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MONOAMINERGIC MECHANISMS IN MOOD-ASSOCIATED BEHAVIOURS AND NEUROCHEMISTRY IN RATS

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

III **Häidkind, R.**, Eller, M., Harro, M., Kask, A., Rinken, A., Oreland, L., Harro, J. (2003) Effects of partial locus coeruleus denervation and chronic mild stress on behaviour and monoamine neurochemistry in the rat. *European Neuropsychopharmacology 13, 19–28*.

IV **Häidkind, R.**, Eller, M., Kask, A., Harro, M. Rinken, A., Oreland, L., Harro, J. (in press). Increased behavioural activity of rats in forced swimming test after partial denervation of serotonergic system by para-chloroamphetamine treatment. *Neurochemistry International*.

ABBREVIATIONS

CRF	corticotropin-releasing factor
CSF	cerebrospinal fluid
D_2	dopamine receptor 2 subtype
DA	dopamine
DOPAC	3,4-dihydroxyphenylacetic acid
DRN	dorsal raphe nucleus
DSP-4	*N*(2-chloroethyl)-*N*-ethyl-2-bromobenzylamine
5-HIAA	5-hydroxyindoleacetic acid
HPA	hypothalamic-pituitary-adrenal axis
5-HT	serotonin
5-HT_{1A}	serotonin receptor 1A subtype
HVA	homovanillic acid
i.p.	intraperitoneal
LC	locus coeruleus
MHPG	3-methoxy-4-hydrophenylglycol
MRN	median raphe nucleus
NA	noradrenaline
NPY	neuropeptide Y
PCA	p-chloroamphetamine
PVN	paraventricular nucleus
SSRI	selective serotonin reuptake inhibitor

1. INTRODUCTION

1.1. Mood disorders with focus on depression

Mood disorders include unipolar and bipolar affective disorders that range from chronic dysthymia to irregular bursts of recurrent brief depression to minor and major depression and to hypomania and full-blown mania (Post, 2003). The diagnosis of major depression has evolved over the past 40 years, with progressively more precise definitions in each edition of the Diagnostic and Statistical Manual of Mental Disorders – Fourth Edition (DSM-IV; American Psychiatric Association, 1994) and International Statistical Classification of Diseases and Related Health Problems (ICD-10, World Health Organization, 1992). A disease such as major depression that manifests symptoms in many different neurobehavioural domains – including mood and emotion, cognition, perception, autonomic function, homeostatic function and stress responsiveness – would be expected to include disruption of several neurochemical systems that regulate these diverse processes at the symptomatic level.

Studies of biological factors in major depression have largely relied on serendipitous discovery of antidepressants and the determination of their mechanism of action. Virtually all of the major neurotransmitter systems (serotonin, noradrenaline, dopamine, gamma aminobutyric acid (GABA), neuropeptides, etc.) have been scrutinized for a role. Monoaminergic theory of depression (Schildkraut, 1965; Bunney and Davis, 1965; Prange, 1964; Coppen, 1967; Lapin and Oxenkrug, 1969) is still the most popular explanation for the neurobiology and pathophysiology of depression (Harro and Oreland, 2001).

In its classic form the monoaminergic theory of depression has suggested that one or another of monoamine neurotransmitters is in deficit in the synaptic clefts in case of the disorder. Later the attention switched to changes in receptor characteristics and function (Vetulani and Sulser, 1975; Baker and Greenshaw, 1989). Several authors have suggested that dysregulation rather than an increase or decrease in the monoaminergic neurotransmission underlies the pathophysiology of depression and hence advocated a more functional approach to the disorder (Carroll and Mendels, 1976; Goodwin and Bunney, 1973; Maas, 1979; Siever and Davis, 1985). Despite 40 years of concerted research, and several more recent developments in understanding the neurochemistry and pathophysiology of mood disorders the primary etiopathology of major depression has not been identified (Barchas and Altemus, 1999; Nestler et al. 2002a; Nemeroff and Owens, 2002; Garlow and Nemeroff, 2003; Holden, 2003). In the monoamine model there are numerous features of depression still unexplained, but even more so in other, less elaborated neurobiological hypotheses implying other mechanisms (Harro and Oreland, 2001).

1.2. Animal models of depression

One of the characteristic properties of antidepressant drugs, which differentiates them from other groups of psychotropic agents, is that they are effective in depressed patients, but devoid of mood-elevating effects in normal (i.e. non-depressed) human subjects. This fact has at least two serious consequences: first, it raises doubts to the clinical relevance of the numerous changes found in normal animals undergoing chronic antidepressant therapy; second, any attempt to predict clinical actions from behavioural effects in normal animals is fraught with uncertainty. Therefore, it is more and more commonly accepted that the best strategy for studying psychobiological mechanisms of depression and its treatment is to employ animal models of the disorder.

There are quite many animal models of psychopathology relevant to mood disorders (for review Katz, 1981; Weiss and Kilts, 1998; Nestler et al. 2002b). Most of them are assay models for drug screening, which have remained very imperfect approximations of the target pathology (Willner, 1990). The important aim has always been to find models, which would allow direct penetrations into disease mechanisms and underlying pathological processes in the brain. The behavioural phenomena used in animal models have been validated mainly with pharmacological means; they react reliably to antidepressant pharmacotherapy (Willner, 1990). Behavioural validation, at the level of constructs, has not been very popular. It appears however appropriate to consider that animal models of depression should favour analysis of a wide array of behaviours, which may simulate the matrix of symptoms characterizing depression. This is the reason why in this dissertation several different behavioural models were used in a hope to validate them against each other and against existing neurochemical knowledge. Attempts to simulate depression in animals have relied on stressful experiences, social isolation or brain damage (Willner 1990). The largest group of animal models of depression involves abnormal behaviours elicited by acute or chronic stress exposure (Willner, 1990; 1993).

1.3. The role of stress in affective disorders

The age-old adage that stress leads to illness is likely to contain an important nugget of truth, although it is unlikely that there is a simple, unidirectional relationship. Stress precipitates major depression and influences its incidence, severity and course (Kendler et al. 1994; 1995). In the biomedical literature stress is commonly conceptualized as a state of threatened homeostasis and a stressor as any event that disrupts the organism's balance or homeostasis (Chrousos and Gold, 1992). The stress response itself consists of alterations in levels of anxiety, a loss of cognitive and affective flexibility, activation of the

hypothalamic-pituitary-adrenal (HPA) axis and autonomic nervous system, and inhibition of vegetative processes that are likely to impede survival during a life-threatening situation (e.g. sleep, sexual activity, and endocrine programs for growth and reproduction) (Gold and Chrousos, 2002).

The stress response and major depression share many features because of similar brain circuitries and mediators and the classic symptoms of depression involve stress system dysregulation (Gold and Chrousos, 2002). The core stress system consists of the CRF system and the locus coeruleus-noradrenaline (LC-NA) systems and the peripheral mediators, adrenaline and cortisol. A key adaptive aspect of the central stress response contains neurochemical responses to challenge and is performed by CRF, noradrenergic and serotonergic systems.

Stressors increase the cellular activity and neurotransmitter turnover in locus coeruleus as well as utilization and synthesis of NA (Berridge and Waterhouse, 2003). Repeated exposure to a stressor attenuates LC neuronal responsivity and NA release to the same (homotypic) stressor, but enhances it in response to novel stressor (Nisenbaum et al. 1991; Finlay et al. 1995). Although chronic/ repeated stressors may not result in an increased release of NA, they do result in an increased capacity of the system to release NA, due to elevated rates of NA synthesis (Irwin et al. 1986). During prolonged exposure to a particular stressor the LC-NA system develops an increased capacity to respond to additional challenges. The development of tolerance to the LC activating effects of repeated presentation of certain stressors may involve alterations in CRF neurotransmission.

Stressors also activate serotonin turnover and thus activate a system that has both anxiogenic and anxiolytic pathways within the forebrain (Deakin and Graeff, 1991). During stress circulating glycocorticoids acutely facilitate the activity of the whole 5-HT system, but chronic exposure to stress tips the balance in favour of 5-HT_2-mediated actions of 5-HT, at least in cerebral cortex, and suppress 5-HT_{1A}-mediated responses in hippocampus (McEwen and Seeman, 2003). Dorsal raphe innervation of amygdala and hippocampus is believed to have anxiogenic effects, via 5-HT_2 receptors; whereas median raphe innervation of hippocampus activates 5-HT_{1A} receptors, stimulation of which facilitates the disconnection of previously learned associations with aversive events, or suppresses formation of new associations, thus providing a resilience to aversive events (Graeff, 1993).

Discrete analyses of specific brain regions in mesocorticolimbic system indicate that stressors such as foot shock and tail pinch increase the synthesis and utilization of central DA (Zacharko and Anisman, 1991). Anteromedial prefrontal cortex reacts quickly with pronounced increase, striatum is not very sensitive (Dunn and File, 1983). An acute stressor in medial prefrontal cortex may proactively influence the response to a subsequently applied stressor (Zacharko and Anisman, 1991). In contrast to the effects of an acute stressor, the mesocorticolimbical DA reductions may be absent following a chronic stressor regimen, in the same way as DOPAC accumulation following chronic

stress exposure is less pronounced than after an acute stressor. DA-ergic reaction to chronic stressors is mainly associated with a decrease in rewarded processes and anhedonia (Zacharko and Anisman, 1991).

Stress schedules used in experimental depression research involve very different regimens, specific stressors or combinations of them (Willner, 1993). Many animal models of depression induce anxiety by imposing heightened stress in test environment. The prenatal, early postnatal and life-enduring stress concepts are used to elicit post-traumatic or chronic disease states in animals. The reported studies repeat once again the ongoing knowledge, that stress per se is not sufficient to cause depression. There are considerable individual differences in coping with challenges, based upon interacting genetic, developmental and experiential factors in an organism's stress reactivity profile.

1.4. Monoamine systems in mood-associated behaviours

1.4.1. Noradrenaline

Noradrenaline (NA) is produced in the same biosynthetic pathway as dopamine and adrenaline. The amino acid tyrosine is converted to L-dopa by tyrosine hydroxylase. L-dopa is converted to DA by aromatic amino acid decarboxylase. Noradrenaline is produced from DA by dopamine β-hydroxylase. The principal metabolite of NA is 3-methoxy-4-hydrophenylglycol (MHPG). The majority of noradrenergic cells in the central nervous system (CNS) are located in the locus coeruleus (LC). Axons from LC neurons project throughout the CNS and are the major source of forebrain NA neurotransmission.

Noradrenaline regulates mood, attention and alertness and is a substrate for many commonly used antidepressants. Neurotransmission in the NA-ergic system is mediated by a number of different neurotransmitter receptors, grouped into classes known as α and β. The α_2- and β-adrenergic receptors have been the focus of considerable research into their putative roles in the biology of depression.

In the NA-based monoaminergic theory of depression (Schildkraut, 1965; Bunney and Davies, 1965) it is commonly assumed that NA levels are reduced and this results in upregulation of ß-adrenoceptors but there are many inconsistencies in experimental evidence (Harro and Oreland 2001). The fact that NA-ergic neurotransmission in the brain is affected by most of antidepressant therapies is unquestionable. The first generation of clinically effective antidepressants had a common action on NA- and 5-HT-ergic neurons. Recent advances in drug design have resulted in very selective NA reuptake inhibitors, such as reboxetine, which are potent antidepressant drugs. Also the classic antidepressant drugs have been believed to exert their therapeutic effect on the molecular level via increases in NA availability. Many studies (Meana and

Garcia-Sevilla, 1987; Meana et al. 1992; Gonzales et al. 1994; Ordway et al. 1994; De Paermentier et al. 1997; Callado et al. 1998) have found increased α_2-adrenoceptors binding in depressed suicide victims in different brain regions (frontal cortex, hippocampus, temporal cortex, hypothalamus and locus coeruleus). Radioligand binding studies of α_2-adrenoceptor following chronic antidepressant treatment have given an impression that there is a NA-dependent downregulation in these receptors (Smith et al. 1981; Nomura et al. 1987; Menargues et al. 1990; Kovachich et al. 1995). It is generally acknowledged that ß-adrenoceptors are upregulated in the brains of suicide victims (Mann et al. 1986; Biegon and Israeli, 1988) Clinically efficient antidepressant drugs decrease the density of brain ß-adrenoceptors in the brains of animals after chronic treatment (Frazer et al. 1974; Wolfe et al. 1978; Sulser, 1984; Grigoriadis et al. 1989; Ordway et al. 1991).

1.4.2. Serotonin

Serotonin is synthesized from the amino acid tryptophan. Tryptophan hydroxylase converts tryptophan to 5-hydroxytryptophan (5-HTP), which in turn is converted to serotonin (5-hydroxytryptamine, 5-HT) by aromatic amino acid decarboxylase. All the serotonin in the CNS is synthesized in neurons of the raphé nuclei. Serotonin is metabolised into 5-hydroxyindoleacetic acid (5-HIAA) which can be used to estimate synaptic activity of serotonergic neurons.

Serotonin is critically involved in many brain functions and is a target for many commonly used antidepressants. In addition, most drugs that are currently used for the treatment of other psychiatric disorders (e.g., mania, schizophrenia, autism, obsessive-compulsive disorder, anxiety disorders) are thought to act, at least partially, through serotonergic mechanisms (Lucki, 1998; Aghajanian and Sanders-Bush, 2002). Serotonin has actually been implicated in almost every conceivable physiologic or behavioral function – affect, aggression, appetite, cognition, emesis, endocrine function, gastrointestinal function, motor function, neurotrophism, perception, sensory function, sex, sleep, and vascular function (Bloom and Kupfer, 1995) and its polyfunctionality seems to depend on the anatomy of serotonergic system.

