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.

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1. Zusammenfassung

Schädigende Umwelteinflüsse können zu massiven Störungen der Hirnreifung führen. Störungen im *limbo-präfrontalen System*, welches als das höchste assoziative System des Gehirns gilt, werden mit verschiedenen psychischen Erkrankungen in Verbindung gebracht. An unserem Tiermodell (*Meriones unguiculatus*) konnte durch frühere Arbeiten gezeigt werden, dass sowohl eine chronische Schädigung in Form der restriktiven Isolationsaufzucht als auch eine akute Schädigung in Form der einmaligen Methamphetamin- (MA) Intoxikation am postnatalen Tag 14 Einfluss nehmen auf die Reifung der Monoamine Dopamin (DA) und Serotonin (5HT) im präfrontalen Kortex und Nucleus accumbens.

Ziel meiner Arbeit war es, den Einfluss dieser beiden experimentellen Parameter auf die postnatale DA und 5HT Reifung in der *Formatio hippocampalis* zu untersuchen und darüber hinaus ein Gesamtbild der Veränderungen dieser Monoamine innerhalb des limbo-präfrontalen Systems zu erstellen. Für die *Formatio hippocampalis* wurden folgende Resultate erhoben:

- Die restriktive Aufzucht führt zu einer leichten (nicht signifikanten) Anhebung der DAergen Faserdichte im *entorhinalen Kortex*. Die einmalige frühkindliche MA-Intoxikation zeigt eine signifikante Erhöhung der DAergen Faserdichte im *ventrolateralen entorhinalen Kortex* der rechten Hemisphäre von Tieren aus restriktiver Aufzucht sowie der linken Hemisphäre von Tieren aus semi-natürlicher Aufzucht. Die Faserdichte des *Subiculums* wird dagegen durch keinen der beiden experimentellen Parameter beeinflusst (Busche et al., 2004).
- Die 5HTergen Faserdichten werden durch die restriktive Isolationsaufzucht und die einmalige MA-Intoxikation bei semi-natürlicher Aufzucht in beiden untersuchten Arealen der *Formatio hippocampalis*, dem *Gyrus dentatus* und dem *lateralen entorhinalen Kortex*, signifikant erhöht. Eine zusätzliche Erhöhung der Faserdichte wurde bei MA-behandelten Tieren aus restriktiver Aufzucht im rechten *septalen Gyrus dentatus* und im linken *lateralen entorhinalen Kortex* gefunden. Dagegen führt die MA-Intoxikation bei restriktiv aufgewachsenen Tieren in den weiteren untersuchten Arealen der *Formatio hippocampalis* tendenziell (nicht signifikant) zur Absenkung der 5HTergen Faserdichte (Busche et al., 2002; Neddens et al., 2003, submitted).

Im Rückblick auf früheren Studien unserer Arbeitsgruppe werden beide Transmitter, DA und 5HT, auch in allen weiteren untersuchten Arealen des limbo-präfrontalen Systems in ihrer postnatalen Reifung stark beeinflusst. Insbesondere die strukturellen Veränderungen der DAergen Projektionen im limbo-präfrontalen System weisen auf eine, sich im Laufe des Reifungsprozesses entwickelten, Imbalance innerhalb dieses Systems hin, die in Form einer Hypoinnervation des präfrontalen Kortex und einer Hyperinnervation des entorhinalen Kortex und der Amygdala auftritt. Lokale Veränderungen der monoaminergen Innervation können sowohl die Aktivitäten innerhalb der einzelnen Regionen als auch die Konnek-tivität zwischen den einzelnen Arealen beeinträchtigen und somit die Funktion des Gesamtsystems erheblich stören. Unsere Befunde unterstützen aktuelle Modellvorstellungen zur Schizophrenie, die eine Hypoaktivierung des präfrontalen Kortex und eine Hyperaktivierung limbischer Areale postulieren.

2. Einleitung

Lernen, Gedächtnisbildung, Entwicklung von kompetentem Sozialverhalten und Strategienbildung sind komplexe Fähigkeiten, die unser Gehirn gewährleistet. Diese Leistungen beruhen darauf, dass das Gehirn kein funktionell und morphologisch starres System darstellt, sondern enorme plastische Kompetenzen aufweist, die gerade im Kindesalter besonders ausgeprägt sind. Ein Kind lernt ganz „unfreiwillig“ durch die intensive Auseinandersetzung mit seiner Umwelt während der Entwicklung. Dieses bringt zum Ausdruck, dass Lernen und Entwicklung zwei Seiten einer Medaille darstellen, also zwei Prozesse, die gar nicht voneinander getrennt werden können. Auf anatomischer und hirnphysiologischer Ebene bedeutet dies, dass während der Entwicklung die neuronalen Verbindungen selektiv reifen und sich stabilisieren. Dabei ist die Interaktion mit der Umwelt von entscheidender Bedeutung für die Ausbildung leistungsfähiger und sinnvoll strukturierter Nervennetze in den verschiedensten sensorischen, motorischen und assoziativen Systemen.

Mit der Geburt wird ein Säugling von einer Flut neuer Sinneseindrücke überschwemmt, die zu diesem Zeitpunkt nicht alle adäquat verarbeitet werden können. Erst nach und nach reifen einzelne Hirnareale heran, wobei die primär sensorischen und motorischen Gebiete früher reifen als die assoziativen Areale, wodurch mit zunehmendem Alter immer komplexere Verhaltensweisen ermöglicht werden (siehe Teuchert-Noodt und Lehmann, 2003). Dabei bedeutet die adaptive Reifung neuronaler Nervennetze nicht etwa ausschließlich eine sukzessive Zunahme von Verbindungen, vielmehr finden in einem hohen Maße Umbauprozesse bzw. Reorganisationen von neuronalen Verbindungen statt. Beispielsweise werden die Anzahl synaptischer Kontakte verändert, bestimmte synaptische Verknüpfungen verstärkt oder abgeschwächt sowie prä- und postsynaptische Rezeptordichten verändert (Wolff, 1982; Edelman, 1993). Neurotransmitter sind durch ihre morphogene Wirkung entscheidend an diesen Prozessen beteiligt (Mattson, 1988; Lauder 1988, 1993).

In den **kritischen Reifungsphasen** finden in einem besonderen Umfang plastische Umbauprozesse neuronaler Verbindungen in einzelnen Funktionssystemen bzw. Hirnarealen statt (Wolff, 1982). Am Ende einer solchen kritischen Reifungsphase hat sich ein relativ stabiles neuronales Netz herausgebildet. Innerhalb dieser kritischen Phasen liegen jedoch noch sehr instabile neuronale Verbindungen vor, die in dieser Zeit maßgeblich durch die Umwelt beeinflusst werden. Erfahrungen, die ein Individuum im Laufe dieser Entwicklungsphasen macht, prägen die Struktur und Funktion neuronaler Verknüpfungen im Gehirn. Somit hinterlassen auch negative Erfahrungen ihre Spuren im Gehirn, und traumatische Erlebnisse gerade im Kindesalter können demzufolge sogar zu einer dysfunktionalen Reifung des Gehirns führen, die sich noch viel später im Wesen und Verhalten des erwachsenen Menschen widerspiegeln kann.

Umwelt-induzierte Veränderungen der Struktur und Funktion während der Entwicklung des Gehirns sind auch Gegenstand der heutigen medizinisch-psychiatrischen Forschung, die eben

Störungen in der prä- und postnatalen Hirnreifung als eine Ursache für das Auftreten späterer psychischer Erkrankungen sieht (Weinberger, 1987; Weinberger et al., 1992; Egan und Weinberger, 1997; Raedler et al., 1998; Bogerts, 2002).

