist uudiskastis viiel järjestikusel päeval, koletsüstokiniin (CCK) 1-tüüpi retseptori antagonist devasepiid (10 μg/kg) blokeeris ja CCK_2 retseptori antagonist L-365,260 (10 μg/kg) suurendas amfetamiinist tingitud aktiivsuse stimulatsiooni. Devasepiidi manustamine koos amfetamiiniga hoidis ära käitumusliku sensitisatsiooni kujunemise amfetamiinile, L-365,260 suurendas seda. Seega, uudistamise ajal vabanev endogeenne CCK kujundab reageerivust amfetamiinile toimides mõlema retseptoritüübi kaudu ning moduleerib käitumusliku sensitisatsiooni kujunemist amfetamiinile. Individuaalsed erinevused uudistavas käitumises olid püsivad ja ennustasid hilisemat amfetamiinist-stimuleeritud käitumist. Ainult väheuudistavatel (LE – low explorer) loomadel kujunes käitumuslik sensitisatsioon amfetamiini korduvmanustamisele, mis viitab võimalusele, et LE ja paljuuudistavatel (HE – high explorer) loomadel on erinev haavatavus sõltuvusainete suhtes. HE ja LE loomad ei erinenud lokaalsest depolarisatsioonist ega amfetamiinist tingitud dopamiini vabanemise suhtes naalduvas tuumas. Locus coeruleus'e (LC) noradrenergiline süsteem reguleerib dopamiini vabanemist ventraalses tegmentumis nii α_1- kui α_2-retseptorite kaudu. Osaline LC denervatsioon selektiivse neurotoksiini DSP-4-ga (10 mg/kg) hoidis ära uudistamisaktiivsuse suurenemise ainult LE loomadel, kuid ei mõjutanud HE loomade käitumist. DSP-4 vähendas amfetamiinist tingitud liikumisaktiivsust ainult HE loomadel. Seega on LC noradrenergiline närviülekande osa uudistavas- ja amfetamiinist-sõltuvas käitumises erinev sõltuvalt loomade spontaansest uudistamisaktiivsuse tasemest. DSP-4 vähendas dopamiini ja selle metaboliitide sisaldust naalduvas tuumas, kuid ainult LE loomadel. Osaline LC denervatioon vähendas D_2 retseptorite funktsiooni juttkehas HE loomadel, kuid suurendas seda LE loomadel.

Märksõnad: uudistav käitumine, amfetamiin, dopamiin, naalduv tuum, juttkeha, D_2 retseptorid, koletsüstokiniin (CCK), noradrenaliin, locus coeruleus (LC), DSP-4, mikrodialüüs

TABLE OF CONTENTS

1. INTRODUCTION

1.1. Exploratory behaviour

Exploratory behaviour allows detecting of and responding to novel stimuli, and, as such, is crucial for survival because it enhances the opportunities to find food, water, shelter etc. As Crucio and van Abeelen (1986) put it: "exploration 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".

The tests used to measure exploration in rodents are based on the animals' natural tendency to explore novel stimuli, but also their initial tendency to avoid unfamiliar places. Thus, an animal's behaviour in a novel environment is a combination of its curiosity and neophobia (see Harro, 1993 for a review).

A classic example for an exploration task, and the most widely used one, is the open-field test. Large open areas present an aversive stimulus for rodents, and Exner and Clark (1993) have argued that active behaviour in the inescapable open field might represent two aspects of novelty-related behaviour: 'exploration' and 'escape'. Thus, using drugs to influence behaviour in the open-field, it is possibly not the exploration that is truly manipulated on, but the animal's response to a stressful context (Prut and Belzung, 2003). Indeed, exposure to an inescapable environment elevates the levels of stress hormones in rats (Dantzer and Mormede, 1983). The true measure of novelty-seeking would be obtained if the animal had an opportunity to actively choose a novel environment over a familiar one.

1.1.1. The exploration box test

The exploration box test was originally designed to assess the behavioural changes after the denervation of noradrenergic projections from the locus coeruleus (Harro et al., 1995). It contains elements of a free-choice exploration test, where the animal has a chance to hide in a small chamber or explore an open arena with novel objects placed in it. Repeated testing in the exploration box enables to assess the different phases of novelty-related behaviour – avoidance, approach and habituation (Otter et al., 1997).

1.2. The nucleus accumbens dopamine system in exploratory behaviour

1.2.1. The anatomy of mesencephalic dopamine system

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

Dopamine exerts its action via five G-protein coupled receptor subtypes, but more is known about the pharmacology and physiology of the D_1 and the D_2 receptors. Especially the D_2 receptors have been the focus of interest because of their involvement in a number of neurological diseases, psychotic disorders and drug-related behaviour (Singer et al., 1990; Seeman et al., 1987; Self and Nestler, 1998). The D_2-like receptors are located both post- and presynaptically throughout the brain, with the highest densities in the projection areas of the midbrain dopamine system – the striatum, nucleus accumbens, olfactory tubercles and the substantia nigra (for a review, see Kalivas, 1993; Levant, 2002).

1.2.2. The nucleus accumbens

The nucleus accumbens is located in the ventral part of the striatum and receives dense input from the dopaminergic neurons in the VTA. The dopaminergic neurotransmission, particularly in the nucleus accumbens, is increased in response to natural rewards (Heffner et al., 1980; Pfaus et al., 1990, Young et al., 1992) and has also been implicated in the mechanism of action of drugs with addictive properties (Di Chiara and Imperato, 1988) and psychotic disorders (Davis et al., 1991 for review).

Novel stimuli initiate investigatory behaviour and orientation, increase the firing rate of dopaminergic neurons in the VTA (Horvitz et al., 1997), and dopamine release in the nucleus accumbens (Rebec et al., 1997). The fact that both rewarding and aversive novel stimuli are able to increase dopaminergic neurotransmission in the nucleus accumbens has led researchers to hypothesize that the functional role of the nucleus

accumbens dopamine system might be the attribution of incentive salience to novel stimuli (Berridge and Robinson, 1998; Ikemoto and Panksepp, 1999).

Although most studies modelling reward and incentive motivation focus on the nucleus accumbens dopaminergic system, reward-related changes in dopaminergic activity have been shown in the striatum as well (Zald et al., 2004).

1.2.3. Cholecystokinin-dopamine interaction in the nucleus accumbens

A subpopulation of the mesolimbic dopaminergic neurons innervating the nucleus accumbens contains cholecystokinin as a cotransmitter (Hökfelt et al., 1980). This gut-brain peptide acts via two receptor subtypes – CCK_1 and CCK_2 – (Noble et al., 1999) and modulates the dopaminergic activity in the nucleus accumbens, thus possibly contributing to the development or expression of drug abuse or psychosis. It has been demonstrated that CCK modulates dopaminergic activity depending on the CCK receptor subtype involved. For example, CCK acting via CCK_1 receptors in the medial posterior nucleus accumbens potentiates dopaminergic activity while CCK acting on CCK_2 receptors in the anterior nucleus accumbens either has no effect or inhibits dopaminergic activity (Crawley, 1991; Marshall et al., 1991). Similarly, behavioural studies have demonstrated that intracerebrally administered CCK potentiates dopamine-dependent behaviour in a CCK_1 receptor-mediated manner while inhibits it via CCK_2 receptors (Crawley, 1991; Crawley, 1992; Crawley, 1994; Vaccarino and Rankin, 1989).

The selective CCK receptor antagonists have been shown to be ineffective in modulating baseline locomotor behaviour, but they alter the behavioural changes induced by dopamine or dopamine agonists, e.g. the indirect agonist amphetamine (Josselyn and Vaccarino, 1995; Philips et al., 1993; Tieppo et al., 2000). CCK receptors also seem to contribute to the development and expression of behavioural sensitization to amphetamine (DeSousa et al., 1999; Wunderlich et al., 2000), a phenomenon known to occur after repeated exposure to psychostimulants (Segal and Mandell, 1974; Pierce and Kalivas, 1997). Nevertheless, there are some inconsistencies in the studies describing the specific roles the CCK receptor subtypes may have in modulating the different behavioural effects of amphetamine, particularly when CCK receptor antagonists have

been used to block the modulating role of endogenous CCK. In the rat, CCK_1 receptor blockade was reported not to influence amphetamine-induced hyperlocomotion (DeSousa et al., 1999) and stereotypy (Tieppo et al., 2000). Neither did a nonpeptide CCK_1 receptor antagonist affect amphetamine-induced disruption of prepulse inhibition (Shilling and Feifel, 2002). Somewhat inconsistently, rats with the naturally-occurring CCK_1 receptor-deficiency were less sensitive to locomotor activity enhancing effects of amphetamine (Feifel et al., 2001), and devazepide, a CCK_1 receptor antagonist, reduced the locomotor stimulant effect of amphetamine in mice (Vasar et al., 1991). CCK_1 receptor blockade by devazepide treatment has been found to block the expression of amphetamine sensitization, but not the development of it (Wunderlich et al., 2000). Interestingly, devazepide has been shown to antagonise the acquisition of amphetamine-conditioned (Josselyn et al., 1996) as well as cocaine-conditioned activity (Josselyn et al., 1997), suggesting differential involvement of CCK_1 receptors in these behavioural phenomena. Regarding CCK_2 receptor blockade, the effect of an antagonist, L-365,260, on the development of amphetamine sensitization was found to be either reducing or potentiating depending upon dose (Wunderlich et al., 2000). In other studies, L-365,260 has been reported to enhance amphetamine-facilitated responding for conditioned rewards (Josselyn and Vaccarino, 1995) and to reduce amphetamine stereotypy (Tieppo et al., 2000).

One possible factor contributing to the role CCK has in behaviour is the relative novelty of the environment (Blacker et al., 1997). Therefore the effects of CCK receptor antagonists may differ when given in an acute experiment or repeatedly, and may also depend upon the contingency between drug administration and testing environment.

1.3. The locus coeruleus noradrenergic system and exploratory behaviour

The locus coeruleus (LC) is a group of noradrenergic neurons, located in the brain stem, next to the fourth ventricle. Although a small cell group, it is the major source of noradrenergic innervation in the forebrain and the only source of noradrenaline in the hippocampus and neocortex (see Berridge and Waterhouse, 2003, for a review). The activity of LC neurons is reduced in the state of low arousal (e.g. sleep), but also during

certain activities when the animal is awake, but pays little attention to its surroundings (Foote et al., 1983). LC neurons are activated in state of vigilance and orientation to stimuli, particularly novel stimuli. Noradrenaline release in the prefrontal cortex is increased when rats are exposed to novelty (Harro and Oreland, 2001). Further, near maximal noradrenergic depletion with the selective noradrenergic neurotoxin DSP-4 does not produce gross changes in spontaneous behaviour in familiar context (Jonsson et al., 1982), but denervated animals show deficits in coping with environmental changes (Archer, 1983; Harro et al., 1995).

Despite the specificity of the primary DSP-4 effect on the noradrenergic terminals, pretreatment with this neurotoxin seems to result in functional changes in other monoamine systems. For example, basal or stimulated dopamine release in the nucleus accumbens is decreased (Lategan et al., 1992; Häidkind et al., 2002), and D_2 receptors are upregulated in the striatum after LC denervation (Harro et al., 2000).

1.4. Noradrenaline-dopamine interaction

There is evidence of a noradrenergic input from the LC to the dopaminergic neurons in the ventral tegmental area, a cell body region of the ascending dopaminergic pathways (Grenhoff et al., 1993; Grenhoff and Svensson, 1989; Herve et al., 1982; Tassin et al., 1979). Dopaminergic cells in the VTA are locally either excited or inhibited by noradrenergic compounds (Grenhoff et al., 1995) and α_1-receptor antagonist modulates the firing pattern of midbrain dopaminergic neurons (Grenhoff and Svensson, 1993).

The noradrenaline-dopamine coupling is seen in the medial prefrontal cortex, where administration of known antipsychotic drugs induces similar increases in extracellular levels of both catecholamines (Westerink et al., 1998). Whether this is achieved by common reuptake (Carboni et al., 1990; Moron et al., 2002) or co-release (Devoto et al., 2001; 2003), as suggested, has so far remained unclear, but it further shows the connection of the VTA and LC systems.

1.5. Dopamine and noradrenaline in behavioural effects of amphetamine

Amphetamine is a prototypical psychostimulant that increases dopaminergic neurotransmission, locomotor activity and has rewarding properties for animals and humans. One of the main actions of amphetamine and the substrate for its behavioural effects is its ability to increase extracellular dopamine in the dopaminergic pathways in the midbrain, primarily via blocking the dopamine transporter (Wise and Bozarth, 1987).

Repeated amphetamine administration sensitises the dopaminergic system and re-exposure to the drug after a withdrawal period produces enhanced behavioural effects – this phenomenon is referred to as behavioural sensitization (for a review, Vezina, 2004). However, the induction and expression of behavioural sensitization to repeated amphetamine treatment is highly dependent on the context in which the drug is given (Badiani et al, 1995).

The extent to which noradrenaline is involved in the central and behavioural effects of psychostimulants is not entirely clear. Darracq et al. (1998) argue that while both noradrenaline and dopamine systems are activated following systemic amphetamine administration, only a part of dopamine release in the nucleus accumbens is functional in relation to the psychostimulant effects of amphetamine and this functional dopamine release is controlled by noradrenergic stimulation of α_1-receptors in the prefrontal cortex. Similarly, Snoddy and Tessel (1985) and Dickinson et al. (1988) demonstrated a role for α_1-receptor activation in behavioural effects of amphetamine. The studies investigating the effects of LC lesions on drug-induced changes in behaviour have yielded conflicting results. For example, DSP-4 treatment has been demonstrated to antagonize dose-dependently the amphetamine-induced hyperactivity (Ögren et al., 1983; Archer et al., 1986), but failed to modify amphetamine-induced behavioural changes in another study (Di Lullo and Martin-Iversen, 1991). Some contradictions may be explained by differences in novelty and level of habituation, which influence the effect of amphetamine in LC-denervated animals (Harro et al., 2000).

Even though lesions of the noradrenergic system are accompanied by compensatory changes in the remaining nerve terminals which are able to maintain extracellular noradrenaline levels as measured by in vivo microdialysis (Abercrombie & Zigmond, 1989; Kask et al., 1997), there is sufficient evidence that changes occur in

dopaminergic neurotransmission which are compatible with reduced noradrenergic function (Gesi et al., 2000).

While most pharmacological or neurogenetic studies have used a near maximal noradrenaline depletion, even a partial LC denervation, which may more closely resemble naturally-occurring variability in noradrenergic function, has also been shown to affect dopaminergic neurotransmission. The number of D_2 binding sites in the striatum was increased (Harro et al., 2003) and dopamine release potential in the nucleus accumbens was decreased (Häidkind et al., 2002) following such partial noradrenaline depletion with DSP-4 at a dose of 10 mg/kg. However, the increased density of D_2 binding sites might not be solely responsible for the behavioural effects seen in noradrenaline-depleted animals. Weinshenker et al. (2002) demonstrated that dopamine β-hydroxylase knockout mice are hypersensitive to amphetamine and a D_2 agonist quinpirole, but this was not due to the upregulation of D_2 receptors, suggesting possible changes in intracellular mechanisms of receptor function.

1.6. Individual differences in exploratory behaviour

Exploratory activity of animals in any given exploration test depends on many factors, including sex, age, strain of rats and the method to measure exploration. Animals can be differentiated on the basis of their locomotor response to a novel environment, and these differences may be related to the neural correlates of the pathogenesis of drug addiction. Individual differences in exploratory activity in rats have been found to predict their subsequent responsivity to psychostimulant drugs, e.g. amphetamine (Piazza et al., 1989). Similarly, individuals with high scores on sensation seeking trait in personality tests tend to use (and abuse) psychoactive substances, especially amphetamine (Zuckerman, 1996; Gerra et al., 2000). Because both novelty and drugs of abuse increase dopamine release in the nucleus accumbens, it could be speculated that exploratory behaviour and drug addiction share, at least partly, a common pathway.

