
PERMANENT PAPER
ISO 9706

Age-dependent and reactive changes in dopaminergic and GABAergic structures in the prefrontal-limbic system of the gerbil (*Meriones unguiculatus*)

Dissertation

zur Erlangung des

Doktorgrades der Naturwissenschaften

der Fakultät für Biologie

der Universität Bielefeld

vorgelegt von

Susanne Brummelte

Bielefeld, Januar 2007

Table of contents

1. Summary

The postnatal development is probably the most important phase during the maturation process of a living creature. External circumstances and influences will stamp the initial wiring of the nervous system and therefore contribute to the establishment of cognitive functions and behavioral repertoires. Disturbances during this crucial time can have deleterious effects on the whole system and can lead to alterations in the neural networks and even to the formation of neurological diseases.

Our group has established an animal model of an early systemic challenge during development using a 2-step approach of impoverished rearing (IR) conditions and a pharmacological intoxication with methamphetamine (MA) on postnatal day (PD) 14. Previous work already revealed that this model induces severe and complex alterations in various transmitter systems and areas and even reflects some findings from schizophrenic patients.

The current work was conducted to clarify some further points concerning this potential animal model of psychosis.

- First, are these changes totally due to the immature networks during development or can an adult challenge with MA cause similar alterations, particularly in the dopaminergic system?
- The second question concerns the variations between the areas after an early challenge and if their developmental patterns might play a role in mediating this effect.
- Finally, I was interested in the contribution of the GABAergic system to the reactive or compensative mechanisms within the disturbed neural networks.

To address these questions we applied a comparable dose of MA to adult gerbils as a start and investigated the long-term effects on the dopaminergic system, which appeared to be quite

different from the early challenge, with only a slight oversprouting of fibers in the nucleus accumbens shell (Brummelte et al., 2006a).

Further, we investigated the postnatal development of dopaminergic and GABAergic fibers in a long-term study in the prefrontal cortex (PFC), amygdala and entorhinal cortex (EC) from PD 14 until high age (PD720) to account for potential varying maturation patterns or ageing-sensibility of these areas or transmitter systems. We found that the different patterns might indeed contribute to the observed imbalance within the neural networks and that only the prefrontal dopaminergic fiber density is revealing ageing-related alterations (Brummelte and Teuchert-Noodt, 2006; Brummelte et al., accepted; Brummelte and Teuchert-Noodt, submitted).

To eventually estimate the participation of the GABAergic system in the rearrangements after the early disturbances, we quantified GABAergic fibers as well as boutons around pyramidal somata in the PFC and revealed that GABA is apparently undergoing a shift from strong somatic inhibition to more moderate dendritic inhibition of pyramidal neurons and therewith derogating the synchronization of whole pyramidal populations (Brummelte et al., 2007).

Thus, our results further strengthen our hypothesis that all transmitter systems show a high neuronal plasticity, partially even in adulthood and that our approach of an early systemic stress leads to several severe and complex alterations in the neuroanatomical networks, which underlines the high interdependency of the various transmitter systems and might resemble some of the changes and deficits seen in schizophrenic individuals.

1.1 Zusammenfassung (deutsch)

Die postnatale Entwicklung ist wahrscheinlich die wichtigste Phase während des Reifungsprozesses eines jeden Lebewesens. Äußere Verhältnisse und Einflüsse wirken auf die anfängliche Verschaltung des Nervensystems ein und tragen so zur Bildung von kognitiven

Fähigkeiten und Verhaltensweisen bei. Störungen während dieser entscheidenden Zeit können schädliche Effekte auf das ganze System haben, da sie zu Modifizierungen in den Nervennetzen oder sogar zur Bildung von neurologischen Krankheiten führen können. Unsere Arbeitsgruppe hat ein Tiermodell einer frühkindlichen Schädigung entwickelt, das aus einem 2-Stufen Modell besteht mit reizarmen Aufzuchtsbedingungen einerseits und einer einzelnen frühen Methamphetamin-Intoxikation (MA) am postnatalen Tag (PD) 14 andererseits. Vorherige Arbeiten konnten bereits zeigen, dass dieses Modell schwerwiegende und komplizierte Veränderungen in verschiedenen Transmittersystemen und Gebieten verursacht und sogar einige Befunde von schizophrenen Patienten widerspiegelt. Die gegenwärtige Arbeit wurde durchgeführt, um weitere Aspekte bezüglich dieses potenziellen Tiermodells zur Psychose zu klären.

- Erstens: Beruhen diese Veränderungen ausschließlich auf den unausgereiften Nervennetzen während der Entwicklung, oder kann eine MA-Intoxikation im Erwachsenenalter ähnliche Modifizierungen, besonders im dopaminergen System, verursachen?
- Die zweite Frage betrifft die unterschiedliche Betroffenheit verschiedener limbischer Gebiete nach der frühen Störung, und ob die möglicherweise unterschiedlichen Entwicklungsmuster der Areale dabei eine Rolle spielen könnten.
- Schließlich interessierte ich mich für den Beitrag des GABAergen Systems zu den reaktiven oder kompensatorischen Mechanismen innerhalb der gestörten Nervennetze.

Um diese Fragen zu klären, haben wir zunächst eine vergleichbare Dosis von MA erwachsenen Rennmäusen verabreicht, um die langfristigen Effekte auf das dopaminerge System zu untersuchen. Im Gegensatz zu der frühen Intoxikation zeigte sich jedoch nur ein leichter Faserüberschuss im Nucleus accumbens shell (Brummelte et al., 2006a).

Daraufhin untersuchten wir die postnatale Entwicklung von dopaminergen und GABAergen Fasern in einer Langzeitstudie im präfrontalen Kortex (PFC), in der Amygdala und im entorhinalen Kortex (EC) vom PD 14 bis zum hohen Alter (PD720), um potenziell

unterschiedliche Reifungsmuster oder alterungsbedingte Veränderungen der entsprechenden Gebiete und ihrer Transmittersysteme aufzuzeigen. Die Ergebnisse zeigen, dass diese verschiedenen Muster tatsächlich zur beobachteten Unausgewogenheit innerhalb der Nervennetze beitragen könnten, und dass nur die dopaminerge Faserdichte im PFC von Alterungsprozessen betroffen ist. (Brummelte and Teuchert-Noodt 2006; Brummelte et al. akzeptiert, Brummelte and Teuchert-Noodt, eingereicht).

Um schließlich den Einfluss des GABAergen Systems bei den Umorganisationen nach den frühen Störungen zu beurteilen, untersuchten wir einerseits GABAerge Fasern und andererseits GABAerge ‚Boutons' an nicht angefärbten pyramidale Zellkörpern im PFC und konnten zeigen, dass die GABAerge Inhibition anscheinend eine Verschiebung von einer starken somatischen Hemmung zu einer eher mäßigen dendritischen Hemmung der pyramidalen Neuronen erlebt, wodurch die Synchronisation ganzer pyramidaler Populationen verringert sein könnte (Brummelte et al., 2007).

Daher bestätigen diese neuen Ergebnisse weiter unsere Hypothese, dass viele Transmittersysteme eine hohe neuronale Plastizität aufweisen, und dies teilweise sogar im Erwachsenenalter. Weiterhin unterstreicht unser Ansatz einer frühkindlichen systemischen Störung die hohe Interdependenz der verschiedenen Transmittersysteme, da er zu vielen komplizierten Veränderungen in den neuroanatomischen Netzwerken führt, die wiederum zum Teil einigen beobachteten Veränderungen und Defiziten von schizophrenen Personen ähneln.

2. Introduction

The mammalian brain is capable of tremendous accomplishments, which are in part due to the fact that the main structural and functional patterns mature postnatally. Right after birth, the nervous system is like a pool of infinite possibilities in form of an endless number of potential connections, which need to be directed to eventually form a well functioning system. The environment plays a fundamental role in the subsequent development of neuronal structures and functions. This so called experience-dependent plasticity was already shown in the striking experiments by Wiesel and Hubel in the 1960ies, when they revealed that the monocular deprivation of kittens during a critical phase of development leads to differences in the cortical wiring and subsequently to a functional loss of the deprived eye (Hubel and Wiesel, 1964; Wiesel and Hubel, 1965). The anatomical changes comprised of variances in the volume of the representing domains of the particular eye and the pattern of the ocular dominance columns (Hubel et al., 1977; Shatz and Stryker, 1978). This demonstrates the interconnectivity of structural arrangements and the corresponding functional or behavioral outcome. Thus, external influences are essential for a natural maturation of the cortical connectivity, including the connectivity of the various transmitter systems.

Usually, a child learns quite unconsciously and mechanically how to use its motor and cognitive capacities as the proceeding maturation of the necessary neuronal networks is a genetically programmed process (Jacobson, 1991). The guidance of particular fibers and connections depends on morphogenetic factors and guidance cues, which lead the way to the target innervation side and thus determine the initial wiring of the nervous system (Sperry, 1963). Thereby, the progression generally follows an inferior to superior and posterior to anterior pattern, with sensory motor structures maturing earlier than associative ones, so that the prefrontal cortices are the last regions to reach their adult appearance. Considering that the prefrontal areas are high associative centers, which are responsible for complex cognitive functions as decision making or the evaluation of new situations and circumstances, it appears quite reasonable that these structures gain their fine tuning only late in adolescence, especially, as these functions mainly depend on extrinsic influences. This crucial neuroplasticity assures a high amount of adaptation to the extrinsic environment, which might be the reason why the important part of the maturation takes place postnatally. However, one should keep in mind that some structures such as the dentate gyrus of the hippocampus or the

olfactory bulb continue to 'mature' throughout the whole life-span, due to the neurogenesis taking place in these areas, i.e. the ingrowth of new neurons into the existing cell assemblies.

2.1 Neurotransmitter systems and plasticity

The first neurotransmitter, acetylcholine, was already discovered in 1914 by Henry Hallett Dale and its function as a transmitter in the nervous system was proved by Otto Loewi in 1921. However, it took another quarter of a century with passionate and controversial arguments until the existence of the chemical messengers was generally acknowledged (rev. in Valenstein, 2002). Today there is no doubt that acetylcholine, serotonin (5-HT), dopamine (DA), gamma-aminobutyric acid (GABA) and glutamate are some of the main neurotransmitters in the mammalian nervous system.

Neurotransmitters are essential for the normal functioning of neural networks and their interdependency of excitatory and inhibitory influences on neuronal cells eventually determines our behavior (Birkmayer et al., 1989). The effectiveness of neurotransmitter action thereby depends on several factors. On the one hand there are the postsynaptic components as receptor types, densities or sensibilities or the responsiveness of the postsynaptic cell to the neurotransmitter message. On the other hand presynaptic factors also contribute significantly to the transmission process. For instance, the position of the synapse on the postsynaptic cell, e.g. on a dendrite or the soma, plays an important role for the magnitude of the 'message'. Also the amount of transmitter released by a particular stimulation can be variable.

Besides these direct factors, there are also several indirect measures to modulate the neurotransmitter function. Thus, the different neurotransmitter systems can influence each other e.g. by terminating on the other ones' synapses or by competing for a particular innervation site. In summary, the interconnectivity of the different transmitter systems is highly complex and a disturbance within one neural system might therefore eventually affect the whole network.

Recently it has been shown that neurotransmitters also exhibit morphogenetic properties and can therefore regulate the proliferation, growth, migration, differentiation and survival of neural precursor cells during development (for review see: Nguyen et al., 2001). However, transmitter systems are themselves affected by drastic changes during the maturation process. For instance, it has been assumed that GABA-A receptor ligands can induce imbalances in monoaminergic versus GABAergic transmission in the developing brain (Lauder et al., 1998).

So, when considering the maturation of fibers and connections of particular transmitter systems, one should keep in mind that their properties and functions might on the one hand be subject to change, too and on the other hand depend on the postsynaptic properties as e.g. receptor densities.
In addition, external influences can have an important impact on the structural arrangement and the interconnectivity of neuronal structures and therefore also on the development and plasticity of neurotransmitter systems.

2.2 The animal model and previous results

Our laboratory has investigated the neuroanatomical distribution and reactive neuroplasticity of several transmitters using immunohistochemistry to stain cells, fibers or spines containing these chemical messengers. The animal of choice for these investigations was the Mongolian gerbil (Meriones unguiculatus), as the genetic variability of these animals is very small (Thiessen and Yahr, 1977). In addition, their behavioral repertoire and, thus, neuronal background is considered to resemble the wild form more than that of mice or rats, since they have not been so intensively domesticated (Rosenzweig and Bennett, 1969).
Animals were either bred in standard makrolon cages (type IV) under impoverished rearing (IR) conditions or in semi-naturally structured compounds (1m x 1m) containing branches and hiding opportunities (enriched rearing = ER) and kept in these conditions until weaning (postnatal day (PD) 30). Afterwards animals from IR conditions were transferred to makrolon (type III) cages, where they were kept individually until further usage, while animals from ER were transferred to another semi-natural compound and kept together with their siblings. All animals received food and water *ad libitum* and were kept on natural day/night cycles (Fig.1).
Enriched environment has since long been known to cause morphological changes in the brain (Diamond et al., 1964; Diamond et al., 1976). In addition, animals from enriched environment reveal better learning and memory skills (Paylor et al., 1992; Nilsson et al., 1999). In contrast, animals from impoverished environments often reveal pathologic stereotypic behaviors and cognitive impairments and can be used as animal models for diverse neurological diseases (Winterfeld et al., 1998).

Fig. 1: Different rearing conditions. Left: Enriched environment: Animals live in huge semi-naturally structured compounds with opportunities to hide and play. Right: Impoverished environment: Animals are kept in standard makrolon cages with nothing but sawdust.

However, it is important to distinguish between rearing and keeping conditions. The impoverished environment during development has a strong influence during the maturation of the brain, while its effect is less devastating after the main neuronal networks have been established. Thus, the restricted rearing conditions used by our laboratory are particularly essential to introduce disturbances during the establishments of important initial connections.

The second part of our animal model consisted of an early methamphetamine (MA) intoxication on PD 14. Thus animals from IR or ER conditions either received an i.p. application of 50mg/kg MA or an application of saline. MA is a dopamine agonist which causes a massive release of DA into the synaptic cleft as well as a blockage of monoamine oxidase (Ricaurte et al., 1982), thus leading to the formation of neurotoxins as oxygen species and reactive nitrogen species (Itzhak et al., 1998; Cadet and Brannock, 1998; Lau et al., 2000; Gluck et al., 2001; Kita et al., 2003), which in turn cause the degeneration of synaptic terminals.

This 2-step approach of an early challenge during development via IR and the MA intoxication leads to several alterations in various transmitter systems in particular areas. For instance, the 5-HT innervation is affected by IR in the central and basolateral amygdala and in parts of the hippocampus and the entorhinal cortex (EC), while frontal and prefrontal cortices show no significant alterations (Busche et al., 2002; Lehmann et al., 2003; Neddens et al., 2003; Neddens et al., 2004). A MA intoxication however, causes an increase of 5-HT fibers in the nucleus accumbens and the septal dentate gyrus in IR animals (Busche et al., 2002; Lehmann et al., 2003; Lesting et al., 2005a) and even more widely spread effects comparing ER MA to ER gerbils (Neddens et al., 2003; Neddens et al., 2004).

Transmitter		5-HT			ACh			Glu		
Area	**Sub-region**	**IR**	**IR MA**	**ER MA**	**IR**	**IR MA**	**ER MA**	**IR**	**IR MA**	**ER MA**
PFC	Medial	n.s.	n.s.	+ 49%	n.s.	n.s.	n.s.	-	-	-
	Orbital	n.s.	n.s.	+ 23%	n.s.	n.s.	n.s.	-	-	-
Cortex	Frontal	n.s.	n.s.	n.s.	-	-	-	I: 3: n.s. / 5:↓ V: 3: n.s. / 5:↓ VI: 3: ↓ / 5:n.s.	I: 3: n.s. / 5: n.s. V: 3: n.s. / 5: ↑ VI: 3: :n.s. / 5: ↑	I: 3: n.s. / 5: ↓ V: 3: n.s. / 5: ↓ VI: 3: n.s. / 5: ↓
	Insular	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	I: 3: ↓ / 5: n.s. V: 3: ↓ / 5: n.s. VI: 3: ↓ / 5: n.s.	I: 3: n.s. / 5: n.s. V: 3: n.s. / 5: n.s. VI: 3: n.s. / 5: ↑	I: 3: ↓ / 5: n.s. V: 3: n.s. / 5:n.s. VI: 3: n.s. / 5:n.s.
	Parietal	n.s.	n.s.	n.s.	-	-	-	I: 3: ↓ / 5: ↓ V: 3: ↓ / 5: ↓ VI: 3: n.s. / 5:↓	I: 3: n.s. / 5: ↑ V: 3: n.s. / 5: ↑ VI: 3: n.s. / 5: ↑	I: 3: ↓ / 5: ↓ V: 3: ↓ / 5: ↓ VI: 3: n.s. / 5: ↓
Ncl. Acc	Core	n.s.	+ 14%	+ 13%	-	-	-	-	-	-
	Shell	n.s.	+ 23%	+ 23%	-	-	-	-	-	-
Amyg-dala	BLA	+ 10%	n.s.	n.s.	-	-	-	-	-	-
	CE	+ 30%	n.s.	n.s.	-	-	-	-	-	-
Ent. Cortex	Ventral	+ 49%	n.s.	+ 58%	+ 10%	n.s.	n.s.	-	-	-
Dent. Gyrus	Temp.	+ 44%	n.s.	+ 54%	+ 41%	- 13%	+ 44%	-	-	-

Table 1: Summary of previous results for the serotonin (5-HT), acetylcholine (ACh) and glutamate (Glu) transmitter systems coming from our 2-step animal model. The percentage values or arrows indicate an increase (black) or decrease (red) in fiber densities of the according transmitter between the groups; animals from impoverished rearing with placebo injection (IR) or enriched rearing with methamphetamine challenge (ER MA) are compared to animals from enriched environment with saline treatment (ER = control), while animals from IR conditions with a MA challenge (IR MA) are compared to animals from IR condition without the intoxication (IR). Noteworthy, all treatments appear to have a rather increasing effect on the 5-HT and ACh fiber densities, while the 2-step approach draws a more complicated picture for the glutamatergic innervations. Here, an imbalance between projections from lamina III (3) and lamina V (5) pyramidal neurons to the different cortices is clearly visible in MA intoxicated IR animals (indicated by the numbers 3 and 5 in red color) throughout most of the investigated layers (I, V and VI are only representative examples). Presented values are based on results for the right hemispheres from the following studies: Busche et al., 2002; Neddens et al., 2003; Lehmann et al., 2004, Neddens et al., 2004; Lesting et al., 2005a; Bagorda et al., 2006; Busche et al., 2006. PFC: prefrontal cortex; Ncl. Acc.: Nucleus accumbens; Ent. Cortex: Entorhinal cortex; Dent. Gyrus temp: temporal dentate gyrus; n.s.: not significant.

Acetylcholine fibers exhibited an increase in prefrontal areas of the left hemisphere and the EC after IR (Lehmann et al., 2004), but showed no effect after MA treatment. A different picture was revealed for the temporal dentate gyrus, where the MA challenge led to a lower amount of fibers in IR animals but at the same time to a higher amount in animals from ER conditions (Busche et al., 2006). A similar reverse effect for MA considering IR and ER animals was also found for the glutamatergic projections from the PFC. Fibers from lamina V revealed a denser innervation in their projection fields in IR MA animals, while projection from lamina III and V revealed a lesser innervation in ER MA animals (Bagorda et al., 2006).

In summary, the reactive changes after an early pharmacological treatment are highly diverse and complex and also depend on the animals' external environment, whereby IR animals generally showed stronger reactions than ER animals. Table 1 gives an overview over the most important findings from previous works concerning 5-HT, acetylcholine and glutamate.

2.3 The dopaminergic and GABAergic transmitter systems

The above mentioned variances in the effects of rearing conditions and especially MA treatment are in part due to the direct effect on the dopaminergic system, which shows severe alterations after both, IR and MA intoxication. As MA is a dopamine agonist it appears quite likely that the most deleterious effects are seen within the DA system, especially since DA is particularly vulnerable to oxidative stress and can even be a source of reactive oxygen species itself (Ueda et al., 2002; Cantuti-Castelvetri et al., 2003). However, after impoverished rearing similar effects can be observed, underlining the suggestion that the dopaminergic transmitter system is indeed exceptionally vulnerable to both, extrinsic and intrinsic challenges. In addition, it is frequently associated with ageing-related changes and neurodegenerative diseases such as Parkinson (Chinta and Andersen, 2005). Interestingly, caudal and rostral areas of the prefrontal-limbic system seem to be affected in opposite directions, with the amygdala or the EC showing an overshoot of dopaminergic fiber densities after the MA challenge or IR (Busche et al., 2004), while the densities are dramatically diminished in the PFC (Dawirs et al., 1994; Winterfeld et al., 1998; Neddens et al., 2001), which points to the complexity of the MA neurotoxicity.

Therefore, the focus of this work will generally be on two neurotransmitter systems: on the one hand on the dopaminergic system, which is mainly directly affected in our animal model and is further believed to regulate multiple brain functions and to be involved in several developmental and neurodegenerative diseases (Nieoullon, 2002) and on the other hand on the GABAergic system, which provides the most important inhibitory control within the nervous system (Bowery and Smart, 2006) and is believed to be able to influence the development of monoaminergic structures (Lauder et al., 1998). In addition, GABA appears basically in local inhibitory interneurons throughout the brain and is therefore assumed to play an important role concerning compensatory or aggravating reactions after disturbances in the local networks (Teuchert-Noodt, 2000; Magnusson et al., 2002; Nishimura et al., 2005). Further, GABA is assumed to play a considerable role in the establishments and consolidation of neuronal networks, in particular as it is known to undergo a shift during development: it

exhibits depolarizing effects until early postnatal stages (Cherubini et al., 1991; Ganguly et al., 2001; Ben-Ari, 2002), while it then changes to an inhibitory transmitter due to the delayed expression of the chloride exporter and the according inverted electrochemical gradient for Cl^- in neonatal neurons (Ben-Ari, 2002). Thus, DA and GABA are both essentially important for a normal maturation of neuroanatomical circuits and their following functional integration.

DA is almost exclusively found in projection neurons, which are nearly all located in a few nuclei in the brain stem. From here, three major pathways of dopaminergic projections evolve which are associated with various functions of the brain. The mesocortical pathway connects the ventral tegmental area (VTA) with the frontal cortices and is therefore involved in cognitive functions as motivation, attention or memory processes. The mesolimbic pathway also originates in the VTA and leads to limbic structures in the midbrain, among others to the nucleus accumbens and amygdala and therefore this pathway is associated with the emotional and reward system of the brain (Fig. 2A). These two pathways are often named together as the mesocorticolimbic projection, as both ascend mainly from the VTA and innervate parts of the big limbic circuit (Fallon et al., 1978; Swanson, 1982; Björklund and Lindvall, 1984). The third major pathway from the brainstem nuclei is the nigrostriatal, which connects the substantia nigra with the basal ganglia loop, especially with the striatum, and thus plays a role in motor function. Another dopaminergic pathway ascends from the arcuate nucleus of the mediobasal hypothalamus and projects to the median eminence, where it inhibits the secretion of prolactin from the adenohypophysis. Thus, DA even plays a role in hormone regulations, which underlines the wide variety of functions of this neurotransmitter in the brain.

It has been shown before that the mesocortical DA pathway exhibits a prolonged maturation until adulthood (Kalsbeek et al., 1988; Dawirs et al., 1993a), while more caudal positioned areas are assumed to reach their adult pattern earlier during development (Busche et al., 2004). Therefore, it seems likely that the various pathways and the according areas may be affected differently, which in turn might explain the apparent imbalance in the dopaminergic system after the early pharmacological challenge.

The GABAergic cell population consists of several subpopulations which can be distinguished on the basis of their cell properties, distribution, shape, synaptic contacts or the content of particular substances within the cell, as for instance calcium-binding proteins.

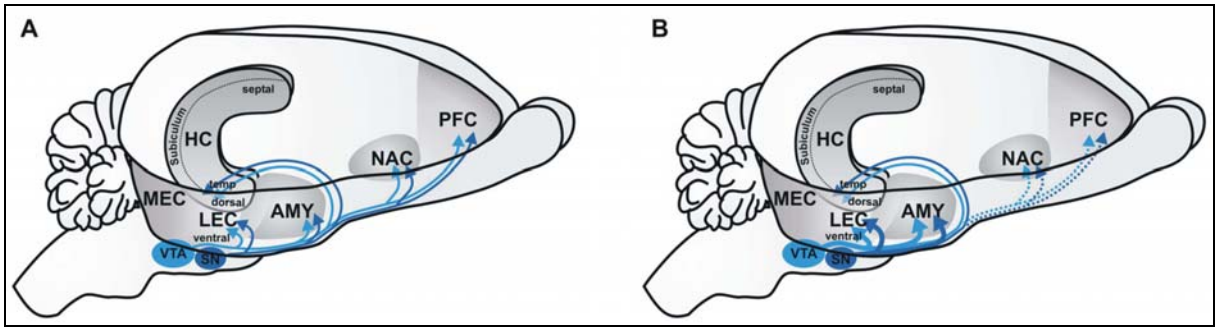


Fig. 2: Dopaminergic pathways in the rodent (A) and the imbalance after an early MA challenge (B). PFC: prefrontal cortex; NAC: Nucleus accumbens; AMY: amygdala; HC: hippocampus; MEC: medial entorhinal cortex; LEC: lateral entorhinal cortex; SN: substantia nigra; VTA: ventral tegmental area (taken from Busche, 2004).

Some of these cells have a rather strong influence on the postsynaptic cells due to their somatic contact while others are more likely to innervate the dendrites of other cells and thus have a more modulatory effect. Noteworthy, GABA is believed to provide the synchronization of whole pyramidal populations via the strong somatic input, which is in turn believed to be the basis for a normal functioning of the brain (Traub et al., 1996; Freund, 2003), as it enables a target-orientated firing of the cortical output neurons. Interestingly, the fast-spiking subpopulation, which exhibits these contacts, shows a slower developmental pattern than the other GABAergic cells, as their establishment of axo-somatic synapses continues well into adolescence (Lewis et al., 2005). In contrast to DA, GABA acts always inhibitory once the chloride exporters have been expressed. Its appearance is almost limited to interneurons with only a few exemptions of GABAergic projection neurons as e.g. in the basal ganglia or in the cerebellum. Due to this local but overall occurrence of GABAergic cells their innervation fields usually only extend to the close proximity. Within the GABAergic population, several subpopulations can be distinguished with the aid of calcium-binding protein markers such as calbindin (CB) or parvalbumin (PV). These proteins are only expressed in particular subgroups of cells and can therefore be used to further specify the potential effects on the GABAergic systems (Celio, 1990).

DA and GABA have been shown to exert a high interaction and interdependency. GABAergic interneurons receive direct dopaminergic input (Goldman-Rakic et al., 1989; Verney et al., 1990; Benes et al., 1993), whereas it can provide both, inhibitory (Retaux et al., 1991) and excitatory (Gorelova et al., 2002) effects and different innervation patterns and receptor distributions, respectively, concerning the various GABAergic subpopulations (Sesack et al., 1995; Le Moine and Gaspar, 1998). In addition, DA terminals also directly innervate the pyramidal neurons (Jay et al., 1995; Davidoff and Benes, 1998) and can thus directly and

indirectly, via the GABAergic interneurons, modulate the firing pattern of the cortical output neurons.

GABA in turn has an influence on the dopaminergic neurons in the brainstem via striatonigral neurons or local circuit neurons in the midbrain (Gale and Guidotti, 1976; Racagni et al., 1977; Grace and Bunney, 1985) and maybe it can even modulate the dopaminergic impact on neuronal networks by innervating dopaminergic terminals. Thus, the interconnection of the GABAergic and dopaminergic system is highly complex and is still influenced by the contribution of the remaining transmitter systems, such as serotonin or acetylcholine. Figure 3 shows the schematic connectivity of the GABAergic subpopulations with the dopaminergic projections and the pyramidal output neurons exemplarily in the prefrontal cortex.

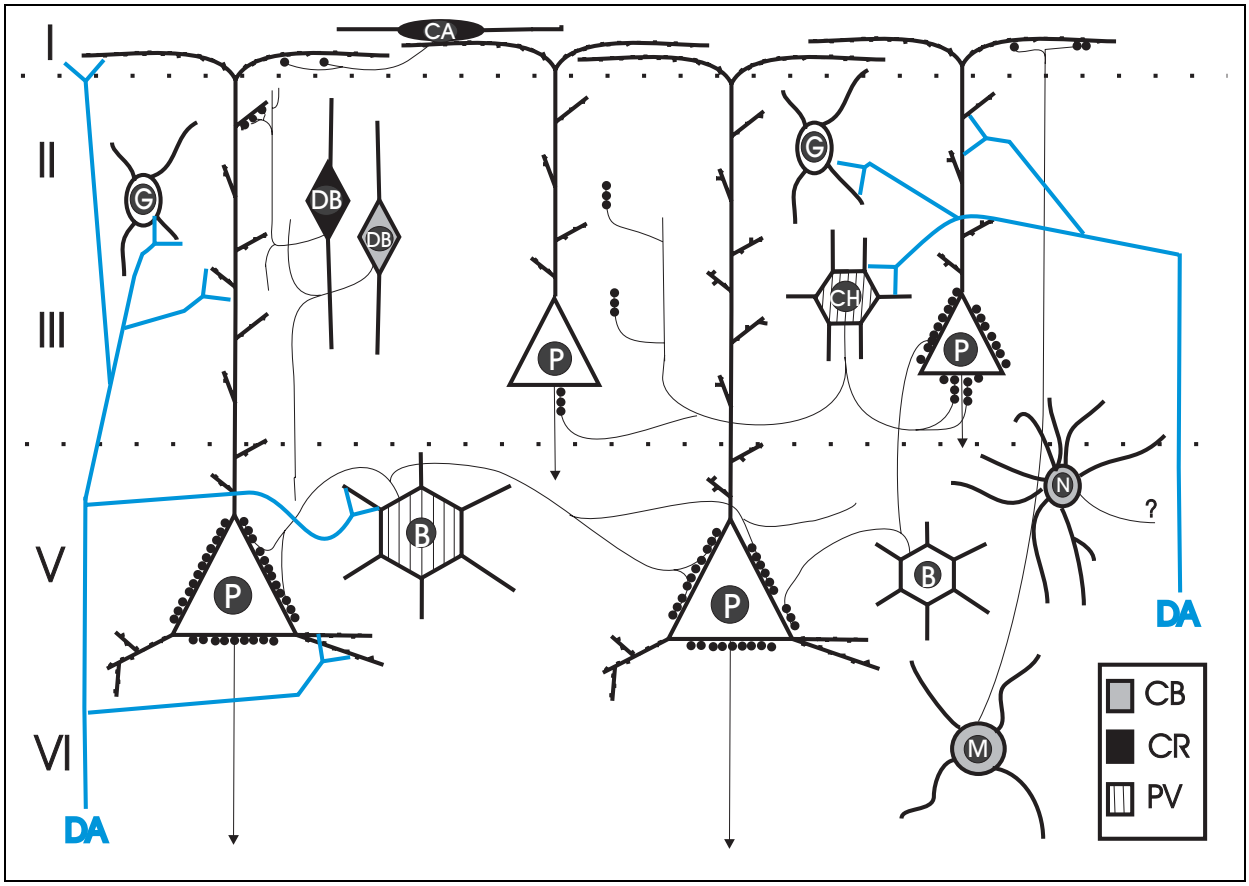


Fig. 3: Schematic illustration of the potential interconnectivity of the different GABAergic subpopulations with dopaminergic projections and the pyramidal neurons. The GABAergic subpopulations can be classified with the aid of different calcium-binding proteins. Calbindin (CB) is found in double bouquet cells (DB), in Martinotti (M) and neuroglia (N) cells. Parvalbumin (PV) in basket (B) and chandelier neurons (CH) and calretinin (CR) can be found in Cajal-Retzius cells (CA) and double bouquet cells, although it is sometimes co-expressed with one of the others. Dopamine (DA) innervates pyramidal cells (P) as well as different GABAergic cells such as basket cells or chandelier neurons and GABAergic cells in lamina II (G), while it can exert excitatory or inhibitory properties.

3. Long-term effects of a single (adult) methamphetamine challenge

Considering the huge amount of data on alterations in the neurotransmitter networks after the early MA challenge, there were still a few questions which remained unanswered. First, are these changes totally due to the immature networks during development or can an adult challenge with MA cause similar alterations, particularly in the dopaminergic system? Second, why are different areas affected in opposite ways? Is there any relationship with their developmental pattern? And last but not least, how is the GABAergic system involved in the reactive or compensative mechanisms within the disturbed neural networks?

It has been shown before that a single *adult* MA challenge can induce reactive changes in the prefrontal cortex of gerbils like an increase of spine density on pyramidal neurons (Dawirs et al., 1991). However, this increase turned out to be only transient and there was actually a slight decrease in density compared to control levels 30 days after the application (Dawirs et al., 1993b). Further, the GABAergic innervation in the prefrontal cortex was elevated 30 days after an adult MA treatment (Dawirs et al., 1997), which points to the plastic capacity of the GABAergic neuron population. Thus, the MA induced degeneration of dopaminergic terminals also impairs other transmitter systems during adulthood. Although the deleterious effect of adult MA is believed to be at least in part reversible (Meredith et al., 2005), the dopaminergic fiber densities after an adult MA challenge have not been investigated in our animal model so far.

Therefore, we wanted to know, if a challenge with MA during adulthood would have similar effects on the dopaminergic system as the early intoxication or if a mature system will be affected differently. Hence, we applied an adjusted dose of MA to adult gerbils (PD 180) and checked the long-term effects 180 days later (Brummelte et al., 2006a). The dose, which was chosen, was actually smaller than the one used for the juvenile animals, but as previous studies have shown that adults appear to be more sensible to MA than youngsters, these two different doses were more likely to reveal comparable mortality rates and similar concentrations in the brain than the same dose would have been (Teuchert-Noodt and Dawirs, 1991; Kokoshka et al., 2000). This already underlines the hypothesis that the neurotoxicity of MA varies between young and adult rodents.

The dopaminergic fiber densities were investigated in various brain areas. Most of them have previously revealed impacts after the early intoxication, namely the prefrontal cortex, the amygdala, the olfactory tubercle and the nucleus accumbens. Interestingly, despite a slight

increase in the shell region of the nucleus accumbens, no alterations could be detected. Table 2 gives an overview over the divergent effects on the dopaminergic system of early compared to adult intoxication. Interestingly, the drug challenge led to an increase in fiber density, not to a decrease, so that it is assumed that the degeneration after the pharmacological challenge was followed by a regeneration of fibers, which resulted in an oversprouting in the shell. In fact, it has been shown before that the destruction of substantia nigra neurons can induce a sprouting of dopaminergic fibers in particular areas (Finkelstein et al., 2000), which proves that there is a high plasticity within the transmitter networks not only during development but also later in adulthood.

To account for potential reactive or compensative effects of the local GABAergic system in the affected area, the cell densities of calbindin (CB) and parvalbumin (PV) neurons in the nucleus accumbens were additionally investigated, but revealed no differences. We used the markers for different subpopulations as in fact, the two subregions of the nucleus accumbens are characterized by different cell populations: CB neurons are mainly located in the core region, while PV neurons are predominantly found in the shell.

		Adult (current study) MA 1x 25mg/kg i.p., PD180	Juvenile MA 1x 50mg/kg i.p., PD14
Prefrontal cortex	Medial Orbital	↔ n.s. ↔ n.s.	↓ - 38% ** ↓ - 50% **
Nucleus accumbens	Core Shell	↔ n.s. ↑ +11% *	↓ - 28% * ↔ n.s.
Amygdala	Basolateral Central	↔ n.s. ↔ n.s.	↑ +18% ** ↔ n.s.
Olfactory tubercle		↔ n.s	No data

Table 2: Comparison of age-related long-term effects of a single methamphetamine intoxication (MA) on the dopamine innervation in limbic-cortical areas of the gerbil brain. Based on the studies: Dawirs et al., 1994; Busche et al., 2004; Lesting et al., 2005a; Brummelte et al., 2006a. Significance values: * $p<0.05$, ** $p<0.01$.

4. Postnatal development of dopaminergic and GABAergic structures in the limbic system

Although the complex neurotoxicity of MA is still not completely clear, the present results strongly indicate that developmental alterations must play a role in mediating the effect of this pharmacological drug. Thus, it is suggested that the maturation patterns of the different areas and according developmental alterations contribute essentially to the varying impact. For instance, the two subregions of the nucleus accumbens, shell and core, exhibit a quite divergent development of their dopaminergic innervation, with the core showing a decrease in

fiber density between PD 14 and 30 and then a slow but steady increase until well into adulthood, while the shell region shows a very steep increase between PD 70-90 (Lesting et al., 2005b). It is conceivable that the significant regression of fibers in the core region is a vulnerable process, which takes place during a sensitive period, so that the MA treatment on PD 14 causes a reduction in adult DA innervation of approximately 20%, while the shell region appears to be spared from these deleterious effects.

The question then arose, if the developmental patterns can also account for the imbalance observed within the dopaminergic system after the early pharmacologic challenge with an oversprouting of fibers in caudal limbic areas and an alleviation in fiber density in frontal areas (Fig. 2B).

For the prefrontal region it had already been shown that it reveals a prolonged development concerning the dopaminergic fiber densities until adulthood, both, for rats (Kalsbeek et al., 1988) and for gerbils (Dawirs et al., 1993a). However, for caudal limbic areas it has only been assumed that they mature relatively early (Busche et al., 2004), but the exact development of the DA innervation in the Mongolian gerbil has so far been neglected. Hence, we designed a long-term study in which we investigated dopaminergic and GABAergic structures in animals from different age stages starting on PD 14 until high age to account for potential alterations during development as well as during ageing (Brummelte and Teuchert-Noodt, 2006; Brummelte et al., accepted). We restricted the study to animals from impoverished rearing since these showed stronger effects after the pharmacological challenge and since the animal husbandry did not allow sufficient space to keep animals from enriched environment for up to two years.

The results revealed that neither the dopaminergic nor the GABAergic fiber densities have reached their complete mature pattern on PD 14 in all the caudal limbic areas. However, there were remarkable differences between the areas. Thus, DA fibers in the EC showed no differences at all between PD 14 and 720, while fibers still increased after PD 14 in the amygdala and even revealed a tendency for an oversprouting during PD 20 (Fig. 4). GABA fiber densities were measured in the PFC and the Amygdala, while the EC was not really suitable for measuring fiber densities due to high background staining. In addition to the GABAergic fibers, CB fibers were also measured in these areas which generally showed a similar developmental course with only minor deviations. In the PFC, GABA and CB fibers increased until PD 30, afterwards the CB fiber density decreased again slightly, while GABA revealed a further increase between PD 70 and PD 540, indicating a potential enhancement of a different GABAergic subpopulation. In the amygdala GABA and CB fibers reached their

maximum already around PD 20, and GABA showed a later decrease between PD 70 and PD540 (Fig. 5 and 6). Taken together, the results underline the feature of the frontal areas to mature later than caudal limbic ones, with the GABAergic fibers reaching their adult pattern in the PFC before the dopaminergic fibers, while the development within the amygdala appears quite similar.

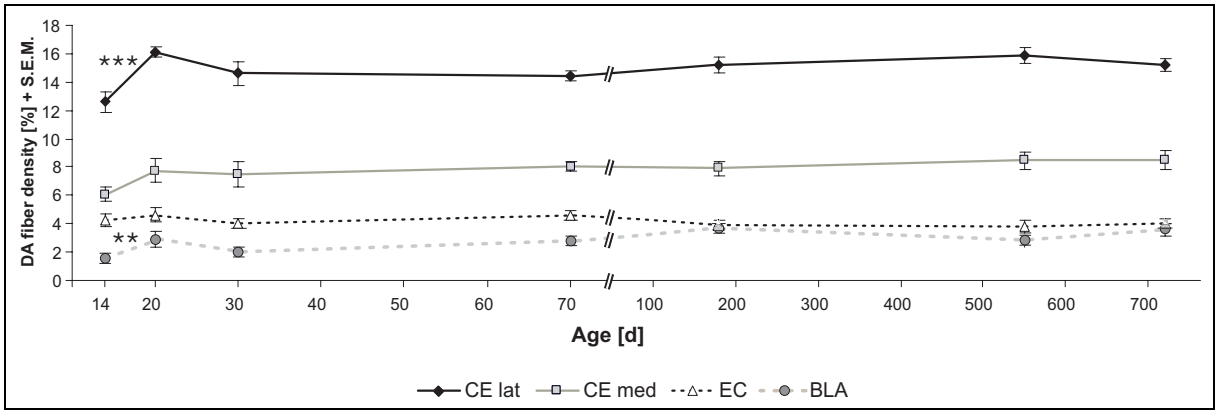


Fig. 4: Postnatal development of dopaminergic fiber densities in the amygdala and the entorhinal cortex. Only the lateral part of the central amygdala (CE lat) and the basolateral amygdala (BLA) show a significant increase between PD 14 and PD 20, and also a tendency for a subsequent decline until PD 30 (p<0.07). The medial part of the central amygdala (CE med) and the entorhinal cortex (EC) revealed no alterations. * p<0.05, ** p<0.01, *** p<0.001.

