Neurochemical consequences of juvenile separation stress: studies in the limbic structures of trumpet-tailed rat (*Octodon degus*)

Dissertation

zur Erlangung des akademischen Grades

DOCTOR RERUM NATURALIUM

(Dr.rer.nat.)

genehmigt durch die Fakultät für Naturwissenschaften

der Otto-von-Guericke-Universität Magdeburg

von M.Sc. Grzegorz Jezierski

geb. am 29.11.1976 in Gdańsk (Danzig), Polen

Gutachter:

Prof. Dr. Katharina Braun, Otto-von-Guericke Universität Magdeburg

Prof. Dr. Rainer Schwarting, Philipps-Universität Marburg

eingereicht am: 24.08.2007

verteidigt am: 21.01.2008

Erklärung

Hiermit erkläre ich, dass ich die von mir eingereichte Dissertation zum dem Thema:

Neurochemical consequences of juvenile separation stress: studies in the limbic structures of trumpet-tailed rat (*Octodon degus*)

selbstständig verfasst, nicht schon als Dissertation verwendet habe und die benutzten Quellen und Hilfsmittel vollständig angegeben wurden.

Weiterhin erkläre ich, dass ich weder diese noch eine andere Arbeit zur Erlangung des akademischen Grades *doctor rerum naturalium* (Dr. rer. nat.) an anderen Einrichtungen eingereicht habe.

Danzig, den 24.08.2007

Grzegorz Jezierski

ACKNOWLEDGEMENT

Here, I wish to thank all the people, who were helping me during my stay in Germany.

First and foremost of all, I am especially thankful to my supervisor, Prof. Katharina Braun, for hosting me in her group in Magdeburg for 3 years, for her guidance and help.

I would like to express my thanks to Prof. Georg Reiser, the coordinator of my German scholarschip, for our stimulating meetings and support.

To all wonderful people, whom I met in Germany – Ewa Ostrowska, Meena and Reena Murmu, Ania Łaszcz, Milind Joshi, Kasia Marcinkiewicz, Edyta Samela, Magda Błażejczyk, Rowena Antemano, Ela Dharmalingam for their friendship.

To Steffi Zehle – for cooperation in our methylphenidate project.

To all my Polish friends, whom I left back in Poland for 3 years, for their constant support and for all the meetings and visits in between.

And last but not least – to Dr. Michael Gruss, for his patience, help and guiding me into the field of brain chemistry.

DEDYKUJĘ TĘ PRACĘ MOJEJ RODZINIE

I DEDICATE THIS WORK TO MY FAMILY

ABSTRACT

Environmental influences during certain early life periods, particularly those provided by the mother or both parents, are generally considered to have a strong impact on the development of brain and behavior of the offspring. Using the semi-precocial South American species *Octodon degus*, a rodent becoming increasingly popular in different laboratory research fields, in the first part of this dissertation I aimed to examine the developmental pattern of serotonergic, dopaminergic and amino acid neurotransmitting systems. Moreover, the consequences of disturbance of the parent-offspring interaction induced by parental separation on the serotonergic neurotransmission were assessed. Based on a quantitative neurochemical approach using brain homogenates obtained from cortical regions and the hippocampus my results revealed that (*i*) levels of monoamines and amino acids reach adult-like levels relatively early in ontogeny, i.e. mainly between postnatal day (PND) 3 and 21, depending on the brain region and substance examined, indicating a relatively matured neurotransmission in cortical regions and hippocampus at birth. In addition, an age-, region- and sex-specific pattern of changes in the serotonergic system has been found induced by (*ii*) an acute stress challenge early in life (parental separation at PND 3, 8, 14 and 21) with the most pronounced effects at earlier ages (PND 3 – PND 14) in the female cortex, and (*iii*) repeated stress exposure (measured at PND 21) with the most pronounced effects in the cortex of both sexes. Taken together, these data indicate that early life stress (i.e., parental separation) influences the developing serotonergic system in the semi-precocial *Octodon degus*, even if the brain is relatively well matured at the early stages of postnatal development.

The second part of my dissertation presents the pattern of dopaminergic responses to methylphenidate in the prestressed, juvenile, immature and still developing brain of *Octodon degus*, which mimics the clinical situation in human children and the use of MP treatment much more appropriately than studies performed in normal adult rodent brains. Methylphenidate (MP) is a drug of choice in the treatment of attention-deficit hyperactivity disorder (ADHD) in human children. Previous studies performed by other members of our group have shown, that exposing the newborn animal to repeated episodes of emotional stress (=separation from the family for one hour per day from PND 1-21) can induce hyperactive behavior and inattentiveness towards maternal

vocalizations in juvenile *Octodon degus*. Using *in vivo* microdialysis I measured the levels of dopamine in the medial prefrontal cortex and nucleus accumbens of awake, normal control and hyperactive degus. These results revealed that (*i*) methylphenidate induces minute response in the mPFC of control animals at PND 22-24 (juveniles), whereas in age-matched prestressed degus dopamine levels significantly decline after acute MP injection (10 mg/kg); (*ii*) chronic injection of methylphenidate between PND 22 and 45 results in the sensitization to the drug; in unstressed control animals pretreated with MP the dopamine levels were elevated to a higher extent in response to MP injection than in the vehicle pretreated controls, (*iii*) at the age of PND 46-48 (adolescent animals) the prestressed, hyperactive animals, which were chronically MP-treated, show potentiated dopamine increases in response to MP administration, compared to the unstressed controls. My study indicates that methylphenidate acts differently in the non-fully developed and mature brain. Moreover, early emotional experience as well as chronic drug treatment strongly influences the action of MP in the brain.

Taken together, these results indicate that experience-induced modulation of limbic structures during development may influence their neurochemical responsiveness later in life.

TABLE OF CONTENTS

1 INTRODUCTION

1.1 Early life stress and its consequences

How experience sculpts the developing neural circuits is one of the most intriguing questions in developmental neurobiology. There is accumulating evidence that epigenetic factors affect the development of brain and behavior in a much more pronounced way than previously appreciated. Such adaptive plasticity makes the developing neuronal circuits sensitive to several environmental stimuli.

One of the very first experiences which the offspring faces immediately after birth is the contact with mother or both parents and establishing an emotional attachment with them. The quality of such interaction, environmental influence, learning, experiences affect significantly the maturation of the brain and thus the normal development of whole organism. The intimate relationship between the infant and parents, in most cases particularly the mother, is a basic factor for the development of the organism.

Early adverse emotional life events in humans has been associated with the development of pathological behavior as well as the etiology of several neurological diseases, such as depression and/or anxiety disorders (Downs and Harrison, 1998; Parker et al., 2000; Heim and Nemeroff, 2001; Wainwright and Surtees, 2002; Pryce et al., 2005), alcoholism (Anda et al., 2002), neuroticism and nicotine dependence (McFarlane et al., 2005), psychopathic behavior (Marshall and Cooke, 1999) and schizophrenia (Agid et al., 1999). Additionally, sexual and physical abuse in childhood has been also found to be associated with the suicidal behavior in adulthood (Ystgaard et al., 2004). Such strong consequences may result from the sensitization and repeatedly altered activity of the hypothalamic-pituitary-adrenocortical (HPA) system.

Early life stress, in particular when it affects the parent-child interaction, can be detrimental for the development of the individual´s physiology, endocrine function, neuroanatomy as well as behavior. There is a body of evidence from studies in rodents, that the physiological responses such as heart rate, growth hormone production and the duration of sleep/wake cycles of an infant are regulated by the mother (Levine, 2001). Consequently, the separation of infant from the mother results in specific physiological changes in the infant, for instance the reduction of heart rate, the decline in growth

hormone secretion (Schanberg et al., 1984) as well as the decrease of the ornithine decarboxylase levels, the tissue enzyme responsible for the regulation of growth and differentiation (Kuhn et al., 1978). On the endocrine level, investigations of Gruss et al. (2006) revealed that repeated postnatal stress in *Octodon degus* may result in an attenuated separation-induced increase of glucocorticoids (females) and a higher behavioral activity at the age of 3 weeks (both sexes). In rats exposed to repeated stress elevated basal adrenocorticotropic hormone (ACTH) and glucocorticoids (GC) levels were observed in adulthood, which somehow suggest the development of hyperactivity in the hypothalamic-pituitary-adrenocortical (HPA) axis, a key neuroendocrine stress axis (De Kloet et al., 1998). On the neuroanatomical level, studies have demonstrated that early sensory, motor and functional experience significantly affects the morphological maturation of neuronal networks (Rosenzweig and Bennett, 1996; Greenough et al., 1987). In extension of these studies on sensory and motor systems development, it was demonstrated that emotional experience results in quite dramatical neuroanatomical alterations in various species. For instance, in neonate domestic chicks, positive emotional experience (filial imprinting) induces the pruning of spine synapses, presumably excitatory, in an analogue of the mammalian prefrontal cortex (Bock and Braun, 1998; 1999), whereas early aversive learning induces increased synaptic densities (Patel and Stewart, 1998). In rodents, early adverse emotional experience, i.e. maternal or parental separation, induces increased spine densities on pyramidal cells in the anterior cingulate and infralimbic cortex and the CA1 subfield of the hippocampus (Helmeke et al., 2001a; 2001b; Ovtscharoff and Braun, 2001; Poeggel et al., 2003a; Bock et al., 2005) as well as affects the morphology of hippocampal dendritic trees (Bartesaghi et al., 2003). In addition, neonatally stressed animals show lower levels of corticotrophin releasing factor (CRF) positive fibers or neurons in central amygdala, dentate gyrus, CA1 region and somatosensory cortex, paralleled by the opposite effect in basolateral amygdala (Becker et al., 2007). At the behavioral level, maternal or parental separation can induce hyperactivity (Kalinichev et al., 2002; Braun et al., 2003), anxiety (Daniels et al., 2004; Renard et al., 2005), elevated exploratory behavior (Becker et al., 2007) and learning deficits (Lehmann and Feldon, 2000; Huang et al., 2002). Early adverse experience have been also found to increase voluntary ethanol consumption (Huot et al., 2001; Vazquez et al. 2002; Ploj et al., 2003a; 2003b; Roman et al., 2004) and exaggerate the behavioral responses to psychostimulants (Rots et al., 1996; Matthews et al., 1996a; 1996b; 1999; 2001; Campbell and Spear, 1999; Meaney

et al., 2002; Matthews and Robbins, 2003; Ploj and Nylander, 2003; Ploj et al., 2003a; 2003b), which provides the evidence that such emotional stress disrupts the development of systems mediating reward-related behaviors.

1.2 Early stress and its experimental paradigms

The laboratory studies in the animal models required the development of early life stress paradigms. Any comparisons between various experimental approaches might be complex, since different laboratories use several experimental stress designs, but also different control groups and, in addition, miscellaneous animal species and strains. In the laboratory rodents major paradigms are as follows (Plotsky and Meaney, 1993; Liu et al., 1997; McCormick et al., 1998; Lehman et al., 1999; Lehman and Feldon, 2000; Pryce et al., 2002):

- **Maternal separation** – this general term refers to several experimental approaches, all of which involve the separation of pups from the dam for at least 1 hour, however it may last for as long as 24 hours. In some laboratories it is the mother, which is removed from the nest and the pups are left in their homecage. The second approach is considered to be less stressful for the young animals, in other words any change in the experimental paradigm results in different stress levels. The term "maternal" refers to rats and mice, however in cases where both parents look after their offspring (as *Octodon degus* parents do) the term "parental" should be used – as in this study. Such separation might be single or repeated for shorter periods e.g. 1-6 hours/day (repeated maternal/parental separation). Maternal (or parental) separation is followed by screening of the effects of the manipulation at specific time points between the manipulation and adulthood.
- **Early handling** – daily handling of the pups to separate them from the mother and the littermates for a short period (max. 15 min/day). Such treatment is not always considered as stress, since it has been described that frequently after reunion of pups and the dam, infants receive more attention and nursing than non-treated animals.

- **Social separation** – separation of pup from the dam and the littermates for a certain time amount (1-6 h).
- **Social rearing** – in this paradigm animals do not experience any direct human disturbance during early life, therefore some researchers consider them as a control group. However, in some laboratories control animals are subjected to occasional handling, due to the need of every day routine cage cleaning without any further manipulations.

1.3 Neurotransmitting systems and early life stress

As opposed to behavioral and endocrine data, still relatively little is known about the neurochemical outcomes of postnatal stress, in particular the disturbance of parents – infant relationship. Monoamines (dopamine and serotonin) as well as inhibitory amino acid GABA have been found to be involved in the regulation of emotions and cognition (Murphy et al., 1996a; 1996b; Sokolowski et al., 1998; Myhrer, 2003; Castrén, 2005). There is evidence that stressful experience such as maternal or parental separation and the exposure of an infant to an unfamiliar environment induce changes in particular of aminergic (Hall et al., 1999; Braun et al., 2000; Miura et al., 2002; Gartside et al., 2003; Poeggel et al., 2003b; van Riel et al., 2004) but also amino acid (mostly GABA) neurotransmission (Plaut and Davies, 1972; Hsu et al., 2003; Jaworski et al., 2005; Alvarez et al., 2006).

It has been shown that parental separation leaves persistent traces in the dopaminergic as well as serotonergic neurotransmission systems. On the cellular level, repeated brief parental separation in an unfamiliar environment can induce an up-regulation of D1, 5-HT1A, but also NMDA receptors in prefrontal cortex, hippocampus and amygdala (Ziabreva et al., 2000; 2003a; 2003b). Moreover, early social deprivation leads to the enhanced density of dopaminergic and serotonergic fibers in subregions of the medial and lateral prefrontal cortex of *Octodon degus* (Braun et al., 2000; Poeggel et al., 2003b). The increase in serotonergic fibers density has been also described for subregions of nucleus accumbens (core), dentate gyrus (stratum moleculare) and amygdala (central nucleus), whereas same fibers decreased in dentate subgranular layer and in the stratum lacunosum of the hippocampal cornu ammonis region 1.

Dopaminergic fibers have been shown to increase in the core and shell region of the nucleus accumbens, stratum granulosum and subgranular layer of early deprived degus, while they decreased in the hilus of the dentate gyrus (Gos et al., 2006). Taken these data together, one can conclude that changes in the monoaminergic fiber densities are not only region- but also strictly transmitter-specific.

Neurochemical measurements have shown, that periodic maternal separation as well as handling of neonatal rats result in increased dopamine levels in the striatum and decreased dopamine turnover in the medial prefrontal cortex (mPFC). These changes are paralleled by reduced serotonin levels in the hippocampus and mPFC of adult rats (Smythe et al., 1994; Matthews et al., 2001). In juveniles, an increased amphetamine-induced release of dopamine in the ventral striatum was detected in 10-day old (Kehoe et al., 1998a) as well as 4- to 5-weeks old rats (Kehoe et al., 1996), suggesting that the influence of repeated maternal separation can be observed already in still developing animals. In addition, the application of the same paradigm is detectable in adulthood – repeated maternal separation leads to altered extracellular 5-HT levels in nucleus accumbens of adult female rats, depending on the estrous stage (Zhang et al., 2006). Extensive studies in the serotonergic system of rat performed by Gartside et al. (2003) showed that early life adversity leaves alterations in two main inhibitory and excitatory regulatory systems of 5-HT activity on neurons: 5-HT_{1A} and α_1-adrenoceptors.

In addition to their function as neurotransmitters, dopamine as well as serotonin and noradrenaline have been shown to act as trophic factors, influencing the functional state of neurons during ontogeny (Pendleton et al., 1998; Herlenius and Lagercrantz, 2001; Whitaker-Azmitia, 2001; Alvarez et al., 2002). In other words, the signaling role of monoamines would be not only the linking between two neurons, but also morphogenic role in developing embryonic tissues. For instance, noradrenaline influences the correct proliferation of glial cells in cerebellum. The neonatal administration of 6-hydroxydopamine (6-OHDA), neurotoxin which depletes noradrenergic stores in nerve endings, leads to abnormal location of the Bergmann glial cells and their structural perturbations (e.g., no intimate associations with Purkinje cells). In addition, 6-OHDA treated rats expressed changes in behavior, in particular in their orientation to a novel environment, searching and skills performance (Podkletnova and Alho, 1998; Podkletnova et al., 2001; Djatchkova-Podkletnova and Alho, 2005). Furthermore, noradrenaline has been found to regulate the development of Cajal-Retzius cells, which are playing an important role in the migration of neurons and

laminar formation in the cerebral cortex (Berger-Sweeney and Hohmann, 1997; Naqui et al., 1999). Dopamine, which appears very early in human development (Sundstrom et al., 1993), may act in the perinatal period via stimulation of D1 receptors, which regulate the transcription of other genes (Boyson and Adams, 1997). Dopamine depletion in rats leads to several developmental abnormalities, such as dyskinesia, dystonia, tics and abnormal movements of the eye (Zhou et al., 1995). Serotonin, which appears already in the fertilized egg, takes part in cell proliferation, migration and differentiation in neuronal, heart and epithelial tissues (Lauder, 1990; Liu and Lauder, 1991; Nebigil et al., 2000; Buznikov et al., 2001; Menegola et al., 2004). Moreover, the action of this monoamine provides the normal development of the somatosensory cortex (Cases et al., 1996).

Apart from monoamines, amino acid transmitters also play a role in the development of the nervous system. Glutamate terminals occur in high excess during first 2 years of life in humans, which might be related to the formation of high number of synapses at this time (Herlenius and Lagercrantz, 2001; 2004). GABA, which normally works as a main inhibitory neurotransmitter in the developing brain, during embryogenesis acts as an excitatory agent (Miles, 1999; Herlenius and Lagercrantz, 2001; 2004; Howard et al., 2007). Disturbances in GABA function at this time may cause the damages to the neuronal wiring, plasticity and neural organization (Belhage et al., 1998).

1.4 Limbic system

In 1937 Papez postulated that limbic lobe together with some of the subcortical areas forms a system, responsible for the behavioral aspects of instincts, emotions and cognition. Thereby, limbic system (Figure 1) would be a functional set containing not only cortical, but also subcortical brain structures. Limbic system, according to several researchers, is composed of the following subunits:

- **Amygdala -** involved in regulation of emotions, aggression and fear
- **Cingulate gyrus** - regulates the functions of cardiovascular system (heart rate, blood pressure) as well as the processing of cognition and attention
- **Hippocampus** - a store of long-term memory

- **Hypothalamus** - regulates (via hormone release) several autonomic functions such as heart rate, blood pressure, sleep/wake cycle, hunger, thirst, sexual arousal
- **Mamillary body** - responsible for the formation of memory
- **Nucleus accumbens** - is implicated in the reward and pleasure and might be involved in addiction
- **Prefrontal cortex** - regulates the complex cognitive behaviors and moderates correct social behavior

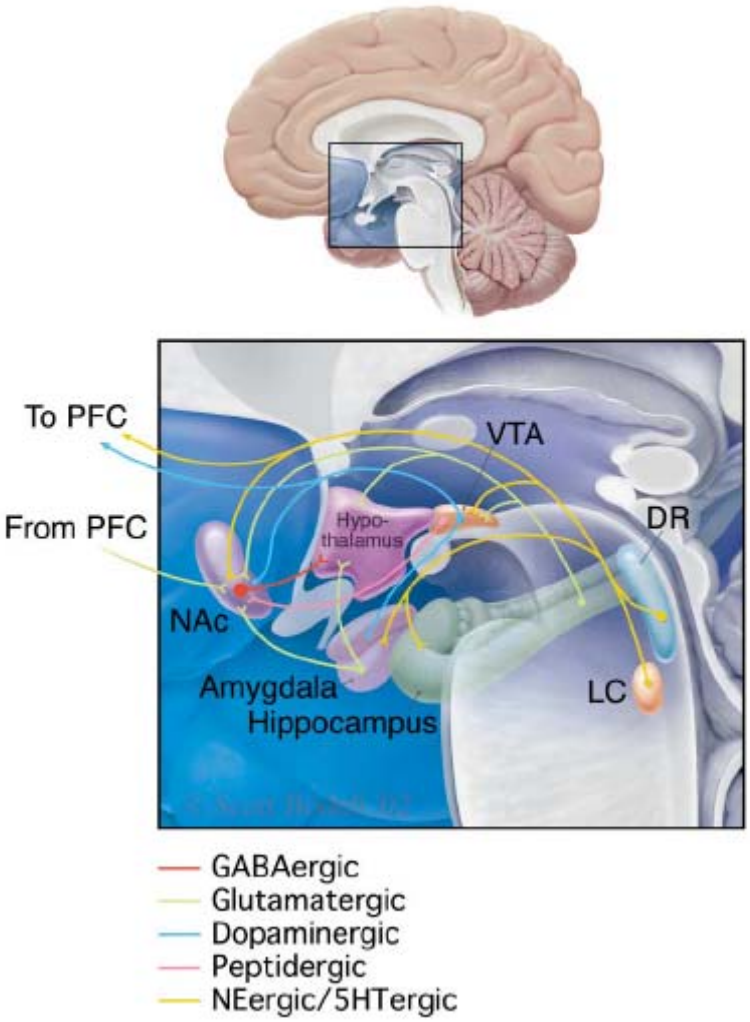

Figure 1. Schematic representation of some of the limbic structures and their connections (from Nestler et al., 2002). PFC – prefrontal cortex, NAc – nucleus accumbens, VTA – ventral tegmental area, LC – locus coeruleus, DR – dorsal raphe, NEergic - noradrenergic.

1.4.1 Prefrontal cortex

Prefrontal cortex (PFC) is the part of the limbic system responsible for the cognitive control of behavior (Goldman-Rakic, 1996; Wise et al., 1996; Miller, 2000; Miller and Cohen, 2001; Tanji and Hoshi, 2001; Funahashi, 2001). Rodent PFC consists of medial, orbital and lateral prefrontal cortex. Medial PFC (mPFC), which is involved in attention, working memory, social behavior, can be divided into four regions: medial precental, anterior cingulate, prelimbic and infralimbic area. PFC is considered to classify the items in short-term memory to organize, plan, and manipulate the information required to generate future thought or action, in other words, this particular structure allows the organism to plan action based on memory (Fuster, 1990; Seamans and Yang, 2004). The neonatal lesions in medial PFC in rats result in severe deficits in spatial learning, cognition and motor behaviors, in the opposite to orbital frontal cortex, where only mild abnormalities have been found as a result of local lesions (Kolb et al., 2004). Working memory performance as well as task-dependent neuronal activity within the PFC is strongly modulated by dopamine. Local injections of D1 receptor antagonist have been shown to disrupt performance on delayed-response tasks (Sawaguchi and Goldman-Rakic, 1991; 1994; Seamans et al., 1998). Prefrontal cortex is also involved in food-reward and olfactory learning tasks (Ragozzino and Kesner, 1999; Rolls, 2000).

In human patients lesions in PFC result in deficits in cognitive behaviors. Schizophrenic patients may express some prefrontal cortical alterations, for instance the dysfunctional dopaminergic input to this structure and a decreased density of dopaminergic fibers (Daniel et al., 1991; Okubo et al., 1997; Akil et al., 1999). In schizophrenia, the prefrontal atrophy has been also described (Ragland et al., 2004; Molina et al., 2005). Moreover, schizophrenics express altered GABAergic neurotransmission, elevated neuronal densities and reduced spine density in PFC (Selemon et al., 1995; 1998; Glantz and Lewis 1997; 2000; Karson et al. 1999; Lewis et al. 1999; Halim et al. 2003). In addition, altered function of PFC might participate in formation of obsessive-compulsive disorder (Szeszko et al., 1999), post-traumatic stress (Rickert et al., 2006), depression (Lai et al., 2000) and personality disorders (Tebartz et al., 2003).

1.4.2 Nucleus accumbens

In 1954 Olds and Milner described so called "pleasure area" of the brain, found in the experiment where rats were self-stimulating it via electrode implanted into septal area. Later on, this particular structure of the nervous system has been called nucleus accumbens (Nac). Nucleus accumbens, which together with some of dopaminergic afferent fibers of ventral tegmental area (VTA) forms the reward circuit, is most likely taking part in the drug dependence and the control of motivations. Nac acquisits and expresses most Pavlovian stimulus-reward learning (Day and Carelli, 2007; Di Chiara and Bassareo, 2007). Nucleus accumbens is composed of core involved in motor functions and shell responsible for processing of emotions and motivations (Zhou et al., 2002). It collects the inputs from amygdala, hippocampus, perirhinal and prefrontal regions, which altogether form a system responsible for receiving, acquisition and coding of aversive learning and memory (Levita et al., 2002). The outputs from nucleus accumbens project to substantia nigra and indirectly to prefrontal cortex (via ventral pallidum and dorsal thalamus).

Action of nucleus accumbens is based on dopamine, promoting the feeling of desire, as well as serotonin, providing satiation. Therefore, many addictive drugs enhance dopamine and in parallel decline serotonin in Nac. The dopaminergic terminals in Nac which originate in ventral tegmental area are the target for addictive drugs, such as amphetamine or cocaine.

1.4.3 Hippocampus

Hippocampus (Hpc) is the limbic structure responsible for the formation and storage of long-term memory. In general, it plays an essential role in the transfer of experienced events into an autobiographic memory. Hippocampus stores the information about episodes and its damage results in difficulties to form new memories, however some of the aspects may remain unaffected, such as the ability to learn new skills (Nakazawa et al., 2004; O'Kane et al., 2004). In addition, hippocampus takes a part in navigation, e.g. finding shortcuts between known places. The individuals who are more skilled in navigation exhibit more active and larger hippocampi than the average ones, which was proved by Macguire et al. (2000) in an excellent London cab drivers study.

The acute preparation of hippocampal slices has been also used to study the activity-dependent forms of synaptic plasticity: long-term potentiation (LTP) and long-term depression (LTD) (Navakkode et al., 2004; 2005; Sajikumar et al., 2005a; 2005b).

1.5 Attention-deficit/hyperactivity disorder – etiology, symptoms and treatment

As known from behavioral studies in our group, repeated episodes of emotional stress are potent to develop hyperactive behavior as well as inattentiveness towards maternal vocalizations (Braun et al., 2003; Gruss et al., 2006). Such symptoms resemble those typical for attention-deficit/hyperactivity disorder (ADHD). ADHD is a clinically heterogenous disorder, which is typically diagnosed during early childhood and characterized by the presence of some basic symptoms: moderate-to-severe distractibility, short attention span, motoric hyperactivity, emotional lability, and impulsivity. Additionally, the patients might suffer from cognitive problems. Finally, all these symptoms may result in either aggression or anxiety and depression (Oades, 1998). Inattention, i.e. problems with attention focusing, distractibility and completing tasks, has been documented using questionnaire ratings by teachers and parents (Solanto, 2002). Neuropsychological investigations as well as neuroimaging studies revealed that ADHD might be caused by dysfunctions of the prefrontal cortex as well as connected subcortical areas (Berger and Posner, 2000; Sowell et al, 2003; Carboni and Silvagni, 2004). Anatomic MRI revealed, that ADHD children matched with healthy same-age controls, show smaller total volume of the brain (around 4% difference), smaller caudate, globus pallidus, anterior frontal cortex and cerebellar vermis (Solanto, 2002). High-resolution MRI and surface-based, computational image analysis performed by Sowell et al. (2003) showed reduced regional brain size localized mainly to inferior portions of dorsal prefrontal cortices, reduced brain size in anterior temporal cortices as well as prominent increases in gray matter found in posterior temporal and inferior parietal cortices. All mentioned brain changes were recorded in both hemispheres.

It is believed that one of the most important factors causing hyperactive behavior in ADHD is dopaminergic dysfunction of the cortical regulation of the dorsal striatum.

Such conclusions are based on the neuropharmacology of drugs used in the treatment of ADHD (Higgins, 1999; Popper, 2000; Greydanus et al., 2002), molecular genetic investigations, which relate the symptoms of ADHD to the polymorphism of genes encoding dopamine transporters (DAT1 gene) and D4 receptors (DRD4 gene) (Cook et al., 1995; LaHoste et al., 1996; Rowe et al., 1998; Swanson et al., 1998; 2000; Faraone et al., 1999) and neuroimaging studies of ADHD patients (Lou et al., 1989; Dougherty et al., 1999; Dresel et al., 2000; Krause et al., 2000). The most affected dopaminergic pathway in ADHD seems to be the one which projects from PFC through the external segment of globus pallidus and ends on the inhibitory projections from the subthalamic nucleus to the internal globus pallidus. The lack of dopamine in this pathway will result in the "inhibition of the inhibition" and therefore in motoric hyperactivity of the patient (Castellanos, 1997). The cognitive dysfunctions in the patients suffering from ADHD are probably caused by the alterations in prefrontal cortex, which is normally responsible for attention, organization and planning. PFC is extremely sensitive to any oscillations of dopamine levels, for instance its insufficient stimulation may lead to the development of working memory deficits (Arnsten, 1997). Children suffering from ADHD require an extended, complex psychological care, which should be provided by parents collaborating with the doctor of appropriate specialty and teachers (Rappley, 2005). Nevertheless, psychotherapy at least in some cases should be additionally supported by the pharmacological treatment.

At present, there are few drugs commonly used in the treatment of ADHD. One of these is atomoxetine (Strattera®, IUPAC name: (3S)-N-methyl-3-(2-methylphenoxy)-3-phenyl-propan-1-amine), which selectively blocks the reuptake of noradrenaline from the synapse, by blocking the noradrenaline presynaptic transporter (Michelson et al., 2001; Bymaster et al., 2002; Kratochvil et al., 2003). The other one is dextroamphetamine (Dexedrine, IUPAC name: 1-phenylpropan-2-amine), blocking the re-entry of dopamine into the neuron (Glaser et al., 2005). However, the drug of choice in the treatment of ADHD is currently methylphenidate.

Methylphenidate (MP, Ritalin®, IUPAC name: methyl-a-phenyl-2-piperidineacetate, Figure 2) is an integral part of the treatment programs for ADHD symptoms, which typically also include other remedial measures (psychological, educational, and social) to stabilize and normalize children diagnosed for ADHD. However, still little is known about the action of MP in the juvenile, still developing brain, which is known to respond differently, quite often more sensitively to

pharmaceutical drugs compared to the mature, adult brain. So far all microdialysis studies to analyze the action of MP were performed in adult rats and revealed that MP increases extracellular dopamine for instance in nucleus accumbens, striatum or prefrontal cortex (Kuczenski and Segal, 1999; Gerasimov et al., 2000; Huff and Davies, 2002; Marsteller et al., 2002), most likely by blocking dopamine transporters and thereby suppressing the re-uptake of dopamine from the synaptic cleft (its re-entry into the neuron). Animal microdialysis data was confirmed by imaging studies in the human brain, where methylphenidate has been shown to raise extracellular dopamine in the striatum of healthy men (Volkow et al., 2001).

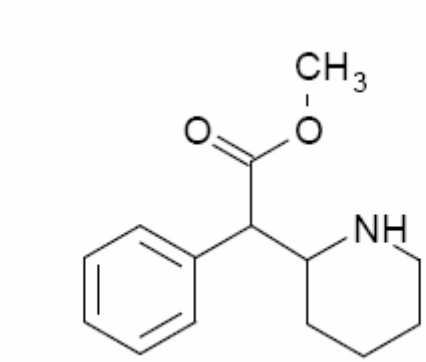

Figure 2. The chemical structure of methylphenidate. Drawn with BKchem software.

The question is how is a drug which elevates extracellular dopamine in the brain downregulating psychomotor activity in hyperactive children, although dopamine is known to enhance motor activity? During normal nerve activity dopamine rises 60-fold outside the cell. After administration of low therapeutic doses of psychostimulants the locomotion in both humans and animals is reduced. The hypothetical explanation would be that the drug raise resting extracellular levels of dopamine several-fold, but reduce the extent to which dopamine is released with nerve impulses, compared to the impulse-associated release in the absence of the drug (Figure 3). This reduced amplitude of impulse-associated dopamine release would finally result in less activation of post-synaptic dopamine receptors which control psychomotor activity (Robbins, 2002; Seeman and Madras, 2002). Additionally, the number of functional D1 and D2 receptors seems to be reduced by the elevated dopamine in the synaptic cleft – in other words, D1 and D2 which are characterized by high-affinity state for dopamine, in this

case would be less stimulated due to their decrease in amount. Such state might be caused by constant occupation of some pool of the receptors by enhanced dopamine during rest, and thereby by desensitization of dopaminergic subcellular system (Seeman and Madras, 2002).