High responders and low responders to novelty are usually differentiated by their locomotor activity in an inescapable exploration task. In particular, animals classified as high responders to novelty based on their open-field behaviour, acquire amphetamine

self-administration more readily than low responders to novelty (Piazza et al., 1989), and develop stronger behavioural sensitization to repeated amphetamine treatment (Hooks et al., 1991). Individual differences in responding to novel stimuli have been shown to be correlated with certain neurochemical features, such as higher basal and evoked dopamine release in the nucleus accumbens (Hooks et al., 1992), and lower 5-HT concentration in the medial prefrontal cortex (Thiel et al., 1999) in high responders compared to low responders to novelty. Rosario and Abercrombie (1999) have shown that the locomotor response to novelty is highly correlated with the magnitude of hippocampal noradrenaline release in stressful conditions, suggesting that the activation of the LC noradrenergic system may also be involved in determining the individuals' locomotor response to novelty.

1.7. Aims of the thesis

This thesis consists of five studies, four of which deal with the neurochemical regulation of exploratory behaviour in a free-choice exploration test (**Study I, II, III** and **V)**. **Study IV** is a pharmacological study attempting to explore how noradrenaline and dopamine interaction is regulated at the level of the VTA.

The aims of the thesis were as follows:

- To study the effect of cholecystokinin-dopamine interaction in the nucleus accumbens on rat exploratory behaviour and on amphetamine-induced behavioural changes and behavioural sensitization to amphetamine administered in association with the testing environment (**Study I**);
- To examine whether rats can be differentiated as high and low explorers based on their response to exposure to the exploration box, whether these differences, if found, persist in time and whether high and low exploring rats differ in their locomotor activity after amphetamine administration, and monoamine neurochemistry *ex vivo* (**Study II**);
- To further characterize the high and low exploring rats using *in vivo* microdialysis to study their possible differences in basal or stimulated dopamine release in the nucleus accumbens (**Study III**);

- To determine whether noradrenergic regulation of dopaminergic neurotransmission in the VTA contributes to the noradrenaline-dopamine interaction in the medial prefrontal cortex (**Study IV**);
- To study how partial noradrenergic denervation with the selective neurotoxin DSP-4 (10 mg/kg) affects exploratory and amphetamine-induced behaviour and D_2 receptor function and whether these neurotoxin effects depend on the animals' spontaneous exploratory activity levels (**Study V**).

2. MATERIALS AND METHODS

2.1. The exploration box test

The exploration box test was conducted as described previously (Harro et al., 1995; Otter et al., 1997). The exploration box was made of metal and consisted of a 0.5 x 1 m open area (side walls 40 cm) with a 20 x 20 x 20 cm small compartment attached to one of the shorter sides of the open area. The open area was divided into eight squares of equal size. In the open area, four objects, three novel and one familiar (a glass jar, a cardboard box, a wooden handle and a food pellet) were situated in certain places (which remained the same throughout the experiment). The small compartment, which had its floor covered with wood shavings, was directly linked to the open area through an opening (size 20 x 20 cm). The apparatus was cleaned with dampened cloth after each animal. The exploration test was initiated by placing a rat into the small compartment, which was then covered with a lid for the exploration time. The following behavioural parameters were registered: 1) latency (of entering open area with all four paws), 2) number of entries into the open area, 3) time spent exploring on the open area, 4) line crossings, 5) rearings and 6) number of unfamiliar object investigations. To provide an index of exploration the scores of line crossing, rearing and object investigation were summed for each animal and thus 7) the sum of exploratory events obtained. A single test session lasted 15 min.

In experiments, designed to study the effects of amphetamine on locomotor activity (**Study I**, **II** and **V**), the passage between the open area compartment and the

small compartment was closed, the animal was placed on the open area and for 15 minutes line crossings, rearings and the number of object investigations were registered. The sum of these measures was also calculated.

2.2. Monoamine tissue content measurements

Monoamines and their metabolites were assayed by HPLC with electrochemical detection. The rat brain tissues were homogenized with Bandelin Sonoplus ultrasonic homogenizer (Bandelin Electronic, Germany) in ice cold solution of 0.1 M perchloric acid (10-30 µl/mg) containing 5 mM sodium bisulfite and 0.4 mM EDTA to avoid oxidation. The homogenate was then centrifuged at 17000 x g for 10 min at 4ºC. Aliquots (10 µl) of the obtained supernatant were chromatographed on a Lichrospher 60 RP Select B column (250x3 mm; 5 µm). The separation was done in isocratic eluation mode at column temperature 30ºC 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% acetonitrile. The chromatography system consisted of a Hewlett Packard HP 1100 series isocratic pump, a thermostatted autosampler, a thermostatted column compartment and an HP 1049 electrochemical detector (Hewlett Packard, Germany) with glassy carbon electrode. The measurements were done at an electrode potential of +0.6 V versus the Ag/AgCl reference electrode.

2.3. Study I: Effect of CCK_1 and CCK_2 receptor blockade on amphetamine-stimulated exploratory behaviour and sensitization to amphetamine

The aim of this study was to characterize the effects of CCK receptor blockade on amphetamine-elicited changes in rat exploratory behaviour, and on the development of behavioural sensitization to amphetamine administered in association with the environment of testing for exploration.

2.3.1. Animals

Male Wistar rats (n = 72, weighing 260 – 390 g, from National Laboratory Animal

Centre, Kuopio, Finland) were housed four per cage in standard polypropylene cages in a light controlled room (12 h light: 12 h dark cycle, lights on at 7 a.m.) maintained at 22°C. Food and water were available *ad libitum.* All experiments were conducted between 13.00 and 19.00.

2.3.2. General procedure and drug administration

All animals were tested in the exploration box on five consecutive days. In order to assess sensitization to amphetamine, nine days after the last day of the 5-day exploration box test all animals received a challenge dose of amphetamine (0.5 mg/kg), and were tested on the open area compartment of the exploration box.

The following drugs were used: a selective CCK_1 receptor antagonist devazepide, a selective CCK_2 receptor antagonist L-365,260 (ML Laboratories/Panos Therapeutics, UK), *d*-amphetamine sulphate (Sigma, St. Louis, MO). CCK antagonists were suspended in a few drops of Tween-85® and diluted in distilled water. Amphetamine was dissolved in distilled water. Fresh solutions were prepared for each day of experiments.

Animals were randomly assigned to four groups: 1) control group (vehicle + distilled water); 2) CCK antagonist (devazepide or L-365,260) group (CCK antagonist + distilled water); 3) amphetamine group (vehicle + amphetamine); 4) amphetamine and CCK antagonist (amphetamine/devazepide or amphetamine/L-365,260) group (n = 9 per group).

The doses of CCK antagonists used in this study were selected to ensure selectivity for the specific receptor type (Harro and Vasar, 1991; Harro et al., 1996). CCK_1 receptor antagonist devazepide (10 μg/kg i.p.) and CCK_2 receptor antagonist L-365,260 (10 μg/kg i.p.) or vehicle was administered 30 minutes prior to behavioural testing on five consecutive days. The dose of amphetamine (0.5 mg/kg i.p.) was selected on the basis of previous studies (Otter et al., 1997) demonstrating that this dose had an exploration-enhancing effect in the exploration box. Amphetamine or distilled water was administered 15 minutes prior to behavioural testing on five consecutive days. All drugs were administered in the volume of 1 ml/kg.

2.4. Study II: Locomotor activity after amphetamine in rats with high or low spontaneous exploratory activity

The purpose of this study was to test the stability of exploratory activity in the exploration box, and further, whether rats with different levels of spontaneous exploration also differ in their response to amphetamine treatment.

2.4.1. Animals

Male Wistar rats (n = 12, weighing 218 – 275 g at the beginning of the experiment, from National Laboratory Animal Centre, Kuopio, Finland) were housed four per cage in standard polypropylene cages in a light controlled room (12 h light: 12 h dark cycle, lights on at 7 a.m.) maintained at 22°C. Food and water were available *ad libitum.* All experiments were conducted between 13.00 and 19.00.

2.4.2. General procedure and drug administration

Previous work form our laboratory has shown that activity during the second testing predicts the animals' general exploratory behaviour in the exploration box test. Thus, the animals were classified as high or low explorers (HE or LE, respectively) based on the median split of their summed exploratory activity on the second exposure to the exploration box. Then, the animals were tested on the open area compartment of the exploration box on five consecutive days to assess the effects of amphetamine (0.5 mg/kg) on their locomotor activity. Nine days after the end of the 5-day test, all animals received a challenge dose of amphetamine (0.5 mg/kg i.p.) and were tested for the expression of behavioural sensitization to repeated amphetamine treatment. In order to study the stability of individual differences in exploratory activity, seven days after the sensitization experiment the animals were tested again in the exploration box test.

D-Amphetamine sulphate (Sigma, St. Louis, MO) (0.5 mg/kg) was dissolved in distilled water and injected intraperitoneally in the volume of 1 ml/kg 15 min prior to behavioural testing.

2.5. Study III: Basal and stimulated dopamine release in the nucleus accumbens in rats with high or low exploratory behaviour: an in vivo microdialysis study

The aim of this study was to investigate whether rats with high or low exploratory activity in the exploration box differ in the basal or stimulated dopamine release in the nucleus accumbens. Local depolarization by infusing high-potassium perfusion solution or systemic administration of amphetamine was used to induce dopamine release.

2.5.1. Animals

Male Wistar rats (n = 32, weighing 310 – 430 g at the beginning of the experiment, from National Laboratory Animal Centre, Kuopio, Finland) were housed four per cage in standard polypropylene cages in a light controlled room (12 h light: 12 h dark cycle, lights on at 7 a.m.) maintained at 22°C. After the surgery all animals were housed individually. Food and water were available *ad libitum.* All experiments were conducted between 13.00 and 19.00.

2.5.2. Behavioural testing

In the experiment using local depolarisation to stimulate dopamine release, the animals were classified as high or low explorers based on the median split of the sum of exploratory events in the exploration box one day prior to the microdialysis experiment. In the amphetamine experiment, the animals were tested on two consecutive days on the exploration box prior to the microdialysis experiment and the high and low explorers were differentiated on the basis of the median split of the sum of exploratory events on the second exposure to the exploration box.

2.5.3. Surgery and microdialysis

The animals were anaesthetized with chloral hydrate (350 mg/kg IP) and mounted in a Kopf stereotactic frame. Guide cannulas for the microdialysis probes (MAB 6; AgnThos AB, Sweden) were implanted above the left nucleus accumbens shell according to the following coordinates relative to bregma: AP: +1.7, ML: +1.2, and DV: -2.8 (the final DV coordinate after probe insertion -8.2; according to Paxinos and Watson (1986). Three stainless steel screws and dental acrylic was used to fix the cannula to the scull. After the

surgery the animals were given 9 days to recover. During this period the rats were handled and weighed daily.

Microdialysis was conducted in awake, freely moving rats. In the morning of the experiment day the animals were transported to a separate experiment room. The animals remained in their individual home cages throughout the experiment. Microdialysis probes with 2 mm active polyethersulphone membrane, cut-off 15 kD (MAB 6, AgnTho's AB, Sweden) were connected to a syringe pump (World Precision Instruments, USA) and a CMA/142 microsampler (CMA, Sweden) via a two-channel swivel. Probes were inserted in the morning of the day of experiment and perfused with artificial cerebrospinal fluid (147 mM NaCl, 3.0 mM KCl, 1.3 mM $CaCl_2$, 1.0 mM $MgCl_2$, 1 mM $Na_2H\ PO_4$, and 0.2 mM NaH_2PO_4; pH 7.3–7.4) at a constant rate 1 μl/min. After the probe insertion the perfusate was discarded during the first 120 min. This was followed by collection of 15 dialysate fractions (each for 20 min) into the vials prefilled with 10 μl of 0.3 M perchloric acid. In experiments using local depolarization to stimulate dopamine release, after the eighth sample the system was switched to the perfusion solution containing 50 mM KCl and left so for 40 min. In separate experiments, after the sixth sample, the animals received an intraperitoneal injection of amphetamine (0.5 mg/kg). After the completion of the experiment the animals were deeply anaesthetized with chloral hydrate (350 mg/kg IP) and decapitated; the brains were removed, immediately frozen in ice cold acetone, 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 nucleus accumbens shell were excluded from the analysis.

2.6. Study IV: Intrategmental infusion of noradrenergic compounds – effect on noradrenaline and dopamine release in the prefrontal cortex and ventral tegmental area: a dual-probe microdialysis study

This study is a result of my study stay in the University of Groningen, primary purpose of which was to enhance my skills in *in vivo* microdialysis and in particular dual-probe microdialysis, which has a major advantage in that it enables to measure extracellular

concentrations of neurotransmitters in the cell body region and its projection area simultaneously. The experiment was designed so as to satisfy the research interests of both our laboratory and that of prof. Ben Westerink.

2.6.1. Animals

Male Wistar rats (285 – 320 g, from Harlan, Zeist, the Netherlands) were used for the experiments. The rats were housed individually in plastic cages (35 × 35 × 40 cm) and had free access to food and water. 4 animals were used for each experiment.

2.6.2. Drugs

The following drugs were used: noradrenaline (10^{-5} M), α_1-adrenoceptor agonist phenylephrine (10^{-4} M) and α_2-adrenoceptor antagonist idazoxan (10^{-4} M). All drugs were dissolved in Ringer's solution and, after collection of six baseline samples, applied for 120 min by retrograde dialysis via the VTA probe.

2.6.3. Surgery and microdialysis

Under isoflurane anaesthesia one I-shaped home made microdialysis probe (from polyacrylonitrile/sodium sulphonate copolymer, i.d. 0.22 mm; o.d. 0.31 mm; AN69 HF, Hospal, Bologna, Italy) was implanted in the ventral tegmental area (VTA; exposed length 1.0 mm), the other in the ipsilateral medial prefrontal cortex (PFC; exposed length 5.0 mm). Both probes were used to record dopamine and noradrenaline, the VTA probe was also used to deliver drugs by retrograde dialysis. Coordinates of the implantation were as follows, PFC: AP 3.3 mm, ML 0.8 mm, DV 5.0 mm; VTA: AP -5.3 mm; ML 2.5 mm, DV -8.4 mm, implanted under an angle of 12º, from bregma and dura, according to Paxinos and Watson (1986). Microdialysis experiments were carried out 24-48 h after surgery. Both probes were perfused with Ringer's solution at a flow rate of 1.5 µl/min and 15-min fractions were collected. The composition of the Ringer's solution was (in mM): NaCl 140.0, KCl 4.0, $CaCl_2$ 1.2, and $MgCl_2$ 1.0. The PFC probe was perfused with Ringer's solution containing 2.3 mM $CaCl_2$ to improve detection limit of noradrenaline and dopamine. When the experiments were terminated, the rats were given an overdose of chloral hydrate and brains were fixed with 4% paraformaldehyde. The brains were

sectioned and the localization of the probes was determined according to Paxinos and Watson (1986).

2.6.4. Measurement of noradrenaline and dopamine in the microdialysates

Noradrenaline and dopamine in the dialysates were quantified by HPLC with electrochemical detection. A Shimadzu LC-10AD pump (Kyoto, Japan) was used in conjunction with an electrochemical detector (ESA; potential first cell: + 175 mV; potential second cell: –250 mV). A reverse-phase column (150 × 4.6 mm; Supelco LC18, Belleofonte, PA, USA) was used. The mobile phase consisted of a mixture of 4.1 g sodium acetate adjusted to pH 4.1, 50 mg Na_2EDTA and 140 mg octanesulphonic acid in 890 ml H_2O, and 110 ml/l methanol. The flow rate was 1.0 ml/min.

2.7. Study V: Effects of partial locus coeruleus denervation on exploratory and amphetamine-induced behaviour, and D_2 receptor function in rats with high or low exploratory activity

The aim of this study was to investigate whether partial denervation of locus coeruleus projections with DSP-4 at a low dose (10 mg/kg) affects exploratory behaviour, amphetamine-induced behavioural changes (Kõiv, 2003) and the D_2 receptor interaction with G proteins in the striatum and the nucleus accumbens, and further, whether these neurotoxin effects depend on the animals' spontaneous exploratory activity levels.