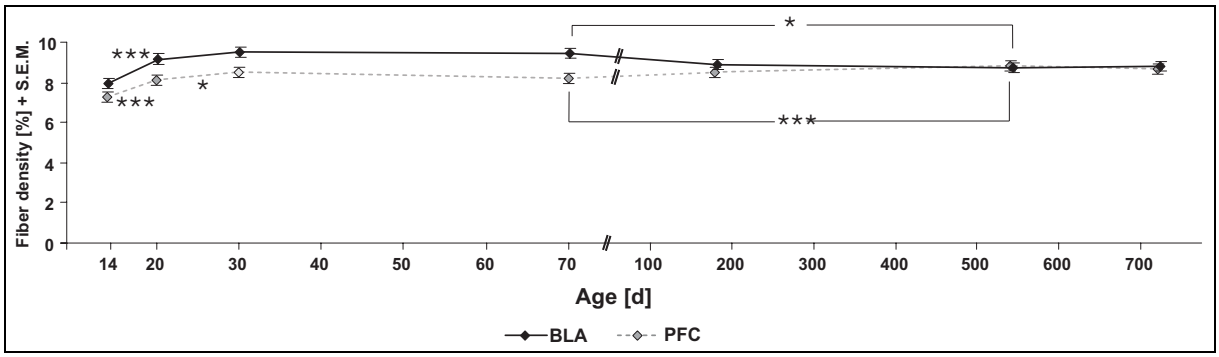


Fig. 5: Postnatal development of GABAergic fiber densities in the prefrontal cortex (PFC) and the basolateral amygdala (BLA). Both areas reveal an early increase, while the fiber densities of the PFC diminish after postnatal day (PD) 70 in the PFC, but show a further augmentation in the BLA between PD70-PD 540.
* p<0.05, ** p<0.01, *** p<0.001.

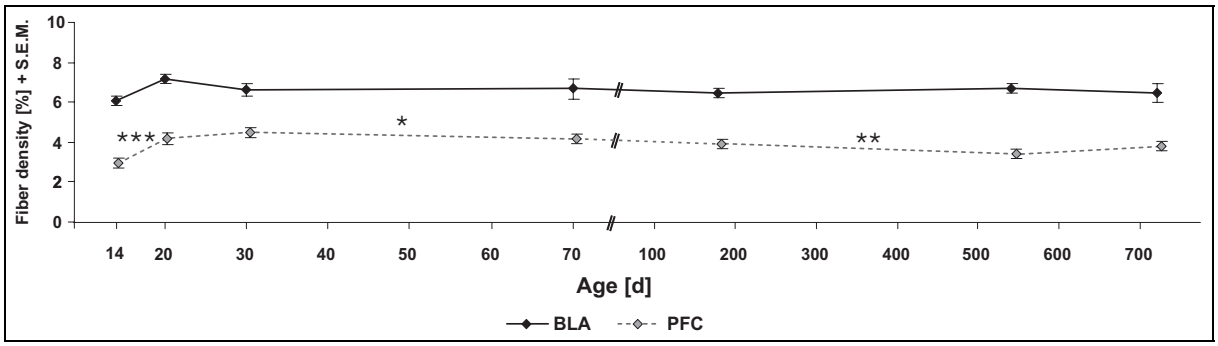


Fig. 6: Postnatal development of calbindin fiber densities in the prefrontal cortex (PFC) and the basolateral amygdala (BLA). CB fibers increase in the PFC until PD 20 and decrease slightly after PD 30, while there are no significant differences in the BLA.. * p<0.05, ** p<0.01, *** p<0.001.

Thus, one could imagine that during development the initial wiring of subcortical limbic areas takes place quite simultaneously concerning GABAergic and dopaminergic structures, while prefrontal areas experience a basic innervation, which is then continuously adapted to the ingrowth of dopaminergic and other fiber systems and to extrinsic influences. This would be in line with results showing that the glutamatergic projections from the medial PFC only reach their adult pattern late during adolescence, too (Witte, Brummelte and Teuchert-Noodt, submitted). In addition, these projections are assumed to provide a control over subcortical structures such as the amygdala. Therefore it seems likely that the generally high emotionality and impulsivity of juveniles is due to the early maturation of caudal limbic areas, which are then slowly put under the control of the prefrontal cortex, so that eventually cognitive and reasonable thoughts and behaviors gain the lead.

During this crucial process when different instances within the brain are striving for power, both, on the microcircuit and on the macrocircuit level, every external disturbance can essentially influence their success in finding a functioning balance. Transmitter systems and especially the slowly maturing ones such as DA are again highly involved in this critical process due to their morphogenetic influence and because of their consistently increasing number of connections during this phase. In fact, it has been proposed that experience during a sensitive period modifies the architecture of a circuit in fundamental ways, causing certain patterns of connectivity to become highly stable and, therefore, energetically preferred (Knudsen, 2004). It is further assumed that after this sensitive period, plasticity can only alter the connectivity pattern within this initial architectural constraints (Knudsen, 2004. However, the concrete distribution pattern of synapses of the various transmitter systems is far from being a stable arrangement. Even in adulthood there is a continuing reorganization of connections, which is believed to play a fundamental role in adaptation processes to extrinsic influences and is also assumed to participate in learning and memory. During development however, the neuroplasticity is still higher and there is an unlimited multitude of external influences which contribute essentially to the shaping and arranging of neuronal networks. This is in concert with our observations and conclusions from the long-term study of an adult MA challenge, that the adult treatment is likely to cause a *regeneration* of fibers, while the early application probably causes a *rearrangement* of fibers (Brummelte et al., 2006a). Plasticity during development is therefore very essential to adapt to external circumstances but also bears the risk of irreversible mismatches.

It has been assumed before that the two main dopaminergic limbic pathways, the meso-cortical and the meso-limbic one can influence each other during development (Le Moal and

Simon, 1991). Thus, one could imagine that the overshoot of fibers in the amygdala and EC on the one hand and the decrease of fibers in the PFC on the other hand are coherent and depend on each other. It is conceivable that usually the increasing control from the PFC somehow regulates the innervation density of the caudal structures, but if this control is retarded, the amygdala or the EC might end up with higher innervation densities while less fibers remain to reach the PFC. In fact, it had already been suggested that a deficiency in mesocortical DA function might cause a disinhibition of mesolimbic DA activity (Weinberger, 1987). In summary, our results suggest that the different maturation patterns might indeed contribute to the observed imbalance within the neural networks and that the incision in the dopaminergic development on PD 14 might therefore even cause a vicious circle, which is also affecting the plastic potentials of the other transmitter systems.

4.1 Ageing-related changes

Interestingly, none of the investigated areas showed ageing-related changes in the dopaminergic, GABAergic or calbindin fiber density. This is in contrast to other studies, which found for instance a prominent reduction of calbindin cells in the basal forebrain (Geula et al., 2003; Wu et al., 2003) with ageing, but also metabolic alterations concerning GABA and also DA (Del Arco et al., 2001; Gluck et al., 2001; Vicente-Torres et al., 2001; Segovia et al., 2001). As the PFC is assumed to be particularly vulnerable to ageing effects, we additionally analyzed the prefrontal fiber density of DA in adult to old gerbils from PD 180 to 720, as this has not been investigated before. Here we found a significant decrease in fiber density after 12 months with a 26% decrease compared to 18 month or 24 month old animals (Fig.7; Brummelte and Teuchert-Noodt, submitted).

The lack of age-related alterations in the remaining areas or transmitters might be due to the fact that 720 days is the average age of gerbils, while individuals might even get older (Troup et al., 1969). However, this fact underlines the vulnerability and sensibility of the dopaminergic system concerning neurodegenerative processes. DA has frequently been associated with age-related alterations, although the focus has been on striatal or brainstem regions (Roth and Joseph, 1994). More recently, the attention has shifted to other areas and it has been revealed that frontal cortices are also strongly affected concerning metabolic or morphological changes (Kaasinen et al., 2000; Inoue et al., 2001). In fact, it has been proposed that the mesolimbic pathways are more vulnerable to ageing than the nigrostriatal one (Cruz-Muros et al., 2006). It has also been assumed that the depletion of DA in the PFC

might contribute to age-related cognitive deficits (Arnsten et al., 1995). Our study provides additional data for neuroanatomical alterations within the prefrontal dopaminergic system with a quite early decline of fibers. Interestingly, the GABA fiber density shows a slight increase until PD 540 in the PFC, although the CB fiber density diminishes at the same time. So, despite a potential decrease of the calcium-binding protein in the fibers, which has been postulated as the probable reason for the observed age-related changes in CB structures (Kishimoto et al., 1998), one is tempted to hypothesize on a highly speculative level that GABAergic fibers might try to compensate the vanishing input from dopaminergic fibers.

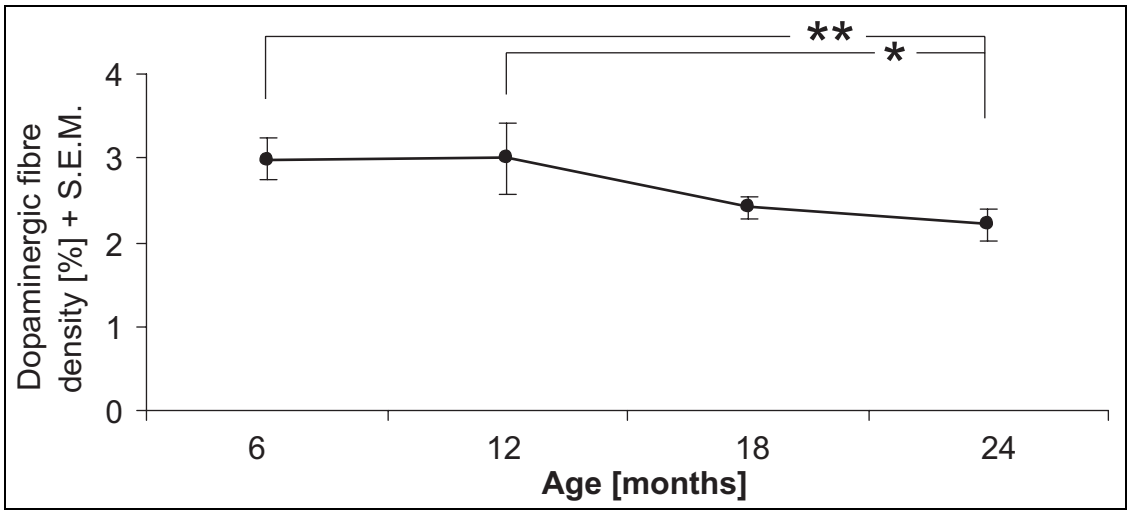


Fig. 7: Ageing-related decrease in the dopaminergic fiber density in the prefrontal cortex.
* $p<0.05$, ** $p<0.01$.

5. Alterations in the GABAergic system

To scrutinize this issue, we wanted to investigate the effect of the early disturbance of the dopaminergic system on GABAergic structures in the most sensitive PFC. As GABA is located mainly in interneurons in the PFC, we thought that these local cells might somehow react to the missing input from DA. As mentioned above, GABA appears in several subpopulations, which serve different functions within the local networks. The calcium-binding protein CB, for instance, is found in neuroglia, Martinotti or double bouquet cells. All these cells mainly innervate distal dendrites of pyramidal cells and thus have a rather modulating influence on the pyramidal activity (Conde et al., 1994; Gabbott and Bacon, 1996). Then again, there are cells which predominantly innervate the somata of pyramidal cells and even build their synapses so densely that they look like a basket around the pyramidal soma; this is the reason why they are named basket cells (DeFelipe and Fairen,

1982; Hendry et al., 1983). These axo-somatic connections have a particularly powerful influence on the firing activity of the pyramidal neurons. Basket cells are also classified as 'fast-spiking' neurons and often contain the calcium-binding protein PV (Kawaguchi and Kubota, 1997). Considering that one basket cell can innervate about a thousand pyramidal cells, it becomes clear that these GABAergic neurons can regulate whole populations of cells. Together with the so-called chandelier neurons, which build axo-axonic contacts at the initial axon segments of the pyramidal neurons, they are further believed to provide the indispensable synchronization of the cortical output neurons (Somogyi et al., 1982; Tamas et al., 1997). This synchronization again, is believed to provide the essential frame for cognitive functions such as working memory and for target-orientated behaviors (Constantinidis et al., 2002; Lewis et al., 2005). Thus, it becomes clear that these somatic contacts are essentially important for regulating the activity of local microcircuits and even macrocircuits and subsequently for assuring a normal working of functional networks.

Therefore, we were particularly interested, if these structures might be influenced by the early MA challenge or the IR conditions, and hence measured on the one hand the overall GABAergic fiber densities in particular laminae (Fig. 8 B.1) and on the other hand the density of GABAergic boutons (Fig. 8 A.1) around unstained pyramidal neurons (Brummelte et al., 2007).

Results revealed that IR led to a 19% decrease of GABAergic boutons round lamina III pyramidal neurons, but only to a tendency for a decrease around lamina V neurons. A MA intoxication however, led to a further decrease in both laminae of more than 20% compared to IR animals, so that the bouton densities of IR MA animals reached only 62 and 67%, respectively, of the control (ER) values. Interestingly, the fiber densities exhibit an augmentation in laminae I/II and V only in IR MA animals, but not in any other group (cf. Fig. 8).

These reactive changes in the GABAergic transmitter system are rather in contrast with our initial expectations, as they reveal alterations within the system which are not very likely to provide a compensating effect. Quite the contrary is the case, since a reduced bouton density can indicate a reduced somatic inhibition, which in turn might cause a loss in synchronization. This lessened synchronization again might explain the observed deficits in cognitive functions such as working memory seen in our animal model (Dawirs et al., 1996). In addition, the increase in fiber density can be a sign of an increase in dendritic expansion, or can be interpreted as an enlargement of axonal fibers, which then in turn would entail an increase in dendritic innervation of the distal parts of the pyramidal neurons. Considering previous results

from Nossoll and colleagues (1997) from our laboratory, who found an increase in non-somatic GABAergic profiles in the PFC after a MA intoxication using electron microscopy and a study showing that pyramidal cells increase their dendritic range and spine density (Blaesing et al., 2001), we find it tempting to suggest that the observed increase in fiber density in the current study may indeed be a sign for a partially ascent of the dendritic innervation. Thus, our results point to a potential shift within the GABAergic inhibition pattern from a strong and powerful inhibition at the somatic site to a more moderate influence at the dendritic sites after the MA intoxication of animals reared under impoverished conditions.

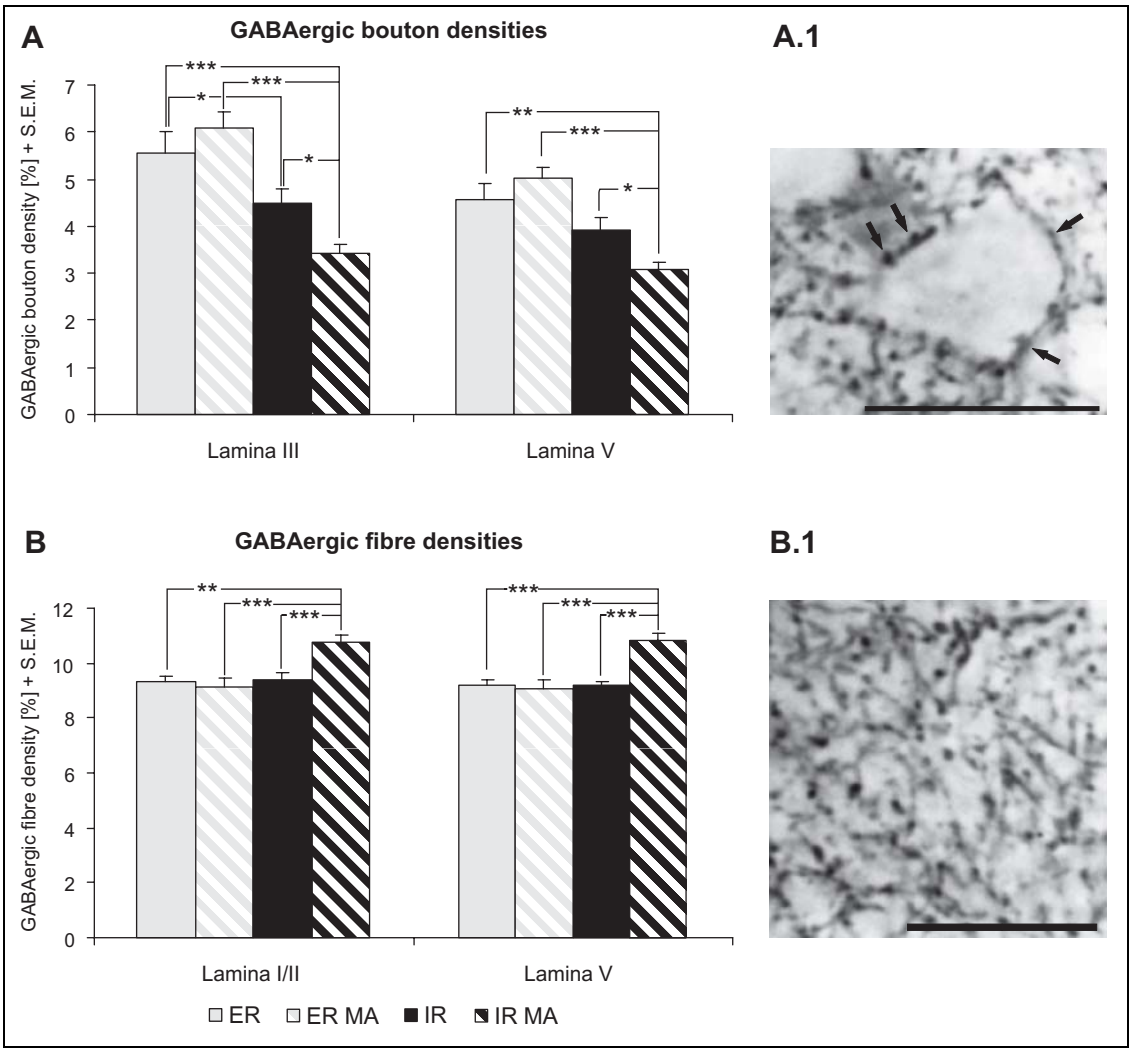


Fig. 8: GABAergic bouton (A) and fiber densities (B) and representative photomicrographs (A.1, B.1) in the analyzed layers of the PFC of gerbils from enriched (ER) and impoverished rearing (IR) conditions treated with either methamphetamine (MA) or saline given by means + standard error (S.E.M.). Bouton density (arrows A.1) is significantly reduced by IR in lamina III and in both laminae after additional MA intoxication. Fiber densities show an augmentation in IR MA animals only. * $p<0.05$, ** $p<0.01$, *** $p<0.001$, scale bar: 20µm.

Taking into account that the maturation of GABAergic synapses in general proceeds until early adulthood (Huang et al., 1999; Chattopadhyaya et al., 2004) and that dopaminergic afferents especially continue to form synapses on prefrontal GABAergic interneurons during the prolonged maturation (Benes et al., 1996b), it is conceivable that the early systemic disturbance has a detrimental influence on the GABAergic system, too. The calcium-binding protein PV, which is used as a marker for fast-spiking neurons such as basket or chandelier cells, and which is believed to function as a buffer protein against high and toxic calcium concentrations within the cell, is not expressed in the gerbil PFC before PD 14 (unpublished data). Despite the fact that a lack of this protein might result in a higher vulnerability of these neurons against high excitation (Heizmann, 1992) it is also considered to be a marker for functional maturity of the cell (Seto-Ohshima et al., 1990; Solbach and Celio, 1991). Thus, the potentially immature fast-spiking GABAergic neurons might be negatively affected by the early impact on the dopaminergic system and thus contribute to the variances in the transmitter connectivity since especially fast-spiking neurons are believed to essentially contribute to the shaping of receptive and spatial memory fields (Jones, 1993; Rao et al., 1999; 2000). In addition, the ability to synchronize pyramidal cell activity is assumed to be in substantial flux until adulthood (Lewis et al., 2005) and although the proliferation and formation of the typical somatic basket terminals seems to be a stereotyped process, it also depends on neuronal activity within cortical circuits (Marty et al., 2000; Chattopadhyaya et al., 2004). Thus, extrinsic and intrinsic influences during this critical period can have vehement consequences on the establishment of functional systems, including the ability of basket cells to properly synchronize pyramidal activity.

Taken together, the early systemic impact causes also severe alterations within the GABAergic system, with a potential shift from somatic to dendritic inhibition, which might contribute to a functional miswirirng of neuronal networks, which in turn might account for the observed cognitive impairments. Figure 9 gives a schematic overview of altered morphologies and potential connections in the PFC of IR animals, which received additionally the MA intoxication.

6. Consequences of early developmental disturbances (implications for schizophrenia)

The remarkable revelation of these studies is that a single disturbance during development which actual primarily affects the dopaminergic system, can have such a wide-spread impact

on miscellaneous local and far-reaching networks. It is obvious that particular critical windows or periods exist during which external influences can be exceptionally formative. However, pups are usually believed to be relatively irresponsive to stressful events during the first few weeks of their life, in the so-called 'stress hyporesponsive period' (Sapolsky and Meaney, 1986), which underlines the potential complexity of diverse extrinsic impacts. Thus, it has been shown that even the maternal care such as licking behavior can essentially contribute to the behavioral and emotional outcome and stress responsiveness of the offspring (Caldji et al., 1998; Francis et al., 1999; Meaney, 2001).

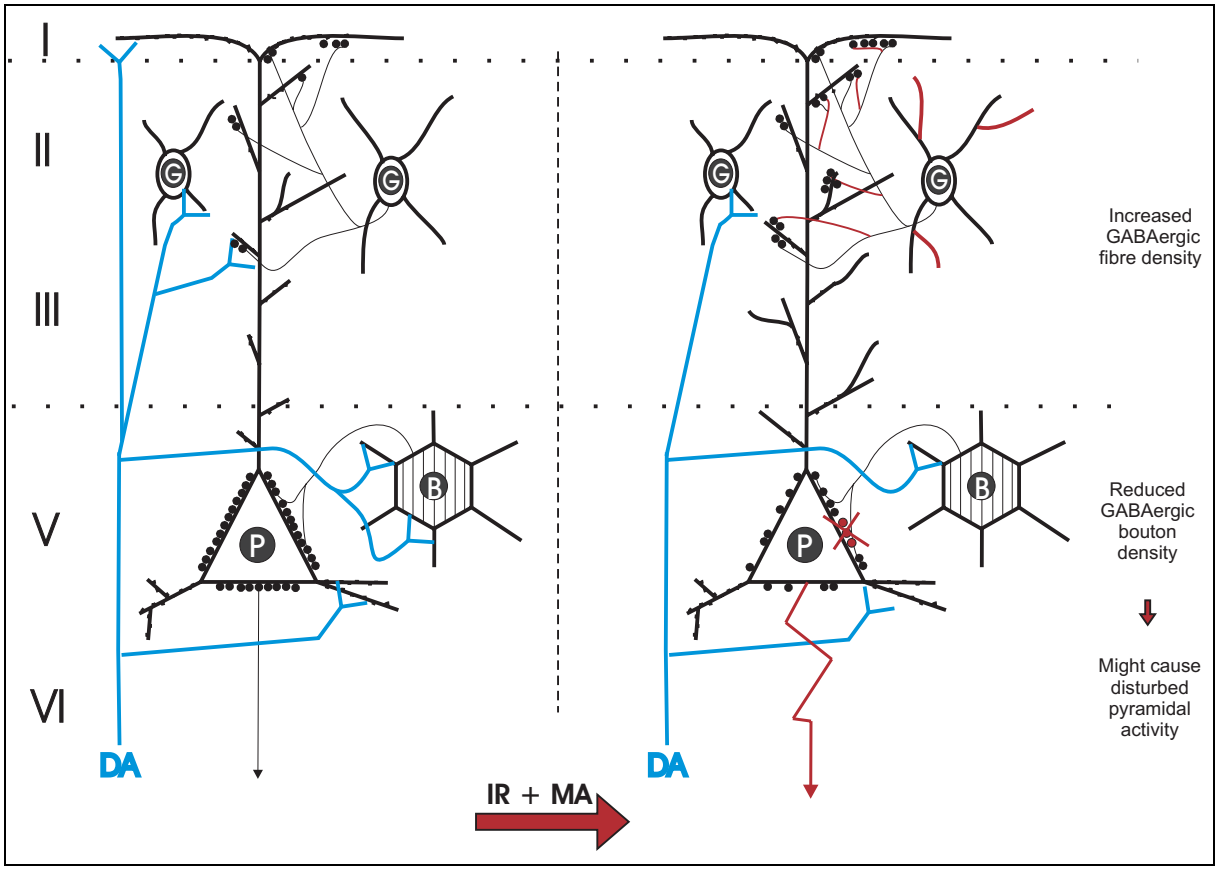


Fig. 9: Schematic illustration of the potential alterations within the prefrontal network after the IR MA challenge. After impoverished rearing (IR) combined with a methamphetamine (MA) intoxication, the dopaminergic fiber density (DA) is reduced (Dawirs et al., 1994), while the pyramidal cells expand their dendrites and spine densities (Blaesing et al., 2001). The current study revealed that the GABA fiber density is also increased, possibly due to an enlargement of dendrites and to a spreading of axonal fibers since a previous study has shown an increase in dendritic GABAergic profiles (Nossoll et al., 1997). At the same time, the bouton density around the pyramidal soma is decreased. Thus, there is an apparent shift in the GABAergic inhibition with diminished somatic inhibition and increased dendritic inhibition probably leading to a disturbed firing pattern of the pyramidal cells due to a lessened synchronization. G: GABA interneuron, B: basket cell, P: pyramidal cell; I-VI: laminae

High-licking or low-licking behavior of the dams can even alter neurogenesis in the hippocampus (Bredy et al., 2003). Therefore, it is no surprise that also the mood and the stress level of the mother, respectively, can have an influence on the progeny. For instance, high

levels of corticosterone, a stress hormone, during the lactation period can cause differences in hippocampal cell proliferation and can evoke signs of hyperactive behavior in the offspring (Brummelte et al., 2006b). However, it is clear that the type and degree of the external stress is important for determining the morphological, behavioral and cognitive consequences.

Our 2-step animal model of using combined early MA intoxication as an acute stressor and IR as a chronic stress factor has so far revealed several morphological changes in neuroanatomical brain networks and some cognitive impairments, which resemble some of the changes and deficits seen in schizophrenic individuals. Thus, Akil and colleagues (1999) found a decrease in dopaminergic fibers in the prefrontal cortex of schizophrenic individuals, comparable with the reduction in our animal model (Dawirs et al., 1994). In addition, the imbalance of the DA system between cortical and subcortical areas, has not only been observed after our IR and MA challenge (Busche et al., 2004) but was reported for the schizophrenic human brain (Laruelle et al., 2003; Abi-Dargham, 2004). Besides, low prefrontal DA levels are associated with negative or cognitive symptoms of schizophrenia, while a hyperactivity of the mesolimbic pathway is assumed to be responsible for the positive symptoms (Crow, 1980; Davis et al., 1991). Furthermore, our animal model revealed a miswiring of prefrontal pyramidal projections (Bagorda et al., 2006), which corroborates the dysconnection hypothesis of schizophrenia from Weinberger and Lipska (1995). In addition, this miswiring, resulting from the different impact on lamina III compared to lamina V pyramidal neurons in IR MA animals might help to explain the discrepancy of human studies, paradoxically reporting either a hypofunction (Volz et al., 1999) or a hyperfunction (Manoach et al., 1999) of the glutamatergic system in schizophrenic patients.

Intriguingly, the results of the current study reveal some resemblances with changes in schizophrenia, too. Thus, a reduction of pyramidal GABAergic synapses has also been observed in schizophrenic patients (Blum and Mann, 2002), with a reduction in PV-immunoreactive structures being one of the most prevalent observations in post-mortem studies (Woo et al., 1998; Pierri et al., 1999; Lewis et al., 1999). In addition, the $GABA_A$ receptor density was upregulated at the cell bodies of pyramidal neurons (Benes et al., 1996a), possibly compensating for a reduced number of inhibitory terminals (Lewis et al., 2005). These indices for a reduced GABAergic somatic inhibition are in line with recent neurophysiological studies, which revealed that some cognitive dysfunctions in schizophrenic patients, as e.g. working memory deficits are associated with an abnormal neural synchronization (Spencer et al., 2003; Lee et al., 2003; Spencer et al., 2004; Uhlhaas and

Singer, 2006). This again is in concert with the impairment of working memory in our animal model (Dawirs et al., 1996).

In summary, our results indicate that a single early pharmacological stress is effectual to induce severe morphological changes in the neuronal networks of the whole limbic system of animals from IR conditions, which resemble at least some of the changes seen in schizophrenic individuals. Taking the observed cognitive impairments into account, one is tempted to suggest that our 2-step approach provides a useful animal model of psychoses and schizophrenia.

Noteworthy, schizophrenia usually does not appear before early adulthood, even though it is assumed to have at least partially developmental etiologic reasons. Thus, one could speculate that the high plasticity during maturation of neuronal networks might somehow prevent the outbreak of the disease but with the omission of this high plastic capacities, the miswiring becomes more stable and starts to unfold its deleterious effects.

Interestingly, a treatment with clinical doses of methylphenidate (e.g. Ritalin®) for 30 consecutive days about two weeks after the noxious application of MA leads to a partially 'recovery' of the diminished dopaminergic fiber densities in adulthood (Grund et al., 2006; Grund et al., revision submitted). Thus the deleterious impact of MA can be influenced by another pharmacological interference, but apparently not by enriched environment (Brummelte et al., in prep.). Methylphenidate is a stimulant drug which selectively blocks the reuptake of DA and noradrenaline by binding to the according transporters (Gatley et al., 1996) and is momentarily the drug of choice for the treatment of attention-deficit/hyperactivity disorder (ADHD). The enhanced concentration of DA in the synaptic cleft must somehow trigger an elevated sprouting of dopaminergic fibers, however, this sprouting is only evident when the animals received the early MA challenge and not when they received the control injection of saline. This is again a sign for the high plastic potentials of the neuronal networks during development.

7. Conclusion and future perspectives

Taken together, this work provides additional evidence for a high plasticity of GABAergic and dopaminergic structures during the maturation process, but in part also during adulthood and ageing. The different extrinsic and intrinsic influences during postnatal development and their interactions essentially contribute to the establishment of functional networks, whereby

the various transmitter systems play an indispensable role. Disturbances during critical periods in the development lead to neuroanatomical alterations of the local networks and thus also of the macrocircuits of the limbic system. The results from our 2-step animal model have shown that especially DA appears to be particularly vulnerable to interfering effects and can then subsequently affect all the connected other transmitter systems. The attempt of microcircuits to compensate the altered innervation patterns probably results in a compromise, which might provide an equilibrium of local connections, but which in turn might cause a decompensation and subsequent imbalance of greater circuits and networks. The tendency of every cell to counterbalance its excitability and its excitatory and inhibitory inputs, e.g. via regulating the feedback loops, might contribute to the alterations seen at the local level (Lehmann et al., 2005). However, the effect on the overall networks might be devastating. Thus, the reactive changes in the morphology cause a different pattern of connectivity and thus imply functional changes and differences in the behavioral and cognitive outcome. This again might help to better understand the complex and individually divergent symptomatic pathology of schizophrenia.

Another important conclusion of these works is the fact that there is not only a high neuroplasticity of the various transmitter systems, and this during development as well as to a lesser extent during adulthood, but also a very high interconnectivity and interdependency of the transmitters. For instance, it has been revealed by others that 5-HT can directly regulate the cortical DA release, probably via the expression of 5-HT_{2A} receptors at the presynaptical site (Miner et al., 2000; 2003; Alex and Pehek, 2006; Pehek et al., 2006). Similar intensive interactions can be assumed for the GABA-DA relationship considering the prominent alterations within the GABAergic system after the early challenge of the DA system (Brummelte et al., 2007). In fact, DA is not only innervating pyramidal and GABAergic cell bodies and dendrites (Sesack et al., 1995; Davidoff and Benes, 1998), but can also act inhibitory or excitatory at GABAergic axon terminals (Geldwert et al., 2006). Moreover, Liu and colleagues recently published their intriguing discovery of a direct protein-protein coupling of the functionally and structurally different $GABA_A$ and DA D_5 receptors, which suggests a functional interaction of these two transmitter types (Liu et al., 2000). Hence, one is tempted to suggest that the dopaminergic system as the main specific modulator and the GABAergic system as the main inhibitor and thus coordinator of neuronal network activities, are especially interwoven and interdependent. However, this relationship needs to be further investigated, since e.g. a direct innervation of GABAergic synapses on cortical dopaminergic nerve terminals has to our knowledge not been revealed to date. In addition, it would be

interesting to further examine the contribution and specific roles of the divergent GABAergic subpopulations in these networks.

In summary, the interconnectivity of the various transmitter systems, in particular of DA and GABA, appears to be highly complex and might therefore trigger or contribute to the reactive processes after external or internal interferences. During development a disturbance of one neurotransmitter system might additionally cause an imbalance in the temporal coordination of the various connected maturation processes. Thus, one should keep in mind that pharmacologic interventions will never only affect one transmitter system, even though they are, e.g. selective 5-HT reuptake inhibitors (SSRIs) or only affecting the $GABA_A$ receptors (benzodiazepines). This high interdependency and plasticity even during adulthood might also help to explain, why the effect of neurological drugs is so unpredictable in the individual case. Therefore, our results lead to the assumption that treatment with pharmaceuticals, especially during the high phase of neuroplasticity during development, but also during the critical and vulnerable period of ageing, should always be considered with care, as despite the acute improvement, there might be hidden long-term side-effects, which might alter the neuronal networks in perpetuity.

8. References

Abi-Dargham A (2004) Do we still believe in the dopamine hypothesis? New data bring new evidence. Int J Neuropsychopharmacol 7 Suppl 1:S1-S5.

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.

Alex KD, Pehek EA (2006) Pharmacologic mechanisms of serotonergic regulation of dopamine neurotransmission. Pharmacol Ther. (Epub ahead of print)

Arnsten AF, Cai JX, Steere JC, Goldman-Rakic PS (1995) Dopamine D2 receptor mechanisms contribute to age-related cognitive decline: the effects of quinpirole on memory and motor performance in monkeys. J Neurosci 15:3429-3439.

Bagorda F, Teuchert-Noodt G, Lehmann K (2006) Isolation rearing or methamphetamine traumatisation induce a "dysconnection" of prefrontal efferents in gerbils: implications for schizophrenia. J Neural Transm 113:365-379.

Ben-Ari Y (2002) Excitatory actions of gaba during development: the nature of the nurture. Nat Rev Neurosci 3:728-739.

Benes FM, Vincent SL, Molloy R (1993) Dopamine-immunoreactive axon varicosities form nonrandom contacts with GABA-immunoreactive neurons of rat medial prefrontal cortex. Synapse 15:285-295.

Benes FM, Vincent SL, Marie A, Khan Y (1996a) Up-regulation of GABAA receptor binding on neurons of the prefrontal cortex in schizophrenic subjects. Neuroscience 75:1021-1031.

Benes FM, Vincent SL, Molloy R, Khan Y (1996b) Increased interaction of dopamine-immunoreactive varicosities with GABA neurons of rat medial prefrontal cortex occurs during the postweanling period. Synapse 23:237-245.

Birkmayer W, Riederer P, Blau K (1989) Understanding the Neurotransmitters. Key to the Working of the Brain. Springer-Verlag Kg.

Björklund A, Lindvall O (1984) Dopamine-containing systems in the CNS. In: Handbook of chemical neuroanatomy - Classical transmitters in the CNS, Part 1 (Björklund A, Hökfelt T, eds), pp 55-122. Amsterdam: Elsevier.

Blaesing B, Nossoll M, Teuchert-Noodt G, Dawirs RR (2001) Postnatal maturation of prefrontal pyramidal neurones is sensitive to a single early dose of methamphetamine in gerbils (Meriones unguiculatus). J Neural Transm 108:101-113.

Blum BP, Mann JJ (2002) The GABAergic system in schizophrenia. Int J Neuropsychopharmacol 5:159-179.

Bowery NG, Smart TG (2006) GABA and glycine as neurotransmitters: a brief history. Br J Pharmacol 147 Suppl 1:S109-S119.

Bredy TW, Grant RJ, Champagne DL, Meaney MJ (2003) Maternal care influences neuronal survival in the hippocampus of the rat. Eur J Neurosci 18:2903-2909.

Brummelte S, Grund T, Czok A, Teuchert-Noodt G, Neddens J (2006a) Long-term effects of a single adult methamphetamine challenge: minor impact on dopamine fibre density in limbic brain areas of gerbils. Behav Brain Funct 2:12.

Brummelte S, Pawluski JL, Galea LA (2006b) High post-partum levels of corticosterone given to dams influence postnatal hippocampal cell proliferation and behavior of offspring: A model of post-partum stress and possible depression. Horm Behav 50:370-382.

Brummelte S, Teuchert-Noodt G (2006) Postnatal development of dopamine innervation in the amygdala and the entorhinal cortex of the gerbil (*Meriones unguiculatus*). Brain Res 1125: 9-16.

Brummelte S, Neddens J, Teuchert-Noodt G (2007) Alteration in the GABAergic network of the prefrontal cortex in a potential animal model of psychosis. J Neural Transm (Epub ahead of print).

Brummelte S., Witte A.V. and Teuchert-Noodt G.: Postnatal development of GABA and Calbindin cells and fibers in the prefrontal cortex and basolateral amygdala of gerbils (*Meriones unguiculatus*). Int J Dev Neurosci (accepted).

Busche A, Neddens J, Dinter C, Dawirs RR, Teuchert-Noodt G (2002) Differential influence of rearing conditions and methamphetamine on serotonin fibre maturation in the dentate gyrus of gerbils (Meriones unguiculatus). Dev Neurosci 24:512-521.

Busche A (2004) Zur Entstehung einer Imbalance im limbo-präfrontalen System bei *Meriones unguiculatus*: Der Einfluss restriktiver Isolationsaufzucht und einer postnatalen Methamphetamin-Intoxikation auf die monoaminergen Transmitter Dopamin und Serotonin in limbischen Regionen. Eine Bewertung quantitativer Datenerhebungen. Dissertation.

Busche A, Polascheck D, Lesting J, Neddens J, Teuchert-Noodt G (2004) Developmentally induced imbalance of dopaminergic fibre densities in limbic brain regions of gerbils (Meriones unguiculatus). J Neural Transm 111:451-463.

Busche A, Bagorda A, Lehmann K, Neddens J, Teuchert-Noodt G (2006) The maturation of the acetylcholine system in the dentate gyrus of gerbils (Meriones unguiculatus) is affected by epigenetic factors. J Neural Transm 113:113-124.

Cadet JL, Brannock C (1998) Free radicals and the pathobiology of brain dopamine systems. Neurochem Int 32:117-131.

Caldji C, Tannenbaum B, Sharma S, Francis D, Plotsky PM, Meaney MJ (1998) Maternal care during infancy regulates the development of neural systems mediating the expression of fearfulness in the rat. Proc Natl Acad Sci U S A 95:5335-5340.

Cantuti-Castelvetri I, Shukitt-Hale B, Joseph JA (2003) Dopamine neurotoxicity: age-dependent behavioral and histological effects. Neurobiol Aging 24:697-706.

Celio MR (1990) Calbindin D-28k and parvalbumin in the rat nervous system. Neuroscience 35:375-475.

Chattopadhyaya B, Di CG, Higashiyama H, Knott GW, Kuhlman SJ, Welker E, Huang ZJ (2004) Experience and activity-dependent maturation of perisomatic GABAergic innervation in primary visual cortex during a postnatal critical period. J Neurosci 24:9598-9611.

Cherubini E, Gaiarsa JL, Ben-Ari Y (1991) GABA: an excitatory transmitter in early postnatal life. Trends Neurosci 14:515-519.

Chinta SJ, Andersen JK (2005) Dopaminergic neurons. Int J Biochem Cell Biol 37:942-946.

Conde F, Lund JS, Jacobowitz DM, Baimbridge KG, Lewis DA (1994) Local circuit neurons immunoreactive for calretinin, calbindin D-28k or parvalbumin in monkey prefrontal cortex: distribution and morphology. J Comp Neurol 341:95-116.

Constantinidis C, Williams GV, Goldman-Rakic PS (2002) A role for inhibition in shaping the temporal flow of information in prefrontal cortex. Nat Neurosci 5:175-180.

Crow TJ (1980) Positive and negative schizophrenic symptoms and the role of dopamine. Br J Psychiatry 137:383-386.

Cruz-Muros I, fonso-Oramas D, Abreu P, Barroso-Chinea P, Rodriguez M, Gonzalez MC, Gonzalez HT (2006) Aging of the rat mesostriatal system: Differences between the nigrostriatal and the mesolimbic compartments. Exp Neurol. (Epub ahead of print)

Davidoff SA, Benes FM (1998) High-resolution scatchard analysis shows D1 receptor binding on pyramidal and nonpyramidal neurons. Synapse 28:83-90.

Davis KL, Kahn RS, Ko G, Davidson M (1991) Dopamine in schizophrenia: a review and reconceptualization. Am J Psychiatry 148:1474-1486.

Dawirs RR, Teuchert-Noodt G, Busse M (1991) Single doses of methamphetamine cause changes in the density of dendritic spines in the prefrontal cortex of gerbils (Meriones unguiculatus). Neuropharmacology 30:275-282.

Dawirs RR, Teuchert-Noodt G, Czaniera R (1993a) Maturation of the dopamine innervation during postnatal development of the prefrontal cortex in gerbils (Meriones unguiculatus). A quantitative immunocytochemical study. J Hirnforsch 34:281-290.

Dawirs RR, Teuchert-Noodt G, Molthagen M (1993b) Indication of methamphetamine-induced reactive synaptogenesis in the prefrontal cortex of gerbils (Meriones unguiculatus). Eur J Pharmacol 241:89-97.

Dawirs RR, Teuchert-Noodt G, Czaniera R (1994) The postnatal maturation of dopamine innervation in the prefrontal cortex of gerbils (Meriones unguiculatus) is sensitive to an early single dose of methamphetamine. A quantitative immunocytochemical study. J Hirnforsch 35:195-204.