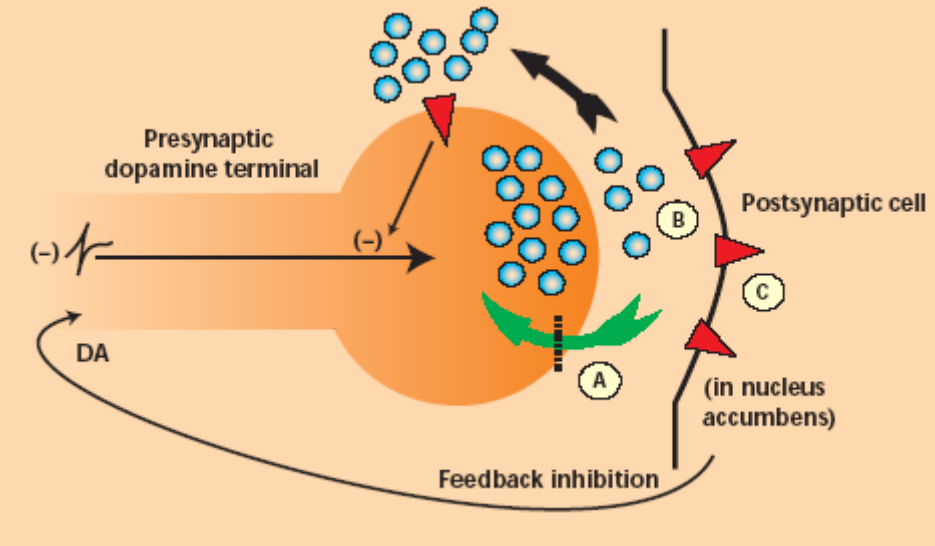

Figure 3. Hypothetical action of methylphenidate at the synapse (picture from Robbins, 2002). Methylphenidate blocks the dopamine transporter (A), which results in the increase of dopamine (blue circles, B) in the synaptic cleft. Dopamine can stimulate its receptors (red triangles, C), but due to the feedback inhibition it could lead at the end to the decline of dopamine release and thereby to the suppression of hyperactive behavior.

Methylphenidate produces effects not only on dopamine, but also on noradrenaline. Low density of dopamine transporters may lead to the inactivation of extracellular dopamine by its uptake into noradrenaline nerve terminals (Tanda et al., 1997; Wayment et al., 2001). The administration of clinical doses of MP and subsequent rise in extracellular noradrenaline may result in increase of cortical dopamine and as a consequence cortical dopaminergic transmission may be facilitated

at doses of the stimulant that do not affect accumbens dopamine (Kuczenski and Segal, 2002).

There is also some evidence, that the action of methylphenidate is somehow linked to the serotonergic activity of central nervous system, although serotonin levels themselves are not affected by this particular drug (Kuczenski and Segal, 1997), nor the affinity of methylphenidate for the serotonin transporter is very low (Gatley et al., 1996). The induction of serotonin increase by citalopram (5-HT re-uptake inhibitor) in rat hippocampus is enhanced by methylphenidate, however MP attenuates the 5-HT citalopram mediated release in prefrontal cortex (Weikop et al., 2007). The study of Gainetdinov et al. (1999) showed that hyperactivity of dopamine transporter gene knock out mice (DAT-KO) might be reduced by serotonergic drugs (fluoxetine). Anyway, the data on serotonin and its role in ADHD need further extensive studies.

The use of methylphenidate in children remains controversial. Some of the contras raised by the opponents of this particular medicine are: short term help provided by MP as well as the risk of cross-sensitization to cocaine and amphetamine (which are relatively similar to MP in terms of pharmacological effects) and thereby the possibility of developing the long-term sensitization to psychostimulants and drug addiction (Huss and Lehmkuhl, 2002; Robbins, 2002).

1.6 *Octodon degus* – an animal model for the study of early life stress

Octodon degus (trumpet-tailed rat or degu) is a semi-precocial, diurnal, caviomorph, hystricomorph rodent originating from central Chile (Figure 4). This particular species has been chosen for extensive psychobiological experiments on account of several interesting features, which make it different from other popular laboratory animals such as rats and mice (Wright and Kern, 1992). For instance, degus as semi-precocial animals are born with functional sensory systems, e.g. open eyes and ears, which allow them, like human babies, to establish an intensive contact with the mother immediately after birth. Moreover, they display relatively advanced behavior – they live in complicated family units, where they communicate with each other using an elaborated set of sounds (Fulk, 1976; Braun and Scheich, 1997; Ebensperger and Bozinovic, 2000). In the field they usually live in groups of around 10 adult animals

(mostly females, which are closely related, and 1-3 males). In the wild degus breed once per year, at the beginning of rainy cold season but a second litter might be also observed (depending on the length of the winter season). The pregnancy lasts for 90-95 days, young animals suckle for the next 28-35 days (Lee, 2004). Finally, not only the mother, but also the father takes care of the pups during early development. Interestingly, in the home environment degu female kin have been found to nest the pups communally, also the non-offspring nursing exists in this species (Ebensperger et al., 2002; 2004). Taken together, these features were quite encouraging to use the degu as an animal model to study the effects of parental separation in this semi-precocial species living in an elaborated family structure.

Besides of being an animal model for investigations of parents' role in early development, *Octodon degus* is also used for studying the mechanisms of visual adaptations (Jacobs et al., 2003), diabetes and cataract development (Datiles and Fukui, 1989), circadian rhythms and recovery from Jet Lag (Garcia-Allegue et al., 1999; Kas and Edgar, 1999; Lee, 2004; Mohawk et al., 2005), drug research (Pellissier et al., 1989) as well as Alzheimer's disease (Inestrosa et al., 2005).

Figure 4. Adult *Octodon degus* couple with 1-day-old pups in the cage. Picture taken with Konica-Minolta Dimage Z6 digital camera.

1.7 Aims of the dissertation

The advanced development of the degu pups at birth in relation to the altricial species (rat and mouse), the crucial role of early life experience and neurotransmitters in normal development and physiology as well as pathophysiology, and the severe neurochemical consequences induced by disturbing the mother-offspring interaction in several rodents, the degu and primates, led me to the working hypothesis that in degus (*i*) the neurotransmission systems are showing adult-like characteristics early in ontogeny, (*ii*) acute separation acts as a severe stressor and induces alterations in neurotransmission, and (*iii*) repeated separation stress has a persistent impact on monoaminergic neurotransmission. To test these hypotheses systematically the first part of my dissertation:

Part I. **Epigenetic influences on neurotransmission in *Octodon degus* during early postnatal development: study in brain homogenates**

was designed to address the following questions:

(i) What is the developmental pattern of serotonin (5-HT) and its metabolite 5-hydroxyindole-3-acetic acid (5-HIAA), dopamine (DA) and its metabolites 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA), as well as brain amino acids: aspartate, glutamate, GABA and taurine in the cortex and hippocampus of the semi-precocial *Octodon degus*? Is the advanced developmental stage at birth of this species paralleled by relatively matured neurotransmitting systems?

(ii) What is the impact of a single, acute parental separation at different stages during early development (at postnatal day (PND) 3, 8, 14, or 21) or

(iii) repeated parental separation during the first 3 weeks of life, i.e. the phase of the most intensive emotional bounding between parents and offspring, on the serotonergic transmission in cortical regions and the hippocampus at PND 21? Previous extensive experiments of our working group on dopaminergic transmission showed, that neither dopamine nor its metabolites (DOPAC and HVA) does not produce any clear pattern of changes, in both experimental designs, therefore these data are not shown.

The second part of my dissertation:

Part II. Neurochemical consequences of juvenile stress and chronic methylphenidate treatment on dopaminergic function in *Octodon degus*: *in vivo* microdialysis study

focused on the dopaminergic neurotransmission and was designed based on the finding that repeatedly separated degus develop hyperactive and inattentive behavior. Therefore, based on this result I proposed that repeatedly stressed, hyperactive degu pups would be a potential animal model for the study of ADHD and the action of methylphenidate in the brain. My working hypothesis for this part of my dissertation was that repeatedly separated (prestressed) animals with hyperactive symptoms respond differently to this drug, compared to controls. This was tested for the dopaminergic responses in two limbic structures: medial prefrontal cortex and nucleus accumbens. Moreover, I hypothesized that the age of the animal as well as the duration of drug treatment would be also the critical factors for methylphenidate action in the brain. Since the effects of this drug as well as the underlying mechanisms on dopaminergic function in the juvenile brain have not yet been investigated on the neurochemical level, this part of the dissertation using a quantitative neurochemical approach was designed to address the following questions:

(i) What is the effect of social separation stress and acute methylphenidate treatment on extracellular dopamine levels in medial prefrontal cortex (mPFC) and nucleus accumbens (Nac) at post-weaning (PND 22-24) and puberty (PND 46-48) ages in unstressed and repeatedly stressed male *Octodon degus*?

(ii) What is the effect of chronic methylphenidate pretreatment on extracellular dopamine levels in the mPFC and Nac at puberty age (PND 46-48) in unstressed and repeatedly stressed male *Octodon degus* during acute social separation stress and methylphenidate challenge?

2 MATERIALS AND METHODS

2.1 Chemicals

Sigma: Methylphenidate, DA, HVA, DOPAC, 5-HT, 5-HIAA, EDTA, NaCl, KCl, $CaCl_2$, $MgCl_2$, CH_3COONa, ortho-phtaldialdehyde, sodium metabisulfite and octanesulfonic acid.

Serva: cresyl violet, aspartate, glutamate, taurine and GABA.

Merck: NaH_2PO_4, acetic acid, perchloric acid, phosphoric acid, acetonitrile, xylol and methanol.

Life Sciences: Histomount

Eurim-Pharm: Halothane

Roth: 2-propanol

Harvard Dental: dental cement

B. Braun Melsungen AG: saline

2.2 Animals: housing and rearing conditions

The degus (*Octodon degus*) used in both experimental approaches were bred in our colony at the Leibniz Institute of Neurobiology, Magdeburg. Family groups consisting of an adult couple and their offspring were housed in wire cages (length x height x depth: 49 x 68 x 40 cm) located in temperature- (22 ± 2°C) and humidity-controlled (55 ± 3%) rooms (Figure 5). The degus were exposed to a 12 h light – 12 h

dark cycle with light on at 06:00 a.m. and had free access to food (rat diet pellets, SSNIFF Spezialdiäten GmbH, Soest, Germany) and water.

All experiments were performed in accordance with the European Communities Council Directive of November 1986 (86/609/EEC) and according to the German guidelines for the care and use of animals in laboratory research and the experimental protocols were approved by an ethical committee.

Figure 5. The degus colony at Leibniz-Institute of Neurobiology, Magdeburg. Each cage contains one family (1 couple and its offspring). Picture taken with Konica-Minolta Dimage Z6 digital camera.

Each breeding couple was checked for litters daily, and the day of birth was defined as postnatal day (PND) 0. To avoid litter effect - differential parental treatment within a litter, as well as genetical similarity of siblings, the whole litter was randomly assigned to one of the following rearing conditions:

unstressed controls: The litters were left undisturbed with their parents until PND 21 (day of weaning), after which the parents were removed from the home cage. The siblings remained together until PND 45, and were thereafter raised as sex-matched groups of two to three siblings from PND 46 until the experiment.

repeated separation stress: From PND 1 to PND 20, the pups were removed from their parents and home cage. In a separate room (temperature: 22 ± 2°C), the pups were kept individually in opaque rodent cages (length x height x depth: 39 x 10 x 10 cm) for one hour daily (starting between 09:00 a.m. and 12:00 a.m.). Thus, during separation the pups had acoustic and olfactory but no visual and social contact with their siblings. After one hour separation pups were returned to their parents and home cage and left undisturbed until next separation period.

Part I. Epigenetic influences on neurotransmission in *Octodon degus* during early postnatal development: study in brain homogenates

2.3 Preparation of brain homogenates

Animals were sacrificed by decapitation, the brains were quickly removed from the skull and placed on an ice-cold dissection plate. Four brain regions were dissected out of each brain: medial prefrontal cortex (mPFC), frontal cortex (including the orbital, primary motor and frontal part of somatosensory cortex), caudal cortex (including the caudal part of somatosensory cortex and visual cortex), and hippocampus. The tissue from both hemispheres was pooled and frozen in liquid nitrogen and stored at -80°C until assayed.

To determine the levels of neurotransmitters in the brain homogenates, the tissue was weighted (= tissue wet weight), homogenized in ice-cold homogenization buffer (0.1 M perchloric acid, 0.1 mM EDTA, 2 mM sodium metabisulfite) and sonicated (Sonoplus HD60, Bandelin, Germany). Subsequently, each sample was centrifuged at 20,800 x g for 15 min at 4°C (Eppendorf centrifuge 5417R, Germany), supernatants were filtered through a syringe filter (0.2 μm pore size, Whatman, USA) and immediately analysed by high performance liquid chromatography (HPLC).

Animals of both rearing groups were used for the following experiments (for the number of animals and litters used at each age see Table I). Each animal was used in a single experiment only. All experiments were carried out during the light phase, i.e. during the activity phase of this day-active species.

2.4 Experiment 1: Postnatal development of neurotransmission

To examine the maturation of neurotransmission, monoaminergic neurotransmitters and their related metabolites as well as amino acids were determined in brain homogenates of unstressed control male and female degus. At PND 3, 8, 14, 21, 45 or 90 (considered as adulthood), the animals were removed from their home cage and immediately sacrificed for the preparation of brain tissue as described above. A maximum of one male/ one female was used from each litter.

2.5 Experiment 2: Age-dependent impact of an acute separation stressor on serotonergic neurotransmission

To examine the effect of an acute, single stress challenge on serotonergic neurotransmission during early development, the levels of serotonin and its metabolite 5-HIAA were examined in previously unstressed control male and female degu pups at different ages (PND 3, 8, 14, or 21). To measure basal as well as stress-evoked neurotransmitter levels, the pups were removed from the home cage and either *(i)* immediately sacrificed (= basal, control values) or *(ii)* subjected to an acute stress-challenge (i.e., a single episode of 1 hour parental separation stress, under the conditions described for repeated parental separation stress) and thereafter immediately sacrificed for the preparation of brain tissue as described above. A maximum of one male/ one female for basal measurement and one male/ one female for the measurement after stress challenge was used from each litter.

2.6 Experiment 3: The impact of repeated separation stress on basal and stress-evoked serotonergic neurotransmission at PND 21

To reveal the impact of repeated parental separation during the first three weeks of life, the levels of serotonin and 5-HIAA in brain homogenates of previously unstressed controls and male and female pups exposed to repeated separation stress were analyzed. At PND 21, animals from both groups were removed from their home cage and either *(i.)* immediately sacrificed (= basal values) or *(ii.)* subjected to an acute stress-challenge (i.e., an episode of 1 hour parental separation, under the conditions described for repeated parental separation stress) and thereafter immediately sacrificed for the preparation of brain tissue as described above. Thus, for the control animals this stress-challenge at PND 21 was the 1st stress exposure (i.e., novel situation), whereas for the repeatedly separated animals this stress challenge was the 21st stress exposure (i.e., a potentially familiar situation). A maximum of one male/ one female for basal measurement and one male/ one female for the measurement after stress challenge was used from each litter.

Table I. Number of animals (males/females) and litters [given in square brackets] used in Part I, Experiments 1- 3. ***** - same animals as in Experiment 1.

Age	**Experiment 1**	**Experiment 2**		**Experiment 3**			
	unstressed controls	unstressed controls *	acute separation stress challenge	unstressed controls	unstressed rearing + acute separation stress challenge	repeated separation stress	repeated separation stress + acute separation stress challenge
PND 3	8/8 [14]	8/8 [14]	9/7 [14]	-	-	-	-
PND 8	8/8 [10]	8/8 [10]	8/8 [10]	-	-	-	-
PND 14	8/8 [8]	8/8 [8]	8/7 [8]	-	-	-	-
PND 21	8/8 [9]	8/8 [9]	7/7 [9]	8/8 [11]	8/8 [11]	9/8 [12]	8/10 [12]
PND 45	7/7 [10]	-	-	-	-	-	-
PND 90	6/6 [7]	-	-	-	-	-	-

2.7 HPLC analysis of brain homogenates

Monoaminergic neurotransmitters DA and its metabolites 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) as well as 5-HT and its metabolite 5-hydroxyindole-3-acetic acid (5-HIAA) (Figure 6) were quantified under isocratic conditions (flow rate: 0.7 ml/min) using a reversed phase HPLC column (100x3 mm, packed with YMC ODS-AQ®, 5 μm, YMC, Germany) with the use of electrochemical detection. The mobile phase consisted of 75 mM NaH_2PO_4 (pH 4.6) with 1 mM EDTA, 0.2 mM octanesulfonic acid including 3.5% (v/v) acetonitrile. A glassy carbon working electrode (electrochemical flowcell: VT-03, ANTEC, The Netherlands) was set at +780 mV against an Ag/AgCl reference electrode. The amounts of the measured substances were quantified using an external calibration curve and expressed as ng/g wet weight.

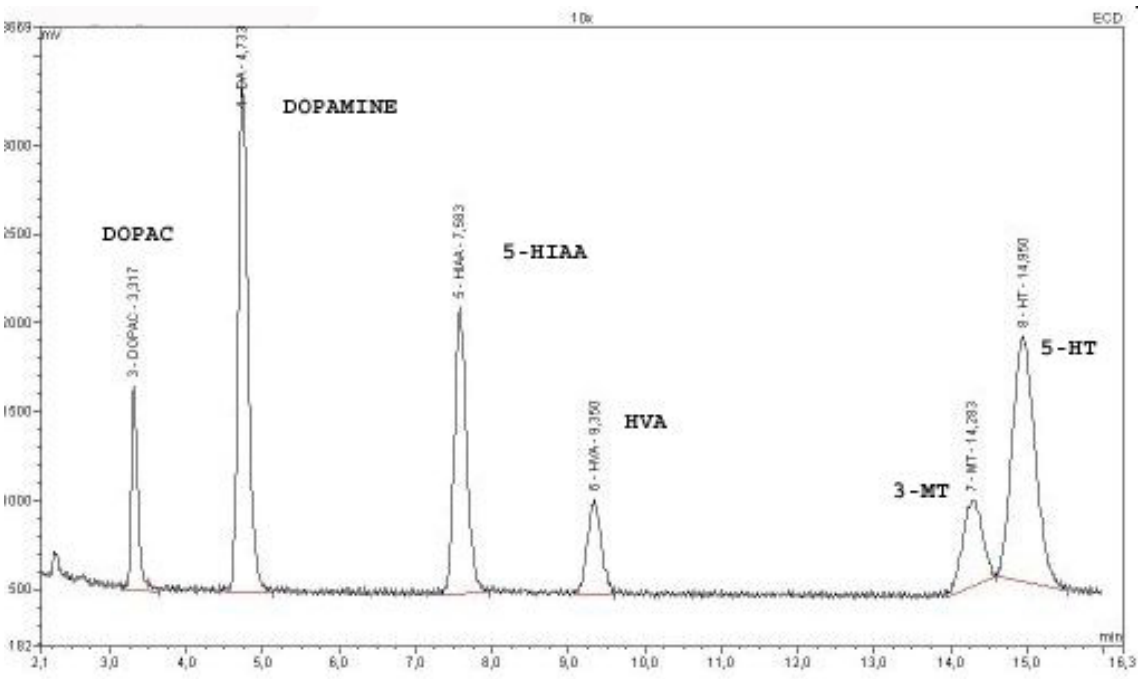

Figure 6. The chromatogram representing the separation of monoamines standards: DOPAC, dopamine, 5-HIAA, HVA, 3-MT (3-methoxytyramine, metabolite of dopamine) and 5-HT.

Amino acids (Figure 7) were measured after an automatic pre-column-derivatization procedure using ortho-phtaldialdehyde (10 µl of the sample + 50 µl ortho-phtaldialdehyde- reagent, reaction time: 2 minutes) following the modified method of Lindroth and Mopper (1979). Aspartate, glutamate, taurine, and GABA were separated using a reversed-phase HPLC column (125x4 mm, packed with LiChrospher®, 5 µm, Merck, Germany) by a non-linear gradient elution method (flow rate: 1.5 ml/min). The mobile phase consisted of 0.15 M phosphate buffer (pH 7.0) including 5-75 % methanol. With the use of fluorescence detection (RF2000 detector, Dionex, Germany) with excitation wavelength set at 330 nm and emission wavelength set at 450 nm, the amounts of amino acids were quantified using an external calibration curve and expressed as µg/g wet weight.

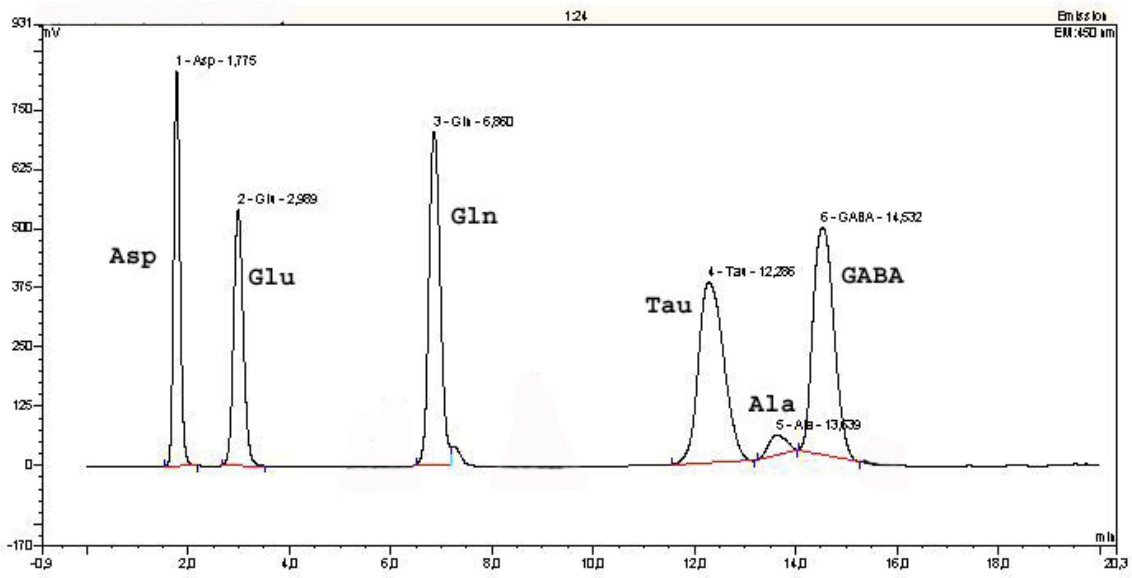

Figure 7. The chromatogram presenting the separation of amino acids standards: aspartate (Asp), glutamate (Glu), glutamine (Gln), taurine, alanine (Ala) and GABA.

2.8 Statistics

For statistical analysis (SigmaStat 2.0, Jandel; Germany) non-parametric tests were used, as most of the data failed to show normality and/or equal variance (even after several transformation procedures). The changes in tissue wet weights and levels of neurotransmitters during development (Experiment 1) were analysed using Kruskal-Wallis one-way analysis of variance (ANOVA) followed by *post hoc* Dunn's test (with PND 90 as the control group). All other comparisons were performed by using the Mann-Whitney U-test. For all statistical tests the level of significance was set at $p<0.05$.

Part II. Neurochemical consequences of juvenile stress and chronic methylphenidate treatment on dopaminergic function in *Octodon degus*: *in vivo* microdialysis study

2.9 Animals

Degus used for the microdialysis experiments were housed and reared under the conditions given in Materials and methods, Part I. Animals of two rearing groups were used for the microdialysis experiment at two developmental time points: postweaning age (PND 23-24) or puberty (PND 47-48) (for schematic representation of the experimental groups see Figure 8). Only male animals were used in microdialysis experiments.

PND 22-24: at PND 22 (i.e. for the repeatedly separation stressed animals the last episode of parental separation took place at PND 21), between 12:00 a.m. – 01:00 p.m. 3 animals (siblings) were removed from the home cage and placed in the microdialysis rodent bowl (height: 35.56 cm, diameter: 40.64 cm, Bioanalytical Systems, USA) equipped with bedding and with free access to food and water. Those 3 animals, familiar to each other, were supposed to form the familiar environment, which was subsequently disturbed in the forthcoming experiment by the application of separation stress (see below). The rodent bowl was placed in a separate room (temperature: 22 ± 2°C, 12 h light – 12 h dark cycle with light on at 06:00 a.m.) for the whole experiment. Two animals from each litter were used at two consecutive days for the microdialysis experiments (see below).

PND 46-48: From PND 22 the animals were injected daily either with saline (200 µL, i.p.) or methylphenidate (MP) solution (10 mg/kg, in saline, 200 µL, i.p.). At PND 46 between 12:00 a.m. – 01:00 p.m. 3 animals (siblings) were removed from the home cage and subjected to the same procedure as the degus at PND 22. From each litter one animal repeatedly injected with saline and one repeatedly injected with MP solution was used.

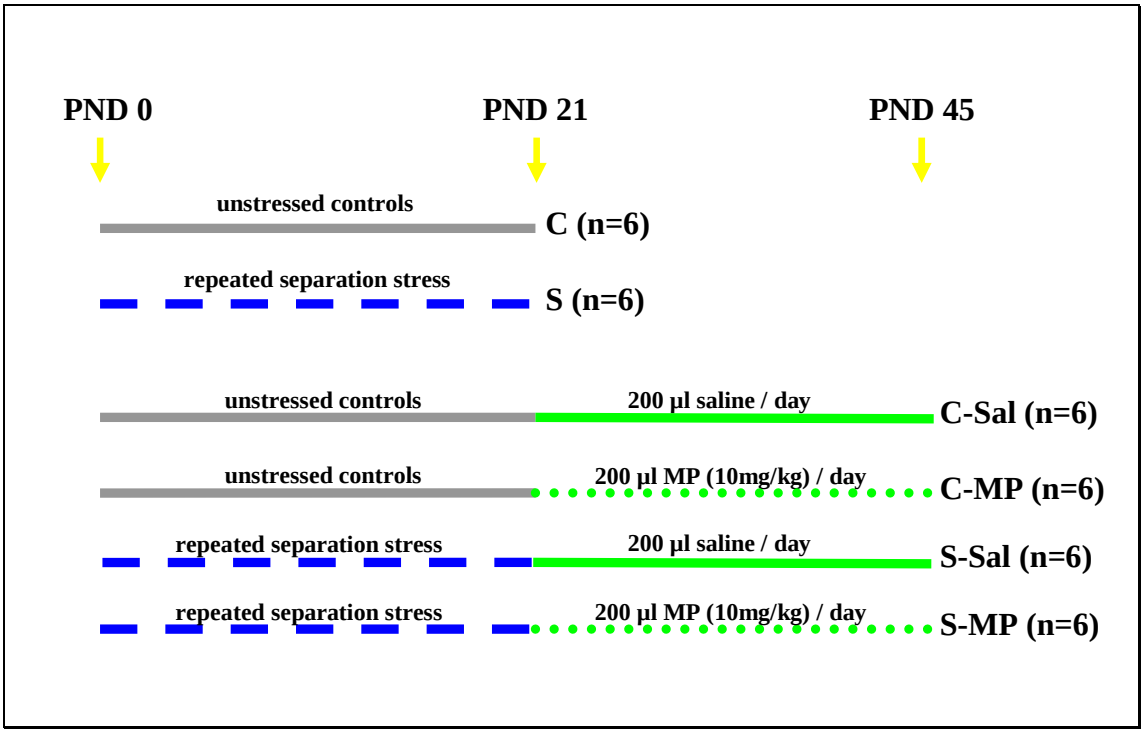

Figure 8. Schematic representation of experimental groups used for *in vivo* microdialysis experiments.

2.10 Microdialysis

2.10.1 The principles of microdialysis

Microdialysis is a unique technique which enables monitoring of the concentration of numerous substances in the living tissue. It was introduced in the 1970's as a useful tool for measuring the release of particular substances in the brain tissue. The principles of microdialysis are very simple and based on free diffusion of substances between two solutions of different concentration (concentration gradient).

Microdialysis can be applied to determine either the pharmacokinetic profile of administered drug in the area of interest (target tissue) (Kreilgaard, 2002) or the amounts of substances of natural origin, such as neurotransmitters in brain tissue (Timmerman and Westerink, 1997). Moreover, when applied to the living, conscious,

freely moving animal, microdialysis enables the researcher to combine the quantitative neurochemical approach with the behavioral or pharmacological experiment (Westerink, 2002; Weikop et al., 2004). In addition, with the use of reverse microdialysis one can administer pharmacological agents locally through microdialysis probe, and measure neurochemical changes in parallel.

The design of a microdialysis probe, as it was used in this study, is presented in Figure 9. Each probe has an inlet, where the infusate (physiological solution, for instance artificial cerebrospinal fluid, which may additionally contain the drug of interest) is slowly delivered with the use of microinjection pump. The microdialysis semi-permeable membrane is exposed to the particular location of the brain, where the measurements take place. Semi-permeable means that it allows the passage of molecules with volumes smaller than the pores in the membrane ("cut-off" value). The substances smaller than the "cut-off" value of the membrane are slowly exchanged between the infusate and the tissue. During the dialysis, the infusate is collected via the probe's outlet into vials and subjected subsequently to the further analysis.

One of the parameters characterizing the microdialysis technique is relative recovery. Relative recovery provides the information on the percentage of substance that can be recovered from extracellular space in particular time unit. The relative recovery of a substance from the tissue depends on:

1. The flow rate of perfusate
2. The dimensions of the membrane – its length and diameter
3. The "cut-off" value of the membrane, i.e. the size of the pores
4. The temperature of tissue
5. The diffusion coefficient of the compounds through the extracellular fluid
6. The analysed sample – its molecular weight, charge, structure
7. The unspecific binding of the substance to the membrane and/or connecting tubings.

Relative recovery increases with the slow down of the flow. Whet the flow stops the recovery reaches 100%.