In the 5-HT-centered monoaminergic theory of depression (Coppen, 1968; Lapin and Oxenkrug, 1969) the deficiency in serotonergic neuronal function is assumed to be the underlying cause of depression. Research involving serotonergic parameters has, however, given inconsistent evidence for serotonergic hypofunction in depression. The most reproducible finding indicates to violent and impulsive, in some cases suicidal behaviour, independent of psychiatric diagnosis (Arango et al. 1995; Åsberg et al. 1987).

There are at least 14 distinct serotonin receptors from seven different families (Ordway et al. 2002). Prior to the discovery of other serotonin receptors the

5-HT_{1A} and 5-HT_2 receptors were the subject of the majority of serotonin receptor research in depression. Increases in the 5-HT_{1A} receptor density in the prefrontal cortex or dorsal raphe of suicide victims have been reported (Matsubara et al. 1991; Arango et al. 1995; Stockmeier et al. 1998), but imaging studies in depressives have revealed contrasting findings (Drevets et al. 1999). In addition to the efficacy of SSRI-s, positive effects of 5-HT_{1A} receptor agonists in depression have been found in clinical studies (Stahl et al. 1992) and administration of various 5-HT_{1A} receptor agonists has an antidepressant effect in animal models. Despite the effectiveness of SSRI-s the induced monoamine deficiency studies by Delgado et al. (1990) demonstrate that deficiency in serotoninergic processes does not induce relapse in all depressive patients.

1.4.3. Dopamine

Dopamine (DA) is synthesized from the amino acid tyrosine, which is converted to L-DOPA by tyrosine hydroxylase. L-DOPA is converted to DA by aromatic amino acid decarboxylase. DA neurotransmission is estimated by its major metabolites homovanillic acid (HVA) and 3,4-dihydroxyphenylacetic acid (DOPAC).

Dopamine plays a critical role in movement and reward and is a common target for most drugs of abuse. Dopamine release in forebrain regions such as the nucleus accumbens is enough to reinforce new behaviours, but dopamine has been shown also to elicit reward-seeking, making it important in the realm of motivation, reward-associated learning and energy behind behaviours (Phillips et al. 2003).

The DA hypothesis of depression (Randrup and Braestrup, 1977) emerged third among the monoamine hypotheses. However, consistent abnormality in DA-ergic neurotransmission has not identified in patients with depressive disorders (Kapur and Mann, 1992). DA turnover appears to be decreased in depression but low cerebrospinal fluid HVA may be secondary to psychomotor retardation in depression (Post et al. 1973). Several antidepressive agents with different primary actions have the ability to functionally enhance DA-ergic neurotransmission in the mesostriatal, mesolimbic and mesocortical systems, either by increasing presynaptic output or upregulating postsynaptic receptors (Ainsworth et al. 1998; Klimek and Nielsen, 1987).

1.5. Models of weaknesses in noradrenergic and serotonergic neurotransmission

Neurochemical depletion studies are a useful way to address the role of particular transmitters in animal models of pathophysiology of depression and in the action of antidepressants. The effects of selective neurochemical damages in animals can be studied at different time intervals after impairment and that makes possible to follow the course of putative pathological process. Fortunately there are neurochemical toxins specific enough that can be used to model components of inherent weaknesses in monoamine based neurochemical processes.

DSP-4 [*N*(2-chloroethyl)-N-ethyl-2-bromobenzylamine] has been described as a selective noradrenergic neurotoxin highly specific to the nerve terminals ascending from locus coeruleus (LC) (Ross, 1976; Jonsson et al. 1981; Fritschy and Grzanna, 1989). Peripheral administration of DSP-4 depletes NA in the brain regions, which are innervated by the LC, and has either zero or little direct effect on serotonin and dopamine levels. Dudley et al. (1990) have reviewed some opinions about the role of noradrenaline system in depression and biochemical effects of NA depletions. They propose that a realistic model of depression might be a chronic, partial depletion of noradrenaline without loss of terminal integrity that can be provided by a low dose of xylamine, which is similar to DSP-4 but less specific to noradrenergic neurons. Archer et al. (1984a) suggested that the DSP-4-treated rat, if not a "depressed" rat, is more easily made depressed by unpleasant and uncontrollable environmental events. The term 'depression' was used to encompass a state in which the animal fails to engage in behaviour either to alter or to avoid an unpleasant or a potentially unpleasant environment.

As a consequence of central DSP-4 denervation α- and β-adrenoceptors are upregulated forming basis for an animal model of adrenergic supersensitivity (Jonsson et al. 1981; Mogilnicka, 1986; Dooley et al. 1983b; Wolfman et al. 1994). Stone and Platt (1982) have studied the relationship between brain beta-adrenergic receptors and adaptation to stress in rats subjected to repeated restraint. They propose that chronic stressor exposure may provoke postsynaptic β-adrenergic receptor downregulation in normal animals similarly to the effect of antidepressants on these receptors. Organismic or experiential variables that interfere with and delay appearance of NA receptor subsensitivity may favour development of the illness and pharmacological intervention may be necessary to provoke downregulation in the NA system (Stone, 1979).

Para-chloroamphetamine (PCA) is a selective serotonergic neurotoxin, which potently reduces 5-HT and 5-hydroxyindoleacetic acid (5-HIAA) levels in the brain (Pletcher et al. 1964; Fuller et al. 1965) without having significant effects on catecholamines (Massari et al. 1978; Leonard, 1976). The marked reduction in the brain levels of 5-HT after PCA persists for months after a

single dose (Sanders-Bush et al. 1975) and with histological techniques it has been established that PCA has a selective toxic effect on 5-HT-ergic neurons (Harvey et al. 1975). Further selectivity in the action of PCA was proposed after findings that the fine axon terminals that arise from the dorsal raphe nucleus (DRN) are vulnerable to PCA but that beaded axons (with large, spherical varicosities) from the median raphe nucleus (MRN) are resistant to the neurotoxin (Mamounas and Molliver, 1988; Mamounas et al. 1991).

2. AIMS OF THE STUDY

The dissertation addresses the effects of following manipulations on the animal behaviour and monoamine neurochemistry:

– extensive and partial noradrenergic denervation of projections originating from the locus coeruleus (LC) by DSP-4 [*N*(2-chloroethyl)-N-ethyl-2-bromobenzylamine]
– chronic mild stress
– chronic mild stress after extensive or partial noradrenergic denervation
– extensive and partial serotonergic denervation by PCA (p-chloroamphetamine).

3. MATERIALS AND METHODS

Animals

Male Wistar rats weighing 280–405 g were used in the experiments. After arrival from Grindex Breeding Centre (Riga, Latvia) or the Finnish National Laboratory Animal Centre (Kuopio, Finland) the animals were left to habituate to the housing conditions at the animal house for at least 7 days. The rats were housed in groups of five (**Article I**) or singly (**Articles I, II, III and IV**) after the start of experiments. The animal room had a light schedule 12:12 (lights on at 7 or 8 am). Food and water were available ad libitum, except for 17 h (**Article I**) or 18 h (**Article III**) before each sucrose intake test.

Neurotoxin treatment

DSP-4 [*N*(2-chloroethyl)-N-ethyl-2-bromobenzylamine] (Astra, Sweden) in the doses of 10 (**Articles II and III**), or 50 mg/kg (**Articles I and II**) was injected in the volume of 2 ml/kg intraperitoneally. To avoid the effects of rapid hydrolysis of the neurotoxin each dose was weighed separately, dissolved in distilled water, and injected immediately. PCA (p-chloroamphetamine, Sigma) (**Article IV**) in the doses of 1, 2, 4, 6 or 10 mg/kg was injected intraperitoneally in the volume of 2 ml/kg. All doses are expressed as for hydrochloride. Control animals received a vehicle injection.

Chronic mild stress procedure (Articles I and III)

Rats belonging to the Stress group were submitted to the CMS procedure in a separate room during 15 (**Article I**) or 29 (**Article III**) days. The CMS procedure was based on the procedure described by Willner et al. (1992). All stressors have been classified as ‘mild’ under the terms of relevant UK legislation. Attempts have been made to elucidate the key elements of this chronic mild stress regime and it has been concluded that no single element appears to be necessary or sufficient; and that a varied and frequent presentation of stressors is most effective (Muscat and Willner, 1992). Thus, some stressors were chosen based on the original procedure and some were used for the purpose of increased variability. In the study reported in **Article I** various stressors were changed in a random order after each 12 h. Some stressors – wet bedding, change of cagemates, and isolation in a small cage were repeated four, some stressors – water deprivation, water and food deprivation, cage tilt 45° and replacement of sawdust with sand – three, and some stressors – stroboscopic

light, strong illumination during the predicted dark phase and noise alternated with silence – were repeated two times during the whole CMS period. As in the original procedure stressors were applied for periods varying between 30 min and 20 h at any time of day intermittently, we made some changes considering this difference between **Article I** and Muscat and Willner (1992). In **Article III** the duration of individual stressors was varied and stressors were changed sequentially on the basis of a full week schedule. The stressors included (once per week unless indicated otherwise) food and water deprivation (18 h), cage tilt 45° (1 × 5 and 1 × 15 h), wet bedding (1 × 15 and 1 x 8 h), an intruder rat (1 × 8 and 1 × 15 h), water deprivation (8 h), stroboscopic light (10 Hz, 1 × 15 and 1 × 8 h), movement restriction in a small cage (11 × 16 × 7cm, 8 h), replacement of sawdust with sand (8 h), strong illumination (850–1000 lx) during the predictive dark phase (15 h) and 60 dBA noise alternated with silence after every 10 min (15 h). Control rats remained undisturbed in their cages.

Open field test (Articles I and III)

The rats were placed at the center of a rectangular arena 100 × 100 cm with 40 cm high sidewalls. The arena was divided into 16 equal squares. The parameters registered during 4 min were the number of squares visited (line crossed with all four feet), the number of rearings and the number of fecal boli left on the arena.

Forced swimming test (Articles I, III and IV)

The technique first characterized by Porsolt et al. (1978) was used after validation in our laboratory. Rats were forced to swim in a vertical glass box (floor 19x29 cm; height 39 cm) containing 25 cm of water (**Article I**) or in a vertical glass cylinder (diameter 22.5 cm and height 60 cm) containing 35 cm of water (**Articles III and IV**) maintained at 25°C. On the first day of experiments, the rats were forced to swim 15 min and were thereafter dried with laboratory tissues. On the following day, the rats were re-exposed to the forced swimming for 5 min. The total duration of immobility was measured on both days, judging the rat to be immobile whenever it remained floating passively in the water in a slightly hunched but upright position, its head just above the surface. In **Article IV** the forced swimming behaviour was recorded on videotape and active components of behaviour (Armario et al. 1988) were measured separately. The rat was judged to struggle whenever it made intense movements of all the four limbs with the two front paws breaking the surface of the water or touching the walls of the tank. The rat was judged to swim whenever it was making active swimming motions, more than necessary to merely maintain its head above the water, e.g., moving around in the cylinder.

Sucrose solution intake (Articles I and III)

Rats were, before the CMS procedure, trained to consume 1% sucrose solution, and sucrose consumption was monitored at weekly intervals. In experiments reported in **Article I** the training consisted of initial 48 h sucrose solution exposure without any other food or water available, and five additional consecutive 1 h per day periods of sucrose availability. Sucrose consumption tests took place once a week at regular times. In **Article I** the animals were housed in groups of five. In the context of individual sucrose consumption measurement temporary test cages were used. Rats were placed into test cages in the morning, while tests took place at late afternoon. Sucrose consumption was measured for a period of 1 h after food and water deprivation for 17 h by weighing preweighed bottles at the end of the test. In the experiments reported in **Article III** the animals were individually housed all the time and sucrose consumption was measured in home cages. At training phase food was available ad libitum. Before each sucrose intake test the animals were food and water deprived for 18 h. During the CMS period, sucrose intake/preference tests were carried out once every week using the two-bottle technique, i.e., water was freely available during the test.

In vivo microdialysis (Article II)

One week after the toxin treatment the animals were anaesthetised with chloral hydrate (350 mg/kg IP) and mounted in a Kopf stereotactic frame. Guide cannulas for the microdialysis probes 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 were used to fix the cannula to the scull. After the surgery the animals were given 1 week 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 (at about 08:00 h) the animals were transported to a separate experiment room. The animals remained in their individual home cages throughout the experiment, though the metal cage covers were replaced with plastic ones having an open ceiling. 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) via a two-channel swivel. Probes were inserted in the morning of the day of the 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_2HPO_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 20 min) into the vials

prefilled with 10 µl of 0.3 M perchloric acid. After the tenth sample the system was switched to the perfusion solution containing 50 mM KCl and left so for 40 min. Upon 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) after the whole frontal cortex had been taken for separate analysis; 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.

Measurement of monoamines and their metabolites in brain tissue and microdialysates

In **Article I** noradrenaline levels were measured in cerebellum and cerebral cortex in the laboratory of Pharmacia & Upjohn, Sweden. The sample preparation was performed as described by Felice et al. (1978) and the analytical procedure as by Sharp and Zetterström (1992). In brief, approximately 50 mg of brain tissue was homogenized in 1.25 ml solution containing 1 ml 0.4 M $HClO_4$, 100 µl 10% EDTA and 50 µl 5% $Na_2S_2O_5$. Solution samples (100 µl) contained 100 ng of 3,4-dihydroxybenzylamine as internal standard. After centrifugation and decanting, catecholamines were extracted by alumina adsorption. The analytes were desorbed by addition of 0.125 M boric-citric acid solution and, after centrifugation, separated by reversed-phase HPLC and measured by electrochemical detection. All values were corrected for the recovery of the internal standard.