Dawirs RR, Teuchert-Noodt G, Czaniera R (1996) Ontogeny of PFC-related behaviours is sensitive to a single non-invasive dose of methamphetamine in neonatal gerbils (Meriones unguiculatus). J Neural Transm 103:1235-1245.

Dawirs RR, Teuchert-Noodt G, Nossoll M (1997) Pharmacologically induced neural plasticity in the prefrontal cortex of adult gerbils (Meriones unguiculatus). Eur J Pharmacol 327:117-123.

De Felipe J, Fairen A (1982) A type of basket cell in superficial layers of the cat visual cortex. A Golgi-electron microscope study. Brain Res 244:9-16.

Del Arco A, Segovia G, Mora F (2001) Dopamine release during stress in the prefrontal cortex of the rat decreases with age. Neuroreport 12:4019-4022.

Diamond MC, Ingham CA, Johnson RE, Bennett EL, Rosenzweig MR (1976) Effects of environment on morphology of rat cerebral cortex and hippocampus. J Neurobiol 7:75-85.

Diamond MC, Krech D, Rosenzweig MR (1964) The effects of an enriched environment on the histology of the rat cerebral cortex. J Comp Neurol 123:111-120.

Fallon JH, Koziell DA, Moore RY (1978) Catecholamine innervation of the basal forebrain. II. Amygdala, suprarhinal cortex and entorhinal cortex. J Comp Neurol 180:509-532.

Finkelstein DI, Stanic D, Parish CL, Tomas D, Dickson K, Horne MK (2000) Axonal sprouting following lesions of the rat substantia nigra. Neuroscience 97:99-112.

Francis DD, Champagne FA, Liu D, Meaney MJ (1999) Maternal care, gene expression, and the development of individual differences in stress reactivity. Ann N Y Acad Sci 896:66-84.

Freund TF (2003) Interneuron Diversity series: Rhythm and mood in perisomatic inhibition. Trends Neurosci 26:489-495.

Gabbott PL, Bacon SJ (1996) Local circuit neurons in the medial prefrontal cortex (areas 24a,b,c, 25 and 32) in the monkey: I. Cell morphology and morphometrics. J Comp Neurol 364:567-608.

Gale KN, Guidotti A (1976) GABA-mediated control of rat neostriatal tyrosine hydroxylase revealed by intranigral muscimol. Nature 263:691-693.

Ganguly K, Schinder AF, Wong ST, Poo M (2001) GABA itself promotes the developmental switch of neuronal GABAergic responses from excitation to inhibition. Cell 105:521-532.

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.

Geldwert D, Norris JM, Feldman IG, Schulman JJ, Joyce MP, Rayport S (2006) Dopamine presynaptically and heterogeneously modulates nucleus accumbens medium-spiny neuron GABA synapses in vitro. BMC Neurosci 7:53.

Geula C, Bu J, Nagykery N, Scinto LF, Chan J, Joseph J, Parker R, Wu CK (2003) Loss of calbindin-D28k from aging human cholinergic basal forebrain: relation to neuronal loss. J Comp Neurol 455:249-259.

Gluck MR, Moy LY, Jayatilleke E, Hogan KA, Manzino L, Sonsalla PK (2001) Parallel increases in lipid and protein oxidative markers in several mouse brain regions after methamphetamine treatment. J Neurochem 79:152-160.

Goldman-Rakic PS, Leranth C, Williams SM, Mons N, Geffard M (1989) Dopamine synaptic complex with pyramidal neurons in primate cerebral cortex. Proc Natl Acad Sci U S A 86:9015-9019.

Gorelova N, Seamans JK, Yang CR (2002) Mechanisms of dopamine activation of fast-spiking interneurons that exert inhibition in rat prefrontal cortex. J Neurophysiol 88:3150-3166.

Grace AA, Bunney BS (1985) Opposing effects of striatonigral feedback pathways on midbrain dopamine cell activity. Brain Res 333:271-284.

Grund T, Lehmann K, Bock N, Rothenberger A, Teuchert-Noodt G (2006) Influence of methylphenidate on brain development - an update of recent animal experiments. Behav Brain Funct 2:2.

Heizmann CW (1992) Calcium-binding proteins: basic concepts and clinical implications. Gen Physiol Biophys 11:411-425.

Hendry SH, Houser CR, Jones EG, Vaughn JE (1983) Synaptic organization of immunocytochemically identified GABA neurons in the monkey sensory-motor cortex. J Neurocytol 12:639-660.

Huang ZJ, Kirkwood A, Pizzorusso T, Porciatti V, Morales B, Bear MF, Maffei L, Tonegawa S (1999) BDNF regulates the maturation of inhibition and the critical period of plasticity in mouse visual cortex. Cell 98:739-755.

Hubel DH, Wiesel TN (1964) Effects of Monocular deprivation in kittens. Naunyn Schmiedebergs Arch Exp Pathol Pharmakol 248:492-497.

Hubel DH, Wiesel TN, LeVay S (1977) Plasticity of ocular dominance columns in monkey striate cortex. Philos Trans R Soc Lond B Biol Sci 278:377-409.

Inoue M, Suhara T, Sudo Y, Okubo Y, Yasuno F, Kishimoto T, Yoshikawa K, Tanada S (2001) Age-related reduction of extrastriatal dopamine D2 receptor measured by PET. Life Sci 69:1079-1084.

Itzhak Y, Gandia C, Huang PL, Ali SF (1998) Resistance of neuronal nitric oxide synthase-deficient mice to methamphetamine-induced dopaminergic neurotoxicity. J Pharmacol Exp Ther 284:1040-1047.

Jacobson M (1991) Developmental Neurobiology. New York: Plenum Press.

Jay TM, Glowinski J, Thierry AM (1995) Inhibition of hippocampoprefrontal cortex excitatory responses by the mesocortical DA system. Neuroreport 6:1845-1848.

Jones EG (1993) GABAergic neurons and their role in cortical plasticity in primates. Cereb Cortex 3:361-372.

Kaasinen V, Vilkman H, Hietala J, Nagren K, Helenius H, Olsson H, Farde L, Rinne J (2000) Age-related dopamine D2/D3 receptor loss in extrastriatal regions of the human brain. Neurobiol Aging 21:683-688.

Kalsbeek A, Voorn P, Buijs RM, Pool CW, Uylings HB (1988) Development of the dopaminergic innervation in the prefrontal cortex of the rat. J Comp Neurol 269:58-72.

Kawaguchi Y, Kubota Y (1997) GABAergic cell subtypes and their synaptic connections in rat frontal cortex. Cereb Cortex 7:476-486.

Kishimoto J, Tsuchiya T, Cox H, Emson PC, Nakayama Y (1998) Age-related changes of calbindin-D28k, calretinin, and parvalbumin mRNAs in the hamster brain. Neurobiol Aging 19:77-82.

Kita T, Wagner GC, Nakashima T (2003) Current research on methamphetamine-induced neurotoxicity: animal models of monoamine disruption. J Pharmacol Sci 92:178-195.

Knudsen EI (2004) Sensitive periods in the development of the brain and behavior. J Cogn Neurosci 16:1412-1425.

Kokoshka JM, Fleckenstein AE, Wilkins DG, Hanson GR (2000) Age-dependent differential responses of monoaminergic systems to high doses of methamphetamine. J Neurochem 75:2095-2102.

Laruelle M, Kegeles LS, bi-Dargham A (2003) Glutamate, dopamine, and schizophrenia: from pathophysiology to treatment. Ann N Y Acad Sci 1003:138-158.

Lau JW, Senok S, Stadlin A (2000) Methamphetamine-induced oxidative stress in cultured mouse astrocytes. Ann N Y Acad Sci 914:146-156.

Lauder JM, Liu J, Devaud L, Morrow AL (1998) GABA as a trophic factor for developing monoamine neurons. Perspect Dev Neurobiol 5:247-259.

Le Moal MM, Simon H (1991) Mesocorticolimbic dopaminergic network: functional and regulatory roles. Physiol Rev 71:155-234.

Le Moine C, Gaspar P (1998) Subpopulations of cortical GABAergic interneurons differ by their expression of D1 and D2 dopamine receptor subtypes. Brain Res Mol Brain Res 58:231-236.

Lee KH, Williams LM, Breakspear M, Gordon E (2003) Synchronous gamma activity: a review and contribution to an integrative neuroscience model of schizophrenia. Brain Res Brain Res Rev 41:57-78.

Lehmann K, Lesting J, Polascheck D, Teuchert-Noodt G (2003) Serotonin fibre densities in subcortical areas: differential effects of isolated rearing and methamphetamine. Brain Res Dev Brain Res 147:143-152.

Lehmann K, Hundsdorfer B, Hartmann T, Teuchert-Noodt G (2004) The acetylcholine fiber density of the neocortex is altered by isolated rearing and early methamphetamine intoxication in rodents. Exp Neurol 189:131-140.

Lehmann K, Butz M, Teuchert-Noodt G (2005) Offer and demand: proliferation and survival of neurons in the dentate gyrus. Eur J Neurosci 21:3205-3216.

Lesting J, Neddens J, Busche A, Teuchert-Noodt G (2005a) Hemisphere-specific effects on serotonin but not dopamine innervation in the nucleus accumbens of gerbils caused by isolated rearing and a single early methamphetamine challenge. Brain Res 1035:168-176.

Lesting J, Neddens J, Teuchert-Noodt G (2005b) Ontogeny of the dopamine innervation in the nucleus accumbens of gerbils. Brain Res 1066:16-23.

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.

Lewis DA, Hashimoto T, Volk DW (2005) Cortical inhibitory neurons and schizophrenia. Nat Rev Neurosci 6:312-324.

Liu F, Wan Q, Pristupa ZB, Yu XM, Wang YT, Niznik HB (2000) Direct protein-protein coupling enables cross-talk between dopamine D5 and gamma-aminobutyric acid A receptors. Nature 403:274-280.

Magnusson AK, Ulfendahl M, Tham R (2002) Early compensation of vestibulo-oculomotor symptoms after unilateral vestibular loss in rats is related to GABA(B) receptor function. Neuroscience 111:625-634.

Manoach DS, Press DZ, Thangaraj V, Searl MM, Goff DC, Halpern E, Saper CB, Warach S (1999) Schizophrenic subjects activate dorsolateral prefrontal cortex during a working memory task, as measured by fMRI. Biol Psychiatry 45:1128-1137.

Marty S, Wehrle R, Sotelo C (2000) Neuronal activity and brain-derived neurotrophic factor regulate the density of inhibitory synapses in organotypic slice cultures of postnatal hippocampus. J Neurosci 20:8087-8095.

Meaney MJ (2001) Maternal care, gene expression, and the transmission of individual differences in stress reactivity across generations. Annu Rev Neurosci 24:1161-1192.

Meredith CW, Jaffe C, ng-Lee K, Saxon AJ (2005) Implications of chronic methamphetamine use: a literature review. Harv Rev Psychiatry 13:141-154.

Miner LA, Backstrom JR, Sanders-Bush E, Sesack SR (2003) Ultrastructural localization of serotonin2A receptors in the middle layers of the rat prelimbic prefrontal cortex. Neuroscience 116:107-117.

Miner LH, Schroeter S, Blakely RD, Sesack SR (2000) Ultrastructural localization of the serotonin transporter in superficial and deep layers of the rat prelimbic prefrontal cortex and its spatial relationship to dopamine terminals. J Comp Neurol 427:220-234.

Neddens J, Brandenburg K, Teuchert-Noodt G, Dawirs RR (2001) Differential environment alters ontogeny of dopamine innervation of the orbital prefrontal cortex in gerbils. J Neurosci Res 63:209-213.

Neddens J, Bagorda F, Busche A, Horstmann S, Moll GH, Dawirs RR, Teuchert-Noodt G (2003) Epigenetic factors differentially influence postnatal maturation of serotonin (5-HT) innervation in cerebral cortex of gerbils: interaction of rearing conditions and early methamphetamine challenge. Brain Res Dev Brain Res 146:119-130.

Neddens J, Dawirs RR, Bagorda F, Busche A, Horstmann S, Teuchert-Noodt G (2004) Postnatal maturation of cortical serotonin lateral asymmetry in gerbils is vulnerable to both environmental and pharmacological epigenetic challenges. Brain Res 1021:200-208.

Nguyen L, Rigo JM, Rocher V, Belachew S, Malgrange B, Rogister B, Leprince P, Moonen G (2001) Neurotransmitters as early signals for central nervous system development. Cell Tissue Res 305:187-202.

Nieoullon A (2002) Dopamine and the regulation of cognition and attention. Prog Neurobiol 67:53-83.

Nilsson M, Perfilieva E, Johansson U, Orwar O, Eriksson PS (1999) Enriched environment increases neurogenesis in the adult rat dentate gyrus and improves spatial memory. J Neurobiol 39:569-578.

Nishimura T, Schwarzer C, Gasser E, Kato N, Vezzani A, Sperk G (2005) Altered expression of GABA(A) and GABA(B) receptor subunit mRNAs in the hippocampus after kindling and electrically induced status epilepticus. Neuroscience 134:691-704.

Nossoll M, Teuchert-Noodt G, Dawirs RR (1997) A single dose of methamphetamine in neonatal gerbils affects adult prefrontal gamma-aminobutyric acid innervation. Eur J Pharmacol 340:R3-R5.

Paylor R, Morrison SK, Rudy JW, Waltrip LT, Wehner JM (1992) Brief exposure to an enriched environment improves performance on the Morris water task and increases hippocampal cytosolic protein kinase C activity in young rats. Behav Brain Res 52:49-59.

Pehek EA, Nocjar C, Roth BL, Byrd TA, Mabrouk OS (2006) Evidence for the preferential involvement of 5-HT2A serotonin receptors in stress- and drug-induced dopamine release in the rat medial prefrontal cortex. Neuropsychopharmacology 31:265-277.

Pierri JN, Chaudry AS, Woo TU, Lewis DA (1999) Alterations in chandelier neuron axon terminals in the prefrontal cortex of schizophrenic subjects. Am J Psychiatry 156:1709-1719.

Racagni G, Bruno F, Cattabeni F, Maggi A, Di Giulio AM, Parenti M, Groppetti A (1977) Functional interaction between rat substantia nigra and striatum: GABA and dopamine interrelation. Brain Res 134:353-358.

Rao SG, Williams GV, Goldman-Rakic PS (1999) Isodirectional tuning of adjacent interneurons and pyramidal cells during working memory: evidence for microcolumnar organization in PFC. J Neurophysiol 81:1903-1916.

Rao SG, Williams GV, Goldman-Rakic PS (2000) Destruction and creation of spatial tuning by disinhibition: GABA(A) blockade of prefrontal cortical neurons engaged by working memory. J Neurosci 20:485-494.

Retaux S, Besson MJ, Penit-Soria J (1991) Opposing effects of dopamine D2 receptor stimulation on the spontaneous and the electrically evoked release of [3H]GABA on rat prefrontal cortex slices. Neuroscience 42:61-71.

Ricaurte GA, Guillery RW, Seiden LS, Schuster CR, Moore RY (1982) Dopamine nerve terminal degeneration produced by high doses of methylamphetamine in the rat brain. Brain Res 235:93-103.

Rosenzweig MR, Bennett EL (1969) Effects of differential environments on brain weights and enzyme activities in gerbils, rats, and mice. Dev Psychobiol 2:87-95.

Roth GS, Joseph JA (1994) Cellular and molecular mechanisms of impaired dopaminergic function during aging. Ann N Y Acad Sci 719:129-135.

Sapolsky RM, Meaney MJ (1986) Maturation of the adrenocortical stress response: neuroendocrine control mechanisms and the stress hyporesponsive period. Brain Res 396:64-76.

Segovia G, Porras A, Del AA, Mora F (2001) Glutamatergic neurotransmission in aging: a critical perspective. Mech Ageing Dev 122:1-29.

Sesack SR, Snyder CL, Lewis DA (1995) Axon terminals immunolabeled for dopamine or tyrosine hydroxylase synapse on GABA-immunoreactive dendrites in rat and monkey cortex. J Comp Neurol 363:264-280.

Seto-Ohshima A, Aoki E, Semba R, Emson PC, Heizmann CW (1990) Appearance of parvalbumin-specific immunoreactivity in the cerebral cortex and hippocampus of the developing rat and gerbil brain. Histochemistry 94:579-589.

Shatz CJ, Stryker MP (1978) Ocular dominance in layer IV of the cat's visual cortex and the effects of monocular deprivation. J Physiol 281:267-283.

Solbach S, Celio MR (1991) Ontogeny of the calcium binding protein parvalbumin in the rat nervous system. Anat Embryol (Berl) 184:103-124.

Somogyi P, Freund TF, Cowey A (1982) The axo-axonic interneuron in the cerebral cortex of the rat, cat and monkey. Neuroscience 7:2577-2607.

Spencer KM, Nestor PG, Niznikiewicz MA, Salisbury DF, Shenton ME, McCarley RW (2003) Abnormal neural synchrony in schizophrenia. J Neurosci 23:7407-7411.

Spencer KM, Nestor PG, Perlmutter R, Niznikiewicz MA, Klump MC, Frumin M, Shenton ME, McCarley RW (2004) Neural synchrony indexes disordered perception and cognition in schizophrenia. Proc Natl Acad Sci U S A 101:17288-17293.

Sperry RW (1963) Chemoaffinity in the orderly growth of the nerve fiber patterns and connections. Proc Natl Acad Sci U S A 50:703-710.

Swanson LW (1982) The projections of the ventral tegmental area and adjacent regions: a combined fluorescent retrograde tracer and immunofluorescence study in the rat. Brain Res Bull 9:321-353.

Tamas G, Buhl EH, Somogyi P (1997) Fast IPSPs elicited via multiple synaptic release sites by different types of GABAergic neurone in the cat visual cortex. J Physiol 500 (Pt 3):715-738.

Teuchert-Noodt G (2000) Neuronal degeneration and reorganization: a mutual principle in pathological and in healthy interactions of limbic and prefrontal circuits. J Neural Transm Suppl315-333.

Teuchert-Noodt G, Dawirs RR (1991) Age-related toxicity in prefrontal cortex and caudate-putamen complex of gerbils (Meriones unguiculatus) after a single dose of methamphetamine. Neuropharmacology 30:733-743.

Thiessen D, Yahr P (1977) The gerbil in behavioral investigations. Mechanisms of territoriality and olfactory communication. Austin: University of Texas Press.

Traub RD, Whittington MA, Stanford IM, Jefferys JG (1996) A mechanism for generation of long-range synchronous fast oscillations in the cortex. Nature 383:621-624.

Troup GM, Smith GS, Walford RL (1969) Life span,chronologic disease patterns and age-related changes in relative spleen weights for the mongolian gerbil (Meriones unguiculatus). Exp Gerontol 4:139-143.

Ueda S, Sakakibara S, Watanabe E, Yoshimoto K, Koibuchi N (2002) Vulnerability of monoaminergic neurons in the brainstem of the zitter rat in oxidative stress. Prog Brain Res 136:293-302.

Uhlhaas PJ, Singer W (2006) Neural synchrony in brain disorders: relevance for cognitive dysfunctions and pathophysiology. Neuron 52:155-168.

Valenstein ES (2002) The discovery of chemical neurotransmitters. Brain Cogn 49:73-95.

Verney C, Alvarez C, Geffard M, Berger B (1990) Ultrastructural Double-Labelling Study of Dopamine Terminals and GABA-Containing Neurons in Rat Anteromedial Cerebral Cortex. Eur J Neurosci 2:960-972.

Vicente-Torres MA, Munoz E, Davila D, Gil-Loyzaga P (2001) Changes in the cochlear dopaminergic system of the aged rat. Brain Res 917:112-117.

Volz H, Gaser C, Hager F, Rzanny R, Ponisch J, Mentzel H, Kaiser WA, Sauer H (1999) Decreased frontal activation in schizophrenics during stimulation with the continuous performance test--a functional magnetic resonance imaging study. Eur Psychiatry 14:17-24.

Weinberger DR (1987) Implications of normal brain development for the pathogenesis of schizophrenia. Arch Gen Psychiatry 44:660-669.

Weinberger DR, Lipska BK (1995) Cortical maldevelopment, anti-psychotic drugs, and schizophrenia: a search for common ground. Schizophr Res 16:87-110.

Wiesel TN, Hubel DH (1965) Comparison of the effects of unilateral and bilateral eye closure on cortical unit responses in kittens. J Neurophysiol 28:1029-1040.

Winterfeld KT, Teuchert-Noodt G, Dawirs RR (1998) Social environment alters both ontogeny of dopamine innervation of the medial prefrontal cortex and maturation of working memory in gerbils (Meriones unguiculatus). J Neurosci Res 52:201-209.

Woo TU, Whitehead RE, Melchitzky DS, Lewis DA (1998) A subclass of prefrontal gamma-aminobutyric acid axon terminals are selectively altered in schizophrenia. Proc Natl Acad Sci U S A 95:5341-5346.

Wu CK, Nagykery N, Hersh LB, Scinto LF, Geula C (2003) Selective age-related loss of calbindin-D28k from basal forebrain cholinergic neurons in the common marmoset (Callithrix jacchus). Neuroscience 120:249-25

9. Publications

Brummelte S., Grund T., Czok A., Teuchert-Noodt G. and Neddens J. (2006a): Long-term effects of a single adult methamphetamine challenge: Minor impact on dopamine fibre density in limbic brain areas of gerbils. Behav Brain Funct. 2: 12 ('highly accessed')

Brummelte S. and Teuchert-Noodt G. (2006): Postnatal development of dopamine innervation in the amygdala and the entorhinal cortex of the gerbil (*Meriones unguiculatus*). Brain Res.1125: 9-16

Brummelte S., Witte A.V. and Teuchert-Noodt G.: Postnatal development of GABA and Calbindin cells and fibers in the prefrontal cortex and basolateral amygdala of gerbils (*Meriones unguiculatus*). (accepted)

Brummelte S. and Teuchert-Noodt G.: Density of dopaminergic fibres in the prefrontal cortex of gerbils (*Meriones unguiculatus*) is sensitive to aging. (Short Communication, submitted)

Brummelte S., Neddens J., and Teuchert-Noodt G. (2007): Alteration in the GABAergic network of the prefrontal cortex in an animal model of psychosis. J Neural Trans (Epub ahead of print)

Behavioral and Brain Functions

BioMed Central

Research

Open Access

Long-term effects of a single adult methamphetamine challenge: Minor impact on dopamine fibre density in limbic brain areas of gerbils

Susanne Brummelte[1], Thorsten Grund[1], Andrea Czok[1], Gertraud Teuchert-Noodt[1] and Jörg Neddens*[1,2]

Address: [1]Department of Neuroanatomy, Faculty of Biology, University of Bielefeld, Universitätsstr. 25, D-33615 Bielefeld, Germany and [2]National Institutes of Health, NICHD, Section on Molecular Neurobiology, Bldg. 35, Rm. 2C-1004, Bethesda, MD 20892-3714, USA

Email: Susanne Brummelte - susanne.brummelte@uni-bielefeld.de; Thorsten Grund - thor.grund@gmx.de; Andrea Czok - andrea.czok@uni-bielefeld.de; Gertraud Teuchert-Noodt - g.teuchert@uni-bielefeld.de; Jörg Neddens* - neddensj@mail.nih.gov

* Corresponding author

Published: 28 March 2006

*Behavioral and Brain Functions*2006, **2**:12 doi:10.1186/1744-9081-2-12

Received: 10 November 2005
Accepted: 28 March 2006

This article is available from: http://www.behavioralandbrainfunctions.com/content/2/1/12

Abstract

Background: The aim of the study was to test long-term effects of (+)-methamphetamine (MA) on the dopamine (DA) innervation in limbo-cortical regions of adult gerbils, in order to understand better the repair and neuroplasticity in disturbed limbic networks.

Methods: Male gerbils received a single high dose of either MA (25 mg/kg i.p.) or saline on postnatal day 180. On postnatal day 340 the density of immunoreactive DA fibres and calbindin and parvalbumin cells was quantified in the right hemisphere.

Results: No effects were found in the prefrontal cortex, olfactory tubercle and amygdala, whereas the pharmacological impact induced a slight but significant DA hyperinnervation in the nucleus accumbens. The cell densities of calbindin (CB) and parvalbumin (PV) positive neurons were additionally tested in the nucleus accumbens, but no significant effects were found. The present results contrast with the previously published long-term effects of early postnatal MA treatment that lead to a restraint of the maturation of DA fibres in the nucleus accumbens and prefrontal cortex and a concomitant overshoot innervation in the amygdala.

Conclusion: We conclude that the morphogenetic properties of MA change during maturation and aging of gerbils, which may be due to physiological alterations of maturing vs. mature DA neurons innervating subcortical and cortical limbic areas. Our findings, together with results from other long-term studies, suggest that immature limbic structures are more vulnerable to persistent effects of a single MA intoxication; this might be relevant for the assessment of drug experience in adults vs. adolescents, and drug prevention programs.

Background

Methamphetamine (MA) is a common illicit drug, which abuse is currently reaching epidemic proportions. According to the 2002 SAMHSA National Household Survey on Drug Abuse, 12.4 million Americans age 12 and older had tried methamphetamine at least once in their lifetimes

(5.3 percent of the population). This increasing number is especially alarming since it has been extensively shown that MA exerts acute neurotoxic effects on the monoaminergic transmitter systems, and thus leads to characteristic cognitive impairments like deficits in memory and learning, psychomotor speed and information processing [1]. It is especially affecting the dopamine (DA) neurons, leading to dramatic loss of fibres and other DAergic structures in certain brain areas within a few days [2,3], even after a single exposure [4].

Some evidence exists that monoaminergic fibres are able to recover to some extend from this damage during long time course [5-9]. Moreover, even reactive overshoot was found for serotonergic fibres in several limbic areas of the brain, including left entorhinal cortex [10] and the septal pole of the hippocampal dentate gyrus [11]. For DA fibres, an early MA treatment produces hyperinnervation in amygdaloid nuclei and ventral entorhinal cortex [12] and a restraint of the maturation in prefrontal cortex [13,14]. This lab has further shown that the single early MA intoxication produces a loss of DA fibres and concomitant hyperinnervation of serotonin fibres in the nucleus accumbens (NAC) [15].

Taken together, our recent studies indicate severe changes in the maturation of the limbo-cortical network following an early single MA challenge. However, a primary study has already shown that the neuroplasticity that follows MA treatment might relate on the age of the animals [4]. Since the functional maturation and aging of the brain is based on various structural and physiological changes, the present study was carried out to question whether the remodelling of neural networks that is induced by the neurotoxic effects of MA may alter during the lifespan of gerbils. For that purpose, 6 months old adult gerbils received a single high dose of MA. At the age of 12 months the DA innervation was examined in prefrontal cortex, olfactory tubercle, NAC, and amygdala to check for long-time effects on the fibre density.

Methods

All experimental procedures were approved by the appropriate committee for animal care in accordance with the guidelines of the European Communities Council Directive. Breeding gerbils *(Meriones unguiculatus)* were obtained from Harlan Winkelmann (Borchen, Germany). From offspring, a total of 18 males (weight 66–91 g; age 331–348 days) were used in this study. Young animals were weaned at postnatal day 30 and subsequently separated in standard cages (Macrolon® type 4) without any content except of sawdust. All animals had free access to food and water and were kept on natural day/night cycles. On postnatal day 180, a total of 9 gerbils received a single systemic injection of (+)-methamphetamine hydrochloride (Sigma, M 8750; 25 mg/kg, i.p.). The other 9 animals were sham-treated by an i.p. injection of saline. This dose was chosen due to our former experiences, which have shown that juvenile gerbils can tolerate higher doses (50 mg/kg) than older ones. Notably, the rate of mortality is similar at both ages receiving the different doses (unpublished data), indicating physiological changes during the postnatal maturation of the brain.

The methods used for sectioning and DA immunohistochemistry have been published recently [15]. For the immunohistochemistry of calbindin and parvalbumin cells, 50 μm thick vibratome sections were taken from the same animals (perfused with 100 ml 0.1 M sodium cacodylate pH 6.2, followed by 750 ml 5% glutaraldehyde in 0.1 M sodium cacodylate pH 7.6) and treated as follows: Every third section was collected in 0.05 M Tris-HCL buffered saline [TBS (pH 7.5)] at 4°C; rinsed 3 × 10 min in TBS; incubated 10 min with 1% H2O2 in TBS; rinsed again 3 × 10 min in TBS; blocked in 10% normal goat serum and 0.4% Triton X-100 (Sigma) for 30 min; incubated with the primary antibody (1:3,000 mouse anti-calbindin, Sigma; 1:2,000 mouse anti-parvalbumin, Sigma) in 1% normal goat serum and 0.4% Triton X-100 for 18 h; rinsed 3 × 10 min in TBS; incubated for 30 min in biotinylated goat-anti-mouse antibody (Sigma) diluted 1:20 with 1% normal goat serum; rinsed 3 × 10 min in TBS; incubated with ExtraAvidin-Peroxidase (Sigma) diluted 1:20 for 30 min; rinsed 3 × 10 min in TBS; stained in 0.05% 3.3-diaminobenzidine (Sigma) with 0.01% H2O2 for 4 min. Finally, the sections were rinsed 5 × 10 min in TBS, mounted on glass slides, dried overnight, dehydrated with ethanol, cleared with xylene and cover slipped with DePeX (Serva, Heidelberg, Germany). To avoid deviations due to probably lateralised innervation densities of DA or calcium-binding proteins only right hemispheres were used for quantification.

For quantification of fibre and cell densities, brain sections were chosen in areas of interest by means of anatomical characteristics according to brain atlases of the rat [16] and the mouse [17]. The identification of the brain region follows the nomenclature of the atlas of the rat. The average number of analysed sections was 18 per animal for DA, with a range of 4 up to 6 sections in single regions. In the defined region of each section (cf. Fig. 1) all detectable fibre fragments and cells were visualised in standard test fields (2,080 × 1,544 pixel; 0.22 mm^2) using a bright field microscope (BX61, Olympus, Hamburg, Germany) and a digital camera for microscopy (ColorView II, SIS, Münster, Germany) at 200-fold magnification. Cells and fibres were quantified by software for image analysis (KS300, Jenoptik, Jena, Germany). Immunoreactive DA fibres of different diameter were standardised to identical thickness and visualised using a combination of Gauss filter

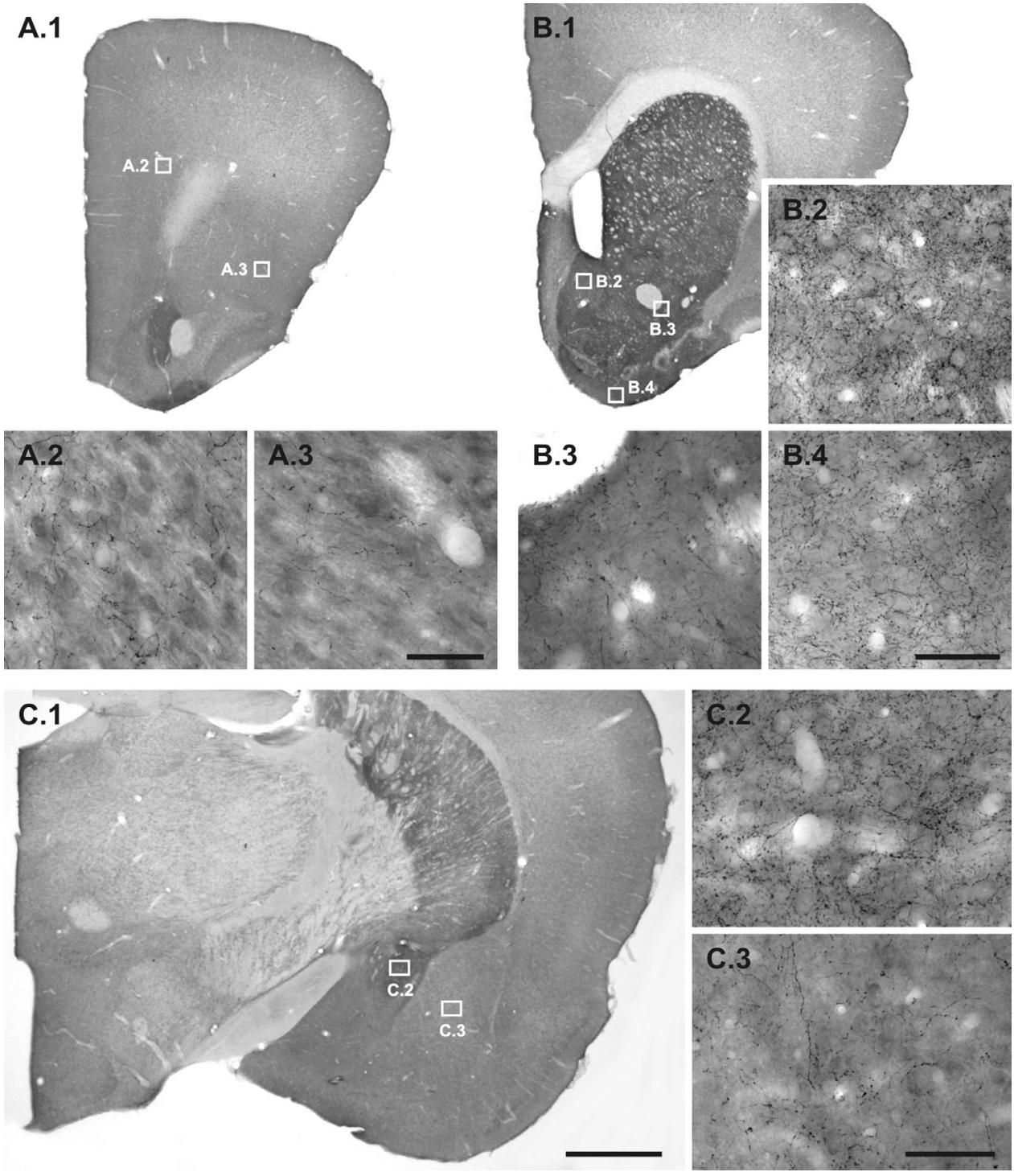


Figure 1

Dopamine immunoreactive fibres in each of the quantified regions. Representative photomicrographs, taken from a saline control, of dopamine (DA) immunoreactive fibres of each of the quantified regions. A.1: Prefrontal cortex; A.2: Layer VI of the prelimbic area; A.3: Layer IV of the lateral orbital and agranular insular areas. B.1: Nucleus accumbens (NAC); B.2: Medial shell of NAC; B.3: Lateral core of NAC; B.4: Olfactory tubercle. C.1: Amygdala (AMY); C.2: Central nucleus of AMY; C.3: Basolateral nucleus of AMY. Note the differential innervation pattern and density of DA fibres in the respective regions. Scale bars: 1000 μm (A.1, B.1, C.1); 50 μm (A.2-3, B.2-4, C.2-3).

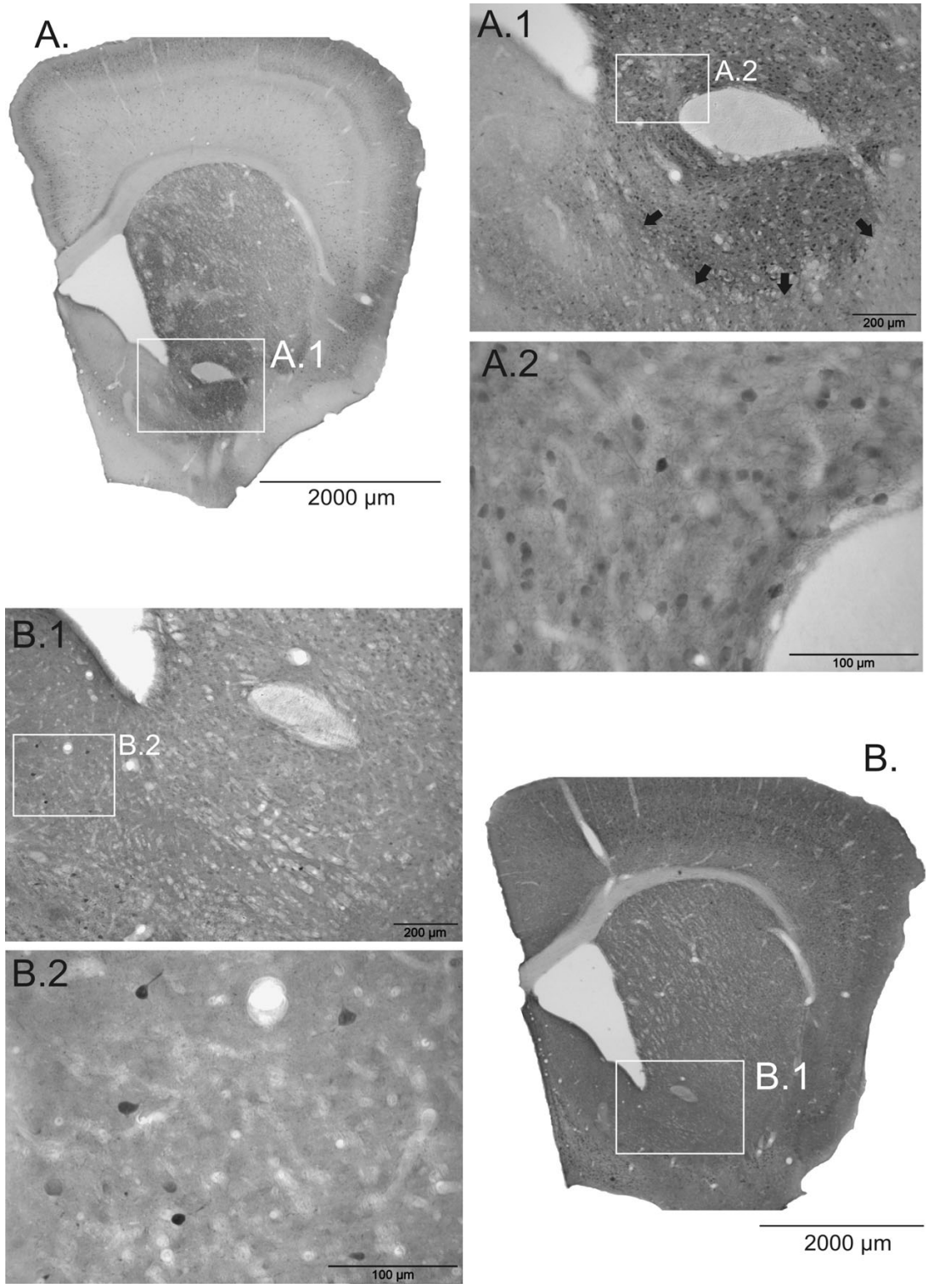


Figure 2

Photomicrographs of Calbindin and Parvalbumin immunoreactive neurons in the nucleus accumbens. Overview (A) and higher magnifications (A1, A2) of the Calbindin innervation of the NAC. The majority of CB+ cells is located in the core, which border to the shell is detectable (black arrows). PV+ cells are almost exclusively located in the shell (B1, B2), however, the overall density is much lower compared to CB+ cells. Scale bars: 2000 μm (A, B); 200 μm (A1, B1); 100 μm (A2, B2).