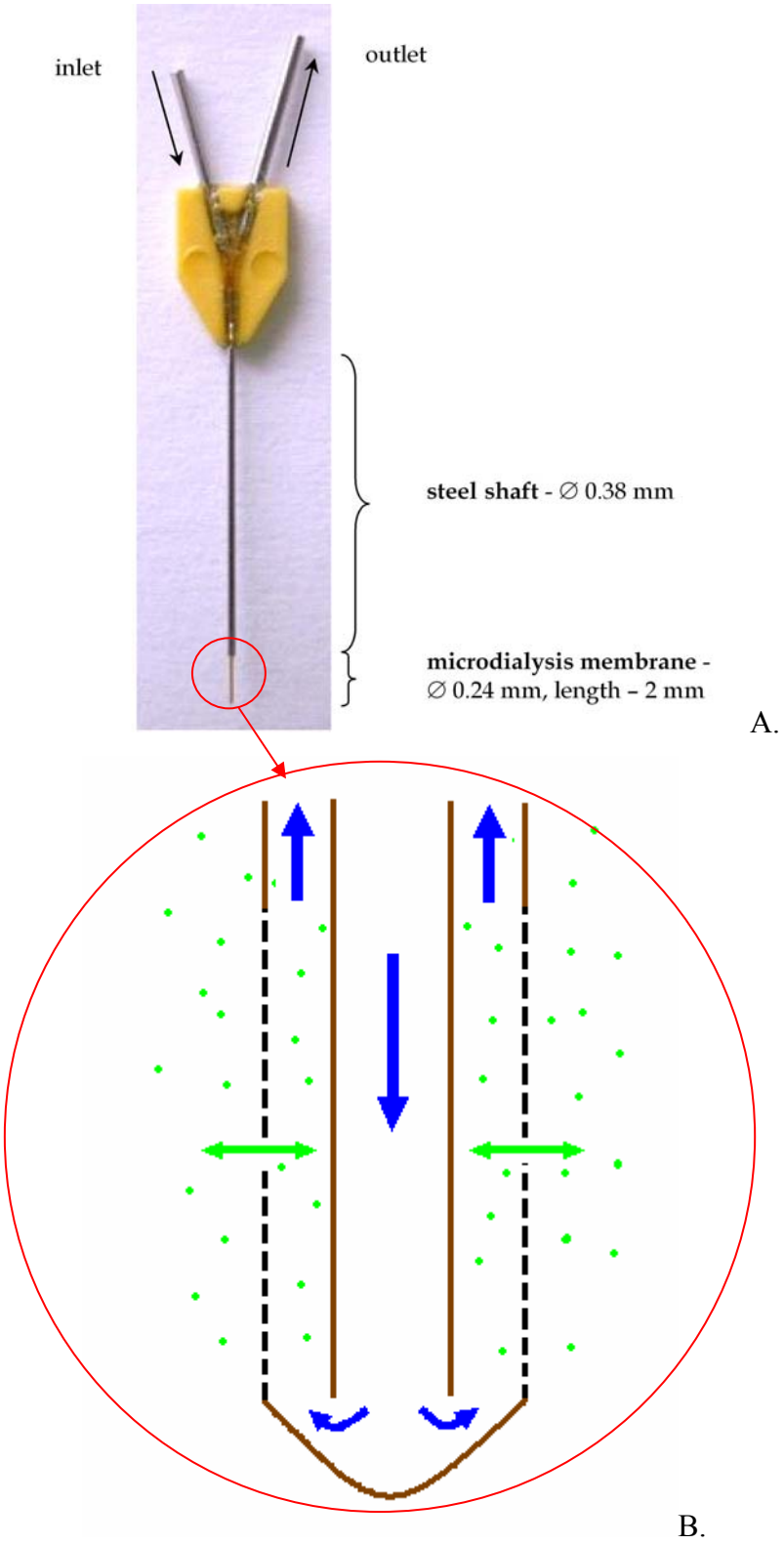

Figure 9. A. The photograph of entire microdialysis probe used in this study (picture taken with Konica-Minolta Dimage Z6 digital camera). **B.** Schematic representation of the tip of microdialysis probe (membrane) – blue arrows indicate the flow direction, green arrows – the diffusion of substance of interest (green dots).

2.10.2 The microdialysis experiments

For the implantation of microdialysis probes (CMA/11, 2 mm, Cuprophan, CMA Microdialysis, Sweden) the animals were anesthetized with 3% halothane and fixed in the stereotaxic apparatus (starting between 03:00 pm – 04:00 pm, on the day of the removal from the home cage or at the next day). Two microdialysis probes were implanted into the left prefrontal cortex (PND 22-24: 1.4 mm anterior to bregma, 0.4 mm lateral, and 2.8 mm below dura; PND 46-48: 2.3 mm anterior to bregma, 0.5 mm lateral, and 2.8 mm below dura) and right nucleus accumbens (PND 22-24: 0.4 mm posterior to bregma, 1.4 mm lateral, and 7.5 mm below dura; PND 46-48: 1.3 mm posterior to bregma, 1.4 mm lateral, and 7.8 mm below dura) according to the stereotaxic atlas of the degu brain (Wright and Kern, 1992, modified). For the schematic representation of the probes placement see Figure 10. The two probes had to be implanted into different hemispheres due to space limitations on the small skulls of young animals. After insertion, the microdialysis probes were secured to the skull using dental cement (Harvard Dental, Germany). The degus were allowed to recover from anesthesia and surgery for approximately 16 h in the presence of 2 siblings to avoid the stress due to social separation. The siblings were also present during the whole experiment (except for the separation procedure, see below).

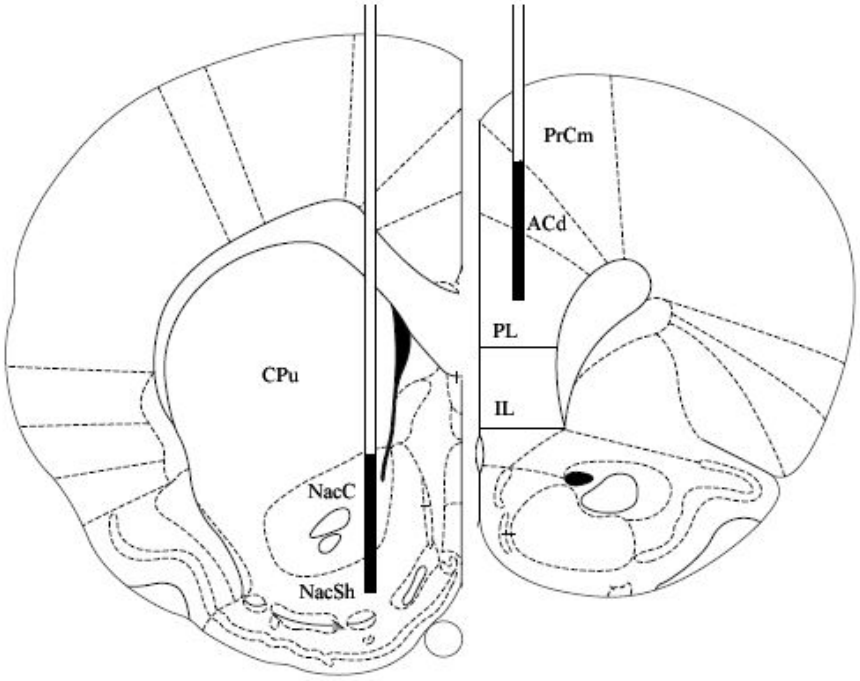

Figure 10. Schematic illustration of microdialysis probes placement in left prefrontal cortex (right) and right nucleus accumbens (left), according to Wright and Kern (1992). Figures reproduced from Paxinos and Watson (1998). PrCm – precentral medial cortex, ACd – anterior cingulate cortex, PL – prelimbic cortex, IL – infralimbic cortex, NacC – nucleus accumbens core, NacSh – nucleus accumbens shell, CPu – caudate putamen.

The infusion of the artificial cerebrospinal fluid (aCSF) was started on the day of implantation. The composition of aCSF was as follows: 140 mM NaCl, 4 mM KCl, 2.4 mM $CaCl_2$, and 1 mM $MgCl_2$. On the day of the experiment the baseline, defined as six consecutive samples with <10% variation in levels of DA, was established (0' – 120'). The microdialysates were collected every 20 (separation procedure) or 10 minutes (methylphenidate injection), with the flow rate of 1.2 μL/min, using a microinjection pump (CMA/100, CMA Microdialysis, Sweden). For the separation procedure the samples were collected only every 20 min in order not to stress an animal additionally with picking up the sample from microdialysis room. Collecting samples every 10 min after MP injection allowed to obtain better time resolution and monitor the effect of MP in more detailed way. After establishing the baseline the animal was placed alone in a novel environment where it was subjected to 1 hour social separation from its siblings (acute separation stress), with parallel counting of animal's vocalizations (121' – 180'),

as a marker of emotional state of the animal. After separation, each subject was brought back to the siblings and the samples were collected for the next 1 h (181' – 240'). In the second part each implanted animal was injected with methylphenidate (10 mg/kg, in saline, i.p.) and the samples were collected for the next 3 h (241' – 420'). For the schematic representation of experimental approach see Figure 11.

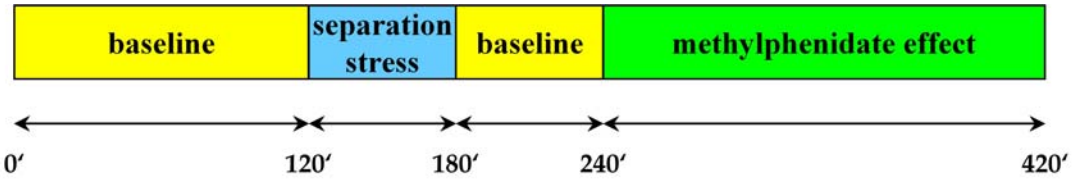

Figure 11. Schematic design of the *in vivo* microdialysis experiment.

2.11 HPLC analysis of microdialysates

Dopamine was quantified under isocratic conditions (flow rate: 0.5 ml/min) using a reversed phase HPLC column (100x2.1 mm, packed with YMC ODS-AQ®, 5µm, YMC, Germany) by electrochemical detection. The mobile phase consisted of 75 mM phosphate buffer (pH 4.6) with 1 mM EDTA, 1.7 mM octanesulfonic acid including 7.5% (v/v) acetonitrile. A glassy carbon working electrode (electrochemical flowcell: VT-03, ANTEC, The Netherlands) was set at +650 mV against an in situ Ag/AgCl reference electrode (3 mM NaCl in the mobile phase). The detection limit of 0.1 nM was routinely achieved. 5-HT and noradrenaline were not measurable under these conditions.

2.12 Histology

The correct placement of the microdialysis probes in the PFC and Nac was histologically verified in each animal by examining series of transversal 40-µm cryostat (Micron, Walldorf, Germany) sections, which were counterstained with cresyl violet (Nissl stain). Each slide containing brain sections was incubated in the following solutions in the given order:

- acetate buffer (50 mM CH_3COONa, 50 mM CH_3COOH, pH 4.6) – 3 min
- 0.5 % cresyl violet – 5 min
- distilled H_2O – 1 min
- acetate buffer – 5 min
- 50 % ethanol – 4 min
- 70 % ethanol – 4 min
- 96 % ethanol – 2-4 min
- 2-propanol – 4 min x2
- xylol – 10 min x2

Finally, the stained sections were mounted with coverslips with appropriate Histomount (Life Sciences) medium.

2.13 Statistics

For the within-group statistical analysis, the effects of acute social separation stress and acute MP-injection were compared with mean baseline values using a repeated measurement ANOVA on ranks followed by a Dunn´s test (SigmaStat 2.0, Jandel; Germany). For the between-group statistical analysis, area-under-curves (AUC´s) were calculated for periods of acute social separation stress ($t_{120\text{-}180}$) as well after acute MP-injection ($t_{250\text{-}280}$, $t_{280\text{-}310}$, $t_{310\text{-}340}$), and either a t-test (two experimental groups) or the General Linear Model including Bonferroni's adjustment for multiple comparisons followed by a t-test was applied (SPSS 8.0.0.). In Experiment 1 (postweaning age), data were analyzed by a 2x3- design (acute MP-injection) with the main factor of rearing conditions (controls/early stressed) and a repeated measurement factor of time following acute MP injection ($t_{250\text{-}280}$, $t_{280\text{-}310}$, $t_{310\text{-}340}$). In Experiment 2

(puberty), either a 2x2-design (acute social separation stress) with the main factors of rearing conditions and pretreatment conditions (saline/MP), or a 2x2x3- design (acute MP-injection) with the main factors of rearing conditions as well as pretreatment conditions, and a repeated measurement factor of time following MP injection was applied. To reveal the relationship between number of distress calls and the increase in DA, the Pearson´s correlation procedure was carried out. In all tests, the significance level was set at $p<0.05$.

3 RESULTS

Part I. Epigenetic influences on neurotransmission in *Octodon degus* during early postnatal development: study in brain homogenates

3.1 Changes of tissue wet weights during postnatal development

As an indicator of the overall brain maturation, the postnatal development of tissue wet weights were determined (Figure 12). In general, an increase in tissue wet weights during development was observed for males and females in the mPFC ($H_{(5)}$=37.5, p<0.001, and $H_{(5)}$=46.2, p<0.001, respectively; Kruskal-Wallis ANOVA), frontal cortex ($H_{(5)}$=37.5, p<0.001, and $H_{(5)}$=36.0, p<0.001, respectively), caudal cortex ($H_{(5)}$=64.4, p<0.001, and $H_{(5)}$=59.3, p<0.001, respectively) and hippocampus ($H_{(5)}$=63.7, p<0.001, and $H_{(5)}$=67.2, p<0.001, respectively). At the earliest time point measured (PND 3) the tissue wet weights reached in both sexes already 54/ 54 % (males and females, respectively) in mPFC, 58/ 59 % in the frontal cortex, 45/ 50 % in the caudal cortex and 49/ 53 % in the hippocampus of adult values (PND 90). Whereas the mPFC reached adult-like wet weights already at PND 21 and the frontal cortex at PND 45, the caudal cortex and hippocampus showed a continuous increase until adulthood (PND 90). In addition, no sex differences in tissue wet weights were found (except for the caudal cortex at PND 90 with males higher (p=0.028, Mann-Whitney U-test) than females).

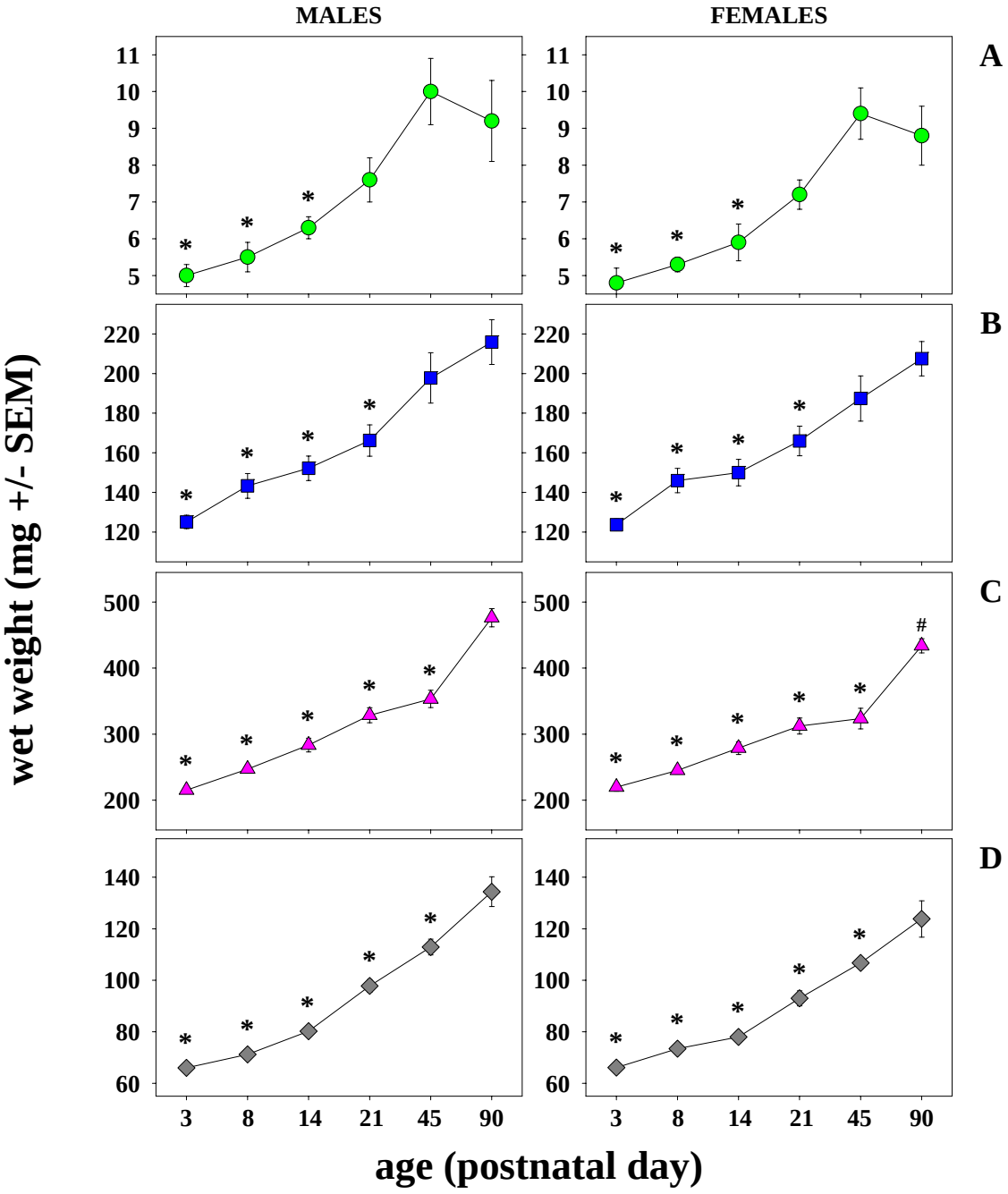

Figure 12. The postnatal development of the tissue wet weights (mg ± SEM) of the mPFC (A), frontal cortex (B), caudal cortex (C), and hippocampus (D) of male (left) and female (right) *Octodon degus*.

***** $p<0.05$ vs. PND 90

\# $p<0.05$ vs. males of the same age

3.2 Experiment 1: Postnatal development of neurotransmission

3.2.1 Dopaminergic system

3.2.1.1 Dopamine, DOPAC and HVA

The developmental changes in DA, DOPAC and HVA levels are presented in Figure 13. In general, the levels of DA increased in male mPFC ($H_{(5)}$=21.5, p<0.001, Kruskal-Wallis-ANOVA) and male and female frontal cortex ($H_{(5)}$=24.0, p=0.023 and $H_{(5)}$=15.9, p<0.001, respectively). The same was found for DOPAC in male and female mPFC ($H_{(5)}$=23.3, p<0.001 and $H_{(5)}$=19.8, p=0.007, respectively), frontal cortex ($H_{(5)}$=23.5, p<0.001, and $H_{(5)}$=20.2, p<0.001, respectively) and female caudal cortex ($H_{(5)}$=19.2, p=0.009). The levels of DOPAC and HVA decreased in male hippocampus ($H_{(5)}$=19.1, p=0.031 and $H_{(5)}$=25.4, p=0.003, respectively). All 3 substances reached adult-like levels relatively early during ontogeny, i.e. until PND 21.

In more detail, in the **mPFC** in females, adult-like levels of DA were reached earlier than in males (PND 3 and PND 8, respectively). For DOPAC in males an increase starting at PND 3 (20 % of adults, p<0.05) was observed until adulthood. In females, the levels of DOPAC expressed no statistical difference from adult-like levels at PND 3 (36 % in comparison to adulthood). At PND 45 the levels of DOPAC in females were significantly higher than in males (p=0.011, Mann-Whitney U-test). The levels of HVA at PND 3 reached in males 90 % (p<0.05, Dunn´s test) and in females 100 % of adult-like amounts, in both sexes this developmental point showed no statistical difference from the adults. At PND 21 the levels of HVA in females were significantly lower than in males (p=0.021, Mann-Whitney U-test).

In the **frontal cortex**, at PND 3, the levels of DA reached 75 % of adult-like amounts in males and 78 % in females. Adult-like levels of DA were observed already at PND 3. For DOPAC an increase starting at PND 3 (males: 18 %, females: 36 % of adults; p<0.05) was observed reaching adult-like levels at PND 8 (both sexes). At PND 14 the levels of DOPAC in females were significantly lower than in males (p=0.038, Mann-Whitney U-test). The levels of HVA at PND 3 reached in males 94 % and in

females 104 % of adult-like amounts, with no developmental significance. At PND 8 the levels of HVA in females were significantly higher than in males ($p=0.015$, Mann-Whitney U-test).

In the **caudal cortex**, the DA values reached 52 % of adult-like levels in males and 45 % ($p<0.05$) in females at PND 3. In males, DA reached adult-like amounts at PND 3, in females it increased until PND 8. Moreover, DOPAC (at PND 3: 35 % and 44 % ($p<0.05$) compared to adults in males and females, respectively) reached the adult-like levels at PND 3 in males and at PND 14 in females. HVA at PND 3 showed 60 % and 67 % of PND 90 amounts in males and females, respectively. In both sexes at the earliest developmental point tested, HVA reached already adult-like levels.

The **hippocampus**, among the regions analyzed, was characterized by the lowest concentrations of DA and DOPAC. At PND 3, DA reached 72 % of adult-like levels in males and females. The concentrations of this monoamine did not change during development significantly in any of the sexes. The levels of DOPAC (at PND 3: 103 % and 48 % compared to adults in males and females, respectively) reached adult-like values at PND 3 in both sexes. HVA amounts were higher at PND 3 (males: 128 %, females: 110 %) than at PND 90 and, comparable to DOPAC, did not change significantly during development.

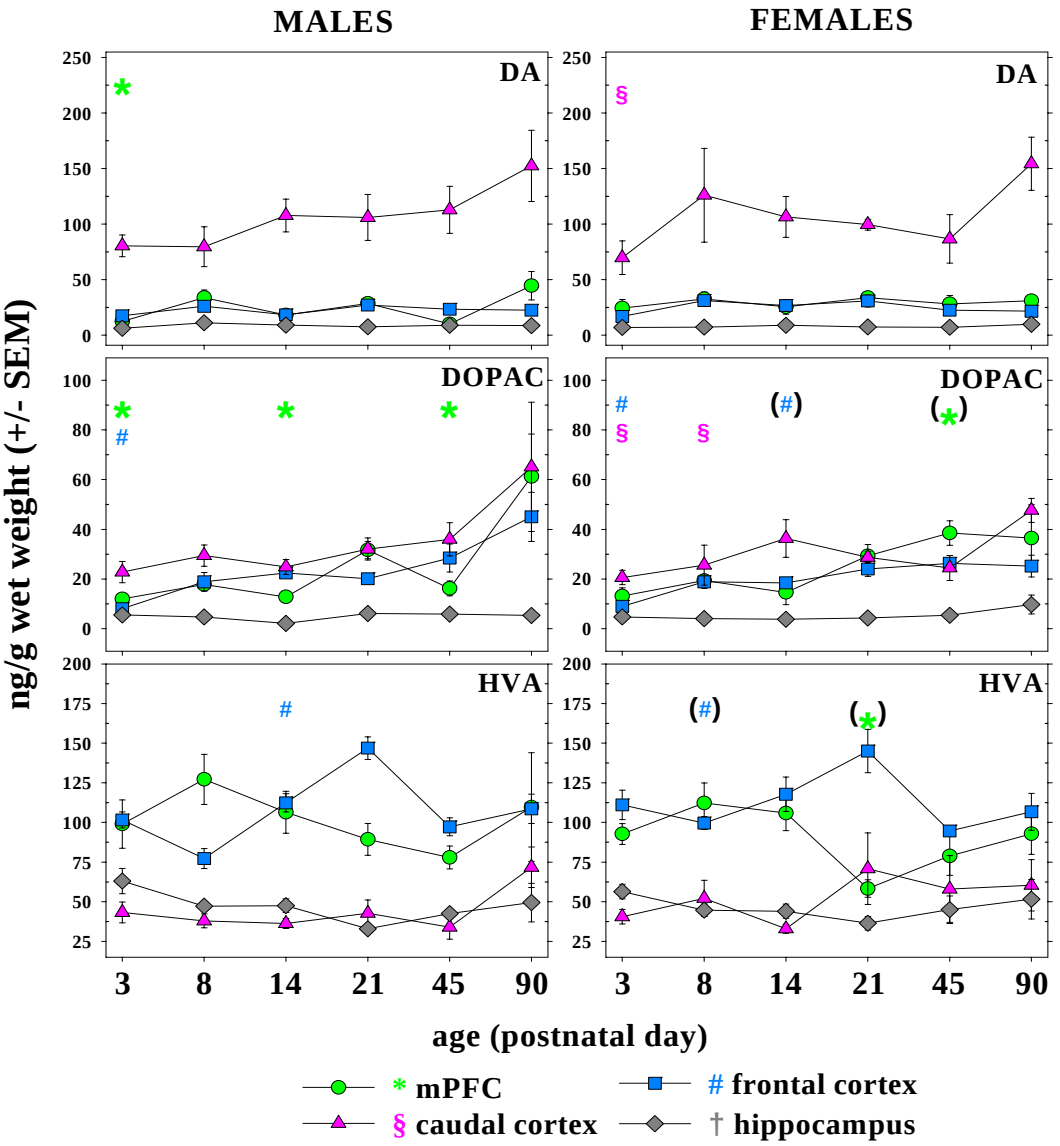

Figure 13. The postnatal development of DA, DOPAC and HVA concentrations (ng/g wet weight ± SEM) of the mPFC, frontal cortex, caudal cortex, and hippocampus of male and female *Octodon degus*. ***** $p<0.05$ vs. PND 90 in mPFC, $^{\#}$ in frontal cortex, § in caudal cortex, † in hippocampus (Dunn's test). $^{(*)}$, $^{(\#)}$ $p<0.05$ vs. males of the same age (Mann-Whitney U-test).

3.2.1.2 Dopamine turnover

The developmental changes in dopamine turnover ratios are presented in Figure 14. In general, the DOPAC/DA ratios displayed no significant changes during development in any of the regions examined. HVA/DA ratio decreased in male mPFC ($H_{(5)}$=22.5, p=0.004, Kruskal-Wallis-ANOVA) and frontal cortex ($H_{(5)}$=31.5, p<0.001). An increase was found for (DOPAC+HVA)/DA ratio in male frontal cortex ($H_{(5)}$=28.4, p=0.002).

In more detail, in the **mPFC** at PND 3 the levels of DOPAC/DA ratio reached in males 94 % and in females 111 % of adult-like values, with no further significant developmental changes. For HVA/DA ratio in males and females a decrease starting at PND 3 (664 %, p<0.05 and 240 % of adults, respectively) was observed reaching levels comparable to adults at PND 8 and 3, respectively. At PND 21 the levels of HVA/DA ratio in females were significantly lower than in males (p=0.029, Mann-Whitney U-test). Similarly to HVA/DA ratio, for (DOPAC+HVA)/DA a decrease was observed for males and females (at PND 3: 446 % and 116 % of adults) with no significant developmental changes except for a peak in males at PND 45 (p<0.05 vs. adults).

In the **frontal cortex**, at PND 3, the levels of DOPAC/DA ratio reached 79 % of adult-like amounts in males and 102 % in females. Adult-like levels of dopamine turnover were observed already at PND 3. The levels of HVA/DA ratio at PND 3 reached 125 % of adult-like amounts in males and 134 % in females and remained stable from PND 14 (males) or 3 (females). (DOPAC+HVA)/DA ratio at PND 3 reached 94 % of adult levels in males (slight increase until PND 14) and 116 % in females (no further significant changes).

At PND 3 in the **caudal cortex**, in males and females respectively, DOPAC/DA ratio reached 106 % and 105 % of adult-like levels, HVA/DA ratio 124 % and 168 %, and (DOPAC+HVA)/DA 106 % and 137 %. None of the ratios expressed significant developmental changes in this brain area, which suggests that adult-like levels are reached already at PND 3. At PND 45 the levels of HVA/DA ratio in females were significantly higher than in males (p=0.023, Mann-Whitney U-test).

At PND 3 in the **hippocampus**, in males and females respectively, DOPAC/DA ratio reached 78 % and 90 % of adult-like levels, HVA/DA ratio 202 % and 220 %, and (DOPAC+HVA)/DA 194 % and 204 %. Similarly to caudal cortex, adult-like comparable levels were reached at PND 3.

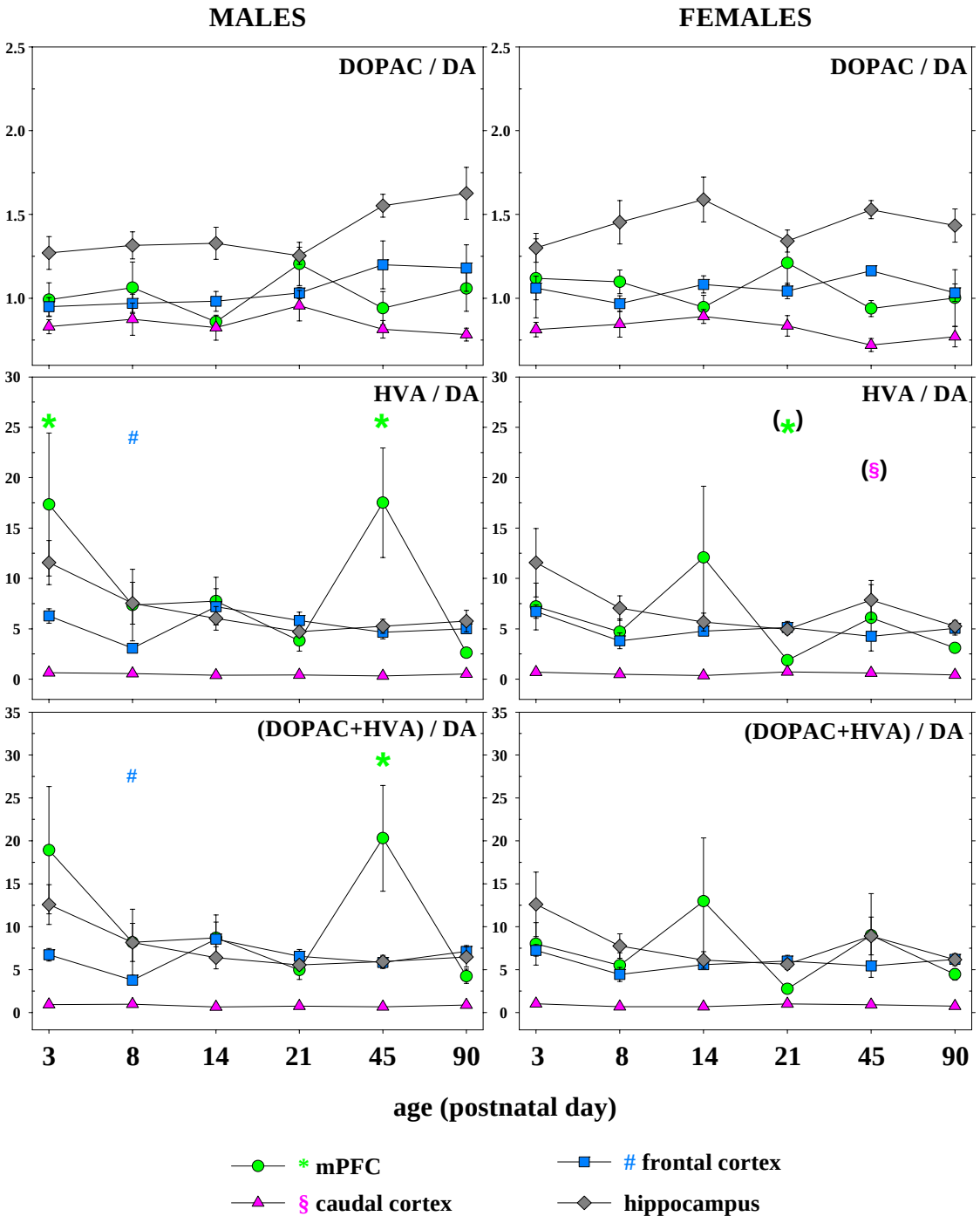

Figure 14. Postnatal development of dopamine turnover in the mPFC, frontal cortex, caudal cortex, and hippocampus of male and female *Octodon degus*. ***** $p<0.05$ vs. PND 90 in mPFC, $^{\#}$ in frontal cortex, § in caudal cortex, (Dunn's test). $^{(*)}$, $^{(\S)}$ $p<0.05$ vs. males of the same age (Mann-Whitney U-test).