In **Articles II, III and IV** the content of monoamines and their metabolites in brain tissue was determined as first described in Harro et al. (2001). The rat brain tissues (frontal cortex in **Article II**, frontal cortex and hypothalamus in **Article III** and frontal cortex, hippocampus, striatum, cerebellum, hypothalamus, septum and cerebral cortex in **Article IV**) were homogenized with a Bandelin Sonoplus ultrasonic homogenizer (Bandelin Electronic, Germany) in ice-cold solution (5–30 µl/mg tissue) of 0.09 M perchloric acid containing 5 mM $Na_2S_2O_5$ and 0.04 mM EDTA to avoid oxidation. The homogenate was then centrifuged at 17000x g for 20 min at 4°C. In **Article III** the aliquots (10–20 µl) of the supernatant obtained were chromatographed on a LiChrospher 100 RP-18 column (250 × 3 mm; 5 µm) protected by a Supersphere RP18 (10 × 2 mm; 4 µm) guard column. In **Article IV** a Hypersil ODS column (125 × 2 mm; 5 µm) was used. The separation was done in an isocratic elution mode at a column temperature of 30°C using a mobile phase containing 0.05 M citric acid buffer at pH 3.9, 0.9 mM sodium octylsulfonate, 0.3 mM triethylamine, 0.02 mM EDTA, 1 mM KCl and 8% acetonitrile or 5 % methanol (**Article IV**). For the assay of MHPG the supernatants were heated at 95°C for 20 min before

chromatography and the hydrolysate was in both studies chromatographed on a LiChrospher 100 RP-18 column (250x3mm; 5 μm) with the described mobile phase containing 8 or 10% acetonitrile. The chromatography system consisted of Hewlett-Packard HP 1100 series isocratic pump, thermostatted autosampler, a thermostatted column compartment and HP 1049 electrochemical detector (Hewlett-Packard, Germany) with glassy carbon electrode. The measurements were done at electrode potential +0.6 V versus and Ag/AgCl reference electrode. Results were expressed as ng/g tissue in **Article I**, pmol/mg tissue in **Article II, III and IV**.

In **Article II** the quantity of DA in the microdialysates was determined by high-performance liquid chromatography with electrochemical detection. The chromatography system consisted of Hewlett Packard series 1100 pump and autosampler, a LiChrospher 100 RP-18 column (250 × 3 mm, 5 μm), an ESA 5021 conditioning cell (+300 mV), an ESA 5011 analytical cell (first electrode –300 mV, second electrode +400 mV) and an ESA Coulochem II controller unit. The mobile phase composition was: 0.05 M sodium citrate buffer, pH 5.5, 0.02 mM EDTA, 2 mM sodium octylsulphonate, 0.3 mM triethylamine, 12% methanol. The results were expressed as fmol per 25 μl of sample.

Binding studies (Articles I, III and IV)

β-adrenoceptors and [^{3}H]-dihydroalprenolol binding (Articles I, III and IV)

Brain tissue (in **Article I** frontal and cerebral cortex and hippocampus, in **Article III and IV** cerebral cortex only) was homogenized in ice-cold Tris-HCl (50 mM, pH 7.4) using a Potter-S glass-teflon homogenizer (1000 rpm, 10 passes). The membranes were washed three times in the same buffer by centrifugation (20 000 g at 4˚C) and resuspension. In the studies reported in **Article I**, the final pellet was rehomogenized in Tris-HCl buffer (50 mM; pH 8.0). In later **Articles (III, IV)** the final homogenization buffer was changed to sodium phosphate (25 mM, pH 7.4) because Tris buffer yields a lower number of binding sites and a lower binding affinity of adrenoceptors (Deaupree et al. 1996 and our observations). β-adrenoceptor labeling was carried out in the presence of 0.05–2.8 nM tritiated ligand dihydroalprenolol ([^{3}H]-DHA, spec. act. 88 or 94 Ci/mmol, Amersham Radiochemicals) at room temperature with 2 mg w/w tissue per tube in a total incubation volume of 0.3 ml (**Article I**). In later **Articles (III and IV)** the concentration range was 0.05–3.2 nM and specific activity of the ligand 95 or 94 Ci/mmol. Propranolol (5 μM) was added to determine nonspecific binding. In some experiments the incubation medium contained 10 μM 5-HT and 10 μM pargyline (a MAO inhibitor) in order to prevent binding of the tracer to 5-HT receptors. In the experiments reported in **Articles I and III** the incubation was terminated after 40 min by rapid filtration over Whatman GF/B filters using Brandel Cell Harvester (M-24S). In **Article IV** the incubation lasted 60 min. The filters were washed with 9 ml cold

incubation buffer, dried and assayed for radioactivity by liquid scintillation spectrometry (Beckman LS scintillation counter).

α_2-adrenoceptors and [^{3}H]-RX 821002 binding (Articles III and IV)

α_2-adrenoceptors measurement was carried out as described by Uhlén and Wikberg (1991) with minor modifications by using [^{3}H]-RX 821002 (spec. act. 49 Ci/mmol, Amersham Radiochemicals) in the concentration range 0.075–7 nM. Incubation was carried out in sodium phosphate buffer (25 mM, pH 7.4) for 45 min at room temperature with 2 mg wet weight (w/w) tissue per test tube in a total incubation volume of 0.3 ml. Atipamezole (1 μM) was added to determine nonspecific binding. The filters were washed with 9 ml cold incubation buffer.

5-HT$_{1A}$ receptors and [^{3}H]-8-OH-DPAT binding (Articles III and IV)

Hippocampal membranes (25 mg tissue/ml) were incubated in a buffer containing 20 mM Tris-HCl, 5 mM $MgCl_2$, 30 mM NaCl, 1 mM EDTA (pH=7.4) and 0.4–10 nM [^{3}H]-8-hydroxy-2-(di-*n*-propylamino)tetraline ([^{3}H]-8-OH-DPAT, 216 Ci/mmol, Amersham Radiochemicals) for 60 min at 25˚C in a total volume of 130 μl. The reaction was terminated by rapid filtration through glass-fiber filters (GF/B, Whatman Int. Ltd, UK) with three washings of 3 ml of ice-cold washing buffer, containing 20 mM K-phosphate and 100 mM NaCl (pH 7.4). 5-HT (10 μM) was used to determine nonspecific binding.

D_2 receptors and [^{3}H]-raclopride binding (Article III)

Brain tissue samples (striatum) of each rat were homogenized in ice-cold Tris-HCl (50 mM, pH 7.4) containing NaCl (100 mM, $MgCl_2$ (5mM) and EDTA (1mM) by sonification using Bandelin Sonoplus GM 2070 sonicator (Bandelin Electronic, Germany), two passes for 10s at 50 W. After a centrifugation at 25 000xg for 20 min and rehomogenization, the membranes were incubated at 30˚C for 15 min. The membranes were precipitated by centrifugation and washed through homogenization in fresh buffer and centrifugation. The final pellet was rehomogenized in the same buffer at concentration 17 mg tissue (ww)/ml. The suspension of membranes (100 μl) was incubated with different concentrations of [^{3}H]-raclopride (0.3–7 nM, 76 Ci/mmol, NEN Life Sciences) for 60 min at 25˚C and the incubation was terminated by rapid filtration through GF/B filters using a Brandel cell harvester with three washings of 5 ml of ice-cold washing buffer (20 mM K-phosphate, 100 mM NaCl, pH 7.4). The specific binding of [^{3}H]-raclopride was defined as the difference between total and non-

specific binding, which were measured in the absence and presence of 10 μM (+)–butaclamol, respectively.

DATA ANALYSIS

All binding data were analyzed by nonlinear least-squares regression analysis using a commercial program GraphPad PRISM™ 2.0 (GraphPad Software, San Diego, USA). Statistical analysis was carried out with the StatView 4.5 package (Abacus Concepts, Berkeley, USA) for Macintosh. For statistical evaluation of the behavioural and biochemical data, two-way analysis of variance (ANOVA) was used with Pretreatment and Treatment (or Housing and Treatment) as independent factors. For such measures as body weight, sucrose intake and immobility time in the forced swimming test, as well as extracellular DA in microdialysates from nucleus accumbens, a third, repeated measures factor was added. Group differences after significant ANOVAs were measured by post hoc Fisher's PLSD test. In the microdialysis study a post-hoc Fisher's analysis was carried out per time point after significant repeated measures analysis of variance. Analysis of covariance was used to reveal the contribution of body weight and weight gain to immobility time. Pearson correlations were used for the determination of associations as well as in presenting data from linear regression.

4. RESULTS AND DISCUSSION

4.1. The effect of noradrenergic denervation on brain noradrenaline levels (Articles I, II and III)

As expected, there was a significant reducing effect of DSP-4 treatment on the noradrenaline levels in comparison to vehicle groups. DSP-4 in dose of 50 mg/kg depleted NA in cerebral cortex and in cerebellum by 70–80% three weeks after administration (**Article I, Figure 4**). In comparison to this large denervation, DSP-4 in dose of 10 mg/kg elicited a partial denervation of locus coeruleus (**Article III, Table 4**): 6 weeks after administration of the toxin noradrenaline levels were reduced in frontal cortex (by 34%), but not in the hypothalamus, which has limited input from the LC. In the microdialysis study (**Article II, Table 1**) two weeks after DSP-4 administration the ex vivo NA levels in frontal cortex were reduced by 14% with the dose 10 mg/kg and 70% with 50 mg/kg.

DSP-4 is used by most investigators in a standard dose of 50 mg/kg to reach nearly maximal depletion of tissue NA levels. In using this dose, Jaim-Etcheverry and Zieher (1980) reported marked (by 82%) and prolonged (unchanged between day 7 until day 32) reduction in noradrenaline levels in cerebral cortex after injecting it i.p. to adult rats. It is believed that DSP-4 impairs brain noradrenergic neurons permanently by triggering the degeneration of noradrenergic terminals (Ross, 1976; Ross and Renyi, 1976), although gradual recovery of NA levels occurs after degeneration elicited by DSP-4 treatment (Jonsson et al. 1981; Wolfman et al. 1994). For example, Jonsson et al. (1981), reported recovery of NA levels in cerebral cortex of mice in which NA levels were reduced initially by 80–90%, but 6 months after an i.p. injection of DSP-4 in the dose of 50 mg/kg the reduction in comparison to controls was only 43%. Wolfman et al. (1994) have described gradual, but slow recovery in noradrenaline levels at 90, 240 and 300 days after DSP-4 injection and found almost complete recovery in brain regional noradrenaline stores one year after neurotoxin treatment. Compensatory responses to the damage in LC-NA transmission have been observed at the level of release, post-synaptic receptor number, second-messenger systems and fiber number (Fritschy and Grzanna, 1992; Hallman and Jonsson, 1984; Harik et al. 1981; Logue et al. 1985).

The standard dose (50 mg/kg) has yielded similar results in all studies carried out in our laboratory. In frontal cortex the NA levels have been reduced e.g., by 71% nine days after treatment (Harro et al. 1999), by 70% two weeks after treatment (**Article II**) and by 75% one week after DSP-4 treatment (Kask et al. 2000). The results with partial noradrenergic denervation (10 mg/kg) have been more variable and may be dependent on time past from toxin administration as well as on environmental demands the animals have been exposed to.

In the study of Eller and Harro (2002) only a 10% reduction in the ex vivo NA levels in the frontal cortices of rats after DSP-4 (10 mg/kg) was found one week later, in contrast to paper by Harro et al. (1999) where the NA levels were decreased more remarkably – by 56% nine days after administration. It should be mentioned that in the last study the forced swimming experiment was carried out before sacrifice, and this may have contributed to NA depletion found in ex vivo noradrenaline measurements. In the experiments with partial denervation reported in the dissertation (**Articles II and III**) the NA levels in frontal cortex were reduced by 14% two weeks and by 34% 6 weeks after neurotoxin treatment. Harro et al. (2003) found a complete recovery of NA levels in frontal cortex one month after DSP-4 (10 mg/kg), although 3 days after treatment NA levels were reduced by 22%. In the case of low dose the extent of NA depletion seems to be larger in the studies where increased environmental demands are imposed on the animals in the context of behavioural studies. Namely, in studies of Eller and Harro (2002), Harro et al. (2003) and in **Article II** the animals were left undisturbed after neurotoxin treatment, whereas in other studies (Harro et al. 1999 and **Article III**) animals were subjected to several behavioural tests.

Six weeks after partial denervation of ascending noradrenergic nerve endings the NA turnover (expressed as a MHPG/NA ratio) was increased in frontal cortex by 70% (**Article III, Table 4**). As MHPG levels were not reduced in the DSP-4 treated animals, but there was rather a tendency of an increase, and frontal NA levels were reduced by about one-third, NA turnover was increased. Increases in the forebrain NA metabolism after extensive lesions of the LC system, despite of the reduced number of nerve terminals, have been described previously (Fluharty et al., 1984; Gage et al., 1983) and could be specific to the cortex and hippocampus (Logue et al., 1985). Noradrenergic neurons with rapid discharge rates take up DSP-4 more efficiently than those that are more quiescent. This increase in NA turnover that is compatible with microdialysis studies (Kask et al. 1997; Hughes and Stanford, 1998) is probably related to increased synthesis of NA in the remaining nerve terminals, together with the preferential release of newly synthesized NA (Carlson, 1975). The increased NA turnover can then be observed several weeks after denervation even when it is only partial.

4.2. The effect of extensive noradrenergic denervation on behaviour (Article I)

Open field test

Behaviour in the open field test was affected by the extensive noradrenergic denervation. The number of squares crossed, rearings and total activity were decreased in denervated animals three weeks after treatment (**Article I, Figure 3**). No change in defecations was found, but the defecation rate in general was rather low in this study. The observation is in accordance with behavioural effects of increased neophobia and fearfulness or similar reductions in locomotor and exploratory activities after extensive noradrenergic denervation found to develop in previous studies (Delini-Stula et al. 1984; Berridge and Dunn, 1990; Harro et al. 1995, 2000, 2001; Archer and Fredriksson, 2001).