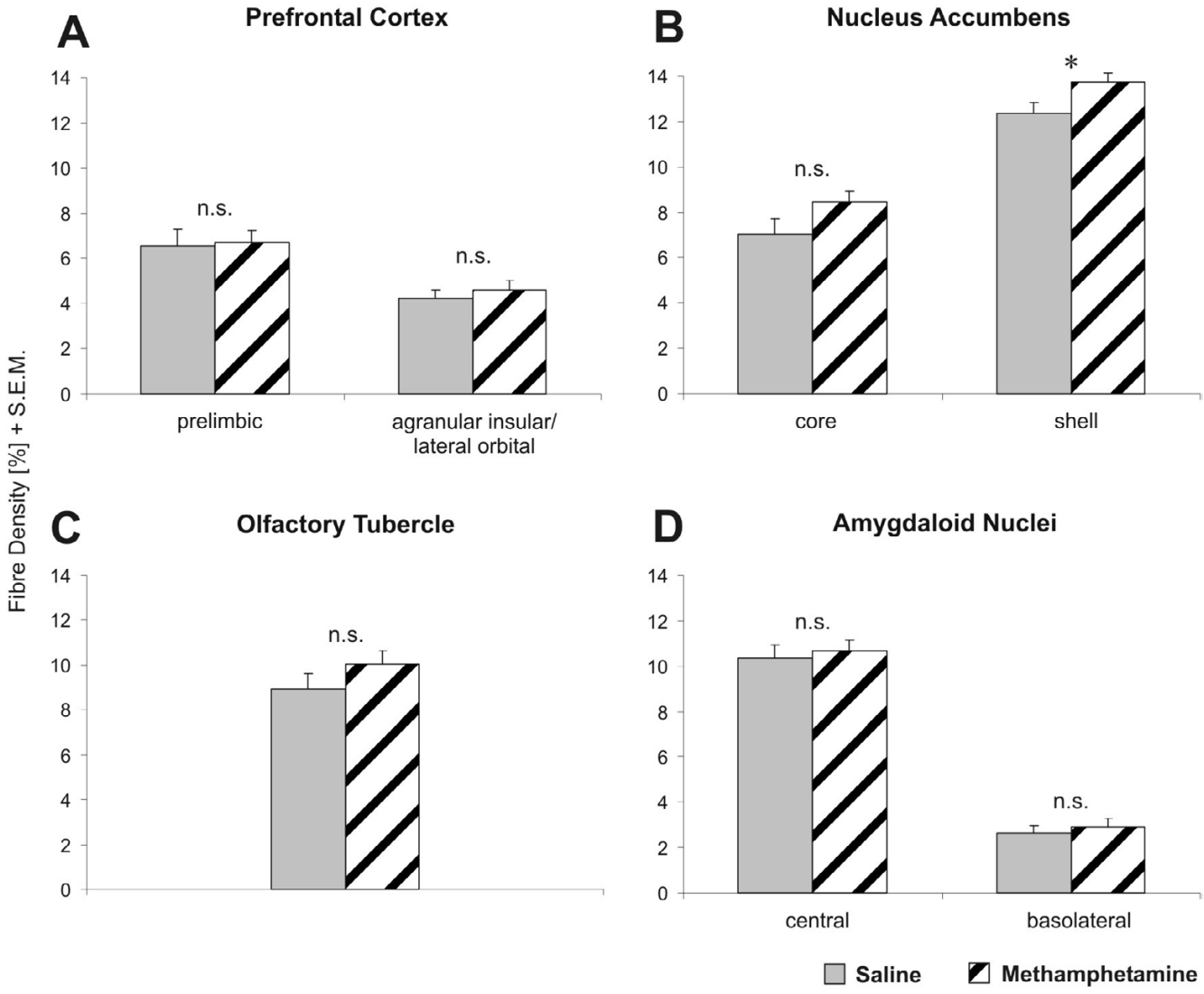


Figure 3

Dopamine innervation density in four regions of the gerbil brain. Dopamine (DA) innervation density ± S.E.M. is presented in four regions of the gerbil brain, namely agranular insular and lateral orbital as well as prelimbic areas of the prefrontal cortex, the olfactory tubercle, core and shell areas of the nucleus accumbens (NAC), and the central and basolateral nuclei of the amygdala complex. Methamphetamine treatment generally tends to increase the DA innervation. However, a significant region-specific change in response to a single adult methamphetamine treatment exclusively occurs in the shell of the NAC (+11%; $p = 0.0332$). The difference in the core appears somewhat more pronounced but is not significant due to higher variance (+21%; $p = 0.1011$). Student's t-Test, significance value: * $p < 0.05$. Following methamphetamine treatment, ANOVA detected a significant overall increase of DA innervation in core and shell of the NAC ($F(1,16) = 4.7316$; $p = 0.0472$).

and Gerig operator that depicts differences of grey values of adjacent pixels and transforms the result into binary images. The DA fibre density was computed as a percentage of the evaluated test area. Calbindin and parvalbumin positive cells were detected by use of a threshold to the grey value, followed by automatic sorting of adequate shape and minimal size (250 pixels) of the structures. Remaining structures were classified as cells, the size of the structures (cell area) being measured cumulatively and the according cell density calculated by proportion of cell number per test field area. Calbindin-positive cells are located almost exclusively in the core region of the NAC and were measured only in this part of the NAC, whereas medium-sized PV-positive cells are specific to the shell and were counted only in this area. All analyses were done by a person blind to the pharmacological treatment of individual animals.

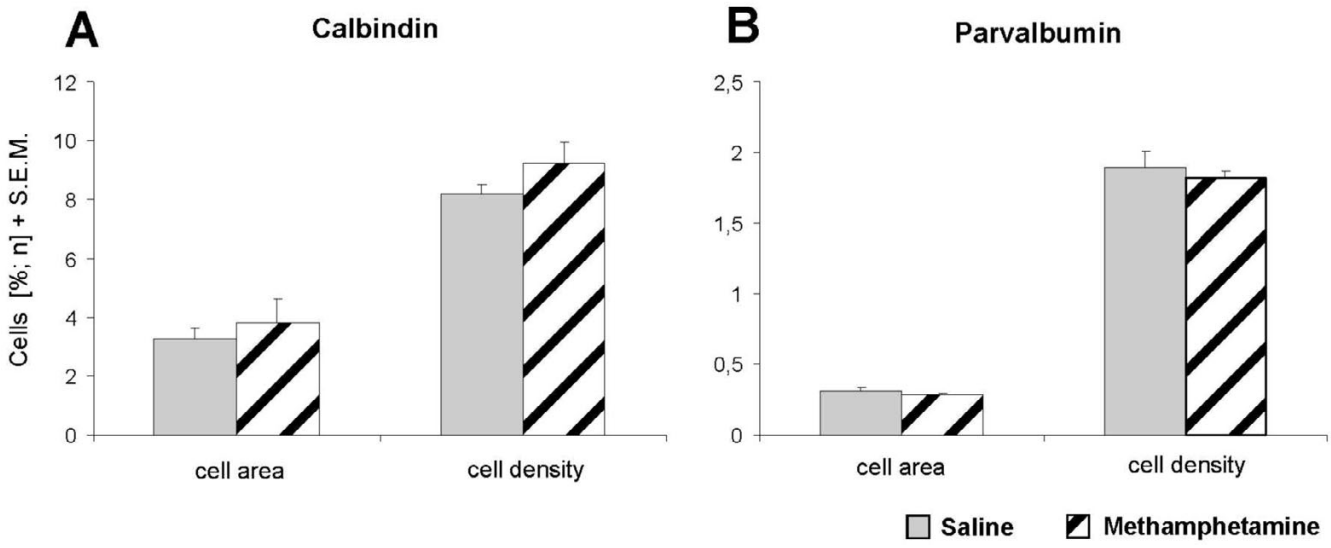


Figure 4

Calbindin and parvalbumin cell densities and cell areas in the nucleus accumbens. Calbindin (CB) and parvalbumin (PV) cell densities and cell areas ± S.E.M. are presented for the nucleus accumbens (NAC). PV-positive cells and CB-positive cells are predominantly located in the shell and in the core of the NAC, respectively, where they were quantified. No statistically significant effect of a single adult methamphetamine challenge could be detected for either number (cell density) or cumulative size (cell area) of both PV and CB cells. Generally, the number of CB cells is considerably higher and their average size is doubled compared to PV cells.

The measurements were computed as arithmetic means by-case and by-group ± S.E.M. of the respective regions (Fig. 3). Statistical analysis revealed regional effects of MA treatment by the use of Student's t-test. General alterations in the NAC were additionally investigated by use of 2-way analysis of variance (ANOVA), which checked for area-specific and group-specific effects [18]. Data analysis was computed with Statistica 6 (StatSoft, Tulsa, USA). The levels of significance were set at * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$.

Results

The innervation pattern of DA immunoreactive fibres in gerbils is generally in line with the results of rats. The innervation pattern and density of DA immunoreactive fibres in the gerbil forebrain are region-specific (Figs. 1 and 3). Representative photographs of the differential DA innervation densities and patterns of the four regions that were subsequently studied in more detail are provided in Fig. 1, taken from a male gerbil of the saline group.

Quantitative DA data were obtained from a total of 327 sections that derived from 18 gerbils of two experimental groups (Saline n = 9, MA n = 9). The adult single systemic MA challenge induces no general alteration of DA innervation pattern in the investigated regions of the gerbil brain (Fig. 3). The overall DA fibre density in the NAC is selectively increased by MA [(ANOVA, $F(1,16) = 4.7316$, $p = 0.0472$) please note that ANOVA included comparison of 8 vs. 8 animals only, because some NAC sections were damaged in one animal of each experimental group]. However, the significant increase (+11%) is limited to the shell (Student's t-test, $p = 0.0332$), whereas alteration in the core misses statistical significance (Student's t-test, $p = 0.1011$). No change in DA fibre density was found in the prefrontal cortex, olfactory tubercle and amygdala.

To determine other potential alterations within the NAC, CB- and PV- positive structures were additionally investigated in this area. The distribution of CB- and PV-positive subpopulations in the gerbil generally resembles the distribution in the NAC of rats and primates (Fig. 2) [19-21]. A single adult MA intoxication caused no significant alteration in the cell density of either CB- positive neurons or PV-positive neurons in the NAC. Neither was there a difference in the cell areas (Fig. 4).

Discussion

According to our results, a single adult MA challenge induces minor long-term changes of the DA innervation

Table 1: Comparison of age-related long-term effects of a single methamphetamine intoxication on the dopamine innervation in limbocortical areas of the gerbil brain.

		Adult (current study) MA 1 × 25 mg/kg i.p., p180	Juvenile [Reference] MA 1 × 50 mg/kg i.p., p14
Prefrontal cortex	Medial	↔ n.s.	↓ – 38% ** [14]
	Orbital	↔ n.s.	↓ – 50% ** [14]
Nucleus accumbens	Core	↔ n.s.	↓ – 28% * [15]
	Shell	↑ +11% *	↔ n.s. [15]
Amygdala	Basolateral	↔ n.s.	↑ +18% ** [12]
	Central	↔ n.s.	↔ n.s. [12]
Olfactory Tubercle		↔ n.s	No data

Significance values: * $p < 0.05$, ** $p < 0.01$.

in the NAC, whereas other regions of the limbocortical circuitry are apparently unaffected. These results contrast with previously published data on the long-term effects of early postnatal MA treatment that demonstrated a restraint maturation of DAergic fibres in the NAC and the prefrontal cortex [14,22] and a concomitant overshoot innervation in the amygdala [12]. Table 1 provides a comparison between MA effects on DA innervation in juvenile and adult gerbils.

Postnatal development and vulnerability to methamphetamine

The age-related and region-specific alterations that are triggered by a single MA-treatment of gerbils might reveal the complexity of MA neurotoxicity. It has to be pointed out that the different doses that were administered to juvenile (50 mg/kg) [10-15,22] vs. adult (25 mg/kg, current study) animals may be even more adequate for comparing age-related effects than using the same dose in both ages, because a lethal dose of MA is approximately also twice as high in juvenile gerbils compared to adults (unpublished data), indicating physiological changes during postnatal maturation of the brain. The reasons for the age-dependent differences in vulnerability to MA in gerbils are currently not clear. However, it appears reasonable to assume that this is related to physiological alterations in maturing vs. mature monoaminergic neurons. Generally, the high amount of MA that is required to induce such effects might be specific to gerbils, probably due to species-specific metabolic enzymes.

Although the exact molecular mechanism of MA neurotoxicity is still not completely understood, it is clear that developmental alteration must play an important role in mediating the MA-induced effects [23]. This is demonstrated by the finding that the application of MA results in higher mortality and stronger reactions of adult gerbils compared to juveniles or adolescents [4,24-29], which may be understandable by reports that, in rats, higher MA concentrations occur in the brains of 90 days old versus 40 days old animals after receiving the same dose [25,29]. However, Kokoshka and colleagues published some intriguing results which on the one hand confirmed previous studies concerning the lack of medium-term (7 days) deficits in the DA systems after MA treatment in adolescent rats, but on the other hand showed that there were acute short-term (1 hour) consequences in adolescent (40 days) and adult (90 days) rats [25]. Further, MA-induced behavioural sensitization, which is a prominent feature of MA administration [30,31], seems to be age-dependent [32]. It does not occur within a crucial postnatal period, which in turn seems to correspond to the time of presynaptic DA autoreceptor formation in the brain [33].

Several parameters of the DA system underlie developmental changes, e.g. DAT expression [34,35], expression of DA receptors and DA concentration [36], and activity of the vesicular monoamine transporter-2 (VMAT-2) [29]. The mechanism underlying the modifications seen in adult animals after MA challenge is therefore thought to vary from the one mediating the neurotoxic effect in juvenile animals. The ability of a single early MA challenge to selectively induce a restraint of the maturation of DA fibres in the prefrontal cortex and the NAC [14,22,37] as well as a concomitant excessive maturation in several amygdaloid nuclei and the entorhinal cortex [12] might be due to a special vulnerability of immature fibre systems [38]. As DA transmission in the NAC seems to play a critical role in an input selection mechanism that regulates the influence of certain inputs over neural activity [39], the reactive changes that occur within local circuits following the MA challenge might cause a new and different innervation pattern of these fibres and thus a neuroanatomical restructuring [40,41]. The severe impairment of the brain architecture induced by a single early MA treatment clearly demonstrates that despite the apparent higher resistance of younger animals, MA is indeed a potent drug capable of inducing extensive structural alterations in the juvenile brain that persist into adulthood.

Effects of methamphetamine on different neurotransmitters and brain regions

It was reported that the mechanism of MA neurotoxicity includes the formation of reactive oxygen [42-44] and nitrogen [42,45,46] species, which damage monoaminergic neurons. However, several other factors may also contribute in mediating the neurotoxic effect of MA, leading to region-specific and neuron-specific differences in vulnerability. Fumagalli and colleagues have shown that rats lacking the dopamine transporter (DAT) are protected against the MA-related neurotoxicity in the striatum [47]. Interestingly, impairment in the function of VMAT-2, which accumulates cytoplasmic DA into synaptic vesicles as seen in mice heterozygous for this transporter, increases the MA neurotoxicity [48]. It has also been demonstrated that the blockage of either DA D1 or D2 receptors prevents the damage of repeated doses of MA to striate DA terminals [49] and that there are regional differences in sensitivity of these terminals to the MA [4,50]. It seems likely that DAT and DA receptors may be factors limiting the severity of neurotoxic effects of MA, presumably by influencing the concentration and distribution of DA.

MA-induced alterations have also been found in other neuronal elements like 5-HT fibres [10,15,51,52], GABAergic neurons [53], and the morphology of cortical pyramidal cells [54]. Our animal model has also revealed that glutamatergic projection fibres from the mediodorsal prefrontal cortex to several other cortical areas are significantly reduced after an early single MA intoxication [55]. Interestingly, in the present study the shell of the NAC is the only area that reacts to a single adult MA challenge, and it is also almost the only area we have studied lacking any effect of the DA fibre density in response to a single postnatal drug treatment [12,14,15] (cf. Table 1). In contrast, the core region of the NAC exhibits a strong decline in DA fibre density after an early single MA administration [15]. This is in line with results from Broening and colleagues, who, after repeated MA treatment of rats, found an almost completely loss of tyrosine hydroxylase immunoreactivity in the core while the shell was almost spared [56]. In addition, most drugs increase extracellular DA levels preferentially in the shell region of the NAC [57], which coincides with differences in DA baseline levels [57,58], and different time-course of the maturation of the DA innervation in the core and shell areas [59]. We may conclude that the DA fibre systems of the brain are far from being homogeneous; thus, statements on the general effects of MA intoxication on DA fibres are misleading.

Regeneration and reorganisation of neural networks: implications for psychiatric diseases

It has been shown before that DA fibres can be rebuilt within 24 weeks after a lesion of the NAC with 6-OHDA [60]. Furthermore, Finkelstein and colleagues could show that a lesion to the substantia nigra causes sprouting of DA fibres in the striatum of rats [61]. Thus, the increased fibre density we have found in the NAC might probably be caused by a specific regeneration rather than a reorganisation of fibres.

Several studies in humans and rodents have shown that the effects of MA are to a large extend reversible, although this process might last many years and may strongly depend on the severity and duration of the drug abuse [1]. We have shown that a single administration of MA on postnatal day 90 leads to a transient increase of the dendritic spine density of prefrontal pyramidal neurons, which regain an almost normal level within 30 days post-treatment [62]. In the present study, we apparently provided sufficient time for the impaired system to recover from MA intoxication and to eventually regain normal DA fibre densities. Our model using only a single administration of MA may therefore be more useful to mimic the effects on first time users rather than on chronically abusers of the drug. In addition, it has been shown that intermittent treatment with MA can lead to the development of tolerance to its neurotoxic effects [63-65]. Thus the paradigm of repeated administration of MA as used in the majority of studies might either conceal or modify the deleterious effects of the psychostimulant. In fact, some studies have shown opposed or stronger effects of single versus chronic administration of MA [66,67].

Chronic MA use is known to cause psychotic symptoms that mimic that of schizophrenia [68-70]. Further, we could recently show that an early single intoxication leads to a 'dysconnection' of prefrontal efferents [55], thus providing an anatomical correlate of schizophrenia [71,72]. This is consistent with results from Chen and colleagues who revealed an association between earlier and larger use of MA with higher risk of psychosis in humans [68].

Conclusion

The results of this study show that even a single application of MA to adult gerbils may induce long-term physical alterations in limbic brain areas, although the effects are not as severe as seen after an early drug challenge. The increased DAergic fibre density in the NAC indicates reactive over-sprouting, which possibly is a response to altered network requirements after MA treatment. It remains to be investigated whether other brain areas would reveal short-term modifications, which might be concealed by recovery in this approach. While it cannot be excluded that the different effects in adult vs. juvenile gerbils are, at least in part, due to the different doses of MA, several other studies also indicate changes in MA effects that depend on the age of rats and mice. We thus may conclude that, in rodents, MA not only acts age-dependent, but also highly region-specific. We have sufficient evi-

dence to suggest that early contact with this psychotropic substance during childhood might increase the risk of persistent severe structural changes of the brain architecture and may result in long-term cognitive and psychiatric disturbances.

Competing interests

The author(s) declare that they have no competing interests.

Authors' contributions

SB participated in the interpretation of the data and drafted the manuscript.

TG contributed to the benchwork, analysis and interpretation of the data.

AC contributed to the acquisition and interpretation of data.

GT contributed to the design of the study and the critical reviewing of the manuscript.

JN handled the animals, contributed to the benchwork, analysis and interpretation of the data, participated in the design of the study, and the drafting and revision of the manuscript.

Acknowledgements

The authors would like to thank Ulrike Schroeder and Francesco Bagorda for excellent technical assistance. The study was supported by grants of the deutsche Parkinson Vereinigung (dPV).

References

1. Meredith CW, Jaffe C, Ang-Lee K, Saxon AJ: **Implications of chronic methamphetamine use: a literature review.** *Harv Rev Psychiatry* 2005, **13:**141-154.
2. Ricaurte GA, Guillery RW, Seiden LS, Schuster CR, Moore RY: **Dopamine nerve terminal degeneration produced by high doses of methylamphetamine in the rat brain.** *Brain Res* 1982, **235:**93-103.
3. Ricaurte GA, Schuster CR, Seiden LS: **Long-term effects of repeated methylamphetamine administration on dopamine and serotonin neurons in the rat brain: a regional study.** *Brain Res* 1980, **193:**153-163.
4. Teuchert-Noodt G, Dawirs RR: **Age-related toxicity in prefrontal cortex and caudate-putamen complex of gerbils (Meriones unguiculatus) after a single dose of methamphetamine.** *Neuropharmacology* 1991, **30:**733-743.
5. Cass WA, Manning MW: **Recovery of presynaptic dopaminergic functioning in rats treated with neurotoxic doses of methamphetamine.** *J Neurosci* 1999, **19:**7653-7660.
6. Cass WA: **Attenuation and recovery of evoked overflow of striatal serotonin in rats treated with neurotoxic doses of methamphetamine.** *J Neurochem* 2000, **74:**1079-1085.
7. Friedman SD, Castaneda E, Hodge GK: **Long-term monoamine depletion, differential recovery, and subtle behavioral impairment following methamphetamine-induced neurotoxicity.** *Pharmacol Biochem Behav* 1998, **61:**35-44.
8. Woolverton WL, Ricaurte GA, Forno LS, Seiden LS: **Long-term effects of chronic methamphetamine administration in rhesus monkeys.** *Brain Res* 1989, **486:**73-78.
9. Sabol KE, Richards JB, Yung K: **The effects of high-dose methamphetamine in the aging rat: differential reinforcement of low-rate 72-s schedule behavior and neurochemistry.** *J Pharmacol Exp Ther* 2000, **294:**850-863.
10. Neddens J, Dawirs RR, Bagorda F, Busche A, Horstmann S, Teuchert-Noodt G: **Postnatal maturation of cortical serotonin lateral asymmetry in gerbils is vulnerable to both environmental and pharmacological epigenetic challenges.** *Brain Res* 2004, **1021:**200-208.
11. Busche A, Neddens J, Dinter C, Dawirs RR, Teuchert-Noodt G: **Differential influence of rearing conditions and methamphetamine on serotonin fibre maturation in the dentate gyrus of gerbils (Meriones unguiculatus).** *Dev Neurosci* 2002, **24:**512-521.
12. Busche A, Polascheck D, Lesting J, Neddens J, Teuchert-Noodt G: **Developmentally induced imbalance of dopaminergic fibre densities in limbic brain regions of gerbils (Meriones unguiculatus).** *J Neural Transm* 2004, **111:**451-463.
13. Dawirs RR, Teuchert-Noodt G, Nossoll M: **Pharmacologically induced neural plasticity in the prefrontal cortex of adult gerbils (Meriones unguiculatus).** *Eur J Pharmacol* 1997, **327:**117-123.
14. Dawirs RR, Teuchert-Noodt G, Czaniera R: **The postnatal maturation of dopamine innervation in the prefrontal cortex of gerbils (Meriones unguiculatus) is sensitive to an early single dose of methamphetamine. A quantitative immunocytochemical study.** *J Hirnforsch* 1994, **35:**195-204.
15. Lesting J, Neddens J, Busche A, Teuchert-Noodt G: **Hemisphere-specific effects on serotonin but not dopamine innervation in the nucleus accumbens of gerbils caused by isolated rearing and a single early methamphetamine challenge.** *Brain Res* 2005, **1035:**168-176.
16. Paxinos G, Watson C: *The rat brain in stereotaxic coordinates* New York: Academic Press; 1986.
17. Valverde F: *Golgi atlas of the postnatal mouse brain* Wien, New York: Springer-Verlag; 1998.
18. Stahel WA: *Statistische Datenanalyse: eine Einführung für Naturwissenschaftler* Braunschweig: Vieweg; 1999.
19. Cote PY, Sadikot AF, Parent A: **Complementary Distribution of Calbindin D-28k and Parvalbumin in the Basal Forebrain and Midbrain of the Squirrel Monkey.** *Eur J Neurosci* 1991, **3:**1316-1329.
20. Meredith GE, Pattiselanno A, Groenewegen HJ, Haber SN: **Shell and core in monkey and human nucleus accumbens identified with antibodies to calbindin-D28k.** *J Comp Neurol* 1996, **365:**628-639.
21. Zahm DS, Brog JS: **On the significance of subterritories in the "accumbens" part of the rat ventral striatum.** *Neuroscience* 1992, **50:**751-767.
22. Neddens J, Lesting J, Dawirs RR, Teuchert-Noodt G: **An early methamphetamine challenge suppresses the maturation of dopamine fibres in the nucleus accumbens of gerbils: on the significance of rearing conditions.** *J Neural Transm* 2002, **109:**141-155.
23. Frost DO, Cadet JL: **Effects of methamphetamine-induced neurotoxicity on the development of neural circuitry: a hypothesis.** *Brain Res Brain Res Rev* 2000, **34:**103-118.
24. Gomes-da-Silva J, de MR, Fernandez-Ruiz J, Summavielle T, Tavares MA: **Effects of neonatal exposure to methamphetamine: catecholamine levels in brain areas of the developing rat.** *Ann N Y Acad Sci* 2004, **1025:**602-611.
25. Kokoshka JM, Fleckenstein AE, Wilkins DG, Hanson GR: **Age-dependent differential responses of monoaminergic systems to high doses of methamphetamine.** *J Neurochem* 2000, **75:**2095-2102.
26. Miller DB, O'Callaghan JP, Ali SF: **Age as a susceptibility factor in the striatal dopaminergic neurotoxicity observed in the mouse following substituted amphetamine exposure.** *Ann N Y Acad Sci* 2000, **914:**194-207.
27. Cappon GD, Morford LL, Vorhees CV: **Ontogeny of methamphetamine-induced neurotoxicity and associated hyperthermic response.** *Brain Res Dev Brain Res* 1997, **103:**155-162.
28. Pu C, Vorhees CV: **Developmental dissociation of methamphetamine-induced depletion of dopaminergic terminals and astrocyte reaction in rat striatum.** *Brain Res Dev Brain Res* 1993, **72:**325-328.
29. Truong JG, Wilkins DG, Baudys J, Crouch DJ, Johnson-Davis KL, Gibb JW, Hanson GR, Fleckenstein AE: **Age-dependent methamphetamine-induced alterations in vesicular monoamine trans-**

porter-2 function: implications for neurotoxicity. *J Pharmacol Exp Ther* 2005, **314:**1087-1092.
30. Akiyama K, Kanzaki A, Tsuchida K, Ujike H: **Methamphetamine-induced behavioral sensitization and its implications for relapse of schizophrenia.** *Schizophr Res* 1994, **12:**251-257.
31. Chiu CT, Ma T, Ho IK: **Attenuation of methamphetamine-induced behavioral sensitization in mice by systemic administration of naltrexone.** *Brain Res Bull* 2005, **67:**100-109.
32. Kuribara H, Yasuda H, Uchihashi Y: **Age-dependent induction and maintenance of sensitization to methamphetamine-induced hyperactivity in mice.** *Psychiatry Clin Neurosci* 1996, **50:**299-303.
33. Fujiwara Y, Kazahaya Y, Nakashima M, Sato M, Otsuki S: **Behavioral sensitization to methamphetamine in the rat: an ontogenic study.** *Psychopharmacology (Berl)* 1987, **91:**316-319.
34. Coulter CL, Happe HK, Murrin LC: **Dopamine transporter development in postnatal rat striatum: an autoradiographic study with [3H]WIN 35,428.** *Brain Res Dev Brain Res* 1997, **104:**55-62.
35. Moll GH, Mehnert C, Wicker M, Bock N, Rothenberger A, Ruther E, Huether G: **Age-associated changes in the densities of presynaptic monoamine transporters in different regions of the rat brain from early juvenile life to late adulthood.** *Brain Res Dev Brain Res* 2000, **119:**251-257.
36. Noisin EL, Thomas WE: **Ontogeny of dopaminergic function in the rat midbrain tegmentum, corpus striatum and frontal cortex.** *Brain Res* 1988, **469:**241-252.
37. Neddens J, Brandenburg K, Teuchert-Noodt G, Dawirs RR: **Differential environment alters ontogeny of dopamine innervation of the orbital prefrontal cortex in gerbils.** *J Neurosci Res* 2001, **63:**209-213.
38. Benes FM: **The role of stress and dopamine-GABA interactions in the vulnerability for schizophrenia.** *J Psychiatr Res* 1997, **31:**257-275.
39. Floresco SB, Blaha CD, Yang CR, Phillips AG: **Modulation of hippocampal and amygdalar-evoked activity of nucleus accumbens neurons by dopamine: cellular mechanisms of input selection.** *J Neurosci* 2001, **21:**2851-2860.
40. Teuchert-Noodt G: **Neuronal degeneration and reorganization: a mutual principle in pathological and in healthy interactions of limbic and prefrontal circuits.** *J Neural Transm Suppl* 2000:315-333.
41. Bennay M, Gernert M, Schwabe K, Enkel T, Koch M: **Neonatal medial prefrontal cortex lesion enhances the sensitivity of the mesoaccumbal dopamine system.** *Eur J Neurosci* 2004, **19:**3277-3290.
42. Cadet JL, Brannock C: **Free radicals and the pathobiology of brain dopamine systems.** *Neurochem Int* 1998, **32:**117-131.
43. Gluck MR, Moy LY, Jayatilleke E, Hogan KA, Manzino L, Sonsalla PK: **Parallel increases in lipid and protein oxidative markers in several mouse brain regions after methamphetamine treatment.** *J Neurochem* 2001, **79:**152-160.
44. Kita T, Wagner GC, Nakashima T: **Current research on methamphetamine-induced neurotoxicity: animal models of monoamine disruption.** *J Pharmacol Sci* 2003, **92:**178-195.
45. Imam SZ, Newport GD, Itzhak Y, Cadet JL, Islam F, Slikker W Jr, Ali SF: **Peroxynitrite plays a role in methamphetamine-induced dopaminergic neurotoxicity: evidence from mice lacking neuronal nitric oxide synthase gene or overexpressing copper-zinc superoxide dismutase.** *J Neurochem* 2001, **76:**745-749.
46. Itzhak Y, Gandia C, Huang PL, Ali SF: **Resistance of neuronal nitric oxide synthase-deficient mice to methamphetamine-induced dopaminergic neurotoxicity.** *J Pharmacol Exp Ther* 1998, **284:**1040-1047.
47. Fumagalli F, Gainetdinov RR, Valenzano KJ, Caron MG: **Role of dopamine transporter in methamphetamine-induced neurotoxicity: evidence from mice lacking the transporter.** *J Neurosci* 1998, **18:**4861-4869.
48. Fumagalli F, Gainetdinov RR, Wang YM, Valenzano KJ, Miller GW, Caron MG: **Increased methamphetamine neurotoxicity in heterozygous vesicular monoamine transporter 2 knock-out mice.** *J Neurosci* 1999, **19:**2424-2431.
49. O'Dell SJ, Weihmuller FB, Marshall JF: **Methamphetamine-induced dopamine overflow and injury to striatal dopamine terminals: attenuation by dopamine D1 or D2 antagonists.** *J Neurochem* 1993, **60:**1792-1799.
50. Eisch AJ, Gaffney M, Weihmuller FB, O'Dell SJ, Marshall JF: **Striatal subregions are differentially vulnerable to the neurotoxic effects of methamphetamine.** *Brain Res* 1992, **598:**321-326.
51. Lehmann K, Lesting J, Polascheck D, Teuchert-Noodt G: **Serotonin fibre densities in subcortical areas: differential effects of isolated rearing and methamphetamine.** *Brain Res Dev Brain Res* 2003, **147:**143-152.
52. Neddens J, Bagorda F, Busche A, Horstmann S, Moll GH, Dawirs RR, Teuchert-Noodt G: **Epigenetic factors differentially influence postnatal maturation of serotonin (5-HT) innervation in cerebral cortex of gerbils: interaction of rearing conditions and early methamphetamine challenge.** *Brain Res Dev Brain Res* 2003, **146:**119-130.
53. Nossoll M, Teuchert-Noodt G, Dawirs RR: **A single dose of methamphetamine in neonatal gerbils affects adult prefrontal gamma-aminobutyric acid innervation.** *Eur J Pharmacol* 1997, **340:**R3-R5.
54. Blaesing B, Nossoll M, Teuchert-Noodt G, Dawirs RR: **Postnatal maturation of prefrontal pyramidal neurones is sensitive to a single early dose of methamphetamine in gerbils (Meriones unguiculatus).** *J Neural Transm* 2001, **108:**101-113.
55. Bagorda F, Teuchert-Noodt G, Lehmann K: **Isolation rearing or methamphetamine traumatisation induce a "dysconnection" of prefrontal efferents in gerbils: implications for schizophrenia.** *J Neural Transm* 2006, **113:**365-379.
56. Broening HW, Pu C, Vorhees CV: **Methamphetamine selectively damages dopaminergic innervation to the nucleus accumbens core while sparing the shell.** *Synapse* 1997, **27:**153-160.
57. Zocchi A, Girlanda E, Varnier G, Sartori I, Zanetti L, Wildish GA, Lennon M, Mugnaini M, Heidbreder CA: **Dopamine responsiveness to drugs of abuse: A shell-core investigation in the nucleus accumbens of the mouse.** *Synapse* 2003, **50:**293-302.
58. King D, Zigmond MJ, Finlay JM: **Effects of dopamine depletion in the medial prefrontal cortex on the stress-induced increase in extracellular dopamine in the nucleus accumbens core and shell.** *Neuroscience* 1997, **77:**141-153.
59. Lesting J, Neddens J, Teuchert-Noodt G: **Ontogeny of the dopamine innervation in the nucleus accumbens of gerbils.** *Brain Res* 2005, **1066:**16-23.
60. Vos PE, Steinbusch HW, van Ree JM: **Reinnervation after destruction of the dopaminergic system in the rat nucleus accumbens: a quantitative immunohistochemical analysis.** *Neurosci Lett* 1996, **207:**21-24.
61. Finkelstein DI, Stanic D, Parish CL, Tomas D, Dickson K, Horne MK: **Axonal sprouting following lesions of the rat substantia nigra.** *Neuroscience* 2000, **97:**99-112.
62. Dawirs RR, Teuchert-Noodt G, Molthagen M: **Indication of methamphetamine-induced reactive synaptogenesis in the prefrontal cortex of gerbils (Meriones unguiculatus).** *Eur J Pharmacol* 1993, **241:**89-97.
63. Segal DS, Kuczenski R, O'Neil ML, Melega WP, Cho AK: **Escalating dose methamphetamine pretreatment alters the behavioral and neurochemical profiles associated with exposure to a high-dose methamphetamine binge.** *Neuropsychopharmacology* 2003, **28:**1730-1740.
64. Segal DS, Kuczenski R: **Human Methamphetamine Pharmacokinetics Simulated in the Rat: Single Daily Intravenous Administration Reveals Elements of Sensitization and Tolerance.** *Neuropsychopharmacology* 2005 in press.
65. Thomas DM, Kuhn DM: **Attenuated microglial activation mediates tolerance to the neurotoxic effects of methamphetamine.** *J Neurochem* 2005, **92:**790-797.
66. Ishikawa K, Nitta A, Mizoguchi H, Mohri A, Murai R, Miyamoto Y, Noda Y, Kitaichi K, Yamada K, Nabeshima T: **Effects of single and repeated administration of methamphetamine or morphine on neuroglycan C gene expression in the rat brain.** *Int J Neuropsychopharmacol* 2005 in press.
67. Zhu JP, Xu W, Angulo N, Angulo JA: **Methamphetamine-induced striatal apoptosis in the mouse brain: Comparison of a binge to an acute bolus drug administration.** *Neurotoxicology* 2005.
68. Chen CK, Lin SK, Sham PC, Ball D, Loh EW, Hsiao CC, Chiang YL, Ree SC, Lee CH, Murray RM: **Pre-morbid characteristics and co-morbidity of methamphetamine users with and without psychosis.** *Psychol Med* 2003, **33:**1407-1414.

69. Machiyama Y: **Chronic methamphetamine intoxication model of schizophrenia in animals.** *Schizophr Bull* 1992, **18:**107-113.
70. Sato M: **A lasting vulnerability to psychosis in patients with previous methamphetamine psychosis.** *Ann N Y Acad Sci* 1992, **654:**160-170.
71. Feinberg I: **Schizophrenia: caused by a fault in programmed synaptic elimination during adolescence?** *J Psychiatr Res* 1982, **17:**319-334.
72. Weinberger DR, Lipska BK: **Cortical maldevelopment, anti-psychotic drugs, and schizophrenia: a search for common ground.** *Schizophr Res* 1995, **16:**87-110.

available at www.sciencedirect.com

ScienceDirect

www.elsevier.com/locate/brainres

BRAIN RESEARCH

Research Report

Postnatal development of dopamine innervation in the amygdala and the entorhinal cortex of the gerbil (*Meriones unguiculatus*)

Susanne Brummelte, Gertraud Teuchert-Noodt*

Department of Neuroanatomy, Faculty of Biology, University of Bielefeld, Universitätsstr. 25, D-33615 Bielefeld, Germany

ARTICLE INFO

Article history:
Accepted 3 October 2006
Available online 16 November 2006

Keywords:
Immunohistochemistry
Development
Aging
Limbic system

ABSTRACT

Dopamine (DA) projections from the mesencephalon are believed to play a critical role during development and are essential for cognitive and behavioral functions. Since the postnatal maturation patterns of these projections differ substantially between various brain regions, cortical, limbic or subcortical areas might exhibit varying vulnerabilities concerning developmental disorders. The dopaminergic afferents of the rodent prefrontal cortex show an extremely prolonged maturation which is very sensitive to epigenetic challenges. However, less is known about the development of the DA innervation of caudal limbic areas. Therefore, immunohistochemically stained DA fibers were quantitatively examined in the basolateral (BLA) and central amygdaloid nucleus (CE) and the ventrolateral entorhinal cortex (EC) of the Mongolian gerbil (*Meriones unguiculatus*). Animals of different ages, ranging from juvenile [postnatal day (PD) 14, 20, 30)] to adolescent (PD70), adult (6, 18 months) and aged (24 months), were analyzed. Results show a significant increase of fibers between PD14 and PD20 in the BLA and lateral part of the CE, with a trend for a subsequent decline in fiber densities until PD30. The EC and medial part of the CE showed no developmental changes. Interestingly, none of the investigated areas showed significant reductions of DA fibers during aging.

1. Introduction

The neurotransmitter dopamine (DA) is known to play a fundamental role in regulating cortical activity during development and in adulthood. The dopaminergic innervation of distinct areas in the mammalian brain descends from different pathways which have their origin in the mesencephalon of the brainstem. The mesostriatal projection has its source mainly in the pars compacta of the substantia nigra (SN; A9) and the nucleus retrorubralis (A8) and innervates the dorsal striatum (caudate putamen) (Gerfen et al., 1987; Hu et al., 2004). Thus, it plays a considerable role in the maintenance of motoric functions. Dopaminergic fibers of the mesolimbic and mesocortical projections, which are often named together as the mesocorticolimbic projection, largely originate from the ventral tegmental area (VTA; A10) (Fallon et al., 1978; Swanson, 1982) and innervate several subregions of the dispersedly organized corticolimbic circuitry, including the amygdala and entorhinal cortex (Bjorklund and Lindvall, 1984; Descarries et al., 1987; Yoshida et al., 1988).

Remarkably, each DA projection field is characterized by its own pattern of time sequence of development in postnatal

* *Corresponding author*. Fax: +49 521 106 6470.
E-mail address: sbrummelte@uni-bielefeld.de (S. Brummelte).

0006-8993/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.brainres.2006.10.006

life. Generally, caudal areas mature before rostral areas, and thus the dopaminergic innervation follows the caudorostral gradient, which also accounts for many other structures and functions.

The amygdala is one of the main targets of the mesolimbic DA pathway. It receives its input from the lateral VTA and medial half of the SN (Fallon et al., 1978; Hasue and Shammah-Lagnado, 2002). Within the amygdaloid complex, several nuclei can be distinguished due to their different connectivity, function and derivation. The central amygdaloid nucleus (CE) and the basolateral amygdala (BLA) are both considered output entities of the amygdaloid complex (Petrovich et al., 1996; Petrovich and Swanson, 1997), with each being connected to a unique set of brain areas (Pitkanen, 2000). Thus, differential developmental patterns may also affect or even be affected by other cortical and subcortical regions. In fact, the prefrontal cortex (PFC), which exhibits a prolonged maturation of its dopaminergic innervation (Kalsbeek et al., 1988; Dawirs et al., 1993), has been assumed to stabilize DA subsystems depending on its postnatal development (Busche et al., 2004; Bennay et al., 2004).

It has been demonstrated that the DA fiber densities of caudal limbic areas in the rodent brain reach their adult levels relatively early (Verney et al., 1985; Erickson et al., 1998) compared to prefrontal cortices (Kalsbeek et al., 1988; Dawirs et al., 1993) or the nucleus accumbens (Lesting et al., 2005), which continue to increase until adulthood. This prolonged maturation of the rostral parts of the brain has been assumed to play an essential role in the maintenance of neural plasticity during development, but according to an animal model of psychosis, it might also be involved in developmentally induced diseases such as schizophrenia (Teuchert-Noodt, 2000; Dawirs and Teuchert-Noodt, 2001).

However, less is known about the long-term postnatal development and aging of the DA fiber densities in the caudal limbic system. The divergent developmental patterns of cortical and subcortical areas are of particular interest as various neurodevelopmental and degenerative diseases such as Parkinson's disease or schizophrenia are assumed to exhibit imbalances on several levels concerning the monoaminergic neurotransmitter systems between these areas (Laruelle et al., 2003; Abi-Dargham, 2004). In fact, a recent study from our laboratory, using an early postnatal traumatization by methamphetamine as an animal model of psychoses, revealed a disequilibrium in dopaminergic fiber densities between the prefrontal cortex and caudal limbic areas (Busche et al., 2004). Therefore, this study was conducted to look closely at the long-term maturation of DA fiber densities in different areas of the limbic circuit, namely the central amygdala, the basolateral amygdala and the ventrolateral entorhinal cortex (EC).

2. Results

The DA innervation pattern in gerbils' amygdala and entorhinal cortex resembles that described for the rat, which in turn has been reported to be similar to that of monkeys (Sadikot and Parent, 1990).

The lateral part of the CE receives the densest dopaminergic innervation followed by its medial neighbor (Fig. 1C). The basolateral amygdala shows only a light DA innervation (Fig. 1C). DA fibers of the ventrolateral EC appear to be arranged in clusters (Figs. 1D, G1–G4).