2.1. Zum Einfluss schädigender Faktoren auf das reifende Gehirn

Es ist hinreichend belegt, dass sowohl extrinsische (über die Umwelt) als auch intrinsische Aktivitäten (innerhalb eines neuronalen Netzes), insbesondere während der kritischen Reifungsphasen, sehr starken Einfluss auf die Struktur und Funktion des Gehirns nehmen. Schon vor nunmehr fast 30 Jahren konnten Hubel und Wiesel (1977) an Primaten zeigen, dass eine Reizdeprivation in der kritischen Reifungsphase die Struktur des visuellen Kortex entscheidend prägt. Die restriktive Isolationsaufzucht stellt seitdem ein bewährtes Tiermodell dar, an dem der Einfluss der Umwelt auch auf die Reifung anderer höherer Hirnareale des Gehirns untersucht werden kann. Zum Beispiel weisen isoliert aufgewachsene Tiere an Neuronen des *Hippocampus* (HC), des *Gyrus dentatus* (GD), des *entorhinalen Kortex* (EC) und des *präfrontalen Kortex* (PFC) morphologische Veränderungen der Dendriten auf, insofern als die Dendritenlänge, die Anzahl dendritischer Verzweigungen und die Spinedichten der Neurone zum Teil stark abgesenkt sind (Bartesaghi und Serrai, 2001; Bartesaghi und Severi, 2002; Bartesaghi et al., 2003a, b; Silva-Gómez et al., 2003). Weiterhin ist *in vitro* gezeigt worden, dass der entorhinale Eingang in den HC die Dendritenlänge der Körnerzellen im GD beeinflussen kann, und die neuronale Aktivität des EC entscheidend an der Reifung der dendritischen Spines mitwirkt (Drakev et al., 1999; Frotscher et al., 2000).

Die Monoamine Dopamin (DA) und Serotonin (5HT) scheinen besonders empfindlich auf Umwelteinflüsse zu reagieren. Für beide Transmitter konnte gezeigt werden, dass die Aufzucht unter reizarmen isolierten Bedingungen zu Veränderungen ihrer Physiologie – beispielsweise der Rezeptordichten, der Transmitterausschüttung und der -umsatzrate – führt (Jones et al., 1992; Hall, 1998; Heidbreder et al., 2000; Lapiz et al., 2003). Auf Grund ihrer morphogenen Eigenschaft spielen sie für die Neurogenese, die Differenzierung der Neurone und die Synaptogenese in der prä- und postnatalen Entwicklung eine besondere Rolle (Lanier et al., 1976; Mattson, 1988; Lauder, 1988; 1993; Lipton und Kater, 1989; Nguyen et al., 2001). Störungen in diesen Transmittersystemen sollten sich demnach sowohl auf die Struktur der Neurone als auch auf deren Konnektivität auswirken. In diesem Zusammenhang konnte gezeigt werden, dass eine Reduktion der DAergen präfrontalen Innervation zu einer Verringerung der basalen Dendritenlänge und der dendritischen Verzweigung präfrontaler Pyramidenzellen führt (Kalsbeek et al., 1989). Ebenso geht beispielsweise eine Schädigung der 5HTergen Innervation bei juvenilen Ratten mit einer Verringerung der Synapsendichte im HC adulter Tiere einher (Mazer et al., 1997; Yan et al., 1997).

Darüber hinaus stehen DA und 5HT in der prä- und postnatalen Reifungsphase in enger Wechselwirkung zueinander (Lauder, 1988; Benes et al., 2000). Es konnte gezeigt werden, dass 5HT das Einwachsen DAerger Fasern in den medialen PFC beeinflusst, insofern als eine Läsion der Raphekerne in neugeborenen Ratten eine Hyperinnervation DAerger Fasern in jung erwachsenen Tieren induziert (Taylor et al., 1998). Verhindert man andererseits bei neugeborenen Ratten das Einwachsen DAerger Fasern in ihre Terminationsgebiete, so sprouten an deren Stelle 5HTerge Fasern vermehrt aus und resultieren in einer 5HTergen Hyperinnervation in diesen Gebieten (Berger et al., 1985; Towle et al., 1989).

Störungen der strukturellen Reifung des Gehirns sind häufig gekoppelt mit einer Reihe von Verhaltensauffälligkeiten. Tiere aus Isolationsaufzucht zeigen beispielsweise eine höhere motorische Aktivität (Wright et al., 1991; Hall et al., 1998; Heidbreder et al., 2000) und Ängstlichkeit (Hall et al., 1998; Molina-Hernandez et al., 2001). Ebenso ist mehrfach nachgewiesen worden, dass diese Tiere deutlich schlechter mit Stresssituationen umgehen können (Klein et al., 1994; Anisman et al., 1998) und in verschiedenen Lerntests erhebliche Defizite aufweisen im Vergleich zu ihren Artgenossen, die in einer reizreichen Umgebung mit der Möglichkeit zu sozialen Kontakten aufgewachsen sind (Juraska et al., 1984; Park et al., 1992; aber Wongwitdecha und Marsden, 1996). Diese Komplexität der Verhaltensauffälligkeiten werden auch als „social isolation syndrome“ bezeichnet (Heidbreder et al., 2000).

2.1.1. Unser Tiermodell

Für unsere Versuche werden ausschließlich männliche Individuen genommen. Um schädigende Einflüsse auf die Reifung des Gehirns an einem „nicht-invasiven“ Tiermodell untersuchen zu können, bedient sich unsere Arbeitsgruppe folgender zwei Ansätze:

(1) **Restriktive Isolationsaufzucht**: die Gerbils werden in Makrolonkäfigen (Typ IV) geboren und

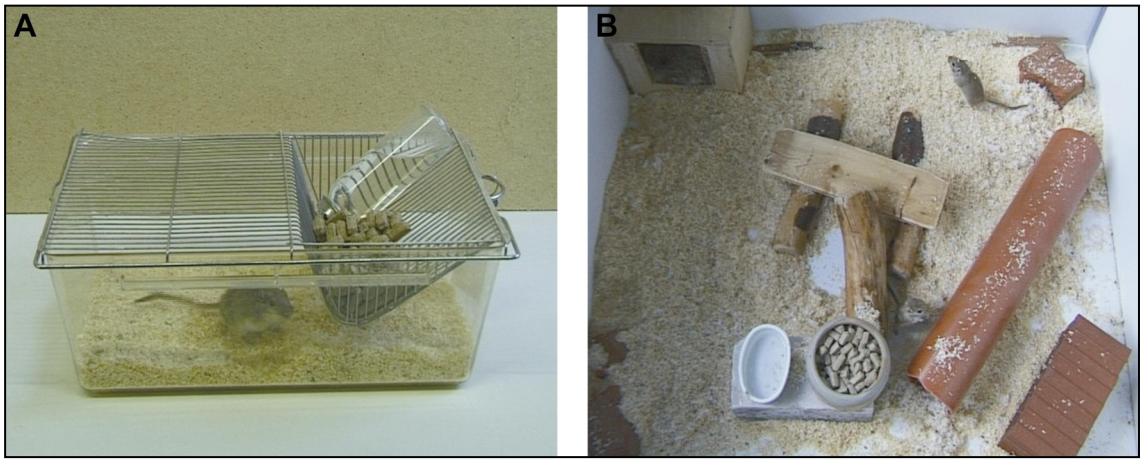

Abb.1: Aufzuchtbedingungen
Bei der restriktiven Isolationsaufzucht (**A**) wachsen die Gerbils nach ihrer Entwöhnung einzeln in Käfigen auf, die lediglich mit Einstreu versehen sind. Die semi-natürliche Aufzucht (**B**) bietet den Gerbils dagegen ein 1x1m großes Gehege, welches mit Spiel- und Versteckmöglichkeiten ausgestattet ist und ihnen die Möglichkeit zu sozialen Kontakten mit Geschwistertieren gibt.