Behavioural effects of extensive noradrenergic denervation by DSP-4 related to learning deficits in avoidance tasks and to impaired attentive abilities of rats in a taste-aversion procedure were first studied by Ögren et al. (1980), Archer et al. (1982a) and Archer et al. (1982b). Increased irritability (Mogilnicka et al. 1983) and impaired acquisition of aversively motivated behaviours (Spyraki et al. 1982) have also been found in DSP-4 treated animals. Dooley et al. (1983a) studied exploratory behaviour (10 days of DSP-4 63 mg/kg) and found no difference in head dips in a hole-board of a rectangular box compared to controls.

Open field has been a traditional model in experimental psychopharmacology for studying emotionality aroused by exposure to the new environment (Hall, 1934). Current opinion about open field test holds that it is useful test for complex individual behavioural strategies in which general arousal and exploratory activities compete with fear-related behavioural inhibition (Roth and Katz, 1979). Open field test cannot be classified into an animal model of depression, but has been often used in studies of antidepressive drugs as well as in studies of the effects of acute or chronic stress in association to anxiety-like and locomotorily activated behaviours (Prut and Belzung, 2003). Although the results of open field tests are often reported as changes in exploration, the test exposes the reaction of subjects to a stressful event which can be modulated by anxiolytic drugs.

LC-NA system has been found to be particularly sensitive to novelty and exert a strong modulatory influence on exploration of and interaction with novel aspects of the environment (Vankov et al. 1995, Berridge and Waterhouse, 2003). Harro et al. (1995) showed that DSP-4-treated rats do not emerge from the starting chamber into a large open field unless they are exposed to the same situation repeatedly; and even then, their activity in the open area remains lower than in control animals. The paradigms for studying exploration in rodents may be remarkably different (Harro, 1993, for a review) which makes direct

comparison of results rather difficult. Dooley et al. (1983a) concluded in referring to supersensitive noradrenergic receptors that exploratory behaviour may be unaffected due to sufficient noradrenergic function. We have found increased binding affinity of ß-adrenoceptors in frontal cortex. The open field activity correlated significantly positively with K_D value of [^{3}H]-dihydroal-prenolol binding in individual animals indicating that the noradrenergic deficit may affect exploration through the supersensitivity mechanism.

Sucrose solution intake, body weight gain and adrenal weight

No effect of the extensive noradrenergic denervation by DSP-4 in dose of 50 mg/kg was found in sucrose intake tests seven and fourteen days later (**Article I, Table 2**). There is a possibility that depletion of noradrenaline from the pathways originating from LC elicits abnormalities in ingestive behaviour at the level of the paraventricular hypothalamic nucleus in the rats since it has been found that the application of NA and NPY directly to the PVN stimulates carbohydrate intake (Tempel and Leibowitz, 1993). The NA-containing cells, which project to the PVN, are located in the A2 and A6 (locus coeruleus) nuclei of the brain stem. Ammar et al. (2001) used two consecutive i.p. injection of 50 mg/kg DSP-4 and found lower intraoral sucrose intake throughout the 7-day test period, the effect was still apparent on day 14 post-injection. Food consumption was low only on days 1–5 and recovered later. Protein intake in addition to carbohydrates was attenuated in the study of Ammar et al. (2001). In this line of reasoning DSP-4 in a singe dose of 50 mg/kg could have had an effect on voluntary sucrose intake as well, but as our results show it did not. Archer et al. (1982b) described saccharin (0.2%) intake and preference in taste-aversion learning three weeks after DSP-4 treatment (50 mg/kg) and found less saccharin consumption in DSP-4 treated rats during extinction trials, which was conceptualized as impairment in rat's ability to attend to all stimuli associated with the presentation and absence of the reinforcement. For example, Morley et al. (1988) found in their study, that chemical denervation of the LC projections by DSP-4 treatment reduced the effectiveness of sucrose (20%) reinforcement. The decreased saccharin intake in the study of Archer et al. (1982b), however, does not seem to be anhedonia, since the reduced intake and preference of sweet solution is not evident throughout the other phases of experiment, where the intake measures of DSP-4 treated rats obviously react to the conditioning context. Archer et al. (1983) found as well a transient decrease in food and water intake after DSP-4 injection in a single dose of 50 mg/kg, but these behaviours recovered completely within 7 days. Thus, it has to be concluded, that extensive noradrenergic denervation alone does not induce sustained anhedonic state in animals, at least when the low concentrations of sweet rewards are used.

Tombaugh et al. (1983) have not found the resistance to extinction effect after dorsal bundle lesion with 6-OHDA, but demonstrated gustatory neophobia associated with 0.1% saccharine consumption in these animals. The animals with 6-OHDA lesions consumed significantly less saccharin when it was offered as a new taste. Our animals were extensively accustomed with the sweet solution before any measurements were carried out. So NA-ergic denervations play a role in consumption of sweet solutions rather via neophobia than anhedonia.

The body weight gain and adrenal weights (**Article I, Table 1**) were not affected by the extensive denervation of LC three weeks after neurotoxin treatment. It has been shown previously that DSP-4 treatment at this dose prevents the weight gain within the week after treatment (Harro et al. 1992). As a rule, in a few days after administrating the toxin the body weights of animals decrease (Archer et al. 1983; Dooley et al. 1984; Morley et al. 1988; Al-Zahrani et al. 1997), reflecting probably reduced food and water intake, but this effect has been transient in all our longer studies at all doses of DSP-4. The DSP-4 effect on the weight gain of animals obviously depends on time, because most animals recover after a week and start gaining weight normally.

Dooley et al. (1984) have found an increase in the adrenal weight 10 days after DSP-4 treatment (63 mg/kg), without any marked effect on the normal and stress-evoked functioning of the HPA-axis. This finding of normal HPA function in the presence of marked NA depletion is in general agreement with the results of other researchers (Martin-Iverson et al. 1982), but as no other reports about the DSP-4 effect on adrenals are available and our results 3 and 6 weeks after either extensive or partial LC denervation indicate no effect on adrenal weight, the Dooley's result remains unrepeated.

Forced swimming test

Extensive noradrenergic denervation did not induce any behavioural alterations in the forced swimming test (**Article I, Figure 1)** in group-housed animals either on the first or the second exposure to the situation. Similarly, Esposito et al. (1987) found no difference between control rats and DSP-4 treated animals in immobility times three weeks after denervation.

Since its introduction by Porsolt et al. (1978) the forced swimming test has been widely used to predict the clinical efficacy of antidepressant drugs (Borsini and Meli, 1988). Acute or short-term treatment with most antidepressants increases the latency to immobility and decreases the amount of immobility time, and in most cases this occurs at drug doses that are not activating on their own (Nestler et al. 2002b). A critical question in association to this model remains to be whether any antidepressant, regardless of its mechanism, would be active in the forced swim test or whether the test can only detect drugs with known (e.g., monoamine-based) actions.

In our laboratory Harro et al. (1999) have found earlier a dose-dependent effect of DSP-4 on the forced swimming activity of animals. One week after the DSP-4 treatment, the extensive denervation elicited by high doses (30 and 50 mg/kg) of the neurotoxin reduced the immobility of the animals exposed to forced swimming. Animals were similarly group-housed in these studies and the effect of the neurotoxin dose on the NA levels in the frontal cortex were comparable, so only the time past from the lesioning event may explain the discrepancies in findings. After denervation gradual adaptations will occur in denervated NA-ergic nerve cells as well as in other transmitter systems and receptors, which will probably induce a different reaction profile to the acute severe stress.

Kostowski et al. (1984) have found that the electrical stimulation of the LC increases the activity of rats in the forced swim paradigm. On the other hand, LC destruction by electrolytic lesions, 6-OHDA as well as DSP-4 (65 mg/kg) significantly reduced the action of single dose of desipramine in forced swim situation in rats (Danysz et al. 1986) indicating that there is a role of noradrenaline neurotransmission in the context of forced swimming test. Hughes and Stanford (1998) have demonstrated in freely-moving rats that after a lesion of LC projection by DSP-4 treatment (75% reduction of tissue NA levels) basal extracellular NA levels were twofold greater. A depolarizing pulse of K+ induced a proportionally larger NA release response after DSP-4 treatment in both anesthetised (Kask et al. 1997) and awake (Hughes and Stanford, 1998) animals. On the basis of these findings Harro and Oreland (2001) proposed accordingly that in the LC-denervated animals, at mild stimulation (awake animals in a familiar environment) the tonic release of NA can be somewhat lower and a postsynaptic supersensitivity develops over time; at moderate stimulation (novel environment), the increase in NA release, which acts at the supersensitive adrenoceptors, brings about anxiety, whereas at strong stimulation (forced swimming), the stores of NA will be finally depleted but due to the postsynaptic supersensitivity the necessary NA-ergic neurotransmission is maintained. Harro et al. (1999) found increased cortical ß-adrenoceptor binding after DSP-4 treatment in doses of 30 and 50 mg/kg. Here we found no change in the number of binding sites in frontal cortex, but rather increased binding affinity in these receptors.

4.3. The effect of partial noradrenergic denervation on behaviour (Article III)

Open field test

Open field behavior, which was observed four weeks after neurotoxin treatment (10 mg/kg), was not affected by partial noradrenergic denervation. The number of fecal boli was increased, but the effect became significant only in interaction with chronic mild stress (see below section 4.6) (**Article III, Table 3**).

Archer et al. (1984b) have reported the effect of DSP-4 on avoidance learning, also after partial denervation (at doses 3 and 6.25 mg/kg), a week after administration. The lowest dose caused an effect after 4, but not after 10 weeks. The study shows that only marginal reductions in NA in brain regions that are sensitive to DSP-4 induce the functional deficit that is great enough to impair avoidance performance. In our study we did not find any deficiencies in open field activity and this may be associated either with housing or test-specific factors. Lapiz et al. (2000) used DSP-4 in a lower dose than usual (25 mg/kg) and found no effect on exploratory activity in isolation-reared DSP-4 treated rats two weeks later. In comparing DSP-4 effects in group-housed and isolation-reared rats, they found that isolation rearing influences the behavioural effects of central NA depletion. Group-reared DSP-4 treated rats made more zone transitions and travelled longer distances than group-reared saline-controls, but no such differences were found in isolation-reared rats. The question, why partial denervation stimulated group-housed rats in exploration test and did not affect behaviour of isolation-reared animals, is separate and very interesting one for future studies. Lapiz et al. (2000) suppose that the central NA, which is manipulated by neurotoxin treatment, is involved in hyperactivity, which characterizes exploratory behaviour in isolation-reared rats without any treatment and so the partial denervation in the system makes group-housed animals similar to them. It is possible that isolated male rats are so much more activated by new environment, that there is no room for additional behavioural stimulation in their brain neurochemical systems affected by deficient noradrenergic neurons. On the other hand, the deficiency induced by the low dose of noradrenergic neurotoxin may not be sufficient to induce neophobia and behavioural inactivation independent of housing factors.

In the same study Lapiz et al. (2000) observed some interesting effects of partial LC denervation on selective attention. In object discrimination test DSP-4 decreased inspective exploration related both to familiar and novel objects, but increased rearings in individually housed rats. According to the opinion of the authors (Lapiz et al. 2000) DSP-4 treatment may have decreased inspective exploration while enhancing inquisitive exploration reflected by these rearings and hyperactivity shown by animals during the second exposure to the locomotor activity cages. Our rats were also singly housed for even a longer

period of time (five weeks although not from weaning) and we did not use any objects on the open field, which could stimulate the appearance of these DSP-4 induced behavioural changes. Even then, although the behavioural change after extensive and partial LC denervation appears to be different, it is not absent after partial denervation.

Sucrose solution intake, body weight gain and adrenal weight

Neither sucrose intake nor preference was influenced by partial denervation in the noradrenergic system in any of five weekly observations (**Article III, Table 2**). As we found no indication of anhedonia in the animals with extensive noradrenergic denervation (see section 4.2) and the literature about the behavioural effects of partial noradrenergic denervation is scarce, it only remains to conclude that anhedonic state is not a characteristic of partial noradrenergic denervation either.

The adrenal weights were not affected by partial noradrenergic denervation 43 days later, but the body weight gain was smaller in interaction with chronic mild stress (**Article III, Table 1**). See discussion 4.6.

Forced swimming test

Partial noradrenergic denervation with DSP-4 in dose of 10 mg/kg reduced immobility in the forced swimming test on both testing days (**Article III, Figure 1**). The result was quite surprising in the context of previous studies. Harro et al. (1999) reported a biphasic effect dependent of the dose of DSP-4 on the forced swimming behaviour. Larger denervation achieved by DSP-4 in dose 50 mg/kg was found to reduce immobility and partial denervation to prolong it. However, these studies have been carried out in group-housed rats only a week after DSP-4 treatment, but the study reported in the dissertation was performed in individually housed rats as long as 6 weeks after partial noradrenergic denervation. Opposite results dependent upon time after DSP-4 treatment have previously been found regarding exploratory behavior (Berridge and Dunn, 1990).

The increase in immobility during the second testing on the following day has been interpreted alternatively as behavioural despair (Porsolt et al. 1978; Thiebot et al., 1992) or learning to conserve energy (De Pablo et al., 1989; West, 1990; Jefferys and Funder, 1996). Forced swimming behaviour during the initial exposure to the water cannot be easily interpreted in this way, and instead active versus passive coping strategies have been implicated (e.g. Liebsch et al., 1998). An alternative interpretation is that more active swimming (and, hence, less immobility) immediately after immersion to water reflects more reactive or

impulsive behaviour similarly to the effect of chronic mild stress and partial 5-HT denervation (**Article I**; Harro, 2002; **Article IV**).