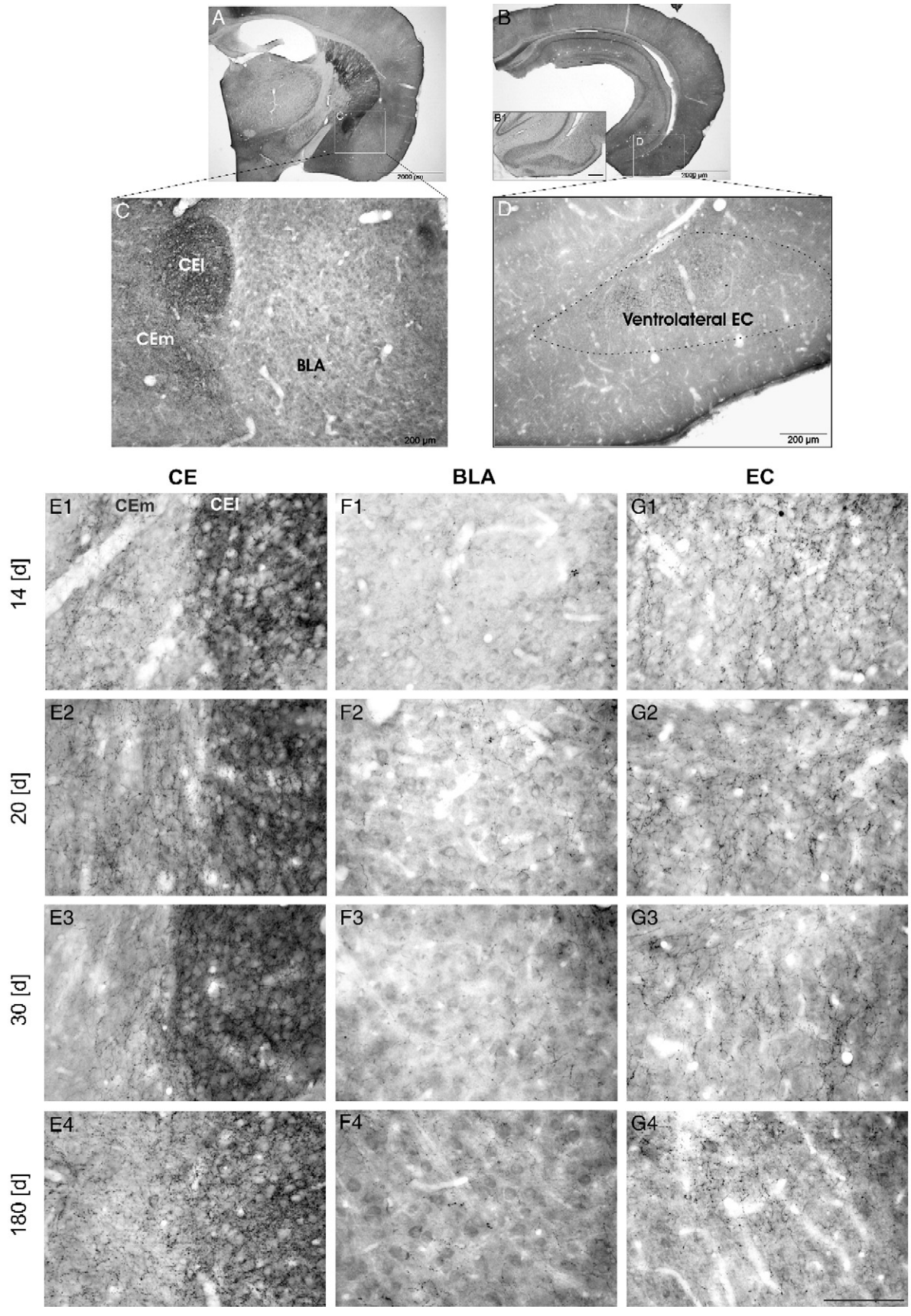
An analysis of variance (ANOVA) with age as the independent variable and the DA fiber densities of the different areas as dependent variables revealed a significant effect of age ($F(24,144)=2.34$, $p=0.0011$). The following Fisher's LSD post hoc test showed a highly significant increase between PD14 and PD20 in the lateral part of the CE (28%; $p=0.0003$) and a tendency for a subsequent decrease between PD20 and PD30 ($p=0.070$). There was no such peak in the development of the medial CE or EC (all p's >0.05), although there was a tendency for an increase after PD14 in the medial part of the CE ($p=0.079$). For the BLA, the post hoc test revealed a similar increase between PD14 and PD20 (90%; $p=0.009$) and also a trend for a subsequent decline in fiber density ($p=0.059$). During aging, no statistically significant alterations were seen in any of the investigated areas. However, the lateral part of the CE showed a tendency ($p=0.088$) for a decline between PD540 and PD720.

3. Discussion

The present data reveal that the development of the dopaminergic innervation varies between different structures of the caudal limbic system of the gerbil. Thus, the ventrolateral entorhinal cortex (EC) and the medial part of the central nucleus of the amygdala (CE) show no significant alterations at all between PD14 and PD720, while there is an increase in density between PD14 and PD20 and a subsequent trend for a decrease until PD30 in the lateral part of the CE and the basolateral nucleus of the amygdala (BLA). Interestingly, none of the structures demonstrates any statistically significant decline in DA fiber densities in high age (Fig. 2).

Beside the dopaminergic fiber densities, there are other elements of the DA system that show differential developmental patterns in different brain regions. Thus, Coulter et al. (1996) demonstrated that the maturation of the dopamine transporter (DAT) follows an anterior-to-posterior and lateral-to-medial gradient, with the prefrontal cortex (PFC) and nucleus accumbens being two of the last areas to reach adult

Fig. 1 – Representative photomicrographs of the dopaminergic innervation of the amygdala and entorhinal cortex at different age stages. A, B: photomicrographs of representative coronal sections at the level of the amygdala and entorhinal cortex (EC), respectively. Picture (B1) shows a Nissl staining of the ventrolateral EC. The areas of the rectangles are magnified in panels C and D. The dotted line in panel D marks the approximate innervation field of the dopamine (DA) projection, which is arranged in clusters (cf. G1–4). Within the amygdala, most fibers are found in the lateral part of the central amygdala (CEl), which is surrounded by the moderate innervation of the medial part (CEm). The BLA shows the slightest innervation density of DA fibers (cf. F1–4). Pictures (E1)–(G4) show examples of DA innervation at juvenile age stages (PD14–PD30) with a comparative section from an adult animal (PD180). Scale bars: 500 μm (B1), 100 μm (E1–G4).


A
B
B1
C
D
CEl
CEm
BLA
200 µm
Ventrolateral EC
200 µm
CE
BLA
EC
14 [d]
20 [d]
30 [d]
180 [d]
E1
CEm
CEl
F1
G1
E2
F2
G2
E3
F3
G3
E4
F4
G4

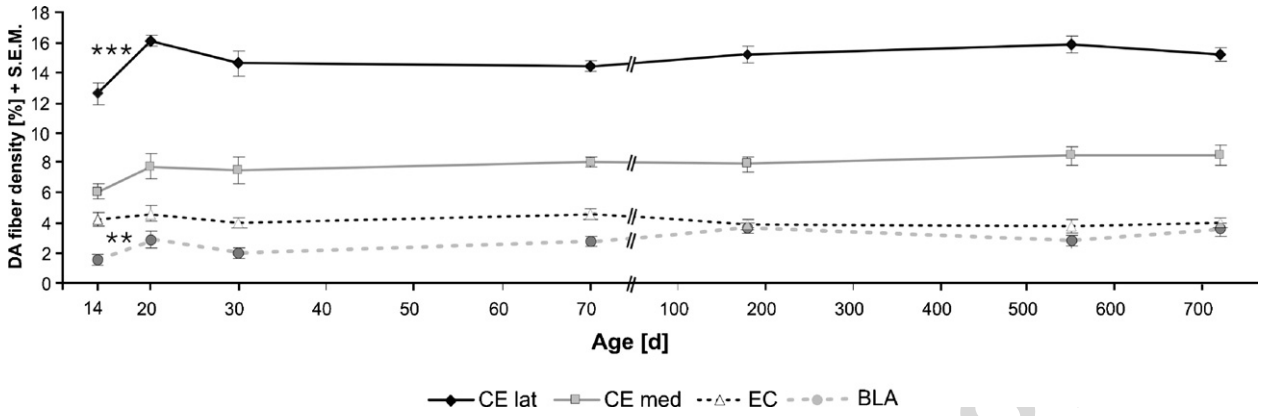


Fig. 2 – Development of DA fiber densities in the amygdala and entorhinal cortex of the gerbil. Shown are the DA fiber density means as percentage [%] of the reference area + SEM at postnatal day (PD) 14, 20, 30, 70, 180, 540 and 720. The lateral part of the central amygdala (CE lat) and the basolateral amygdala (BLA) show significant increases between PD14 and PD20, while the entorhinal cortex (EC) and medial part of the CE (CE med) display no alterations during maturation or aging. The double bar in the middle marks a break in the scaling of the x-axis. $^{**}p<0.01$, $^{***}p<0.001$.

levels. However, in the striatum, DAT density rather declines after an early peak during development (Moll et al., 2000). Similar reorganization processes have been observed for DA varicosities, synapses and DA receptor types in the nucleus accumbens and caudate putamen (Tarazi et al., 1998, 1999; Antonopoulos et al., 2002), while there is no such peak and subsequent elimination in receptor densities in the frontal cortex or limbic areas (Tarazi and Baldessarini, 2000). Furthermore, DA axons appear to pass through a peak in density in particular areas of the striatum (Hu et al., 2004; Lesting et al., 2005) which has been assumed to be due to the reorganization and elimination of non-specific targeting from the dopaminergic pathways (Hu et al., 2004). However, none of the investigated caudal limbic areas from the current study reveals this 'pruning effect', at least not at a statistically significant level.

Remarkably, there was also no detectable alteration in DA fiber densities in old animals compared to adolescent or adult ones in the current study. This is in line with previous observations in the nucleus accumbens shell and core in the gerbil, which also showed no age-related changes in DA fiber densities (Lesting et al., 2005). But it is in contrast with several studies, which have reported about metabolic changes within the monoaminergic system in older animals, as e.g. differences in the turnover rates, biosynthesis or concentration of DA or its metabolites (Miguez et al., 1999; Miura et al., 2002). However, none of these alterations suggests a correlated change in fiber densities as a reduced DA concentration or metabolism does not necessarily imply a reduced number or density of axons in the particular area. It is rather likely that these metabolic differences are mediated by an altered receptor physiology or density (Cross et al., 1984, 1988; Sweet et al., 2001), although we have to admit that we cannot exclude alterations in animals older than 24 months as this is only the mean survival of the Mongolian gerbil, whereas individuals might get older (Troup et al., 1969).

Due to the caudorostral maturation gradient, it seems likely that the entorhinal cortex and amygdala develop relatively early during the postnatal period. Erickson and co-workers (1998) have shown that the densities of DA axons increased until 7 months of age in monkeys in layer III of the rostral entorhinal cortex. However, we could not detect such an age-dependent alteration in the dopaminergic fiber densities in the gerbil EC after PD14. This might be due to the fact that there are apparently some discrepancies concerning the exact classification of the entorhinal area. A recent anatomical study suggests that the amygdalopiriform transition area, which was so far often considered part of the EC, should be viewed as a separate anatomical entity (Santiago and Shammah-Lagnado, 2005). In order to show the boundaries of the investigated area in the current study, a picture of a Nissl staining of the gerbil EC is provided, to be compared with the dopaminergic innervation and measurement field (Fig. 1B1).

Dopaminergic fibers are arranged in clusters or "islands" in the adult rodent EC (Fallon et al., 1978; Busche et al., 2004), which can already be seen during early development (cf. Figs. 1G1–G3). DA terminals directly innervate excitatory and inhibitory entorhinal neurons, and thus the innervation pattern resembles that of neocortical or amygdaloid projections (Asan, 1998; Erickson et al., 2000). Forming the main input to the hippocampus and being intensively connected with the BLA and the CE (McDonald and Mascagni, 1996; Pitkanen, 2000), the EC is in close association to the limbic system and is even considered a part of it by some authors (Amaral and Witter, 1995). Disturbances during the maturation process of the EC might therefore have devastating consequences for the orderly information flow. In concert with this, neonatally induced structural abnormalities in the entorhinal cortex have been shown to affect DA transmission in the limbic regions at the adolescent stage (Uehara et al., 2000). Furthermore, a reduced density of tyrosine hydroxylase-immunoreactive axons has been observed in the entorhinal cortex of schizophrenic patients (Akil et al., 2000).

Compared to the ventrolateral EC, the amygdala seems to lag behind in the maturation of the innervation density of DA. The increase in the lateral part of the CE after PD14 might be due to a sprouting of axon collaterals or further elaboration of local arbors within the nucleus, which might also account for

the non-significant rise in density in the medial part, just to a lesser extent. The CE, with its several distinct subdivisions, receives intensive input from cortical areas as well as from limbic structures as the hippocampus or EC. Interestingly, the CE has no reciprocal connections with the aforementioned regions (Pitkanen, 2000) but sends a strong input to the dopaminergic system in the mesencephalon, thus influencing the DA innervation of several other areas and modulating behavioral responses (Fudge and Haber, 2000). It is further the nucleus with the highest density of DA synapses and axons with the lateral part being in turn more dense than the medial part (Asan, 1997, 1998). This strong interaction with the dopaminergic cells in the mesencephalon might be a reason why especially the lateral part seems to be relatively resistant to early postnatal environmental and pharmacological challenges (Busche et al., 2004). In addition, the catecholaminergic innervation of the medial part is assumed to serve more modulatory functions due to the fact that it is directed preferentially at peripheral neuronal structures (Asan, 1998).

The BLA is characterized by its strong reciprocal connection with the prefrontal cortex (Pitkanen, 2000) and is thought to mediate affective behavior via its DA innervation (Kroner et al., 2005). As this connectivity with the PFC matures relatively late and is considered to influence the development and integration of normal or abnormal emotional behavior during adolescence (Cunningham et al., 2002), it is not surprising that the BLA is particularly sensitive to early environmental or pharmacological challenges (Busche et al., 2004; Grund et al., 2006).

We have recently shown that an early single methamphetamine (MA) intoxication on PD14 can cause a surplus of DA fibers in the adult ventrolateral EC. An even stronger increase was found in the BLA, but not in the lateral part of the CE and only to a lesser extent in the left hemisphere of the medial part of the CE (Busche et al., 2004). Considering the marked decrease of dopaminergic fibers in rostral areas such as the prefrontal cortex using the same pharmacological approach (Dawirs et al., 1994), it seems likely that the different dopaminergic pathways influence each other, thus causing an imbalance between cortical and caudal limbic innervation areas (Busche et al., 2004). Interestingly, such long-term alterations in fiber densities are not seen in the PFC or amygdala, when MA is applied at adult age, underlining the sensitivity of these areas during development compared to adulthood (Brummelte et al., 2006).

BLA and the lateral part of the CE are affected differently by the MA challenge, although both structures show a further increase in DA fibers after PD14. It is assumed that the PFC is able to stabilize DA subsystems depending on its postnatal development (Busche et al., 2004; Bennay et al., 2004). However, the PFC is also supposed to be exceedingly damageable and accident-sensitive during development with the period of this vulnerability lasting long until early adulthood (for reviews see: Diamond, 1996; Lewis, 1997; Sullivan and Brake, 2003; Adriani and Laviola, 2004). As the BLA exhibits particularly strong reciprocal connections with the PFC, the observed peak in fiber density during development may be particularly vulnerable to changes resulting in dysfunctional connections with the PFC. In fact, extracellular recording studies have observed that a prestimulation of the medial PFC reduces the responsiveness of CE neurons to inputs from the BLA, thus contributing to the idea that the PFC even gates transmission within the amygdala (Quirk et al., 2003). This inhibitory control by the cortex, probably mediated via GABAergic neurons, can be diminished by the release of DA (Marowsky et al., 2005). Thus, the DA innervation of the amygdala contributes essentially to a balanced output of this structure and consequently regulates the modulation of affective behavior (Asan, 1997; Marowsky et al., 2005; Kroner et al., 2005).

Naturally, not only DA plays a significant role during its own pathway and general development. Thus, the interaction with other transmitter systems, e.g. serotonin or glutamate and their receptors, might considerably contribute to the divergent developmental patterns of cortical and subcortical areas and the according differences in vulnerability during maturation. In fact, it has often been assumed that the putative DA imbalance in schizophrenia might be secondary to alterations of other resources, as e.g. an NMDA hypofunction or alteration in the GABAergic or glutamate system (Laruelle et al., 2003; Abi-Dargham, 2004). Consistent with this hypothesis, we have recently demonstrated a dysconnection within macrocircuits of the glutamatergic system (Bagorda et al., 2006; Witte et al., 2006) and a shift within the prefrontal GABAergic innervation pattern (unpublished data) in our animal model of psychosis. Therefore, the authors are tempted to suggest that the mutual impact and interdependency of the areas and their corresponding transmitter systems during development all contribute essentially to a normal and healthy maturation and that disturbances in one of the integrated features might cause various adaptations and alterations in several systems.

4. Experimental procedures

4.1. *Animals*

All experimental procedures were approved by the appropriate committee for animal care in accordance with the European Communities Council Directive. Male Mongolian gerbils (*M. unguiculatus*) were kept under natural day/night cycles with food and water being provided *ad libitum*. Until weaning (PD30), animals were kept in standard Macrolon® cages (type 4). Afterwards, they were reared individually in Macrolon® type 3 cages. A total of 51 male Mongolian gerbils were used for this study. Seven experimental animal groups of different ages were investigated to cover convincing periods of the life span of gerbils: PD14 ($n=6$), PD20 ($n=6$), PD30, ($n=9$), PD70 ($n=9$), PD180 ($n=6$), PD540 ($n=7$) and PD720 ($n=8$). Gerbils were chosen due to their wild-type like behavioral and neuronal repertoire as they have not been so intensively domesticated compared to rats or mice (Rosenzweig and Bennett, 1969). In addition, the present data can be considered against a huge amount of previously published data of the gerbil brain from our laboratory.

4.2. *Dopamine immunohistochemistry*

Animals under deep chloralhydrate anesthesia (1.7 g/kg, i.p.) were transcardially perfused with 0.1 M sodium cacodylate pH

6.2 followed by 5% glutaraldehyde in 0.1 M sodium cacodylate pH 7.5. Immediately after perfusion, the brains were dissected and 50 μm thick frontal sections of the right hemisphere were cut with a vibratome (Leica VT 1000S). For immunostaining, the slices were rinsed in wash buffer followed by a pre-incubation in 10% normal goat serum and 0.4% Triton X-100 (Sigma). Then the slices were incubated with the primary antibody (rabbit anti-dopamine, DiaSorin, Stillwater, MN) diluted 1:600 with 1% normal goat serum and 0.4% Triton X-100 for 40 h. The following rinses were done with 0.05 M Tris–HCl buffered saline (pH 7.5). The slices were rinsed and incubated in the biotinylated goat–anti-rabbit antibody (Sigma) diluted 1:20 with 1% normal goat serum, rinsed again and incubated with ExtraAvidin–Peroxidase (Sigma) diluted 1:20. After another rinse, the slices were stained in 0.05% 3.3-diaminobenzidine (DAB, Sigma) with 0.01% H_2O_2. Then the slices were washed, mounted on glass slides, dried overnight, dehydrated with ethanol, cleared with xylene and coverslipped with DePeX (Serva, Heidelberg, Germany). For more details on animal preparation and dopamine immunohistochemistry, see Lesting et al., 2005.

4.3. *Quantification of DA innervation*

DA fiber densities were measured in three to four consecutive coronal slices of the corresponding brain sections, which were assigned by means of anatomical characteristics according to brain atlases of the rat (Paxinos and Watson, 1986) and the mouse (Valverde, 1998). Fibers were visualized using a bright-field microscope (BX61, Olympus, Hamburg, Germany) and 400-fold magnification and a digital camera for microscopy (ColorView II, SIS, Münster, Germany). All pictures were adjusted in contrast and brightness for better conspicuity of DA fibers. The lateral and medial part of the CE, the BLA and the entorhinal cortex were encircled by an experimenter blind to the animals' age, and all detectable fragments were quantified using software for image analysis (KS300, Jenoptik, Jena, Germany; for details of the quantification process, see Lesting et al., 2005). Fiber densities were calculated as a percentage of the reference area. We abstained from additionally measuring the growth of the according areas in each individual as the potential increase of the area during development is already accounted for by this method.

4.4. *Data analysis*

Measurements were computed as arithmetic means by-case and by-group ± SEM. An analysis of variance (ANOVA) with age (7 levels) as an independent variable and area (4 levels) as dependent variable was used to check for statistical significance between groups followed by LSD post hoc test for multiple comparisons. Statistical analysis was computed with Statistica 6 (StatSoft, Tulsa, USA). The levels of significance were set at $^{*}p<0.05$, $^{**}p<0.01$ and $^{***}p<0.001$.

Acknowledgments

We would like to thank Dr. Francesco Bagorda and Ulrike Schroeder for excellent technical assistance. Furthermore, we wish to thank Dr. Jorg Neddens for his help with this work and Beatrix Ahlswede for correcting the English. This study was supported by grants of the 'Deutsche Parkinson Vereinigung'.

REFERENCES

Abi-Dargham, A., 2004. Do we still believe in the dopamine hypothesis? New data bring new evidence. Int. J. Neuropsychopharmacol. 7 (Suppl 1), S1–S5.

Adriani, W., Laviola, G., 2004. Windows of vulnerability to psychopathology and therapeutic strategy in the adolescent rodent model. Behav. Pharmacol. 15, 341–352.

Akil, M., Edgar, C.L., Pierri, J.N., Casali, S., Lewis, D.A., 2000. Decreased density of tyrosine hydroxylase-immunoreactive axons in the entorhinal cortex of schizophrenic subjects. Biol. Psychiatry 47, 361–370.

Amaral, D.G., Witter, M.P., 1995. Hippocampal formation. In: Paxinos, G. (Ed.), The Rat Nervous System. Academic Press, San Diego, pp. 443–493.

Antonopoulos, J., Dori, I., Dinopoulos, A., Chiotelli, M., Parnavelas, J.G., 2002. Postnatal development of the dopaminergic system of the striatum in the rat. Neuroscience 110, 245–256.

Asan, E., 1997. Ultrastructural features of tyrosine-hydroxylase-immunoreactive afferents and their targets in the rat amygdala. Cell Tissue Res. 288, 449–469.

Asan, E., 1998. The catecholaminergic innervation of the rat amygdala. Adv. Anat. Embryol. Cell Biol. 142, 1–118.

Bagorda, F., Teuchert-Noodt, G., Lehmann, K., 2006. Isolation rearing or methamphetamine traumatisation induce a "dysconnection" of prefrontal efferents in gerbils: implications for schizophrenia. J. Neural Transm. 113, 365–379.

Bennay, M., Gernert, M., Schwabe, K., Enkel, T., Koch, M., 2004. Neonatal medial prefrontal cortex lesion enhances the sensitivity of the mesoaccumbal dopamine system. Eur. J. Neurosci. 19, 3277–3290.

Bjorklund, A., Lindvall, O., 1984. Dopamine-containing systems in the CNS. In: Bjorklund, A., Hokfelt, T. (Eds.), Classical Transmitters in the CNS. Elsevier, Amsterdam, pp. 55–122.

Brummelte, S., Grund, T., Czok, A., Teuchert-Noodt, G., Neddens, J., 2006. Long-term effects of a single adult methamphetamine challenge: minor impact on dopamine fibre density in limbic brain areas of gerbils. Behav. Brain Funct. 2, 12.

Busche, A., Polascheck, D., Lesting, J., Neddens, J., Teuchert-Noodt, G., 2004. Developmentally induced imbalance of dopaminergic fibre densities in limbic brain regions of gerbils (*Meriones unguiculatus*). J. Neural Transm. 111, 451–463.

Cortes, R., Probst, A., Palacios, J.M., 1988. Decreased densities of dopamine D1 receptors in the putamen and hippocampus in senile dementia of the Alzheimer type. Brain Res. 475, 164–167.

Coulter, C.L., Happe, H.K., Murrin, L.C., 1996. Postnatal development of the dopamine transporter: a quantitative autoradiographic study. Brain Res. Dev. Brain Res. 92, 172–181.

Cross, A.J., Crow, T.J., Ferrier, I.N., Johnson, J.A., Markakis, D., 1984. Striatal dopamine receptors in Alzheimer-type dementia. Neurosci. Lett. 52, 1–6.

Cunningham, M.G., Bhattacharyya, S., Benes, F.M., 2002. Amygdalo-cortical sprouting continues into early adulthood: implications for the development of normal and abnormal function during adolescence. J. Comp. Neurol. 453, 116–130.

Dawirs, R.R., Teuchert-Noodt, G., 2001. A novel pharmacological concept in an animal model of psychosis. Acta Psychiatr. Scand. 10–17 (Suppl.).

Dawirs, R.R., Teuchert-Noodt, G., Czaniera, R., 1993. Maturation of the dopamine innervation during postnatal development of the prefrontal cortex in gerbils (*Meriones unguiculatus*). A quantitative immunocytochemical study. J. Hirnforsch. 34, 281–290.

Dawirs, R.R., Teuchert-Noodt, G., Czaniera, R., 1994. The postnatal maturation of dopamine innervation in the prefrontal cortex of gerbils (*Meriones unguiculatus*) is sensitive to an early single dose of methamphetamine. A quantitative immunocytochemical study. J. Hirnforsch. 35, 195–204.
Descarries, L., Lemay, B., Doucet, G., Berger, B., 1987. Regional and laminar density of the dopamine innervation in adult rat cerebral cortex. Neuroscience 21, 807–824.
Diamond, A., 1996. Evidence for the importance of dopamine for prefrontal cortex functions early in life. Philos. Trans. R. Soc. Lond., B Biol. Sci. 351, 1483–1493.
Erickson, S.L., Akil, M., Levey, A.I., Lewis, D.A., 1998. Postnatal development of tyrosine hydroxylase- and dopamine transporter-immunoreactive axons in monkey rostral entorhinal cortex. Cereb. Cortex 8, 415–427.
Erickson, S.L., Sesack, S.R., Lewis, D.A., 2000. Dopamine innervation of monkey entorhinal cortex: postsynaptic targets of tyrosine hydroxylase-immunoreactive terminals. Synapse 36, 47–56.
Fallon, J.H., Koziell, D.A., Moore, R.Y., 1978. Catecholamine innervation of the basal forebrain. II. Amygdala, suprarhinal cortex and entorhinal cortex. J. Comp. Neurol. 180, 509–532.
Fudge, J.L., Haber, S.N., 2000. The central nucleus of the amygdala projection to dopamine subpopulations in primates. Neuroscience 97, 479–494.
Gerfen, C.R., Herkenham, M., Thibault, J., 1987. The neostriatal mosaic: II. Patch- and matrix-directed mesostriatal dopaminergic and non-dopaminergic systems. J. Neurosci. 7, 3915–3934.
Grund, T., Lehmann, K., Bock, N., Rothenberger, A., Teuchert-Noodt, G., 2006. Influence of methylphenidate on brain development—An update of recent animal experiments. Behav. Brain Funct. 2, 2.
Hasue, R.H., Shammah-Lagnado, S.J., 2002. Origin of the dopaminergic innervation of the central extended amygdala and accumbens shell: a combined retrograde tracing and immunohistochemical study in the rat. J. Comp. Neurol. 454, 15–33.
Hu, Z., Cooper, M., Crockett, D.P., Zhou, R., 2004. Differentiation of the midbrain dopaminergic pathways during mouse development. J. Comp. Neurol. 476, 301–311.
Kalsbeek, A., Voorn, P., Buijs, R.M., Pool, C.W., Uylings, H.B., 1988. Development of the dopaminergic innervation in the prefrontal cortex of the rat. J. Comp. Neurol. 269, 58–72.
Kroner, S., Rosenkranz, J.A., Grace, A.A., Barrionuevo, G., 2005. Dopamine modulates excitability of basolateral amygdala neurons in vitro. J. Neurophysiol. 93, 1598–1610.
Laruelle, M., Kegeles, L.S., Abi-Dargham, A., 2003. Glutamate, dopamine, and schizophrenia: from pathophysiology to treatment. Ann. N. Y. Acad. Sci. 1003, 138–158.
Lesting, J., Neddens, J., Teuchert-Noodt, G., 2005. Ontogeny of the dopamine innervation in the nucleus accumbens of gerbils. Brain Res. 1066, 16–23.
Lewis, D.A., 1997. Development of the prefrontal cortex during adolescence: insights into vulnerable neural circuits in schizophrenia. Neuropsychopharmacology 16, 385–398.
Marowsky, A., Yanagawa, Y., Obata, K., Vogt, K.E., 2005. A specialized subclass of interneurons mediates dopaminergic facilitation of amygdala function. Neuron 48, 1025–1037.
McDonald, A.J., Mascagni, F., 1996. Cortico-cortical and cortico-amygdaloid projections of the rat occipital cortex: a Phaseolus vulgaris leucoagglutinin study. Neuroscience 71, 37–54.
Miguez, J.M., Aldegunde, M., Paz-Valinas, L., Recio, J., Sanchez-Barcelo, E., 1999. Selective changes in the contents of noradrenaline, dopamine and serotonin in rat brain areas during aging. J. Neural Transm. 106, 1089–1098.
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.
Moll, G.H., Mehnert, C., Wicker, M., Bock, N., Rothenberger, A., Ruther, E., Huether, G., 2000. Age-associated changes in the densities of presynaptic monoamine transporters in different regions of the rat brain from early juvenile life to late adulthood. Brain Res. Dev. Brain Res. 119, 251–257.
Paxinos, G., Watson, C., 1986. The Rat Brain in Stereotaxic Coordinates. Academic Press, New York.
Petrovich, G.D., Swanson, L.W., 1997. Projections from the lateral part of the central amygdalar nucleus to the postulated fear conditioning circuit. Brain Res. 763, 247–254.
Petrovich, G.D., Risold, P.Y., Swanson, L.W., 1996. Organization of projections from the basomedial nucleus of the amygdala: a PHAL study in the rat. J. Comp. Neurol. 374, 387–420.
Pitkanen, A., 2000. Connectivity of the rat amygdaloid complex. In: Aggleton, J.P. (Ed.), The Amygdala. Oxford Univ. Press, New York, pp. 31–116.
Quirk, G.J., Likhtik, E., Pelletier, J.G., Pare, D., 2003. Stimulation of medial prefrontal cortex decreases the responsiveness of central amygdala output neurons. J. Neurosci. 23, 8800–8807.
Rosenzweig, M.R., Bennett, E.L., 1969. Effects of differential environments on brain weights and enzyme activities in gerbils, rats, and mice. Dev. Psychobiol. 2, 87–95.
Sadikot, A.F., Parent, A., 1990. The monoaminergic innervation of the amygdala in the squirrel monkey: an immunohistochemical study. Neuroscience 36, 431–447.
Santiago, A.C., Shammah-Lagnado, S.J., 2005. Afferent connections of the amygdalopiriform transition area in the rat. J. Comp. Neurol. 489, 349–371.
Sullivan, R.M., Brake, W.G., 2003. What the rodent prefrontal cortex can teach us about attention-deficit/hyperactivity disorder: the critical role of early developmental events on prefrontal function. Behav. Brain Res. 146, 43–55.
Swanson, L.W., 1982. The projections of the ventral tegmental area and adjacent regions: a combined fluorescent retrograde tracer and immunofluorescence study in the rat. Brain Res. Bull. 9, 321–353.
Sweet, R.A., Hamilton, R.L., Healy, M.T., Wisniewski, S.R., Henteleff, R., Pollock, B.G., Lewis, D.A., DeKosky, S.T., 2001. Alterations of striatal dopamine receptor binding in Alzheimer disease are associated with Lewy body pathology and antemortem psychosis. Arch. Neurol. 58, 466–472.
Tarazi, F.I., Baldessarini, R.J., 2000. Comparative postnatal development of dopamine D(1), D(2) and D(4) receptors in rat forebrain. Int. J. Dev. Neurosci. 18, 29–37.
Tarazi, F.I., Tomasini, E.C., Baldessarini, R.J., 1998. Postnatal development of dopamine D4-like receptors in rat forebrain regions: comparison with D2-like receptors. Brain Res. Dev. Brain Res. 110, 227–233.
Tarazi, F.I., Tomasini, E.C., Baldessarini, R.J., 1999. Postnatal development of dopamine D1-like receptors in rat cortical and striatolimbic brain regions: an autoradiographic study. Dev. Neurosci. 21, 43–49.
Teuchert-Noodt, G., 2000. Neuronal degeneration and reorganization: a mutual principle in pathological and in healthy interactions of limbic and prefrontal circuits. J. Neural 315–333.
Troup, G.M., Smith, G.S., Walford, R.L., 1969. Life span, chronologic disease patterns and age-related changes in relative spleen weights for the Mongolian gerbil (*Meriones unguiculatus*). Exp. Gerontol. 4, 139–143.
Uehara, T., Tanii, Y., Sumiyoshi, T., Kurachi, M., 2000. Neonatal lesions of the left entorhinal cortex affect dopamine metabolism in the rat brain. Brain Res. 860, 77–86.
Valverde, F., 1998. Golgi Atlas of the Postnatal Mouse Brain. Springer-Verlag, Wien, New York.
Verney, C., Baulac, M., Berger, B., Alvarez, C., Vigny, A., Helle, K.B.,

1985. Morphological evidence for a dopaminergic terminal field in the hippocampal formation of young and adult rat. Neuroscience 14, 1039–1052.
Witte, A.V., Bagorda, F., Teuchert-Noodt, G., Lehmann, K., 2006. Contralateral prefrontal projections in gerbils mature abnormally after early methamphetamine trauma and isolated rearing. J. Neural Transm. (electronic publication ahead of print).
Yoshida, M., Sakai, M., Kani, K., Nagatsu, I., Tanaka, M., 1988. The dopaminergic innervation as observed by immunohistochemistry using anti-dopamine serum in the rat cerebral cortex. Experientia 44, 700–702.

Accepted by 'International Journal of Developmental Neuroscience'

Manuscript Number: DN-D-06-00062R1

(Date of acceptance: January 4, 2007)

Postnatal development of GABA and Calbindin cells and fibers in the prefrontal cortex and basolateral amygdala of gerbils (*Meriones unguiculatus*)

Susanne Brummelte[1], Veronica Witte[1,2] and Gertraud Teuchert-Noodt[1]

[1]Department of Neuroanatomy, Faculty of Biology, University of Bielefeld, Universitätsstr. 25, D-33615 Bielefeld, Germany

[2] Current address: Department of Neurology, University of Münster, Albert-Schweitzer-Straße 33, 48129 Münster, Germany

Corresponding author:
Susanne Brummelte, Department of Neuroanatomy, Faculty of Biology, University of Bielefeld, Universitaetsstr. 25, D-33615 Bielefeld, Germany, Phone: 0049 521 106 5704 Fax: 0049 521 106 6470 e-mail: sbrummelte@uni-bielefeld.de

Running title: Late development of GABA cells and fibers in the gerbil

Keywords: gamma-amino-butyric acid; calbindin; calcium-binding proteins; immunohistochemistry; development; limbic system

Abbrevations used: GABA: gamma-amino-butyric acid; CB: calbindin; PFC: prefrontal cortex; mPFC: medial prefrontal cortex; BLA: basolateral amygdala; PD: postnatal day; PV: parvalbumin

Abstract

The postnatal maturation of immunohistochemically stained gamma-amino-butyric acid (GABA) and calbindin (CB) cells and fibers were quantitatively examined in the prefrontal cortex (PFC) and the basolateral amygdala (BLA) of the Mongolian gerbil (*Meriones unguiculatus*). Animals of different ages, ranging from juvenile (postnatal day (PD)14, PD20, PD30), to adolescent (PD70), adult (PD180, PD540) and aged (PD720) were analyzed. Results reveal an increase in GABAergic fiber densities between PD14-20 in the PFC and the BLA with a concomitant decrease in cell density. After PD70 GABA fiber density slightly decreases again in the BLA, while there is a further slow but significant increase in the PFC between PD70-PD540. Fibers immunoreactive for the calcium binding-protein CB, which is predominantly localized in particular GABAergic subpopulations, also accumulate between PD14-PD20 in the PFC and BLA, while a concomitant decrease in cell density is only seen in the BLA. Both areas reveal a decrease of CB cells between PD30-PD70, which parallels with a decrease of CB fibers in the PFC. However, there is no particular 'aging-effect' in the fiber or cell densities of GABA or CB in any of the investigated areas in old animals.

In conclusion, we here demonstrate long-term dynamics in cell and fiber densities of the GABAergic system until late in development which might correspond to the prolonged maturation of other neuroanatomical and functional systems.

1. Introductory statement

Gamma-amino-butyric acid (GABA) is probably the most important inhibitory neurotransmitter in the mammalian nervous system. It is usually expressed in local interneurons, which can modulate and even control the neuronal activity of cortical and subcortical output neurons. Further, GABA has been shown to exert important morphogenetic influences during development (Chronwall and Wolff, 1980; Nguyen et al., 2001) and to play an essential role in reactive plasticity and reorganization processes during development and adulthood (Dawirs et al., 1997; Hensch, 2005; Merzenich et al., 1983; Zito and Svoboda, 2002). Thus, GABA has a central part in shaping and maintaining of neuronal networks.

Within the GABAergic population several classes of subpopulation can be distinguished according to their content of calcium-binding proteins (Baimbridge et al., 1992) and corresponding different maturation patterns. One of these proteins is calbindin (CB) which is e.g. found in Marinotti, Neuroglia and Double Bouquet cells within the cortex, i.e. in cells, that primarily innervate distal parts and spines of pyramidal dendrites (Conde et al., 1994; DeFelipe et al., 1989; Gabbott and Bacon, 1996; Lund and Lewis, 1993) and appear and mature relatively early (Alcantara et al., 1993). In the amygdala, CB cells are distributed differently in the various nuclei (Kemppainen and Pitkanen, 2000), but in contrast to the prefrontal cortex (PFC), CB varicosities are found in the basolateral amygdala (BLA) to form basket-like structures around unlabelled projection neuron somata (Berdel and Morys, 2000; Kemppainen and Pitkanen, 2000; Legaz et al., 2005; Muller et al., 2003). This points to the particularly interesting role of CB in this subcortical area, as it is widely known, that axo-somatic synapses have an exceptionally powerful control over target neurons compared to distal dendritic or spine contacts. In the cortex, these baskets are usually built by GABAergic cells containing parvalbumin, another calcium-binding protein, or other substances such as cholecystokinin (Conde et al., 1994; Kawaguchi and Kubota, 1998).

Despite this difference, the GABAergic innervation patterns of the PFC and the BLA bear marked resemblances (Carlsen, 1988; Muller et al., 2006), although the origin and function of the PFC and the BLA are quite diverse, which is a reason for choosing these particular two structures for investigation in the current study. Further reasons are the high interconnection and thus potential interrelation of the PFC and BLA during development and their distant positions in the brain, which imply divergent developmental patterns. In addition, both areas belong to one main circuit, characterized by the mesolimbic prefrontal dopamine projections, which originate in the ventral tegmental area and the substantia nigra (Björklund and Lindvall, 1984; Fallon and Ciofi, 1992). This dopamine fiber innervation is of particular interest as it shows a prolonged maturation until adulthood in the rodent medial PFC (mPFC) (Dawirs et al., 1993; Kalsbeek et al., 1988), while it stays relatively stable after PD 20 in the gerbil amygdala or entorhinal cortex (Brummelte and Teuchert-Noodt, 2006). This is in line with the general developing pattern, with the PFC being one of the last areas to reach adult stages (Mrzljak et al., 1990; Van Eden et al., 1990), while the amygdala maturates relatively early after birth (Joseph, 1999; Morys et al., 1999).

The prenatal and early postnatal maturation of the GABAergic population in the cortex, with particular emphasis on the visual cortex, has been intensively investigated in the last two decades (Chronwall and Wolff, 1981; Del Rio et al., 1992; Parnavelas, 1992; Van Eden et al., 1989; Wolff et al., 1984). However, less research has been done concerning the late postnatal development and aging effects of GABAergic and CB fibers and concerning different cortical or subcortical areas. It is assumed that GABA exhibits a high synaptic plasticity and can help to reorganize, shape and modulate neuronal circuits not only during development (Chen et al., 2002; Teuchert-Noodt, 2000). This compensatory effect in plastic processes might be reflected in changes of the GABAergic or CB fiber densities even during adulthood and aging. As it is further supposed, that the cortex might continuously adapt to new situations and experiences by (re)arranging neuronal networks (Bagorda et al., 2006; Holtmaat et al.,

2006; Trachtenberg et al., 2002), the current study was conducted to examine the life long progression of GABAergic and CB structures in two areas of the mesolimbocortical circuit, the mPFC and BLA.

2. Experimental procedures

A total of 60 male Mongolian gerbils (*Meriones unguiculatus*) were used for this study. Breeding gerbils were obtained from Harlan Winkelmann (Borchen, Germany). The animals were bred in standard cages (Macrolon type 4) and, after weaning on postnatal day (PD) 30, were reared individually in standard cages (Macrolon type 3). All gerbils were kept under natural day/night cycles with food and water being provided *ad libitum*. Seven experimental animal groups of different ages were investigated to cover convincing periods of the life span of gerbils: PD14 (n=11), PD 20 (n=6) (juvenile), PD30 (n=12) (weaning), PD70 (n=11) (young adult), PD180 (n=8), PD540 (n=8) (adult) and PD720 (n=4) (aging). Gerbils were chosen due to their very small genetic variability (Thiessen and Yahr, 1977), and their rich wild-type like behavioral repertoire (Rosenzweig and Bennett, 1969). All experimental procedures were approved by the appropriate committee for animal care in accordance with the European Communities Council Directive.

Immunohistochemistry

Animals were transcardially perfused under deep chloralhydrate anesthesia (1.7g/kg, i.p.). The perfusion was performed with 200ml 0.05M phosphate buffer (pH 6.2), containing 1% sodium metabisulfite, followed by 750ml 5% glutaraldehyde with 1% sodium metabisulfite in 0.1M phosphate buffer (pH 7.5), with appropriate amounts of solutions for younger animals. Immediately after perfusion, the brains were removed and postfixed for 30 min. Coronar sections of 50µm were cut with a vibratome (Vibratome Series 1000, Technical Products International

Inc.) of which every 3rd was used for GABA and CB immunostaining, respectively. For GABA staining sections were collected in wash buffer at 4°C and rinsed 3x 10min followed by a preincubation in 10% normal goat serum and 0.4% Triton X-100 (Sigma) for 30min. Subsequently, the sections were incubated with rabbit anti-GABA (ImmunoStar, Hudson, WI), diluted 1: 5000 with 1% normal goat serum and 0.4% Triton X- 100 for 48h. Sections used for CB staining were treated in almost the same manner, but collected and rinsed in 0.05M tris- HCL buffered saline (pH 7.5, TBS), and were additionally incubated in 1% H_2O_2 for 10 min. The primary antibody was mouse anti-calbindin (Sigma, diluted 1:3000, for 18h). The following rinses, all three times for 10min, and dilutions were all done in TBS. The sections were rinsed and incubated for 30min in biotinylated goat anti-rabbit antibody (Sigma) for GABA and biotinylated goat-anti-mouse antibody (Sigma) for CB staining, respectively, diluted 1:20 with 1% normal goat serum, rinsed again and incubated with ExtraAvidin-Peroxidase (Sigma) diluted 1:20 for 30min. After another rinse the sections were stained in 0.05% 3.3-diaminobenzidine (Sigma) with 0.01% H_2O_2 for 4min. Then the sections were washed, mounted on glass slides, dried overnight, dehydrated with ethanol, cleared with xylene and cover slipped with DePeX (Serva, Heidelberg, Germany). To avoid deviations due to possibly lateralized innervation densities of GABA and CB only right hemispheres were used for analyses.