3.2.2 Serotonergic system

The developmental changes in 5-HT and 5-HIAA levels as well as 5-HT turnover ratios are presented in Figure 15. In general, the levels of 5-HT increased in male and female mPFC ($H_{(5)}$=15.1, p=0.010 and $H_{(5)}$=22.0, p<0.001, respectively; Kruskal-Wallis-ANOVA), frontal cortex ($H_{(5)}$=14.5, p=0.013 and $H_{(5)}$=27.0, p<0.001, respectively), caudal cortex ($H_{(5)}$=22.5, p<0.001, and $H_{(5)}$=24.7, p<0.001, respectively) and hippocampus ($H_{(5)}$=14.8, p=0.011 and $H_{(5)}$=20.5, p<0.001, respectively). The same was found for 5-HIAA in male and female mPFC ($H_{(5)}$=16.3, p<0.001, and $H_{(5)}$=21.5, p<0.001, respectively), frontal cortex ($H_{(5)}$=31.3, p=0.021 and $H_{(5)}$=29.5, p=0.022, respectively), caudal cortex ($H_{(5)}$=13.7, p<0.001, and $H_{(5)}$=19.8, p<0.001, respectively) and hippocampus ($H_{(5)}$=15.1, p=0.031 and $H_{(5)}$=12.7, p=0.013, respectively). Both substances reached adult-like levels relatively early during ontogeny, i.e. mostly between PND 3 and 21. The 5-HIAA/5-HT ratios displayed no significant changes during development in any of the regions examined. In addition, no differences in 5-HT and 5-HIAA levels between males and females were found in the developmental pattern.

In more detail, in the **mPFC** at PND 3 the levels of serotonin reached in males 43 % (p<0.05, Dunn´s test) and in females 40 % (p<0.05) of adult-like amounts. In males, adult-like levels of 5-HT were reached much earlier than in females (PND 8 and PND 45, respectively). For 5-HIAA an increase starting at PND 3 (males: 37 %, females: 39 % of adults; p<0.05) was observed reaching adult-like levels at PND 21 (males) or PND 14 (females).

The **frontal cortex**, among the regions analyzed, was characterized by the lowest levels of 5-HT and 5-HIAA. At PND 3, the levels of 5-HT reached 64 % of adult-like levels in males and 56 % in females (p<0.05, respectively). Comparable to the mPFC, adult-like levels of 5-HT were observed much earlier in males (at PND 8) than in females which showed a gradual increase lasting until adulthood. For 5-HIAA an increase starting at PND 3 (males: 55 %, females: 57 % of adults; p<0.05) was observed reaching adult-like levels at PND 14 (males) or PND 21 (females).

In the **caudal cortex**, the 5-HT values reached 60 % (p<0.05) of adult-like levels in males and 48 % (p<0.05) in females at PND 3. In males, 5-HT increased to adult-like amounts until PND 14, in females until PND 21. Moreover, 5-HIAA (at PND 3: 65 %

and 52 % compared to adults in males and females, respectively; $p<0.05$) reached the adult-like levels at PND 3 in males and at PND 8 in females.

The **hippocampus**, among the regions analyzed, was characterized by the highest concentrations of 5-HT and 5-HIAA. At PND 3, 5-HT reached 77 % and 58 % ($p<0.05$, respectively) of adult-like levels in males and females, respectively. Whereas in males the concentrations of 5-HT did not change during development significantly, in females adult-like levels were reached at PND 21. The levels of 5-HIAA (at PND 3: 59 % and 52 % compared to adults in males and females, respectively; $p<0.05$) reached adult-like values at PND 8 in both sexes.

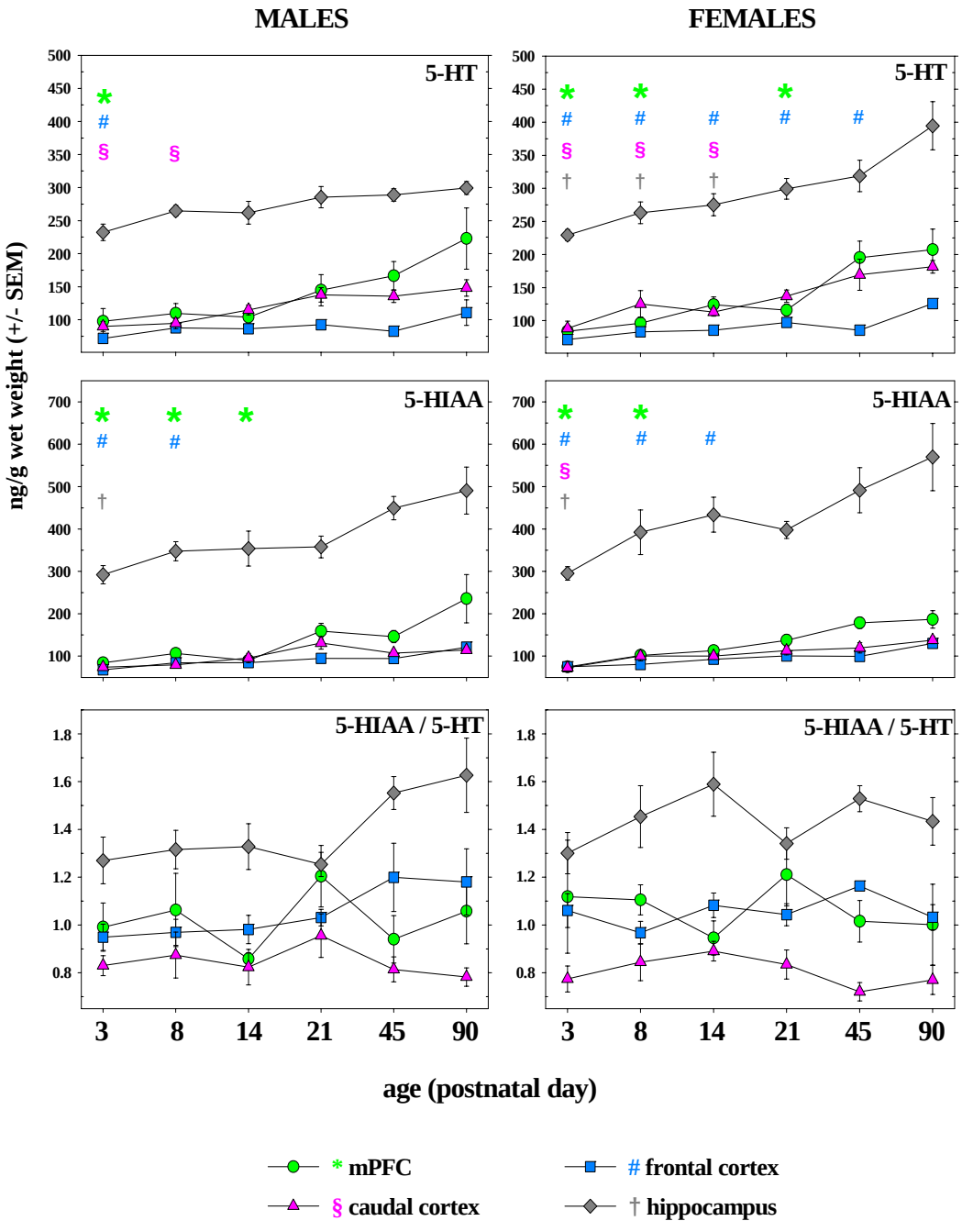

Figure 15. Postnatal development of 5-HT and 5-HIAA concentrations (ng/g wet weight ± SEM) as well as 5-HIAA/5-HT ratio of the mPFC, frontal cortex, caudal cortex, and hippocampus of male and female *Octodon degus*. ***** $p<0.05$ vs. PND 90 in mPFC, $^{\#}$ in frontal cortex, § in caudal cortex, † in hippocampus (Dunn's test).

3.2.3 Amino acids

The developmental changes in amino acids levels are presented in Figure 16. In general, the levels of aspartate decreased in male and female caudal cortex ($H_{(5)}$=32.2, p=0.003, and $H_{(5)}$=33.2, p=0.004, respectively; Kruskal-Wallis-ANOVA) and female hippocampus ($H_{(5)}$=30.1, p=0.004). Glutamate increased in male and female hippocampus ($H_{(5)}$=24.5, p=0.031, and $H_{(5)}$=26.6, p=0.007, respectively) and in female caudal cortex ($H_{(5)}$=28.9, p<0.001). Taurine decreased in male and female mPFC ($H_{(5)}$=19.1, p<0.001, and $H_{(5)}$=22.2, p<0.001, respectively), frontal cortex ($H_{(5)}$=25.3, p<0.001, $H_{(5)}$=24.7, p<0.001, respectively), caudal cortex ($H_{(5)}$=23.2, p<0.001, and $H_{(5)}$=28.5, p<0.001, respectively) and hippocampus ($H_{(5)}$=29.2, p<0.001, and $H_{(5)}$=30.6, p<0.001, respectively). GABA increased significantly only in female hippocampus ($H_{(5)}$=29.2, p=0.008). In addition, no sex differences were found in the developmental pattern.

In more detail, in the **mPFC** at PND 3 the levels of aspartate reached in males 76 % and in females 80 % of adult-like amounts. In both sexes adult-like levels were reached already at PND 3. The same was found for glutamate, with 73 % and 71 % of PND 90 levels at PND 3 in males and females, respectively. Taurine gradually decreased from PND 3 (males: 215 %, p<0.05, females: 273 %, p<0.05 of adult-like concentrations), reaching stable levels PND 14 (males) and PND 21 (females). GABA did not change significantly during development, and at PND 3 it made 93 % and 82 % of adult-like amounts in males and females, respectively.

In the **frontal cortex** at PND 3 the levels of aspartate reached in males 100 % and in females 115 % of adult-like amounts. In both sexes adult-like levels were reached already at PND 3. The same was found for glutamate, with 109 % and 98 % of PND 90 levels at PND 3 in males and females, respectively. Taurine gradually decreased from PND 3 (males: 329 %, p<0.05, females: 325 %, p<0.05 of adult-like concentrations), reaching stable levels PND 21 (both sexes). GABA did not change significantly during development, and at PND 3 it made 105 % and 100 % of adult-like amounts in males and females, respectively.

In the **caudal cortex** at PND 3 the levels of aspartate reached in males 110 % and in females 113 % of adult-like amounts. Stable levels of this amino acid were reached at PND 45 (males) and PND 21 (females) after crossing a peak in the middle of developmental curve. For glutamate, 95 % and 86 % of PND 90 levels at PND 3 in

males and females, respectively, were found. The concentrations typical for adulthood were detected already at PND 3 for males and PND 14 for females. Taurine gradually decreased from PND 3 (males: 325 %, $p<0.05$, females: 285 %, $p<0.05$ of adult-like concentrations), reaching stable levels PND 21 (males) and PND 45 (females). GABA did not change significantly during development, and at PND 3 it made 87 % and 106 % of adult-like amounts in males and females, respectively.

In the **hippocampus** at PND 3 the levels of aspartate reached in males 104 % and in females 112 % of adult-like amounts. Stable levels of this amino acid were reached at PND 3 (males) and PND 21 (females) after crossing a peak at PND (14). For glutamate, 77 % ($p<0.05$) and 78 % ($p<0.05$) of PND 90 levels at PND 3 in males and females, respectively, were found. The concentrations typical for adulthood were reached at PND 21 for males and PND 14 for females. Taurine gradually decreased from PND 3 (males: 192 %, $p<0.05$, females: 190 %, $p<0.05$ of adult-like concentrations), reaching stable levels PND 21 (both sexes). GABA did not change significantly during development in males, and at PND 3 it made 81 % of adult-like amounts. In females, at PND 3 concentration of this amino acid reached 79 % of PND 90 levels and increased until PND 14.

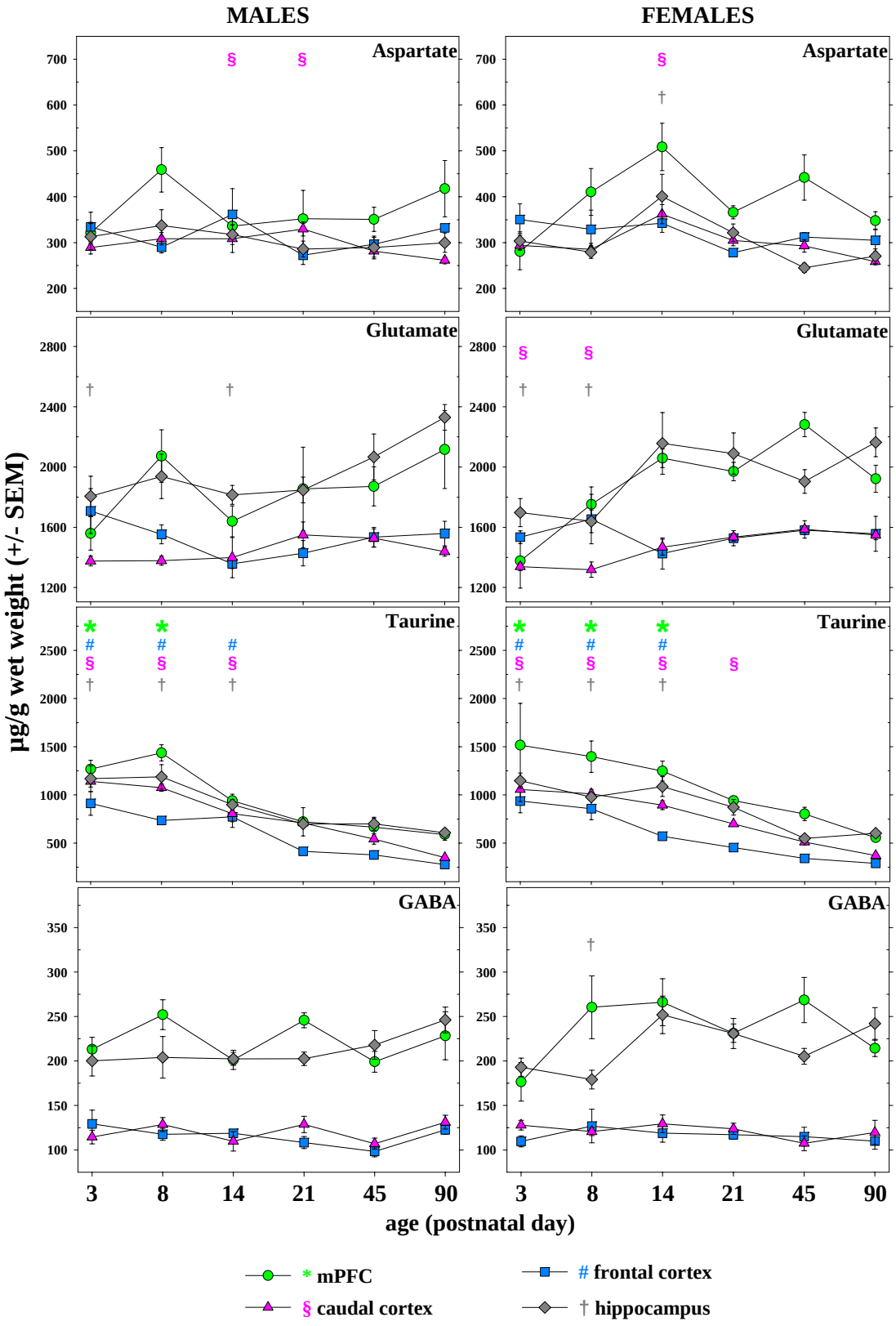

Figure 16. Postnatal development of brain amino acids concentrations (µg/g wet weight ± SEM) in mPFC, frontal cortex, caudal cortex, and hippocampus of male and female *Octodon degus*. ***** $p<0.05$ vs. PND 90 in mPFC, $^{\#}$ in frontal cortex, § in caudal cortex, † in hippocampus (Dunn's test).

3.3 Experiment 2: Age-dependent impact of an acute separation stressor on serotonergic neurotransmission

The basal and acute stress-evoked levels of 5-HT as well as the 5-HIAA/5-HT ratios are summarized in Table II. Since 5-HIAA was not affected in any of the brain regions by acute stress, the data are not shown.

In general, the stress challenge (i.e. separation from parents and from siblings) induced an age-, region- and sex-specific pattern of changes in the serotonergic system. The most pronounced effects were found in the frontal cortex of females.

In more detail, in the **mPFC**, except of an increased 5-HIAA/5-HT ratio after acute separation stress at PND 14 in males ($p=0.009$; Mann-Whitney U-test) as well as in females ($p=0.013$), there was no effect of the acute stressor.

In the **frontal cortex**, an acute separation stress induced a decrease of 5-HT at PND 3 ($p<0.001$; Mann-Whitney U-test), PND 8 ($p=0.010$) and PND 14 ($p=0.039$) in males and at PND 3 ($p=0.024$) and PND 8 ($p=0.005$) in females. The 5-HIAA/5-HT ratios were increased by the acute separation stress at PND 3 ($p<0.001$) and PND 8 ($p<0.001$) in males and at PND 3 ($p=0.023$), PND 8 ($p=0.010$), PND 14 ($p=0.006$) and PND 21 ($p=0.014$) in females.

In the **caudal cortex**, no impact of the acute separation stress on the neurochemical parameters was observed.

In the **hippocampus**, except of an elevated 5-HIAA/5-HT ratio at PND 21 in males ($p=0.008$) as well as in females ($p=0.008$), there was no effect of the acute stressor on serotonergic neurotransmission.

Table II. The impact of acute parental separation stress on 5-HT levels (ng/g wet weight ± SEM) and 5-HIAA/5-HT ratios in previously unstressed male and female *Octodon degus*.

Region	PND	5-HT				5-HIAA/5-HT			
		Males		Females		Males		Females	
		unstressed controls	acute separation stress challenge	unstressed controls	acute separation stress challenge	unstressed controls	acute separation stress challenge	unstressed controls	acute separation stress challenge
mPFC	**3**	91.6 ± 17.8	85.8 ± 16.7	84.1 ± 15.1	83.8 ± 13.0	0.99 ± 0.10	1.12 ± 0.14	1.12 ± 0.23	1.23 ± 0.14
	8	109.7 ± 14.7	103.3 ± 15.7	96.1 ± 9.4	100.1 ± 15.3	1.06 ± 0.15	1.04 ± 0.05	1.10 ± 0.06	1.10 ± 0.19
	14	103.9 ± 8.8	107.6 ± 15.4	123.9 ± 11.8	113.8 ± 14.6	**0.86 ± 0.02**	**1.27 ± 0.17 #**	**0.94 ± 0.07**	**1.13 ± 0.11 #**
	21	144.6 ± 23.6	124.8 ± 15.2	115.8 ± 9.1	112.2 ± 16.6	1.20 ± 0.12	1.39 ± 0.15	1.21 ± 0.13	1.32 ± 0.12
frontal cortex	**3**	**71.7 ± 2.4**	**48.3 ± 3.9 #**	**71.4 ± 2.1**	**66.1 ± 8.1 #**	**0.95 ± 0.05**	**1.30 ± 0.13 #**	**1.06 ± 0.07**	**1.38 ± 0.07 #**
	8	**87.6 ± 3.3**	**73.3 ± 3.5 #**	**83.2 ± 3.4**	**75.3 ± 5.4 #**	**0.96 ± 0.05**	**1.23 ± 0.02 #**	**0.96 ± 0.04**	**1.21 ± 0.05 #**
	14	**86.4 ± 6.0**	**62.1 ± 9.1 #**	85.4 ± 5.4	97.5 ± 11.2	0.98 ± 0.05	1.10 ± 0.09	**1.08 ± 0.05**	**1.30 ± 0.12 #**
	21	92.5 ± 3.6	95.3 ± 7.5	97.3 ± 6.0	91.3 ± 4.1	1.03 ± 0.03	1.12 ± 0.09	**1.04 ± 0.04**	**1.32 ± 0.13 #**
caudal cortex	**3**	89.9 ± 7.5	117.8 ± 15.4	88.3 ± 4.1	109.4 ± 17.9	0.83 ± 0.04	0.86 ± 0.05	0.77 ± 0.05	0.90 ± 0.05
	8	94.6 ± 8.0	101.5 ± 4.6	125.2 ± 20.3	100.1 ± 3.2	0.87 ± 0.09	0.98 ± 0.03	0.84 ± 0.07	0.94 ± 0.04
	14	114.9 ± 7.4	108.3 ± 6.7	112.9 ± 6.2	112.3 ± 8.4	0.82 ± 0.07	0.99 ± 0.02	0.89 ± 0.04	0.95 ± 0.03
	21	137.9 ± 11.1	130.8 ± 13.6	136.9 ± 9.2	136.4 ± 14.7	0.95 ± 0.09	0.81 ± 0.04	0.83 ± 0.06	0.79 ± 0.05
hippocampus	**3**	232.3 ± 12.4	244.7 ± 5.4	229.4 ± 8.3	240.4 ± 8.6	1.27 ± 0.09	1.34 ± 0.26	1.30 ± 0.08	1.28 ± 0.26
	8	264.8 ± 8.1	264.5 ± 13.7	263.0 ± 16.5	260.7 ± 15.5	1.31 ± 0.08	1.53 ± 0.48	1.45 ± 0.12	1.65 ± 0.40
	14	261.8 ± 14.2	269.3 ± 8.9	275.2 ± 16.6	267.0 ± 8.9	1.32 ± 0.09	0.84 ± 0.19	1.58 ± 0.13	0.76 ± 0.15
	21	285.3 ± 16.1	287.5 ± 14.6	299.3 ± 15.5	265.7 ± 17.3	**1.25 ± 0.05**	**1.66 ± 0.17 #**	**1.34 ± 0.06**	**1.70 ± 0.15 #**

# $p<0.05$ vs. levels in unstressed controls (Mann-Whitney U-test)

3.4 Experiment 3: The impact of repeated separation stress on basal and stress-evoked serotonergic neurotransmission at PND 21

The impact of repeated stress exposure, i.e. repeated parental separation during the first three postnatal weeks, on the basal and stress-evoked levels of 5-HT, 5-HIAA and 5-HIAA/5-HT ratios at PND 21 is summarized in Figure 17.

In general, repeated stress exposure had sex- and region-specific effects on the serotonergic system with the most pronounced effects in the frontal (only females) and caudal cortex (both sexes).

In more detail, in the **mPFC**, except of an increase of the basal levels of 5-HIAA in males ($p<0.001$ compared to unstressed control animals; Mann-Whitney U-test) and females ($p<0.001$), no effects of repeated separation stress were detected.

In the **frontal cortex** of females, acute separation stress resulted in an increase of 5-HIAA/5-HT ratio in unstressed controls ($p=0.001$, compared to the basal levels) and repeatedly stressed animals ($p<0.001$). Consequently, there was an attenuated responsiveness (-18 % vs. acutely stressed, control animals, $p=0.038$) of the serotonergic system in the repeatedly stressed females. In contrast, no stress evoked changes of 5-HIAA/5-HT ratio were found either in unstressed controls (comparable to Experiment 2) or repeatedly stressed males.

In the **caudal cortex**, repeated separation stress resulted in an up-regulation of the basal levels of 5-HIAA in males ($p<0.001$ compared to unstressed control animals) and females ($p=0.021$). In all experimental groups, a tendency towards decreased 5-HIAA levels after acute stress was observed. This decrease was more pronounced in repeatedly stressed females than in males ($p=0.033$).

In the **hippocampus**, a stress-induced increase of 5-HT ($p=0.043$ compared to basal levels) and an increase of the basal 5-HIAA/5-HT ratio ($p=0.028$ compared to unstressed control animals) was observed in repeatedly stressed males. Acute stress resulted in an increase of 5-HIAA/5-HT ratio in unstressed control males ($p=0.019$, compared to the basal levels) and females ($p=0.030$, respectively), but not in repeatedly stressed animals.

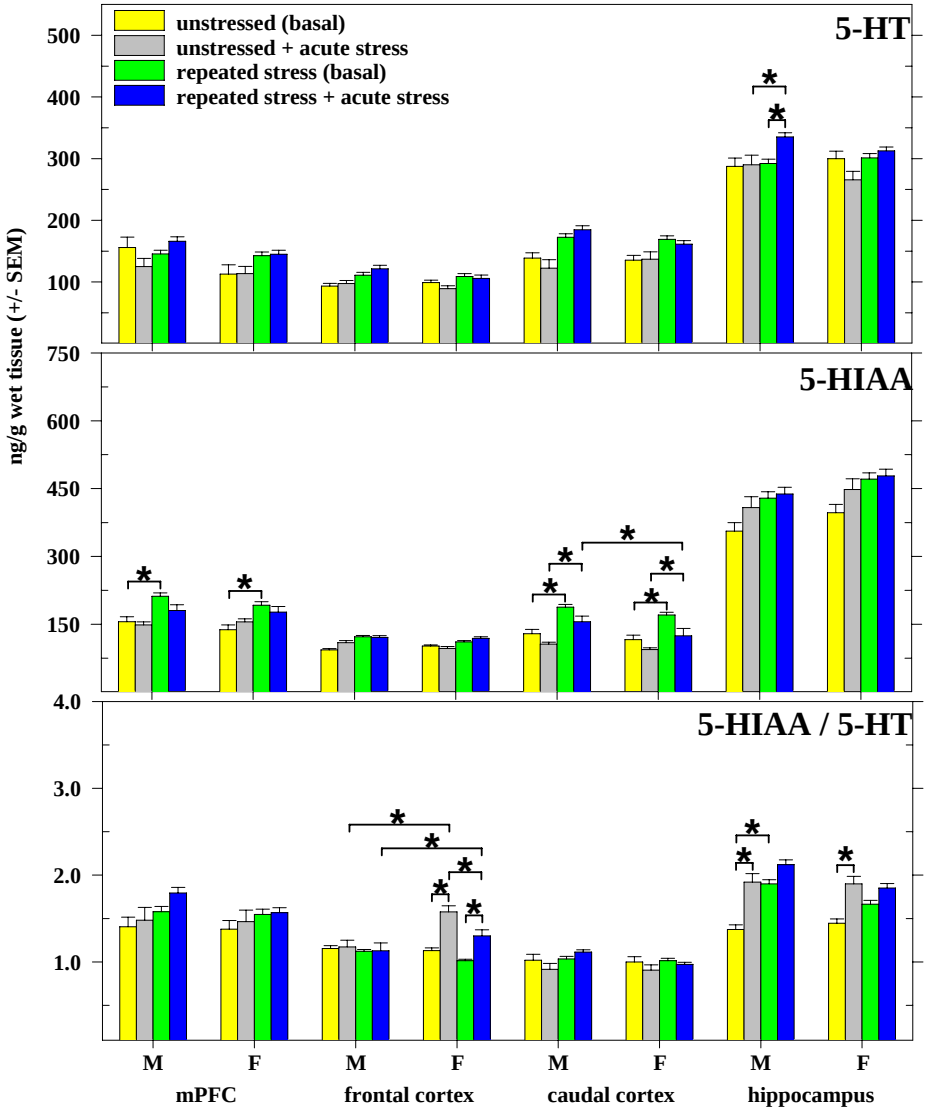

Figure 17. The impact of repeated parental separation stress on basal and stress-induced (acute, 1 h parental separation) levels of serotonin, 5-HIAA and 5-HIAA/5-HT ratio in mPFC, frontal and caudal cortex and hippocampus of male (M) and female (F) *Octodon degus* at PND 21. Data are given as ng/g wet tissue (mean ± SEM). * $p<0.05$ (Mann-Whitney U-test)

Part II. Neurochemical consequences of juvenile stress and chronic methylphenidate treatment on dopaminergic function in *Octodon degus*: *in vivo* microdialysis study

3.5 Influence of repeated separation stress and methylphenidate injection on body weights and basal levels of dopamine

Table III. Body weights and basal extracellular levels of dopamine. Values are given as mean ± SD. #: $p<0.05$ vs. C; §: $p<0.05$ vs. C-Sal; *: $p<0.05$ vs. S-Sal; †: $p<0.05$ vs. C-MP;

	Experimental group	Body weights (g)	mPFC (nM)	Nac (nM)
postweaning age	C	47.6 ± 3.2	0.908 ± 0.002	11.259 ± 0.001
	S	50.3 ± 2.5	0.992 ± 0.002 #	13.981 ± 0.003 #
puberty	C-Sal	119.3 ± 9.1	0.871 ± 0.001	11.440 ± 0.001
	C-MP	129.0 ± 3.3	0.829 ± 0.001 §	11.471 ± 0.001
	S-Sal	128.1 ± 5.2	1.049 ± 0.002 §	12.872 ± 0.004 §
	S-MP	131.0 ± 2.6	1.010 ± 0.001 * †	10.731 ± 0.001 * †

At *postweaning age* (Table III), no difference in body weights ($p=0.123$) was found between repeatedly stressed (S) and unstressed control (C) animals. Comparing the basal DA concentrations, increased levels were observed in repeatedly stressed animals compared to controls in both the mPFC ($p<0.001$) and Nac ($p<0.001$).

At *puberty*, increased body weights were observed in repeatedly stressed and/or MP- pretreated animals, as indicated by an effect of rearing ($F_{(1,20)}=5.393$, $p=0.031$) and pretreatment ($F_{(1,20)}=7.35$, $p=0.013$), but no rearing x pretreatment- interaction was detected. Comparing the basal DA levels, a significant effect of rearing ($F_{(1,20)}=118282.1$, $p<0.001$), pretreatment ($F_{(1,20)}=5877.0$, $p<0.001$), and a rearing x pretreatment- interaction ($F_{(1,20)}=4.4$, $p=0.048$) was observed in the mPFC. Post-hoc analysis revealed increased DA levels in repeatedly stressed animals (C-Sal vs. S-Sal:

$p<0.05$; C-MP vs. S-MP: $p<0.05$), but decreased levels of DA after chronic MP-pretreatment (C-Sal vs. C-MP: $p<0.05$; S-Sal vs. S-MP: $p<0.05$). In the Nac, a significant effect of rearing ($F_{(1,20)}=181572.3$, $p<0.001$), pretreatment ($F_{(1,20)}=1690651.7$, $p<0.001$), and a rearing x pretreatment- interaction ($F_{(1,20)}=1789955.9$, $p<0.001$) was observed on basal DA levels. Post-hoc analysis revealed increased DA levels in saline- pretreated (C-Sal vs. S-Sal: $p<0.05$), but decreased DA levels in MP- pretreated, repeatedly stressed animals (C-MP vs. S-MP: $p<0.05$). In addition, decreased levels of DA were observed after chronic MP-pretreatment in repeatedly stressed animals (S-Sal vs. S-MP: $p<0.05$), but not in unstressed controls.

3.6 Distress calls during social separation stress

Postweaning age: During acute social separation stress four (unstressed controls) and two (repeatedly stressed) out of six animals displayed distress vocalization (Figure 18), a difference which tended towards significance ($p = 0.070$). A correlation analysis revealed no relationship between the number of distress calls and the accumulated increase of dopamine (as indicated by the AUC- values) in mPFC or Nac in any of the experimental groups (data not shown).

Puberty: During acute separation stress, two (unstressed controls; unstressed controls + chronically MP-pretreated; repeatedly stressed) or three (repeatedly stressed + chronically MP-pretreated) out of six animals displayed distress calls (Figure 18), however this difference was not significant. Furthermore, there was no correlation between the number of distress calls and the increase of dopamine (as indicated by the AUC- values) in mPFC or Nac (data not shown).

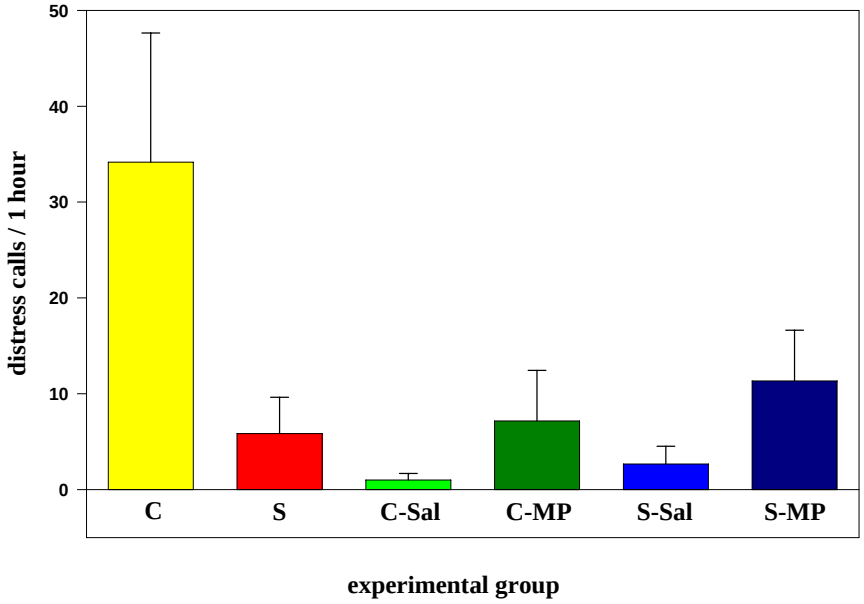

Figure 18. Distress calls during period of separation from the siblings. Data are given as mean ± SEM.