The immobility reducing effect of partial noradrenergic denervation was evident already on the first exposure to the forced swimming test. All rats had longer immobility times on the second day of testing compared to the first 5-min period on the first day and there was no difference between the groups regarding the increase in immobility. However, the similar more active behavioural reaction to the second exposure to the forced swimming stress differentiated denervated animals from the controls in the same way as in the first session, exposing the reduction of immobility in partially denervated rats as a stable behavioural profile. This can be interpreted as an overall increase in behavioural reactivity to the acute stress, which may depend on the reduced apparent density of ß-adrenergic receptors found in cerebral cortex.

4.4. The effect of noradrenergic denervation on brain monoamine systems (Articles II and III)

4.4.1. Serotonin and dopamine and their main metabolites ex vivo (Article II and Article III)

The extensive noradrenergic denervation (**Article II, Table 1**) reduced only the NA levels in frontal cortex two weeks after treatment. All the other monoamine measures taken from the same region (DA, HVA, DOPAC, 5-HT and 5-HIAA) remained unchanged. The partial noradrenergic denervation in the same study increased HVA level in frontal cortex compared to controls and the animals with extensive denervation. The change in HVA levels is an indication of increased DA metabolism in the frontal cortex in animals treated with a low (10 mg/kg) but not with a high dose (50 mg/kg) of DSP-4.

Six weeks after administration the partial noradrenergic denervation (**Article III, Table 4**) did not affect DA, DOPAC and HVA levels or DA turnover in frontal cortex or hypothalamus. 5-HT-ergic measures were also not affected with an exception of reduced 5-HIAA concentrations in hypothalamus. Post hoc tests indicate however that this effect is mainly a result of combining partial noradrenergic denervation and chronic mild stress (see discussion in section 4.6).

DSP-4 is a noradrenergic neurotoxin highly specific to the nerve terminals originating from the LC (Fritschy and Grzanna, 1989; Ross, 1976), while it has no direct effect on DA- or 5-HT-ergic neurons (Ögren et al. 1980; Chrobak et al. 1985; Harro et al. 1992; Somboonthum et al. 1997; Al-Zahrani et al. 1998; Hughes and Stanford, 1998), even though some investigators have found small but significant reductions in 5-HT levels (Jonsson et al. 1982; Ohno et al. 1996).

Zimelidine or some other inhibitor of 5-HT uptake has been administered before DSP-4 in many studies to avoid the 5-HT depleting effect of DSP-4 (Ross et al. 1976, Jonsson et al. 1981, Jonsson et al. 1982), but the majority of researchers, including the work from our laboratory, have consistently found no effect of DSP-4 on 5-HT levels or 5-HT reuptake. For example, the increase in cortical 5-HT found by Jonsson et al. (1981) one week after DSP-4 (50 mg/kg) in mice was not considered to be a neurotoxic effect, because no long-term alterations in either [^{3}H]-5-HT uptake or 5-HT levels were observed.

Despite the selectivity of DSP-4 in its primary effect on the LC projections, there appear to be long-lasting functional consequences in other monoamine systems after treatment with this neurotoxin. Eller and Harro (2002) found that DSP-4 (10 mg/kg) did not influence 5-HT content a week after treatment, but decreased 5-HIAA in the cerebral cortex and hippocampus. Indeed there is a complex interaction between the LC system and 5-HT neurons at multiple levels (Harro and Oreland, 2001; Mongeau et al. 1997; Szabo and Blier, 2001).

DA utilization and 3,4-dihydroxyphenylacetic acid (DOPAC) production rates have been found to be lower after DSP-4 treatment (Hallman and Jonsson, 1984; Hatip-al-Khatib et al. 2001), DA release has been reduced in both neostriatum and nucleus accumbens (Lategan et al. 1992), and also striatal DA D_2 receptors have been upregulated as well as behavioural supersensitivity to the effects of amphetamine after DSP-4 treatment (Harro et al. 2000). These findings are in line with the fact that there is a stimulatory input from LC to the mesencephalic DA cell groups (see Harro and Oreland 2001, for references).

Eller and Harro (2002), similarly to the observation by Hallman and Jonsson (1984), found a week after DSP-4 treatment (10 mg/kg) significantly reduced DOPAC levels in cerebral cortex and hippocampus, while DA levels were unaffected. Two months after low dose of DSP-4, no significant differences in catecholamine levels were observed. In Harro et al. (2003) the levels of monoamines and their metabolites were measured both after partial and extensive LC denervation 3 days and one month after DSP-4 treatment. 3 days after extensive denervation there was an increase in DA, decrease in DOPAC in frontal cortex and an increase in DOPAC levels in striatum. One month after extensive LC denervation the increase in frontal cortical DA was not significant and DOPAC levels were also recovered (Harro et al. 2003).

Thus, the increased DA metabolism found in animals with partially denervated LC two weeks after neurotoxin administration may be an indication of increased sensitivity to stress and suggests differential regulation of DA in the forebrain by the LC lesions. Increases in frontal cortical HVA have been suggested to be strongly indicative as a reaction of stress (Roth et al. 1988; Thierry et al. 1976), which has previously also been observed in this laboratory (Harro et al. 1999). Thus, it is tempting to speculate that partial LC denervation (10 mg/kg) renders animals more susceptible to stress at least for some time after the lesion.

4.4.2. Dopamine release potential in the nucleus accumbens shell in conscious rats (Article II)

Pretreatment with DSP-4 two weeks earlier had no effect on baseline extracellular dopamine (DA) levels in conscious rats, but reduced dose-dependently the dopamine response to depolarization induced by 50 mM KCl (**Article II, Figure 1**). The peak extracellular DA was significantly reduced after the higher (50 mg/kg) dose of DSP-4, whereas the effect of the lower dose (10 mg/kg) missed the conventional level of significance.

Lategan et al. (1992) observed a 28% reduction in basal DA overflow in anaesthetised rats using in vivo microdialysis in the nucleus accumbens after pre-treatment with DSP-4. In a recent study of Hatip-al-Khatib et al. (2001) in conscious rats no decrease in the extracellular DA in the nucleus accumbens core was found after DSP-4 treatment. Similarly, we did not find any significant reduction of extracellular DA in the nucleus accumbens shell without KCl induced depolarization in conscious rats.

The reduced DA release potential in the nucleus accumbens after DSP-4 treatment suggests that weakening of the LC input to DA nerve cells may translate into lower DA release in the nucleus accumbens in response to stimuli activating the LC. This may be a way, by which chemical denervation of the LC projections reduces the effectiveness of positive reinforcers (Morley et al. 1988). Thus, there is indeed a lower DA release potential after LC denervation, at least in the mesolimbic projections that could elicit other long-term changes in DA-ergic neurotransmission and serve as a substrate for psychiatric disorders – such as depression and drug addiction. Dopaminergic neurotransmission, particularly DA release in the nucleus accumbens, has been implicated in the mechanism of action of addictive drugs (Di Chiara and Imperato, 1988; Gerrits et al. 2002), and reductions in the DA-ergic neurotransmission in the accumbens may serve as the basis for anhedonia and loss of energy in depression (Harro and Oreland, 2001).

4.4.3. The effect of noradrenergic denervation on monoamine receptors implicated in depression

ß-adrenoceptors in frontal cortex, hippocampus and cerebral cortex

After the decrement of NA contents in cerebral cortex and in cerebellum (**Article I, Figure 4**) an increase of the maximal apparent number of binding sites (B_{max}) of [^{3}H]-DHA binding in hippocampus, but not in frontal cortex, was detected 22 days after DSP-4 treatment in dose 50 mg/kg (**Article I, Table 3**). [^{3}H]-DHA binding affinity was increased in frontal cortex. Thus, DSP-4 caused an upregulation of β-adrenoceptors both in hippocampus and frontal cortex. Previous studies (Dooley et al. 1983b; Suzdak and Gianutsos, 1985; Wolfman et

al. 1994) have found increased numbers of [^{3}H]-DHA binding sites in the neocortex and hippocampus after DSP-4 treatment (in doses 63 or 50 mg/kg). It has also been shown that β-adrenoceptors are functionally supersensitive after DSP-4 treatment (Dooley et al. 1983a) because clenbuterol inhibited open field exploratory behaviour in DSP-4-treated animals at lower doses than in controls. In our study the β-adrenoceptor binding in the frontal cortex of individual animals was negatively associated with their open field activity. [^{3}H]-DHA binding in the hippocampus correlated with immobility in the forced swimming test. Immobility on the first swimming session correlated negatively with the number of [^{3}H]-DHA binding sites. Thus, the state of β-adrenoceptors regulates behavioural activity in the open field and forced swimming tests and is dependent of extensive LC denervation both in hippocampus and frontal cortex three weeks later.

Partial noradrenergic denervation (DSP-4 in dose 10 mg/kg) (**Article III, Table 5**) reduced the density of β-adrenoceptors (B_{max}) in the cerebral cortex six weeks later. The increased turnover of NA still observable 6 weeks after partial LC denervation may be responsible for the down-regulation of β-adrenoceptors in neurotoxin-treated animals. Contrary to the previous results with extensive LC denervation, after partial denervation, which reduced frontal NA levels by one-third, there was a decrease in β-adrenoceptor binding. Significant positive correlations were found between the K_D value of [^{3}H]-DHA binding in cerebral cortex and the number of squares crossed and rearings made in the open field test after partial denervation that was similar to the extensive denervation.

α_2-Adrenoceptors in cerebral cortex after partial LC denervation

Six weeks after partial denervation of noradrenergic nerve endings (**Article III, Table 5**) no changes in [^{3}H]-RX 821002 binding to α_2-adrenoceptors in cerebral cortex were found.

We calculated the ratio between the apparent maximal numbers of binding sites for [^{3}H]-RX 821002 and [^{3}H]-DHA, because during writing of this paper, the group of García-Sevilla reported that there is a stable positive relationship between ß- and α_2-adrenoceptors in the human frontal cortex and the ratio between ß- and α_2-adrenoceptors is increased in the brains of the suicide victims (Sastre et al., 2001). Neurotoxin treatment increased the ratio between the apparent maximal numbers of binding sites for [^{3}H]-RX-821002 and [^{3}H]-DHA in cerebral cortex 1.3 fold. When plotting [^{3}H]-DHA binding against [^{3}H]-RX-821002 binding, a second-order polynomial regression revealed a significant model (**Article III, Figure 2**). Using the data obtained in the present study, we found that even in the rat cortex, there is a correlation between ß- and α_2-adrenoceptors, even though our data are different to some extent. Thus, there would be a significant linear relationship between these measures, exactly as reported for the humans, if four cases with the highest α_2-adrenoceptor density

were omitted. Inclusion of these cases, however, retains a significant association between these measures, but it becomes more complex. The possibility that the relationship between ß- and α_2-adrenoceptors would not be linear over the entire range should be kept in mind in further studies on humans.

Dooley et al. (1983b) performed α_2-adrenoceptor binding with [^{3}H]-PAC ([^{3}H] *p*-aminoclonidine) 10 days after DSP-4 treatment (63 mg/kg) and found lower K_D values across CNS regions together with significantly decreased B_{max} in corpus striatum and hippocampus. At the same time in neocortex only the K_D value was lowered, B_{max} was not changed. Assessment of adrenergic receptors in neocortex 50 days after injection indicated stability in this affinity change of the presumably postsynaptic α_2-adrenoceptor. However, PAC is an agonist of the α_2-adrenoceptors and can bind to e.g. the imidazoline binding sites. So the differences between the studies may originate from using different ligand and its binding to other binding sites but also from higher dose of neurotoxin.

5-HT$_{1A}$ receptors in hippocampus after partial LC denervation

Partial LC denervation did not induce any changes in [^{3}H]8-OH-DPAT binding to 5-HT$_{1A}$ receptors in hippocampus (**Article III, Table 5**). Thus, partial denervation in LC system alone does not induce changes in 5-HT$_{1A}$ receptors in hippocampus, which mediate adaptation to stress, allowing animals become tolerant to chronic aversive stimuli (Deakin et al. 1992). Recent animal models have suggested a role of 5-HT$_{1A}$ in the development of chronic anxiety (Overstreet et al. 2003) and have helped to generate animal models of anxiety-related disorders. The state of 5-HT$_{1A}$ receptors in hippocampus may be used as a correlate of the regulation of neuroendocrine functions related to stress. The absence of changes in 5-HT neurotransmission and no evidence of behaviourally depressive symptomatology in animals with partially denervated LC system 6 weeks after administration, are well consistent with no change of 5-HT$_{1A}$ receptors in hippocampus.

D$_2$ receptors in striatum after partial LC denervation

Partial LC denervation did not affect D_2 receptors in striatum in this study. However, significant upregulation of D_2 receptors in striatum has been found by Harro et al. (2000) two weeks after extensive noradrenergic denervation as the increase in the maximal number of binding sites for [^{3}H]-raclopride. Harro et al. (2003) found the increase in the apparent number of D_2 binding sites in striatum also one month, but not 3 days after both partial and extensive LC denervation. These data suggest that an upregulation of D_2 binding sites may develop in striatum even after minor denervation of the LC projections. The difference in D_2 receptor binding a month compared to six weeks after partial LC denervation

may find some explanation by the use of behavioural experiments, which in this study interfered with the restoring of NA levels in frontal cortex. An intact LC-NA-ergic innervation exerts a tonic facilitation of DA-ergic transmission (Hallman et al. 1984), and the reduced activity of the LC neurons after DSP-4 treatment (Olpe et al. 1983) could be the basis for reduced DA-ergic output in the striatum. The upregulation of D_2 binding sites in the striatum after DSP-4 pretreatment is compatible with the reduction of basal DA release. Behavioural experiments involving such stressful incidents as forced swimming test might have potentiated NA and subsequently DA release and contributed to the normalized status of D_2 receptors in the brain region measured.

4.5. The effect of chronic mild stress (CMS) on behaviour and monoamines in the brain (Articles I and III)

4.5.1. Behavioural effects of chronic mild stress

Open field test

In the open field experiment 15 days of CMS reduced explorative behaviour in the form of the number of rearings. The number of fecal boli left on the arena was also reduced, which is a sign of reduced emotionality or anxiety felt in new environments (**Article I, Figure 3**) referring to an unexpected effect of chronic stress.