For quantification of fiber densities, brain sections were chosen in areas of interest by means of anatomical characteristics according to brain atlases of the rat (Paxinos and Watson, 1986) and the mouse (Valverde, 1998). The BLA and mPFC subregions Cg1 and Cg3, with the latter being further divided into layer III and layer V, were chosen for investigation due to the clear presence of GABAergic and CB fibers and cells. The average number of analyzed sections was 5 per animal and region. In the defined region of each section all detectable fiber fragments were visualized in standard test fields using a bright field microscope (BX61, Olympus, Hamburg, Germany) and a digital camera for microscopy (ColorView II, SIS,

Münster, Germany). Calbindin sections were investigated using 200-fold magnification, GABA sections at 600-fold magnification.

To account for a possible interaction of fiber density and cell density or size of the investigated area, these parameters were measured additionally for the PFC and BLA at 200-fold and 20-fold magnification, respectively. Digital images were adjusted in contrast and intensity before fibers, cells or the size of the area were quantified by software for image analysis (KS300, Jenoptik, Jena, Germany). For further details of the quantification process see (Brummelte et al., 2006a; Brummelte and Teuchert-Noodt, 2006). The fiber density was calculated as a percentage of the evaluated test area, the cell density as number of cells per test area. Lightly stained cells (cf. qualitative results) were excluded in the counting by a minimum threshold of gray values for cell recognizing. All analyses were done by a person blind to the age of individual animals.

Data analysis

Measurements were computed as arithmetic means by-case and by-group ± S.E.M. The overall size of the particular area in which fiber densities were measured as well as the number of GABA or CB cells were integrated as covariates in the statistical analysis to account for a possible interaction of an augmentation of volume or cells and fiber sprouting. For the PFC, a two-way analysis of covariance (ANCOVA) with age (7 levels) and area (3 levels) as independent variables, GABA or CB as dependent variable and area size and GABA or CB cell number as covariates were used to check for statistical significance between groups, followed by Fisher LSD post-hoc test for multiple comparisons if appropriate. For the BLA, the ANCOVA comprised only one area level. As the covariates revealed some significant effects on the fiber development, these parameters were also statistically analyzed using an ANCOVA (cell number, size as covariate) or ANOVA (size) and subsequent LSD post-hoc tests. Statistical analysis was computed with Statistica 6

(StatSoft, Tulsa, USA). The levels of significance were set at * $p<0.05$, ** $p<0.01$ and *** $p<0.001$.

3. Results

Qualitative observations

The GABAergic fiber innervation is equally dense in all investigated areas, while the CB fibers are more present in the BLA compared to the mPFC. The overall distribution *pattern* of GABAergic and CB fibers, however, is similar in animals from all age stages (cf. Fig. 1). PFC and BLA contain a population of lightly stained CB pyramidal neurons, which has been previously observed in rats, too (Celio, 1990; Kemppainen and Pitkanen, 2000). In the PFC these cells are arranged in a bundle throughout lamina II (Fig.1 B.1). In concert with data from other species (Hof et al., 1999) hardly any CB or GABA cells were seen in lamina I. In the BLA, GABAergic and CB cells were distributed quite equally through the nucleus. We could not detect clear CB basket-like structures in the BLA at light microscope level, although these have been described for rats (Berdel and Morys, 2000).

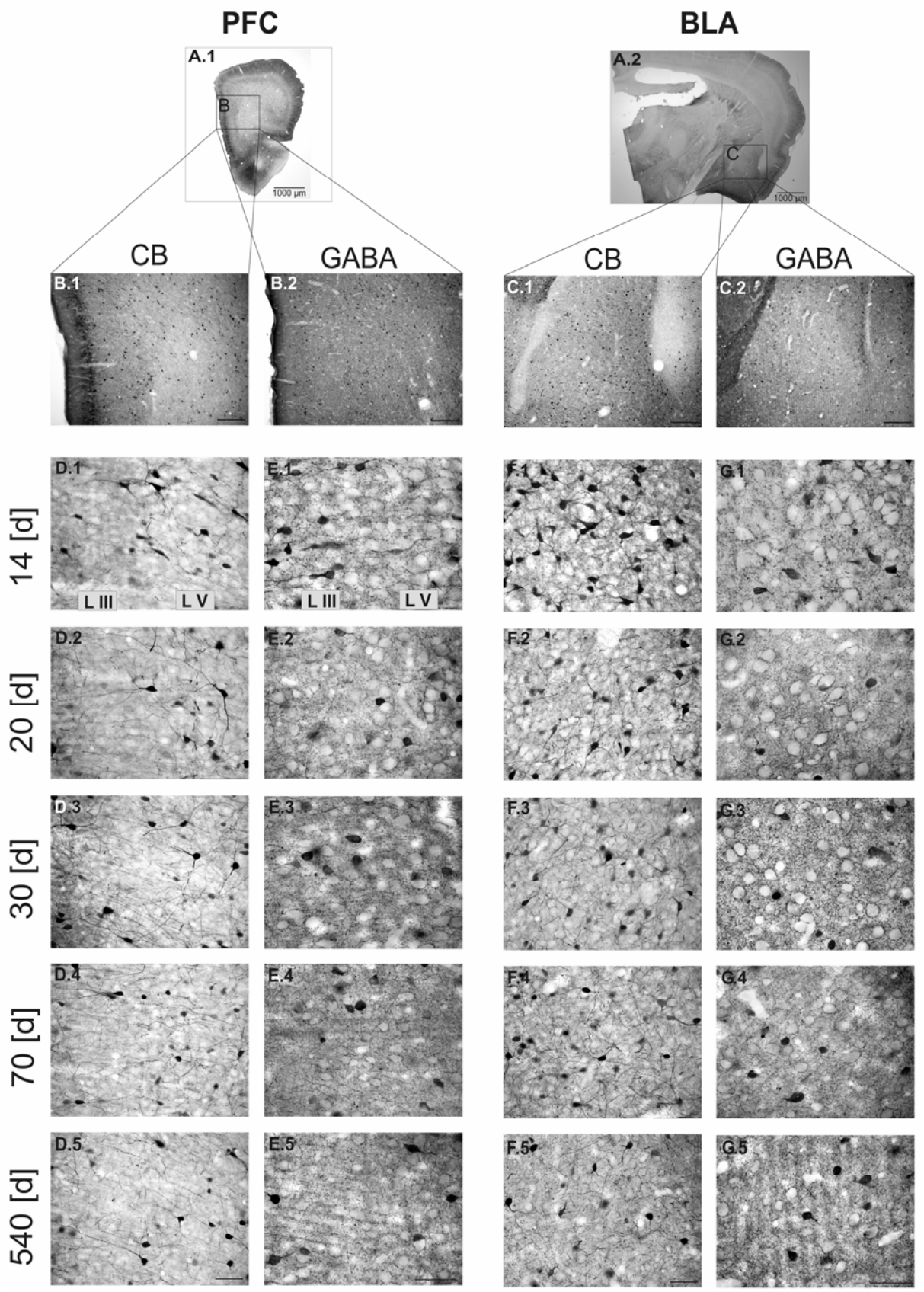


Fig. 1: Representative photomicrographs of the GABAergic and Calbindin (CB) distribution of the medial prefrontal cortex (mPFC) and the basolateral amygdala (BLA) at different age stages.
A.1 and A.2 are photomicrographs of representative coronal sections at the level of the PFC and amygdala, respectively. The areas of the rectangles are magnified in panels B.1 – C.2 for CB and GABA. Pictures (D1)–(G5) show examples of GABA and CB structures at juvenile age stages (PD14–

PD30), adolescence (PD 70) and a comparative section from an adult animal (PD540) in lamina III and V of the Cg3 region and in the BLA. Scale bars: 200 μm (B.1-C.2), 50 μm (D1–G5).

Quantitative analysis

PFC

For the GABAergic fiber density in the PFC the two-way analysis for variance reveals a highly significant effect of age ($F(6,147) = 16.67$; $p<.001$), but not for area or interaction of age and area ($F(12,147) = .168$; $p=.99$). Both covariates show a significant effect on the fiber development (GABA cells: $p=.007$; size: $p=.016$). A subsequent Fisher LSD test exhibits a significant increase in fiber density between PD14-PD20 (+11%; $p<.001$) and between PD20-PD30 (+5%; $p=.037$) and a further trend for an increase between PD70-PD180 ($p=.078$), which becomes significant compared to PD 540 (PD70-PD540: +7%; $p<.001$) (Fig. 2A).

For the CB fibers in the PFC the ANCOVA reveals a significant effect of age ($F(6,148) = 23.94$; $p<.001$) and area ($F(2, 148) = 6.26$; $p=.002$) but not for the interaction of age and area. Size as a covariate shows a significant effect ($p=.005$), while the CB cell number narrowly fails to reach a significant level ($p=.054$). The subsequent LSD test for the area effect reveals a significant difference in innervation density between the Cg1 area and Cg3 lamina III and between lamina III and lamina V within the Cg3 region (both p's<.001). Further, a post-hoc test shows a highly significant increase of fibers between PD14-PD20 (+43%; $p<.001$), followed by slight decrease between PD30-PD70 (-8%; $p=.017$) and PD180-PD540 (-13%; $p=.003$) (Fig. 2B).

Due to the significant effect of the covariates, the cell numbers were additionally analyzed and a two-way analysis of covariance reveals a significant effect of area ($F(4, 294) = 85.38$; $p<.001$), with all areas being significantly different from each other concerning GABA and CB cells (all p's <.05). Age also shows a significant effect ($F(12, 294) =17.0$; $p<.001$) with GABA cells exhibiting a decrease between PD14-PD20 (-41%; $p<.001$), followed by an

increase between PD20-PD30 (+26%; p=.001) and another decrease after PD30 (-11%; p=.035). The last effect could also be seen in the CB cell density (-21%; p<.001) (Fig. 2).

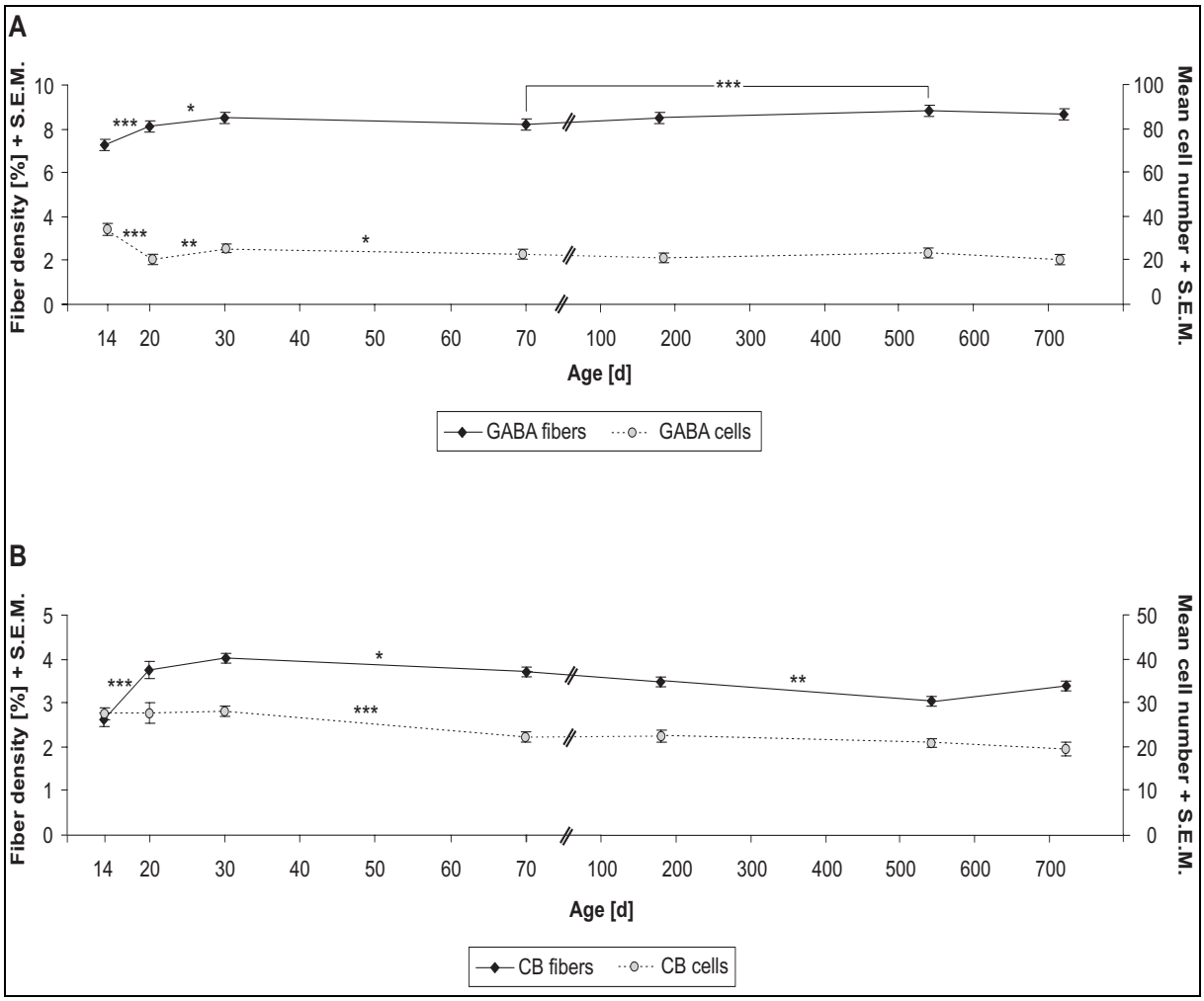


Fig. 2: Postnatal development of GABA (**A**) and Calbindin (CB) (**B**) cells and fibers in the **prefrontal cortex (PFC)**.
Shown are the fibers density means as percentage [%] and the cell number means, respectively, of the reference area + S.E.M at postnatal day 14, 20, 30, 70, 180, 540 and 720. The double bar in the middle marks a break in the scaling of the x-axis. * < 0.05, **p <0.01, ***p <0.001.

Size as a covariate has again a significant influence (p=.013) and when therefore analyzed separately it reveals a reverse peak with an increase between PD14-PD20 (+8%; p<.001) and a subsequent decline until PD30 (-7%; p=.003) (Table 1). As there was no interaction effect for age and area in any of the analyses, the line plots (Fig. 2 and 3) show the overall values for

the PFC. The separate data for the different investigated areas of the PFC are presented in table 2 and 3.

BLA

GABAergic fibers in the BLA show a significant effect of age ($F(6, 49) = 8.84$; $p<.001$) with none of the covariates showing a significant contribution. The LSD post-hoc test reveals a highly significant increase between PD14-PD20 (+15%; $p<.001$) and a tendency for a decline after PD70 (PD70-PD180: $p=.073$), showing a significance compared to PD540 (-7%; $p=.012$). CB fibers in the BLA reveal no significant age-effect ($p=.21$), however, the CB cell number as a covariate exhibits a significance ($p=.031$).

An analysis of covariance of the cell numbers in the BLA shows a significant effect of age ($F(12, 98) = 8.85$; $p<.001$) with no effect of size. A following LSD test further reveals a decrease of GABAergic cells (-14%; $p=.025$) and CB cells (-18%; $p=.001$) between PD14-PD20 with a further slow decrease until PD70 in the GABAergic population (PD20-PD70 -21%; $p=.007$) and a more steep decline in the CB cell density (PD30-PD70: -23%; $p<.001$). In addition, the CB cell number decreased between PD180-PD540 (-14%; $p=.036$) (Fig. 3).

AGE	PFC	BLA
14 [d]	8241.93 ± 349.47 µm	3949.28 ± 36.90 µm
20 [d]	8367.58 ± 561.35 µm ***	4045.24 ± 98.24 µm
30 [d]	8248.79 ± 365.93 µm **	4019.70 ± 30.63 µm
70 [d]	8226.65 ± 389.70 µm	4106.80 ± 78.18 µm
180 [d]	8307.00 ± 340.07 µm	4193.11 ± 112.2 µm
540 [d]	8262.83 ± 382.16 µm	4325.24 ± 60.86 µm
720 [d]	8225.94 ± 491.41 µm	4218.94 ± 85.76 µm

Table 1: Circumferences of the two analyzed areas: the prefrontal cortex (PFC) and the basolateral amygdala (BLA) + S.E.M.
The PFC reveals a peak in volume on postnatal day 20, while there is no age-dependent effect in the BLA. Levels of significance, compared to the age stage before: **p <0.01, ***p <0.001.

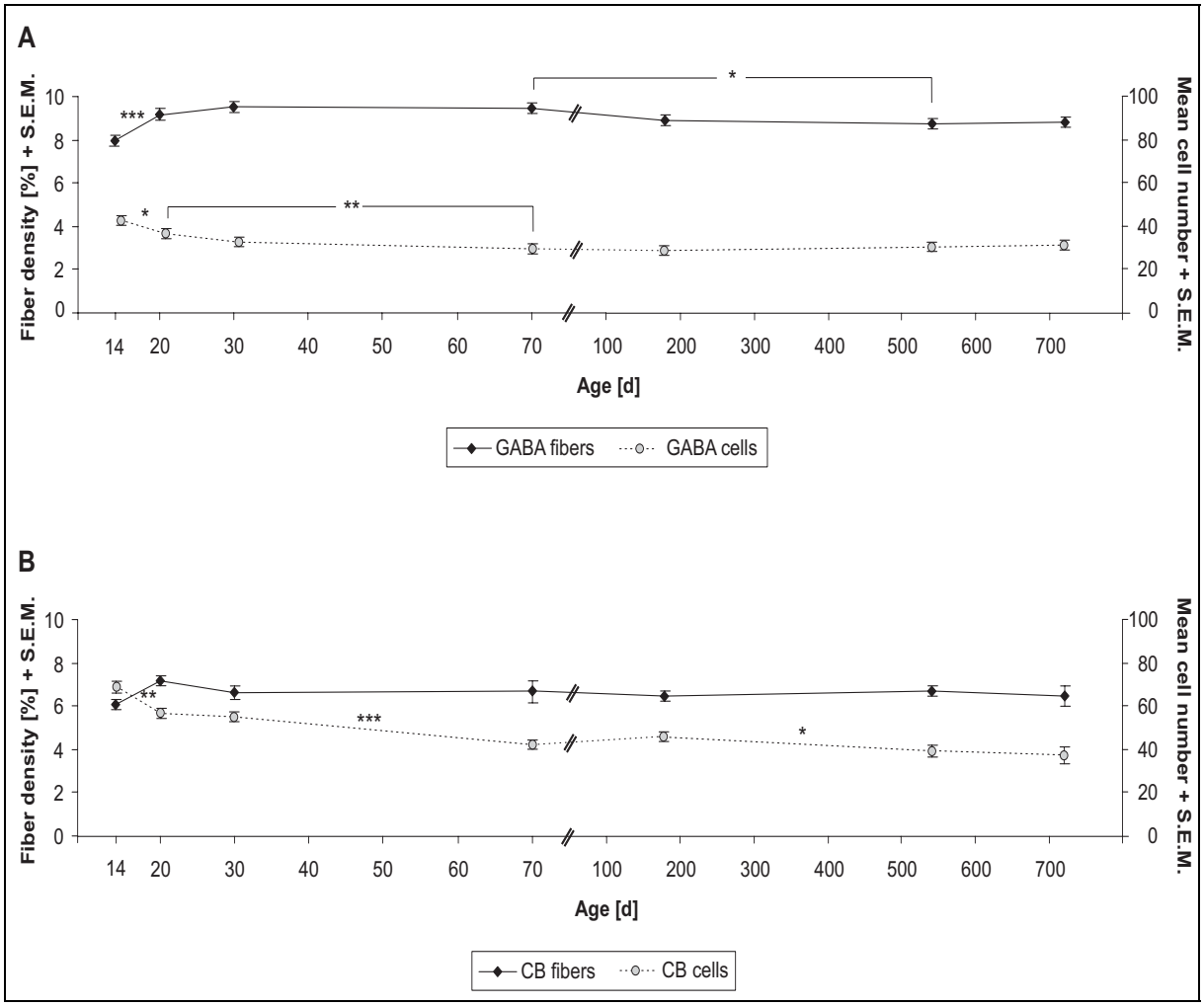


Fig. 3: Postnatal development of GABA (**A**) and Calbindin (CB) (**B**) cells and fibers in the **basolateral amygdala (BLA)**.
Shown are the fibers density means as percentage [%] and the cell number means, respectively, of the reference area + S.E.M at postnatal day 14, 20, 30, 70, 180, 540 and 720. The double bar in the middle marks a break in the scaling of the x-axis. * < 0.05, **p <0.01, ***p <0.001.

AGE	GABA				CB		
	Cg1	Cg3 L III	Cg3 L V		Cg1	Cg3 L III	Cg3 L V
14 [d]	7.31 ± 0.19	7.04 ± 0.27	7.52 ± 0.21		2.76 ± 0.26	2.02 ± 0.16	3.07 ± 0.33
20 [d]	8.16 ± 0.25	7.84 ± 0.26	8.26 ± 0.14		3.75 ± 0.24	3.29 ± 0.21	4.22 ± 0.38
30 [d]	8.52 ± 0.25	8.35 ± 0.23	8.57 ± 0.17		3.95 ± 0.15	3.80 ± 0.10	4.29 ± 0.19
70 [d]	8.22 ± 0.24	8.15 ± 0.28	8.34 ± 0.19		3.84 ± 0.19	3.50 ± 0.16	3.76 ± 0.17
180 [d]	8.59 ± 0.15	8.29 ± 0.15	8.71 ± 0.11		3.62 ± 0.11	3.57 ± 0.13	3.30 ± 0.12
540 [d]	8.88 ± 0.16	8.75 ± 0.09	8.83 ± 0.19		3.35 ± 0.09	3.02 ± 0.09	2.79 ± 0.05
720 [d]	8.82 ± 0.07	8.58 ± 0.04	8.63 ± 0.12		3.59 ± 0.09	3.29 ± 0.11	3.34 ± 0.07
Mean	**8.31 ± 0.11**	**8.09 ± 0.11**	**8.38 ± 0.09**		**3.55 ± 0.09**	**3.21 ± 0.10***	**3.56 ± 0.11**

Table 2: GABA and CB fiber densities in the various areas within the prefrontal cortex, namely the Cg1 region and lamina (L) III and V of the Cg3 region + S.E.M.
There was a difference in the mean innervation density concerning the CB fibers in lamina III of the Cg3 region compared to the other two areas (* p < 0.001).

AGE	GABA				CB		
	Cg1	Cg3 L III	Cg3 L V		Cg1	Cg3 L III	Cg3 L V
14 [d]	26.42 ± 6.97	23.54 ± 8.54	47.03 ± 10.2		23.86 ±1.57	22.14 ± 1.12	36.72 ± 1.69
20 [d]	15.90 ± 2.65	10.90 ± 2.11	29.65 ± 5.99		23.38 ± 1.56	21.18 ± 2.11	38.65 ± 3.27
30 [d]	18.99 ± 3.69	16.21 ± 3.27	36.23 ± 5.97		24.42 ± 0.62	22.86 ± 1.09	37.09 ± 0.99
70 [d]	16.21 ± 4.05	14.52 ± 4.98	32.86 ± 5.02		19.16 ± 1.16	18.11 ± 1.37	29.27 ± 2.06
180 [d]	16.26 ± 1.50	10.68 ± 1.95	29.21 ± 3.23		20.35 ± 1.42	18.35 ± 1.76	28.76 ± 1.49
540 [d]	16.66 ± 1.83	16.29 ± 2.89	29.91 ± 3.14		21.57 ± 1.09	16.42 ± 0.76	24.83 ± 1.70
720 [d]	14.78 ± 1.85	12.50 ± 1.58	26.81 ± 6.04		18.54 ± 2.10	16.60 ± 2.24	23.77 ± 3.22
Mean	**18.53 ± 5.43***	**15.70 ± 6.17***	**34.42 ± 8.80***		**21.93 ± 0.54***	**19.78 ± 0.60***	**31.96 ± 0.97***

Table 3: GABA and CB cell densities in the various areas within the prefrontal cortex.
Lamina V of the Cg3 region revealed the highest cell density and lamina III the lowest. All areas were significantly different from each other (* $p < 0.01$).

4. Discussion

The current study provides first data for GABAergic and Calbindin (CB) cell and fiber densities in two prominent structures of the mesolimbocortical circuit from the juvenile period to aging in the Mongolian gerbil. The fluctuations in fiber densities might in part be due to variances in the cell numbers or expansions of the reference area as these parameters reveal significant contributions in the analyses of covariance. For instance, the PFC exhibits a peak in volume around PD20, which has been reported for rats before (Van Eden and Uylings, 1985), and which is accompanied by a low level of GABAergic cells. Interestingly, fiber densities in general tend to increase, although the according cell densities decrease during development. We additionally report long-term dynamic variations of the GABAergic fiber system in the gerbil brain, which are probably independent of the early changes in cell number or volume.

Species- and area-specific maturation of GABAergic cell and fiber densities

Previous prenatal, and early postnatal investigations have shown, that GABAergic cells appear in the rodent visual or somato-sensory cortex as early as embryonic day (ED) 14-16 (Chronwall and Wolff, 1980; Del Rio et al., 1992) and that there is no apparent change in cell density after the third postnatal week (Chronwall and Wolff, 1980). CB cells have also been observed to appear prenatally in the rat cortex and increase until PD 8-11, but their number seems to decrease notably between PD11-15 while reaching adult levels around the end of the third postnatal week (Alcantara et al., 1993). In the amygdala first CB cells appeared around ED13 in the mouse (Legaz et al., 2005), and were observed on ED 20 in the rat, where they reached adult levels around PD20 (Berdel and Morys, 2000).

These previous observations are in part at variance with our present results from the Mongolian gerbil. One first explanation for this might be the different developmental pattern of gerbils compared to rats or mice. The gerbil is known to develop its auditory and visual capacity later than the rat (Seto-Ohshima et al., 1990) and to lag behind about 2 weeks in reaching its sexual maturity. In addition, the dopaminergic innervation of the mPFC shows a prolonged maturation until PD 60 in the rat (Kalsbeek et al., 1988), while dopaminergic afferents continue to grow until PD 90 in the gerbil (Dawirs et al., 1993). Thus, the partially highly significant increase in GABAergic and CB fiber densities until PD30 and the fluctuations in the cell densities we observed in the mPFC and the BLA in the present study might indicate the postponed maturation of the gerbil nervous system compared to other rodents and the later onset of functional systems.

The apparent discrepancy of former and our present results might further be due to the developmental differences of particular areas. Thus, calcium-binding proteins occur several days later in the associative cortices compared to the primary visual cortex (Alcantara et al., 1993). In addition, Wolff and colleagues (1984) found no notable difference in GABA cell proportion after P3 in the layers II-VI in the visual cortex, while Vincent and colleagues

(1995) found a decrease of GABA cell density until PD 15 in the mPFC of rats. They further suggest that this decrease is associated with an expansion of the cortex. The severe decrease of GABA cells between PD14-PD20 in the gerbil might therefore be due to the peak in mPFC volume around PD20. Intriguingly, we did not observe such a decline in CB cell density during this particular time. This might depend on a variation of the amount of the protein within the cells, so that despite the decreasing number of GABAergic cells more of the remaining cells expressed enough CB to reach the minimum gray value for cell counting. The inverse effect might also account for the later decline in the CB number (PD30-PD70), which would be in line with the hypotheses that one population of CB cells only expresses the protein transitorily, while the other neurons are permanently immunoreactive for CB (Alcantara et al., 1993).

The maturation of GABAergic fibers in the BLA seems to differ slightly from the cortex. One reason for this could be the different targets and functions of the subpopulations in the cortex and the amygdala, as for instance, CB cells have been shown to build basket like structures in the BLA (Berdel and Morys, 2000) but not in the cortex. Further, CB interneurons in the cortex rather present a minor subpopulation (Celio, 1990), while they constitute almost 60% of the GABA-containing population in the BLA (McDonald and Mascagni, 2001) which would be in line with our observation of a higher CB fiber density in the BLA compared to the cortex. In addition, another 60% of these CB neurons have been shown to also coexpress another calcium-binding protein, parvalbumin (PV), permanently (McDonald and Betette, 2001), while such a coexpression is only transiently observed in the cortex (Alcantara et al., 1996). This might explain the existence of CB baskets around unlabelled pyramidal neurons in the BLA and further hints to the particular role of the various calcium-binding proteins in different subpopulations of the brain.

GABA plasticity from adolescence to aging

Regardless of fluctuations in cell densities, there are prolonged variations in fiber densities and thus in the inhibitory networks of the particular areas during adolescence and even adulthood. Generally it is thought that local circuit neuron connections mature relatively late compared to projections from efferent neurons (Miller, 1988). In addition, GABA appears to exert direct and indirect trophic action and thus initiate the establishment of synaptic contacts such as excitatory synapses, which usually appear 1-4 days after the GABA cells (Wolff et al., 1978; 1993). The synaptogenesis of inhibitory GABAergic boutons seems to be even further delayed and continues well into adulthood (Bahr and Wolff, 1985; Lewis et al., 2005), which would be in line with a continuing augmentation of fibers.

GABA is known to undergo a shift from an excitatory transmitter before birth into an inhibitory transmitter after birth (Ben-Ari, 2002; Cherubini et al., 1991; Ganguly et al., 2001). Further, there is a potential postnatal shift between the different GABAergic subpopulations with a decrease in CB immunoreactivity, which is usually accompanied by the appearance of PV-positive structures in various areas of the brain (Cruz et al., 2003; Davila et al., 2005; Erickson and Lewis, 2002; Legaz et al., 2005). PV cells mature considerably late during development (Alcantara et al., 1993), e.g. in the gerbil, first PV neurons appear around PD 14 in the mPFC, though their number is very small (unpublished data), and are known to build axo-somatic contacts and basket like boutons around pyramidal somata, which likewise appear considerably late (Bahr and Wolff, 1985). These types of connections have a particularly powerful influence on the firing activity and synchronization of target neurons (Freund, 2003; Gibson et al., 1999; Klausberger et al., 2003; Miles et al., 1996; Tamas et al., 1997; Tamas et al., 2000). Such oscillatory (rhythmic) synchronization is for instance generated by a BLA PV network during emotional arousal (Muller et al., 2005) and is further believed to create the necessary temporal and spatial frame for functions such as working memory in the PFC (Constantinidis et al., 2002; Lewis et al., 2005) or consolidation of

emotional memories in the amygdala (McDonald and Mascagni, 2004). In addition, it has been assumed that morphological changes in response to learning stimuli may include a shift of synapses nearer to neuronal somata (Murakami et al., 1988). Taken together, these evidences underline the importance of somatic and axonic inhibitory synapses, although the majority of GABAergic contacts terminate on dendrites or spines of the postsynaptic cells (Beaulieu et al., 1992; Beaulieu and Somogyi, 1990; Nitsch and Riesenberg, 1995), which in turn emphasizes the general importance of understanding the involvement of the GABAergic system and its different subpopulations in neuronal circuits and plasticity.

Different transmitter systems have been shown to exhibit high plastic potentials during adolescence and adulthood and thus contribute to the shaping or remodeling of neuronal circuits. For instance, the dopaminergic innervation modulates neuronal out-put activity by directly terminating on glutamatergic projection neurons in the PFC and amygdala and indirectly via GABAergic interneurons (Asan, 1998; Brinley-Reed and McDonald, 1999; Sesack et al., 1995) and thus may have a particularly important part in shaping neuronal connectivity. A similar innervation pattern was revealed for the BLA input to the PFC, which connects to pyramidal spines as well as to GABAergic local circuit neurons (Bacon et al., 1996; Gabbott et al., 2006) and thus may also be essential for the establishment of neuronal circuits. It becomes apparent that irrespective of the art of input, the GABAergic transmitter system seems to be generally perfectly positioned to mediate between the various incoming projections and the efferents. As the dopaminergic innervation continues to grow into the PFC during adolescence (Dawirs et al., 1993; Kalsbeek et al., 1988) and the connections from the BLA to the PFC also mature relatively late compared to other connections arising from the amygdala (Diergaarde et al., 2005), it seems likely that local interneurons might continue to adapt to the changing input by enlarging or rearranging their fiber densities. It has already been assumed, that the late development of the local circuit neurons and the subsequent remodeling of networks may provide a morphological basis for functional plasticity in mature

cortical neurons (Miller, 1988) and thus it might even contribute to complex processes as long-term learning and memory.

As we could recently show in our laboratory using an animal model of early traumatized gerbils, epigenetic disturbances during development can cause a shift within the GABAergic system, with a loss of GABAergic boutons around pyramidal somata and an increase in lamina I/II GABAergic fibers in the mPFC of adult animals (Brummelte et al., 2006b). The lessening of somatic inhibition and the potential subsequent interference of the synchronization of whole pyramidal populations might contribute to the observed deficits in PFC-related behaviors and functions such as working memory after this early developmental disturbance (Dawirs et al., 1996). In addition, GABA has also been shown to exhibit a high plasticity when challenged in adult animals (Dawirs et al., 1997). Therefore the question arose, if GABA keeps its natural neuroplastic potential even up to adulthood, especially as it is believed that disturbances in the GABAergic inhibitory regulation of cortical networks contribute considerably to cognitive impairments as seen in schizophrenia (Benes and Berretta, 2001), which's onset is usually in young adulthood. We here demonstrated that there are indeed long-term variations in the GABAergic system during adolescence.

Intriguingly, there was no aging-related change in the fiber densities of GABA or CB in neither the PFC nor the BLA. Several studies have reported about an age-related decrease in CB immunoreactivity in basal forebrain cholinergic cells (Geula et al., 2003; Wu et al., 1997; 2003) and also about age-related changes of CB structures in some cortical and subcortical areas (Bu et al., 2003; Hwang et al., 2002; Kishimoto et al., 1998). Further, several studies have shown alteration in the CB immunoreactivity in Alzheimer patients compared to controls (Ichimiya et al., 1988; Lally et al., 1997; McLachlan et al., 1987). However, further investigations suggest, that it might rather be a decrease in the expression of the protein than a decline of whole cells or branches (Kishimoto et al., 1998). Nevertheless, a decline in CB within the cell might cause a diminished capacity to buffer high levels of calcium, thus

leading to a higher vulnerability towards pathological processes that might cause the degeneration of the cell in the end (Bu et al., 2003). However, to our knowledge, there is no study revealing a significant age-related effect in CB immunoreactivity for the PFC or BLA. But there are other hints for alterations within the GABAergic system as e.g. differences in GABA activity in specific hypothalamic areas (Jarry et al., 1999) or age-related changes in GABA receptor compositions (Caspary et al., 1999). Such changes can not be excluded in the old gerbil referring to our data, but there is apparently no alteration in fiber densities. However, we have to admit, that 110 weeks is the mean survival of a male gerbil, meaning that in the individual case the maximum age lies higher (Troup et al., 1969), thus our results of stable GABAergic and CB fiber densities up to PD720 do not exclude variations in still older animals.

Nevertheless, GABA seems to appear relatively consistent against the deleterious effects of age compared to other transmitters as e.g. dopamine, which is believed to play a role in various age-related diseases such as Alzheimer, Parkinson's or Huntington disease (reviewed in: Backman and Farde, 2001; Morgan et al., 1987; Ossowska, 1993) and shows a decline of fibers in 720 days old gerbils in the PFC (unpublished data) but not in the amygdala (Brummelte and Teuchert-Noodt, 2006). On a highly speculative level it might be assumed that neurodegenerative diseases are likely to appear when the GABAergic plasticity finally vanishes during aging.

Acknowledgments

The authors wish to thank Dr. F. Bagorda and U. Schroeder for excellent technical assistance.

Reference List

Alcantara,S., de,Lecea.L., Del Rio,J.A., Ferrer,I., and Soriano,E. (1996). Transient colocalization of parvalbumin and calbindin D28k in the postnatal cerebral cortex: evidence for a phenotypic shift in developing nonpyramidal neurons. Eur. J. Neurosci. *8*, 1329-1339.

Alcantara,S., Ferrer,I., and Soriano,E. (1993). Postnatal development of parvalbumin and calbindin D28K immunoreactivities in the cerebral cortex of the rat. Anat. Embryol. (Berl) *188*, 63-73.

Asan,E. (1998). The catecholaminergic innervation of the rat amygdala. Adv. Anat. Embryol. Cell Biol. *142*, 1-118.

Backman,L., and Farde,L. (2001). Dopamine and cognitive functioning: brain imaging findings in Huntington's disease and normal aging. Scand. J. Psychol. *42*, 287-296.

Bacon,S.J., Headlam,A.J., Gabbott,P.L., and Smith,A.D. (1996). Amygdala input to medial prefrontal cortex (mPFC) in the rat: a light and electron microscope study. Brain Res. *720*, 211-219.

Bagorda,F., Teuchert-Noodt,G., and Lehmann,K. (2006). Isolation rearing or methamphetamine traumatisation induce a "dysconnection" of prefrontal efferents in gerbils: implications for schizophrenia. J Neural Transm. *113*, 365-379.

Bahr,S., and Wolff,J.R. (1985). Postnatal development of axosomatic synapses in the rat visual cortex: morphogenesis and quantitative evaluation. J. Comp Neurol. *233*, 405-420.

Baimbridge,K.G., Celio,M.R., and Rogers,J.H. (1992). Calcium-binding proteins in the nervous system. Trends Neurosci *15*, 303-308.

Beaulieu,C., Kisvarday,Z., Somogyi,P., Cynader,M., and Cowey,A. (1992). Quantitative distribution of GABA-immunopositive and -immunonegative neurons and synapses in the monkey striate cortex (area 17). Cereb. Cortex *2*, 295-309.

Beaulieu,C., and Somogyi,P. (1990). Targets and Quantitative Distribution of GABAergic Synapses in the Visual Cortex of the Cat. Eur. J Neurosci *2*, 296-303.

Ben-Ari,Y. (2002). Excitatory actions of gaba during development: the nature of the nurture. Nat. Rev Neurosci *3*, 728-739.

Benes,F.M., and Berretta,S. (2001). GABAergic interneurons: implications for understanding schizophrenia and bipolar disorder. Neuropsychopharmacology *25*, 1-27.

Berdel,B., and Morys,J. (2000). Expression of calbindin-D28k and parvalbumin during development of rat's basolateral amygdaloid complex. Int J Dev. Neurosci *18*, 501-513.

Björklund,A., and Lindvall,O. (1984). Dopamine-containing systems in the CNS. In Handbook of chemical neuroanatomy - Classical transmitters in the CNS, Part 1, A. Björklund, and T. Hökfelt, eds. (Amsterdam: Elsevier), pp. 55-122.

Brinley-Reed,M., and McDonald,A.J. (1999). Evidence that dopaminergic axons provide a dense innervation of specific neuronal subpopulations in the rat basolateral amygdala. Brain Res. *850*, 127-135.

Brummelte,S., and Teuchert-Noodt,G. (2006). Postnatal development of dopamine innervation in the amygdala and the entorhinal cortex of the gerbil (Meriones unguiculatus). Brain Res. (Epub ahead of print)

Brummelte,S., Grund,T., Czok,A., Teuchert-Noodt,G., and Neddens,J. (2006a). Long-term effects of a single adult methamphetamine challenge: Minor impact on dopamine fibre density in limbic brain areas of gerbils. Behav. Brain Funct. *2*, 12.

Brummelte,S., Neddens,J., and Teuchert-Noodt,G. (2006b). Alteration in the GABAergic network of the prefrontal cortex in a potential animal model of psychosis. J. Neural Transm. (in press)

Bu,J., Sathyendra,V., Nagykery,N., and Geula,C. (2003). Age-related changes in calbindin-D28k, calretinin, and parvalbumin-immunoreactive neurons in the human cerebral cortex. Exp. Neurol. *182*, 220-231.

Carlsen,J. (1988). Immunocytochemical localization of glutamate decarboxylase in the rat basolateral amygdaloid nucleus, with special reference to GABAergic innervation of amygdalostriatal projection neurons. J Comp Neurol. *273*, 513-526.

Caspary,D.M., Holder,T.M., Hughes,L.F., Milbrandt,J.C., McKernan,R.M., and Naritoku,D.K. (1999). Age-related changes in GABA(A) receptor subunit composition and function in rat auditory system. Neuroscience *93*, 307-312.

Celio,M.R. (1990). Calbindin D-28k and parvalbumin in the rat nervous system. Neuroscience *35*, 375-475.

Chen,R., Cohen,L.G., and Hallett,M. (2002). Nervous system reorganization following injury. Neuroscience *111*, 761-773.

Cherubini,E., Gaiarsa,J.L., and Ben-Ari,Y. (1991). GABA: an excitatory transmitter in early postnatal life. Trends Neurosci *14*, 515-519.

Chronwall,B., and Wolff,J.R. (1980). Prenatal and postnatal development of GABA-accumulating cells in the occipital neocortex of rat. J Comp Neurol. *190*, 187-208.

Chronwall,B.M., and Wolff,J.R. (1981). Development of GABA-accumulating neurons and glial cells in the rat visual cortex. Adv. Biochem. Psychopharmacol. *29*, 453-458.