2.7.1. Animals

Male Wistar rats (n = 44; weighing 222 – 312 g, Kuopio, Finland) were housed four per cage in standard polypropylene cages in a light controlled room (12 h light:12 h dark cycle, lights on at 7 a.m.) maintained at 22°C. Food and water were available *ad libitum.* The experiments were conducted in four sets, with 12, 12, 8, and 12 animals per set. All experiments were carried out between 13.00 and 19.00.

2.7.2. General procedure and drug administration

DSP-4 [*N*-(2-chloroethyl)-*N*-ethyl-2-bromobenzylamine] (AstraZeneca, Sweden) was administered intraperitoneally in the dose of 10 mg/kg (expressed as for hydrochloride). Each dose was weighed separately, dissolved in distilled water, and injected immediately. Control animals received an injection of distilled water. Each animal in one cage received a similar pretreatment. *D*-Amphetamine sulphate (Sigma, St. Louis, MO) was dissolved in distilled water and administered in the dose of 0.5 mg/kg intraperitoneally 15 min prior to the testing on the open area compartment of the exploration box.

Animals were classified as high or low explorers (HE or LE, respectively) based on the median split of the sum of exploratory events on the second exposure to the exploration box. Unpublished results from our laboratory have suggested that the animals' behaviour throughout repeated tests of exploratory behaviour can be best predicted by their behaviour on the second exposure to the exploration box. On day 3, the animals were administered amphetamine (0.5 mg/kg) and their locomotor activity was tested. On day 6, the animals were administered either DSP-4 (10 mg/kg) (HE, $n = 13$; LE, $n = 10$) or vehicle (HE, $n = 11$; LE, $n = 10$). Fourteen days after the neurotoxin or vehicle treatment, the animals were once again tested in the exploration box. The next day another amphetamine experiment was carried out.

The rats were sacrificed by decapitation 4 – 6 days after the last behavioural experiment. The brains were quickly dissected on ice and the brain tissue was stored at -80ºC in a deep freezer.

Rats from the first three test sessions ($n = 32$; LE + vehicle: $n = 10$; LE + DSP-4: $n = 9$; HE + vehicle: $n = 7$; HE + DSP-4: $n = 6$) were included in the biochemical analysis.

2.7.3. D_2 receptor-related [^{35}S]GTPγS binding assays

Guanosine-5'-(γ-thio)-triphosphate ([^{35}S]GTPγS) was purchased from Perkin Elmer Life Sciences, guanosine diphosphate sodium salt (GDP), (+)-butaclamol hydrochloride and 3-hydroxytyramine hydrochloride (dopamine) were from Sigma-Aldrich Fine Chemicals.

Rat striatal and nucleus accumbens membranes were prepared as described previously (Lepiku et al., 1996). Particularly, the tissues were homogenized in 100 vol

(ww/v) of homogenization buffer (HB, 50 mM Tris-HCl , pH=7.4) by Bandelin Sonoplus sonificator (2 passes, á 10 sec). The membranes were collected by centrifugation at 40,000×g for 20 min at 4°C and was washed by homogenization in HB and centrifuged two more times. The final pellet of striatal membranes were homogenized in 900 vol (ww/v) and nucleus accumbens membranes in 450 vol (ww/v) of the incubation buffer (IB, 20 mM K-Hepes, 7 mM $MgCl_2$, 100 mM NaCl, 1 mM EDTA, 1 mM DTT, pH = 7.4 and was used directly for binding experiments.

Binding of [^{35}S]GTPγS in IB was carried out as described earlier (Rinken et al., 1999; Uustare et al., 2004). In brief, the membranes (500 μg of striatal or 150 μg of nuc. accumbens membranes per tube) in IB were incubated with 0.2 nM [^{35}S]GTPγS and different concentrations of GDP (3 mM – 1 μM) and 1mM dopamine or 10μM butaclamol for 90 minutes at 30 ^{o}C. The reaction was stopped by rapid filtration through GF/B glass-fibre 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) by Beckman LS 1800 scintillation counter.

All binding data were analysed by computer modelling, fitting it to appropriate formulas using nonlinear least-squares regression, using GraphPad PRISM™ (GraphPad Software, San Diego, CA, USA).

2.8. Statistical analysis

All statistical analysis was performed with Statview for Windows (SAS Institute Inc., USA). For statistical analysis of the behavioural and biochemical data, two-way analysis of variance (ANOVA) was used. Where appropriate, a third, repeated measures factor was added. Group differences after significant ANOVAs were measured by post hoc Fisher's PLSD test. Pearson correlations were used for the determination of associations.

3. RESULTS AND DISCUSSION

3.1. Study I: Effect of CCK_1 and CCK_2 receptor blockade on amphetamine-stimulated exploratory behaviour and sensitization to amphetamine

The results of this study are published in detail in Alttoa and Harro (2004) and the figures referred to here are included in this paper. Briefly, amphetamine stimulated exploratory behaviour in the exploration box; while coadministration of the CCK_1 antagonist devazepide blocked the effect of amphetamine (Fig. 1), the CCK_2 antagonist L-365,260 potentiated it (Fig. 2). Further, it appeared that at least using the exploration box, repeated coadministration of L-365,260 with amphetamine is necessary for the expression of the additive effect of amphetamine and CCK_2 blockade. Neither CCK antagonist alone modified exploratory behaviour. Devazepide tended to block the development of behavioural sensitization to repeated amphetamine treatment (Fig. 3), and L-365,260 augmented it (Fig. 4). More detailed discussion of the results can be found in the attached paper.

Thus, endogenous CCK bidirectionally modulates amphetamine-induced exploratory behaviour in the exploration box and behavioural sensitization to amphetamine, dependent on the receptor subtype involved.

3.2. Study II: Locomotor activity after amphetamine in rats with high or low spontaneous exploratory activity

3.2.1. Results

3.2.1.1. Individual differences in exploratory activity in the exploration box

The animals were classified as high or low explorers (HE and LE, respectively) based on the median split of the sum of exploratory events on the second exposure to the exploration box. HE rats had shorter latency entering the open area ($F(1,10)=34.52$; $p<0.001$), made more line crossings ($F(1,10)=10.42$; $p<0.01$), object investigations ($F(1,10)=8.24$; $p<0.05$), rearings ($F(1,10)=5.50$; $p<0.05$), entries into the open area ($F(1,10)=15.21$; $p<0.01$), explored longer on the open area ($F(1,10)=11.05$; $p<0.01$), and

had a larger sum of exploratory events ($F(1,10)=9.80$; $p<0.05$) than LE animals (data not shown).

3.2.1.2. The stability of individual differences in exploratory activity

The animals' activity in the exploration box test on the second exposure to the apparatus was significantly correlated to their activity in the same test conducted 22 days later (r=.78 - .92; $p<0.05$ for the measured parameters of exploratory activity, excl. latency).

3.2.1.3. Locomotor activity after amphetamine in high or low exploring rats

On all five consecutive days of testing for locomotor activity after amphetamine in the open area compartment (Fig. 1), the HEs had higher activity on all measures of

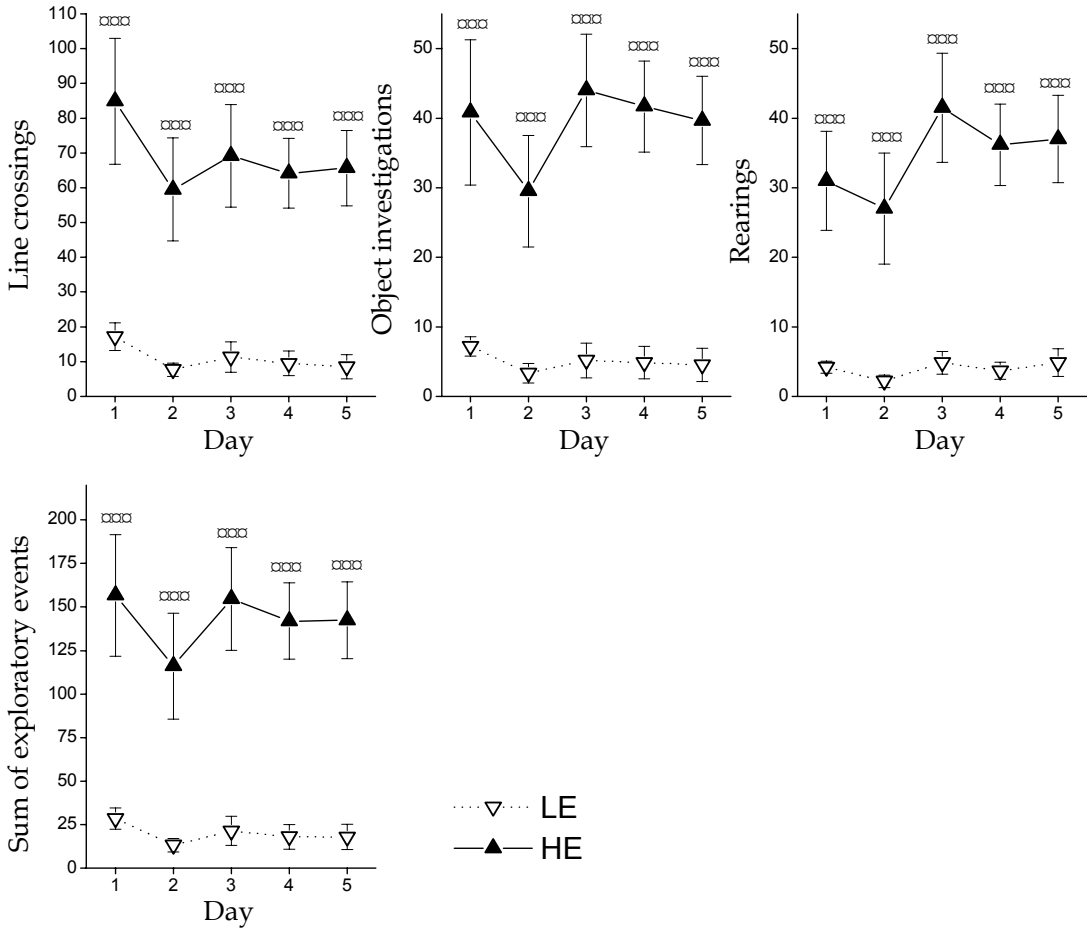

Figure 1. Effect of amphetamine (0.5 mg/kg, i.p.) on rat locomotor activity on the open area (data expressed as means ± S.E.M.). ¤¤¤ - $p<0.001$ vs. LE group.

exploration – line crossings ($F(1,10)=19.10$; $p<0.01$), object investigations ($F(1,10)=20.11$; $p<0.01$), rearings ($F(1,10)=22.92$; $p<0.001$**)** and sum of exploratory events ($F(1,10)=21.06$; $p<0.001$) compared to the LE animals.

3.2.1.4. Expression of behavioural sensitization after repeated amphetamine treatment

The behavioural data obtained in the sensitization experiment was compared to the animals' locomotor activity on the first day of the five-day amphetamine test (Fig. 2).

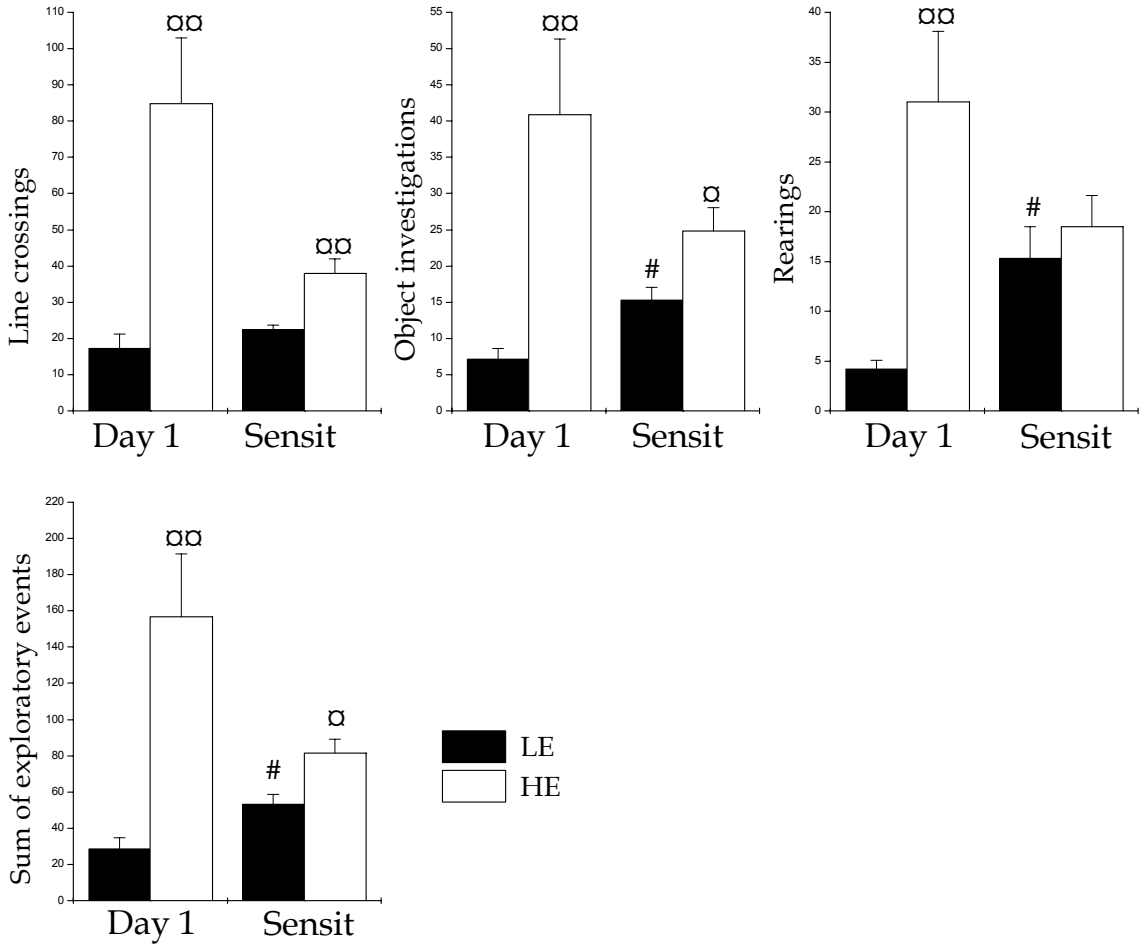

Figure 2. The expression of behavioural sensitization to repeated amphetamine pretreatment (sensit) (data expressed as means ± S.E.M.). ¤¤ - $p<0.01$; ¤ - $p<0.05$ vs. LE group; # - $p<0.05$ vs. Day 1.

The HEs were significantly more active than the LEs on all exploration parameters independent of the day of testing [Activity effect ($F(1,10)=17.16$; $p<0.01$) for line crossings, ($F(1,10)=15.29$; $p<0.01$) for object investigations, ($F(1,10)=11.65$; $p<0.01$) for rearings and ($F(1,10)=17.86$; $p<0.01$) for the sum of exploratory events].

There was a significant Test (Day 1 vs. Sensitization experiment) x Activity interaction for line crossings ($F(1,10)=8.55$; $p<0.05$), rearings ($F(1,10)=8.51$; $p<0.05$) and the sum of exploratory events ($F(1,10)=7.56$; $p<0.05$). According to *post hoc* tests, only the amphetamine-stimulated activity of the LE rats was significantly increased, but the activity of the HE rats rather tended to be reduced in the sensitization experiment.

3.2.1.5. Tissue concentrations of monoamines

Compared to the LE rats, the HE animals had lower levels of serotonin (5-HT) in the right frontal cortex ($F(1,10)=29.91$; $p<0.001$) (Table 1). The HEs also tended to have lower noradrenaline content in the right and left frontal cortex and DOPAC/dopamine and 5-HIAA/5-HT ratio in the striatum ($p=0.06 – 0.09$), but these differences from the LEs failed to reach the level of significance.