nach ihrer Entwöhnung am Tag 30 in Makrolonkäfige (Typ III) vereinzelt, wo sie bis zum Tag der Perfusion verbleiben. Als Kontrolltiere dienen Gerbils, die im Gehege geboren werden und nach ihrer Entwöhnung im Geschwisterverband in 1x1m großen Gehegen aufwachsen, die mit Spiel- und Versteckmöglichkeiten ausgestattet sind (Abb.1). Die Isolationsaufzucht stellt demnach eine chronische soziale Deprivation für die Tiere dar. Bei isoliert aufgewachsenen Gerbils sind die DAergen Faserdichten im PFC, und im dorsalen Striatum signifikant abgesenkt (Winterfeld et al., 1998; Neddens et al., 2001; Lehmann et al., 2002). Im Gegensatz führt die restriktive Aufzucht zu einer Erhöhung der 5HT Faserdichten in mehreren Areale des Gehirns (Neddens et al., 2003; Lehmann et al., 2003). Diese Strukturveränderungen sind gekoppelt mit einer erhöhten motorischen Aktivität im open field und deutlichen Schwächen des Arbeitsgedächtnisses (Winterfeld et al., 1998).

(2) Einmalige, frühkindliche Gabe von **Methamphetamin** (MA; 50mg/kg, i.p.) am postnatalen Tag 14: Im Vergleich zur Isolationsaufzucht stellt die MA-Injektion eine punktuelle starke Beeinträchtigung dar, die zu einem sehr kritischen Zeitpunkt der Reifung passiert. Methamphetamin wirkt selektiv neurotoxisch auf DA und 5HT im ZNS (Seiden et al., 1988; zusammengefasst in Seiden und Sabol, 1996). Durch MA wird vermehrt DA und 5HT von den Terminalien ausgeschüttet und gleichzeitig der Reuptake in die Präsynapse gehemmt, so dass es zu einer Akkumulation der Transmitter im synaptischen Spalt kommt. Dieses forciert die nicht-enzymatische Bildung von 6-Hydroxydopamin bzw. 5,7-Dihydroxytryptamin (Seiden und Vosmer, 1984), die wieder in die Präsynapse aufgenommen werden und dort wahrscheinlich degenerative Prozesse auslösen (Ricaurte et al., 1982; Marek et al., 1990). Es hat sich herausgestellt, dass sogar eine einmalige hoch dosierte Injektion von MA ausreichend ist, um DAerge (im Striatum) und 5-HTerge (im HC) Terminalien zu zerstören (Seiden und Vosmer, 1984; Commins et al., 1987; Fukumura et al., 1998). Für Gerbils konnte nachgewiesen werden, dass die einmalige MA-Intoxikation bei juvenilen Tieren selektiv neurotoxisch im PFC wirkt, während sich bei adult behandelten Tieren die MA-Toxizität im Striatum zeigt (Teuchert-Noodt und Dawirs, 1991). Kombiniert wird die MA-Intoxikation mit den zwei verschiedenen Aufzuchtbedingungen.

Die MA-Intoxikation am postnatalen Tag 14 erfolgt zu einem Zeitpunkt, an dem die beiden Transmitter DA und 5HT noch nicht voll ausgereift sind (Lanier et al., 1978; Mattson, 1988). Unsere bisherigen Ergebnisse deuten darauf hin, dass MA „in Abhängigkeit der Aufzucht-bedingungen zu einer selektiven Veränderung jeweils unterschiedlicher Transmittersysteme führt“ (Neddens, 2002). Methamphetamin induziert bei isoliert aufgewachsenen Gerbils eine weitere Absenkung der präfrontalen DAergen Faserdichte (Dawirs et al., 1994), die einhergeht mit einer ebenfalls deutlichen Verschlechterung des Arbeitsgedächtnisses (Dawirs et al., 1996). Keine Veränderung der Faserdichte zeigt sich dagegen bei MA-behandelten Gehegeaufzuchten (Neddens, 2002). Für das 5HTerge System zeigt sich ein genau entgegen gesetztes Bild: MA-

behandelte Tiere aus Gehegeaufzucht zeigen eine deutliche Erhöhung der präfrontalen Faserdichten, während kein Unterschied bei den isoliert aufgewachsenen Tieren zu finden ist (Neddens et al., 2003). Im Nucleus accumbens (NAC) sind die DAergen und 5HTergen Faserdichten sowohl bei den MA-behandelten isoliert aufgewachsenen Gerbils als auch den behandelten Gehegeaufzuchten betroffen (Neddens et al., 2002; Lehmann et al., 2003). Wobei die DAergen Faserdichten generell abgesenkt und die 5HTergen Faserdichten im NAC erhöht sind.

Beide Versuchsparameter – die restriktive Isolationsaufzucht und die einmalige frühkindliche MA-Intoxikation – haben weitere adaptive Veränderungen im Gehirn von Gerbils zur Folge. Der durch MA hervorgerufene Verlust DAerger präfrontaler Fasern wird beispielsweise durch ein lokales Sprouten GABAerger Profile kompensiert (Nossoll et al., 1997). Gleichzeitig kommt es zu einem chronischen Anstieg synaptischer Spines an präfrontalen Pyramidenzellen (Blaesing et al., 2001). Über diese lokalen Veränderungen hinaus, hier speziell im PFC, geben anterograde Tracerstudien erste Hinweise darauf, dass sich ganze Nervennetze umorganisieren. Sowohl die Isolationsaufzucht als auch die zusätzliche MA-Intoxikation verändern die präfrontalen efferenten Projektionen in kortikale (Bagorda F, in Vorbereitung) sowie subkortikale Regionen (Lehmann, 2001).

Unsere Resultate und die anderer Autoren zeigen deutlich, dass Umwelt-induzierte Störungen der Reifungsprozesse zu komplexen adaptiven strukturellen Veränderungen im Gehirn führen. Eine Fülle von Verhaltensauffälligkeiten bei kognitiven, mnemotischen, motorischen und emotionalen Tests lässt erahnen, dass diese Strukturveränderungen nicht nur lokal in einzelnen Hirngebieten, sondern in einem, mehrere Hirnareale umfassenden, funktionellen neuronalen Netzwerk auftreten. Dieses umfasst im Gehirn von Säugetieren und dem Menschen speziell den PFC und verschiedene limbische Areale, die zusammen auch als **limbo-präfrontales System** bezeichnet werden.