Conde,F., Lund,J.S., Jacobowitz,D.M., Baimbridge,K.G., and Lewis,D.A. (1994). Local circuit neurons immunoreactive for calretinin, calbindin D-28k or parvalbumin in monkey prefrontal cortex: distribution and morphology. J Comp Neurol. *341*, 95-116.

Constantinidis,C., Williams,G.V., and Goldman-Rakic,P.S. (2002). A role for inhibition in shaping the temporal flow of information in prefrontal cortex. Nat. Neurosci *5*, 175-180.

Cruz,D.A., Eggan,S.M., and Lewis,D.A. (2003). Postnatal development of pre- and postsynaptic GABA markers at chandelier cell connections with pyramidal neurons in monkey prefrontal cortex. J Comp Neurol. *465*, 385-400.

Davila,J.C., Real,M.A., Olmos,L., Legaz,I., Medina,L., and Guirado,S. (2005). Embryonic and postnatal development of GABA, calbindin, calretinin, and parvalbumin in the mouse claustral complex. J Comp Neurol. *481*, 42-57.

Dawirs,R.R., Teuchert-Noodt,G., and Czaniera,R. (1993). Maturation of the dopamine innervation during postnatal development of the prefrontal cortex in gerbils (Meriones unguiculatus). A quantitative immunocytochemical study. J Hirnforsch. *34*, 281-290.

Dawirs,R.R., Teuchert-Noodt,G., and Czaniera,R. (1996). Ontogeny of PFC-related behaviours is sensitive to a single non-invasive dose of methamphetamine in neonatal gerbils (Meriones unguiculatus). J Neural Transm. *103*, 1235-1245.

Dawirs,R.R., Teuchert-Noodt,G., and Nossoll,M. (1997). Pharmacologically induced neural plasticity in the prefrontal cortex of adult gerbils (Meriones unguiculatus). Eur. J. Pharmacol. *327*, 117-123.

DeFelipe,J., Hendry,S.H., and Jones,E.G. (1989). Synapses of double bouquet cells in monkey cerebral cortex visualized by calbindin immunoreactivity. Brain Res. *503*, 49-54.

Del Rio,J.A., Soriano,E., and Ferrer,I. (1992). Development of GABA-immunoreactivity in the neocortex of the mouse. J Comp Neurol. *326*, 501-526.

Diergaarde,L., Gerrits,M.A., Brouwers,J.P., and van Ree,J.M. (2005). Early amygdala damage disrupts performance on medial prefrontal cortex-related tasks but spares spatial learning and memory in the rat. Neuroscience *130*, 581-590.

Erickson,S.L., and Lewis,D.A. (2002). Postnatal development of parvalbumin- and GABA transporter-immunoreactive axon terminals in monkey prefrontal cortex. J Comp Neurol. *448*, 186-202.

Fallon,J.H., and Ciofi,P. (1992). Distribution of monoamines within the amygdala. In The amygdala: Neurobiological aspects of emotion, memory, and mental dysfunction, Aggleton JP, ed. (New York: Wiley-Liss, Inc.), pp. 97-114.

Freund,T.F. (2003). Interneuron Diversity series: Rhythm and mood in perisomatic inhibition. Trends Neurosci *26*, 489-495.

Gabbott,P.L., and Bacon,S.J. (1996). Local circuit neurons in the medial prefrontal cortex (areas 24a,b,c, 25 and 32) in the monkey: II. Quantitative areal and laminar distributions. J Comp Neurol. *364*, 609-636.

Gabbott,P.L., Warner,T.A., and Busby,S.J. (2006). Amygdala input monosynaptically innervates parvalbumin immunoreactive local circuit neurons in rat medial prefrontal cortex. Neuroscience.

Ganguly,K., Schinder,A.F., Wong,S.T., and Poo,M. (2001). GABA itself promotes the developmental switch of neuronal GABAergic responses from excitation to inhibition. Cell *105*, 521-532.

Geula,C., Bu,J., Nagykery,N., Scinto,L.F., Chan,J., Joseph,J., Parker,R., and Wu,C.K. (2003). Loss of calbindin-D28k from aging human cholinergic basal forebrain: relation to neuronal loss. J. Comp Neurol. *455*, 249-259.

Gibson,J.R., Beierlein,M., and Connors,B.W. (1999). Two networks of electrically coupled inhibitory neurons in neocortex. Nature *402*, 75-79.

Hensch,T.K. (2005). Critical period mechanisms in developing visual cortex. Curr. Top. Dev. Biol. *69*, 215-237.

Hof,P.R., Glezer,I.I., Conde,F., Flagg,R.A., Rubin,M.B., Nimchinsky,E.A., and Vogt Weisenhorn,D.M. (1999). Cellular distribution of the calcium-binding proteins parvalbumin, calbindin, and calretinin in the neocortex of mammals: phylogenetic and developmental patterns. J Chem. Neuroanat. *16*, 77-116.

Holtmaat,A., Wilbrecht,L., Knott,G.W., Welker,E., and Svoboda,K. (2006). Experience-dependent and cell-type-specific spine growth in the neocortex. Nature *441*, 979-983.

Hwang,I.K., Kang,T.C., Lee,J.C., Lee,I.S., Park,S.K., An,S.J., Jeong,Y.G., Seo,J.G., Oh,Y.S., and Won,M.H. (2002). Age-related change of calbindin D-28k immunoreactive neurons in the rat main olfactory bulb. Neurosci Lett. *326*, 159-162.

Ichimiya,Y., Emson,P.C., Mountjoy,C.Q., Lawson,D.E., and Heizmann,C.W. (1988). Loss of calbindin-28K immunoreactive neurones from the cortex in Alzheimer-type dementia. Brain Res. *475*, 156-159.

Jarry,H., Wise,P.M., Leonhardt,S., and Wuttke,W. (1999). Effects of age on GABA turnover rates in specific hypothalamic areas in female rats. Exp. Clin. Endocrinol. Diabetes *107*, 59-62.

Joseph,R. (1999). Environmental influences on neural plasticity, the limbic system, emotional development and attachment: a review. Child Psychiatry Hum. Dev. *29*, 189-208.

Kalsbeek,A., Voorn,P., Buijs,R.M., Pool,C.W., and Uylings,H.B. (1988). Development of the dopaminergic innervation in the prefrontal cortex of the rat. J Comp Neurol. *269*, 58-72.

Kawaguchi,Y., and Kubota,Y. (1998). Neurochemical features and synaptic connections of large physiologically-identified GABAergic cells in the rat frontal cortex. Neuroscience *85*, 677-701.

Kemppainen,S., and Pitkanen,A. (2000). Distribution of parvalbumin, calretinin, and calbindin-D(28k) immunoreactivity in the rat amygdaloid complex and colocalization with gamma-aminobutyric acid. J Comp Neurol. *426*, 441-467.

Kishimoto,J., Tsuchiya,T., Cox,H., Emson,P.C., and Nakayama,Y. (1998). Age-related changes of calbindin-D28k, calretinin, and parvalbumin mRNAs in the hamster brain. Neurobiol. Aging *19*, 77-82.

Klausberger,T., Magill,P.J., Marton,L.F., Roberts,J.D., Cobden,P.M., Buzsaki,G., and Somogyi,P. (2003). Brain-state- and cell-type-specific firing of hippocampal interneurons in vivo. Nature *421*, 844-848.

Lally,G., Faull,R.L., Waldvogel,H.J., Ferrari,S., and Emson,P.C. (1997). Calcium homeostasis in ageing: studies on the calcium binding protein calbindin D28K. J. Neural Transm. *104*, 1107-1112.

Legaz,I., Olmos,L., Real,M.A., Guirado,S., Davila,J.C., and Medina,L. (2005). Development of neurons and fibers containing calcium binding proteins in the pallial amygdala of mouse, with special emphasis on those of the basolateral amygdalar complex. J Comp Neurol. *488*, 492-513.

Lewis,D.A., Hashimoto,T., and Volk,D.W. (2005). Cortical inhibitory neurons and schizophrenia. Nat. Rev. Neurosci. *6*, 312-324.

Lund,J.S., and Lewis,D.A. (1993). Local circuit neurons of developing and mature macaque prefrontal cortex: Golgi and immunocytochemical characteristics. J Comp Neurol. *328*, 282-312.

McDonald,A.J., and Betette,R.L. (2001). Parvalbumin-containing neurons in the rat basolateral amygdala: morphology and co-localization of Calbindin-D(28k). Neuroscience *102*, 413-425.

McDonald,A.J., and Mascagni,F. (2001). Colocalization of calcium-binding proteins and GABA in neurons of the rat basolateral amygdala. Neuroscience *105*, 681-693.

McDonald,A.J., and Mascagni,F. (2004). Parvalbumin-containing interneurons in the basolateral amygdala express high levels of the alpha1 subunit of the GABAA receptor. J Comp Neurol. *473*, 137-146.

McLachlan,D.R., Wong,L., Bergeron,C., and Baimbridge,K.G. (1987). Calmodulin and calbindin D28K in Alzheimer disease. Alzheimer Dis. Assoc. Disord. *1*, 171-179.

Merzenich,M.M., Kaas,J.H., Wall,J., Nelson,R.J., Sur,M., and Felleman,D. (1983). Topographic reorganization of somatosensory cortical areas 3b and 1 in adult monkeys following restricted deafferentation. Neuroscience *8*, 33-55.

Miles,R., Toth,K., Gulyas,A.I., Hajos,N., and Freund,T.F. (1996). Differences between somatic and dendritic inhibition in the hippocampus. Neuron *16*, 815-823.

Miller,M.W. Development of Projection and Local Circuit Neurons in Neocortex. Peters, A. and Jones, E. G. Cerebral Cortex: Development and Maturation of Cerebral Cortex. [7], 133-166. 1988. New York and London, Plenum Press.

Morgan,D.G., May,P.C., and Finch,C.E. (1987). Dopamine and serotonin systems in human and rodent brain: effects of age and neurodegenerative disease. J. Am. Geriatr. Soc. *35*, 334-345.

Morys,J., Berdel,B., Jagalska-Majewska,H., and Luczynska,A. (1999). The basolateral amygdaloid complex--its development, morphology and functions. Folia Morphol. (Warsz.) *58*, 29-46.

Mrzljak,L., Uylings,H.B., Van Eden,C.G., and Judas,M. (1990). Neuronal development in human prefrontal cortex in prenatal and postnatal stages. Prog. Brain Res. *85*, 185-222.

Muller,J.F., Mascagni,F., and McDonald,A.J. (2003). Synaptic connections of distinct interneuronal subpopulations in the rat basolateral amygdalar nucleus. J Comp Neurol. *456*, 217-236.

Muller,J.F., Mascagni,F., and McDonald,A.J. (2005). Coupled networks of parvalbumin-immunoreactive interneurons in the rat basolateral amygdala. J Neurosci *25*, 7366-7376.

Muller,J.F., Mascagni,F., and McDonald,A.J. (2006). Pyramidal cells of the rat basolateral amygdala: synaptology and innervation by parvalbumin-immunoreactive interneurons. J Comp Neurol. *494*, 635-650.

Murakami,F., Oda,Y., and Tsukahara,N. (1988). Synaptic plasticity in the red nucleus and learning. Behav. Brain Res. *28*, 175-179.

Nguyen,L., Rigo,J.M., Rocher,V., Belachew,S., Malgrange,B., Rogister,B., Leprince,P., and Moonen,G. (2001). Neurotransmitters as early signals for central nervous system development. Cell Tissue Res. *305*, 187-202.

Nitsch,C., and Riesenberg,R. (1995). Synaptic reorganisation in the rat striatum after dopaminergic deafferentation: an ultrastructural study using glutamate decarboxylase immunocytochemistry. Synapse *19*, 247-263.

Ossowska,K. (1993). Disturbances in neurotransmission processes in aging and age-related diseases. Pol. J. Pharmacol. *45*, 109-131.

Parnavelas,J.G. (1992). Development of GABA-containing neurons in the visual cortex. Prog. Brain Res. *90*, 523-537.

Paxinos,G., and Watson,C. (1986). The rat brain in stereotaxic coordinates (New York: Academic Press).

Rosenzweig,M.R., and Bennett,E.L. (1969). Effects of differential environments on brain weights and enzyme activities in gerbils, rats, and mice. Dev. Psychobiol. *2*, 87-95.

Sesack,S.R., Snyder,C.L., and Lewis,D.A. (1995). Axon terminals immunolabeled for dopamine or tyrosine hydroxylase synapse on GABA-immunoreactive dendrites in rat and monkey cortex. J Comp Neurol. *363*, 264-280.

Seto-Ohshima,A., Aoki,E., Semba,R., Emson,P.C., and Heizmann,C.W. (1990). Appearance of parvalbumin-specific immunoreactivity in the cerebral cortex and hippocampus of the developing rat and gerbil brain. Histochemistry *94*, 579-589.

Tamas,G., Buhl,E.H., Lorincz,A., and Somogyi,P. (2000). Proximally targeted GABAergic synapses and gap junctions synchronize cortical interneurons. Nat. Neurosci *3*, 366-371.

Tamas,G., Buhl,E.H., and Somogyi,P. (1997). Fast IPSPs elicited via multiple synaptic release sites by different types of GABAergic neurone in the cat visual cortex. J Physiol *500 (Pt 3)*, 715-738.

Teuchert-Noodt,G. (2000). Neuronal degeneration and reorganization: a mutual principle in pathological and in healthy interactions of limbic and prefrontal circuits. J. Neural Transm. Suppl 315-333.

Thiessen,D., and Yahr,P. (1977). The gerbil in behavioral investigations. Mechanisms of territoriality and olfactory communication. (Austin: University of Texas Press).

Trachtenberg,J.T., Chen,B.E., Knott,G.W., Feng,G., Sanes,J.R., Welker,E., and Svoboda,K. (2002). Long-term in vivo imaging of experience-dependent synaptic plasticity in adult cortex. Nature *420*, 788-794.

Troup,G.M., Smith,G.S., and Walford,R.L. (1969). Life span,chronologic disease patterns and age-related changes in relative spleen weights for the mongolian gerbil (Meriones unguiculatus). Exp. Gerontol. *4*, 139-143.

Valverde,F. (1998). Golgi atlas of the postnatal mouse brain (Wien, New York: Springer-Verlag).

Van Eden,C.G., Kros,J.M., and Uylings,H.B. (1990). The development of the rat prefrontal cortex. Its size and development of connections with thalamus, spinal cord and other cortical areas. Prog. Brain Res. *85*, 169-183.

Van Eden,C.G., Mrzljak,L., Voorn,P., and Uylings,H.B. (1989). Prenatal development of GABA-ergic neurons in the neocortex of the rat. J Comp Neurol. *289*, 213-227.

Van Eden,C.G., and Uylings,H.B. (1985). Postnatal volumetric development of the prefrontal cortex in the rat. J. Comp Neurol. *241*, 268-274.

Vincent,S.L., Pabreza,L., and Benes,F.M. (1995). Postnatal maturation of GABA-immunoreactive neurons of rat medial prefrontal cortex. J Comp Neurol. *355*, 81-92.

Wolff,J.R., Balcar,V.J., Zetzsche,T., Bottcher,H., Schmechel,D.E., and Chronwall,B.M. (1984). Development of GABA-ergic system in rat visual cortex. Adv. Exp. Med. Biol. *181*, 215-239.

Wolff,J.R., Chronwall,B.M., and Rickmann,M. (1978). Morphogenetic relations between cell migration and synaptogenesis in the neocortex of rat. Proc. Eur. Soc. Neurochem. *1*, 158-173.

Wolff,J.R., Joo,F., and Kasa,P. (1993). Modulation by GABA of neuroplasticity in the central and peripheral nervous system. Neurochem. Res. *18*, 453-461.

Wu,C.K., Mesulam,M.M., and Geula,C. (1997). Age-related loss of calbindin from human basal forebrain cholinergic neurons. Neuroreport *8*, 2209-2213.

Wu,C.K., Nagykery,N., Hersh,L.B., Scinto,L.F., and Geula,C. (2003). Selective age-related loss of calbindin-D28k from basal forebrain cholinergic neurons in the common marmoset (Callithrix jacchus). Neuroscience *120*, 249-259.

Zito,K., and Svoboda,K. (2002). Activity-dependent synaptogenesis in the adult Mammalian cortex. Neuron *35*, 1015-1017.

Submitted to 'Behavioural and Brain Functions'

Manuscript ID 1553343361124716

Density of dopaminergic fibres in the prefrontal cortex of gerbils is sensitive to aging.

Susanne Brummelte* and Gertraud Teuchert-Noodt

Department of Neuroanatomy, Faculty of Biology, University of Bielefeld, Universitätsstr. 25, D-33615 Bielefeld, Germany, Phone: 0049 521 106 5704; Fax: 0049 521 106 6470, sbrummelte@uni-bielefeld.de, g.teuchert@uni-bielefeld.de

*corresponding author

Abstract

Mesencephalic dopamine (DA) projections are essential for cognitive and behavioral functions and believed to play a critical role during development and aging. The dopaminergic afferents of the rodent prefrontal cortex (PFC) show an extremely prolonged maturation which is very sensitive to epigenetic challenges. However, less is known about the long-term maturation and aging of these DA axons. Therefore, immunohistochemically stained DA fibres were quantitatively examined in the PFC of the Mongolian gerbil (*Meriones unguiculatus*) ranging from 6 to 24 months of age. Results show a decrease in DA fibre densities in the superficial layers of the PFC in 24 month old animals compared to 6 and 12 months.

Findings

Dopamine (DA) has frequently been associated with age-related changes and neurodegenerative diseases such as parkinson. In particular, striatal alterations have been in the focus of many investigations, as these are assumed to contribute to observed cognitive and motor dysfunction in elderly people or parkinson patients [1].

However, recent studies also suggest age-related DA changes in extrastriatal brain regions. Mirura and colleagues [2] observed that the level and turnover of monoamines and their metabolites were reduced in several brain regions as e.g. the prefrontal cortex (PFC), the amygdala, nucleus accumbens and hippocampus of 18 months old rats compared to young animals. For humans, it has been shown that the DA synthesis is lower with age in several extrastriatal regions, including the dorsolateral prefrontal and anterior cingulate cortex [3]. In addition, an age-related decline in D2 receptors was also found in various extrastriatal areas of healthy volunteers suggesting an association with normal aging processes [4]. In fact, it appears that the declines in D1 and D2 receptor binding might even be faster or more pronounced in the frontal cortices compared to striatal or thalamic regions [4-6]. This is in line with other studies reporting a greater loss of DA from the PFC compared to motor areas in aged monkeys [7,8], which underlines the importance of dopaminergic function during aging in this area.

So far, most studies have focused on the metabolic function of the dopaminergic system during aging, but less research has been done concerning neuroanatomical alterations. Our laboratory could recently show, that the dopaminergic fibre densities of the nucleus accumbens, the amygdala and the entorhinal cortex show no age-related changes in 24 month old gerbils (*Meriones unguiculatus*) compared to young animals [9,10]. However, as the PFC has been frequently associated with an age-related decline in cognitive function, this study was conducted to check for alterations in the dopaminergic fibre density in this particularly vulnerable area.

All experimental procedures were approved by the appropriate committee for animal care in accordance with the European Communities Council Directive. Gerbils were chosen due to their wild-type like behavioural and neuronal repertoire, as they have not been so intensively domesticated compared to rats or mice [11]. A total of 33 male Mongolian gerbils were used in this study (6 Mon n=8; 12 Mon n=5; 18 Mon n=11; 24 Mon n=9). Animal rearing and keeping conditions as well as the DA staining procedure have been described elsewhere [9].

Prefrontal DA fibre densities were measured in four consecutive coronal slices of the PFC. Fibre fragments in the upper layers were visualised in standard test fields in the prelimbic cortex (PrL) and in the infralimbic cortex (IL), using a bright-field microscope (BX61, Olympus, Hamburg, Germany) and a digital camera for microscopy (ColorView II, SIS, Münster, Germany) at 400-fold magnification. Fibres were quantified by software for image analysis (KS300, Jenoptik, Jena, Germany). For details of the quantification see [9]. The fibre area was calculated as a percentage of the reference area. All measurements were done by an experimenter blind to the coding of the samples.

Measurements were computed as arithmetic means by-case and by-group ± S.E.M. and a two-way analysis of variance (ANOVA) with age (4 levels) and area (2 levels) as independent variables and the dopaminergic fibre density as the dependent variable was used to check for statistical significance between groups followed by LSD post-hoc test for multiple comparisons. Statistical analysis was computed with Statistica 6 (StatSoft, Tulsa, USA). The levels of significance were set at * $p<0.05$, ** $p<0.01$ and *** $p<0.001$.

Statistical analysis revealed a significant effect of age ($F(3,56)=3.47$; $p=.022$) and area ($F(1,56)=5.53$; $p=.022$), but no interaction effect ($F(3,56)=.184$; $p=.907$). The PrL cortex showed a dense innervation of DA fibres, which was according to a Fisher LSD post-hoc test significantly lower in the IL ($p=.008$). The post-hoc test further revealed a significant age-related decrease in DA fibre density in the superficial layers of the PFC between 12 month

and 24 month old animals (-26%; p=.025), with the significance being even more prominent compared to 6 month old gerbils (-26%; p=.0098) (Fig.1).

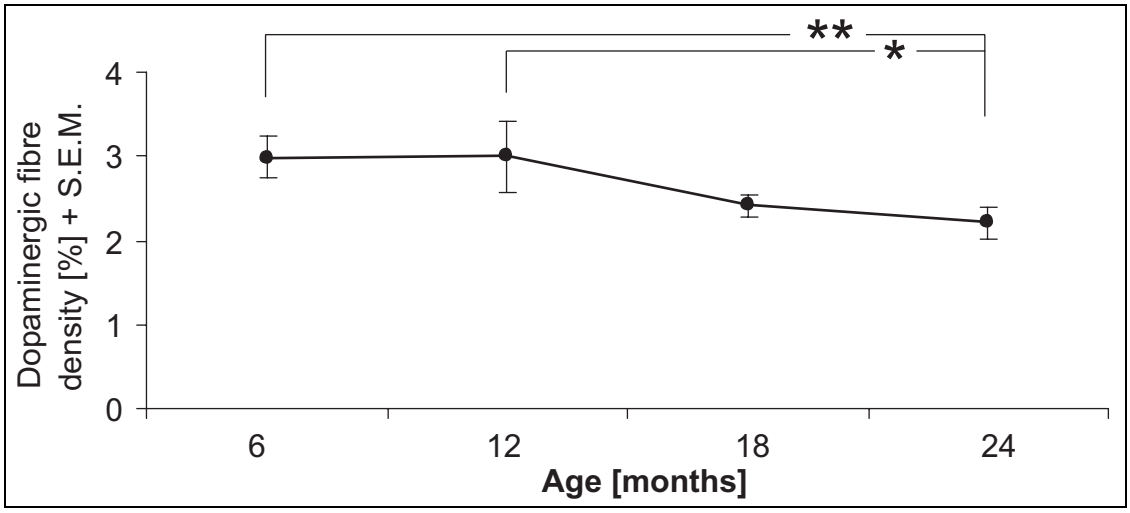


Fig.1: Development of dopaminergic fibre densities in the prefrontal cortex.
There is a significant decline in the density in 24 months old animals compared to 12 months and 6 months old gerbils.

Thus, we here present evidence for age-related anatomical alterations in the dopaminergic innervation pattern of the gerbil PFC. The decrease in DA fibre densities we found in the superficial layers of the PFC is in line with other observations of age-related alterations in the dopaminergic system. For instance, it has been shown, that the stress-related increase of dopamine diminishes with age as well as the dopamine transporter densities [12,13]. Thus, it has been assumed that the dopamine depletion of the PFC might contribute essentially to age-related cognitive declines [14].

As the autoxidation of dopamine leads to the formation of free radicals, which are known to play a major role in neurodegenerative disease and normal aging processes, it seems likely that there might exist a strong relation between the dopaminergic system and neurodegeneration during aging and diseases [15,16]. Alterations within the dopaminergic system have also been frequently associated with the occurrence of lewy bodies in the brain [17,18] and have been observed in alzheimer patients [19].

Remarkably, previous studies in the gerbil could not detect a decline in DA fibre densities in other brain areas than the PFC in old animals compared to adult ones [9,10]. The different vulnerability of DA fibres in distinct areas might be related to varying maturation patterns of the DA fibres. The dopaminergic fibre densities of the PFC reveal a prolonged maturation until early adulthood [20,21] while the innervation patterns of other areas mature relatively early. This ongoing increase in fibre density has been assumed to be associated with a continuing high plasticity within the PFC, but also with a high vulnerability concerning external influences [22]. The observed decline in DA fibres in the gerbil PFC of 24 month-old animals reflects an age-related disturbance in the DA system, which might also be related to the high plasticity in this area, thus possible only reflecting reactive or adaptive processes following other physiological changes. Interestingly, an adult pharmacological challenge only induced significant long-term effects of the dopaminergic fiber densities in the shell region of the Nucleus accumbens, but not in the PFC [23]. However, the present results are in line with observations from Ishida and co-workers [24] who found an early reduction of noradrenergic innervations in the frontal cortex of aging rats. In addition, it has been shown, that aging can change the interaction of different transmitters in the brain [25]. As the PFC is known to have several controlling connection over other brain systems and hence can essentially influence behavioral and cognitive functions, it seems likely that a disturbance within this superior cortex division might have extensive and far-reaching consequences for other areas and their function.

Competing interests

The authors declare that they have no competing interests.

Authors' contribution:

SB contributed to the benchwork, analysis and interpretation of the data and the drafting and revision of the manuscript

GT contributed to the design of the study and the critical reviewing of the manuscript.

Acknowledgements

The authors would like to thank Dr. F. Bagorda and Dr. J. Neddens for technical assistance. The study was supported by grants of the ‘Deutsche Parkinson Vereinigung’ and a doctoral fellowship from the ‘German National Academic Foundation’ to SB.

References

1. Roth GS, Joseph JA: **Cellular and molecular mechanisms of impaired dopaminergic function during aging.** *Ann N Y Acad Sci* 1994, **719:** 129-135.

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

3. Ota M, Yasuno F, Ito H, Seki C, Nozaki S, Asada T *et al.*: **Age-related decline of dopamine synthesis in the living human brain measured by positron emission tomography with L-[beta-11C]DOPA.** *Life Sci* 2006, **79:** 730-736.

4. Inoue M, Suhara T, Sudo Y, Okubo Y, Yasuno F, Kishimoto T *et al.*: **Age-related reduction of extrastriatal dopamine D2 receptor measured by PET.** *Life Sci* 2001, **69:** 1079-1084.

5. Suhara T, Fukuda H, Inoue O, Itoh T, Suzuki K, Yamasaki T *et al.*: **Age-related changes in human D1 dopamine receptors measured by positron emission tomography.** *Psychopharmacology (Berl)* 1991, **103:** 41-45.

6. Kaasinen V, Vilkman H, Hietala J, Nagren K, Helenius H, Olsson H *et al.*: **Age-related dopamine D2/D3 receptor loss in extrastriatal regions of the human brain.** *Neurobiol Aging* 2000, **21:** 683-688.

7. Goldman-Rakic PS, Brown RM: **Regional changes of monoamines in cerebral cortex and subcortical structures of aging rhesus monkeys.** *Neuroscience* 1981, **6:** 177-187.

8. Wenk GL, Pierce DJ, Struble RG, Price DL, Cork LC: **Age-related changes in multiple neurotransmitter systems in the monkey brain.** *Neurobiol Aging* 1989, **10:** 11-19.

9. Lesting J, Neddens J, Teuchert-Noodt G: **Ontogeny of the dopamine innervation in the nucleus accumbens of gerbils.** *Brain Res* 2005, **1066:** 16-23.

10. Brummelte S, Teuchert-Noodt G: **Postnatal development of dopamine innervation in the amygdala and the entorhinal cortex of the gerbil (Meriones unguiculatus).** *Brain Res* 2006.

11. Rosenzweig MR, Bennett EL: **Effects of differential environments on brain weights and enzyme activities in gerbils, rats, and mice.** *Dev Psychobiol* 1969, **2:** 87-95.

12. Del Arco A, Segovia G, Mora F: **Dopamine release during stress in the prefrontal cortex of the rat decreases with age.** *Neuroreport* 2001, **12:** 4019-4022.

13. Lavalaye J, Booij J, Reneman L, Habraken JB, van Royen EA: **Effect of age and gender on dopamine transporter imaging with [123I]FP-CIT SPET in healthy volunteers.** *Eur J Nucl Med* 2000, **27:** 867-869.

14. Arnsten AF, Cai JX, Steere JC, Goldman-Rakic PS: **Dopamine D2 receptor mechanisms contribute to age-related cognitive decline: the effects of quinpirole on memory and motor performance in monkeys.** *J Neurosci* 1995, **15:** 3429-3439.

15. Cantuti-Castelvetri I, Shukitt-Hale B, Joseph JA: **Dopamine neurotoxicity: age-dependent behavioral and histological effects.** *Neurobiol Aging* 2003, **24:** 697-706.

16. Mattila PM, Roytta M, Lonnberg P, Marjamaki P, Helenius H, Rinne JO: **Choline acetytransferase activity and striatal dopamine receptors in Parkinson's disease in relation to cognitive impairment.** *Acta Neuropathol (Berl)* 2001, **102:** 160-166.

17. O'Brien JT, Colloby S, Fenwick J, Williams ED, Firbank M, Burn D *et al.*: **Dopamine transporter loss visualized with FP-CIT SPECT in the differential diagnosis of dementia with Lewy bodies.** *Arch Neurol* 2004, **61:** 919-925.

18. Sweet RA, Hamilton RL, Healy MT, Wisniewski SR, Henteleff R, Pollock BG *et al*.: **Alterations of striatal dopamine receptor binding in Alzheimer disease are associated with Lewy body pathology and antemortem psychosis.** *Arch Neurol* 2001, **58:** 466-472.

19. Storga D, Vrecko K, Birkmayer JG, Reibnegger G: **Monoaminergic neurotransmitters, their precursors and metabolites in brains of Alzheimer patients.** *Neurosci Lett* 1996, **203:** 29-32.

20. Kalsbeek A, Voorn P, Buijs RM, Pool CW, Uylings HB: **Development of the dopaminergic innervation in the prefrontal cortex of the rat.** *J Comp Neurol* 1988, **269:** 58-72.

21. Dawirs RR, Teuchert-Noodt G, Czaniera R: **Maturation of the dopamine innervation during postnatal development of the prefrontal cortex in gerbils (Meriones unguiculatus). A quantitative immunocytochemical study.** *J Hirnforsch* 1993, **34:** 281-290.

22. Teuchert-Noodt G: **Neuronal degeneration and reorganization: a mutual principle in pathological and in healthy interactions of limbic and prefrontal circuits.** *J Neural Transm Suppl* 2000, 315-333.

23. Brummelte S, Grund T, Czok A, Teuchert-Noodt G, Neddens J: **Long-term effects of a single adult methamphetamine challenge: minor impact on dopamine fibre density in limbic brain areas of gerbils.** *Behav Brain Funct* 2006, **2:** 12.

24. Ishida Y, Shirokawa T, Miyaishi O, Komatsu Y, Isobe K: **Age-dependent changes in noradrenergic innervations of the frontal cortex in F344 rats.** *Neurobiol Aging* 2001, **22:** 283-286.

25. Segovia G, Mora F: **Dopamine and GABA increases produced by activation of glutamate receptors in the nucleus accumbens are decreased during aging.** *Neurobiol Aging* 2005, **26:** 91-101.

JNT-0/613
For Author's Correction Only

J Neural Transm (2007) 000: 1–9
DOI 10.1007/s00702-006-0613-4
Printed in The Netherlands

Journal of Neural Transmission

Alteration in the GABAergic network of the prefrontal cortex in a potential animal model of psychosis

S. Brummelte, J. Neddens, G. Teuchert-Noodt

Department of Neuroanatomy, Faculty of Biology, University of Bielefeld, Bielefeld, Germany

Received: April 4, 2006 / Accepted: November 26, 2006 / Published online: • •, 2007

Summary The GABAergic input on cortical pyramidal cells has an important influence on the firing activity of the cortex and thus in regulating the behavioural outcome. The aim of the current study was to investigate the long-term neuroplastic adaptation of the GABAergic innervation pattern after an early severe systemic impact. Therefore 40 Mongolian gerbils (*Meriones unguiculatus*) were either reared under impoverished (IR) or enriched rearing conditions (ER) and received a single early (+)-methamphetamine (MA) challenge (50 mg/kg i.p.) or saline on postnatal day 14. The density of perisomatic immunoreactive GABAergic terminals surrounding layers III and V pyramidal neurons was quantified as well as the overall GABAergic fibre density in layers I/II and V of the medial prefrontal cortex (mPFC) of young adult animals (90 days). We found that IR in combination with an early MA administration led to a significant decrease in GABAergic bouton densities while the overall GABAergic fibre density increased in all investigated layers. The results indicate a shift in inhibition from somatic to dendritic innervation of pyramidal neurons in this potential animal model of psychosis. We conclude that IR combined with early MA trigger changes in the postnatal maturation of the prefrontal cortical GABAergic innervation, which may interfere with proper signal processing within the prefrontal neural network.

Keywords: GABA, ■, ■, ■

Introduction

The interaction of the different transmitter systems plays a decisive role for the functioning of neural circuits throughout the brain. Several transmitters, such as gamma-aminobutyric acid (GABA), serotonin, and dopamine, contribute to the modulation of activity of the cortical pyramidal neurons, and thus have an important influence on the behavioural outcome. Every segment of the pyramidal neuron, from the initial axonal segment and the cell body up to dendritic spines, receives dense GABAergic innervation (Hendry et al., 1983; Houser et al., 1983; Beaulieu et al., 1992). Intriguingly, each of these segments receives its innervation from a distinct subpopulation of GABAergic neurons (Kisvarday et al., 1990).

Somatic GABAergic boutons are mainly build by the basket cell subpopulation, which owe their name to the basket-like arrangement of synapses surrounding pyramidal cell bodies (DeFelipe and Fairen, 1982; Hendry et al., 1983). The majority of cortical basket cells express the calcium-binding protein parvalbumin [PV (Hendry et al., 1989; Kawaguchi and Kubota, 1996)] and they mostly have a fast-spiking firing pattern (Kawaguchi and Kondo, 2002). A second type of cortical GABA cells, the chandelier neurons, are also associated with PV and are known to produce mainly axo-axonic contacts, which form axonal 'cartridges' along the initial axonal segment of the pyramidal neurons (Somogyi et al., 1982; Conde et al., 1994; Gabbott and Bacon, 1996). Beside these two populations of powerful interneurons, there are additional groups of GABAergic cells, which usually contain the calcium-binding proteins Calbindin (CB) or Calretinin (CR) and which are known to innervate primarily the dendritic spines and shafts of the pyramidal neuron (Conde et al., 1994; Gabbott and Bacon, 1996; Radnikow et al., 2002) and are thus less powerful in regulating the firing pattern of pyramidal cells. In summary, the subpopulations of interneurons each participate differently in establishing and maintaining the activity of cortical networks. Disturbances in this inhibitory regulation may result in extensive impairments in cognitive and behav-

Correspondence: Susanne Brummelte, Department of Neuroanatomy, Faculty of Biology, University of Bielefeld, Universitätsstraße 25, 33615 Bielefeld, Germany
e-mail: sbrummelte@uni-bielefeld.de

ioural function, like those seen in schizophrenia (Benes and Berretta, 2001).

Our lab has developed a potential animal model of schizophrenia using a combination of a single early methamphetamine (MA) intoxication on postnatal day 14, which damages monoaminergic fibers (Ricaurte et al., 1980, 1982), and chronically impoverished rearing conditions (IR) of gerbils. Among effects in several areas of the limbic-cortical system, the model impairs the maturation of the prefrontal cortex (PFC) by inducing diminished dopamine innervation (Dawirs et al., 1994; Neddens et al., 2001), increased GABA innervation (Nossoll et al., 1997), altered shape of pyramidal cells (Blaesing et al., 2001), and 'miswiring' of prefrontal efferents (Witte et al., 2006; Bagorda et al., 2006). In effect, the model successfully mimics several characteristics of the schizophrenic human brain (Feinberg, 1982; Weinberger and Lipska, 1995; Akil et al., 1999).

Since numerous alterations concerning the prefrontal GABAergic network have been reported for schizophrenia, as e.g. a particular defect in the parvalbumin-class of interneurons [reviewed in Blum and Mann (2002)], the current study was designed to investigate whether or how the GABAergic system may be affected in our animal model. Therefore, we analyzed different GABAergic structures, namely fibers and somatic terminals in the medial PFC of the developmentally disturbed gerbils.

Material and methods

Animals and rearing conditions

All experimental procedures were approved by the appropriate committee for animal care in accordance with the guidelines of the European Communities Council Directive. Breeding gerbils (*Meriones unguiculatus*) were obtained from Harlan Winkelmann (Borchen, Germany). Gerbils were chosen due to their very small genetic variability (Thiessen and Yahr, 1977), their rich wild-type like behavioural repertoire, and complex social interaction (Rosenzweig and Bennett, 1969).

A total of 40 males (weight 66–90 g) were used in this study. Half of them were bred in standard makrolon cages (type IV) whereas the other half were bred in semi-naturally structured compounds containing branches and hiding opportunities (1×1 m; enriched condition). At weaning (30 days), the gerbils that were born in cages were assigned to impoverished conditions (IR, animals kept alone in standard makrolon cages type III without any content except of sawdust), while the other group grew up as a group of siblings under enriched rearing conditions (ER, kept in compounds similar to those they were born in), both for further 60 days. On postnatal day 14 a total of 20 animals received a single injection of (+)-methamphetamine hydrochloride [Sigma (50 mg/kg; i.p.)], whereas the remaining 20 gerbils were sham-treated with saline, resulting in four experimental groups: ER-Sal, ER-MA, IR-Sal, IR-MA; $n = 10$ for each group. All animals had free access to food and water and were kept on natural day/night cycles during summer season.

Immunohistochemistry

On PD 90, animals were transcardically perfused under deep chloralhydrate anesthesia (1.7 g/kg, i.p.). The perfusion was performed with 200 ml 0.05 M phosphate buffer (pH 6.2), containing 1% sodium metabisulfite, followed by 750 ml 5% glutaraldehyde with 1% sodium metabisulfite in 0.1 M phosphate buffer (pH 7.5). Immediately after perfusion, the brains were removed and postfixed for 30 min. Coronar sections of 50 µm were cut with a vibratome (Vibratome Series 1000, Technical Products International Inc.) of which every 3rd was collected in wash buffer at 4°C. For immunostaining the sections were rinsed 3×10 min in cold wash buffer, followed by a preincubation in 10% normal goat serum and 0.4% Triton X-100 (Sigma) for 30 min. Subsequent the sections were incubated with rabbit anti-GABA (ImmunoStar, Hudson, WI), diluted 1:5000 with 1% normal goat serum and 0.4% Triton X-100 for 48 h.

The following rinses, all three times for 10 min, and dilutions were done in 0.05 M tris–HCL buffered saline pH 7.5 (TBS). The sections were rinsed and incubated for 30 min in biotinylated goat anti-rabbit IgG (Sigma) diluted 1:20 with 1% normal goat serum, rinsed again and incubated with ExtraAvidin-Peroxidase (Sigma) diluted 1:20 for 30 min. After another rinse the sections were stained in 0.05% 3.3-diaminobenzidine (Sigma) with 0.01% H_2O_2 for 4 min. Then the sections were washed, mounted on glass slides, dried overnight, dehydrated with ethanol, cleared with xylene and cover slipped with DePeX (Serva, Heidelberg, Germany). To avoid deviations due to possibly lateralised innervation densities of GABA only right hemispheres were used for quantification.

Quantification of GABAergic profiles

For quantification of bouton and fibre densities, brain sections were chosen in areas of interest (Fig. 1A–E) by means of anatomical characteristics according to brain atlases of the rat (Paxinos and Watson, 1986) and the mouse (Valverde, 1998); identification of the brain regions follows the nomenclature of the atlas of the rat. For the quantification of GABAergic boutons a total number of 3200 cells was analysed, with an average number of 4 analysed sections per animal and an average of 10 clearly identified pyramidal cell somata in standard test fields (0.22 mm^2) per section and layer (layers III and V). A cell was chosen if the unstained soma was clearly lying within the range of layer III or V of the cingular cortex area 3 (Cg3) of the mPFC and had a round to slightly oval shape which was clearly surrounded by darkly stained GABAergic boutons (see Fig. 1G). An experimenter blind to the experimental conditions marked the pyramidal cell soma manually. All boutons in a range of 1.66 µm from this soma were automatically assigned and the density was computed as a percentage of the evaluated test area. The fibre densities were quantified in standard test fields (900 μm^2) in layers V and I/II with an average of 10 test fields per section and layer (see Fig. 1F and H). Layer I/II was chosen due to their high innervation with GABAergic fibres inhibiting distal apical dendrites of pyramidal neurons. All detectable GABAergic boutons and fibres were visualised using a bright field microscope (Olympus BX61, Hamburg, Germany) and a digital camera for microscopy (SIS ColorViewII, Münster, Germany) at 600-fold magnification. Boutons and fibres were quantified by software for image analysis (KS300, Jenoptik, Jena, Germany), which uses a combination of Gauss filter and Gerig operator that depicts differences of grey values of adjacent pixels and transforms the result into binary images. In effect, fibres were depicted as lines of one pixel width, such that different diameters of fibres would not influence the measurement.

Data analysis

The data were computed as arithmetic means by-case and by-group ± S.E.M. of the respective layers and were analysed for the effects of both rearing conditions and pharmacological treatment. To account for possible interactions between the somatic size of the investigated cells and the area being covered by perisomatic GABAergic boutons, the size of the pyramidal cell bodies was used as a covariate in a 2-way analysis of covariance (ANCOVA) of perisomatic terminals.