3.2.2. Discussion

The results of this study indicate that individual differences in the exploratory activity in the exploration box (as determined by the summed activity on the second exposure to the exploration box) are stable and able to predict the animals' subsequent locomotor activity after amphetamine treatment.

Previous studies have reported that individual differences in a free-choice exploration test do not predict the locomotor stimulating, but do predict the rewarding effects of amphetamine (Robinet et al., 1998; Klebaur and Bardo, 1999). Robinet et al. (1998) used approach to a novel object in a cage as a measure of free-choice exploration and in Klebaur and Bardo's (1999) the individual differences in exploratory activity a playground maze was used. Approach to a novel stimulus in an otherwise familiar environment might represent a different aspect of exploration than the summed exploratory activity in the exploration box, in which novel objects are present, but approach to them also requires entrance to a novel environment. In this experiment we

had no vehicle groups, so we cannot conclude that the HEs are more responsive to amphetamine treatment than the LEs; the interpretation of the present results is at this

Table 1. Tissue content of monoamines and their metabolites (means ± S.E.M.) (pmol/mg wet weight tissue). ¤ - $p<0.05$ vs. LE group.

	Dopamine (DA)		DOPAC	
	HE	LE	HE	LE
Right frontal cortex	0.44 ± 0.10	0.46 ± 0.03	0.16 ± 0.03	0.15 ± 0.01
Left frontal cortex	0.46 ± 0.10	0.45 ± 0.03	0.14 ± 0.03	0.17 ± 0.01
Nucleus accumbens	53.41 ± 10.79	50.39 ± 6.18	18.83 ± 3.83	18.61 ± 2.46
Striatum	93.81 ± 20.35	91.45 ± 6.31	14.47 ± 3.20	14.26 ± 0.79

	HVA		DOPAC/DA	
	HE	LE	HE	LE
Right frontal cortex	0.04 ± 0.01	0.09 ± 0.06	0.36 ± 0.08	0.33 ± 0.03
Left frontal cortex	0.05 ± 0.01	0.09 ± 0.06	0.31 ± 0.07	0.40 ± 0.06
Nucleus accumbens	5.11 ± 1.07	4.91 ± 0.49	0.38 ± 0.08	0.38 ± 0.03
Striatum	5.85 ± 1.26	5.17 ± 0.20	0.15 ± 0.04	0.16 ± 0.01

	Noradrenaline		Serotonin (5-HT)	
	HE	LE	HE	LE
Right frontal cortex	2.41 ± 0.55	2.60 ± 0.19	¤ 2.91 ± 0.71	3.42 ± 0.18
Left frontal cortex	2.57 ± 0.58	2.72 ± 0.18	3.35 ± 0.77	3.62 ± 0.22
Nucleus accumbens	3.57 ± 0.75	2.91 ± 0.11	3.52 ± 0.75	3.25 ± 0.21
Striatum	2.08 ± 0.44	1.94 ± 0.15	3.53 ± 0.79	3.43 ± 0.13

	5-HIAA		5-HIAA/5-HT	
	HE	LE	HE	LE
Right frontal cortex	1.84 ± 0.43	2.11 ± 0.15	0.63 ± 0.14	0.62 ± 0.04
Left frontal cortex	1.83 ± 0.44	2.15 ± 0.16	0.55 ± 0.13	0.60 ± 0.04
Nucleus accumbens	3.47 ± 0.81	3.62 ± 0.11	1.01 ± 0.23	1.14 ± 0.08
Striatum	3.28 ± 0.79	3.55 ± 0.07	0.93 ± 0.22	1.04 ± 0.05

point limited to the statement that amphetamine treatment does not abolish the differences in activity levels of the HEs and the LEs.

The expression of behavioural sensitization to psychostimulants is highly dependent on the contextual stimuli during the drug administration regimen. In the present study only the LE animals developed behavioural sensitization to repeated amphetamine treatment. The LEs tend to be more anxious and/or less motivated to explore a novel environment (our unpublished observations) and this might make the low exploring rat an endophenotype of vulnerability to addictive drugs.

The present study did not reveal differences between the HEs and the LEs in the tissue dopamine content or turnover in the nucleus accumbens. The HEs and the LEs differed in the tissue serotonin content in the right frontal cortex, which was lower in the HE animals. Thiel et al. (1999) also found that high responders to novelty had overall lower serotonin content in the frontal cortex and that the serotonin content in their right frontal cortex was lower than in the left frontal cortex. Zuckerman (1996) postulated that the substrate for human sensation-seeking trait is a highly reactive dopaminergic system in combination with weakened noradrenergic and serotonergic system. We also found a tendency for lower noradrenaline content in the frontal cortex of the HE rats compared to the LEs. However, tissue neurotransmitter levels do not reflect the activity of the given neurotransmitter system; also, it is impossible to determine whether the neurochemical differences found in this study are the basis for the individual differences in exploratory activity or brought on by repeated amphetamine treatment.

3.3. Study III: Basal and stimulated dopamine release in the nucleus accumbens in rats with high or low exploratory behaviour: an in vivo microdialysis study

3.3.1. Results

3.3.1.1. Basal levels of dopamine in the nucleus accumbens

The basal levels of dopamine in the nucleus accumbens (Fig. 3) in the local depolarization experiment were 33.29 ± 2.80 and 26.89 ± 1.3 fmol/25 µl/sample (for the HEs and LEs, respectively). The tendency for a difference in average baseline value of dopamine (samples 1 – 8) reached the statistical significance when one HE animal with

exceptionally low baseline dopamine was excluded from the analysis. In the experiment where dopamine release was stimulated with amphetamine administration, the basal levels of dopamine in the nucleus accumbens (samples 1 – 6) were similar in the HEs and LEs (27.17 ± 1.44 and 26.89 ± 1.26 fmol/25 µl/sample, respectively).

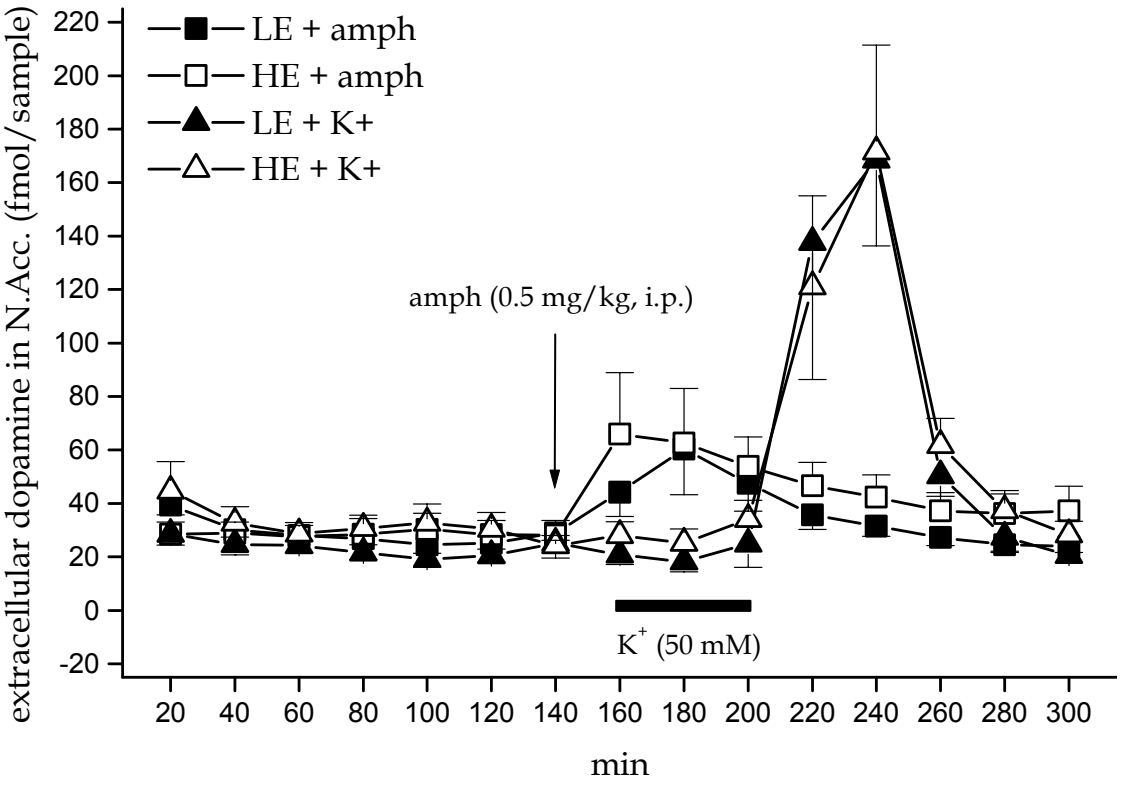

Figure 3. Basal, amphetamine (0.5 mg/kg, i.p.)- and local depolarisation (50 mM KCl in the perfusion solution) induced dopamine release in the nucleus accumbens (N.Acc.) (data expressed as means ± S.E.M.).

3.3.1.2. The effect of local depolarisation on dopamine release in the nucleus accumbens in high or low exploring rats

Local depolarisation with the perfusion solution containing 50 mM KCl significantly increased dopamine release in the nucleus accumbens to about 550% of basal values. This increment was comparable in the HEs and the LEs (Fig. 3).

3.3.1.3. The effect of amphetamine on dopamine release in the nucleus accumbens in high or low exploring rats

Systemic administration of amphetamine (0.5 mg/kg, i.p.) significantly increased dopamine release in the nucleus accumbens to about 250% of basal values. Again, no differences in the magnitude of stimulated dopamine release were seen between the HEs and the LEs (Fig. 3).

3.3.2. Discussion

In the local depolarisation experiment, the basal dopamine release in the nucleus accumbens of the HE rats tended to be higher than that of the LE rats and this tendency turned out to be a significant difference between the two groups, once one of the outliers was excluded from the analysis. This is in accordance with Hooks et al. (1992) who have reported that higher exploratory activity in an inescapable novelty test is associated with higher dopamine release in the nucleus accumbens at baseline conditions. However, in the amphetamine experiment, such a difference was not found. The reason for this discrepancy might be that the selection procedure used to differentiate between the HEs and the LEs in the local depolarisation experiment did not exactly match that of the amphetamine experiment. Specifically, in the local depolarisation experiment the animals were tested only once in the exploration box for their spontaneous exploratory activity levels one day before the microdialysis experiment; but in the amphetamine experiment the animals were tested for two consecutive days prior to the microdialysis experiment.

Microdialysis is an invasive technique and for the conductance of behavioural testing the use of guide cannulas and a post-surgery recovery period is recommended (Westerink, 2000). The experiment in which local depolarisation was used to stimulate dopamine release was the first experiment in our laboratory that combined post-surgery behavioural and microdialysis data. Because of that we had no knowledge at that time whether the exploration box test could be used to test the animals that had been operated on. As mentioned before, the exploratory activity on the first day of testing in the exploration box is not consistently correlated with the activity on the second exposure to the exploration box and may be more dependent on the anxiety factor (unpublished observations).

Local depolarisation in the nucleus accumbens increased dopamine release in both HEs and LEs, but there were no between-group differences. Higher stress- and drug-induced dopamine release has been reported for the high responders to novelty (Saigusa et al., 1999; Rouge-Pont et al., 1993, Hooks et al., 1992). At this point it remains unclear whether the reason for the lack of difference between HEs and LEs in depolarisation-induced dopamine release is 1) the different selection procedure in the studies cited above and the present experiment (forced exploration vs. emergence task), or 2) that the local depolarisation does not reflect the effect of such dopamine releasing stimuli, the primary effect of which originates from other brain regions. However, systemic administration of a low dose of amphetamine (0.5 mg/kg) also increased dopamine release in a comparable manner in the HEs and LEs. Thus, it could be argued that the basis for higher locomotor activity in the HEs after amphetamine treatment (see **Study II**) is not higher drug-induced dopamine release, but rather differences related to dopamine receptor properties. Hooks et al. (1994) have reported that high responders to novelty in the open-field test have fewer D_2 receptors in the nucleus accumbens and the striatum compared to the low responders to novelty, but there are no differences in the binding properties of the receptors.

3.4. Study IV: Intrategmental infusion of noradrenergic compounds – effect on noradrenaline and dopamine release in the prefrontal cortex and ventral tegmental area: a dual-probe microdialysis study

3.4. Results

3.4.1.1. The basal levels of dopamine and noradrenaline

The basal values of extracellular dopamine were 0.79±0.12 and 0.50±0.11 fmol/min for PFC and VTA, respectively; the basal values of extracellular noradrenaline were 6.11±1.18 and 4.43±0.98 fmol/min for PFC and VTA, respectively. The data shown in figures is expressed as percent of baseline. The average concentration of three stable baseline samples was set as 100% (Fig. 4).

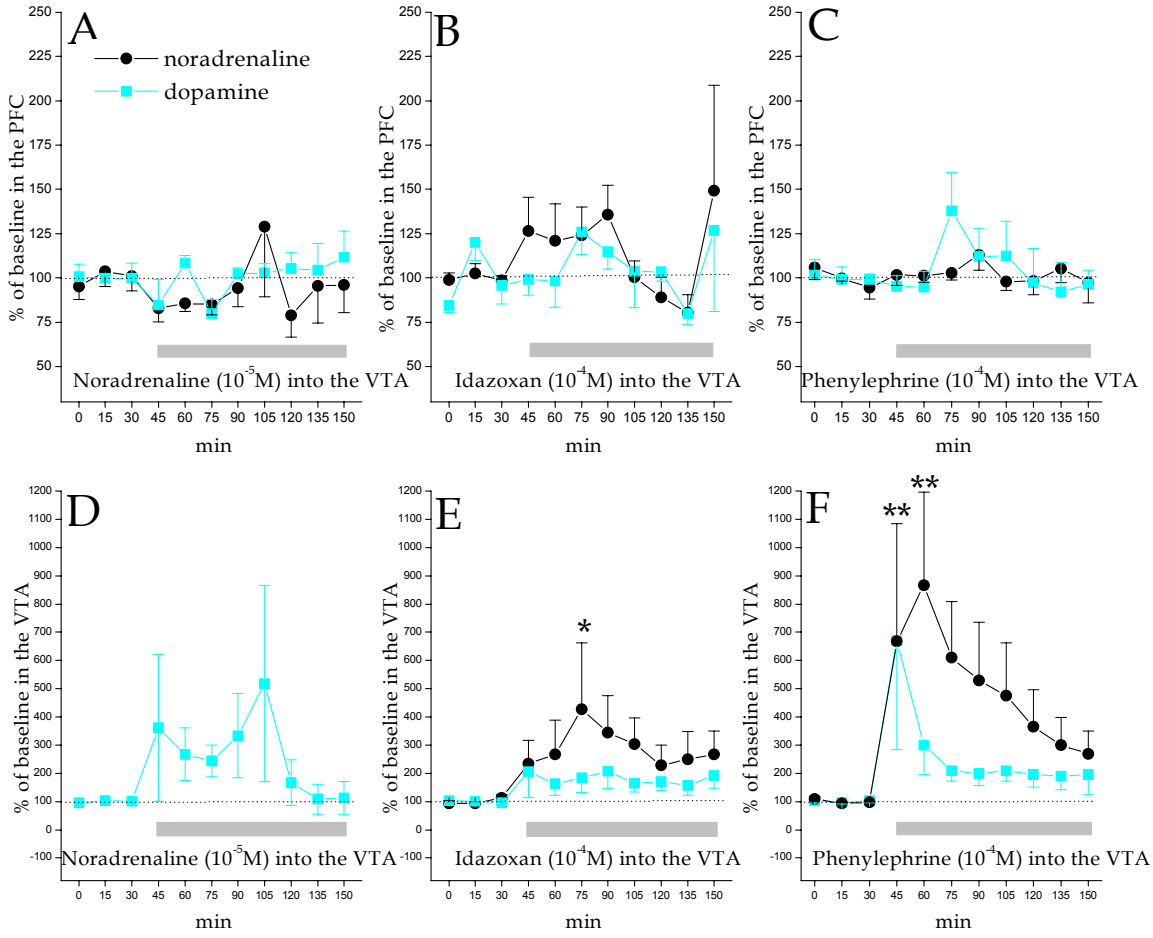

Figure 4. Effect of noradrenaline (10^{-5} M), idazoxan (10^{-4} M) and phenylephrine (10^{-4} M) on noradrenaline and dopamine release in the PFC (A-C) and the VTA (D-F) (n=4 for each experiment). ** - $p<0.01$; * - $p<0.05$ vs. basal values.