3. Das limbo-präfrontale System

Ausgehend von der ersten Beschreibung eines kleinen geschlossenen Neuronenkreislaufes, der für die Emotionsbildung verantwortlich sein sollte (Papez, 1937), wurde der Begriff limbisches System eingeführt. Mittlerweile werden immer mehr neuronale Strukturen diesem System zugerechnet. Unter dem Begriff limbisch sind all jene funktionellen Aspekte subsumiert, die sich von der primären Motorik und Sensorik abgrenzen, wie etwa Motivation, Emotion, Lern- und Gedächtnisfunktionen. Zu den limbischen Arealen zählen insbesondere die Hippocampusformation (HF: umfasst den GD, die CA-Regionen des HC, das Subiculum, sowie Prä- und Parasubiculum und den EC), die Amygdala und der NAC, sowie die im Hirnstamm lokalisierten monoaminergen

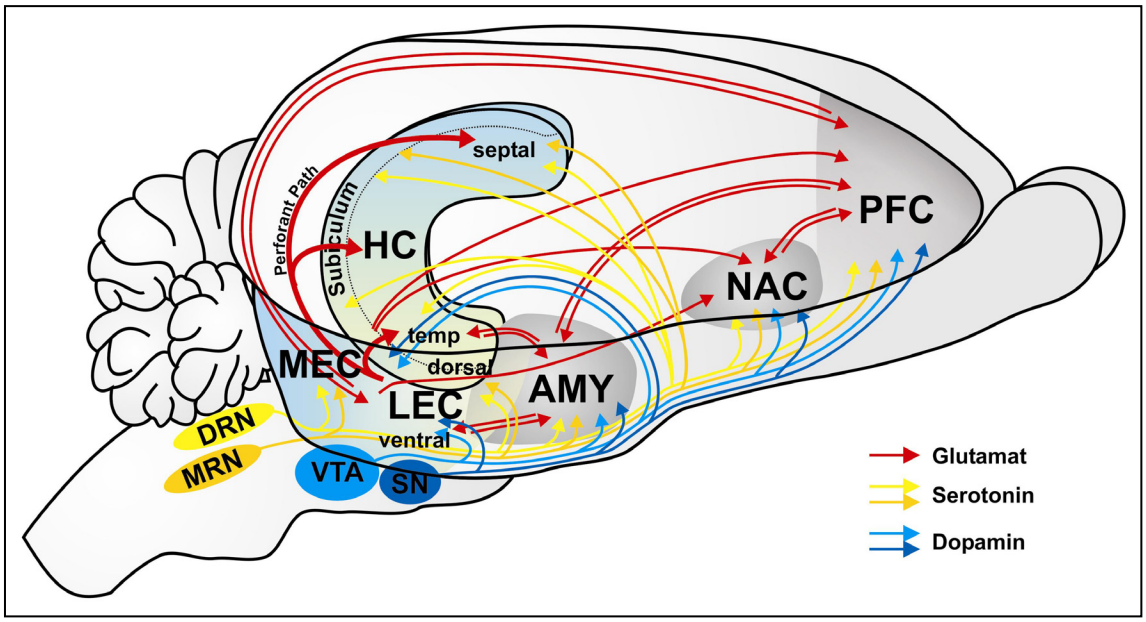

Abb.2: Eine schematische Darstellung des limbo-präfrontalen Systems bei Nagern
Die einzelnen Kortexareale und Kerngebiete des limbo-präfrontalen Systems weisen zahlreiche, zumeist reziproke, Verbindungen miteinander auf und zeichnen sich durch eine starke monoaminerge Innervation aus den Raphe-Kernen und der VTA / SN aus. Dopaminerge Fasern innervieren ganz spezifisch einzelne Subregionen des limbo-präfrontalen Systems, während die serotonergen Neurone sehr gleichmäßig in alle Hirngebiete projizieren.

AMY	Amygdala	MRN	Nucleus raphe medialis
DRN	Nucleus raphe dorsalis	NAC	Nucleus Accumbens
HC	Hippocampus	PFC	präfrontaler Kortex
LEC	lateraler entorhinaler Kortex	SN	Substantia nigra
MEC	medialer entorhinaler Kortex	VTA	ventrale tegmentale Area

Kerngebiete, die alle sowohl anatomisch als auch funktionell eng miteinander verknüpft sind (Krettek und Price, 1977; Cassel et al., 1986; Le Moal und Simon, 1991; Jacobs und Azmitia, 1992; Totterdell und Meredith, 1997; Pikkarainen et al., 1999; Pitkanen, 2000; Pitkanen et al., 2000; Pikkarainen und Pitkanen, 2001). Der PFC unterhält vielfache zum Teil reziproke Verbindungen zu den oben genannten limbischen Arealen (Swanson, 1981; Jay und Witter, 1991; Sesack und Pickel, 1992; Carr und Sesack, 1996; McDonald et al., 1996; Insausti et al., 1997; Rosenkranz und Grace, 2001, 2002), was zu der Prägung des Begriffes limbo-präfrontales System geführt hat (Abb.2; Teuchert-Noodt und Dawirs, 1999).

Insbesondere zwei kortikale Strukturen des limbo-präfrontalen Systems, der PFC und die HF, stellen wahrscheinlich entscheidende Integrationszentren in diesem System dar, indem sie multimodale Afferenzen sowohl aus assoziativen Kortexarealen als auch aus subkortikalen Kerngebieten erhalten und in weite Bereiche des Gehirns zurückprojizieren (Sesack et al., 1989; Amaral und Witter, 1995; Fuster, 1997; Insausti et al., 1997). Da der PFC und die HF in höchste kognitive Funktionen eingebunden sind, die sich erst im Erwachsenen voll entwickelt haben, ist es verständlich, dass sich diese Strukturen zudem durch lange Reifungsphasen auszeichnen. Der PFC (entscheidend für das Sozialverhalten, willentliche Entscheidungen, Raum-Zeit-Verrechnung) reift, insbesondere durch die sehr langsam einwachsende mesopräfrontale DA-Projektion (Kalsbeek et al., 1988; Dawirs et al., 1993; Rosenberg und Lewis, 1995), noch bis ins junge Erwachsenenalter

(Mrzljak et al., 1990; Casey et al., 2000). Die HF (entscheidend für das Kurzzeitgedächtnis, Aufmerksamkeit, Antrieb) verbleibt dagegen auf Grund der anhaltenden Neurogenese im GD (Altman und Das, 1965; Kaplan und Bell, 1984; Altman und Bayer, 1990; Eriksson et al., 1998) sogar ein Leben lang in einem Zustand fortdauernder Entwicklung, wobei an Gerbils gezeigt wurde, dass die Zellproliferation postnatal am höchsten ist und mit zunehmendem Alter immer mehr abnimmt (Dawirs et al., 2000). Die sich ständig in das lokale Netzwerk einbringenden jungen Körnerzellen veranlassen die hohe Plastizität in der HF. Das hohe Maß an Plastizität im PFC sowie in der HF bedingt vermutlich einerseits hohe Lern- und Gedächtnisleistungen, und andererseits eine erhöhte Vulnerabilität gegenüber schädigenden Umwelteinflüssen.

Darüber hinaus beeinflussen sich der PFC und die HF sehr stark gegenseitig in ihrer Entwicklung: Bei Ratten führen neonatale Läsionen des ventralen HC bei jung adulten Tieren zu einer Verringerung der Spinedichte und der Dendritenlänge präfrontaler Pyramidenzellen (Lipska et al., 2000) sowie zu einer Veränderung der glutamatergen Transmission dieser Zellen (Schroeder et al., 1999). Zusätzlich ist die Leistung des Arbeitsgedächtnisses dieser Tiere schlechter im Vergleich zu nicht-lädierten Kontrolltieren, was auch auf eine funktionelle Störung des PFC schließen lässt (Lipska et al., 2002a). Erst kürzlich konnten Lipska und Kollegen (2002b) an Ratten zeigen, dass sogar eine kurzfristige, reversible Inaktivierung des ventralen HC in einer kritischen Entwicklungsphase (P7) später zu komplexen Verhaltensauffälligkeiten bei adulten Tieren führt. Die beobachteten Verhaltensänderungen lassen auch hier auf Störungen funktioneller Circuits schließen, die über den zunächst betroffenen HC hinausgehen. Wurde die kurzfristige Inaktivierung des ventralen HC hingegen bei adulten Tieren durchgeführt, hatte dies keinerlei langfristige Auswirkungen auf das Verhalten. Dies macht noch einmal die besondere Vulnerabilität neuronaler Netze während der Entwicklung deutlich.