Katz et al. (1981), Garcia-Marquez and Armario (1987), Molina et al. (1994a) have demonstrated a decrease in locomotor response to a novel environment after chronic variable stress. In chronic mild stress paradigm, which got its basic idea of modelling anhedonia in animals through decreased sucrose consumption after chronic stress exposure (Katz, 1982; Willner, 1997), significantly milder stressors are used (e.g., no prolonged inescapable electric shocks, restraint, 1 h tail shock, ether anesthesia, cold water immersion, 48 h food/water deprivation, tail pinch). Chronic mild stress procedure consists of sequentially applied series of supposedly relatively mild stressors, none of which is supposed to be either necessary or sufficient to affect behaviour on its own: the essential feature of the model is believed to be variety and unpredictiveness (Muscat and Willner, 1992). This principle allows discrepancies in the details of procedure between laboratories and may be one reason why there are problems with reliability concerning CMS model (Willner, 1997).

In the present investigation, the number of rearings in the open field was indeed reduced after chronic mild stress procedure, whereas horizontal activity was not significantly modified. However, in subsequent experiments we have failed to reproduce the reduction in rearings, suggesting that this is not a stable

effect in the paradigm described here. In addition, the number of defecations was reduced in the stressed rats, suggesting that chronic mild stress reduced emotionality (Hall, 1934; Katz et al. 1981). Such an effect has been observed after an acute loud noise stress and was attributed to a novelty-activated behavioural profile, whereas chronic exposure to variable but severe stressors increased the number of defecations in the novel environment (Katz et al. 1981; Harro et al. 2001). Levine et al. (1967), Fernández-Teruel et al. (1992), Gilad et al. (2000) have described reduced defecations in new environment in rats that were handled in infancy and tested later in adulthood. This handling effect was accompanied with increased activity in the open field and lower corticosterone secretion evident of reduced emotional behaviour towards environmental novelty. It has to be admitted that the chronic mild stress model of depression involves a lot of handling and is only mildly stressful. Roth and Katz (1979) have noted a controversy concerning the degree to which stress alters behaviour, and even the direction of that change. They found, for example, that pre-exposure to moderately intense light and white noise facilitated open field activity as measured by initial activity, lowered defecation scores, and supplementary measures (rearing, grooming, center field penetration).

There are few studies about the CMS effects on the open field behaviour. For example, Harris et al. (1998) reported increased exploration and rearing in CMS rats exposed to novel open field contrary to changes found in the case of CVS exposure. D'Aquila et al. (1994; 1997) found an anxiolytic profile in the elevated plus-maze test in CMS exposed animals and no difference between CMS and control rats in a locomotor activity monitoring system. Papp et al. (1993a,b) found no CMS effect on basal locomotor activity also. Gorka et al. (1996) found that CMS decreased continuously monitored home-cage locomotor activity of animals in the dark phase of the diurnal cycle by approximately 50%, but did not change the activity in the light phase. In contrast to these negative results, D'Aquila et al. (2000) found the exploratory activity in the open field to be reduced by 7 weeks of chronic mild stress.

Longer CMS procedure (29 days) in individually housed rats did not elicit any differences in explorative activity compared to control animals in the open field test (**Article III, Table 3**). CMS exacerbated the effect of partial LC denervation on the number of fecal boli (see discussion 4.6).

Emotional reactivity of chronic mild stressed rats is rather low in the open field test independent of the length of CMS, although the expectation about the effects of stress would be exactly the opposite. Locomotor activity has not been sensitive to chronic stress procedures with varying stressors in our studies, but a more intensive stress procedure leading to physiological and biochemical changes indicative of chronic stress elicited at least an increased defecation rate in the open field (Harro et al. 2001).

Sucrose solution intake, body weight gain and adrenal weight

Chronic mild stress model of depression (Willner et al. 1987; Willner et al. 1992; Willner, 1997) is based on the observation of Katz (1982) about the suppressing effect of a chronic stress regimen on the sweetened fluid intake in rats. The effect of chronic mild stress is mainly used to model anhedonia, an inability to experience pleasure, that is demonstrated by reduced intake or preference of sucrose solution – sucrose consumption or preference test was adopted to our lab. Results from sucrose tests have remained inconclusive at best, but it seems that independent of the variation of chronic mild stress procedure it does not induce the anhedonic state reliably in experimental animals. Sucrose intake in the first study was decreased temporarily after first week of CMS exposure in group-housed rats; the effect however disappeared next week (**Article I, Table 2**). Many investigators have used longer periods of mild stress than 15 days. Moreau et al. (1992) demonstrated a significant increase in the threshold for intracranial self-stimulation already on the 5th day of stress, and indeed maximal effect was observed on Day 16 after the beginning of stress procedure. Furthermore, a reduction in sucrose intake has also been observable from at least 2nd week of stress procedure (Willner, 1997).

Longer period of chronic mild stress (5 weeks) in the context of more traditional individual housing for CMS studies (**Article III, Table 2**) did not reveal any significant effect of stress on sucrose intake or preference. Masi et al. (2001) reported that chronic variable stress could reduce motivation for palatable food, and the associated DA-response in the nucleus accumbens, only if the rats had not acquired the appetitive behaviour before the stress onset. The extensive familiarization of the rats with sucrose intake test before CMS as well as before LC denervation could be one reason why we failed to observe any reduction in this measure. On the other hand, the CMS technique and its behavioural end-point measures are not unanimously accepted and criticisms have been addressed toward several aspects of the method. Since reliability is a problem with the sucrose intake measure in several laboratories (see Willner, 1997; Nielsen et al. 2000) a very detailed description of the procedure from authors being able to observe the reduction of sucrose intake after the CMS is warranted. One aspect about chronic mild stress model, which has never been reported, but has been advised by M. Papp (personal communication), is the preselection of animals according to their sucrose intake. In pharmacological studies, inclusion of only these animals into study, which react to the stress with reduced sucrose solution intake, would not be a big problem. The drug-treated animals will be the anhedonic ones, and if they react to treatment with improved symptomatic profile this predicts a real antidepressant effect. In neurobiological studies, like ours, the CMS effect cannot be detected in such case because of remarkable individual variability in subjects.

In addition to the problems with reliability, it has been suggested that the reduction of sucrose intake after CMS can be accounted for by the reduction of

body weight caused by the procedure (Matthews et al. 1995; Forbes et al. 1996). However, in the **Article III**, the adjustment of the sucrose intake measures to the body weight of animals or to total fluid intake (sucrose preference) did not expose any covert CMS effect in sucrose intake data. The inclusion of food and water deprivation into the CMS paradigm and, in particular, immediately before sucrose intake or preference tests for all animals causes additional problems in interpretation of results. Harris et al. (1997) and Hatcher et al. (1997) have found that preference for sweetened solutions is not reduced after CMS, and it has been reported that intake of saccharin is reduced after CMS only if the procedure had included food deprivation (Hagan and Hatcher, 1997; Hatcher et al. 1997). For example, it is conceivable that when a reduction of sucrose consumption is found in CMS, it may be due to overconsumption of sucrose in control animals after the deprivation period. In both our studies the food and water deprivation has been included, but no reliable CMS effect on sucrose intake or preference has been found, however. In a study by Harro et al. (2001) no food and water deprivation was used but a clear and reliable preference for 1% sucrose over water developed. Although in this study the stress procedure was designed to be stronger variable stress type, still no reduction in sucrose intake or preference was found.

Chronic mild stress reduced the weight gain of animals after 2 weeks of exposure. Four weeks after CMS exposure the weight gain was reduced, but the reduction was significant only in interaction with partial noradrenergic denervation. Still a decrease in the body weight of stressed rats as compared to controls (which is likely to reflect the arrest of the usual weight gain in free-feeding rats) has been observed previously in CMS studies (Moreau et al. 1992; Willner 1997; Nielsen et al. 2000). Each element of stress procedure may exert its own effect on the weight gain. Harro et al. (2001) found significant stress effects on weight gain after wet bedding and overnight illumination. Willner et al. (1992) reported that the most powerful stressors affecting sucrose intake are paired housing, exposure to wet bedding and 45º cage tilt. However all these stressors were used in both our CMS studies. Thus, the stress procedure we applied had an obvious effect on the animals. Apparently animals habituated to the longer stress procedure and started to gain weight again. There is no other obvious reason to consider the effects of CMS procedures used in **Article I** and in **Article III** to be different.

Adrenal weight was unaffected in both studies (**Articles I, Table 1 and Article III, Table 1**). Previously only Muscat and Willner (1992) have reported adrenal hypertrophy after CMS procedure. The lack of significant effect on adrenals in both our studies agrees with the results of Dunčko et al. (2001) and indicates that the effect of stress was indeed with low severity.

Forced swimming

In the forced swimming test group-housed CMS animals did not show any effect of stress at the first exposure. On the next session, which in psychopharmacological studies is used to reveal antidepressant effects, the CMS procedure reduced immobility, as antidepressants would do, but it turned to be significant only in DSP-4 treated animals (**Article I, Figure 1**). In subsequent experiments, there was a similar and significant effect of chronic mild stress on both vehicle- and DSP-4 treated rats (data not shown). Since the finding that chronic mild stress can reduce immobility was unexpected and the other studies on chronic mild stress have, when reported, used single-housed animals, we carried out a separate experiment comparing the effect of chronic mild stress on rats housed either individually or groups of five. Chronic mild stress, again, reduced the immobility period in group-housed rats, but failed to do this in the animals housed individually **(Article I, Figure 2)**. Nevertheless, we have found in other CMS studies reduced immobility in singly housed animals.

Previous studies using the chronic mild stress paradigm have not studied forced swimming behaviour. An early report concerning unpredictable stress effects in forced swimming test is by Rodríguez-Echandía et al. (1988). They treated rats daily with unpredictable emotional or physical stressors over 14-day period and found longer immobility duration in stressed animals. Few investigators have shown an increase in immobility after chronic variable stress (Garcia-Marquez and Armario, 1987; Molina et al. 1994a, b). The severity of stressors probably determines whether the increase in immobility can be demonstrated. These studies have used relatively severe stressors, for example inescapable electric footshock, swimming in cold water, restraint, 1 h tail shock, ether anesthesia, applied repeatedly. Data obtained using the forced swimming test in our study are, however, at variance with what might be expected from an experimental model of depression. It could be argued, that our stress procedure was too short to model depression adequately, since many investigators have used longer periods of mild stress than 15 days. Longer chronic mild stress period (**Article III, Figure 1 A, B**) decreased immobility both on first and second exposure to forced swimming test. On the first day of testing significant between-groups differences were seen only during the behaviour observed in the very first 5 minutes which is taken as a sign of increased behavioural reactivity. The increase in immobility during the second testing on the following day has been interpreted alternatively as behavioural despair (Porsolt et al. 1978; Thiebot et al. 1992) or learning to conserve energy (De Pablo et al. 1989; West, 1990; Jeffreys and Funder, 1996). Forced swimming behaviour during the initial exposure to the water cannot be easily interpreted this way, and instead active versus passive coping strategies have been implicated (e.g. Liebsch et al. 1998). This interpretation would suggest that chronic stress could elicit more active coping strategies. Earlier chronic restraint stress was found to reduce immobility in the forced swimming test (Platt and Stone, 1982). We have also

observed that chronic variable stress reduces the immobility period within the first 5 min after immersion into water in the forced swimming test (Harro et al. 2001). More active swimming (and, hence less immobility) immediately after immersion to water reflects then a more reactive or impulsive behaviour. The decrease in immobility in the forced swimming test and increased defecation in the open field test has previously been observed after presentation of inescapable footshocks and was considered to indicate a state of increased anxiety (van Dijken et al. 1992).

4.5.2. Brain monoamines, their metabolites and receptors in brain

Chronic mild stress did not affect either ex vivo noradrenaline content in cerebral cortex and cerebellum 15 days after exposure (**Article I, Figure 4**), or noradrenaline, serotonin and dopamine and any of their metabolite levels in frontal cortex and hypothalamus 29 days after exposure (**Article III, Table 4**).

Willner et al. (1992) associated CMS effects on sucrose solution intake/ preference with deficiencies in the mesolimbic dopamine projection from the ventral tegmental area (VTA) to the nucleus accumbens, which play a crucial role in mediating the behavioural effects of rewards. Later, however, the mesotelencephalic DA-ergic pathways have been suggested to mediate signals of salience than of reward (Horwitz, 2000). It has been found that three or seven weeks of exposure to CMS increased the concentration of DA and 5-HT, and their metabolites, in limbic areas but not in the caudate nucleus; concentrations of NA in the same time were unaltered (Willner et al. 1991). In an in vivo voltammetric study by Stamford et al. (1991) in anaesthetized animals it was found that CMS increased the electrically-stimulated release of DA in nucleus accumbens but the sensitivity of inhibitory DA autoreceptors was decreased.