3.4.1.2. The effect of infusion of noradrenaline (10^{-5} M) into the VTA on dopamine and noradrenaline release in the PFC and VTA

Noradrenaline (10^{-5} M) infusion into the VTA did not modify basal noradrenaline or dopamine release in either the PFC or VTA (Fig. 4A, D).

3.4.1.3. The effect of infusion of idazoxan (10^{-4} M) into the VTA on dopamine and noradrenaline release in the PFC and VTA

α_2-receptor antagonist idazoxan (10^{-4} M), infused into the VTA, increased noradrenaline

release in the VTA by about 450% of basal values, but failed to modify noradrenaline release in the PFC. Dopamine release in the VTA was increased to about 200% of the basal levels, but this result was statistically non-significant with the given number of observations, dopamine release in the PFC remained unchanged by this treatment (Fig. 4B, E).

3.4.1.4. The effect of infusion of phenylephrine (10^{-4} M) into the VTA on dopamine and noradrenaline release in the PFC and VTA

The infusion of α_1-receptor agonist phenylephrine (10^{-4} M) into the VTA strongly increased noradrenaline and dopamine release in the VTA (by 900% and 700% of baseline values, respectively). This treatment did not modify the basal levels of either neurotransmitter in the PFC (Fig. 4C, F).

3.4.2. Discussion

There is ample evidence that the VTA dopaminergic system and the LC noradrenergic system are anatomically and functionally connected. Several studies have shown that the midbrain dopaminergic neurons receive a stimulatory noradrenergic input from the LC. Both the VTA and the LC pathways project to the medial prefrontal cortex, a region which is strongly implicated in the pathogenesis of several psychiatric disorders and the mechanism of action of the drugs used in their treatment (Tanda et al., 1996). Various antidepressants and atypical antipsychotics cause similar increases in both dopamine and noradrenaline release in the prefrontal cortex (Li et al., 2002, Westerink et al., 1998)– this effect is attributed to an interaction between noradrenergic and dopaminergic nerve terminals. Also, local application of glutamatergic compounds into the LC increases dopamine release in the PFC (Kawahara et al., 2001). The nature of this interaction has remained unclear, but it has been explained by a common reuptake system for noradrenaline and dopamine in the PFC (Carboni et al., 1990; Moron et al., 2002) or co-release of dopamine from the noradrenergic nerve terminals. Moreover, as the LC neurons activate mesencephalic dopamine neurons, they are able to elicit action potential dependent dopamine release in the PFC (Herve et al., 1982; Linner et al., 2001).

In the present study we explored the possibility that the noradrenaline-dopamine coupling in the PFC is regulated by LC noradrenergic neurons at the level of the VTA. Local application of noradrenaline into the VTA did not modify the basal levels of either noradrenaline or dopamine release in the VTA or the PFC. The α_2 antagonist idazoxan increased only noradrenaline release in the VTA but not in the PFC. Idazoxan also produced a slight tendency of augmentation of dopamine release in the VTA as well, but failed to modify dopamine release in either brain region. The α_1 agonist phenylephrine, however, stimulated the release of both noradrenaline and dopamine in the VTA, but not in the PFC.

The lack of effect of noradrenaline infusion in the VTA on either noradrenaline or dopamine release may be explained by its agonistic action at both α_1- and α_2- receptors, located postsynaptically, which would cancel each other out. No effect on dopamine release in the PFC was seen after the phenylephrine treatment, which increased extracellular dopamine in the VTA. However, it could be speculated that dopamine in the VTA (released, for example, somatodendritically) acted agonistically at D_2 autoreceptors, which inhibit the activity of dopamine neurons in the VTA (White and Wang, 1984) and also decrease dopamine release in the nerve terminals (Santiago et al., 1993).

The results of the present study could not confirm or refute that the noradrenaline-dopamine interaction in the PFC is regulated by noradrenergic nerve terminals at the level of VTA, but has given several new ideas for future experiments.

3.5. Study V: Effects of partial locus coeruleus denervation on exploratory and amphetamine-induced behaviour, and D_2 receptor function in rats with high or low exploratory activity

3.5.1. Results

3.5.1.1. Individual differences in exploratory activity in the exploration box

On the second exposure to the exploration box (which was the basis of differentiating between HE and LE animals), there was a significant Activity effect for all parameters measured: latency ($F(1,42)=50.5$; $p<0.0001$), line crossings ($F(1,42)=87.4$; $p<0.0001$), object investigations ($F(1,42)=106.9$; $p<0.0001$), rearings ($F(1,42)=42.5$; $p<0.0001$),

entries into the open area (F(1,42)=47.5; p<0.0001), and sum of exploratory events (F(1,42)=87.7; p<0.0001). Hence, HE animals had a statistically highly significantly shorter latency entering the open area and higher scores on all other measures of exploratory activity compared to the LE animals (Fig. 5, preDSP-4).

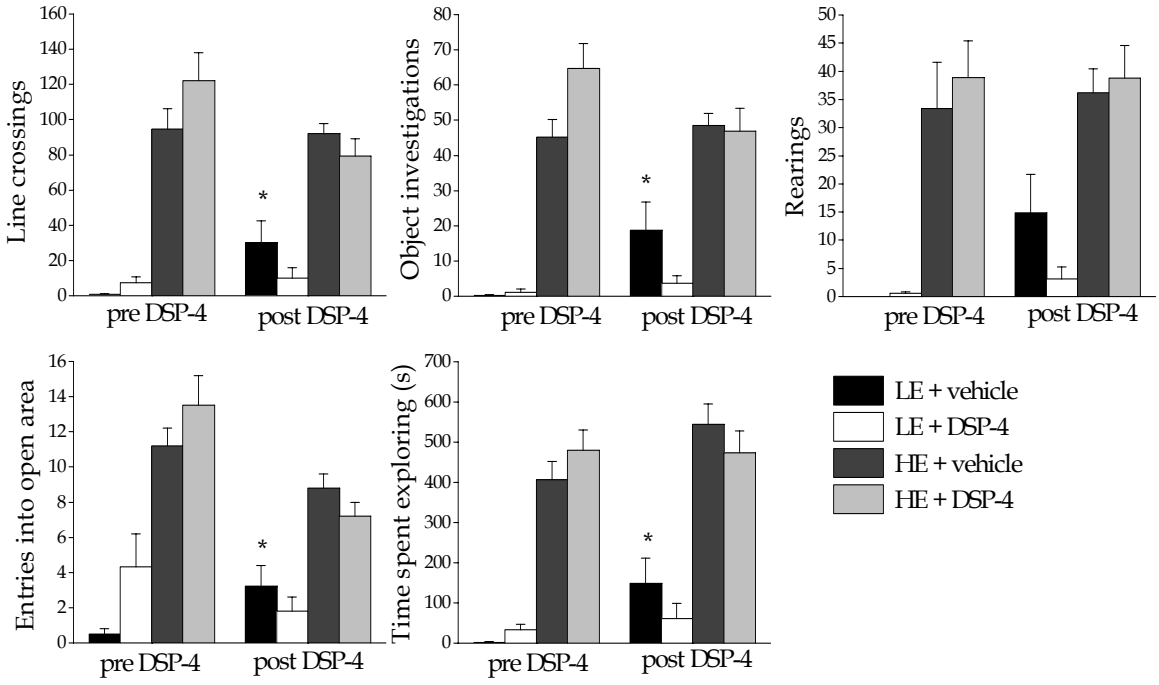

Figure 5. Exploratory behaviour in the exploration box before (pre DSP-4) and after (post DSP-4) DSP-4 (10 mg/kg) treatment. Data expressed as means ± S.E.M. * - p<0.05 vs. activity in pre DSP-4 test.

3.5.1.2. Individual differences in the locomotor activity after amphetamine

There was a significant difference between HE and LE animals in their locomotor activity after amphetamine treatment. Thus, compared to LE animals, HE animals made more line crossings (F(1,42)=23.4; p<0.0001), object investigations (F(1,42)=15.9; p<0.0001), rearings (F(1,42)=13.7; p<0.001), had a larger sum of exploratory events (F(1,42)=21.7; p<0.0001) and fewer faecal boli left on the open area (F(1,42)=11.2; p<0.01) after administration of amphetamine (Fig. 6, preDSP-4).

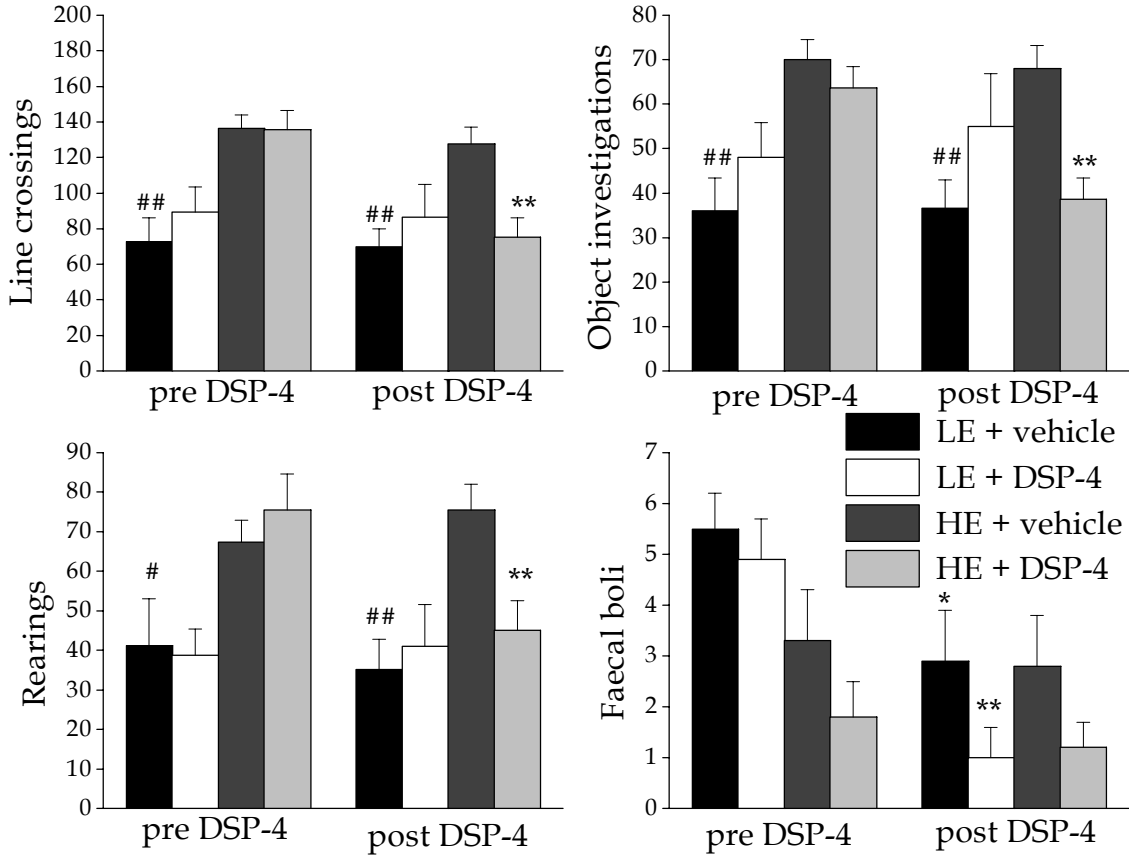

Figure 6. Locomotor activity after amphetamine (0.5 mg/kg) before (pre DSP-4) and after (post DSP-4) DSP-4 (10 mg/kg) treatment. Data expressed as means ± S.E.M. * - $p<0.05$, ** - $p<0.01$ vs. scores on pre DSP-4 test; # - $p<0.05$, ## - $p<0.01$ vs. HR + vehicle group.

3.5.1.3. The exploration box test: changes in behavioural responses after DSP-4 administration

Repeated measures ANOVA revealed a significant difference between HE and LE animals for latency to enter the open area ($F(1,40)=40.5$, $p<0.0001$), line crossings ($F(1,40)=133.2$; $p<0.0001$), object investigations ($F(1,40)=131.2$; $p<0.0001$), rearings ($F(1,40)=52.7$; $p<0.0001$), entries into the open area ($F(1,40)=67.1$; $p<0.0001$), time spent exploring ($F(1,40)=129.9$; $p<0.0001$) and sum of exploratory events ($F(1,40)=118.3$; $p<0.0001$). Thus, the overall higher activity of HE animals in the exploration box, observed in the selection test day, was also apparent after the neurotoxin treatment (Fig. 5 post DSP-4).

There was also a significant Day effect on entries into the open area ($F(1,40)=8.85$; $p<0.01$) and time spent exploring ($F(1,40)=7.02$; $p<0.05$); a significant Activity x Day interaction for line crossings ($F(1,40)=7.79$; $p<0.01$), object investigations ($F(1,40)=6.01$; $p<0.05$), entries into the open area ($F(1,40)=8.85$; $p<0.01$) and sum of exploratory events ($F(1,40)=5.83$; $p<0.05$); a significant Toxin x Day interaction was revealed for latency ($F(1,40)=6.31$; $p<0.05$), line crossings ($F(1,40)=5.74$; $p<0.05$), object investigations ($F(1,40)=6.41$; $p<0.05$), entries into the open area ($F(1,40)=10.3$; $p<0.01$), time spent exploring ($F(1,40)=5.17$; $p<0.05$) and sum of exploratory events ($F(1,40)=4.99$; $p<0.05$).

Post hoc test revealed that only the activity of the vehicle-treated, but not the neurotoxin-treated, LE group had increased over the two testing days, while the activity of the HE groups was not changed.

3.5.1.4. Changes in amphetamine-induced behaviour after DSP-4 administration

Repeated measures ANOVA revealed a significant Activity effect for line crossings ($F(1,40)=22.8$; $p<0.0001$), object investigations ($F(1,40)=10.2$; $p<0.01$), rearings ($F(1,40)=18.1$; $p<0.0001$), sum of exploratory events ($F(1,40)=21.7$; $p<0.0001$) (Fig.6 post DSP-4). Thus, compared to the LE animals, the HE animals exhibited greater exploratory behaviour after amphetamine treatment on both testing sessions. There was also a tendency of HE animals having left fewer faecal boli on the area compared to the LE animals ($F(1,40)=4.07$; $p=0.0503$).

We found a significant Day effect for line crossings ($F(1,40)=4.57$; $p<0.05$) and the number of faecal boli ($F(1,40)=15.6$; $p<0.001$). The number of defecations decreased over the two test sessions. Also, the average number of line crossings was lower on the second amphetamine test, but according to the *post hoc* test the number of line crossings was significantly decreased only in the neurotoxin-treated HE animals.

There was also a significant Activity x Toxin interaction for line crossings ($F(1,40)=7.04$; $p<0.05$), object investigations ($F(1,40)=10.7$; $p<0.01$) and sum of exploratory events ($F(1,40)=6.41$; $p<0.05$). According to *post hoc* test, the neurotoxin treatment decreased activity in the HE, but not in the LE animals.

3.5.1.5. Tissue concentrations of monoamines and their metabolites after partial noradrenergic denervation

There was a significant Toxin effect on noradrenaline content in the frontal cortex ($F(1,24)=13.4$; $p<0.01$) and hippocampus ($F(1,24)=9.79$; $p<0.01$). The DSP-4 administration in the dose of 10 mg/kg caused a decrease in the noradrenaline content in these brain regions (Table 2). More specifically, the noradrenaline levels were reduced by 29 % and 26 % in the frontal cortex of the neurotoxin-treated HE and LE animals, respectively; and by 18 % and 16 % in the hippocampus of the neurotoxin-treated HE and LE animals, respectively. ANOVA revealed a significant Activity x Toxin interaction for noradrenaline ($F(1,26)=2.34$; $p<0.05$) in the nucleus accumbens. Neurotoxin-treated LE animals tended to have lower noradrenaline levels in the nucleus accumbens than their vehicle-treated counterparts.