Zusammenfassend lässt sich aus diesen Befunden ableiten, dass Störungen der postnatalen Reifung eines dieser beiden Areale zu **adaptiven Veränderungen** des gesamten limbo-präfrontalen Systems und möglicherweise sogar zu einer **Diskonnektion** innerhalb dieses funktionalen Nervennetzes führen können (Friston, 1998). In Anbetracht der bereits an unserem Tiermodell gewonnenen Resultate zur adaptiven Reifung der DAergen und 5HTergen Faserdichten im PFC und NAC ergab sich daher unweigerlich die Frage nach vergleichbaren Veränderungen dieser Monoamine in der Formatio hippocampalis, was Thema meiner Dissertation wurde.

3.1. Zur Struktur und Funktion der Formatio hippocampalis

Die HF als multimodales Integrationszentrum ist in viele verschiedene Funktionen eingebunden. Sie spielt eine essentielle Rolle in Bezug auf Lern- und Gedächtnisleistungen und „prüft“ in diesem Zusammenhang eingehende Aktivitäten auf ihren Neuheitswert (Izquierdo und Medina, 1997; Eichenbaum, 1999). Über zahlreiche Verbindungen zur Amygdala (McDonald und

Mascagni, 1997; Pikkarainen et al., 1999; Pitkanen et al., 2000) und zu endokrinen vegetativen Zentren, wie etwa dem Hypothalamus, ist die HF ebenfalls in emotionale, motivationale Funktionen involviert (Amaral und Witter, 1995). Eine prominente Projektion des ventralen Subiculums und des EC zum NAC deutet weiterhin auf eine Beteiligung der HF an motorische Funktionen hin (Witter et al., 1989; Brudzynski und Gibson, 1997; Totterdell und Meredith, 1997; Bardgett und Henry, 1999).

Die Vielschichtigkeit der funktionellen Einbindungen der HF gibt zu der Vermutung Anlass, dass die HF möglicherweise keine funktionelle Einheit darstellt, sondern es wenigstens zwei unterschiedliche Subsysteme in der HF gibt. Für den HC ist eine funktionelle Trennung des dorsalen oder auch septalen Teils von dem ventralen oder auch temporalen Teil schon mehrfach vorgeschlagen worden (Moser und Moser, 1998; Pothuizen et al., 2004). Der dorsale HC scheint für die Gedächtnisleistungen – insbesondere für das räumliche Gedächtnis – essentiell zu sein, während der ventrale HC eher in autonome, endokrine und emotionale Funktionen eingebunden ist. Unterstützt wird diese These der funktionalen Trennung auch durch die topographisch geordneten entorhinalen Projektionen in den HC.

Der EC stellt die Haupteingangspforte in den HC dar, über den die meisten kortikalen sowie einige subkortikale Eingänge verschalten und von dort über den glutamatergen Perforant Path den GD und die CA-Regionen des HC erreichen (siehe Amaral und Witter, 1995). Entorhinale Neurone aus Lamina II projizieren in den GD und die CA3-Region, während die Lamina III-Neurone die CA1-Region und das Subiculum innervieren (Steward und Scoville, 1976; Ruth et al., 1982, 1988; Amaral und Witter, 1995; Dolorfo und Amaral, 1998). Grundsätzlich wird der EC in zwei Subregionen unterteilt: den lateralen EC (LEC) und den medialen EC (MEC) (Brodmann, 1909; Blackstad, 1956). Auf Grund der Konnektivität und unterschiedlicher histochemischer Eigenschaften einzelner entorhinaler Anteile, werden mittlerweile von verschiedenen Autoren Einteilungen von bis zu sechs Subarealen vorgeschlagen (Insausti et al., 1997). Ich werde mich im weiteren Text an der Nomenklatur nach Krettek und Price (1977) orientieren, die innerhalb des LEC noch einen dorsalen, ventralen und ventromedialen Teil unterscheiden (Abb.2). Der dorsale LEC sowie der caudale Teil des MEC erhalten überwiegend neokortikale Afferenzen (Deacon et al., 1983; Burwell und Amaral, 1998), während der ventrale LEC und rostrale Anteile des MEC starke reziproke Verbindungen zur Amygdala und anderen subkortikalen Gebieten aufweisen (Krettek und Price, 1977; McDonald und Mascagni, 1997; Miettinen et al., 1996; Pitkanen, 2000). Die entorhinalen Afferenzen in den GD folgen wiederum einem Gradienten: laterale und caudale Anteile des EC projizieren in den septalen GD, während die Efferenzen der rostromedialen Subregionen im temporalen GD terminieren (Abb.3; Ruth et al., 1982, 1988; Dolorfo und Amaral, 1998). Diese topographische Ordnung unterstützt die funktionale Trennung des septalen vom temporalen HC insofern, als dass neokortikale sensorische Afferenzen verstärkt den septalen HC und subkortikale Afferenzen den temporalen HC erreichen (siehe Dolorfo und Amaral, 1998).