3.7 The impact of acute separation stress and MP injection on extracellular levels of dopamine in the medial prefrontal cortex and nucleus accumbens at postweaning age

Acute social separation stress increased the levels of DA in the **mPFC** (Figure 19) in unstressed control (χ2=30.847, $p<0.001$) as well as repeatedly prestressed animals (χ^2=24.467, $p<0.001$), reaching 171 ± 4% ($p<0.05$ compared to baseline) and 146 ± 3% ($p<0.05$) of baseline, respectively. The between-group comparison revealed an attenuation of the DA increase in repeatedly prestressed compared to unstressed control animals ($p<0.001$). In the **Nac** (Figure 20), DA levels were increased in unstressed controls (χ^2=21.154, p=0.0021) as well as repeatedly prestressed animals (χ^2=15.643, p=0.016) to 169 ± 18% ($p<0.05$) and 150 ± 14% ($p<0.05$) of baseline, respectively. However, the between-group comparison using AUC-values revealed no significant difference in the effect of acute social separation stress on DA release between both groups. Importantly, after cessation of acute social separation stress, DA levels returned to baseline in both brain regions within the next 60 minutes.

A single acute MP injection increased the levels of DA in the **mPFC** (Figure 19) in unstressed controls (χ^2=66.954, $p<0.001$) reaching 116 ± 2% of baseline ($p<0.05$ compared to baseline), but not in repeatedly prestressed animals (the decrease observed failed significance). The between-group comparison using AUC-values revealed a strong tendency towards significance for the main effect of rearing ($F_{(1,10)}$=4.902, p=0.051), an effect of time following injection ($F_{(2,20)}$=11.615, $p<0.001$), and a rearing x time following injection- interaction ($F_{(2,20)}$=11.396, $p<0.001$) on DA release. Post-hoc analysis indicated a difference between both rearing groups during the first (p=0.004) and second (p=0.041), but not during the third half-hour following MP injection. In the **Nac** (Figure 20), DA levels were increased in unstressed controls (χ^2=47.882, $p<0.001$) to 136 ± 6%; ($p<0.05$) of baseline, but not in repeatedly prestressed animals. A between-group comparison revealed no difference in the effect of acute MP injection between both rearing groups.

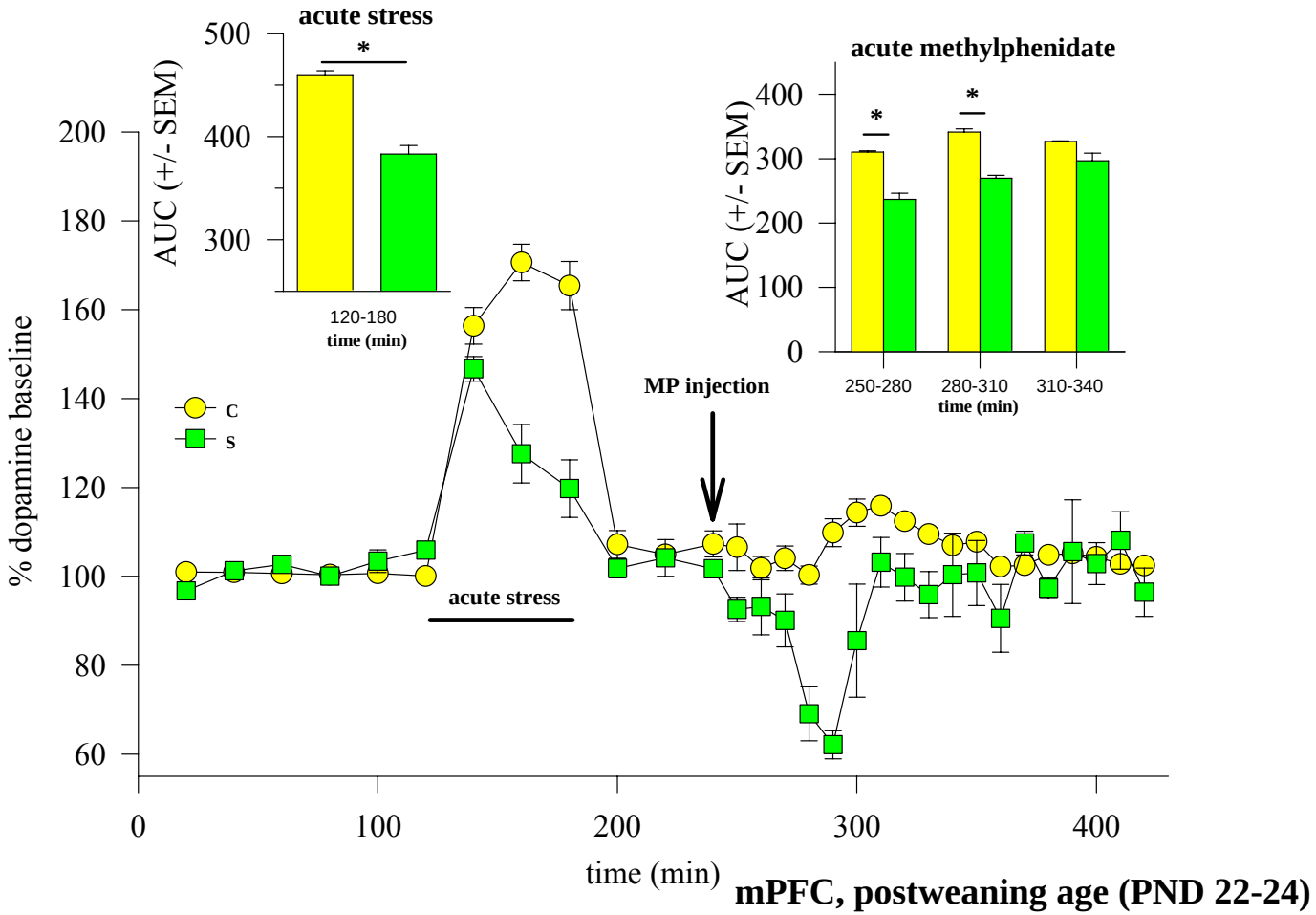

Figure 19. Effect of acute separation stress challenge (horizontal bar) and acute injection of methylphenidate (vertical arrow) on the extracellular levels of dopamine in mPFC at postweaning age (PND 22-24). Data are given as percent of baseline levels (± SEM). The inserts represent changes for the $t_{120\text{-}180}$ (acute separation stress) and $t_{250\text{-}280}$, $t_{280\text{-}310}$, $t_{310\text{-}340}$ (acute MP) sampling period. Statistical differences versus baseline levels in the range of each experimental group are given in the text.

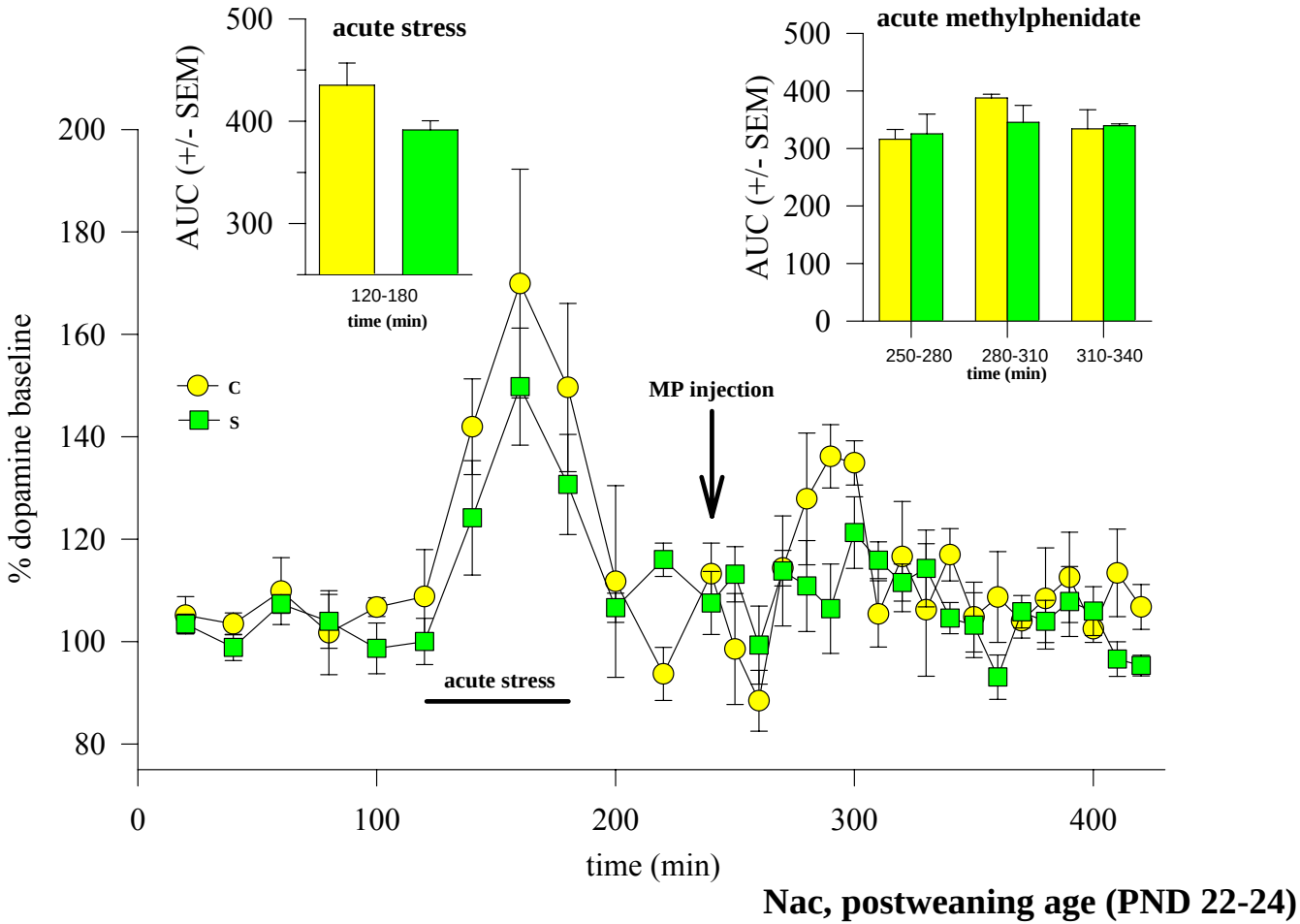

Figure 20. Effect of acute separation stress (horizontal bar) and acute injection of methylphenidate (vertical arrow) on the extracellular levels of dopamine in Nac at postweaning age (PND 22-24). Data are given as percent of baseline levels (± SEM). The inserts represent changes for the $t_{120\text{-}180}$ (acute separation stress) and $t_{250\text{-}280}$, $t_{280\text{-}310}$, $t_{310\text{-}340}$ (acute MP) sampling period. Statistical differences versus baseline levels in the range of each experimental group are given in the text.

3.8 The impact of chronic methylphenidate treatment on acute separation stress and acute MP injection evoked extracellular levels of dopamine in the prefrontal cortex and nucleus accumbens at puberty

Acute social separation stress increased the levels of DA in the **mPFC** (Figure 21) in unstressed controls (χ^2=30.358, p<0.001) to 142 ± 3% of baseline (p<0.05 compared with baseline), in unstressed MP-pretreated (χ^2=35.122, p<0.001) to 170 ± 1% (p<0.05), in repeatedly prestressed (χ^2=24.539, p<0.001) to 148 ± 12% (p<0.05), and in repeatedly prestressed MP-pretreated animals (χ^2=24.867, p<0.001) to 265 ± 52% (p<0.05) of baseline levels. The between-group comparison using AUC-values revealed a main effect of rearing ($F_{(1,20)}$=8.876, p=0.007) and pretreatment conditions ($F_{(1,20)}$=10.050, p=0.005) on DA release in the mPFC, but no interaction between both factors. In the **Nac** (Figure 22), DA was increased in unstressed MP-pretreated (χ^2=31.929, p<0.001) to 186 ± 6% (p<0.05), in repeatedly prestressed (χ^2=16.890, p<0.001) to 125 ± 7% (p<0.05), and in repeatedly prestressed MP-pretreated animals (χ^2=30.162, p<0.001) to 214 ± 26% (p<0.05) of baseline levels, but not in unstressed control animals. The between- group comparison using AUC-values revealed a main effect of pretreatment conditions ($F_{(1,20)}$=32.869, p<0.001) on DA levels during acute social separation stress, but no effect of rearing and no interaction between rearing x pretreatment conditions. Importantly, after cessation of acute separation stress DA levels returned to baseline within the next 60 minutes in both brain regions of all experimental groups.

A single acute MP injection increased the levels of DA in the **mPFC** (Figure 21) in unstressed control animals (χ^2=97.558, p<0.001) to 174 ± 2% of baseline (p<0.05 compared with baseline), in unstressed, chronically MP-pretreated (χ^2=100.859, p<0.001) to 441 ± 5% (p<0.05), in repeatedly prestressed (χ^2=88.042, p<0.001) to 277 ± 13% (p<0.05), and in repeatedly prestressed MP-pretreated animals (χ^2=87.159, p<0.001) to 583 ± 58% (p<0.05) of baseline levels. The between-group comparison using AUC-values revealed a main effect of rearing ($F_{(1,20)}$=73.477, p<0.001) and pretreatment conditions ($F_{(1,20)}$=68.26, p<0.001) on DA release in the mPFC, but no interaction between both factors. Furthermore, there was a main effect of time following injection ($F_{(2,40)}$=777.736, p<0.001), and an interaction between rearing x time

following injection ($F_{(2,40)}$=80.405, p<0.001), pretreatment conditions x time following injection ($F_{(2,40)}$=231.998, p<0.001), and an interaction between rearing x pretreatment conditions x time following injection ($F_{(2,40)}$=4.941, p=0.012). Post-hoc analysis revealed increased DA levels in repeatedly prestressed animals (unstressed controls vs. repeatedly prestressed: p<0.05; unstressed, chronically MP pretreated vs. repeatedly prestressed, MP pretreated: p<0.05) as well as after chronic MP- pretreatment (unstressed controls vs. unstressed, chronically MP pretreated: p<0.05; repeatedly prestressed vs. repeatedly prestressed, MP pretreated: p<0.05) most prominent in the first half-hour following acute MP injection. In the **Nac** (Figure 22), DA was increased in unstressed controls (χ^2=81.654, p<0.001) to 195 ± 10% (p<0.05), in unstressed, chronically MP pretreated (χ^2=93.693, p<0.001) to 275 ± 9% (p<0.05), in repeatedly prestressed (χ^2=87.562, p<0.001) to 204 ± 9% (p<0.05), and in repeatedly prestressed, MP pretreated (χ^2=91.717, p<0.001) to 321 ± 12% (p<0.05) of baseline levels after acute MP injection. The between-group comparison using AUC-values revealed a main effect of pretreatment conditions ($F_{(1,20)}$=31.337, p<0.001) and an interaction between rearing x pretreatment conditions ($F_{(1,20)}$=8.989, p=0.007) on DA release in the Nac, but no effect of rearing conditions. Furthermore, there was a main effect of time following injection ($F_{(2,40)}$=484.118, p<0.001), and an interaction between rearing x time following injection ($F_{(2,40)}$=6.342 p=0.004), pretreatment conditions x time following injection ($F_{(2,40)}$=140.138, p<0.001), and rearing x pretreatment conditions x time following injection ($F_{(2,40)}$=13.884, p<0.001). Post-hoc analysis revealed increased levels of DA in repeatedly prestressed animals (unstressed controls vs. repeatedly prestressed: p<0.05), and decreased DA levels in repeatedly prestressed, chronically MP pretreated animals (unstressed, chronically MP pretreated vs. repeatedly prestressed, MP pretreated: p<0.05) as well as after chronic MP pretreatment (unstressed controls vs. unstressed, chronically MP pretreated: p<0.05; repeatedly prestressed vs. repeatedly prestressed, MP pretreated: p<0.05) most prominent in the first half-hour following acute MP injection.

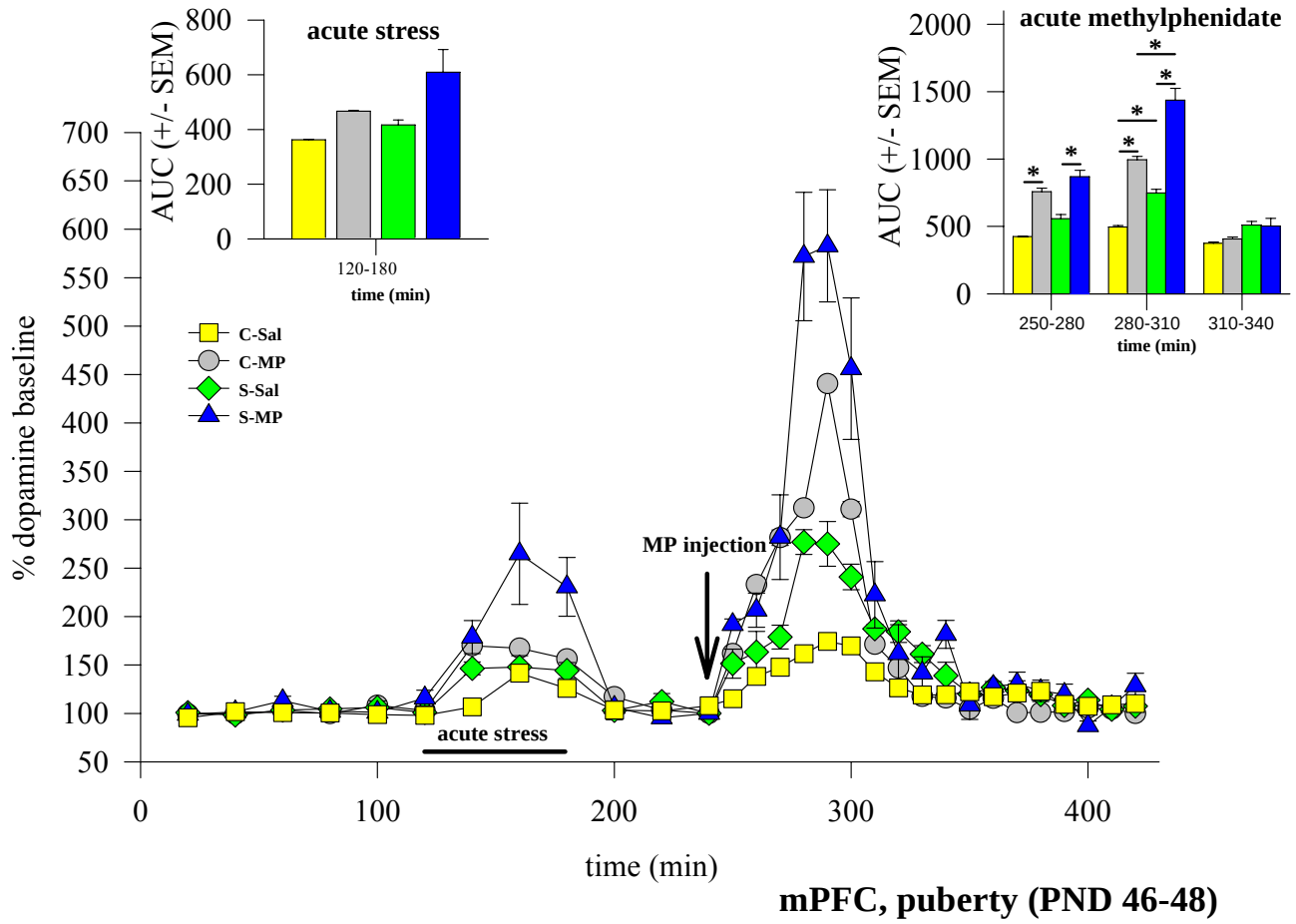

Figure 21. Effect of acute social separation stress (horizontal bar) and acute injection of methylphenidate (vertical arrow) on the extracellular levels of dopamine in mPFC at puberty (PND 46-48). Data are given as percent of baseline levels (± SEM). The inserts represent changes for the $t_{120\text{-}140}$, $t_{140\text{-}160}$, $t_{160\text{-}180}$, $t_{180\text{-}200}$ (acute separation stress) and $t_{250\text{-}280}$, $t_{280\text{-}310}$, $t_{310\text{-}340}$ (acute MP) sampling period. Statistical differences versus baseline levels in the range of each experimental group are given in the text.

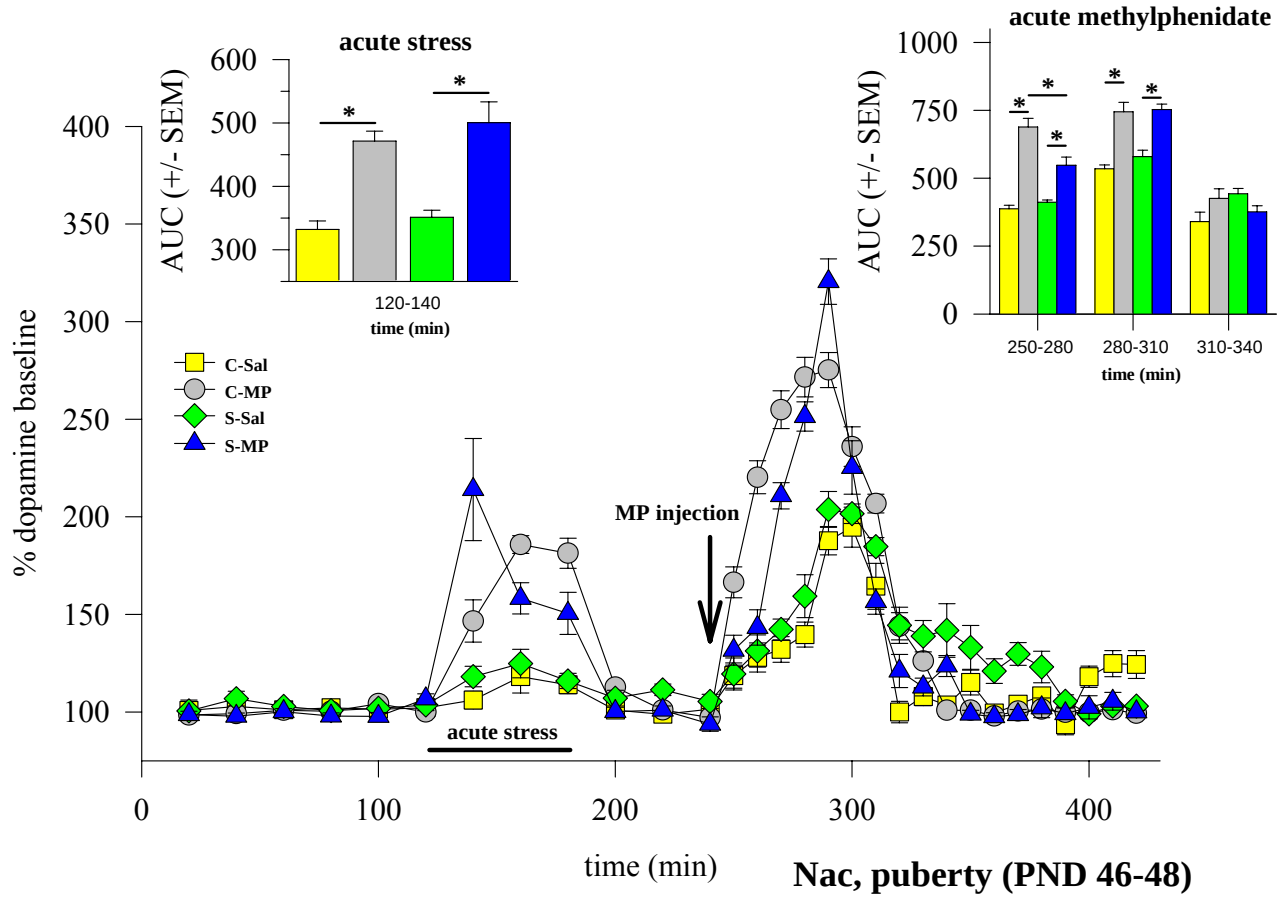

Figure 22. Effect of acute social separation stress (horizontal bar) and acute injection of methylphenidate (vertical arrow) on the extracellular levels of dopamine in Nac at puberty (PND 46-48). Data are given as percent of baseline levels (± SEM). The inserts represent changes for the $t_{120\text{-}140}$, $t_{140\text{-}160}$, $t_{160\text{-}180}$, $t_{180\text{-}200}$ (acute separation stress) and $t_{250\text{-}280}$, $t_{280\text{-}310}$, $t_{310\text{-}340}$ (acute MP) sampling period. Statistical differences versus baseline levels in the range of each experimental group are given in the text.

4 DISCUSSION

Part I. Epigenetic influences on the neurotransmission in *Octodon degus* during early postnatal development

The first part of my dissertation characterizes (*i*) the ontogenetic development of dopaminergic, serotonergic as well as amino acids neurotransmitting systems and the impact of (*ii*) acute and (*iii*) repeated stress exposure early in life on serotonergic neurotransmission in *Octodon degus*. In this semi-precocial species I observed that (*i*) tissue levels of monoamines and amino acids reached adult-like levels relatively early in ontogeny (mainly between PND 3 and 21, depending on the brain region and substance examined), indicating a relatively matured neurotransmission in cortical regions and hippocampus at birth. In addition, an age-, region- and sex-specific pattern of changes were found in the serotonergic system induced by (*ii*) an acute stress challenge early in life (i.e., parental separation at PND 3, 8, 14 or 21) with the most pronounced effects at early age stages (PND 3 – PND 14) in the frontal cortex of females, and (*iii*) repeated stress exposure (i.e., parental separation during the first 3 weeks of life, 1 hour daily) with the most pronounced effects in the frontal (only females) and caudal cortex (both sexes) measured at PND 21. These results support the conclusion that in *Octodon degus* aversive emotional experience alters serotonergic transmission acutely as well as permanently.

4.1 Postnatal development of the brain wet weights, dopaminergic and serotonergic neurotransmission

One major aim of the present study was to determine how the developmental patterns of the neurotransmitter systems differ between semi-precocial and altricial species. It came out that, parallel to the well matured physiology (i.e., functional sensory systems, advanced behavioral activity), the degu pups are characterized by a relatively matured dopaminergic and serotonergic neuronal network around birth.

Whereas in rats, an altricial species, there is a 10-fold increase in brain weight, 14-fold increase in tissue levels of dopamine and 5-fold increase in tissue levels of serotonin from birth until adulthood (Agrawal et al., 1966; Herregodts et al., 1990), a quite different pattern was found in *Octodon degus*, a precocial species, with an average 2-fold increase in brain weight as well as levels of dopamine, serotonin, their metabolites and metabolic ratios. Remarkably, in *Octodon degus* adult-like levels of serotonin, its metabolite 5-HIAA and 5-HIAA/5-HT ratios are reached relatively early during ontogeny, i.e. mostly until weaning, with similar findings for DA, DOPAC and HVA amounts, followed by DA turnover. These results are in line with findings of Braun et al. (2000), who described in male degus an adult-like dopaminergic and serotonergic innervation pattern in the mPFC at about 12th - 14th day of life. Additionally, the adult-like density of serotonergic appositions in the pyramidal cells of prefrontal cortex is reached before 14 day of life in precocial rhesus monkeys (Lambe et al., 2000), nevertheless the density of catecholamine appositions on pyramidal neurons matures very slowly. Another interesting feature found in my study is the sex specific developmental pattern for serotonin. In males, adult-like levels of serotonin are reached much earlier in life, i.e. between PND 8 – 14, than in females, where the process of maturation is extended until adulthood. Whether this sex difference in the maturation of the serotonergic system might be one of the reasons that female degu pups are more susceptible to early life stress (see below) has to be proven in future experiments.

The analysis of brain homogenates gives the opportunity to analyse the whole pool of several neurotransmitters in different brain regions. Not only can those transmitters which are already present in the synaptic cleft be measured, but also already synthesized and not yet released part. The disadvantage of this experimental approach is that only *post mortem* investigation is possible – in other words there is no opportunity to measure the dynamic changes of neurotransmitters during for instance behavioral or pharmacological experiment. Such limitation is counterbalanced by the possibility of investigation of several brain structures at the same time. Moreover, analysis of homogenates allows determining the metabolic ratios of the analyzed substances. As known from previous studies, the concentration of metabolites as well as their ratio to the monoamine may serve as an indicator of monoamine turnover and a parameter of neuronal activity in the brain (Ribary et al., 1986; Herregodts et al., 1990). Taken all the findings together, with particular attention to relatively high dopamine turnover ratios in some regions of the brain, I postulate, that dopamine as well as

serotonin are fully available and act in the degu brain already immediately after birth. However, these monoamines were found to take a part not only in neurotransmission, but also in brain development, namely as a trophic factor modulating the functional state of neurons, from very early stages of life (Pendleton et al., 1998; Herlenius and Lagercrantz, 2001; Whitaker-Azmitia, 2001), by modulating the activity of particular cells. Therefore, some pool of DA and 5-HT might be acting not as the neurotransmitters, but also regulate establishment of synaptic connections in response to environmental influences. In addition, not only the levels of neurotransmitters themselves, but also the presence and amount of receptors, transporters as well as other parts of molecular machinery responsible for the signal transduction, should be taken into account when speaking about the functional state of neurotransmission in the developing brain.

The limitation of this study was that noradrenaline was not measurable under HPLC conditions established in our lab, due to the very short retention time, in comparison to serotonin. The developmental pattern of noradrenaline, its metabolite 3-methoxy-4-hydroxyphenylethyleneglycol (MOPEG) as well as noradrenaline turnover shall be established in future experiments.

4.2 Postnatal development of the amino acids neurotransmitting systems

As described above, the monoamines increase differently in the brain of precocial and altricial species. In contrast, the amino acids represent similar developmental pattern in both groups. For instance, glutamate increases approximately 2-fold in rats, 1.5-fold in *Octodon degus*, from birth until adulthood, however in some brain areas certain amino acids do not change their levels during brain maturation. In principle the development of amino acids in the degu brain is completed at the developmental time window between PND 14 and 21.

Among all the amino acids analyzed only taurine has been found to be the one expressing clear developmental pattern for both sexes in *Octodon degus*. In all the examined regions this brain amino acid decreased constantly from PND 3 until adulthood, which resembles the picture found in altricial mice (Agrawal et al., 1968).

Taurine has been proposed to play a neuromodulating role in the developing, non-mature brain (Sturman, 1993). It takes also a part in neuroprotection (Saransaari and Oja, 2000; 2007) and osmoregulation (Walz and Allen, 1987). Taurine has a specific function in terms of influencing GABA role in the brain. First of all, taurine inhibits GABA transaminase, enzyme metabolizing GABA in neuronal tissue (Frossini et al., 2003; 2006; Ricci et al., 2006). This allows GABA to persist longer in the synapse and bind to postsynaptic receptor. Secondly, taurine is able to mimic the effects of GABA by binding to $GABA_A$ receptor (Louzada et al., 2004; Paula-Lima et al., 2005). By enhancing GABA function, taurine is preventing the excitotoxicity in the brain. Excitotoxicity can occur when glutamate activates NMDA receptor and consequently intracellular Ca^{2+} ions increase, which causes cell excitability. When GABA and taurine activate $GABA_A$ receptor the intracellular Cl^- ions concentration goes up, which reduces the excitability and, as a consequence, prevents the excitotoxicity (El Idrissi, 2006). Therefore, GABA and taurine form a protective system against the excess of excitatory amino acids in the neuronal tissue (Saransaari and Oja, 2007). Taurine has been also found to increase in response to presence of free radicals, which increase glutamate. In this case taurine also modulates the state of the cell by keeping in hyperpolarized, and therefore it prevents it from excitability, acting as an antioxidant (Saransaari and Oja, 2004; Yildirim et al., 2007). Perhaps at the very early (gestational and after birth) stages of development taurine is the main inhibitory amino acid, while at the same time GABA could still play an excitatory role (Leinekugel et al., 1999). That would explain the sudden and constant decline of taurine levels in developing degu brain. However, for GABA no pronounced increase in the amounts in the maturing brain has been found, which would suggest that GABAergic system is relatively well developed already at birth, which is in contrast to findings in altricial species such as rats and mice, where GABA shows a progressive increase in the whole brain content up to PND 30 (Agrawal et al. 1966; 1968).