The HE animals had higher 5-HT and 5-HIAA levels in the frontal cortex ($F(1,24)=4.48$; $p<0.05$ and $F(1,24)=9.16$; $p<0.01$, respectively) and the striatum ($F(1,24)=6.70$; $p<0.05$ and $F(1,24)=7.99$; $p<0.01$, respectively), compared to the LE animals. The neurotoxin treatment decreased 5-HT and 5-HIAA content in the striatum ($F(1,24)=4.40$; $p<0.05$ and $F(1,24)=5.92$; $p<0.05$, respectively).

The neurotoxin treatment also decreased DOPAC and HVA content in the nucleus accumbens ($F(1,24)=7.90$; 0.01 and $F(1,24)=4.39$; $p<0.05$, respectively). According to *post hoc* test, the decrease in the levels of dopamine metabolites after neurotoxin treatment was significant only in the LE animals. There was also a tendency of increased DOPAC+HVA/DA ratio in the frontal cortex after the DSP-4 treatment ($F(1,25)=3.74$; $p=0.064$) that just missed the level of significance – vehicle-treated HE animals had significantly lower DOPAC+HVA/DA ratio in the frontal cortex than the animals belonging to any other group. DOPAC ($F(1,24)=4.49$; $p<0.05$) and HVA ($F(1,24)=4.82$; $p<0.05$) levels in the striatum were higher in the HE animals, than in the LE animals. ANOVA revealed a significant Activity x Toxin interaction for dopamine levels ($F(1,24)=4.90$; $p<0.05$) in the nucleus accumbens. According to the *post hoc* test, neurotoxin-treated LE animals had lower dopamine levels in the nucleus accumbens, compared to their vehicle-treated counterparts.

Table 2. Monoamine levels in the rat brain 19-21 days after DSP-4 (10 mg/kg).[d]

	LE+vehicle	LE+DSP-4	HE+vehicle	HE+DSP-4
Frontal cortex				
NA	1.68 ± 0.12	1.42 ± 0.10	1.92 ± 0.07	1.38 ± 0.08[a]
5-HT	2.38 ± 0.17[b]	2.59 ± 0.15	3.11 ± 0.25	2.78 ± 0.19
5-HIAA	2.01 ± 0.11	1.99 ± 0.08	2.67 ± 0.33	2.60 ± 0.37
DA	0.24 ± 0.01	0.22 ± 0.01	0.40 ± 0.13	0.21 ± 0.01
HVA	0.16 ± 0.01	0.15 ± 0.01	0.16 ± 0.01	0.15 ± 0.01
DOPAC	0.17 ± 0.01	0.17 ± 0.01	0.17 ± 0.02	0.18 ± 0.02
Hippocampus				
NA	2.47 ± 0.09	2.09 ± 0.12[a]	2.34 ± 0.09	1.94 ± 0.13[a]
5-HT	1.91 ± 0.35	3.03 ± 0.12	2.01 ± 1.25	2.05 ± 0.07
5-HIAA	2.16 ± 0.06	2.19 ± 0.10	2.16 ± 0.10	2.01 ± 0.12
DA	0.09 ± 0.01	0.09 ± 0.01	0.10 ± 0.01	0.20 ± 0.11
Striatum				
NA	0.97 ± 0.18	0.92 ± 0.07	1.88 ± 0.08	0.94 ± 0.78
5-HT	2.81 ± 0.25	2.24 ± 0.12	4.07 ± 0.81	3.03 ± 0.25
5-HIAA	5.83 ± 0.50	4.22 ± 0.26[a,c]	7.26 ± 1.01	6.20 ± 0.60
DA	47.0 ± 4.18	44.7 ± 3.61	54.8 ± 5.19	45.2 ± 2.17
HVA	9.08 ± 0.87	7.17 ± 0.72	9.89 ± 0.45	9.39 ± 1.05
DOPAC	36.9 ± 3.58	28.4 ± 2.07[c]	39.1 ± 1.84	39.6 ± 5.02
Nucleus accumbens				
NA	2.69 ± 0.55	1.98 ± 0.21[c]	2.93 ± 0.61	4.51 ± 0.82
5-HT	2.15 ± 0.20	1.80 ± 0.21[c]	2.17 ± 0.33	2.78 ± 0.44
5-HIAA	3.74 ± 0.20	2.72 ± 0.36[a,c]	3.84 ± 0.12	3.96 ± 0.57
DA	38.9 ± 3.10	26.7 ± 1.31[a]	34.5 ± 5.28	36.0 ± 3.57
HVA	5.48 ± 0.41	3.88 ± 0.33[a]	5.43 ± 1.04	4.70 ± 0.39
DOPAC	25.6 ± 1.73	17.3 ± 1.07[a]	24.4 ± 3.79	20.7 ± 1.70

Note: [a] $p<0.05$ vs. respective vehicle group
[b] $p<0.05$ vs. respective HE group
[c] $p<0.05$ vs. HE+DSP-4 group
[d] The values (mean ± S.E.M.) are expressed as pmol/mg wet weight tissue.

3.5.1.6. Effect of partial LC denervation on D_2 receptor function in the striatum

There was a significant Activity x Toxin interaction on dopamine effect on [^{35}S]GTPγS binding in the striatum ($F(1,22)=9.10$; $p<0.01$), which was higher in neurotoxin-treated LE animals and lower in neurotoxin-treated HE animals compared to their respective vehicle-treated counterparts (Fig. 7). There was also a tendency of higher dopamine ability to activate [^{35}S]GTPγS binding in the striatum of the vehicle-treated HEs compared to the vehicle-treated LEs, but the difference did not reach statistical significance ($p=0.10$). The animals' spontaneous activity levels and the neurotoxin treatment also affected the DA effect on GDP binding affinity (Activity x Toxin interaction $F(1,22)=7.34$; $p<0.05$), which was significantly smaller in neurotoxin-treated HE animals compared to the vehicle-treated HE rats. There were no differences in D_2 receptor function in the nucleus accumbens.

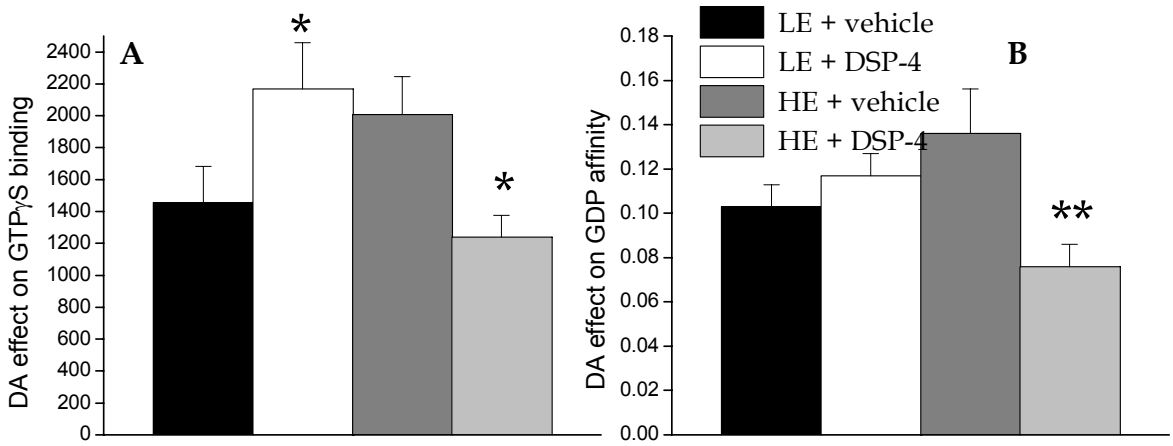

Figure 7. G-protein and D_2-receptor interaction in the rat striatum after DSP-4 (10 mg/kg). Data expressed as means ± S.E.M. of dpm of radioligand specifically bound (A) and log unit shift (B). ** - $p<0.01$; * - $p<0.05$ vs. respective vehicle group.

3.5.2. Discussion

The administration of a low dose (10 mg/kg) of the selective neurotoxin DSP-4 reduced exploratory activity only in the LE group. More specifically, the DSP-4 treated LE animals did not show any sign of habituation to the testing environment, as did their

vehicle treated counterparts. This seems to be consistent with previous studies that have emphasized the role of LC in adapting to environmental changes (Delini-Stula et al., 1984; Berridge and Dunn, 1990; Harro et al., 1995). These data also suggest that partial noradrenergic denervation is sufficient for the expression of deficits in novelty-related behaviour, but only in the animals with low spontaneous exploratory activity levels.

The HE and LE animals also differed in their response to amphetamine (0.5 mg/kg) treatment; locomotor activity after amphetamine was more pronounced in the HE animals. The frequency of defecations, considered to be a measure of emotionality in the open field (Candland et al., 1967), was significantly higher in the LE animals, suggesting a higher anxiety level in these animals. This difference between HE and LE animals disappeared by the second amphetamine test, indicative of a habituation to the procedure. However, as the overall amphetamine-induced activity levels of the LE animals, regardless of whether they received a neurotoxin or vehicle treatment, did not change over the two test sessions, the differences between the HE and LE animals can not be entirely attributed to differences in emotionality.

After the neurotoxin treatment, the DSP-4 treated HE animals (but not the neurotoxin-treated LE animals) displayed a marked decrease in all amphetamine-induced activity measures. This implies that the expression of behavioural responses to amphetamine is dependent on an intact LC noradrenergic system in HE, but not in LE animals.

On the basis of literature, behavioural differences observed in the HE and LE animals have been related to differences mainly in the dopaminergic system, such as higher basal and evoked dopamine release, and higher dopamine tissue content in the nucleus accumbens of the HE animals (Hooks et al., 1992; Rouge-Pont et al., 1993; Thiel et al., 1999). In the present study the HE and LE animals only differed in respect to the 5-HT and 5-HIAA content in the frontal cortex, which were higher in the HE animals. Our result is at variance with Thiel et al.'s (1999) study, which reported higher dopamine levels in the nucleus accumbens and lower 5-HT levels in the medial frontal cortex in the HE animals. One possible explanation for this discrepancy may be the different selection procedure used to classify the animals as high or low responders to novelty (or, in our case, high or low explorers). The basis for differentiation between high and low

responders to novelty in Thiel et al. (1999) was rearing activity in an inescapable open field. The result of this study is also different from the results of **Study II**, where the HEs were characterized by lower 5-HT content in their frontal cortex. These results, however, are not entirely comparable because of the different testing procedures used, specifically, all animals in **Study II** were repeatedly administered amphetamine, which is likely to affect tissue neurotransmitter content.

The administration of a low dose (10 mg/kg) of DSP-4 produced a decrease in noradrenaline levels, comparable in the HE and LE animals, about 20-25% in the frontal cortex and about 15-20% in hippocampus. Thus, it can be concluded that at the dose used in the present study, DSP-4 effectively produces a partial noradrenergic denervation of frontal and hippocampal regions. It remains unclear how the rate of noradrenergic neurotransmission is altered after such treatment because of the possible compensatory responses in postsynaptic receptors (Harro and Oreland, 2001); but the data obtained in this study suggest that the noradrenergic input from LC might be weakened. Although DSP-4 treatment is selective for the noradrenergic nerve terminals and other neurotransmitter systems remain directly unaffected (Ross, 1976), we found that this treatment also decreased the levels of dopamine and its metabolites in the nucleus accumbens, but only in the LE animals. A similar dose (10 mg/kg) of DSP-4 is sufficient to reduce the evoked dopamine release in the nucleus accumbens (Häidkind et al., 2002) and results in D_2 receptor upregulation in the striatum (Harro et al., 2003). However, in Harro et al. (2003) the D_2 receptor upregulation after partial LC denervation was not accompanied by changes in the characteristics of G proteins in rats not preselected for individual differences in exploratory activity. The results of the present study suggest that a partial LC denervation has a differential effect on the intracellular signal transduction, depending on the animals' spontaneous exploratory activity. Thus, the ability of dopamine to activate [^{35}S]GTPγS binding in the striatum was increased in LE animals and decreased in HE animals after the neurotoxin treatment. Therefore, one possible explanation for the DSP-4-induced decrease in locomotor activity after amphetamine treatment in HE animals is the reduced sensitivity of D_2 receptors in the striatum. In neurotoxin-treated LE animals, locomotor activity after amphetamine remained

unchanged, possibly because the effects of the noradrenergic denervation were compensated by the increase in D_2 receptor sensitivity.

The present results suggest that the input of the LC noradrenergic system to the midbrain dopaminergic system is dependent on the animals' spontaneous activity levels.

4. CONCLUSIONS

The nucleus accumbens dopamine system is involved in novelty-, but also drug-related behaviours and its interaction with cholecystokinin and the locus coeruleus noradrenaline system in the regulation of rat exploratory behaviour in the exploration box was studied in four studies described here. Endogenous cholecystokinin contributes differentially to amphetamine-stimulated behaviour and the development of behavioural sensitization to repeated amphetamine treatment, depending on the receptor subtype it acts upon. Spontaneous activity levels in the exploration box appear to be stable and predict subsequent amphetamine-stimulated locomotor behaviour, but only low explorers develop behavioural sensitization to repeated amphetamine treatment. As suggested by **Study III**, the differences between the HEs and the LEs are not due to differential basal or stimulated dopamine release in the nucleus accumbens, which were similar in both groups, thus raising the possibility that other mechanisms, for instance, at dopamine receptor level, are involved. As demonstrated in **Study IV**, dopamine release in the ventral tegmental area is regulated by a noradrenergic mechanism. In **Study V** we used partial locus coeruleus denervation to study the noradrenergic regulation of the exploratory and amphetamine-stimulated behaviour, and D_2 receptor interaction with G-proteins in the nucleus accumbens and the striatum, in animals with high or low spontaneous exploratory activity levels. The locus coeruleus noradrenergic system has a differential role in exploratory and amphetamine-stimulated behaviour in the HEs and the LEs. As partial locus coeruleus denervation has differential effects on the D_2 function in the striatum, this may subserve the changes in amphetamine-stimulated locomotor activity in the HEs after the neurotoxin treatment.

5. ACKNOWLEDGEMENTS

I would like to thank my supervisor Prof Jaanus Harro for his ever-present assistance and advice. I am also grateful to Marika Eller, Kadri Kõiv, Toomas Kivastik, Ivo Kolts, Ago Rinken, Ain Uustare, Yuki Kawahara and Prof Ben Westerink for intellectual and technical contribution to this thesis. We thank Prof Leslie Iversen and ML Laboratories/Panos Therapeutics, UK for the generous donation of devazepide and L-365,260 and Svante Ross and AstraZeneca, Sweden, for the generous donation of DSP-4. The studies presented in this thesis were supported by the Estonian Science Foundation grant No 4531, the Estonian Ministry of Education and Science projects No 0814 and No 2643 and the EU Framework 6 Integrated Project NEWMOOD (LSHM-CT-2003-503474).

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European Neuropsychopharmacology 14 (2004) 324–331

EUROPEAN NEURO-PSYCHOPHARMACOLOGY

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Effect of CCK_1 and CCK_2 receptor blockade on amphetamine-stimulated exploratory behavior and sensitization to amphetamine

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Received 21 January 2003; received in revised form 17 June 2003; accepted 30 September 2003

Abstract

Interactions between dopaminergic neurotransmission and cholecystokinin (CCK) in the CNS may be important in the pathogenesis of psychotic disorders and substance abuse. In this study, the effect of coadministration of the selective CCK receptor antagonists devazepide and L-365,260 (for selectively blocking CCK_1 and CCK_2 receptors, respectively), on the effect of amphetamine on the rat exploratory behavior, and on sensitization of locomotor response to amphetamine, were studied. Amphetamine (0.5 mg/kg) increased exploratory activity in the exploration box for 5 consecutive testing days, while devazepide (10 μg/kg) blocked and L-365,260 (10 μg/kg) enhanced amphetamine-induced stimulation of activity. Devazepide coadministration prevented the development of sensitization to amphetamine, while coadministration of L-365,260 with amphetamine potentiated the locomotor effect of a challenge dose of amphetamine. These results suggest that endogenous CCK, released during exploratory activity, shapes behavioral responses to amphetamine by acting on both receptor subtypes, and modulates the development of sensitization to amphetamine.