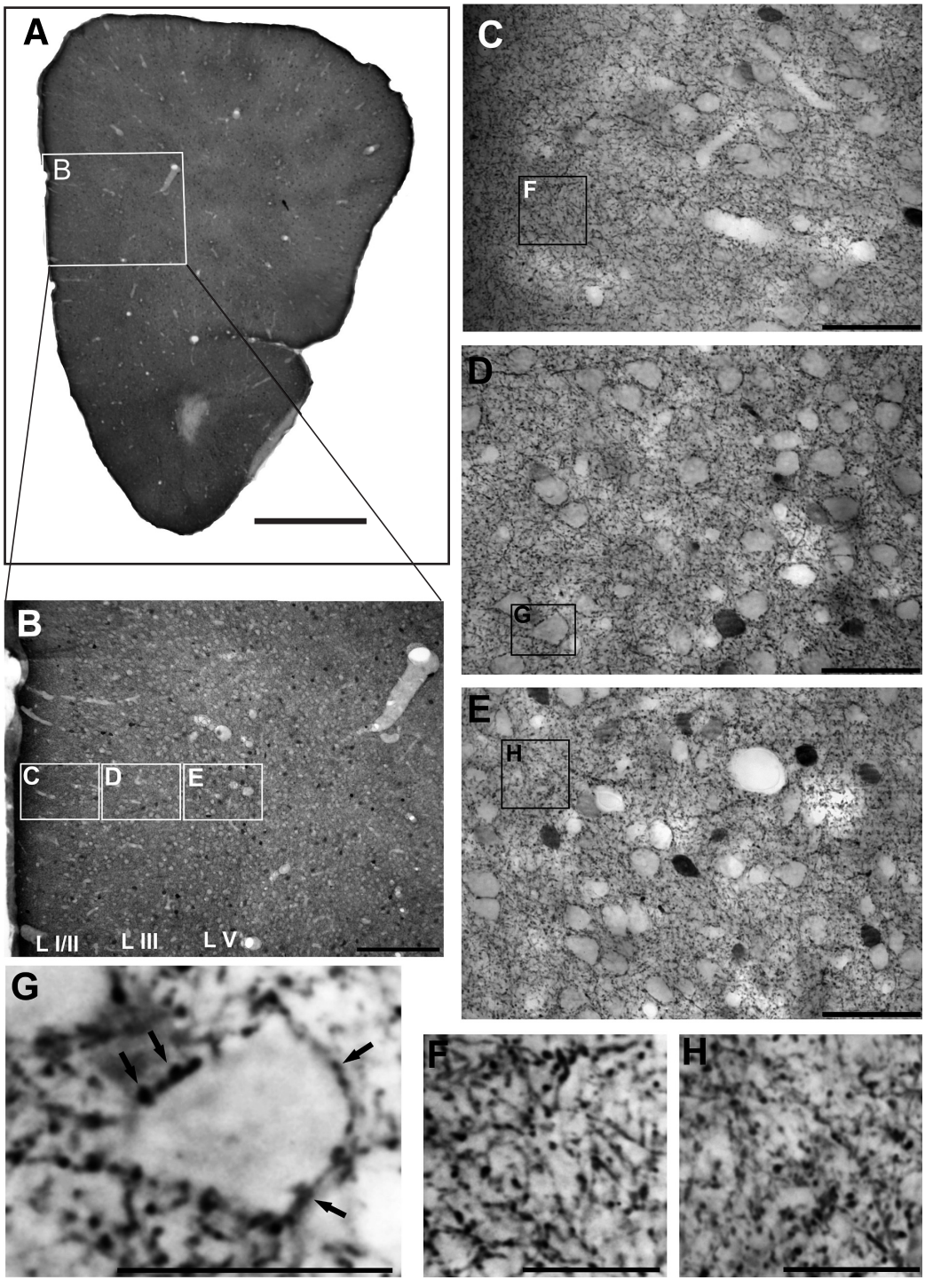


Fig. 1. Brightfield photomicrograph of a representative coronar section of the medial prefrontal cortex (**A**). The rectangle (**B**) shows the analysed section of the Cg3 region with subsequent rectangles for the analysed layers, which are magnified in (**C**, **D** and **E**). The GABAergic fibre density is generally similar in layers I/II and V (**F** and **H**). **G** shows GABAergic boutons (arrows) around an unstained pyramidal soma. Scale bars: 1 mm (**A**), 200 µm (**B**), 50 µm (**C**–**E**) and 20 µm (**G**–**H**)

Statistical analysis of the overall GABAergic fibre densities was done using a factorial ANOVA. Due to technical problems, sections from 6 animals (two from each group except IR-MA) had to be excluded from the study. All statistical analysis was computed with Statistica 6 (StatSoft, Tulsa, USA). The levels of significance were set at $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$.

Results

Qualitative results

The GABAergic innervation pattern is relatively homogenous throughout the cortex of gerbils and is similar to rats (Seto-Ohshima et al., 1990). It is characterised by a dense fibre innervation in all cortical layers with the highest density in the molecular layer. We identified immunonegative pyramidal neurons in layers III and V by their round or oval shape, their size and orientation, and the presence of basket-like GABAergic innervation (Fig. 1G).

Quantitative results

GABAergic bouton densities

The 2-way ANCOVA revealed a highly significant effect of rearing conditions on boutons in layer III [$F(1,29) = 28.59$, $p < 0.001$] and layer V [$F(1,29) = 25.58$, $p < 0.001$], and also a significant interaction between rearing and treatment in both layers [L III: $F(1,29) = 6.35$, $p = 0.0175$; L V: $F(1,29) = 5.0806$, $p = 0.0319$]. *Post-hoc* analysis with Newman-Keuls test showed the following results: Isolation rearing (IR) led to a significant decrease in GABAergic bouton density in layer III (−19%, $p = 0.032$), but not in layer V ($p = 0.093$). An early MA intoxication led to a further decrease in the bouton densities of both layers in IR-MA compared to IR-Sal animals (L III: −24%, $p = 0.031$; L V: −22%, $p = 0.032$). However, such an effect was not seen in animals from enriched rearing conditions (ER-MA vs. ER-Sal). Thus, bouton densities were reduced in the IR-MA group (L III: −38%, $p < 0.001$; L V: −33%, $p = 0.001$) compared to ER-Sal animals (cf. Fig. 2A).

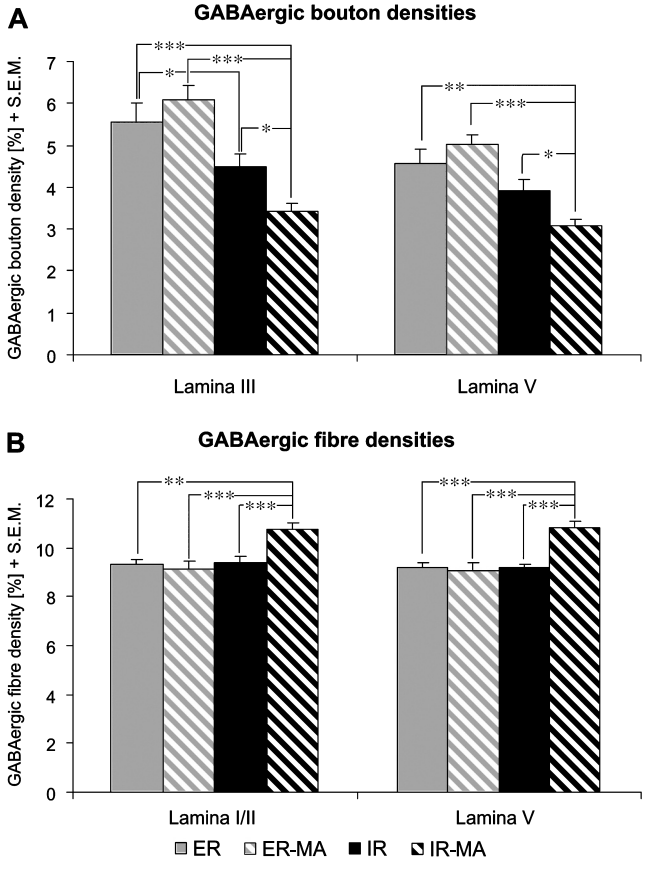


Fig. 2. GABAergic bouton (**A**) and fibre densities (**B**) in the analysed layers of the PFC of gerbils from enriched (*ER*) and impoverished rearing (*IR*) conditions treated with either methamphetamine (*MA*) or saline given by means + standard error (*S.E.M.*). $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$

GABAergic fibre densities

A factorial ANOVA identified a significant interaction of treatment and rearing conditions in both layer V [$F(1,30) = 13.07$, $p = 0.001$] and layers I/II [$F(1,30) = 9.8844$, $p = 0.004$]. *Post-hoc* Newman-Keuls tests revealed a significant increase in layer I/II fibre density of IR-MA animals (+15, +16, +18%; all $p < 0.001$) compared to IR-Sal, ER-Sal, and ER-MA animals, respectively. A similar increase in GABAergic fibre density was found in layer V (+17 to +19%) of the IR-MA group, compared to IR-Sal, ER-Sal, and ER-MA animals [all $p < 0.001$, except ER-Sal: $p = 0.0012$ (cf. Fig. 2B)].

Discussion

We have demonstrated that a single early MA intoxication combined with impoverished rearing (IR) significantly reduces the densitiy of GABAergic boutons that surround layers III and V pyramidal neurons in the prefrontal cortex of the Mongolian gerbil, whereas the overall GABAergic fibre density in layers I/II and V is increased compared to control animals.

Early MA intoxication and impoverished rearing as a model for schizophrenia

The single early high dose of MA on PD 14 in combination with IR used in the current study is effective to disturb normal postnatal development of the dopaminergic system, by triggering a restraint of the maturation of dopamine fibres in the prefrontal cortex and the nucleus accumbens (Dawirs et al., 1994; Neddens et al., 2001, 2002) as well as a concomitant excessive maturation in several amygdaloid

nuclei and the entorhinal cortex (Busche et al., 2004). A similar pattern of cortical-subcortical dopaminergic imbalance has also been observed in the schizophrenic brain (Laruelle et al., 2003; Abi-Dargham, 2004). Early MA treatment additionally impairs PFC-related abilities and behaviours, such as working memory and spatial learning (Dawirs et al., 1996; Williams et al., 2002). Again, deficits in working memory are well known characteristics of schizophrenic patients (Goldman-Rakic, 1995; Lewis and Anderson, 1995). Furthermore, the early drug challenge in combination with IR leads to a miswiring of prefrontal efferents (Bagorda et al., 2006), in accordance with the dysconnection hypothesis of schizophrenia (Weinberger and Lipska, 1995).

Taken together, our approach using combined early MA intoxication and IR leads to several morphological changes in neuroanatomical brain networks and impairs cognitive functions, resembling some of the changes and deficits seen in schizophrenic individuals, and thus provides a potential animal model of the disease. The present study reveals that an early MA intoxication additionally decreases GABAergic boutons that surround pyramidal cell somata, indicating a loss of somatic synapses (Karube et al., 2004) and a concomitant increase in overall GABAergic fibre density in the medial prefrontal cortex. These findings raise the possibility that the local prefrontal cortical inhibitory network may be functionally disorganised.

The role of somatic inhibition

The distinct classes of GABAergic synapses play differential roles in regulating the activity of pyramidal neurons. The majority of GABAergic synapses terminate on dendrites or spines of the postsynaptic cells (Beaulieu and Somogyi, 1990; Beaulieu et al., 1992; Nitsch and Riesenberg, 1995), thus they are likely to control the efficacy and plasticity of excitatory inputs onto the postsynaptic target (Miles et al., 1996; Tamas et al., 1997, 2003). However, somatic inhibition is thought to be particularly effective in controlling the output of pyramidal neurons and, importantly, has been implicated to synchronize activity patterns of whole pyramidal populations (Miles et al., 1996; Tamas et al., 1997, 2000; Gibson et al., 1999; Freund, 2003; Klausberger et al., 2003). Such oscillatory synchronization is further believed to create the necessary temporal and spatial frame for prefrontal functions such as working memory (Constantinidis et al., 2002; Lewis et al., 2005). In addition, cortical interneurons, in particular 'fast-spiking' neurons, have been shown to play an important role in shaping receptive fields as well as spatial memory fields (Jones, 1993; Rao et al., 1999, 2000). GABAergic somatic inhibition is thus exceptionally essential for the maintenance of cortical and cognitive functions and one is tempted to suggest that a decrease in this type of GABAergic inhibition and the potential subsequent deficit in synchronization might contribute to reported working memory dysfunction in schizophrenia (Lewis et al., 2005) and our animal model (Dawirs et al., 1996). In fact, post-mortem studies of schizophrenic patients reveal fewer GABAergic synapses on cortical pyramidal cells (Blum and Mann, 2002) and in addition, recent neurophysiological studies have shown, that some cognitive dysfunctions in schizophrenia are associated with an abnormal neural synchronization (Spencer et al., 2003, 2004; Lee et al., 2003; Uhlhaas et al., 2006).

The maturation and shift of GABAergic inhibition

It is well documented that GABA exhibits depolarizing effects at early postnatal stages (Cherubini et al., 1991; Ganguly et al., 2001; Ben-Ari, 2002), due to an inverted electrochemical gradient for Cl^- in neonatal neurons (Ben-Ari, 2002). The shift from an excitatory to an inhibitory transmitter is assumed to coincide with the first expression of PV in cortical interneurons (Berger et al., 1999) and the calcium-binding protein is therefore considered a marker of functional maturity of the neuron (Seto-Ohshima et al., 1990; Solbach and Celio, 1991). In the gerbil mPFC, the first PV-immunoreactive cells appear around PD 14 (unpublished data), that is, at the time of the MA challenge. Interestingly, the maturation of GABAergic synapses in general proceeds until early adulthood (Huang et al., 1999; Morales et al., 2002; Chattopadhyaya et al., 2004; Lewis et al., 2005), and in that, every subpopulation of presynaptic terminals exhibits a particular developmental pattern (Lewis et al., 2005). Therefore, the ability to synchronize pyramidal cell activity is assumed to be in substantial flux until adulthood (Lewis et al., 2005). Although the proliferation and formation of the typical perisomatic basket terminal seems to be a largely stereotypical process, it is additionally also dependent on neuronal activity within cortical circuits (Marty et al., 2000; Chattopadhyaya et al., 2004).

GABAergic interneurons receive direct dopaminergic input (Goldman-Rakic et al., 1989; Verney et al., 1990; Benes et al., 1993), with D1 and D2 receptor types being most abundantly expressed by PV-neurons (Le Moine and Gaspar, 1998). Dopamine modulates cortical GABA cells; both inhibitory (Retaux et al., 1991) and excitatory (Gorelova et al., 2002) effects on fast-spiking interneurons have been reported. The omission of prefrontal dopaminergic afferent fibres by an early MA challenge (Dawirs et al., 1994;

Neddens et al., 2001) might therefore induce significant alterations in the local GABAergic networks.

The dopaminergic afferents to the prefrontal cortex show a prolonged maturation (Kalsbeek et al., 1988; Dawirs et al., 1993; Rosenberg and Lewis, 1995) and continue to form synapses on GABAergic interneurons until early adulthood (Benes et al., 1996b). Pyramidal neurons are also directly innervated by dopaminergic terminals (Jay et al., 1995; Davidoff and Benes, 1998) which demonstrates the rather complex capacity of dopamine to directly and indirectly regulate the firing pattern of pyramidal neurons. By early MA intoxication we induce a restraint of the maturation of prefrontal dopaminergic afferents, which triggers reactive neuroplastic adaptation of the local network. Anatomical data suggest that pyramidal cells may adapt by increasing their dendritic range and their spine density (Blaesing et al., 2001). Our current findings indicate an increase of GABAergic fibre density, which is in line with an earlier study using electron-microscopy that already revealed an increase in non-somatic GABAergic terminals (Nossoll et al., 1997). Therefore, we find it tempting to suggest that an early MA challenge, by acutely reducing the density of monoaminergic innervation of the PFC, might trigger a reactive shift within the GABAergic networks from somatic to dendritic pyramidal inhibition.

GABAergic dysfunction in schizophrenia

GABAergic dysfunction in schizophrenia has first been proposed by Roberts (1972). Since then, several studies have revealed disturbances of GABAergic networks in schizophrenic patients (for review see Benes and Berretta, 2001) or in animal models of schizophrenia (Cochran et al., 2002, 2003; Keilhoff et al., 2004; Reynolds et al., 2004; Penschuck et al., 2006). A decline in PV-immunoreactive structures, particularly in axon cartridges from chandelier neurons, seems to be one of the most prevalent observations in *post-mortem* brains from schizophrenic individuals (Woo et al., 1998; Pierri et al., 1999; Lewis et al., 1999). Furthermore, the $GABA_A$ receptor density was found to be upregulated at the axon initial segment (Volk et al., 2002) as well as at the cell body of pyramidal neurons (Benes et al., 1996a), possibly compensating for a reduction of inhibitory terminals from chandelier and basket cells (Lewis et al., 2005). In contrast to the alterations in PV-containing neurons, only few studies reported on changes in the subpopulation of CB- or CR-immunopositive cells. Iritani and colleagues (1999) found a fibre disarray from CB-containing cells in the PFC, while Daviss and Lewis (1995) described an increase in the density of CB cells but no change in the CR population in a *post-mortem* study on schizophrenic brains. This would also be in line with our findings, since an increased number of CB cells and an altered fibre pattern are likely to present an elevated GABAergic inhibition of afferent pyramidal parts.

Conclusion

Here we present evidence for a probable dysfunctional reorganization of GABAergic networks in our potential animal model of schizophrenia. GABAergic interneurons critically contribute to the establishment of complex behaviours by controlling and synchronizing the firing patterns of pyramidal neurons. A weakened or altered inhibition may give rise to a broad array of disturbances in cognitive function, like those seen in schizophrenia (Benes and Berretta, 2001). The current study indicates a potential shift from a strong and powerful somatic inhibition to dendritic inhibition, which might attenuate the GABAergic influence on pyramidal activity and thus lead to an uncontrolled firing or abnormal synchronization. Our data coincide with findings of a reduced GABAergic somatic innervation in individuals with schizophrenia. We suggest that, in our animal model, this change in the GABAergic network is secondary, being triggered by the primary impairment of monoaminergic and namely dopaminergic afferents. Further investigations of the separate subpopulations of GABAergic interneurons in the PFC of gerbils are in process to identify the responsible cell classes for the observed alteration in the GABAergic network.

Acknowledgements

The authors wish to thank F. Bagorda, U. Schroeder and S. Graumüller for technical assistance.

References

Abi-Dargham A (2004) Do we still believe in the dopamine hypothesis? New data bring new evidence. Int J Neuropsychopharmacol 7 Suppl 1: S1–S5

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

Bagorda F, Teuchert-Noodt G, Lehmann K (2006) Isolation rearing or methamphetamine traumatisation induce a "dysconnection" of prefrontal efferents in gerbils: implications for schizophrenia. J Neural Transm 113: 365–379

Beaulieu C, Somogyi P (1990) Targets and quantitative distribution of GABAergic synapses in the visual cortex of the cat. Eur J Neurosci 2: 296–303

Beaulieu C, Kisvarday Z, Somogyi P, Cynader M, Cowey A (1992) Quantitative distribution of GABA-immunopositive and -immunonegative neurons and synapses in the monkey striate cortex (area 17). Cereb Cortex 2: 295–309

Ben-Ari Y (2002) Excitatory actions of gaba during development: the nature of the nurture. Nat Rev Neurosci 3: 728–739

Benes FM, Berretta S (2001) GABAergic interneurons: implications for understanding schizophrenia and bipolar disorder. Neuropsychopharmacology 25: 1–27

Benes FM, Vincent SL, Molloy R (1993) Dopamine-immunoreactive axon varicosities form nonrandom contacts with GABA-immunoreactive neurons of rat medial prefrontal cortex. Synapse 15: 285–295

Benes FM, Vincent SL, Marie A, Khan Y (1996a) Up-regulation of GABAA receptor binding on neurons of the prefrontal cortex in schizophrenic subjects. Neuroscience 75: 1021–1031

Benes FM, Vincent SL, Molloy R, Khan Y (1996b) Increased interaction of dopamine-immunoreactive varicosities with GABA neurons of rat medial prefrontal cortex occurs during the postweanling period. Synapse 23: 237–245

Berger B, De GN, Alvarez C (1999) Precocious development of parvalbumin-like immunoreactive interneurons in the hippocampal formation and entorhinal cortex of the fetal cynomolgus monkey. J Comp Neurol 403: 309–331

Blaesing B, Nossoll M, Teuchert-Noodt G, Dawirs RR (2001) Postnatal maturation of prefrontal pyramidal neurones is sensitive to a single early dose of methamphetamine in gerbils (Meriones unguiculatus). J Neural Transm 108: 101–113

Blum BP, Mann JJ (2002) The GABAergic system in schizophrenia. Int J Neuropsychopharmacol 5: 159–179

Busche A, Polascheck D, Lesting J, Neddens J, Teuchert-Noodt G (2004) Developmentally induced imbalance of dopaminergic fibre densities in limbic brain regions of gerbils (Meriones unguiculatus). J Neural Transm 111: 451–463

Chattopadhyaya B, Di CG, Higashiyama H, Knott GW, Kuhlman SJ, Welker E, Huang ZJ (2004) Experience and activity-dependent maturation of perisomatic GABAergic innervation in primary visual cortex during a postnatal critical period. J Neurosci 24: 9598–9611

Cherubini E, Gaiarsa JL, Ben-Ari Y (1991) GABA: an excitatory transmitter in early postnatal life. Trends Neurosci 14: 515–519

Cochran SM, Fujimura M, Morris BJ, Pratt JA (2002) Acute and delayed effects of phencyclidine upon mRNA levels of markers of glutamatergic and GABAergic neurotransmitter function in the rat brain. Synapse 46: 206–214

Cochran SM, Kennedy M, McKerchar CE, Steward LJ, Pratt JA, Morris BJ (2003) Induction of metabolic hypofunction and neurochemical deficits after chronic intermittent exposure to phencyclidine: differential modulation by antipsychotic drugs. Neuropsychopharmacology 28: 265–275

Conde F, Lund JS, Jacobowitz DM, Baimbridge KG, Lewis DA (1994) Local circuit neurons immunoreactive for calretinin, calbindin D-28k or parvalbumin in monkey prefrontal cortex: distribution and morphology. J Comp Neurol 341: 95–116

Constantinidis C, Williams GV, Goldman-Rakic PS (2002) A role for inhibition in shaping the temporal flow of information in prefrontal cortex. Nat Neurosci 5: 175–180

Davidoff SA, Benes FM (1998) High-resolution scatchard analysis shows D1 receptor binding on pyramidal and nonpyramidal neurons. Synapse 28: 83–90

Daviss SR, Lewis DA (1995) Local circuit neurons of the prefrontal cortex in schizophrenia: selective increase in the density of calbindin-immunoreactive neurons. Psychiatry Res 59: 81–96

Dawirs RR, Teuchert-Noodt G, Czaniera R (1993) Maturation of the dopamine innervation during postnatal development of the prefrontal cortex in gerbils (Meriones unguiculatus). A quantitative immunocytochemical study. J Hirnforsch 34: 281–290

Dawirs RR, Teuchert-Noodt G, Czaniera R (1994) The postnatal maturation of dopamine innervation in the prefrontal cortex of gerbils (Meriones unguiculatus) is sensitive to an early single dose of methamphetamine. A quantitative immunocytochemical study. J Hirnforsch 35: 195–204

Dawirs RR, Teuchert-Noodt G, Czaniera R (1996) Ontogeny of PFC-related behaviours is sensitive to a single non-invasive dose of methamphetamine in neonatal gerbils (Meriones unguiculatus). J Neural Transm 103: 1235–1245

DeFelipe J, Fairen A (1982) A type of basket cell in superficial layers of the cat visual cortex. A Golgi-electron microscope study. Brain Res 244: 9–16

Feinberg I (1982) Schizophrenia: caused by a fault in programmed synaptic elimination during adolescence? J Psychiatr Res 17: 319–334

Freund TF (2003) Interneuron diversity series: rhythm and mood in perisomatic inhibition. Trends Neurosci 26: 489–495

Gabbott PL, Bacon SJ (1996) Local circuit neurons in the medial prefrontal cortex (areas 24a, b, c, 25 and 32) in the monkey: I. Cell morphology and morphometrics. J Comp Neurol 364: 567–608

Ganguly K, Schinder AF, Wong ST, Poo M (2001) GABA itself promotes the developmental switch of neuronal GABAergic responses from excitation to inhibition. Cell 105: 521–532

Gibson JR, Beierlein M, Connors BW (1999) Two networks of electrically coupled inhibitory neurons in neocortex. Nature 402: 75–79

Goldman-Rakic PS (1995) Cellular basis of working memory. Neuron 14: 477–485

Goldman-Rakic PS, Leranth C, Williams SM, Mons N, Geffard M (1989) Dopamine synaptic complex with pyramidal neurons in primate cerebral cortex. Proc Natl Acad Sci USA 86: 9015–9019

Gorelova N, Seamans JK, Yang CR (2002) Mechanisms of dopamine activation of fast-spiking interneurons that exert inhibition in rat prefrontal cortex. J Neurophysiol 88: 3150–3166

Hendry SH, Houser CR, Jones EG, Vaughn JE (1983) Synaptic organization of immunocytochemically identified GABA neurons in the monkey sensory-motor cortex. J Neurocytol 12: 639–660

Hendry SH, Jones EG, Emson PC, Lawson DE, Heizmann CW, Streit P (1989) Two classes of cortical GABA neurons defined by differential calcium binding protein immunoreactivities. Exp Brain Res 76: 467–472

Houser CR, Hendry SH, Jones EG, Vaughn JE (1983) Morphological diversity of immunocytochemically identified GABA neurons in the monkey sensory-motor cortex. J Neurocytol 12: 617–638

Huang ZJ, Kirkwood A, Pizzorusso T, Porciatti V, Morales B, Bear MF, Maffei L, Tonegawa S (1999) BDNF regulates the maturation of inhibition and the critical period of plasticity in mouse visual cortex. Cell 98: 739–755

Iritani S, Kuroki N, Ikeda K, Kazamatsuri H (1999) Calbindin immunoreactivity in the hippocampal formation and neocortex of schizophrenics. Prog Neuropsychopharmacol Biol Psychiatry 23: 409–421

Jay TM, Glowinski J, Thierry AM (1995) Inhibition of hippocampoprefrontal cortex excitatory responses by the mesocortical DA system. Neuroreport 6: 1845–1848

Jones EG (1993) GABAergic neurons and their role in cortical plasticity in primates. Cereb Cortex 3: 361–372

Kalsbeek A, Voorn P, Buijs RM, Pool CW, Uylings HB (1988) Development of the dopaminergic innervation in the prefrontal cortex of the rat. J Comp Neurol 269: 58–72

Karube F, Kubota Y, Kawaguchi Y (2004) Axon branching and synaptic bouton phenotypes in GABAergic nonpyramidal cell subtypes. J Neurosci 24: 2853–2865

Kawaguchi Y, Kondo S (2002) Parvalbumin, somatostatin and cholecystokinin as chemical markers for specific GABAergic interneuron types in the rat frontal cortex. J Neurocytol 31: 277–287

Kawaguchi Y, Kubota Y (1996) Physiological and morphological identification of somatostatin- or vasoactive intestinal polypeptide-containing cells among GABAergic cell subtypes in rat frontal cortex. J Neurosci 16: 2701–2715

Keilhoff G, Becker A, Grecksch G, Wolf G, Bernstein HG (2004) Repeated application of ketamine to rats induces changes in the hippocampal expression of parvalbumin, neuronal nitric oxide synthase and cFOS similar to those found in human schizophrenia. Neuroscience 126: 591–598

Kisvarday ZF, Gulyas A, Beroukas D, North JB, Chubb IW, Somogyi P (1990) Synapses, axonal and dendritic patterns of GABA-immunoreactive neurons in human cerebral cortex. Brain 113 (Pt 3): 793–812

Klausberger T, Magill PJ, Marton LF, Roberts JD, Cobden PM, Buzsaki G, Somogyi P (2003) Brain-state- and cell-type-specific firing of hippocampal interneurons in vivo. Nature 421: 844–848

Laruelle M, Kegeles LS, bi-Dargham A (2003) Glutamate, dopamine, and schizophrenia: from pathophysiology to treatment. Ann Acad Sci NY 1003: 138–158

Le Moine C, Gaspar P (1998) Subpopulations of cortical GABAergic interneurons differ by their expression of D1 and D2 dopamine receptor subtypes. Brain Res Mol Brain Res 58: 231–236

Lee KH, Williams LM, Breakspear M, Gordon E (2003) Synchronous gamma activity: a review and contribution to an integrative neuroscience model of schizophrenia. Brain Res Brain Res Rev 41: 57–78

Lewis DA, Anderson SA (1995) The functional architecture of the prefrontal cortex and schizophrenia. Psychol Med 25: 887–894

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

Lewis DA, Hashimoto T, Volk DW (2005) Cortical inhibitory neurons and schizophrenia. Nat Rev Neurosci 6: 312–324

Marty S, Wehrle R, Sotelo C (2000) Neuronal activity and brain-derived neurotrophic factor regulate the density of inhibitory synapses in organotypic slice cultures of postnatal hippocampus. J Neurosci 20: 8087–8095

Miles R, Toth K, Gulyas AI, Hajos N, Freund TF (1996) Differences between somatic and dendritic inhibition in the hippocampus. Neuron 16: 815–823

Morales B, Choi SY, Kirkwood A (2002) Dark rearing alters the development of GABAergic transmission in visual cortex. J Neurosci 22: 8084–8090

Neddens J, Brandenburg K, Teuchert-Noodt G, Dawirs RR (2001) Differential environment alters ontogeny of dopamine innervation of the orbital prefrontal cortex in gerbils. J Neurosci Res 63: 209–213

Neddens J, Lesting J, Dawirs RR, Teuchert-Noodt G (2002) An early methamphetamine challenge suppresses the maturation of dopamine fibres in the nucleus accumbens of gerbils: on the significance of rearing conditions. J Neural Transm 109: 141–155

Nitsch C, Riesenberg R (1995) Synaptic reorganisation in the rat striatum after dopaminergic deafferentation: an ultrastructural study using glutamate decarboxylase immunocytochemistry. Synapse 19: 247–263

Nossoll M, Teuchert-Noodt G, Dawirs RR (1997) A single dose of methamphetamine in neonatal gerbils affects adult prefrontal gamma-aminobutyric acid innervation. Eur J Pharmacol 340: R3–R5

Paxinos G, Watson C (1986) The rat brain in stereotaxic coordinates. Academic Press, New York

Penschuck S, Flagstad P, Didriksen M, Leist M, Michael-Titus AT (2006) Decrease in parvalbumin-expressing neurons in the hippocampus and increased phencyclidine-induced locomotor activity in the rat methylazoxymethanol (MAM) model of schizophrenia. Eur J Neurosci 23: 279–284

Pierri JN, Chaudry AS, Woo TU, Lewis DA (1999) Alterations in chandelier neuron axon terminals in the prefrontal cortex of schizophrenic subjects. Am J Psychiatry 156: 1709–1719

Radnikow G, Feldmeyer D, Lubke J (2002) Axonal projection, input and output synapses, and synaptic physiology of Cajal-Retzius cells in the developing rat neocortex. J Neurosci 22: 6908–6919

Rao SG, Williams GV, Goldman-Rakic PS (1999) Isodirectional tuning of adjacent interneurons and pyramidal cells during working memory: evidence for microcolumnar organization in PFC. J Neurophysiol 81: 1903–1916

Rao SG, Williams GV, Goldman-Rakic PS (2000) Destruction and creation of spatial tuning by disinhibition: GABA(A) blockade of prefrontal cortical neurons engaged by working memory. J Neurosci 20: 485–494

Retaux S, Besson MJ, Penit-Soria J (1991) Synergism between D1 and D2 dopamine receptors in the inhibition of the evoked release of [3H]GABA in the rat prefrontal cortex. Neuroscience 43: 323–329

Reynolds GP, Abdul-Monim Z, Neill JC, Zhang ZJ (2004) Calcium binding protein markers of GABA deficits in schizophreniapostmortem studies and animal models. Neurotox Res 6: 57–61

Ricaurte GA, Schuster CR, Seiden LS (1980) Long-term effects of repeated methylamphetamine administration on dopamine and serotonin neurons in the rat brain: a regional study. Brain Res 193: 153–163

Ricaurte GA, Guillery RW, Seiden LS, Schuster CR, Moore RY (1982) Dopamine nerve terminal degeneration produced by high doses of methylamphetamine in the rat brain. Brain Res 235: 93–103

Roberts E (1972) Prospects for research on schizophrenia. An hypotheses suggesting that there is a defect in the GABA system in schizophrenia. Neurosci Res Program Bull 10: 468–482

Rosenberg DR, Lewis DA (1995) Postnatal maturation of the dopaminergic innervation of monkey prefrontal and motor cortices: a tyrosine hydroxylase immunohistochemical analysis. J Comp Neurol 358: 383–400

Rosenzweig MR, Bennett EL (1969) Effects of differential environments on brain weights and enzyme activities in gerbils, rats, and mice. Dev Psychobiol 2: 87–95

Seto-Ohshima A, Aoki E, Semba R, Emson PC, Heizmann CW (1990) Appearance of parvalbumin-specific immunoreactivity in the cerebral cortex and hippocampus of the developing rat and gerbil brain. Histochemistry 94: 579–589

Solbach S, Celio MR (1991) Ontogeny of the calcium binding protein parvalbumin in the rat nervous system. Anat Embryol (Berl) 184: 103–124

Somogyi P, Freund TF, Cowey A (1982) The axo-axonic interneuron in the cerebral cortex of the rat, cat and monkey. Neuroscience 7: 2577–2607

Spencer KM, Nestor PG, Niznikiewicz MA, Salisbury DF, Shenton ME, McCarley RW (2003) Abnormal neural synchrony in schizophrenia. J Neurosci 23: 7407–7411

Spencer KM, Nestor PG, Perlmutter R, Niznikiewicz MA, Klump MC, Frumin M, Shenton ME, McCarley RW (2004) Neural synchrony indexes disordered perception and cognition in schizophrenia. Proc Natl Acad Sci USA 101: 17288–17293

Tamas G, Buhl EH, Somogyi P (1997) Fast IPSPs elicited via multiple synaptic release sites by different types of GABAergic neurone in the cat visual cortex. J Physiol 500 (Pt 3): 715–738

Tamas G, Buhl EH, Lorincz A, Somogyi P (2000) Proximally targeted GABAergic synapses and gap junctions synchronize cortical interneurons. Nat Neurosci 3: 366–371

Tamas G, Lorincz A, Simon A, Szabadics J (2003) Identified sources and targets of slow inhibition in the neocortex. Science 299: 1902–1905

Thiessen DD, Yahr P (1977) The gerbil in behavioral investigations. Mechanisms of territoriality and olfactory communication. In: University of Texas Press, Austin

Uhlhaas PJ, Linden DE, Singer W, Haenschel C, Lindner M, Maurer K, Rodriguez E (2006) Dysfunctional long-range coordination of neural activity during Gestalt perception in schizophrenia. J Neurosci 26: 8168–8175

Valverde F (1998) Golgi atlas of the postnatal mouse brain. Springer, Wien, New York

Verney C, Alvarez C, Geffard M, Berger B (1990) Ultrastructural double-labelling study of dopamine terminals and GABA-containing neurons in rat anteromedial cerebral cortex. Eur J Neurosci 2: 960–972

Volk DW, Pierri JN, Fritschy JM, Auh S, Sampson AR, Lewis DA (2002) Reciprocal alterations in pre- and postsynaptic inhibitory markers at chandelier cell inputs to pyramidal neurons in schizophrenia. Cereb Cortex 12: 1063–1070

Weinberger DR, Lipska BK (1995) Cortical maldevelopment, anti-psychotic drugs, and schizophrenia: a search for common ground. Schizophr Res 16: 87–110

Williams MT, Vorhees CV, Boon F, Saber AJ, Cain DP (2002) Methamphetamine exposure from postnatal day 11 to 20 causes impairments in both behavioral strategies and spatial learning in adult rats. Brain Res 958: 312–321

Witte AV, Bagorda F, Teuchert-Noodt G, Lehmann K (2006) Contralateral prefrontal projections in gerbils mature abnormally after early methamphetamine trauma and isolated rearing. J Neural Transm. Epub ahead of print, DOI 10.1007/s00702-006-0506-6

Woo TU, Whitehead RE, Melchitzky DS, Lewis DA (1998) A subclass of prefrontal gamma-aminobutyric acid axon terminals are selectively altered in schizophrenia. Proc Natl Acad Sci USA 95: 5341–5346

9.1 Further publications and posters

Refereed Journals

Brummelte S., Teuchert-Noodt G., Grund T., Moll G.H. and Dawirs R.R: Environmental enrichment has no effect on the development of dopaminergic and GABAergic fibres during methylphenidate treatment of early traumatized gerbils. (in prep)

Witte A.V., Brummelte S. and Teuchert-Noodt G.: Pattern changes of prefrontal efferents in the juvenile gerbil *(Meriones unguiculatus)*.(submitted)

Grund T., Teuchert-Noodt G., Busche A., Neddens J., Brummelte S., Moll G.H. and Dawirs R.R.: Oral Methylphenidate during prepuberty prevents pharmacologically-induced (preweaning) suppressive development of dopamine projections into the prefrontal cortex and amygdala. J Child Adolesc Psychopharm (revision submitted)

Brummelte S., Pawluski J.L. and. Galea L.A.M. (2006b): High postpartum levels of corticosterone given to dams influence postnatal hippocampal cell proliferation and behaviour of offspring: A model of post-partum stress and possible depression. Horm Behav. 50(3):370-82

Poster and Presentations

Brummelte S., Pawluski J.L. and Galea L.A.M.: High post-partum levels of corticosterone given to dams influence postnatal hippocampal cell proliferation and behavior of offspring: A model of post-partum stress and possible depression. Focused Meeting of the Physiological Society: New Developments in Stress physiology: From Gene to Man, Bristol, UK, 2006, PC19

Brummelte S., Witte A.V., Graumueller S., and Teuchert-Noodt G.: Adaptive changes in GABAergic innervation pattern of the prefrontal cortex in an animal model of psychosis. FENS Abstr. Vol 3, A080.1, 2006

Witte A.V., Brummelte S., Bagorda F. and Teuchert-Noodt G.: Pattern changes of prefrontal efferents in the neocortex of juvenile gerbils. FENS Abstr. Vol 3, A228.16, 2006

Brummelte S., Pawluski J.L. and. Galea L.A.M: A possible model of post-partum depression based on high post-partum levels of corticosterone. SBN (Society for Behavioral Neuroendocrinology) Annual meeting 2005, Abstr. 50.

10. Acknowledgements

Mein besonderer Dank gilt Frau Prof. Dr. Dr. Teuchert-Noodt, die mir diese Promotion in der Abteilung für Neuroanatomie ermöglichte und sich immer für mich eingesetzt. Ihre durchgehende intensive Betreuung und konstruktive Kritik auch noch nach ihrer Pensionierung waren eine bedeutende Hilfe bei der Erstellung dieser Arbeit.

Auch Herrn Prof. York Winter und seinen Mitarbeitern gilt mein Dank für die freundliche Aufnahme in die Arbeitsgruppe und die hilfreiche Unterstützung.

Außerdem möchte ich mich herzlich bei Herrn Prof. Egelhaaf, für seinen persönlichen Einsatz bezüglich meiner erneuten Aufnahme in die Studienstiftung und für die Übernahme der formellen Betreuung der Doktorarbeit bedanken.

Des Weiteren möchte ich mich bei der gesamten Arbeitsgruppe Neuroanatomie für die gute Zusammenarbeit, die Unterstützung und ein tolles Arbeitsklima bedanken. Besonders erwähnt seien hier Frau Ulrike Schroeder für ihre tatkräftige Hilfe im Labor und ihr offenes Ohr, Herr Dr. Francesco Bagorda für seine Unterstützung bei den Versuchen und dafür, dass er immer da war, wenn man ihn brauchte, und last but not least Frau Dr. Andrea Busche und Herr Dr. Jörg Neddens, die mir trotz z.T. großer geographischer Distanzen immer mit Rat und Tat zur Seite standen. Darüber hinaus gilt auch Herrn Prof. Dawirs mein persönlicher Dank für die partielle finanzielle Unterstützung und die Kooperation bei den Ritalin-Studien.

Ebenso möchte ich mich bei Frau Dipl. Biol. Veronica Witte bedanken für ihre allgegenwärtige Motivation und den gegenseitigen Austausch.

I'd further like to thank Dr. Liisa Galea and the Laboratory of Behavioural Neuroscience at the UBC in Vancouver, Canada for a great project and collaboration and a really nice and successful 7-months stay as a visiting researcher.

Außerdem möchte ich mich bei der Studienstiftung des deutschen Volkes und der Graduiertenförderung der Universität Bielefeld für die ideelle und finanzielle Förderung bedanken, ohne die diese Promotion nicht möglich gewesen wäre.

Mein tiefster Dank gilt meiner Familie und besonders meinen Eltern, die mich auf meinem bisherigen Weg immer begleitet und nach besten Kräften unterstützt haben.

Eidesstattliche Erklärung

Hiermit erkläre ich, dass ich diese Arbeit selbstständig erstellt und nur die angegebenen Hilfsmittel und Quellen verwendet habe. Weiterhin erkläre ich, dass es sich um meinen ersten Promotionsversuch handelt.

Bielefeld, Januar 2007

_____

S u s a n n e B r u m m e l t e