The role of asparatate in neural transmission remains up to now unclear. The study of Gundersen et al. (1998) proves that in stratum radiatum of hippocampal CA1 region, aspartate is colocalized with glutamate in excitatory terminals, concentrated in synaptic vesicles and subject to exocytotic release from the same nerve endings that contain and release glutamate. Therefore perhaps it plays some role in excitatory neurotransmission, however its role in the process of neurotransmission remains controversial and unclear. Study of Ogata et al. (1996) showed that during ischemia

neurons may be damaged by increased release of aspartate from glial cells, although under physiological conditions aspartate is not as potent as glutamate in inducing excitatory transmission. The immunochistochemical measurements showed that aspartate is localized in higher concentration in certain rat brain areas, such as olfactory bulb, hypothalamus, cerebellum and brain stem, but also endocrine structures, for instance adrenal medulla, posterior pituitary and pineal gland (Schell et al., 1997). That would denote aspartate as a substance playing both neuronal and neuroendocrine function. The increased levels of aspartate in cortical regions, striatum, hippocampus, cerebellum and brainstem of juvenile FMR1 knockout mice points to this amino acid as to the signal molecule possibly involved in fragile X mental retardation (Gruss and Braun, 2001; 2004).

4.3 The impact of an acute stress challenge on serotonergic neurotransmission during early postnatal development

There is evidence from experiments using brain homogenates (Noguchi et al., 2001) or *in vivo* microdialysis (Adell et al., 1997; Fujino et al., 2002) that the serotonergic system is involved in the mediation of various kinds of stress including tail pinch, handling, saline injection or forced swimming (Chaouloff et al., 1999). However, to the present knowledge little is known about the effects of strong emotional stress in the immature brain. As it comes from this study, parental separation acts as a stressor which is able to alter serotonergic neurotransmission in a sex-, time- and brain region specific manner in *Octodon degus*. In both sexes, I observed a decrease of serotonin levels (frontal cortex) and an increase in 5-HIAA/5-HT ratios (frontal cortex, hippocampus) which indicates an increased 5-HT turnover in the acutely separated animals. Interestingly, neonatal handling of rat pups (i.e., removal from the mother and the home cage for 15 minutes), a widely used model to study the environmental regulation of neural development and function, also induces increased 5-HIAA/5-HT ratios in frontal cortex and hippocampus (Smythe et al., 1994). Vazquez et al. (2000) had shown that post-synaptic 5-HT receptors in the developing hippocampus and cortex are sensitive to maternal deprivation – such treatment significantly increases the levels of 5-HT mRNA receptors in both structures at the age of PND 6, 9 and 12 in rats. In

addition, early postnatal aversive experience influences the serotonergic system, so that induced changes are still detectable at adulthood (Daniels et al., 2004; Matsumoto et al., 2005). Taken together, these findings indicate that short-term manipulation of the neonate's social environment affects the serotonergic system immediately. Noteworthy, in the frontal cortex of male degu pups the response towards parental separation is restricted to the first two weeks of life, while in females there is a prolonged period of responsiveness, i.e. at least until PND 21. An extended female's vulnerability towards a challenging situation in this species was also shown by Ziabreva et al. (2003a; 2003b). A short-term exposition to brief (3 min) separation from the parents and siblings and exposure to unfamiliar environment early in life resulted in more pronounced effects at the level of D1 and 5-HT1A receptors in females compared to males. This contributes to a growing body of literature that suggests that pre- or post-natal exposure to stress causes sexually dimorphic effects (Patchev and Almeida, 1998; Palanza, 2001; Kudielka and Kirschbaum, 2005; Weinstock, 2005). For instance, Papaioannou et al. (2002) had proven the sex differences in the effects of neonatal handling and the female's increased susceptibility to express ´depressive´ behavior. Furthermore, Carlsson et al. (1985) showed stronger responses towards different drugs involved in the serotonergic transmission in females which are indicative of sex differences in the brain 5-HT neuronal systems.

4.4 The impact of repeated neonatal stress exposure on basal and stress-evoked serotonergic neurotransmission

The present study revealed that repeated separation stress has a region specific permanent impact on the serotonergic system in *Octodon degus* manifested mainly as increased basal 5-HIAA levels or 5-HIAA/5-HT ratios but no changes in 5-HT (except of an increase after acute stress in repeatedly separated males) measured at PND 21. In contrast to most of the previous studies which focused on long-term effects induced by different separation paradigms in adulthood (for review see Lehmann and Feldon, 2000; Holmes et al., 2005; Pryce et al., 2005), this early age stage (i.e., PND 21) was chosen because of the interest in finding indices related to neurological diseases to make the possible therapeutic window as wide as possible. The present results in young animals

contrast with findings of Matthews et al. (2001), who described after maternal separation a decrease of serotonin and no differences in 5-HIAA/5-HT ratios in homogenates of the mPFC and hippocampus of adult rats. Since both studies differ in some aspects of the experimental procedure (i.e., species used, duration of separation, environmental conditions, age of examination), the differences rather highlight the importance of those aspects mentioned here for the final outcome of separation than make these studies comparable directly. However, these results together with the work of Gartside et al. (2003), who determined an array of functional alterations in 5-HT levels and its metabolic activity in adulthood, further strengthen the evidence that early life experience can lead to long-term dysregulation of serotonergic functions in a multi-factorial dependent manner. The question is, whether such changes are pathological or beneficial for the animal's behavior and development? The study of Gruss et al. (2006) showed that repeatedly separated male degus develop enhanced hyperactivity compared to females. In addition, same males express higher endocrine response than females. Braun et al. (2003) proved that parentally deprived degus besides of the developed running hyperactivity remain unaffected by the presence of maternal call, which might suggest that aversive experience can induce reduced sensitivity or attention deficit in these animals. The disturbance of early parent-infant relationship is considered as one of the main factors in development of adult mental and behavioral disorders. Furthermore, clinical as well as basic research implicates altered 5-HT function in such diseases. For instance, serotonin, among other factors, has been shown to play a crucial role in the etiology of depression which is supported by the fact that the administration of 5-HT precursors (i.e. tryptophan, 5-hydroxytryptophan) either suppresses the symptoms or prevents the disease formation (Kalia, 2005). Additionally, several classes of antidepressants have been shown to strengthen serotonergic neurotransmission at pre- and postsynaptic sites (Blier and de Montigny, 1999; Blardi et al., 2005). The second interesting finding of this experiment is the attenuated increase of 5-HIAA/5-HT ratio in the frontal cortex of repeatedly separated females which points to adaptation-like processes in these animals. This is supported by the finding that the same females express attenuated stress evoked cortisol/corticosterone responses under the same stress paradigm (Gruss et al., 2006). This would suggest that female degus might develop stronger endocrine and neurochemical adaptive mechanisms in response to separation stress. However, if this leads to more efficient coping strategies in response to stressful events in later life compared to male animals, remains to be determined in future

experiments. These results stand in the opposite to findings of Smythe et al. (1994) who demonstrated a stronger increase in 5-HIAA/5-HT ratios in chronically compared to acutely handled 7-day old rats. As stated earlier, the difference might be explained by the different species, age and/or environmental situation and last but not least by experimental approach. Some authors consider early handling as a paradigm, which leads to acquisition of more maternal care. In other words, handling itself would be not long enough to induce the disturbance in homeostasis, but would stimulate the mother to nurse the infants more intensively after reunion (Pryce et al., 2002).

In conclusion, the first part of my dissertation revealed an early maturation of the dopaminergic, serotonergic and amino acids systems in the semi-precocial *Octodon degus* compared to altricial species like rats or mice. Furthermore, these findings suggest that repeated one-hour separation periods at different age stages during the first three weeks of life acts as a stressor having an immediate as well as lasting effect on the serotonergic system in *Octodon degus*. Therefore, the current experiments expand the understanding of the neurochemical maturation of those brain regions, which respond to parental separation with changes in serotonergic activity. However, the method used in this study (i.e., brain tissue homogenates) offers no information about the changes in the dynamics of serotonergic neurotransmission which remains to be determined by other techniques, i.e. *in vivo* microdialysis, in future experiments.

Part II. Neurochemical consequences of juvenile stress and chronic methylphenidate treatment on dopaminergic function in *Octodon degus*. *In vivo* microdialysis study

The second part of my dissertation presents the pattern of dopaminergic responses to methylphenidate in the prestressed, juvenile, immature and still developing brain of *Octodon degus.* This paradigm mimics the clinical situation in human children and the use of MP treatment much more appropriately than studies performed in normal and adult brains. My working hypothesis for this part of my dissertation was that repeatedly separated (prestressed) animals with hyperactive symptoms respond differently to this drug, compared to controls. Moreover, I hypothesized that the age of the animal as well as the duration of drug treatment would be also the critical factors for methylphenidate action in the brain. As an outcome of performed *in vivo* experiments I observed that: (*i*) the effect of an acute methylphenidate injection on extracellular DA levels is age dependent, (*ii*) repeatedly stressed animals are less sensitive to acute social separation stress challenge and showed sensitized DA release during an acute methylphenidate injection and (*iii*) chronic methylphenidate pretreatment sensitizes the animals to DA release during acute social separation stress as well as an acute methylphenidate injection with the repeatedly prestressed animals showed more pronounced DA release than unstressed controls. These findings are in line with my hypothesis, that age, rearing conditions as well as drug treatment duration might significantly alter the action of this psychostimulant in the brain.

4.5 Age differences in response to methylphenidate

One of the aims of this study was to determine how the animals tested at two different developmental time points respond to methylphenidate. MP is believed to block dopamine transporters (DAT), which results in inhibition of the re-uptake of neurotransmitter to the presynaptic site. All *in vivo* microdialysis studies performed so far in rats have been investigating just the effects in adult brains (Kuczenski and Segal,

1999; Gerasimov et al., 2000; Huff and Davies, 2002; Marsteller et al., 2002), revealing that the levels of extracellular dopamine rise after administration of the drug. This effect has been also found in striatum of healthy human subjects with the use of PET studies (Volkov et al., 2001). In my experiments I observed that injection of MP does not lead to significant changes in dopamine levels in both mPFC and Nac, at the post-weaning age. The age-dependency towards acute MP application could be due to structural, neuroanatomical, neurochemical or (intra-)cellular immaturity of the dopaminergic system, especially in the late developing prefrontal cortex. One possible explanation would be the immaturity or lack of cellular elements involved in dopamine action at the synapse, mainly dopamine transporters (DAT), which are believed to be engaged in the response to MP. However this interpretation seems unlikely, since it has been found in the altricial rat that DA transporters reach around 70 % of adult levels in the PFC and 70 – 90 % in Nac at PND 21 (Coulter et al., 1996; Tarazi et al., 1998). On the structural level adult like innervation patters in the mPFC of degus are established around the age of PND 14 (Braun et al., 2000). Thus, it appears likely that in the semi-precocial degu similar or even more adult-like dopaminergic function should be found, and therefore cannot be solely accounted to the observed age-dependency of MP-evoked responses. Neurochemically it has been found in the mPFC and Nac that the magnitude of acute stress-evoked DA increase is comparable in juvenile and adolescent animals. Furthermore, injection of amphetamine, which also enhances DA in the extracellular space, but by a different mechanism (Kahling et al., 2005; Sulzer et al., 2005), revealed a comparable increase of DA levels in both, juvenile and adolescent degus (own unpublished observations). The postsynaptic dopamine machinery i.e. the receptors might be also involved in this age-specific reaction. As known from rat development D1 receptor levels in the Nac at PND 21 reach about 60 % of adult amounts, similar to findings in the frontal cortex, while D2 at this developmental point are slightly above adult levels in Nac and 70 % of adult values in frontal cortex (Tarazi and Baldessarini, 2000). Last but not least, one of the mechanisms leading to observed age differences in response to MP could be the immaturity of the cellular machinery that regulates exocytosis, i.e. the release of dopamine into synapse, or some other elements involved in dopaminergic action. Penner et al. (2002) observed age specific c-fos protein distribution in murine striatum after acute MP administration. c-*fos* gene is one of the immediate early genes, which are induced by psychostimulants such as cocaine or amphetamine and subsequently intensively expressed. Fukui et al. (2003) demonstrated

in adult mice, that MP increases Thr34 and decreases Thr75 phosphorylation of DARPP-32 (dopamine- and cAMP-regulated phosphoprotein, 32 kDa). Interestingly, such effect was not observed in young (14-15 days old) animals, which proves that also in this species some of not yet fully developed elements directly related to the vesicular dopamine release may be the cause of differential responses to methylphenidate at different ages.

4.6 The impact of early experience on social separation stress effect and methylphenidate action in the brain

My data showed that repeated exposure to parental separation stress during the first 3 weeks of life significantly affects the response of dopaminergic mechanism to both acute social separation stress and to methylphenidate injection. Since repeatedly stressed animals display a blunted DA release in response to acute separation stressor, this might indicate a habituation or decreased sensitivity towards stressful events. In other words, this experience-induced effect indicates that there are adaptive mechanisms, which might be related to learning, and specifically involves changes in the mPFC, and to a lesser extent also in the Nac. Along this line, an adaptation of the endocrine response was observed in repeatedly separated degu pups (Gruss et al., 2006). This endocrine and neurochemical habituation might allow the animals to cope differentially - perhaps better - with stressful situations.

Another outcome of this study was the observed tendency of prestressed animals to vocalize in a lower extend than controls under stressful event, which would contribute to the adaptation mechanism hypothesis. This confirms previous findings of behavioral aspects in 10-14 day old degus (Braun et al., 2003). As known from other reports, the distress vocalizations might reflect the emotional state of the individual (Panksepp, 1980; Thomas et al., 1983; Knutson et al., 2002; Panksepp and Burgdorf, 2003). In contrast to data from my study, Kehoe showed that exorbitant behavioral responses to environmental challenges are typical for juvenile (Kehoe et al., 1998a) as well as adult (Kehoe et al., 1998b) rats stressed as neonates, which points to the early experience as to the factor which under some conditions might sensitize the animal to the stressful events. Since vocalizations emitted during stress may serve as an anxiety

marker, it is believed that this phenomenon is mediated mostly by the serotonergic and GABAergic systems. Fish et al. (2000) proved that agonists of the 5-HT_1 receptors (8-OH-DPAT, flesinoxan, CP-94,253 and TFMPP) are potent to decrease maternal separation induced distress vocalizations in 7 day old mice. Same was observed for midazolam and allopregnanolone, drugs acting on $GABA_A$ receptors. In my study, serotonin was not measurable under the conditions established for dopamine, however it would be of interest to see the correlation of 5-HT levels and the amount of vocalizations evoked by acute separation stressor. That needs to be determined in future experiments.

My data have shown that basal levels of dopamine differ significantly between unstressed and repeatedly stressed degus, which suggests that in addition to the several morphological changes in the brain (Braun et al., 2000; Helmeke et al., 2001a; 2001b; Ovtscharoff and Braun, 2001; Poeggel et al., 2003a; 2003b) dopamine itself does not appear in stable levels at the synapse after repeated stressful events. This result would be in contrast to findings of Matthews et al. (2001), who described no significant disparities in total (intra- and extracellular) dopamine content of medial PFC between socially reared and repeatedly maternally separated rats, measured at adulthood. On the other hand, microdialysis technique restricts the investigations only to the extracellular pool of measured substance, therefore these results are not fully comparable. In addition, Zhang et al. (2006) described no effect on extracellular dopamine levels after neonatal isolation in rats. None the less, their measurements have been performed in adulthood, i.e. at PND 70-90, and there is no proof that any of the changes are not visible yet at younger ages.

The second effect of early stressful experience described in this study is the enhancement of dopaminergic response to pharmacological challenge, i.e. methylphenidate injection. Repeatedly stressed, adolescent degus respond with increased extracellular dopamine, as it was described in adult animals of other species, while at the age of weaning they show a distinct decline in the prefrontal DA levels after drug injection. Such surprising and new effect would be probably the sum of both early experience and non-finished development of some dopamine-engaged mechanisms. Repeatedly stressed rats, subjected to an amphetamine or cocaine challenge at PND 10 respond with much higher dopamine release in nucleus accumbens or ventral striatum than non stressed controls (Kehoe et al., 1998a; Kosten et al., 2003). Additionally, cross-sensitization to amphetamine may be induced by social defeat stress

in rats (Nikulina et al., 2004; de Jong et at., 2005). Altogether, such findings support my results from adolescent degus. Quantitative developmental analysis of dopaminergic fiber innervation revealed gradually increasing dopaminergic afferent fiber density in the mPFC during the first 2 postnatal weeks, i.e. the time period during which the animals in the present study were exposed to early separation stress (Braun et al., 2000). Together with previous results, which showed changes in the dopaminergic innervation pattern of the mPFC (Braun et al., 2000) and Nac (Gos et al., 2006), these neurochemical data confirm the hypothesis that repeated parental separation stress, acting as a severe stressor in degu pups (Gruss et al., 2006), modifies not only the neuroanatomy of the dopaminergic fiber systems, but also alters dopamine release in response to both, emotional as well as pharmacological challenges. The results in adolescent animals also revealed another developmental time window of brain plasticity in periadolescence (PND 21 - 45), during which the neonatally induced neurochemical changes can be "normalized" by rearing the animals under undisturbed social control conditions. The "opposite" neurochemical response in the mPFC of stressed animals appears to be transient, since as adolescents (i.e. more than three weeks after the last exposure to separation stress) their neurochemical response to MP is back to normal, i.e. similar to age matched control animals.

What underlies the decline of dopamine in juvenile prestressed PFC remains controversial, especially taking into account that this particular model would somehow reflect the human ADHD children, namely the therapeutical target group in ADHD cure and methylphenidate treatment. Previous studies in our group revealed that repeated brief parental separation followed by an exposure of the degu pup to the unfamiliar environment induces an up-regulation of D1 receptors in several subregions of PFC (Ziabreva et al., 2003b). In rats, dopamine transporters have been found to decrease after repeated events early in life in nucleus accumbens and caudate putamen (Meaney et al., 2002). Perhaps, such two alterations would lead together to the observed pattern of dopaminergic responses, however if dopamine transporters are also altered in repeatedly separated degus needs to be investigated in further studies.

Another hypothesis which needs to be resolved by a separate series of experiments is the possible modulation of dopaminergic drug response by the acute stress (social separation) applied just 1 hour beforehand. Handling of adult rats has been shown to attenuate significantly the dopamine release after administration of methylphenidate (Marsteller et al., 2002). However, handling does not necessarily has

to be considered as stress. Moreover, all the observed fluctuations in dopamine levels might be mediated by a network connections in the limbic system, since stimulation of PFC have been shown to inhibit the action of Nac (Jackson et al., 2001) via amygdala regulation (Jackson and Moghaddam, 2001).

Another aspect of methylphenidate effect is the possible mediation of this drug response by serotonergic or noradrenergic systems. It has been shown that application of MP does not influence serotonin levels (Kuczenski and Segal, 1997), moreover the affinity of methylphenidate for the serotonin transporter is very low (Gatley et al., 1996). However, the induction of serotonin increase by citalopram (5-HT re-uptake inhibitor) in rat hippocampus might be enhanced by methylphenidate, in addition MP attenuates the 5-HT citalopram mediated release in prefrontal cortex (Weikop et al., 2007). The study of Gainetdinov et al. (1999) showed that hyperactivity of dopamine transporter gene knock out mice (DAT-KO) might be reduced by serotonergic drug fluoxetine. Noradrenergic neurons, located mainly in locus coeruleus (LC) are considered to mediate the orienting response, selective attention and vigilance (Pribram and McGuinness, 1975; Tucker and Williamson, 1984). Studies in monkeys with high levels of activity, behavioral agitation and worse vigilance performance (Solanto, 1998) have shown that MP is potent to produce a significant reduction in firing of LC neurons. It is also likely, that in ADHD excessive noradrenaline release from LC causes reduced capacity of PFC to respond to phasic stimuli (Mefford and Potter, 1989; McCracken, 1991; Pliszka et al, 1996). Finally, atomoxetine, which selectively blocks the reuptake of noradrenaline from the synapse, by blocking the noradrenaline presynaptic transporter (Michelson et al., 2001; Bymaster et al., 2002; Kratochvil et al., 2003) is considered as one of the most common drugs in ADHD treatment, next to methylphenidate. Still, the data on MP action on noradrenergic and especially on serotonergic system is quite limited, and in order to conclude if MP effects found in this study are related to 5-HT or NA role, a series of additional microdialysis experiments should be performed.

4.7 Neurochemical sensitization to emotional and pharmacological challenge after repeated methylphenidate treatment

In this study I describe that adolescent degus, subjected to over 3-weeks chronic methylphenidate treatment, develop an enhanced dopaminergic response to the drug. Sensitization (the enhancement of the response to consecutive doses of the drug), next to tolerance, has been believed to play the primary role in drug addiction. Sensitization is evoked by such psychostimulants as cocaine, amphetamine, nicotine and the way of drug administration might be important for its development (Vetulani, 2001). In rats, repeatedly withdrawn amphetamine leads to behavioral sensitization following a stress challenge and is used as an animal model of anhedonia, a core symptom of depression in humans (Russig et al., 2006). Exposure to repeated amphetamine induces sensitization of HPA axis or dopaminergic neurons to a subsequent stressor, such as restraint stress (Barr et al., 2002) or foot shock (Robinson et al., 1987). On the subcellular level repeatedly injected methylphenidate has been proved to decrease the amount of dopamine transporters in rostral caudate putamen but not in nucleus accumbens (Izenwasser et al., 1999). Chronic MP pretreatment may also result in cross-sensitization to both cocaine (Andersen et al., 2002; Schenk and Izenwasser, 2002) and amphetamine (Yang et al., 2003b) but not methamphetamine (Kuczenski and Segal, 2002). Concerning locomotor sensitization, the available data are inconsistent, since methylphenidate has been either shown not to produce sensitization (Izenwasser et al., 1999) or to result in an opposite way (McDougall et al., 1999). However, such differences might be an outcome of different drug doses as well as rat strains used (Yang et al., 2003a; Amini et al., 2004). The issue which still needs further investigation is, whether the neurochemical sensitization observed in degus is persistent or abolished after abstinence period. The low, oral doses of methylphenidate may produce the improvement of cognitive function of rat PFC (Arnsten and Dudley, 2005), furthermore oral vs. intraperitoneal administration is much less potent in terms of increasing the dopamine at the synapse (Gerasimov et al., 2000). Therefore, the way of administration as well as the determination of appropriate dose of the drug would be also of interest, as in my study it goes several fold beyond the therapeutic doses used in human children. Interestingly, my data further support the suggestion that not only stressful events are able to modulate the action of MP, but vice versa, also chronic MP treatment can "cross-sensitize" the response of the mesocortical as well as mesolimbic dopaminergic

system towards stress observed in MP-pretreated degus. In line with this interpretation, Marsteller et al. (2002) demonstrated a potentiation of DA increase during an acute stress situation induced by MP. A comprehensive behavioral study suggests that repeated MP application during adolescence resulted in a decreased responsiveness to rewarding stimuli but an increased sensitivity to aversive stimuli in animals tested as adults (Bolanos et al., 2003). The observed sensitization and "cross-sensitization" of behaviorally and pharmacologically induced neurochemical changes are most likely due to complex functional changes which include numerous neurotransmitters and brain regions. The recent investigations of our group have shown that stress-induced elevated spine densities in anterior cingulated region of mPFC at PND 21 can be normalized by chronic methylphenidate treatment during periadolescence (Zehle et al., 2007). However, such effect is found in the prestressed animals, treated with relatively low dose of the drug (1 mg/kg). In addition, repeated injections with higher MP dose (5 mg/kg) in unstressed controls caused the opposite effect. Furthermore, there is evidence for an involvement of calcium/ calmodulin-kinase II-dependent mechanisms in accumbal amphetamine-induced dopamine release potentiated by cocaine pretreatment (Pierce and Kalivas, 1997). The observed pharmacologically induced functional changes might be the result of altered dopaminergic innervation of GABA-ergic neurons in the areas which were measured, as well as altered functions of dopaminergic neurons in the midbrain (Hedou et al., 2001), however the detailed mechanisms underlying these changes need to be analyzed in more details in the future.

4.8 General conclusions and clinical relevance

According to European Society for Child and Adolescent Psychiatry (ESCAP) ADHD is a risk factor of developmental alterations and may constitute a background for plenty of severe psychological complications. The lack of proper knowledge about ADHD as well as little or no help from the closest environment causes the formation of inappropriate demeanours towards the affected child as well as constant critics about its behavior and repulsive attitudes. Thus, it is of great importance for therapists and teachers to understand the background of this particular disease in order to avoid the mistakes in the treatment and therapy.

The finding that the functional maturation of dopaminergic limbic function is significantly altered in stressed, behaviorally altered and/or periadolescent drug-treated animals reveals novel insights into experience-related factors involved in the etiology of ADHD, and into the long-term consequences of pharmacological treatment on brain development. Current results widen the knowledge about the differential action of methylphenidate, which is frequently prescribed to children, in young, non-fully developed brain in comparison to the adult neuronal tissue. Basic information for the therapists would be the fact that early experience can significantly alter the way of methylphenidate action in the brain. Such experience might be related to the absence of parents as it was shown in this study, but one can expect that other stressful stimuli would be also potent to induce neurochemical, neuroanatomical or subcellular changes in the brain that would finally lead to differential pharmacological responses. Therefore, at least in some cases the treatment of ADHD should be preceded with a detailed survey on the patient's family situation and past events that would potentially influence the disease therapy. Another important aspect is the chronic methylphenidate treatment. Here I have shown, that repeated administration of this particular psychostimulant leads to sensitization to both behavioral as well as pharmacological challenges. One can conclude, that extended theraphy in human children or adolescents would potentially induce similar effects, which at last might be disruptive for the entire treatment. However, it has to be taken into account that in this research project relatively high amounts of the drug have been used – 10 times higher than therapeutic doses. Whether such sensitization is persistant and lasts until adulthood should be determined in separate series of experiments. At last, the differential responses to the methylphenidate at different developmental points should be taken into consideration. As it comes from this study, young brain responds differentially to this psychostimulant. Such results point to the need of cooperation of several specialists, who should consider both medical and psychological aspects in the therapy of ADHD.

5 REFERENCES

Adell A, Casanovas JM, Artigas F. **1997**. Comparative study in the rat of the actions of different types of stress on the release of 5-HT in the raphe nuclei and forebrain areas. *Neuropharmacology* 36:735-741.

Agid O, Shapira B, Zislin J, Ritsner M, Hanin B, Murad H, Troudart T, Bloch M, Heresco-Levy U, Lerer B. **1999**. Environment and vulnerability to major psychiatric illness: a case control study of early parental loss in major depression, bipolar disorder and schizophrenia. *Mol Psychiatry* 4:163-172.

Agrawal HC, Davis J, Himwich WA. **1966**. Postnatal changes in free amino acid pool of rat brain. *J Neurochem* 13:607-615.

Agrawal HC, Davis J, Himwich WA. **1968**. Developmental changes in mouse brain: weight, water content and free amino acids. *J Neurochem* 15:917-923.

Akil M, Pierri JN, Whitehead RE, Edgar CL, Mohila C, Sampson AR, Lewis DA. **1999**. Lamina-specific alterations in the dopamine innervation of the prefrontal cortex in schizophrenic subjects. *Am J Psychiatry* 156:1580-1589.

Alvarez C, Vitalis T, Fon EA, Hanoun N, Hamon M, Seif I, Edwards R, Gaspar P, Cases O. **2002**. Effects of genetic depletion of monoamines on somatosensory cortical development. *Neuroscience* 115:753-764.

Alvarez MP, Jimenez V, Cano P, Rebollar P, Cardinali DP, Esquifino AI. **2006**. Circadian rhythms of prolactin secretion in neonatal female rabbits after acute separation from their mothers. *Gen Comp Endocrinol* 146:257-64.

Amini B, Yang PB, Swann AC. **2004**. Differential locomotor responses in male rats from three strains to acute methylphenidate. *Int J Neurosci* 114:1063-1084.

Anda RF, Whitfield CL, Felitti VJ, Chapman D, Edwards VJ, Dube SR, Williamson DF. **2002**. Adverse childhood experiences, alcoholic parents and later risk of alcoholism and depression. *Psychiatr Serv* 53:1001-1009.

Andersen SL, Arvanitogiannis A, Pliakas AM, LeBlanc C, Carlezon WA Jr. **2002**. Altered responsiveness to cocaine in rats exposed to methylphenidate during development. *Nat Neurosci* 5:13-14.

Arnsten AFT. **1997**. Catecholamine regulation of the prefrontal cortex. *J Psychopharmacol* 11:151-162.

Arnsten AFT, Dudley AG. **2005**. Methylphenidate improves prefrontal cortical cognitive function through α2 adrenoreceptor and dopamine D1 receptor actions: relevance to therapeutic effects in attention deficit hyperactivity disorder. *Behav Brain Funct* 1:2.

Barr AM, Hofmann CE, Weinberg J, Phillips AG. **2002**. Exposure to repeated, intermittent d-amphetamine induces sensitization of HPA axis to a subsequent stressor. *Neuropsychopharmacology* 26:286-294.

Bartesaghi R, Severi S, Guidi S. **2003**. Effects of early environment on pyramidal neuron morphology in field CA1 of the guinea pig. *Neuroscience* 116:715-732.

Becker K, Abraham A, Kindler J, Helmeke C, Braun K. **2007**. Exposure to neonatal separation stress alters exploratory behavior and corticotropin releasing factor expression in neurons in the amygdala and hippocampus. *Dev Neurobiol* 67:617-29.

Belhage B, Hansen GH, Elster L, Schousboe A. **1998**. Effects of gamma-aminobutyric acid (GABA) on synaptogenesis and synaptic function. *Perspect Dev Neurobiol* 5:235– 246.

Berger A, Posner MI. **2000**. Pathologies of brain attentional networks. *Neurosci Biobehav Rev* 24:3-5.

Berger-Sweeney J, Hohmann CF. **1997**. Behavioral consequences of abnormal cortical development: insights into developmental disabilities. *Behav Brain Res* 86:121-142.

Blardi P, de Lalla A, Urso R, Auteri A, Dell'Erba A, Bossini L, Castrogiovanni P. **2005**. Activity of Citalopram on adenosine and serotonin circulating levels in depressed patients. *J Clin Psychopharmacol* 25:262-266.

Blier P, de Montigny C. **1999**. Serotonin and drug-induced therapeutic responses in major depression, obsessive-compulsive and panic disorders. *Neuropsychopharmacology* 21:91S-98S.

Bock J, Braun K. **1998**. Differential emotional experience leads to pruning of dendritic spines in the forebrain of domestic chicks. *Neural Plast* 6:17-27.

Bock J, Braun K. **1999**. Filial imprinting in domestic chicks is associated with spine pruning in the associative area, dorsocaudal neostriatum. *Eur J Neurosci* 11:2566-2570.

Bock J, Gruss M, Becker S, Braun K. **2005**. Experience-induced changes of dendritic spine densities in the prefrontal and sensory cortex: correlation with developmental time windows. *Cerebral Cortex* 15:802-808.