Keywords: Amphetamine; CCK; Devazepide; L-365,260; Exploratory behavior; Sensitization

1. Introduction

Cholecystokinin (CCK) is a gut–brain peptide, which acts via CCK_1 and CCK_2 receptor subtypes (Noble et al., 1999). CCK is involved in the regulation of feeding, pain perception, and learning and memory (Crawley and Corwin, 1994; Moran and Schwartz, 1994), and possibly in the pathogenesis of anxiety and psychosis (see Bourin et al., 1996; Harro et al., 1993 for review).

A subpopulation of the dopaminergic neurons in the ventral tegmental area projecting to the nucleus accumbens contains CCK as a cotransmitter (Hökfelt et al., 1980). It has been demonstrated that CCK modulates dopaminergic activity depending on the CCK receptor subtype involved. For example, CCK acting via CCK_1 receptors in the medial posterior nucleus accumbens potentiates dopaminergic activity while CCK acting on CCK_2 receptors in the anterior nucleus accumbens either has no effect or inhibits dopaminergic activity (Crawley, 1991; Marshall et al., 1991). Similarly, behavioral studies have demonstrated that intracerebrally administered CCK potentiates dopamine-dependent behavior in a CCK_1 receptor-mediated manner while inhibits it via CCK_2 receptors (Crawley, 1991, 1992, 1994; Vaccarino and Rankin, 1989). Dopaminergic activity in the nucleus accumbens is increased in response to natural rewards and drugs of abuse (Heffner et al., 1980; Di Chiara and Imperato, 1988; Pfaus et al., 1990; Young et al., 1992) and novelty (Rebec et al., 1997). Given the dopamine involvement in drug reward (Wise and Bozarth, 1985) or attribution of incentive salience to stimuli (Berridge and Robinson, 1998; Ikemoto and Panksepp, 1999) as well as in psychotic disorders (Davis et al., 1991 for review), CCK may contribute to the development or expression of drug abuse or psychosis.

The selective CCK receptor antagonists have been shown to be ineffective in modulating baseline locomotor behavior, but they alter the behavioral changes induced by dopamine or dopamine agonists, e.g. the indirect agonist amphetamine (Josselyn and Vaccarino, 1995; Philips et al., 1993; Tieppo et al., 2000). CCK receptors also seem to contribute to the development and expression of behavioral sensitization to amphetamine (DeSousa et al., 1999; Wunderlich et al., 2000), a phenomenon known to occur after repeated exposure to psychostimulants (Segal and Mandell, 1974; Pierce

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doi:10.1016/j.euroneuro.2003.09.006

and Kalivas, 1997). Nevertheless, there are some inconsistencies in the studies describing the specific roles the CCK receptor subtypes may have in modulating the different behavioral effects of amphetamine, particularly when CCK receptor antagonists have been used to block the modulating role of endogenous CCK. In the rat, CCK_1 receptor blockade was reported not to influence amphetamine-induced hyperlocomotion (DeSousa et al., 1999) and stereotypy (Tieppo et al., 2000). Neither did a nonpeptide CCK_1 receptor antagonist affect amphetamine-induced disruption of prepulse inhibition (Shilling and Feifel, 2002). Somewhat inconsistently, rats with the naturally-occurring CCK_1 receptor-deficiency were less sensitive to locomotor activity enhancing effects of amphetamine (Feifel et al., 2001), and devazepide, a CCK_1 receptor antagonist, reduced the locomotor stimulant effect of amphetamine in mice (Vasar et al., 1991). CCK_1 receptor blockade by devazepide treatment has been found to block the expression of amphetamine sensitization, but not the development of it (Wunderlich et al., 2000). Interestingly, devazepide has been shown to antagonise the acquisition of amphetamine-conditioned (Josselyn et al., 1996) as well as cocaine-conditioned activity (Josselyn et al., 1997), suggesting differential involvement of CCK_1 receptors in these behavioral phenomena. Regarding CCK_2 receptor blockade, the effect of an antagonist, L-365,260, on the development of amphetamine sensitization was found to be either reducing or potentiating depending upon dose (Wunderlich et al., 2000). In other studies, L-365,260 has been reported to enhance amphetamine-facilitated responding for conditioned rewards (Josselyn and Vaccarino, 1995) and to reduce amphetamine stereotypy (Tieppo et al., 2000).

One possible factor contributing to the role CCK has in behavior is the relative novelty of the environment (Blacker et al., 1997). Therefore, the effects of CCK receptor antagonists may differ when given in an acute experiment or repeatedly, and may also depend upon the contingency between drug administration and testing environment. The aim of this study thus was to characterize the effects of CCK receptor blockade on amphetamine-elicited changes in rat exploratory behavior, and on the development of behavioral sensitization to amphetamine administered in association with the environment of testing for exploration.

2. Experimental procedures

2.1. Animals

Male Wistar rats ($n=72$, weighing 260–390 g, from National Laboratory Animal Center, Kuopio, Finland) were housed four per cage in standard polypropylene cages in a light controlled room (12-h light:12-h dark cycle, lights on at 07:00 h) maintained at 22 °C. Food and water were available ad libitum. All experiments were conducted between 13:00 and 19:00 h.

2.2. Behavioral testing

The exploration box test was conducted as described previously (Harro et al., 1995; Otter et al., 1997). The exploration box was made of metal and consisted of a 0.5×1 m open area (side walls 40 cm) with a 20×20×20 cm small compartment attached to one of the shorter sides of the open area. The open area was divided into eight squares of equal size. In the open area, four objects, three novel and one familiar (a glass jar, a cardboard box, a wooden handle and a food pellet) were situated in certain places (which remained the same throughout the experiment). The small compartment, which had its floor covered with wood shavings, was directly linked to the open area through an opening (size 20×20 cm). The apparatus was cleaned with dampened cloth after each animal. The exploration test was initiated by placing a rat into the small compartment, which was then covered with a lid for the exploration time. The following behavioral parameters were registered: (1) latency (of entering open area with all four paws), (2) number of entries into the open area, (3) time spent exploring on the open area, (4) line crossings, (5) rearings and (6) number of unfamiliar object investigations. To provide an index of exploration the scores of line crossing, rearing and object investigation were summed for each animal and thus (7) the sum of exploratory events obtained. A single test session lasted 15 min. All animals were tested in the exploration box on 5 consecutive days.

In order to assess sensitization to amphetamine, 9 days after the last day of the 5-day exploration box test all animals received a challenge dose of amphetamine (0.5 mg/kg), the passage between the open area compartment and the small compartment was closed, the animal was placed on the open area and for 15 min line crossings, rearings and the number of object investigations were registered. The sum of these measures was also calculated.

2.3. Drug administration

Animals were randomly assigned to four groups: (1) control group (vehicle + distilled water); (2) CCK antagonist (devazepide or L-365,260) group (CCK antagonist + distilled water); (3) amphetamine group (vehicle + amphetamine); (4) amphetamine and CCK antagonist (amphetamine/devazepide or amphetamine/L-365,260) group ($n=9$ per group).

The following drugs were used: a selective CCK_1 receptor antagonist devazepide, a selective CCK_2 receptor antagonist L-365,260 (ML Laboratories/Panos Therapeutics, UK), *d*-amphetamine sulfate (Sigma, St. Louis, MO). CCK antagonists were suspended in a few drops of Tween-85® and diluted in distilled water. Amphetamine was dissolved in distilled water. Fresh solutions were prepared for each day of experiments.

The doses of CCK antagonists used in this study were selected to ensure selectivity for the specific receptor type

(Harro and Vasar, 1991; Harro et al., 1996). CCK_1 receptor antagonist devazepide (10 μg/kg i.p.) and CCK_2 receptor antagonist L-365,260 (10 μg/kg i.p.) or vehicle was administered 30 min prior to behavioral testing on 5 consecutive days. The dose of amphetamine (0.5 mg/kg i.p.) was selected on the basis of previous studies (Otter et al., 1997) demonstrating that this dose had an exploration-enhancing effect in the exploration box. Amphetamine or distilled water was administered 15 min prior to behavioral testing on 5 consecutive days. All drugs were administered in the volume of 1 ml/kg.

In the sensitization experiment all animals received a challenge dose of amphetamine (0.5 mg/kg) 15 min before behavioral testing.

2.4. Data analysis

Data from the 5-day exploration box test were analyzed with two-factor ANOVA (CCK antagonist × amphetamine) with repeated measures. Data from the sensitization experiment were analyzed with two-factor ANOVA (CCK antagonist pretreatment×amphetamine pretreatment). Subsequent pairwise comparisons were made with Fisher's LSD test.

3. Results

3.1. Effect of CCK_1 receptor blockade on amphetamine-stimulated exploration

Analysis of data obtained from the 5-day exploration box test with repeated measures ANOVA revealed a significant amphetamine effect on line crossings ($F(1,32)=6.21$; $P<0.05$), number of object investigations ($F(1,32)=7.43$; $P<0.05$), rearings ($F(1,32)=8.08$; $P<0.01$), time spent exploring ($F(1,32)=6.43$; $P<0.05$) and sum of exploratory events ($F(1,32)=7.12$; $P<0.05$). There was also a significant devazepide effect on latency ($F(1,32)=7.15$; $P<0.05$), line crossings ($F(1,32)=7.18$; $P<0.05$), number of object investigations ($F(1,32)=8.12$; $P<0.01$), rearings ($F(1,32)=8.86$; $P<0.01$), number of entries into open area ($F(1,32)=6.62$; $P<0.05$), time spent exploring

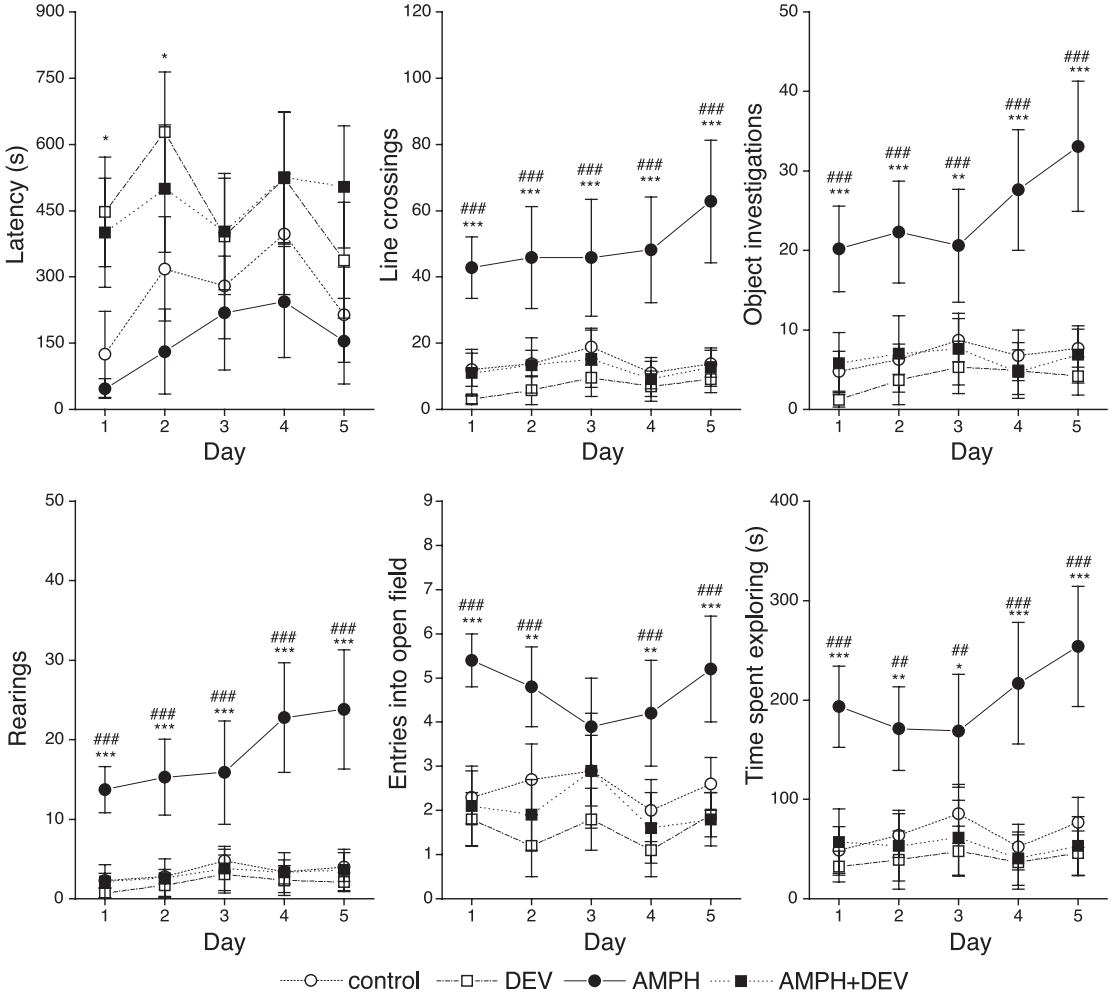

Fig. 1. The effects of amphetamine (0.5 mg/kg, AMPH) and CCK_1 receptor antagonist devazepide (10 μg/kg, DEV) on rat exploratory behavior in the exploration box. *, $P<0.05$; **, $P<0.01$; ***, $P<0.001$ vs. control; #, $P<0.05$; ##, $P<0.01$; ###, $P<0.001$ vs. amphetamine + devazepide (AMPH + DEV).

($F(1,32)=8.74$; $P<0.01$) and sum of exploratory events ($F(1,32)=8.03$; $P<0.01$). Further, ANOVA revealed a significant devazepide and amphetamine interaction on the number of object investigations ($F(1,32)=4.22$; $P<0.05$), rearings ($F(1,32)=5.98$; $P<0.05$), time spent exploring ($F(1,32)=4.41$; $P<0.05$) and sum of exploratory events ($F(1,32)=4.16$; $P<0.05$). There was no Day effect on any of the behavioral parameters registered. Post hoc tests revealed that animals of the amphetamine group were significantly more active than controls in regard to every registered parameter of exploratory activity regardless of the day of testing (Fig. 1). Devazepide (10 μg/kg) had no effect of its own. When coadministered with amphetamine, devazepide blocked amphetamine's stimulating effect on behavior on all days of testing.

3.2. Effect of CCK_2 receptor blockade on amphetamine-stimulated exploration

Repeated measures ANOVA revealed a significant Day effect on latency ($F(4,128)=2.59$; $P<0.05$), line crossings ($F(4,128)=9.90$; $P<0.00001$), object investigations ($F(4,128)=9.96$; $P<0.00001$), rearings ($F(4,128)=6.22$; $P<0.001$), entries into open area ($F(4,128)=5.25$; $P<0.001$), time spent exploring ($F(4,128)=5.21$; $P<0.001$) and sum of exploratory events ($F(4,128)=10.81$; $P<0.00001$). All groups explored less on the first day of the 5-day exploration box test.

There was again a significant amphetamine effect on exploratory behavior [latency ($F(1,32)=12.61$; $P<0.01$), line crossings ($F(1,32)=24.89$; $P<0.0001$), object investigations ($F(1,32)=31.57$; $P<0.00001$), rearings ($F(1,32)=22.16$; $P<0.0001$), entries into open area ($F(1,32)=36.61$; $P<0.00001$), time spent exploring ($F(1,32)=25.05$; $P<0.0001$) and sum of exploratory events ($F(1,32)=26.61$; $P<0.0001$)]. Thus, amphetamine (0.5 mg/kg) decreased latency and stimulated activity in the exploration box (Fig. 2).