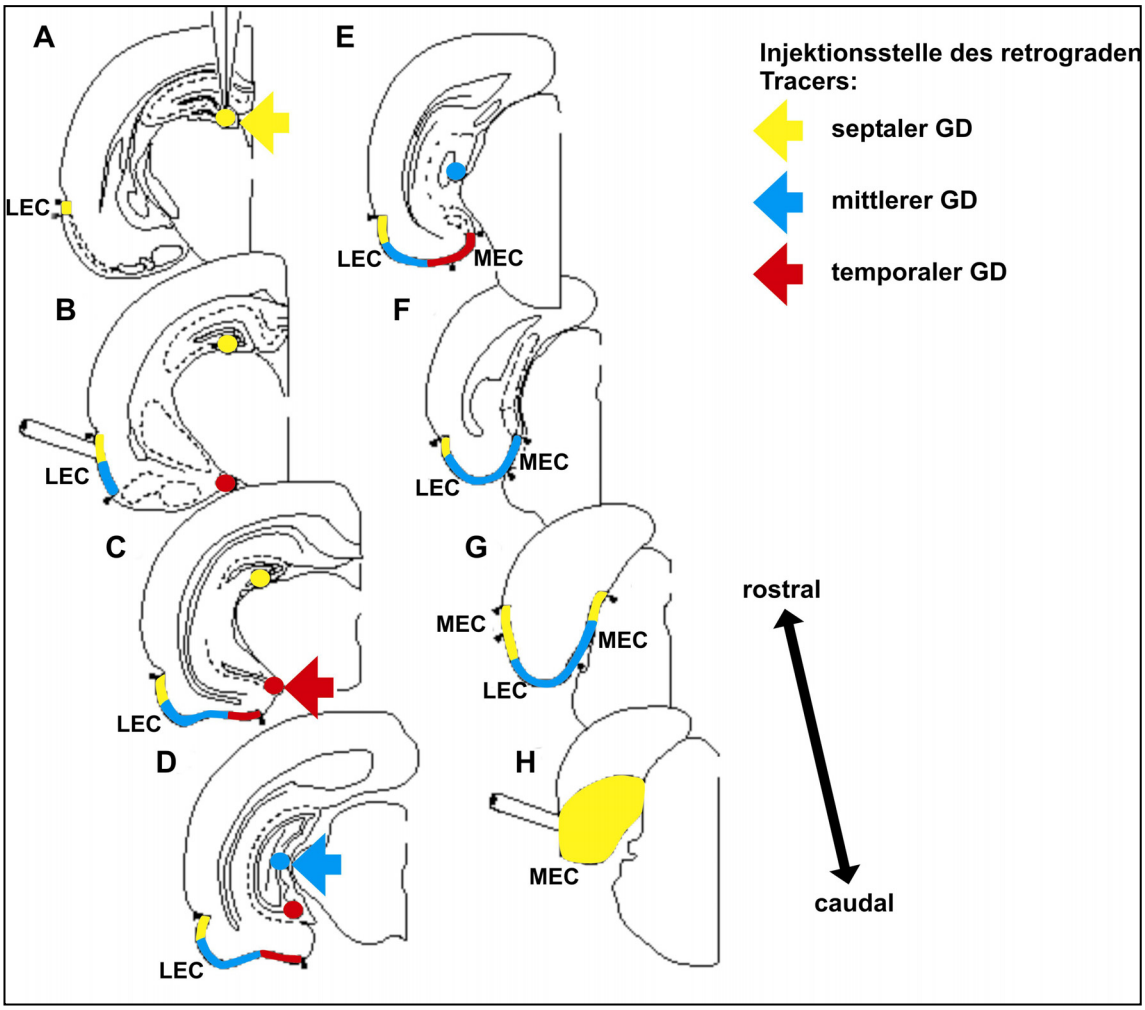

Abb.3: Darstellung der topographisch geordneten Projektionen des entorhinalen Kortex in den Gyrus dentatus
Der am weitesten lateral gelegene Teil des LEC und ganz caudale Subregionen des MEC projizieren in den septalen GD (gelb). Ventrolaterale Anteile des LEC und Teile des MEC senden ihre Efferenzen in eine mittlere Ebene des GD (blau), während der temporale GD Afferenzen aus dem mehr ventromedial gelegenen Teil erhält (rot). Entsprechend der Eingänge in die verschiedenen Subregionen des entorhinalen Kortex erhält der septale GD eher sensorische, neokortikale Informationen, während der temporale GD funktionell stärker mit der Amygdala verbunden ist. (Modifiziert nach Amaral und Dolorfo, 1998)

LEC lateraler entorhinaler Kortex
MEC medialer entorhinaler Kortex

Die Efferenzen verlassen den HC zum größten Teil über das Subiculum via den Fornix bzw. auch über den EC, der Projektionen aus dem Subiculum erhält (Witter und Groenewegen, 1990; Canteras und Swanson, 1992; Amaral und Witter, 1995). Auch im Subiculum findet sich eine topographische Ordnung wieder: Aus dem ventralen Anteil werden überwiegend Efferenzen in den NAC (Groenewegen et al., 1987) und die Amygdala (Price et al., 1987) gesendet, während der dorsale Anteil seine stärkste Projektion in die Mammilarkörper schickt (Witter und Groenewegen, 1990). Im Gegensatz zu der CA1-Region, von der nur der temporale Teil in den PFC projiziert (Jay und Witter, 1991), gibt das Subiculum über seine gesamte septo-temporale Ausdehnung Afferenzen an den PFC ab, jedoch überwiegend aus dem an die CA1-Region angrenzenden proximalen Anteil (Jay und Witter, 1991). Über die Projektion in den EC wird nahezu der gesamte

Kortex von dem Output des Subiculums erreicht (Insausti et al., 1997). Grundsätzlich scheint es so zu sein, dass die Subregionen des EC, die assoziativen sensorischen Input erhalten, entsprechend Efferenzen in occipitale, temporale, parietale, frontale und cinguläre Kortizes entsenden. Intermediäre und mediale Subregionen projizieren demgegenüber eher in den medial frontalen Kortex und in olfaktorische Strukturen (Insausti et al., 1997).

Eine besondere Struktur der HF ist der GD, da in dessen subgranulärer Schicht sich perinatal ein sekundäres Keimlager etabliert, aus welchem ein Leben lang neue Zellen proliferieren (s.o.). Die genaue Funktion dieser anhaltenden Zellproliferation ist noch nicht geklärt, bislang wird über eine Bedeutung im Zusammenhang mit den hippocampalen Lern- und Gedächtnisleistungen spekuliert (Gould et al., 1999; Kempermann und Gage, 1999; Dawirs et al., 2000; Teuchert-Noodt, 2000). Neuere Untersuchungen bringen die Neurogenese auch mit der Kontrolle über bestimmte motorische Verhaltensweisen in Verbindung (Rhodes et al., 2003). Gesichert ist dagegen, dass auf Grund der mitotischen Aktivität immer wieder neue Zellen in das lokale neuronale Netz des GD drängen und integriert werden, wodurch das Gleichgewicht des Mikrocircuits des GD fortwährend gestört wird. Die Integration neuer Körnerzellen in diesen Mikrocircuit zieht einen anhaltenden hohen synaptischen Umbau nach sich und bedingt eine ständige Strukturerneuerung in diesem Gebiet (Dawirs et al., 1992; 2000; Geinisman, 2000; Keller et al., 2000). Gut untersucht sind Faktoren, die die Zellproliferation im GD regulieren. So beeinflussen Steroide (Cameron und Gould, 1994), Stress (Gould et al., 1997), Glutamat (Gould et al., 1994; Cameron et al., 1995), DA (Dawirs et al., 1998, Hildebrandt et al., 1999; Teuchert-Noodt et al., 2000), Acetylcholin (Mohapel et al., 2002) und 5HT (Brezun und Dazuta, 1999) nachweislich die mitogene Aktivität. Auf die Bedeutung von DA und 5HT für die Zellproliferation im GD wird unter Punkt 3.4. noch näher eingegangen.

3.2. Zur dopaminergen Innervation des limbo-präfrontalen Systems

Dopamin ist einer der am besten untersuchten Neurotransmitter des Gehirns. Dieser Transmitter nimmt in der Koordination und Integration motorischer, limbischer und kognitiver Verhaltensaspekte eine Schlüsselrolle ein (siehe Nieoullon, 2002). Über zwei verschiedene Rezeptortypen, D_1- und D_2-Rezeptoren, ist das DAerge System in der Lage, auf vielfältige Weise modulatorisch Einfluss auf unterschiedliche Verhaltensaspekte zu nehmen (siehe Pralong et al., 2002). Die DAergen Bahnen zum Telencephalon entspringen aus drei Kerngebieten des Mesencephalons: (1) der ventralen tegmentalen Area (VTA, A10), (2) der Substantia nigra (SN, A9), und (3) dem retrorubalen Feld (A8) und innervieren sehr spezifisch einzelne Areale des Gehirns (siehe Björklund und Lindvall, 1984; Yoshida et al., 1988). Als mesohippocampale Projektion erreichen DAerge Fasern hauptsächlich aus der VTA den HC und den EC (Fallon et al., 1978; Gasbarri et al., 1994) und zeigen in diesen beiden Regionen ein sehr spezifisches

Innervationsmuster, wobei generell die ventralen Anteile eine höhere DAerge Faserdichte aufweisen als die dorsalen. Im HC des Nagerhirns sind der Übergang der CA1-Region zum Subiculum und die Molekularschicht des Subiculums am dichtesten DAerg innerviert, während in den übrigen hippocampalen Arealen nur vereinzelte Fasern zu finden sind (Verney et al., 1985; siehe auch Busche et al., 2004). Im EC sind der MEC und der dorsale LEC eher moderat von DAergen Fasern durchzogen, die überwiegend in den Laminae III-VI terminieren. Der ventrale LEC ist dagegen sehr dicht DAerg innerviert und die Fasern sind zum Teil in Clustern arrangiert (Fallon et al., 1978; Busche et al., 2004). Gerade die ventralen Subregionen des HC und des EC sind eng mit Teilen des PFC, der Amygdala sowie dem NAC verknüpft, die zusammen ein funktionales Nervennetz bilden, welches Emotion, Motivation und autonome Funktionen repräsentiert (s.o.). Die hohe DAerge Innervation dieser Hirngebiete deutet daraufhin, dass dieses funktionelle System sehr wahrscheinlich unter starkem modulatorischem DAergen Einfluss steht.