Bolanos CA, Barrot M, Berton O, Wallace-Black D, Nestler EJ. **2003**. Methylphenidate treatment during pre- and periadolescence alters behavioral responses to emotional stimuli at adulthood. *Biol Psychiatry* 54:1317-1329.

Boyson SJ, Adams CE. **1997**. D1 and D2 dopamine receptors in perinatal and adult basal ganglia. *Pediatr Res* 41:822-831.

Braun K, Lange E, Metzger M, Poeggel G. **2000**. Maternal separation followed by early social deprivation affects the development of monoaminergic fiber systems in the medial prefrontal cortex of *Octodon degus*. *Neuroscience* 95:309-318.

Braun K, Kremz P, Wetzel W, Wagner T, Poeggel G. **2003**. Influence of parental deprivation on the behavioral development in *Octodon degus*: modulation by maternal vocalizations. *Dev Psychobiol* 42:237-245.

Braun S, Scheich H. **1997**. Influence of experience on the representation of the "mothering call" in frontoparietal and auditory cortex of pups of the rodent *Octodon degus*: FDG mapping. *J Comp Physiol A* 181:697-709.

Buznikov GA, Lambert HW, Lauder JM. **2001**. Serotonin and serotonin-like substances as regulators of early embryogenesis and morphogenesis. *Cell Tissue Res* 305:177-186.

Bymaster FP, Katner JS, Nelson DL, Hemrick-Luecke SK, Threlkeld PG, Heiligenstein JH, Morin SM, Gehlert DR, Perry KW. **2002**. Atomoxetine increases extracellular levels of norepinephrine and dopamine in prefrontal cortex of rat: a potential mechanism for efficacy in attention deficit/hyperactivity disorder. *Neuropsychopharmacology* 27:699-711.

Campbell J, Spear LP. **1999**. Effects of early handling on amphetamine induced locomotor activation and conditioned place preference in the adult rat. *Psychopharmacology (Berl.)* 143: 183-189.

Carboni E, Silvagni A. **2004**. Experimental investigations on dopamine transmission can provide clues on the mechanism of the therapeutic effect of amphetamine and methylphenidate in ADHD. *Neural Plast* 11:77-95.

Carlsson M, Svensson K, Eriksson E, Carlsson A. **1985**. Rat brain serotonin: biochemical and functional evidence for a sex difference. *J Neural Transm* 63:297-313.

Cases O, Vitalis T, Seif I, De Maeyer E, Sotelo C, Gaspar P. **1996**. Lack of barrels in the somatosensory cortex of monoamine oxidase A deficient mice: role of a serotonin excess during the critical period. *Neuron* 16:297–307.

Castellanos F. **1997**. Toward a pathophysiology of attention-deficit/hyperactivity disorder. *Clin Pediatr* 36:381-93.

Castrén E. **2005**. Is mood chemistry? *Nature Rev Neurosci* 6:241:246.

Chaouloff F, Berton O, Mormède P. **1999**. Serotonin and stress. *Neuropsychopharmacology* 21:28S-32S.

Cook EH, Stein MA, Krasowski MD, Cox NJ, Olkon DM, Keiffer JE, Leventhal BL. **1995**. Association of attention deficit disorder and the dopamine transporter gene. *Am J Hum Genet* 56:993–8.

Coulter CL, Happe HK, Murrin LC. **1996**. Postnatal development of the dopamine transporter: a quantitative autoradiographic study. *Dev Brain Res* 92:172-181.

Daniel DG, Weinberger DR, Jones DW, Zigun JR, Coppola R, Handel S, Bigelow LB, Goldberg TE, Berman KF, Kleinman JE. **1991**. The effect of amphetamine on regional cerebral blood flow during cognitive activation in schizophrenia. *J Neurosci* 11:1907-1917

Daniels WM, Pietersen CY, Carstens ME, Stein DJ. **2004**. Maternal separation in rats leads to anxiety-like behavior and a blunted ACTH response and altered neurotransmitter levels in response to a subsequent stressor. *Metab Brain Dis* 19:3-14.

Datiles MB, Fukui H. **1989**. Cataract prevention in diabetic *Octodon degus* with Pfizer's sorbinil. *Curr Eye Res* 8:233-237.

Day JJ, Carelli RM. **2007**. The nucleus accumbens and Pavlovian reward learning. *Neuroscientist* 13:148-159.

De Kloet ER, Vreugdenhil E, Oitzl MS, Joels M. **1998**. Brain corticosteroid receptor balance in health and disease. *Endocr Rev* 19:269-301.

Di Chiara G, Bassareo V. **2007**. Reward system and addiction: what dopamine does and doesn't do. *Curr Opin Pharmacol* 7:69-76.

Djatchkova-Podkletnova I, Alho H. **2005**. Alterations in the development of rat cerebellum and impaired behavior of juvenile rats after neonatal 6-OHDA treatment. *Neurochem Res* 30:1599-1605

Dougherty DD, Bonab AA, Spencer TJ, Rauch SL, Madras BK, Fischman AJ. **1999**. Dopamine transporter density in patients with attention deficit hyperactivity disorder. *Lancet* 354:2132-2133.

Downs WR, Harrison L. **1998**. Childhood maltreatment and the risk of substance problems in later life. *Health Soc Care Community* 6:35-46.

Dresel S, Krause J, Krause KH, LaFougere C, Brinkbaumer K, Kung HF, Hahn K, Tatsch K. **2000**. Attention deficit hyperactivity disorder: binding of [99mTc]TRODAT-1 to the dopamine transporter before and after methylphenidate treatment. *Eur J Nucl Med* 27:1518-1524.

Ebensperger LA, Bozinovic F. **2000**. Communal burrowing in the hystricognath rodent, *Octodon degus*: a benefit of sociality? *Behav Ecol Sociobiol* 47:365-369.

Ebensperger LA, Hurtado MJ, Soto-Gamboa M, Lacey EA, Chang AT. **2004**. Communal nesting and kinship in degus (*Octodon degus*). *Naturwissenschaften* 91:391-395.

Ebensperger LA, Veloso C, Wallem PK. **2002**. Do female degus communally nest and nurse their pups? *J Ethol* 20:143-146.

El Idrissi A. **2006**. Taurine increases mitochondrial buffering of calcium: role in neuroprotection. *Amino Acids* in press.

Faraone SV, Biederman J, Weiffenbach B, Keith T, Chu MP, Weaver A, Spencer TJ, Wilens TE, Frazier J, Cleves M, Sakai J. **1999**. Dopamine D4 gene 7-repeat allele and attention deficit hyperactivity disorder. *Am J Psychiatry* 156:768-770.

Frosini M, Sesti C, Dragoni S, Valoti M, Palmi M, Dixon HB, Machetti F, Sgaragli G. **2003**. Interactions of taurine and structurally related analogues with the GABAergic system and taurine binding sites of rabbit brain. *Br J Pharmacol* 138:1163-1171.

Frosini M, Ricci L, Saponara S, Palmi M, Valoti M, Sgaragli G. **2006**. GABA-mediated effects of some taurine derivatives injected i.c.v. on rabbit rectal temperature and gross motor behavior. *Amino Acids* 30:233-242.

Fujino K, Yoshitake T, Inoue O, Ibii N, Kehr J, Ishida J, Nohta H, Yamaguchi M. **2002**. Increased serotonin release in mice frontal cortex and hippocampus induced by acute physiological stressors. *Neurosci Lett* 320:91-95.

Fukui R, Svenningsson P, Matsuishi T, Higashi H, Nairn AC, Greengard P, Nishi A. **2003**. Effect of methylphenidate on dopamine/DARPP signalling in adult, but not young, mice. *J Neurochem* 87:1391–1401.

Fulk GW. **1976**. Notes on the activity, reproduction, and social behavior of *Octodon degus*. *J Mammal* 57:495-505.

Funahashi S. **2001**. Neuronal mechanisms of executive control by the prefrontal cortex. *Neurosci Res* 39:147–165.

Fuster JM. **1990**. Prefrontal cortex and the bridging of temporal gaps in the perception-action cycle. *Ann N Y Acad Sci* 608:318–329.

Gainetdinov RR, Wetsel WC, Jones SR, Levin ED, Jaber M, Caron MG. **1999**. Role of serotonin in the paradoxical calming effect of psychostimulants on hyperactivity. *Science* 283:397-401.

Garcia-Allegue R, Lax P, Madariaga AM, Madrid JA. **1999**. Locomotor and feeding activity rhythms in a light-entrained diurnal rodent, *Octodon degus*. *Am J Physiol* 277:R523-R531.

Gartside SE, Johnson DA, Leitch MM, Troakes C, Ingram CD. **2003**. Early life adversity programs changes in central 5-HT neuronal function in adulthood. *Eur J Neurosci* 17:2401-2408.

Gatley SJ, Pan D, Chen R, Chaturvedi G, Ding YS. **1996**. Affinities of methylphenidate derivatives for dopamine, norepinephrine and serotonin transporters. *Life Sci* 58:231-239.

Gerasimov MR, Franceschi M, Volkow ND, Gifford A, Gatley SJ, Marsteller D, Molina PE, Dewey SL. **2000**. Comparison between intraperitoneal and oral methylphenidate administration: a microdialysis and locomotor activity study. *J Pharmacol Exp Ther* 295:51-57.

Glantz LA and Lewis DA. **1997**. Reduction of synaptophysin immunoreactivity in the prefrontal cortex of subjects with schizophrenia. Regional and diagnostic specificity. *Arch Gen Psychiatry* 54:660-669.

Glantz LA and Lewis DA. **2000**. Decreased dendritic spine density on prefrontal cortical pyramidal neurons in schizophrenia. *Arch Gen Psychiatry* 57:65-73.

Glaser PE, Thomas TC, Joyce BM, Castellanos FX, Gerhardt GA. **2005**. Differential effects of amphetamine isomers on dopamine release in the rat striatum and nucleus accumbens core. *Psychopharmacology (Berl)* 178:250-258.

Goldman-Rakic PS. **1996**. The prefrontal landscape: implications of functional architecture for understanding human mentation and the central executive. *Philos Trans R Soc Lond Biol* 351:1445–1453.

Gos T, Becker K, Bock J, Malecki U, Bogerts B, Poeggel G, Braun K. **2006**. Early neonatal and postweaning social emotional deprivation interferes with the maturation of serotonergic and tyrosine hydroxylase-immunoreactive afferent fiber systems in the rodent nucleus accumbens, hippocampus and amygdala. *Neuroscience* 140:811-821.

Greenough WT, Black JE, Wallace CS. **1987**. Experience and brain development. *Child Dev* 58:539-559.

Greydanus DE, Sloane MA, Rappley MD. **2002**. Psychopharmacology of ADHD in adolescents. *Adolesc Med* 13:599-624.

Gruss M, Braun K. **2001**. Alterations of amino acids and monoamine metabolism in male Fmr1 knockout mice: a putative animal model of the human fragile X mental retardation syndrome. *Neural Plast* 8:285-298.

Gruss M, Braun K. **2004**. Age- and region-specific imbalances of basal amino acids and monoamine metabolism in limbic regions of female Fmr1 knock-out mice. *Neurochem Int* 45:81-88.

Gruss M, Westphal S, Luley C, Braun K. **2006**. Endocrine and behavioral plasticity in response to juvenile stress in the semi-precocial rodent *Octodon degus*. *Psychoneuroendocrinology* 31:361-372.

Gundersen V, Chaudhry FA, Bjaalie JG, Fonnum F, Ottersen OP, Storm-Mathisen J. **1998**. Synaptic vesicular localization and exocytosis of L-aspartate in excitatory nerve terminals: a quantitative immunogold analysis in rat hippocampus. *J Neurosci* 18:6059-6070.

Hall FS, Wilkinson LS, Humby T, Robbins TW. **1999**. Maternal deprivation of neonatal rats produces enduring changes in dopamine function. *Synapse* 32:37-43.

Halim ND, Weickert CS, McClintock BW, Hyde TM, Weinberger DR, Kleinman JE, Lipska BK. **2003**. Presynaptic proteins in the prefrontal cortex of patients with

schizophrenia and rats with abnormal prefrontal development. *Mol Psychiatry* 8:797-810.

Hedou G, Homberg J, Feldon J, Heidbreder CA. **2001**. Expression of sensitization to amphetamine and dynamics of dopamine neurotransmission in different laminae of the rat medial prefrontal cortex. *Neuropharmacology* 40:366-382.

Heim C, Nemeroff CB. **2001**. The role of childhood trauma in the neurobiology of mood and anxiety disorders: preclinical and clinical studies. *Biol Psychiatry* 49:1023-1039.

Helmeke C, Ovtscharoff jr W, Poeggel G, Braun K. **2001a**. Juvenile emotional experience alters synaptic composition in the anterior cingulate cortex. *Cereb Cortex* 11:717-727.

Helmeke C, Poeggel G, Braun K. **2001b**. Differential emotional experience induces elevated spine densities on basal dendrites of pyramidal neurons in the anterior cingulate cortex. *Neuroscience* 104:927-931.

Herlenius E, Lagercrantz H. **2001**. Neurotransmitters and neuromodulators during early human development. *Earl Hum Dev* 65:21-37.

Herlenius E, Lagercrantz H. **2004**. Development of neurotransmitter systems during critical periods. *Exp Neurol* 190 Suppl 1:S8-21.

Herregodts P, Velkeniers B, Ebinger G, Michotte Y, Vanhaelst L, Hooghe-Peters E. **1990**. Development of monoaminergic neurotransmitters in fetal and postnatal rat brain: analysis by HPLC with electrochemical detection. *J Neurochem* 55:774-779.

Higgins ES. **1999**. A comparative analysis of antidepressants and stimulants for the treatment of adults with attention-deficit hyperactivity disorder. *J Fam Pract* 48:15-20.

Holmes A, le Guisquet AM, Vogel E, Millstein RA, Leman S, Belzung C. **2005**. Early life genetic, epigenetic and environmental factors shaping emotionality in rodents. *Neurosci Biobehav Rev* 29:1335-1346.

Howard MA, Burger RM, Rubel EW. **2007**. A developmental switch to GABAergic inhibition dependent on increases in Kv1-type K+ currents. *J Neurosci* 27:2112-2123.

Hsu FC, Zhang GJ, Raol YS, Valentino RJ, Coulter DA, Brooks-Kayal AR. **2003**. Repeated neonatal handling with maternal separation permanently alters hippocampal GABAA receptors and behavioral stress responses. *Proc Natl Acad Sci U S A* 100:12213-12218.

Huang L-T, Holmes GL, Lai M-C, Hung P-L, Wang C-L, Wang T-J, Yang C-H, Liou C-W, Yang SN. **2002**. Maternal deprivation stress exacerbates cognitive deficits in immature rats with recurrent seizures. *Epilepsia* 43:1141-1148.

Huff JK, Davies MI. **2002**. Microdialysis monitoring of methylphenidate in blood and brain correlated with changes in dopamine and rat activity. *J Pharm Biomed Anal* 29:767-777.

Huot RL, Thrivikraman KV, Meaney MJ, Plotsky PM. **2001**. Development of adult ethanol preference and anxiety as a consequence of neonatal maternal separation in Long Evans rats and reversal with antidepressant treatment. *Psychopharmacology (Berl.)* 158:366-373.

Huss M, Lehmkuhl U. **2002**. Methylphenidate and substance abuse: a review of pharmacology, animal, and clinical studies. *Journal of Attention Disorders* 6:S53-S59.

Inestrosa NC, Reyes AE, Chacón MA, Cerpa W, Villalón A, Montiel J, Merabachvili G, Aldunate R. **2005**. Human-like rodent amyloid-ß-peptide determines Alzheimer pathology in aged wild-type *Octodon degus*. *Neurobiol Aging* 26:1023-1028.

Izenwasser S, Coy AE, Ladenheim B, Loeloff RJ, Cadet JL, French D. **1999**. Chronic methylphenidate alters locomotor activity and dopamine transporters differently from cocaine. *Eur J Pharmacol* 373:187–193.

Jackson ME, Frost AS, Moghaddam R. **2001**. Stimulation of prefrontal cortex at physiologically relevant frequencies inhibits dopamine release in the nucleus accumbens. *J Neurochem* 78:920-923.

Jackson ME, Moghaddam R. **2001**. Amygdala regulation of nucleus accumbens dopamine output is governed by the prefrontal cortex. *J Neurosci* 21:676-681.

Jacobs GH, Calderone JB, Fenwick JA, Krogh K, Williams GA. **2003**. Visual adaptations in a diurnal rodent *Octodon degus*. *J Comp Physiol* A 189:347-361.

Jaworski JN, Francis DD, Brommer CL, Morgan ET, Kuhar MJ. **2005**. Effects of early maternal separation on ethanol intake, GABA receptors and metabolizing enzymes in adult rats. *Psychopharmacology (Berl)* 181:8-15.

de Jong JG, Wasilewski M, van der Vegt BJ, Buwalda B, Koolhaas JM. **2005**. A single social defeat induces short-lasting behavioral sensitization to amphetamine. *Physiol Behav* 83:805-811.

Kahlig KM, Binda F, Khoshbouei H, Blakely RD, McMahon DG, Javitch JA, Galli A. **2005**. Amphetamine induces dopamine efflux through a dopamine transporter channel. *Proc Natl Acad Sci USA* 102:3495-3500.

Kalia M. **2005**. Neurobiological basis of depression: an update. *Metabolism* 54:24-27.

Kalinichev M, Easterling KW, Holtzman SG. **2002**. Early neonatal experience of Long-Evans rats results in long-lasting changes in reactivity to a novel environment and morphine-induced sensitization and tolerance. *Neuropsychopharmacology* 27:518-533.

Karson CN, Mrak RE, Schluterman KO, Sturner WQ, Sheng JG, Griffin WS. **1999**. Alterations in synaptic proteins and their encoding mRNAs in prefrontal cortex in schizophrenia: a possible neurochemical basis for 'hypofrontality'. *Mol Psychiatry* 4:39-45.

Kas MJ, Edgar DM. **1999**. A nonphotic stimulus inverts the diurnal-nocturnal phase preference in *Octodon degus*. *J Neurosci* 19:328-333.

Kehoe P, Shoemaker WJ, Arons C, Triano L, Suresh G. **1998a**. Repeated isolation stress in the neonatal rat: relation to brain dopamine systems in the 10-day-old rat. *Behav Neurosci* 112:1466-1474.

Kehoe P, Shoemaker WJ, Triano L, Callahan M, Rappolt G. **1998b**. Adult rats stressed as neonates show exaggerated behavioral responses to both pharmacological and environmental challenges. *Behav Neurosci* 112:116-125.

Kehoe P, Shoemaker WJ, Triano L, Hoffman J, Arons C. **1996**. Repeated isolation in the neonatal rat produces alterations in behavior and ventral striatal dopamine release in the juvenile after amphetamine challenge. *Behav Neurosci* 110:1435-1444.

Knutson B, Burgdorf J, Panksepp J. **2002**. Ultrasonic vocalizations as indices of affective states in rats. *Psychol Bull* 128:961-977.

Kolb B, Pellis S, Robinson TE. **2004**. Plasticity and functions of the orbital frontal cortex. *Brain Cogn* 15:341-348.

Kosten TA, Zhang XY, Kehoe P. **2003**. Chronic neonatal isolation stress enhances cocaine-induced increases in ventral striatal dopamine levels in rat pups. *Brain Res Dev Brain Res* 141:109-116.

Kratochvil CJ, Vaughan BS, Harrington MJ, Burke WJ. **2003**. Atomoxetine: a selective noradrenaline reuptake inhibitor for the treatment of attention-deficit/hyperactivity disorder. *Expert Opin Pharmacother* 4:1165-1174.

Krause KH, Dresel SH, Krause J, Kung HF, Tatsch K. **2000**. Increased striatal dopamine transporter in adult patients with attention deficit hyperactivity disorder: effects of methylphenidate as measured by single photon emission computed tomography. *Neurosci Lett* 285:107-110.

Kreilgaard M. **2002**. Assessment of cutaneous drug delivery using microdialysis. *Adv Drug Deliv Rev* 54(Suppl. 1):S99-S121.

Kuczenski R, Segal DS. **1997**. Effects of methylphenidate on extracellular dopamine, serotonin, and norepinephrine: comparison with amphetamine. *J Neurochem* 68:2032-2037.

Kuczenski R, Segal DS. **1999**. Dynamic changes in sensitivity occur during the acute response to cocaine and methylphenidate. *Psychopharmacology* 147:96-103.

Kuczenski R, Segal DS. **2002**. Exposure of adolescent rats to oral methylphenidate: preferential effects on extracellular norepinephrine and absence of sensitization and cross-sensitization to methamphetamine. *J Neurosci* 22:7264-7271.

Kudielka BM, Kirschbaum C. **2005**. Sex differences in HPA axis responses to stress: a review. *Biol Psychol* 69:113-132.

Kuhn CM, Butler SR, Schanberg SM. **1978**. Selective depression of serum growth hormone during maternal deprivation in rat pups. *Science* 201:1034-1036.

LaHoste GJ, Swanson JM, Wigal SB, Glabe C, Wigal T, King N, Kennedy JL. **1996**. Dopamine D4 receptor gene polymorphism is associated with attention deficit hyperactivity disorder. *Mol Psychiatry* 1:121-124.

Lai T, Payne ME, Byrum CE, Steffens DC, Krishnan KR. **2000**. Reduction of orbital frontal cortex volume in geriatric depression. *Biol Psychiatry* 48:971-975.

Lambe EK, Krimer LS, Goldman-Rakic PS. **2000**. Differential postnatal development of catecholamine and serotonin inputs to identified neurons in prefrontal cortex of rhesus monkey. *J Neurosci* 20:8780-8787.

Lauder JM. **1990**. Ontogeny of the serotonergic system in the rat: serotonin as a developmental signal. *Ann NY Acad Sci* 600:297-313.

Lee TM. **2004**. *Octodon degus*: a diurnal, social, and long-lived rodent. *ILAR J* 45:14-24.

Lehmann J, Feldon J. **2000**. Long-term biobehavioral effects of maternal separation in the rat: consistent or confusing? *Rev Neurosci* 11:383-408.

Lehmann J, Pryce CR, Bettschen D, Feldon J. **1999**. The maternal separation paradigm and adult emotionality and cognition in male and female wistar rats. *Pharmacol Biochem Behav* 64: 705-715.

Leinekugel X, Khalilov I, McLean H, Caillard O, Gaiarsa JL, Ben-Ari Y, Khazipov R. **1999**. GABA is the principal fast-acting excitatory transmitter in the neonatal brain. *Adv Neurol* 79:189-201.

Levine S. **2001**. Primary social relationships influence the development of the hypothalamic-pituitary-adrenal axis in the rat. *Physiol Behav* 73:255-260.

Levita L, Dalley JW, Robbins TW. **2002**. Nucleus accumbens dopamine and learned fear revisited: a review and some new findings. *Behav Brain Res* 137:115-127.

Lewis DA, Pierri JN, Volk DW, Melchitzky DS, Woo TU. **1999**. Altered GABA neurotransmission and prefrontal cortical dysfunction in schizophrenia. *Biol Psychiatry* 46:616-626.

Lindroth P, Mopper K. **1979**. High performance liquid chromatographic determination of subpicomole amounts of amino acids by precolumn fluorescence derivatisation with o-phtaldialdehyde. *Anal Chem* 51:1667–1674.

Liu D, Diorio J, Tannenbaum B, Caldji C, Francis D, Freedman A, Sharma S, Pearson D, Plotsky PM, Meaney MJ. **1997**. Maternal care, hippocampal glucocorticoid receptors, and hypothalamic-pituitary-adrenal responses to stress. *Science* 277:1659-1662.

Liu J, Lauder JM. **1991**. Serotonin and nialmide differentially regulate survival and growth of cultured serotonin and catecholamine neurons. *Dev Brain Res* 62:297-305.

Lou HC, Henriksen L, Bruhn P, Borner H, Nielsen JB. **1989**. Striatal dysfunction in attention deficit and hyperkinetic disorder. *Arch Neurol* 46:48-52.

Louzada PR, Lima AC, Mendonca-Silva DL, Noël F, De Mello FG, Ferreira ST. **2004** Taurine prevents the neurotoxicity of beta-amyloid and glutamate receptor agonists: activation of GABA receptors and possible implications for Alzheimer's disease and other neurological disorders. *FASEB J* 18:511-518.

Maguire EA, Gadian DG, Johnsrude IS, Good CD, Ashburner J, Frackowiak RS, Frith CD. **2000**. Navigation-related structural change in the hippocampi of taxi drivers. *Proc Natl Acad Sci U S A* 97:4398-4403.

Marshall LA, Cooke DJ. **1999**. The childhood experiences of psychopaths: a retrospective study of familial and societal factors. *J Personal Disord* 13:211-225.

Marsteller DA, Gerasimov MR, Schiffer WK. **2002**. Acute handling stress modulates methylphenidate-induced catecholamine overflow in the medial prefrontal cortex. *Neuropsychopharmacology* 27:163-170.

Matsumoto M, Higuchi K, Togashi H, Koseki H, Yamaguchi T, Kanno M, Yoshioka M. **2005**. Early postnatal stress alters the 5-HTergic modulation to emotional stress at postadolescent periods of rats. *Hippocampus* 15:775-781.

Matthews K, Dalley JW, Matthews C, Tsai TH, Robbins TW. **2001**. Periodic maternal separation of neonatal rats produces region- and gender-specific effects on biogenic amine content in post-mortem adult brain. *Synapse* 40:1-10.

Matthews K, Hall FS, Wilkinson LS, Robbins TW. **1996a**. Retarded acquisition and reduced expression of conditioned locomotor activity in adult rats following repeated early maternal separation: effects of prefeeding, D-amphetamine, dopamine antagonists and clonidine. *Psychopharmacology (Berl.)* 126:75-84.

Matthews K, Robbins TW. **2003**. Early experience as a determinant of adult behavioral responses to reward: the effects of repeated maternal separation in the rat. *Neurosci Biobehav Rev* 27:45-55.

Matthews K, Robbins TW, Everitt BJ, Caine SB. **1999**. Repeated neonatal maternal separation alters intravenous cocaine selfadministration in adult rats. *Psychopharmacology (Berl.)* 141:123-134.

Matthews K, Wilkinson LS, Robbins TW. **1996b**. Repeated maternal separation of preweanling rats attenuates behavioral responses to primary and conditioned incentives in adulthood. *Physiol Behav* 59:99-107.

McCormick CM, Kehoe P, Kovacs S. **1998**. Corticosterone release in response to repeated, short episodes of neonatal isolation: evidence of sensitization. *Int J Dev Neurosci* 16: 175-185.

McCracken JT. **1991**. A two part model of stimulant action on attention-deficit hyperactivity disorder in children. *J Neuropsychiatry Clin Neurosci* 3:201-209.

McDougall SA, Collins RL, Karper PE, Watson JB, Crawford CA. **1999**. Effects of repeated methylphenidate treatment in the young rat: sensitization of both locomotor activity and stereotyped sniffing. *Exp Clin Psychopharmacol* 7:208-218.

McFarlane A, Clark CR, Bryant RA, Williams LM, Niaura R, Paul RH, Hitsman BL, Stroud L, Alexander DM, Gordon E. **2005**. The impact of early life stress on psychophysiological, personality and behavioral measures in 740 non-clinical subjects. *J Integr Neurosci* 4:27-40.

Meaney MJ, Brake W, Gratton A. **2002**. Environmental regulation of the development of mesolimbic dopamine systems: a neurobiological mechanism for vulnerability to drug abuse? *Psychoneuroendocrinology* 27:127–138.

Mefford IN, Potter WZ. **1989**. A neuroanatomical and biochemical basis for attention deficit disorder with hyperactivity in children: a defect in tonic adrenaline mediated inhibition of locus coeruleus stimulation. *Med Hypotheses* 29:33-42.

Menegola E, Broccia ML, Di Renzo F, Massa V, Giavini E. **2004**. Effects of excess and deprivation of serotonin on in vitro neuronal differentiation. *In Vitro Cell Dev Biol – Animal* 40:52-56.

Michelson D, Faries D, Wernicke J, Kelsey D, Kendrick K, Sallee FR, Spencer T and the Atomoxetine ADHD Study Group. **2001**. Atomoxetine in the treatment of children and adolescents with attention-deficit/hyperactivity disorder: a randomized, placebo-controlled, dose-response study. *Pediatrics* 108:E83.

Miller EK. **2000**. The prefrontal cortex and cognitive control. *Nat Rev Neurosci* 1:59–65.

Miller EK, Cohen JD. **2001**. An integrative theory of prefrontal cortex function. *Annu Rev Neurosci* 24:167–202.

Miles R. **1999**. A homeostatic switch. *Nature* 397:215-216.

Miura H, Qiao H, Ohta T. **2002**. Influence of aging and social isolation on changes in brain monoamine turnover and biosynthesis of rats elicited by novelty stress. *Synapse* 46:116–124

Mohawk JA, Cashen K, Lee TM. **2005**. Inhibiting cortisol response accelerates recovery from a photic phase shift. *Am J Physiol Regul Integr Comp Physiol* 288:R221-R228.

Molina V, Sanz J, Munoz F, Casado P, Hinojosa JA, Sarramea F, Martin-Loeches M. **2005**. Dorsolateral prefrontal cortex contribution to abnormalities of the P300 component of the event-related potential in schizophrenia. *Psychiatry Res* 140:17-26.

Murphy BL, Arnsten AFT, Goldman-Rakic PS, Roth RH. **1996a**. Increased dopamine turnover in the prefrontal cortex impairs spatial working memory performance in rats and monkeys. *Proc Natl Acad Sci USA* 93:1325-1329.

Murphy BL, Arnsten AFT, Jentsch JD, Roth RH. **1996b**. Dopamine and spatial working memory in rats and monkeys: pharmacological reversal of stress-induced impairment. *J Neurosci* 16:7768-7775.

Myhrer T. **2003**. Neurotransmitter systems involved in learning and memory in the rat: a meta-analysis based on studies of four behavioral tasks. *Brain Res Brain Res Rev* 41:268-287.

Nakazawa K, McHugh TJ, Wilson MA, Tonegawa S. **2004**. NMDA receptors, place cells and hippocampal spatial memory. *Nat Rev Neurosci* 5:361-372.

Naqui SZH, Harris BS, Thomaidou D, Parnavelas JG. **1999**. The noradrenergic system influences in fate of Cajal-Retzius cells in the developing cerebral cortex. *Dev Brain Res* 113:75-82.

Navakkode S, Sajikumar S, Frey JU. **2004**. The type IV-specific phosphodiesterase inhibitor rolipram and its effect on hippocampal long-term potentiation and synaptic tagging. *J Neurosci* 24:7740-7744.

Navakkode S, Sajikumar S, Frey JU. **2005**. Mitogen-activated protein kinase-mediated reinforcement of hippocampal early long-term depression by the type IV-specific phosphodiesterase inhibitor rolipram and its effect on synaptic tagging. *J Neurosci* 25:10664-10670.

Nebigil CG, Choi DS, Dierich A, Hickel P, Le Meur M, Messaddeq N, Launay JM, Maroteaux L. **2000**. Serotonin 2B receptor is required for heart development. *Proc Natl Acad Sci USA* 97:9508-9513.

Nestler EJ, Barrot M, DiLeone RJ, Eisch AJ, Gold SJ, Monteggia LM. **2002**. Neurobiology of depression. *Neuron* 34:13-25.

Nikulina EM, Covington HE 3rd, Ganschow L, Hammer RP Jr, Miczek KA. **2004**. Long-term behavioral and neuronal cross-sensitization to amphetamine induced by

repeated brief social defeat stress: Fos in the ventral tegmental area and amygdala. *Neuroscience* 123:857-865.

Noguchi T, Yoshida Y, Chiba S. **2001**. Effects of psychological stress on monoamine systems in subregions of the frontal cortex and nucleus accumbens of the rat. *Brain Res* 916:91-100.

Oades RD. **1998**. Frontal, temporal and lateralized brain function in children with attention-deficit hyperactivity disorder: a psychophysiological and neuropsychological viewpoint on development. *Behav Brain Res* 94:83-95.