Further, repeated measures ANOVA revealed a significant amphetamine and Day interaction on latency ($F(4,128)=3.84$; $P<0.01$), line crossings ($F(4,128)=3.83$; $P<0.01$), object investigations ($F(4,128)=3.23$; $P<0.05$),

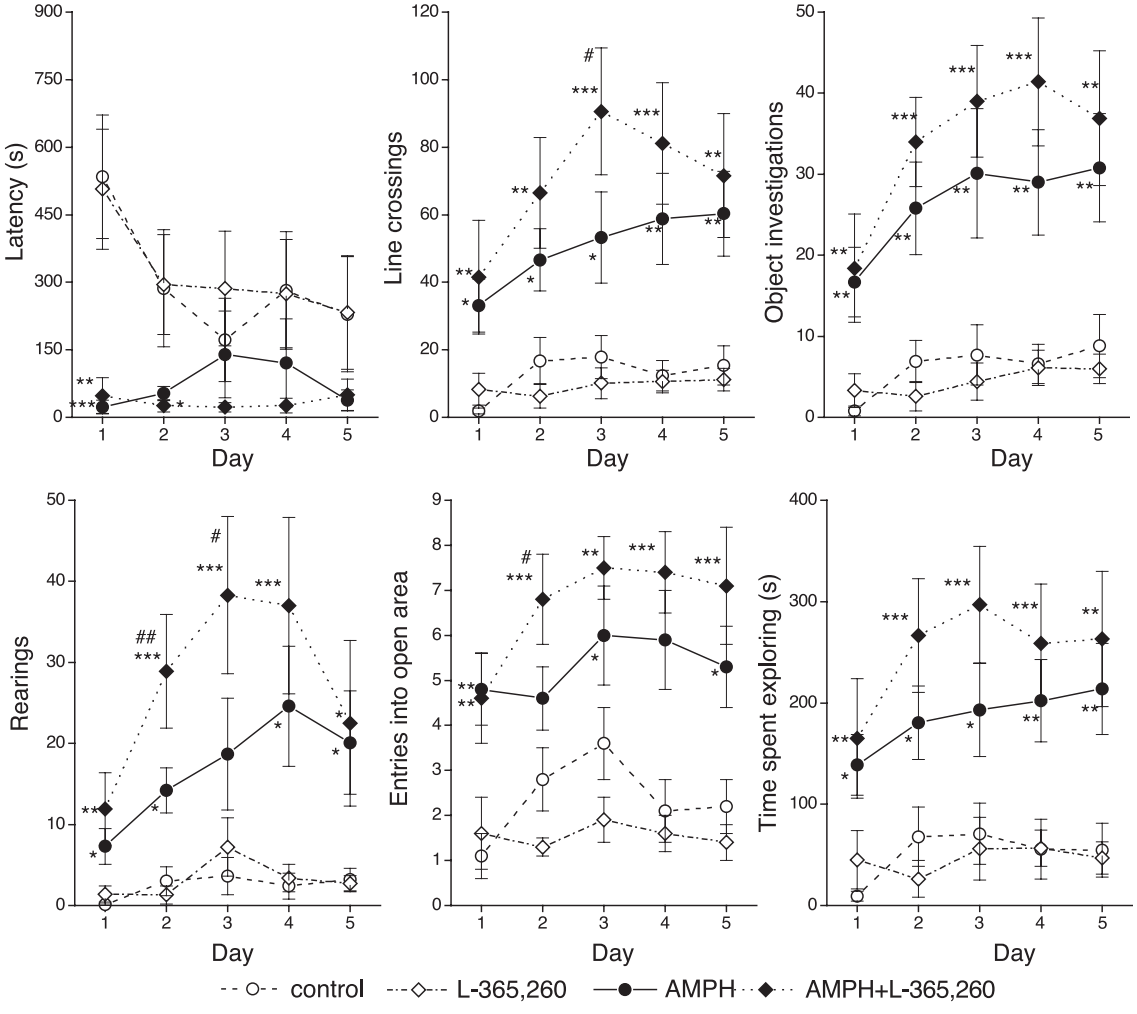

Fig. 2. The effects of amphetamine (0.5 mg/kg, AMPH) and CCK_2 receptor antagonist L-365,260 (10 μg/kg) on rat exploratory behavior in the exploration box. *, $P<0.05$; **, $P<0.01$; ***, $P<0.001$ vs. control; #, $P<0.05$; ##, $P<0.01$ vs. amphetamine + L-365,260.

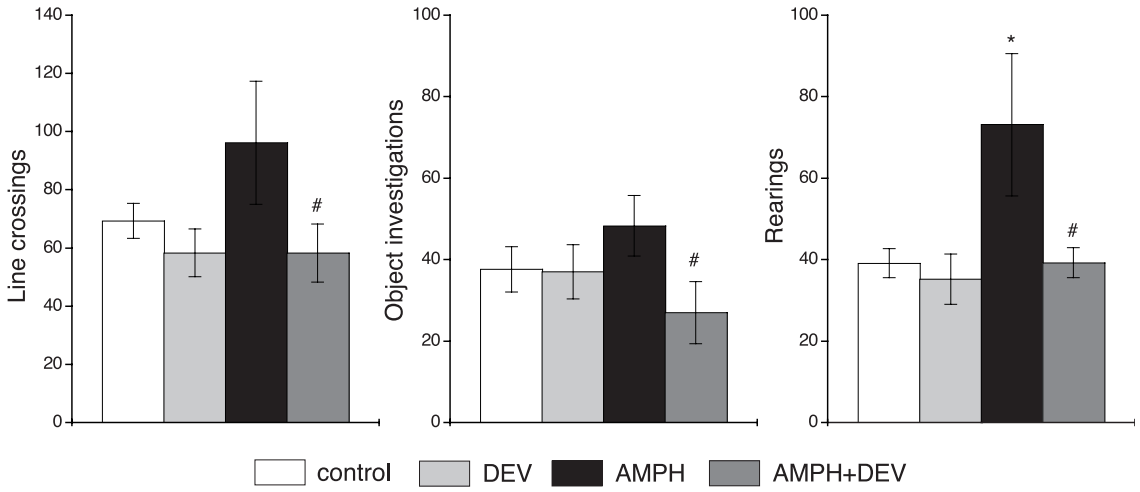

Fig. 3. The effect of amphetamine challenge (0.5 mg/kg) on locomotor activity of rats pretreated with amphetamine (AMPH) and/or devazepide (DEV). *, $P<0.05$ vs. control; #, $P<0.05$ vs. AMPH. Data on sum of the events are not shown.

rearings ($F(4,128)=3.23$; $P<0.05$) and sum of exploratory events ($F(4,128)=4.32$; $P<0.01$). Post hoc tests revealed that compared to controls animals of amphetamine and amphetamine/L-365,260 groups had a shorter latency on the first 2 days of the 5-day experiment. By the third day of the experiment the difference in latency between amphetamine group and controls had disappeared, but latency of amphetamine/L-365,260 group remained unchanged throughout the experiment. Although amphetamine administration whether with or without L-365,260 coadministration stimulated animals' behavior on all days of the 5-day experiment compared to controls, there was a significant difference in activity levels of amphetamine group and amphetamine/L-365,260 group animals on days 2–4 of the experiment. Thus, repeated administration of L-365,260 (10 μg/kg) potentiated the behavioral response to amphetamine. L-365,260 administered alone had no effect on exploratory behavior.

3.3. Behavioral sensitization to amphetamine: effect of CCK_1 receptor blockade

In this experiment there was a statistically not significant tendency of amphetamine (0.5 mg/kg) challenge to produce a higher behavioral response in the amphetamine pretreated group. The effect of amphetamine pretreatment on rearings just missed the level of significance ($P=0.06$). ANOVA revealed devazepide pretreatment effect on the sum of events ($F(1,32)=4.34$; $P<0.05$), and effects on other measures tended to be significant ($P=0.56–0.77$). One-way ANOVA revealed significant differences between the groups regarding rearings ($F(3,32)=3.24$, $P<0.05$). Post hoc tests revealed that the number of rearings in amphetamine pretreatment group was significantly increased compared to other groups and this effect was not present in animals who received devazepide (10 μg/kg) together with amphetamine during the pretreatment phase (Fig. 3).

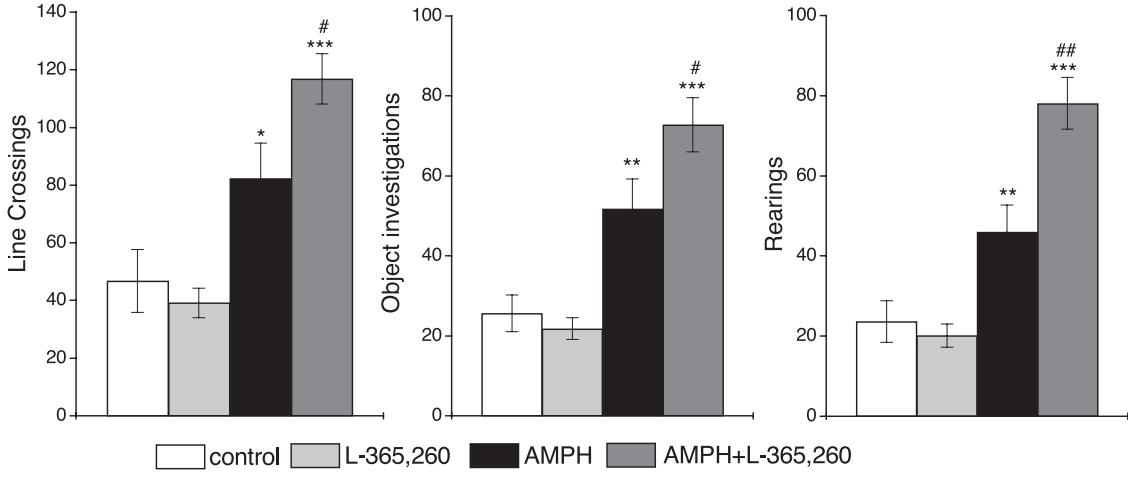

Fig. 4. The effect of amphetamine challenge (0.5 mg/kg) on locomotor activity of rats pretreated with amphetamine (AMPH) and/or L-365,260. *, $P<0.05$; **, $P<0.01$, ***, $P<0.001$ vs. control; #, $P<0.05$; ##, $P<0.01$ vs. AMPH. Data on sum of the events are not shown.

3.4. Behavioral sensitization to amphetamine: effect of CCK_2 receptor blockade

ANOVA revealed a significant amphetamine pretreatment effect on line crossings ($F(1,32)=1.98$; $P<0.0001$), object investigations ($F(1,32)=45.27$; $P<0.0001$), rearings ($F(1,32)=52.51$; $P<0.0001$) and sum of exploratory events ($F(1,32)=45.70$; $P<0.0001$) on the open area compartment of the exploration box. Thus, repeated amphetamine pretreatment sensitized the behavioral response to the challenge dose of amphetamine (Fig. 4). In this experiment, there was also a statistically significant L-365,260 and amphetamine interaction for line crossings ($F(1,32)=4.77$; $P<0.05$), object investigations ($F(1,32)=4.67$; $P<0.05$), rearings ($F(1,32)=10.32$; $P<0.01$) and the sum of exploratory events ($F(1,32)=6.52$; $P<0.05$). L-365,260 administration during repeated amphetamine pretreatment increased the behavioral sensitization observed after the amphetamine challenge.

4. Discussion

The results of this study indicate a substantial involvement of endogenous CCK in amphetamine-dependent behavioral responses in a test of exploratory behavior. While amphetamine-stimulated exploratory behavior in the exploration box, the CCK_1 receptor antagonist devazepide completely blocked this effect of amphetamine, but the CCK_2 receptor antagonist L-365,260 potentiated it. CCK receptor antagonists did not modulate exploratory behavior when administered alone. This is consistent with previous studies that have shown the involvement of both CCK receptor subtypes in the expression of behavioral responses to amphetamine and the inability of the CCK receptor antagonists alone to modify behavior (Josselyn and Vaccarino, 1995; Vasar et al., 1991). Consistently, Otsuka Long Evans Tokushima Fatty (OLETF) rats of the naturally-occurring CCK_1 receptor-deficient rat line have been demonstrated to be less sensitive to locomotor activity enhancing effects of amphetamine (Feifel et al., 2001) and they also exhibit less behavioral sensitization to cocaine (Beinfeld et al., 2001). CCK_2 receptor-deficient mice had an increased locomotor activity in response to amphetamine (Dauge et al., 2001; Kõks et al., 2001) and also a pronounced behavioral sensitization to morphine (Dauge et al., 2001) compared to wild type mice.

The time-dependency of L-365,260 effect on amphetamine's action in this study suggests that at least using the exploration test containing a free-choice element (animals can hide into the small chamber), single administration of the CCK_2 antagonist is not sufficient for the expression of the additive effect of amphetamine and CCK_2 receptor blockade. All groups exhibited the lowest levels of exploratory activity on the first day of the 5-day test. This appears to be related to the anxiogenic effect of the initial exposure to the testing environment in the exploration box (Harro et al., 1995). The present study suggests that CCK_2 receptor blockade induced potentiation of amphetamine-induced stimulation depends upon the level of anxiety.

Together these experiments demonstrate that the effects of amphetamine on exploratory behavior are bidirectionally modulated by endogenous CCK, dependent upon receptor subtype. It has been suggested that the involvement of CCK in exploratory behavior depends upon the time animal has been able to explore the environment (Blacker et al., 1997). Thus, the somewhat conflicting data on the effects of CCK antagonists on different behavioral effects of amphetamine may be related to the specifics of the paradigm, but also to the doses used, because devazepide does behave as a CCK_2 antagonist at higher doses (0.1–1 mg/kg), and L-365,260 has an inverse U-shaped effect on CCK_2 receptor mediated reduction of exploratory behavior (Harro and Vasar, 1991).

Also, CCK receptor blockade during repeated amphetamine administration altered the behavioral response to amphetamine administered after a withdrawal period. Devazepide pretreatment strongly tended to block while L-365,260 pretreatment potentiated the development of behavioral sensitization. Our results are somewhat different compared to DeSousa et al. (1999) and Wunderlich et al. (2000), who have suggested that endogenous CCK via CCK_1 receptors attenuates the expression (not studied in our present investigation) but not the development of behavioral sensitization. Regarding CCK_2 receptor blockade, Wunderlich et al. (2000) found similarly to us that a low dose (1 μg/kg in their study) potentiated the development of amphetamine sensitization; however, higher doses (including 10 μg/kg which was effective in our study) did not. In their study, a higher dose of amphetamine (1.5 mg/kg) was used in the pretreatment phase and perhaps more importantly, the drug treatment, as emphasized by the authors themselves, was carried out in the home cages—the amphetamine pretreatment environment was distinct from the subsequent testing environment. Thus, to the extent present data differ from the previous report, this may be due to the pairing of all amphetamine and CCK antagonist treatments with the environment for testing exploratory behavior. Environmental stimuli have the ability to activate postero-accumbal dopamine neurons (Ladurelle et al., 1995) although the increase in dopamine efflux in response to novelty has been shown to be brief and confined to the shell compartment of the nucleus accumbens (Rebec, 1998). According to Badiani et al. (2000), novelty enhances the behavioral response to amphetamine although it does not alter amphetamine-induced dopamine overflow in the nucleus accumbens. Since the blockade of CCK receptors altered the development of behavioral sensitization to 0.5 mg/kg (i.p.) amphetamine in the present study, it could be hypothesized that the simultaneous exposure to the testing environment in the exploration box and repeated amphetamine treatment produce a stimulation that is sufficient to release both dopamine and CCK.

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

Acknowledgements

This study was supported by grants from the Estonian Science Foundation (No. 4531) and the Estonian Ministry of Education (No. 0814). We are grateful to Professor Leslie Iversen and ML Laboratories/Panos Therapeutics, UK for the generous donation of devazepide and L-365,260.

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