15 days of chronic mild stress exposure elicited a tendency towards an increase in B_{max} of β-adrenoceptor binding in hippocampus but no effect on binding in frontal cortex was found (**Article I, Table 3**). The hippocampal effect of stress is similar to the effect of extensive noradrenergic denervation in the same region but clearly smaller. Stanford et al. (1984) found in brain cerebral cortical synaptosomes after 14 days of mild stress (handling) an increase in noradrenaline synthesis rate and a decrease of α_2- and β-adrenoceptors. Papp et al. (1994a) have previously reported an increase in cortical β-adrenoceptor binding after chronic mild stress as measured by [^{3}H]-DHA binding, whereas they did not study the binding in hippocampus. In the present investigation, we have found a strong tendency of increase in the apparent number of β-adrenoceptors in hippocampus, but not in the frontal cortex or cerebral cortex. In one subsequent study, the effect of chronic mild stress to increase hippocampal [^{3}H]-DHA binding was statistically significant (unpublished). However, it appears that upregulation of [^{3}H]-DHA binding in the brain some time after chronic mild stress is a reproducible phenomenon. In the

investigation of Papp et al. (1994a) the stress procedure lasted 7 weeks, so this might well explain why the results regarding cortex differ. Papp et al. (1994a) also reported that there was no significant correlation between their ß-adrenoceptor binding data and the animals's sucrose intake. In the present study, we have observed significant correlations, firstly positive between [^{3}H]-DHA binding affinity in the frontal cortex (notably not in cerebral cortex) and the locomotor activity in the open field, and secondly, negative between the numbers of [^{3}H]-DHA binding sites and the immobility in the forced swimming test. These preliminary data should be interpreted with caution, but possibly brain ß-adrenoceptor characteristics correlate better with behaviours related to adaptation with the environmental changes than with rewarded behaviour.

More prolonged period of CMS in our laboratory (**Article III, Table 5**) increased K_D values of [^{3}H]-DHA binding in cerebral cortex that is a sign of downregulation of affinity in β-adrenoceptors. Stone (1979) has suggested that adaptation to stress involves desensitisation of adrenoceptors. As we did not observe any behavioural indices of stress (see above open field, sucrose intake, forced swimming), our rats may have habituated to CMS procedure and adapted to stress at the level of β-adrenergic receptors.

Significant positive correlations were found between K_D value of [^{3}H]-DHA-binding and the number of squares crossed and rearings made in the open field test. These correlations are similar to those found in the **Article I**, even though in this case there was a period of 3 weeks between the behavioural test and the sacrifice of the animals. Thus, it is possible that there is an association between frontal cortical β-adrenoceptors and open field behaviour.

No effect of CMS was found on α_2-adrenoceptors in cerebral cortex, D_2 receptors in striatum or 5-HT_{1A} receptors in hippocampus (**Article III, Table 5**).

Upregulation or increased sensitivity of α_2-adrenoceptors has been associated with social stress, chronic variable stress and isolation rearing (Flügge et al. 1997; Garcia-Vallejo et al. 1998; Fulford et al. 1994) and this receptor has regularly been shown downregulated after antidepressant treatments (for review see Harro and Oreland 2001). Papp et al. (1994b) found reduced D_2 receptor binding in "limbic forebrain", but not in striatum after CMS, and explained CMS-induced sucrose intake with these changes in D_2 receptors. We also found no effect of CMS on D_2 receptors in striatum. Previously, Papp et al. (1994a) have described the increase in [^{3}H]8-OH-DPAT binding in hippocampus after CMS (but also after chronic administration of imipramine) that was not found in the present study. Among possible explanations for this discrepancy a major one is methodological: in the previous study, some of the incubation conditions (37ºC, addition of ascorbic acid) were suboptimal for achieving binding stability (Hall et al. 1985; Arro et al. 2001).

4.6. The interaction between noradrenergic denervation and chronic mild stress (Articles I and III)

Archer et al. (1984a) were first to hypothesize on the basis of behavioural maladaptations found in DSP-4 treated rats, that if they are not "depressed" animals, they are more easy to depress by unpleasant and uncontrollable environmental events. It has been further proposed in the spreading neuronal adjustment model of depression by Harro and Oreland (2001) that deficiency in LC noradrenergic system may render animals or affected persons more susceptible to stress. This consideration motivated our special interest in the possible synergistic effect of noradrenergic denervation and chronic stress.

Behaviour after extensive noradrenergic denervation combined with chronic mild stress

Interaction between noradrenergic denervation and CMS was found in open field test. The extensive denervation (DSP-4 50 mg/kg) combined with 2 weeks of chronic mild stress reduced significantly the number of crossed squares and total exploratory activity (**Article I, Figure 3**). Chronic mild stress reversed these effects of denervation and re-established behavioural activity in denervated animals. Since stress also reduced the immobility of rats in the forced swimming test (see discussion 4.5), the results seem comparable to the study of Porsolt et al. (1978) in which rats placed into an "enriched" environment containing several stimulus objects had significantly lower immobility scores in forced swimming test and tended to be more active in open field.

The number of fecal boli in the open field test was also lowered in association with the interaction between denervation and CMS factors. CMS reduced this measure actually independent of treatments and the effect has been interpreted as reduction of emotionality in new environment. In the forced swimming test chronic mild stress reduced significantly the immobility of DSP-4 treated rats on the second exposure to the situation but, again, the stress effect was similar in vehicle-treated animals. Two weeks of chronic mild stress reduced the weight gain in DSP-4 treated animals but the effect of stress alone was quite similar. No effect of the combination of treatments was found on the adrenals.

Plaźnik et al. (1988) studied the interactions between DSP-4 treatment (65 mg/kg) and inescapable shock both in open field and forced swimming tests. DSP-4 alone reduced activity in both tests and interacted significantly with inescapable shock only in reducing further the open field motor activity. Bakke et al. (1986) have interestingly found the protective effect of DSP-4 (50 mg/kg) on stress-induced gastric ulceration in rats 15 days after administration. In these rats the basal corticosterone concentrations in plasma were lower compared to

controls but after 23 h of immobilization stress no difference was found between groups. Iuvone and Dunn (1986) have found that footshock resulted in the similar activation of tyrosine hydroxylase in vehicle or DSP-4 treated mice. These results suggest that interactions between stress and LC denervation depend on multiple factors.

Behaviour after partial noradrenergic denervation combined with chronic mild stress

No interaction between partial denervation of noradrenergic nerve endings ascending from LC and long-lasting chronic mild stress (4 weeks) on locomotor activity in the open field test was found (**Article III, Table 3**). An interaction in the open field appeared to be related to the number of excrements. Partial noradrenergic denervation increased the number of fecal boli left on the open field arena six weeks after treatment, but the effect was significant only for the group in which DSP-4 pretreatment was combined with stress. This synergistic effect of partial LC denervation by DSP-4 pretreatment and chronic stress was one of the most interesting results in this experiment, indicating stronger emotional reaction to novelty. Increased number of fecal boli left on the open field has been one of the most consistent indicators of enhanced emotionality and anxiety provoked by unfamiliar situations in laboratory animals (Hall, 1934, Katz et al. 1981). Lesioning of the ascending NA-ergic innervation has been suggested to reduce tolerance to stress (Tsaltas et al. 1987), and extensive DSP-4 induced LC denervation has been demonstrated to elicit neophobia (Delini-Stula et al. 1984; Berridge and Dunn, 1990; Harro et al. 1995), but the present study provides evidence for an enhancement of the effect of stress by a partial selective LC denervation.

Weight gain after long-lasting chronic mild stress was the smallest in animals with partial LC denervation. Sucrose intake was not affected. In forced swimming test partial noradrenergic denervation and chronic stress together decreased immobility time in both exposures to testing in the same way as both treatments alone.

Interactive effects of noradrenergic denervation and chronic mild stress on brain monoaminergic mechanisms

Noradrenaline, its metabolites, turnover, α_2- and β-adrenoceptors

No interaction on noradrenaline levels either in cerebral cortex or cerebellum were found after extensive LC denervation (DSP-4 50 mg/kg) (**Article I, Figure 4**). Partial LC denervation and CMS did not interact at noradrenaline levels in frontal cortex, but in hypothalamus the tendency towards an interaction was found (**Article III, Table 4**). The concentration of noradrenaline was

decreased in DSP-4 + Control animals, but exposure to stress compensated the deficiency. No interaction was found on the MHPG and NMN levels and noradrenaline turnover in either brain area. α_2- Adrenoceptors were unaffected either by CMS or partial LC denervation or by their interaction.

The binding affinity of β-adrenoceptors in frontal cortex was increased three weeks after extensive noradrenergic denervation but this effect was not present after chronic mild stress (**Article I, Table 3**). Given that ß-adrenoceptor binding in frontal cortex in individual animals was negatively associated with their open field activity in the present study, it is tempting to hypothesize that the increase in [^{3}H]-DHA binding affinity after DSP-4 treatment is linked to the reductions in open field exploration in the locus coeruleus denervated animals, and that chronic mild stress restores the normal behaviour by desensitizing the cortical ß-adrenoceptors. The mechanism of this effect is unknown, but could be related to the noradrenaline-releasing effect of stress. NA release measurements in vivo would be needed to fully test this hypothesis. Noradrenaline release in the frontal cortex after DSP-4 treatment has been described by in vivo microdialysis (Kask et al. 1997). Thus, in anaesthetized rats, the basal extracellular level of noradrenaline was similar to controls, even though tissue levels were much lower. Stimulation of noradrenaline release by administration of atipamezole, a α_2-adrenoceptor antagonist, resulted in considerably lower but a still significant increase in noradrenaline extracellular levels, which suggests that there is some compensatory potential after neurotoxic lesions. On the other hand, longer CMS exposure (**Article III, Table 5**) decreased the β-adrenoceptor affinity in cerebral cortex similarly to the previous study but this effect was not present after partial denervation of LC. Here one could speculate that desensitization of β-adrenoceptors by stress is an adaptive reaction, which is not functioning after DSP-4 treatment. In hippocampal β-adrenoceptors no interactions in statistical sense was found but in the maximal number of binding sites the additive effect of extensive LC denervation and chronic mild stress was seen (**Article I, Table 3**). The effect of co-occurrence was additive, both treatments upregulated the number of [^{3}H]-DHA binding sites in hippocampus.

Serotonin, its metabolites, turnover and 5-HT$_{1A}$ receptors

Partial LC denervation interacted with long-term chronic mild stress in affecting 5-HIAA levels in hypothalamus. Compared with Control + Stress group animals with LC denervation reacted to stress with lowered 5-HIAA levels in hypothalamus. This is possibly an indirect effect derived as an adaptation for coping with the weakness of one monoaminergic system by another neurochemical system or as an alternative secondary deficit developed in the other system. No other aspect of serotonergic neurotransmission was affected by interactions between partial LC denervation and CMS.

Dopamine, its metabolites, turnover and D_2 receptors

Dopaminergic neurotransmission neither in frontal cortex nor hypothalamus was affected by interactions between partial LC denervation and chronic mild stress. Claustre et al. (1986) found increased DOPAC in the frontal cortex after DSP-4 (50 mg/kg) and electric footshock stress but this effect was similar to the effect of stress alone.

4.7. Effect of serotonergic denervation on the levels serotonin, other monoamines and on the forced swimming behaviour (Article IV)

The changes in brain monoamines and behaviour obtained after serotonergic denervation have presented in **Article IV**. We studied both dose- and time-dependency in the effect of PCA and found (one week after 0, 2, 4, 6 mg/kg) a dose-dependent reducing effect on 5-HT levels in all studied brain regions. This effect was statistically significant in cerebral cortex, hippocampus, striatum, frontal cortex, cerebellum and hypothalamus, but missed the conventional level of significance in septum (**Article IV, Figure 2**). The effect of the lowest dose of PCA, 2 mg/kg was stronger in cerebellum, hypothalamus, hippocampus and cerebral cortex, and negligible in striatum, frontal cortex and septum. PCA also reduced, the levels of 5-HIAA in the frontal cortex, cerebral cortex, hippocampus, striatum, hypothalamus, septum and cerebellum (**Article IV, Table 1**). The lowest dose of PCA (2 mg/kg) reduced the 5-HIAA levels only in hippocampus (by about 30%) and in frontal cortex (16%). The turnover of 5-HT was significantly reduced in hippocampus and septum but not in the striatum, hypothalamus and cortex. Two weeks after administration of serotonergic neurotoxin the effects of PCA in lower dose range (0, 1, 2, and 4 mg/kg) were measured in the frontal cortex and septum (**Article IV, Table 4**). 5-HT and 5-HIAA concentrations in frontal cortex were reduced after administration of PCA in doses of 2 and 4 mg/kg. In septum the effect of PCA was significant in the dose of 4 mg/kg. Our data, especially on the effects of smaller doses of PCA, which cause a limited denervation, are compatible with the idea that the regional sensitivity to PCA is not completely explained by its selective effect on dorsal raphe projections (e.g., Marcusson et al. 1988; Invernizzi et al. 1989; Hensler et al. 1994; McQuade and Sharp, 1995; Moorman and Leslie, 1996). Frontal cortex, which contains an extremely dense plexus of fine 5-HT-ergic axons, has a distinct response to PCA. On the other hand, the predicted lower sensitivity of septum can be demonstrated only one week but not two or four weeks (Harro et al. 2001) after treatment. Thus, the effect of PCA was largely similar one and two weeks after treatment, except in septum, where it might have needed more time to develop. Thus, the regional specificity of the neurotoxic effect of PCA is time-dependent.

PCA affected also noradrenergic and dopaminergic neurotransmission one week after administration (**Article IV, Table 2**). NA levels were reduced dose-dependently in cerebral cortex and reduced by the lowest dose of PCA (2 mg/kg) in cerebellum. DA levels were significantly reduced and DA turnover increased in hypothalamus. Two weeks after administration PCA (4 mg/kg) reduced DA levels and increased DA turnover in the frontal cortex, but not in septum. NA levels were not affected two weeks after serotonergic denervation. PCA has been reported to be without significant effect on catecholamines (Massari et al. 1978; Leonard, 1976), so it is probable that the effects in our study are functional in nature. It seems that NA neurotransmission is recovered two weeks after serotonergic denervation and DA levels, which have been measured in different brain areas, exhibit longer vulnerability from serotonergic denervation.