Ogata T, Nakamura Y, Tsuji K, Okumura H, Kataoka K, Shibata T. **1996**. Role of aspartate in ischemic spinal cord damage. *J Orthop Res* 14:504-510.

O'Kane G, Kensinger EA, Corkin S. **2004**. Evidence for semantic learning in profound amnesia: an investigation with patient H.M. *Hippocampus* 14: 417 – 425.

Okubo Y, Suhara T, Suzuki K, Kobayashi K, Inoue O, Terasaki O, Someya Y, Sassa T, Sudo Y, Matsushima E, Iyo M, Tateno Y, Toru M. **1997**. Decreased prefrontal dopamine D1 receptors in schizophrenia revealed by PET. *Nature* 385:634-636.

Olds J, Milner P. **1954**. Positive reinforcement produced by electrical stimulation of septal area and other regions of rat brain. *J Comp Physiol Psychol* 47:419-427.

Ovtscharoff jr W, Braun K. **2001**. Maternal separation and social isolation modulates the postnatal development of synaptic composition in the infralimbic cortex of *Octodon degus*. *Neuroscience* 104:33-40.

Palanza P. **2001**. Animal models of anxiety and depression: how are females different? *Neurosci Biobehav Rev* 25:219-233.

Panksepp J. **1980**. Brief social isolation, pain responsiveness, and morphine analgesia in young rats. *Psychopharmacology (Berl)* 72:111-112.

Panksepp J, Burgdorf J. **2003**. "Laughing" rats and the evolutionary antecedents of human joy? *Physiol Behav* 79:533-547.

Papaioannou A, Dafni U, Alikaridis F, Bolaris S, Stylianopoulou F. **2002**. Effects of neonatal handling on basal and stress-induced monoamine levels in the male and female rat brain. *Neuroscience* 114:195-206.

Papez JW. **1995**. A proposed mechanism of emotion. 1937. *J Neuropsychiatry Clin Neurosci* 7:103-112.

Parker G, Gladstone G, Mitchell P, Wilhelm K, Roy K. **2000**. Do early adverse experiences establish a cognitive vulnerability to depression on exposure to mirroring life events in adulthood? *J Affect Disord* 57:209-215.

Patchev VK, Almeida OF. **1998**. Gender specificity in the neural regulation of the response to stress: new leads from classical paradigms. *Mol Neurobiol* 16:63-77.

Patel SN, Stewart MG. **1998**. Changes in the number and structure of dendritic spines 25 hours after passive avoidance training in the domestic chick, *Gallus domesticus*. *Brain Res* 449:34-46.

Paula-Lima AC, De Felice FG, Brito-Moreira J, Ferreira ST. **2005**. Activation of GABA(A) receptors by taurine and muscimol blocks the neurotoxicity of beta-amyloid in rat hippocampal and cortical neurons. *Neuropharmacology* 49:1140-1148.

Paxinos G, Watson C. The rat brain in stereotaxic coordinates. 4th Edition. Academic Press **1998**.

Pelissier T, Bustamante D, Saavedra H, Tampier L, Vergara V, Paeile C. **1989**. New differences between the Wistar rat and *Octodon degus*, a putative laboratory animal resistant to morphine. *Comp Biochem Physiol C* 93:359-366.

Pendleton RG, Rasheed A, Roychowdhury R, Hillman R. **1998**. A new role for catecholamines: ontogenesis. *TIPS* 19:248-251.

Penner MP, McFadyen MP, Pinaud R, Carrey N, Robertson HA, Brown RE. **2002**. Age-related distribution of c-fos expression in the striatum of CD-1 mice after acute methylphenidate administration. *Dev Brain Res* 135:71-77.

Pierce RC, Kalivas PW. **1997**. Repeated cocaine modifies the mechanism by which amphetamine releases dopamine. *J Neurosci* 17:3254-3261.

Plaut SM, Davies JM. **1972**. Effects of mother-litter separation on survival, growth and brain amino acid level. *Physiol Behav* 8:43-51.

Pliszka SR, McCracken JT, Maas JW. **1996**. Catecholamines in attention-deficit hyperactivity disorder: current perspectives. *J Am Acad Child Adolesc Psychiatry* 35:264-272.

Plotsky PM, Meaney MJ. **1993**. Early, postnatal experience alters hypothalamic corticotropin-releasing factor (CRF) mRNA, median eminence CRF content and stress-induced release in adult rats. *Brain Res Mol Brain Res* 18:195-200.

Ploj K, Nylander I. **2003**. Long-term effects on brain opioid and opioid receptor like-1 receptors after short periods of maternal separation in rats. *Neurosci Lett* 345:195-197.

Ploj K, Roman E, Nylander I. **2003a**. Long-term effects of maternal separation on ethanol intake and brain opioid and dopamine receptors in male Wistar rats. *Neuroscience* 121:787-799.

Ploj K, Roman E, Nylander I. **2003b**. Long-term effects of short and long periods of maternal separation on brain opioid peptide levels in male Wistar rats. *Neuropeptides* 37:149-156.

Podkletnova I, Alho H. **1998**. Neonatal noradrenaline depletion prevents the transition of Bergmann glia in the developing cerebellum. *J Chem Neuroanat* 14:167-173.

Podkletnova I, Rothstein JD, Helen P, Alho H. **2001**. Microglial response to the neurotoxicity of 6-hydroxydopamine in neonatal rat cerebellum. *Int J Dev Neurosci* 19:47-52.

Poeggel G, Helmeke C, Abraham A, Schwabe T, Friedrich P, Braun K. **2003a**. Juvenile emotional experience alters synaptic composition in the rodent cortex, hippocampus, and lateral amygdala. *Proc Natl Acad Sci USA* 100:16137-16142.

Poeggel G, Nowicki L, Braun K. **2003b**. Early social deprivation alters monoaminergic afferents in the orbital prefrontal cortex of *Octodon degus*. *Neuroscience* 116:617-620.

Popper CW. **2000**. Pharmacologic alternatives to psychostimulants for the treatment of attention-deficit/hyperactivity disorder. *Child Adolesc Psychiatr Clin N Am* 9:605-646.

Pribram KH, McGuinness D. **1975**. Arousal, activation, and effort in the control of attention. *Psychol Rev* 82:116-149.

Pryce CR, Rüedi-Bettschen D, Dettling AC, Feldon J. **2002**. Early life stress: long-term physiological impact in rodents and primates. *News Physiol Sci* 17:150-155.

Pryce CR, Rüedi-Bettschen D, Dettling AC, Weston A, Russig H, Ferger B, Feldon J. **2005**. Long-term effects of early-life environmental manipulations in rodents and primates: Potential animal models in depression research. *Neurosci Biobehav Rev* 29:649-674.

Ragland JD, Gur RC, Valdez J, Turetsky BI, Elliott M, Kohler C, Siegel S, Kanes S, Gur RE. **2004**. Event-related fMRI of frontotemporal activity during word encoding and recognition in schizophrenia. *Am J Psychiatry* 161:1004-1015.

Ragozzino ME, Kesner RP. **1999**. The role of the agranular insular cortex in working memory for food reward value and allocentric space in rats. *Behav Brain Res* 98:103-12.

Rappley MD. **2005**. Attention deficit-hyperactivity disorder. *N Eng J Med* 352:165-173.

Renard GM, Suarez MM, Levin GM, Rivarola MA. **2005**. Sex differences in rats: Effects of chronic stress on sympathetic system and anxiety. *Physiol Behav* 85:363-369.

Ribary U, Schlumpf M, Lichtensteiger W. **1986**. Analysis by HPLC-EC of metabolites of monoamines in fetal and postnatal rat brain. *Neuropharmacology* 25:981-986.

Ricci L, Frosini M, Gaggelli N, Valensin G, Machetti F, Sgaragli G, Valoti M. **2006**. Inhibition of rabbit brain 4-aminobutyrate transaminase by some taurine analogues: a kinetic analysis. *Biochem Pharmacol* 71:1510-1519.

Rickert KA, Carrion VG, Karchemskiy A, Reiss AL. **2006**. Regional differences of the prefrontal cortex in pediatric PTSD: an MRI study. *Depress Anxiety* 23:17-25.

van Riel E, van Gemert NG, Meijer OC, Joels M. **2004**. Effect of early life stress on serotonin responses in the hippocampus of young adult rats. *Synapse* 53:11-19.

Robinson TE, Becker JB, Young EA, Akil H, Castaneda E. **1987**. The effects of footshock stress on regional brain dopamine metabolism and pituitary beta-endorphin release in rats previously sensitized to amphetamine. *Neuropharmacology* 26:679-291.

Robbins T. **2002**. ADHD and addiction. *Nature Med* 9:24-25.

Rolls ET. **2000**. The orbitofrontal cortex and reward. *Cereb Cortex* 10:284-94.

Roman E, Ploj K, Nylander I. **2004**. Maternal separation has no effect on voluntary ethanol intake in female Wistar rats. *Alcohol* 33:31-39.

Rosenzweig MR, Bennett EL. **1996**. Psychobiology of plasticity: effects of training and experience on brain and behavior. *Behav Brain Res* 78:57-65.

Rots NY, de Jong J, Workel JO, Levine S, Cools AR, De Kloet ER. **1996**. Neonatal maternally deprived rats have as adults elevated basal pituitary-adrenal activity and enhanced susceptibility to apomorphine. *J Neuroendocrinol* 8:501-506.

Rowe DC, Stever C, Giedinghagen LN, Gard JM, Cleveland HH, Terris ST, Mohr JH, Sherman S, Abramowitz A, Waldman ID. **1998**. Dopamine DRD4 receptor polymorphism and attention deficit hyperactivity disorder. *Mol Psychiatry* 3:419-426.

Russig H, Pryce CR, Feldon J. **2006**. Amphetamine withdrawal leads to behavioral sensitization and reduced HPA axis response following amphetamine challenge. *Brain Res* 1084:185-195.

Sajikumar S, Navakkode S, Frey JU. **2005a**. Protein synthesis-dependent long-term functional plasticity: methods and techniques. *Curr Opin Neurobiol* 15:607-613.

Sajikumar S, Navakkode S, Sacktor TC, Frey JU. **2005b**. Synaptic tagging and cross-tagging: the role of protein kinase Mzeta in maintaining long-term potentiation but not long-term depression. *J Neurosci* 25:5750-5756.

Saransaari P, Oja SS. **2000**. Taurine and neural cell damage. *Amino Acids* 19:509-526.

Saransaari P, Oja SS. **2004**. Characteristics of taurine release induced by free radicals in mouse hippocampal slices. *Amino Acids* 26:91-98.

Saransaari P, Oja SS. **2007**. Taurine release in mouse brain stem slices under cell-damaging conditions. *Amino Acids* 32:439-446.

Sawaguchi T, Goldman-Rakic PS. **1991**. D1 dopamine receptors in prefrontal cortex: involvement in working memory. *Science* 251:947–950.

Sawaguchi T, Goldman-Rakic PS. **1994**. The role of D1-dopamine receptor in working memory: local injections of dopamine antagonists into the prefrontal cortex of rhesus monkeys performing an oculomotor delayed-response task. *J Neurophysiol* 71:515–528.

Schanberg SM, Evoniuk G, Kuhn CM. **1984**. Tactile and nutritional aspects of maternal care: specific regulators of neuroendocrine function and cellular development. *Proc Soc Exp Biol Med* 175:135-146.

Schell MJ, Cooper OB, Snyder SH. **1997**. D-aspartate localizations imply neuronal and neuroendocrine roles. *Proc Natl Acad Sci USA* 94:2013-2018.

Schenk S, Izenwasser S. **2002**. Pretreatment with methylphenidate sensitizes rats to the reinforcing effects of cocaine. *Pharmacol Biochem Behav* 72:651-657.

Seeman P, Madras B. **2002**. Methylphenidate elevates resting dopamine which lowers the impulse-triggered release of dopamine: a hypothesis. *Behav Brain Res* 130:79-83.

Seamans JK, Floresco SB, Phillips AG. **1998**. D1 receptor modulation of hippocampal–prefrontal cortical circuits integrating spatial memory with executive functions in the rat. *J Neurosci* 18:1613–1621.

Seamans JK, Yang CR. **2004**. The principal features and mechanisms of dopamine modulation in the prefrontal cortex. *Prog Neurobiol* 74:1–57.

Selemon LD, Rajkowska G, Goldman-Rakic PS. **1995**. Abnormally high neuronal density in the schizophrenic cortex. A morphometric analysis of prefrontal area 9 and occipital area 17. *Arch Gen Psychiatry* 52:805-818.

Selemon LD, Rajkowska G, Goldman-Rakic PS. **1998**. Elevated neuronal density in prefrontal area 46 in brains from schizophrenia patients: application of a three dimensional, stereologic counting method. *J Comp Neurol* 392:402-412.

Smythe JW, Rowe WB, Meaney MJ. **1994**. Neonatal handling alters serotonin (5-HT) turnover and 5-HT2 receptor binding in selected brain regions: relationship to the handling effects on glucocorticoid receptor expression. *Dev Brain Res* 80:183-189.

Sokolowski JD, Conlan AN, Salamone JD. **1998**. A microdialysis study of nucleus accumbens core and shell dopamine during operant responding in the rat. *Neuroscience* 86:1001-1009.

Solanto MV. **2002**. Dopamine dysfunction in AD/HD: integrating clinical and basic neuroscience research. *Behav Brain Res* 130:65-71.

Sowell ER, Thompson PM, Welcome SE, Henkenius AL, Toga AW, Peterson BS. **2003**. Cortical abnormalities in children and adolescents with attention-deficit hyperactivity disorder. *Lancet* 362:1699-1707.

Sturman JA. **1993**. Taurine in development. *Physiol Rev* 73:119-147.

Sulzer D, Sonders MS, Poulsen NW, Galli A. **2005**. Mechanisms of neurotransmitter release by amphetamines: a review. *Prog Neurobiol* 75:406-433.

Sundstrom E, Kolare S, Souverbie F, Samuelsson EB, Pschera H, Lunell NO, Seiger A. **1993**. Neurochemical differentiation of human bulbospinal monoaminergic neurons during the first trimester. *Brain Res Dev Brain Res* 75:1-12.

Swanson JM, Flodman P, Kennedy J, Spence MA, Moyzis R, Schuck S, Murias M, Moriarity J, Barr C, Smith M, Posner M. **2000**. Dopamine genes and ADHD. *NeurosciBiobehav Rev* 24:21-25.

Swanson JM, Sunohara GA, Kennedy JL, Regino R, Fineberg E, Wigal T, Lerner M, Williams L, LaHoste GJ, Wigal S. **1998**. Association of the dopamine receptor D4 (DRD4) gene with a refined phenotype of attention deficit hyperactivity disorder (ADHD): a family-based approach. *Mol Psychiatry* 3:38-41.

Szeszko PR, Robinson D, Alvir JM, Bilder RM, Lencz T, Ashtari M, Wu H, Bogerts B. **1999**. Orbital frontal and amygdala volume reductions in obsessive-compulsive disorder. *Arch Gen Psychiatry* 56:913-919.

Tanda G, Pontieri FE, Frau R, Di Chiara G. **1997**. Contribution of blockade of the noradrenaline carrier to the increase of extracellular dopamine in the rat prefrontal cortex by amphetamine and cocaine. *Eur J Neurosci* 9:2077–2085.

Tanji J, Hoshi E. **2001**. Behavioral planning in the prefrontal cortex. *Curr Opin Neurobiol* 11:164–170.

Tarazi FI, Tomasini EC, Baldessarini RJ. **1998**. Postnatal devlopment of dopamine and serotonin transporters in rat caudate-putamen and nucleus accumbens septi. *Neurosci Lett* 254:21-24.

Tarazi FI, Baldessarini RJ. **2000**. Comparative postnatal development of dopamine D1, D2 and D4 receptors in rat forebrain. *Int J Dev Neurosci* 18:29-37.

Tebartz van Elst L, Hesslinger B, Thiel T, Geiger E, Haegele K, Lemieux L, Lieb K, Bohus M, Hennig J, Ebert D. **2003**. Frontolimbic brain abnormalities in patients with borderline personality disorder: a volumetric magnetic resonance imaging study. *Biol Psychiatry* 54:163-171.

Thomas DA, Takahashi LK, Barfield RJ. **1986**. Analysis of ultrasonic vocalizations emitted by intruders during aggressive encounters among rats (*Rattus norvegicus*). *J Comp Psychol* 97:201-206.

Timmerman W, Westerink BHC. **1997**. Brain microdialysis of GABA and glutamate: what does it signify? *Synapse* 27:242-262.

Tucker DM, Williamson PA. **1984**. Asymmetric neural control systems in human self-regulation. *Psychol Rev* 91:185-215.

Vazquez DM, Lopez JF, Van Hoers H, Watson SJ, Levine S. **2000**. Maternal deprivation regulates serotonin 1A and 2A receptors in the infant rat. *Brain Res* 855:76-82.

Vazquez DM, Eskandari R, Zimmer CA, Levine S, Lopez JF. **2002**. Brain 5-HT receptor system in the stressed infant rat: implications for vulnerability to substance abuse. *Psychoneuroendocrinology* 27:245-272.

Vetulani J. **2001**. Drug addiction. Part II. Neurobiology of addiction. *Pol J Pharmacol* 53:303-317.

Volkow ND, Wang G, Fowler JS. **2001**. Therapeutic doses of oral methylphenidate significantly increase extracellular dopamine in the human brain. *J Neurosci* 21:RC121.

Wainwright NW, Surtees PG. **2002**. Childhood adversity, gender and depression over the life-course. *J Affect Disord* 72:33-44.

Walz W, Allen AF. **1987**. Evaluation of the osmoregulatory function of taurine in brain cells. *Exp Brain Res* 68:290-298.

Wayment HK, Schenk JO, Sorg BA. **2001.** Characterization of extracellular dopamine clearance in the medial prefrontal cortex: role of monoamine uptake and monoamine oxidase inhibition. *J Neurosci* 21:35–44.

Weikop P, Egestad B, Kehr J. **2004**. Application of triple-probe microdialysis for fast pharmacokinetic/pharmacodynamic evaluation of dopamimetic activity of drug candidates in the rat brain. *J Neurosci Methods* 140:59-65.

Weikop P, Yoshitake T, Kehr J. **2007**. Differential effects of adjunctive methylphenidate and citalopram on extracellular levels of serotonin, noradrenaline and dopamine in the rat brain. *Eur Neuropsychopharmacol*, in press (available on line).

Weinstock M. **2005**. The potential influence of maternal stress hormones on development and mental health of the offspring. *Brain Behav Immun* 19:296-308.

Westerink BHC. **2002**. Can antypsychotic drugs be classified by their effects on a particular group of dopamine neurons in the brain? *Eur J Pharmacol* 455:1-18.

Whitaker-Azmitia PM. **2001**. Serotonin and brain development: role in human developmental diseases. *Brain Res Bull* 15:479-485.

Wise SP, Murray EA, Gerfen CR. **1996**. The frontal cortex-basal ganglia system in primates. *Crit Rev Neurobiol* 10:317–356.

Wright JW, Kern MD. **1992**. Stereotaxic atlas of the brain of *Octodon degus*. *J Morphol* 214:299-320.

Yang PB, Amini B, Swann AC, Dafny N. **2003a**. Strain differences in the behavioral responses of male rats to chronically administered methylphenidate. *Brain Res* 971:139-152.

Yang PB, Swann AC, Dafny N. **2003b**. Chronic pretreatment with methylphenidate induces cross-sensitization with amphetamine. *Life Sci* 73:2899-2911.

Yildirim Z, Kiliç N, Ozer C, Babul A, Take G, Erdogan D. **2007**. Effects of taurine in cellular responses to oxidative stress in young and middle-aged rat liver. *Ann N Y Acad Sci* 1100:553-561.

Ystgaard M, Hestetun I, Loeb M, Mehlum L. **2004**. Is there a specific relationship between childhood sexual and physical abuse and repeated suicidal behavior? *Child Abuse Negl* 28:863-875.

Zehle S, Bock J, Jezierski G, Braun K. **2007**. Methylphenidate treatment recovers stress-induced elevated dendritic spine densities in the rodent dorsal anterior cingulate cortex. *Dev Neurobiol*, in press.

Zhang XY, Kehoe P, Kosten TA. **2006**. Neonatal isolation alters estrous cycle effects on ventral striatal extracellular monoamine levels. *Prog Neuropsychopharmacol Biol Psychiatry* 30:504-511.

Zhou Q-YO, Quaife CJ, Palmitier RD. **1995**. Targeted disruption of the tyrosine hydroxylase gene reveals that catecholamines are required for mouse fetal development. *Nature* 374:640-646.

Zhou FM, Wilson CJ, Dani JA. **2002**. Cholinergic interneuron characteristics and nicotinic properties in the striatum. *J Neurobiol* 53:590-605.

Ziabreva I, Poeggel G, Schnabel R, Braun K. **2003a**. Separation-induced receptor changes in the hippocampus and amygdala of *Octodon degus*: influence of maternal vocalizations. *J Neurosci* 23:5329-5336.

Ziabreva I, Schnabel R, Braun K. **2000**. Parental deprivation induces N-methyl-D-aspartate-receptor upregulation in limbic brain areas of *Octodon degus*: protective role of the maternal call. *Neural Plast* 7:233-244.

Ziabreva I, Schnabel R, Poeggel G, Braun K. **2003b**. Mother's voice "buffers" separation-induced receptor changes in the prefrontal cortex of *Octodon degus*. *Neuroscience* 119:433-441.

6 APPENDICES

6.1 Abbreviations

aCSF – artificial cerebrospinal fluid
ADHD – attention-deficit/hyperactivity disorder
ANOVA – analysis of variance
CP-94,253 – 3-(1,2,5,6-tetrahydro-4-pyridyl)-5-propoxypirolo[3,2-b]pyridine
CRF – corticotropin releasing factor
DA – dopamine
DAT – dopamine transporter
DOPAC – 3,4-dihydroxyphenylacetic acid
EDTA – ethylenediaminetetraacetic acid
GABA – γ-aminobutyric acid
5-HIAA – 5-hydroxyindole-3-acetic acid
HPA – hypothalamic-pituitary-adrenocortical
Hpc – hippocampus
HPLC – high performance liquid chromatography
HVA – homovanillic acid, 4-hydroxy-3-methoxyphenylacetic acid
5-HT – 5-hydroxytryptamine, serotonin
i.p. – intra peritoneal
IUPAC – International Union of Pure and Applied Chemistry
LC – locus coeruleus
MOPEG – methoxy-4- hydroxyphenylethyleneglycol
MP – methylphenidate
mPFC – medial prefrontal cortex
mRNA – messenger ribonucleic acid
MRI – magnetic resonance imaging
3-MT – 3-methoxythyramine
NA – noradrenaline
Nac – nucleus accumbens

NMDA – *N*-methyl-$_D$-aspartate

6-OHDA – 6-hydroxydopamine

8-OH-DPAT – (+)8-hydroxy-2-(di-n-propylamino)tetralin

PET – positron emission tomography

PFC – prefrontal cortex

SD – standard deviation

SEM – standard error of the mean

TFMPP – 1-(m-trifluoromethylphenyl)-piperazine

VTA – ventral tegmental area

6.2 Publications and conference abstracts

Original publications in international journals:

Jezierski G, Zehle S, Braun K, Gruss M. **2007**. Stress and chronic methylphenidate cross-sensitizes dopaminergic responses in the adolescent medial prefrontal cortex and nucleus accumbens. *J Neurochem* 103:2234-2244.

Zehle S, Bock J, Jezierski G, Braun K. **2007**. Methylphenidate treatment recovers stress-induced elevated dendritic spine densities in the rodent dorsal anterior cingulate cortex. *Dev Neurobiol* 67:1891-1900.

Jezierski G, Braun K, Gruss M. **2006**. Epigenetic influences on the serotonergic neurotransmission in the semi-precocial rodent *Octodon degus* during early postnatal development. *Neurochem Int* 48:350-357.

Kędzierska S, Jezierski G, Taylor A. **2001**. DnaK/DnaJ chaperone system reactivates endogenous *E. coli* thermostable FBP aldolase in vivo and in vitro; the effect is enhanced by GroE heat shock proteins. *Cell Stress Chaperones* 6:29-37.

Conference abstracts:

11th European Behavioural Pharmacology Society Meeting, Barcelona, Spain, 09.09 – 12.09.2005. Abstract published in *Behav Pharmacol* 16: S34, 2005

Jezierski G, Gruss M, Braun K. Neurochemical consequences of juvenile stress and chronic methylphenidate treatment on dopaminergic function in the rodent limbic cortex.

4th Forum of European Neuroscience, Lisbon, Portugal, 10.07 – 14.07.2004. Organized by Federation of European Neurosciences Societies (FENS):

Jezierski G, Gruss M, Braun K. Developmental profile of amino acid and monoamine levels in the rodent hippocampus and prefrontal cortex: impact of acute and periodic stress.

Bock J, Zehle S, Jezierski G, Braun K. Stress-induced hyperactivity in trumpet tailed rats (*Octodon degus*): can methylphenidate treatment normalize behaviour and the brain?

37th Annual Meeting of International Society for Developmental Psychobiology, Aix en Provence, France, 25.06 – 28.06.2004:

Zehle S, Bock J, Jezierski G, Braun K. Stress-induced hyperactivity in trumpet-tailed rats (*Octodon degus*): effects of methylphenidate treatment on behavior and brain activity.

6.3 *Curriculum Vitae*

Grzegorz Jezierski

Born: 29th November 1976 in Gdańsk, Poland

Nationality: Polish

2007 – present Application Support Specialist, Applied Biosystems (Applera Polska), Warsaw, Poland

2006 – 2007 Assistant in In Vitro and Molecular Biology Laboratory, Invicta Infertility Treatment Clinic, Gdańsk, Poland

2003 – 2005 PhD student at Department of Zoology/Developmental Neurobiology, Institute of Biology (head: Prof. Katharina Braun), Otto-von-Guericke University Magdeburg, Germany. Member of the working group "Neurochemistry and development of behavior" (head: Dr. Michael Gruss). Supported by Deutsche Forschungsgemeinschaft Graduiertenkolleg 253 "Biologische Grundlagen von Erkrankungen des Nervensystems (Biological Basis of Nervous System Diseases)".

2000 – 2002 Employed at Department of Forensic Medicine, Medical University of Gdańsk, Poland

1995 – 2000 Biology student at the Faculty of Biology, Geography and Oceanology, University of Gdańsk, Poland

Februar 2000 – MSc degree in biology (molecular biology)

1991 – 1995 High school, Gdańsk, Poland

Memberships: Polish Society of Human Genetics, Polish National Chamber of Laboratory Diagnosticians.

6.4 Zusammenfassung

Die Umwelt, und hier insbesondere der während der frühen Lebensphase von der Mutter oder beide Elternteilen ausgehenden Einfluß, hat eine große Bedeutung für die Entwicklung des Gehirns und des Verhaltens der Nachkommen. In der vorliegenden Arbeit habe ich die Konsequenzen einer Störung der Eltern-Kind-Beziehung am Tiermodell der Strauchratte, *Octodon degus*, auf der Ebene der Neurotransmission in ausgewählten Arealen des limbischen Systems untersucht.

Im ersten Teil der Arbeit charakterisierte ich anhand von Gewebehomogenaten kortikaler Areale und des Hippokampus die Veränderungen der *i.)* basalen Gewebekonzentrationen der Monoamine Dopamin und Serotonin sowie der Aminosäuren Aspartat, Glutamat, Taurin und GABA während der Ontogenese der Strauchratte. Darüber hinaus untersuchte ich die Auswirkungen einer *ii.)* akuten bzw. *iii.)* wiederholten Störung der Eltern-Kind-Beziehung durch 1-stündige Separation der Jungtiere von der Familie während der ersten Lebenswochen. Dabei konnte ich zeigen, dass *i.)* die basalen Gewebekonzentrationen der Monoamine und Aminosäuren das für erwachsene Tiere charakteristische Niveau bereits zwischen dem postnatalen Tag (PND) 3 und 21 erreichen. Weiterhin konnte ich nachweisen, dass, abhängig vom untersuchten Hirnareal, dem Geschlecht und Alter, sowohl die *ii.)* akute (mit den deutlichsten Effekten im Kortex von Weibchen zwischen PND 3 und PND 14) als auch die *iii.)* wiederholte Separation von der Familie (mit den deutlichsten Effekten im Kortex beider Geschlechter) starke Veränderungen der serotonergen Neurotransmission verursachen. Diese Ergebnisse zeigen einerseits, dass in einer frühen Lebensphase die Ontogenese der Neurotransmittersysteme der Strauchratte, verglichen mit z.B. der Ratte, bereits sehr weit fortgeschritten ist, und dass andererseits insbesondere das serotonerge System in diesem Zeitfenster vulnerabel für Störungen der Eltern-Kind-Beziehung ist.

Im zweiten Teil der Arbeit untersuchte ich in einem neuropharmakologischen Ansatz unter Verwendung der *in vivo* Mikrodialyse die Wirkung von Methylphenidat (Handelsbezeichnung: Ritalin) auf die dopaminerge Neurotransmission im medialen präfrontalen Kortex (mPFC) und dem Nucleus accumbens (Nac) von männlichen Strauchratten. Methylphenidat (MP) wird hauptsächlich zur medikamentösen

Behandlung der Aufmerksamkeitsdefizit-/Hyperaktivitätsstörung (ADHS) bei Kindern eingesetzt. Vorangegangene Untersuchungen in unserem Labor haben gezeigt, dass die wiederholte 1-stündige Separation von der Familie während der ersten 3 Lebenswochen bei Jungtieren der Strauchratte Symptome von Hyperaktivität und Störungen der Aufmerksamkeit hervorrufen, und somit dieser tierexperimentelle Ansatz potentiell ein Modellsystem zur Untersuchung der neurobiologischen Grundlagen der ADHS darstellt. Der Vergleich von *i.)* juvenilen (PND22/24) und adolescenten (PND46/48), im Sozialverband aufgewachsenen Strauchratten zeigte, dass eine acute Injektion von MP (10 mg/kg; i.p.) in den juvenilen Tieren nur eine vergleichsweise sehr geringe Stimulation der dopaminergen Transmission im mPFC und Nac verursacht. Der Vergleich von *ii.)* im Sozialverband aufgewachsenen und wiederholt separierten Tieren zeigte im mPFC juveniler Tiere eine Verminderung der dopaminergen Transmission, während im Nac keine Unterschiede zwischen beiden Versuchsgruppen nachweisbar waren. In adolescenten, wiederholt separierten Tieren konnte ich, verglichen mit den sozial aufgewachsenen Tieren, eine Sensitivierung der dopaminergen Transmission in beiden untersuchten Hirnregionen nachweisen. Der Vergleich von *iii.)* adolescenten, acut injizierten und chronisch mit MP vorbehandelten Strauchratten (täglich 10 mg/kg MP zwischen PND 22 und PND 46) zeigte eine Sensitivierung der dopaminergen Transmission in beiden untersuchten Hirnregionen von chronisch vorbehandelten Tieren. Weiterhin konnte ich nachweisen, dass eine vorangegangene wiederholte Separation von der Familie zu einer Verstärkung der nach der chronischen Vorbehandlung zu beobachtenden Sensitivierung der dopaminergen Transmission im mPFC führt. Mit diesen Ergebnissen konnte ich erstmals nachweisen, dass MP im juvenilen, sich entwickelnden limbischen System auf der Ebene der dopaminergen Transmission eine deutlich andere Wirkung entfaltet als in der Adolescents. Außerdem konnte ich nachweisen, dass sowohl frühe emotionale Erfahrungen, hervorgerufen durch die Störung der Eltern-Kind-Beziehung in den ersten Lebenswochen, als auch die chronische Gabe von MP in der frühen Ontogenese dramatische Veränderungen der dopaminergen Transmission im limbischen System verursachen.