Die mesohippocampale DAerge Projektion in den HC und den EC ist Teil des **mesokortikolimbischen Systems** (Le Moal und Simon, 1991), zu dem ebenso die mesopräfrontalen, die mesoamygdaloiden und die mesoaccumbalen DAergen Bahnen gezählt werden. Mit Hilfe von selektiven Läsionen hat man herausgefunden, dass diese einzelnen DAergen Bahnen nicht isoliert voneinander ausschließlich in ihren spezifischen Terminationsgebieten regulatorisch wirksam sind, sondern sich in einem komplexen Netzwerk gegenseitig beeinflussen (Le Moal und Simon, 1991). Die Schädigung einer dieser DAergen Projektionen sollte demnach zu Veränderungen in den anderen DA-Subsystemen führen. Entsprechend hat eine neurotoxische Läsion der DAergen präfrontalen Innervation eine erhöhte DAerge Ausschüttung im NAC, insbesondere in Stresssituationen, zur Folge (King et al., 1997). Ein gestörtes Gleichgewicht innerhalb des mesokortikolimbischen Systems wird als eine mögliche Ursache psychischer Erkrankungen, insbesondere der Schizophrenie, gesehen (Davis et al., 1991; Jentsch et al., 2000; Sesack und Carr, 2002; Meyer-Lindenberg et al., 2002). So wird von einer DAergen Hypoaktivität im PFC, die möglicherweise für die Negativsymptomatik (z.B. Antriebslosigkeit, Denkstörungen) der Schizophrenie mitverantwortlich ist, und einer DAergen Hyperaktivität in limbischen Arealen gesprochen, die für die Positivsymptomatik (z.B. Halluzinationen, Wahnvorstellungen) unter anderem ursächlich sein könnte (Crow, 1980; Davis et al., 1991).

3.2.1. Zu den Befunden an unserem Tiermodell

In entsprechender Weise wie für die Schizophrenie auf der physiologischen Ebene vermutet wird, bildet sich in unserem Tiermodell eine Imbalance des mesokortikolimbischen DA Systems auf struktureller Ebene aus. Die quantitative Untersuchung DAerger Faserdichten in den einzelnen Regionen des limbo-präfrontalen Systems hat gezeigt, dass die angewandten experimentellen Parameter (die Isolationsaufzucht und die frühkindliche MA-Intoxikation) die Reifung DAerger

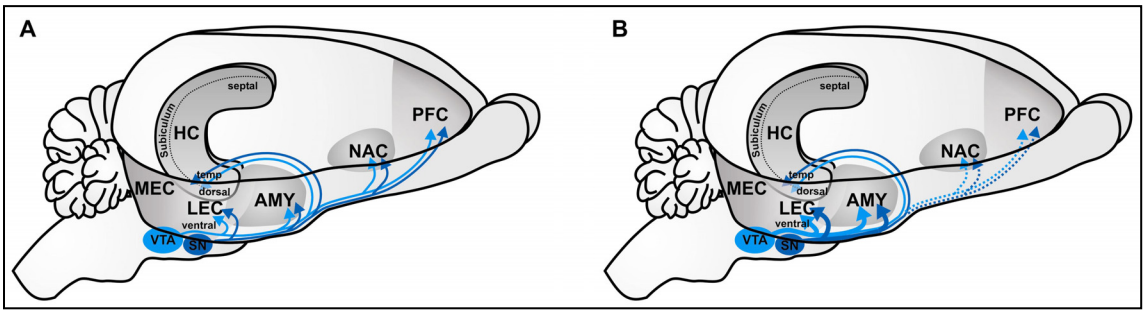

Abb.4: Epigenetisch induzierte Veränderungen des mesokortikolimbischen Systems
(**A**) Schematische Darstellung der DAergen Innervation des limbo-präfrontalen Systems in einem Kontrolltier. (**B**) Durch die restriktive Aufzucht und die einmalige Methamphetamin-Intoxikation (P14) gerät das mesokortikolimbische DAerge System aus der Balance. Während die DAergen Faserdichten in den rostral gelegenen Gebieten – dem PFC und dem NAC – abgesenkt sind, zeigt sich ein Anstieg der Faserdichten in caudalen Terminationfeldern, dem ventralen LEC und der Amygdala.

Bahnen unterschiedlich beeinträchtigen (Abb.4; Busche et al., 2004): Die Isolationsaufzucht bedingt eine suppressive Reifung der mesopräfrontalen Bahn (Winterfeld et al., 1998; Neddens et al., 2001). Dagegen kommt es in den von mir untersuchten Regionen des Subiculums und des LEC zu keinen signifikanten Veränderungen der DAergen Faserdichten, wobei der Mittelwertvergleich tendenziell sogar eher eine Erhöhung der Faserdichten vermuten lässt. Eine signifikante Erhöhung der DAergen Faserdichte wurde im basolateralen Kern der Amygdala (BLA) gefunden (Polascheck, in Vorbereitung; Busche et al., 2004). Die Kombination der Isolationsaufzucht mit der MA-Intoxikation beeinträchtigt die Balance der einzelnen DAergen Projektionen in einem noch erheblicheren Ausmaß. Stark verringerte Faserdichten im PFC und NAC (Dawirs et al., 1994; Neddens et al., 2002) stehen signifikante Erhöhungen der Faserdichten im ventralen LEC und einigen amygdaloiden Kernen (basolateraler Kern, lateraler Kern, medial zentraler Kern) gegenüber (Busche et al., 2004). Ausgehend von unseren gesamten Befunden lassen sich über die aktivitätsabhängige postnatale DA Reifung in einzelnen Gebieten des mesokortikolimbischen Systems für Gerbils folgende Aussagen treffen:

(1) In unserem Tiermodell treten signifikante strukturelle Veränderungen der DAergen Projektionen des mesokortikolimbischen Systems in *rostralen* Terminationsgebieten als eine *Verringerung* und in *caudalen* Terminationsgebieten als eine *Erhöhung* der DAergen Faserdichten in Erscheinung.

(2) Die Stärke der Veränderungen der DAergen Faserdichten in den *frontalen* Gebieten ist *positiv korreliert* mit den Veränderungen in den *caudalen* Gebieten.

Hier ergibt sich die Frage, wie sich derart differenzierte Unterschiede während der postnatalen Reifungsphase einstellen können.