In cerebral cortical noradrenergic receptors no effect of PCA on [^{3}H]-RX 821002 binding parameters was found one week after treatment but [^{3}H]-DHA binding affinity was reduced. Two weeks after administration of the neurotoxin the effect was nonsignificant. Thus, with regard to α_2- and β-adrenoceptors neither partial nor almost complete 5-HT denervation by PCA treatment leads to changes associated with depression (Meana and Garcia-Sevilla, 1987; Garcia-Sevilla et al. 1999). PCA increased binding affinity of [^{3}H]-8-OH-DPAT at all dose levels on week after treatment. These effects could be caused by the PCA-dependent depletion of 5-HT that decreases the levels of endogenous ligand. Nevertheless, since there was no dose-dependency with regard to the effect of PCA, we remain cautious regarding its validity.

The forced swimming test was carried out to measure behavioural effects of the serotonergic denervation. On the first exposure to forced swimming the lowest dose of PCA, 2 mg/kg, reduced immobility during the first five minutes (**Article IV, Figure 1**). On the second day of testing the effect of PCA was statistically not significant. In Experiment 2 (**Article IV, Figure 3**) the swimming time during the first 5 min of the first session of the forced swimming test was significantly increased after PCA (2 mg/kg) compared with controls and also compared with other PCA treated groups (1 mg/kg and 4 mg/kg). At the second exposure to the forced swimming the differences became insignificant. The time the animals spent in struggling or in immobile posture were not different between groups either at the first or the second session of the test.

The effect of 5-HT depletion in Porsolt's forced swimming test, either by inhibition of synthesis or by excessive release and neurodegeneration, has mainly been studied in conditions of near-total depletion (Chojnacka-Wójcik et al. 1991; Cervo et al. 1991; Luscombe et al. 1993; Page et al. 1999). The effects of partial depletion may however, to be of greater theoretical interest with respect to both the physiological regulation of 5-HT function and its implication in, e.g., depression (Datla and Curzon, 1996). In the present study the PCA treatment that elicits a large reduction of 5-HT levels in the rat brain, had no effect in the forced swimming test compatibly with earlier data. Harro et al. (2001) found earlier and this study confirms the anti-immobility effect of a low

dose of PCA (2 mg/kg) that causes after a week of administration a limited 5-HT depletion in hippocampus, cerebral and frontal cortex but does not affect 5-HT levels in striatum and septum. More detailed analysis of forced swimming behaviour two weeks after PCA administration, showed that the reduction of immobility one week after PCA treatment results from increased swimming activity. From the time the active components of behaviour in the forced swimming test started to be measured separately (Weiss et al. 1981; Armario et al. 1988), the reductions in inactivity by noradrenergic and serotonergic agents are known to be achieved through increases in struggling and in swimming, respectively (Detke et al. 1995; Rénéric and Lucki, 1998; Page et al. 2003). The most straightforward interpretation of this behavioural change would be that a partial and regionally selective degeneration, elicited by a small dose of PCA, has an antidepressant effect but such a theoretically inconvenient conclusion would be premature, since the forced swimming test has been validated as a screening test for antidepressants given in two to three doses and never for modeling depression. The effect of partial serotonergic denervation in forced swimming test has been noted only in the first 5 min of the first swimming session similarly in Harro et al. (2001) and this study. This temporary reduction of immobility cannot be interpreted as a reduction of behavioural despair, as an active coping strategy, or as a learning effect, and as a working hypothesis it has been suggested to reflect an increase in reactive or impulsive behaviour (Harro, 2002). Similar effects in the forced swimming test have been noted after chronic stress (Platt and Stone, 1982; van Dijken et al. 1992; **Article III**) and six weeks after partial noradrenergic denervation (**Article III**). Thus, a reduction of immobility or an increase in swimming in the forced swimming test should in certain paradigms be interpreted as enhanced reactivity or impulsivity rather than reduced depressiveness. Together, these data suggest that in studies modeling features of depression, which use neurochemical manipulations other than drugs with known antidepressant efficacy, interpretation of results obtained with forced swimming may need a new conceptualization.

In the present study we have found correlative evidence that variability in struggling in the forced swimming test is associated with NA-ergic neurotransmission and swimming with 5-HT-ergic, which is consistent with previous suggestions (Armario et al. 1988; Detke et al. 1995; Page et al. 2003). Time spent in swimming in the second swimming session correlated negatively with serotonin turnover in frontal cortex (**Article IV, Figure 4**). K_D of the ß-adrenoceptor binding correlated negatively with struggling behaviour on the second exposure to the forced swimming test (**Article IV, Figure 5**). It could be argued that an increase in impulsiveness should be displayed rather as an increase in struggling than in swimming behaviour. Attribution of behaviours in the forced swimming test to specific psychological constructs remains nevertheless speculative for the time being, and it may appear safer to suggest that partial 5-HT-ergic lesions promote behavioural reactivity.

5. CONCLUSIVE REMARKS

Extensive as well as partial noradrenergic denervation elicit time- and activity-dependent changes in monoaminergic neurotransmission and mood-associated behaviours. There are long-lasting reductions in the NA levels to which β- but not α_2- adrenoceptors react with supersensitivity. Deficits in the LC noradrenergic system have functional influence on other monoamine neurotransmitters and their receptors. The reduced DA release potential in the nucleus accumbens after DSP-4 treatments suggests a mechanism by which weakening of the LC input to DA nerve cells may contribute to motivational deficits seen in depression. Partial noradrenergic denervation as well as partial serotonergic denervation may be better models to study monoaminergic mechanisms in depression than extensive denervations, because both increase behavioural reactivity to stress.

We failed to accommodate the chronic mild stress model of depression in our laboratory. My personal point of view tends to be, that chronic mild stress models depression only in animals, that react to stress with anhedonic behavioural profile. If only these animals are taken into the study, then probably it is possible to achieve increased emotionality in open field and increased despair in forced swimming situation. Other animals may need more severe stress. Otherwise studying the general effects of stress in the chronic mild stress model of depression gives nothing more than studying the reactivity to environmental challenges, which may be adaptive or nonadaptive dependent upon individual.

Even if no animal model of depression does induce complete disorder, it seems conceivable to try to find some behavioural, neurochemical and pharmacological consistency between them in relation to existing knowledge about neurobiology of depression. Some researchers consider this to be an unattainable goal, but in the light of basic neurobiological processes that are influenced by antidepressant treatments, it is worthwhile to continue questioning long-held assumptions behind screening tests used in psychopharmacology. For example, the forced swimming test may need a new conceptualization if it is used in other types of studies than pure pharmacological screening of putative antidepressant compounds.

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8. SUMMARY IN ESTONIAN

Meeleolust mõjustatud käitumise monoamiinergilised mehhanismid roti ajus

Depressiooni monoamiinergilise hüpoteesi kohaselt põhineb depressiivne meeleoluhäire monoamiinide (noradrenaliin, serotoniin, dopamiin) nappusel haigestunud inimese ajus.

Töö eesmärgiks oli uurida järgmiste manipulatsioonide mõju loomade käitumisele ja monoamiinide neurokeemiale:

- ulatusliku ja osalise *locus coeruleus*'est lähtuvate noradrenergiliste projektsioonide denervatsioon kasutades selektiivset neurotoksiini DSP-4 [*N*(2-klooretüül)-N-etüül-2-bromobensüülamiin] (**artiklid I, II ja III**).
- krooniline mõõdukas stress (**artiklid I ja III**)
- kroonilise mõõduka stressi mõju ulatuliku ja osalise noradrenergilise denervatsiooniga loomadele (**artiklid I ja III**)
- ulatuslik ja osaline serotonergiline denervatsioon kasutades neurotoksiini PCA (paraklooramfetamiin) (**artikkel IV**).

Ulatuslik noradrenergiline denervatsioon vähendas aktiivsust avarväljal kooskõlas varasemate tähelepanekutega DSP-4 uudsusekartust suurendavast ja uudistamisaktiivsust vähendavast toimest (**I**). Suhkrulahuse tarbimine ja käitumine sundujumise testis polnud noradrenaliini süsteemi ulatuslikust kahjustamisest mõjustatud (**I**). Osaline noradrenergiline denervatsioon mõjustas käitumist avarvälja katses koosmõjus kroonilise mõõduka stressiga (**III**). Ekskrementide arv, mille suurenemist uuele keskkonnale eksponeeritud loomade puhul peetakse elavama emotsionaalse reaktsiooni (ärevuse) märgiks, suurenes. Osaline denervatsioon ei mõjustanud suhkrulahuse tarbimist aga lühendas oluliselt liikumatusaega sundujumise katses (**III**). Mõju sundujumisele oli nähtav esmasel katsesituatsiooni sattumisel ega muutunud järgneval mõõtmisel. Seega, osalise noradrenergilise denervatsiooniga loomadel on üldiselt suurenenud reaktiivsus stressiolukorras.

Nii ulatuslik kui osaline noradrenergiline denervatsioon mõjustas teiste monoamiinergiliste süsteemide talitlust (**I**, **II** ja **III**). Dopamiini vabanemine *nucleus accumbensis* teadvusel ja vabalt ringi liikuvatel ulatusliku denervatsiooniga loomadel osutus stimulatsiooni puhul oluliselt madalamaks (**II**). See muutus dopamiinisüsteemis võib olla mehhanismiks, mille vahendusel nõrgema noradrenergilise närviaktiivsuse foonil ilmneb motivatsiooni defitsiit. Kaks nädalat pärast osalist denervatiooni suurenes dopamiini metaboliidi HVA tase ja kiirenes dopamiini metabolism frontaalkoores (**II**). See on märk suurenenud tundlikkusest stressi suhtes. Ulatuslik noradrenergiline denervatsioon suurendas hippokampuses β-adrenoretseptorite arvu ja otsmikukoores retseptorite tund-

likkust kolm nädalat pärast toksiini manustamist (**I**). Osaline noradrenergiline denervatsioon vähendas β-adrenoretseptorite tihedust ajukoores. Retseptorite aktiivsuse mahareguleerimine toimus oletatavasti noradrenergilise närviülekande võimendumise toimel (**III**). α_2, 5-HT_{1A} ja D_2 retseptorite seisund ei olnud osalisest noradrenergilisest denervatsioonist mõjustatud (**III**).

Krooniline mõõdukas stress vähendas tõusude ja ekskrementide arvu, aga mitte uudistamisaktiivsust üldiselt (**I**). Suhkrulahuse tarbimise testis ilmnes ajutine magusatarbimise langus (**I**). Sundujumise katses vähendas krooniline stress liikumatusaega grupiviisiliselt majutatud loomadel (**I**). Pikema stressiprotseduuri järel ilmnes sarnane liikumatusaega vähendav toime ka üksimajutuse järel (**III**). Krooniline mõõdukas stress ei mõjustanud monoamiinide ja nende metaboliitide tasemeid (**I** ja **III**). Kaks nädalat pärast stressi ilmnes hippokampuse β-adrenotseptorite afiinsuse tõus sarnaselt ulatusliku noradrenergilise denervatsiooni toimele (**I**). Pikem stressiperiood seevastu vähendas β-adrenotseptorite afiinsust ajukoores, mis võib olla seotud stressile habitueerumisega (**III**). Muutusi α_2, 5-HT_{1A} ja D_2 retseptorites ei ilmnenud (**III**).

Ulatuslik noradrenergiline denervatsioon interakteerus kroonilise mõõduka stressiga. Stressi mõju kompenseeris noradrenergilise denervatsiooni toimel tekkinud uudistamisaktiivsuse vähenemise ning suurenenud β-adrenotseptorite tundlikkuse otsmikukoores (**I**). Krooniline mõõdukas stress võimendas osalise noradrenergiline denervatsiooni toimet avarvälja ekskrementide arvule, kaaluiibele ning liikumatusaja pikkusele (**III**). Krooniline mõõdukas stress ei suutnud kompenseerida osalisest noradrenergilisest denervatsioonist tingitud β-adrenotseptorite afiinsuse tõusu, viidates sellele, et stressi β-adrenotseptorite tundlikkust alandav adaptiivne mehhanism ei toimi denervatsiooniga loomadel (**III**).

Artiklis IV uuritud ulatuslik ja osaline serotonergiline denervatsioon parakloorampetamiiniga kutsus esile annusest sõltuvaid muutusi serotonergilises närviülekandes. Sundujumise katses ilmnes oluline erinevus kontrollrühma käitumisest osalise serotonergilise denervatsiooni toimel (PCA annuses 2 mg/kg). See PCA annus vähendas serotoniini taset oluliselt väikeajus, hüpotaalamuses, hippokampuses ja ajukoores. Septumis võttis toime ilmnemine pisut rohkem aega.

Nii osaline noradrenergiline kui osaline serotonergiline denervatsioon osutusid sobivamateks meeleolust mõjutatud käitumise monoamiinergiliste mehhanismide uurimise mudeliteks kui ulatuslikud denervatsioonid. Mõlemat tüüpi osalised denervatsioonid kutsusid esile käitumusliku reaktiivsuse tõusu stressiolukordades.

Võimalik, et krooniline mõõdukas stress modelleerib depressiooni ainult loomadel, kes reageerivad stressile anhedoonilise käitumisprofiiliga. Võimalik, et kui vaid need loomad uuringusse võtta, õnnestuks kroonilise mõõduka stressi järel tuvastada ka suurenenud emotsionaalset reaktsiooni uudsele keskkonnale avarväljal ning suurenud meeleheitereaktsiooni sundujumise katses. Teistsuguse

käitumisprofiiliga loomadel eeldab nende muutuste saavutamine tugevamat stressi.

Kuigi ükski depressiooni loommudel depressiooni kui häiret tervikuna ei modelleeri, tasuks otsida käitumuslikke, neurokeemilisi ja farmakoloogilisi kokkulangevusi nende vahel, toetudes olemasolevatele teadmistele depressiooni neurobioloogiast. Näiteks sundujumise test vajaks uut lahtimõtestamist olukordade tarvis, kus seda kasutatakse teist tüüpi uuringutes kui oletatavate depressioonivastaste ainete sõeltestimine.

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List of publications

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