Als eine Ursache für diese ungleichen Reaktionen der einzelnen DAergen Projektionen könnte ein Sprouting-Effekt in Betracht kommen. Demnach könnte eine Zerstörung DAerger Terminalien in rostralen, von der VTA weit entfernten Gebieten, zu einem homotypen Sprouten DAerger Fasern in caudalen, der VTA näher gelegenen Regionen führen. Ähnliches wird in der Literatur für die Transmitter 5HT und Noradrenalin beschrieben (Jonsson und Hallman, 1982). Eine weitere

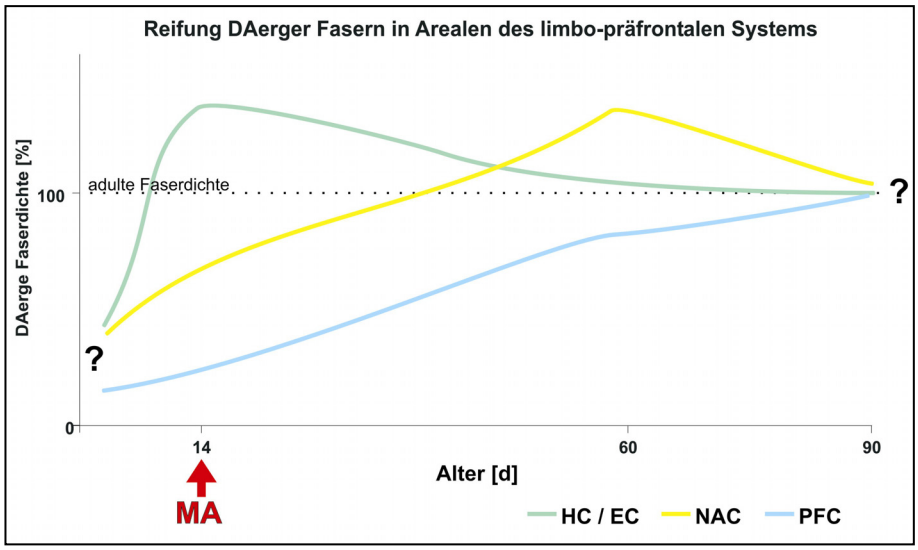

Abb.5: Reifungskurven einzelner DA Projektionen bei Nagern
Vereinfachte schematische Darstellung über den zeitlichen Verlauf der Reifung der mesohippocampalen, mesoaccumbalen und mesopräfrontalen DAergen Bahnen. Zum Zeitpunkt der Methamphetamin Injektion (MA) am Tag 14 liegt die DAerge Faserdichte im HC und EC über der adulten Faserdichte (100%), während die mesoaccumbale und insbesondere die mesopräfrontale Projektionen noch nicht die adulte Faserdichte erreicht haben. Die mesopräfrontale DAerge Bahn ist erst im jungen Erwachsenenalter voll ausgereift.

wahrscheinlichere Ursache könnte in dem unterschiedlichen zeitlichen Verlauf der Reifung der mesohippocampalen, mesoamygdaloiden, mesoaccumbalen und mesopräfrontalen DAergen Bahnen liegen. So ist die Reifung DAerger Fasern in die caudalen Gebiete dadurch charakterisiert, dass sehr früh nach der Geburt (P14 bei der Ratte; 5.-7. Monat bei Primaten) ein transienter Überschuss an DAergen Fasern vorhanden ist, welcher sich im Laufe der weiteren Entwicklung langsam zurückbildet, bis das adulte Innervationsmuster erreicht ist (Abb.5; Verney et al., 1985; Erickson et al., 1998). Die mesoaccumbale DAerge Innervation erreicht bei Ratten ihren Gipfel etwa am postnatalen Tag 60 (Tarazi et al., 1998; Moll et al., 2000). Hingegen wachsen DAerge Fasern noch bis ins junge Erwachsenenalter in den PFC ein (Abb.5; Kalsbeek et al., 1988; Dawirs et al., 1993; Rosenberg und Lewis, 1995) und tragen maßgeblich zu der prolongierten funktionellen Reifung des PFC bei (Lambe et al., 2000). Aus diesen unterschiedlichen Zeitverläufen lässt sich folgendes ableiten: Da postnatale Reifungsprozesse von Transmittern generell aktivitätsabhängig sind, führen durch unser Tiermodell induzierte veränderte extrinsische und intrinsische Aktivitäten vermutlich dazu, dass einerseits die mesopräfrontalen und mesoaccumbalen DAergen Fasern gar nicht erst voll ausreifen können und dadurch andererseits der Faserüberschuss der mesohippocampalen und mesoamygdaloiden DAergen Bahnen nicht abgebaut wird.

3.3. Zur serotonergen Innervation des limbo-präfrontalen Systems

Die 5HTergen Neuronen aus den zwei großen Kerngebieten im Hirnstamm, der dorsalen Raphe (DRN) und der medialen Raphe (MNR), projizieren – im Unterschied zu DA – relativ gleichmäßig in das gesamte Gehirn (Jacobs und Azmitia, 1992). Das ubiquitäre Vorkommen von

5HT im ZNS weist auf eine allgemeine funktionelle Bedeutung dieses Transmitters hin. So moduliert 5HT im Zusammenspiel mit anderen Transmittern, insbesondere Acetylcholin und Noradrenalin, die kortikale Aktivität (Arousal-System) und den Schlaf-Wachrhythmus (Koella, 1984; Geyer, 1996). Darüber hinaus erfährt dieser Transmitter allerdings auch speziellere funktionelle Einbindungen. In der HF spielt 5HT eine essentielle modulatorische Rolle bei Lern- und Gedächtnisleistungen (referiert in Vizi und Kiss, 1998; Gulyás et al., 1999). Verschiedene psychische Erkrankungen wie die Depression, Angststörungen und auch die Schizophrenie sind – offenbar zusätzlich zu weiteren Faktoren – auch auf Störungen des 5HTergen Systems, insbesondere in der HF, dem PFC und der Amygdala, zurückzuführen (Roth und Meltzer, 1995; Liebermann et al., 1998; Blier und Montigny, 1999; Mann, 1999).

Eine herausragende Eigenschaft des 5HTergen Systems ist seine enorme Regenerationsfähigkeit. Untersuchungen am HC adulter Tiere haben mehrfach nachgewiesen, dass sowohl mechanische als auch pharmakologische Zerstörungen 5HTerger Projektionen durch Aussprossen noch intakter 5HTerger Axone teilweise komplett kompensiert werden können (Azmitia et al., 1978; Zhou und Azmitia, 1984, 1986; Sotelo, 1991; Zhou et al., 1995). Dabei scheint je nach Ausmaß der Zerstörung die strukturelle Regeneration der 5HTergen Innervation auch mit einer funktionellen Wiederherstellung bestimmter Verhaltensweisen einherzugehen (Gage et al., 1983). Im Gegensatz dazu führt eine systematische neurotoxische Schädigung des 5HTergen Systems bei jungen Ratten (P10-20) zu vermutlich langfristigen Lerndefiziten (Mazer et al., 1997).

3.3.1. Zu den Befunden an unserem Tiermodell

Die Befunde zu der 5HTergen Innervation in der HF zeigen, dass die Isolationsaufzucht und die MA-Behandlung der Gehegeaufzuchten zu einer Erhöhung der Faserdichten im GD (Busche et al., 2002) und im EC (Neddens et al., 2003, submitted) führt. Die MA-Intoxikation bei isoliert aufgewachsenen Tieren führt zu einer zusätzlichen Erhöhung der 5HTergen Faserdichten im rechten septalen GD und im linken LEC, während es in allen weiteren ausgewerteten Regionen der HF tendenziell zu einer Absenkung der Faserdichten kommt (Busche et al., 2002; Neddens et al., submitted). Systematische Untersuchungen der Reaktion der 5HTergen Innervation auf diese beiden experimentellen Parameter in weiteren kortikalen und subkortikalen Arealen des Gehirns von Gerbils zeigen sehr ähnliche Ergebnisse. So reagiert das 5HTerge System sowohl auf die Isolationsaufzucht als auch auf die MA-Intoxikation überwiegend mit einer Hyperinnervation, während die MA-behandelten Käfigaufzuchten zumeist keine Veränderungen zeigen und die Faserdichten tendenziell sogar abgesenkt sind (Abb. 6; Neddens et al., 2003; Lehmann et al., 2003). Grundsätzlich lassen sich folgende Aussagen zum Einfluss unserer Versuchsparameter auf die 5HTerge Innervation im Gehirn von Gerbils treffen:

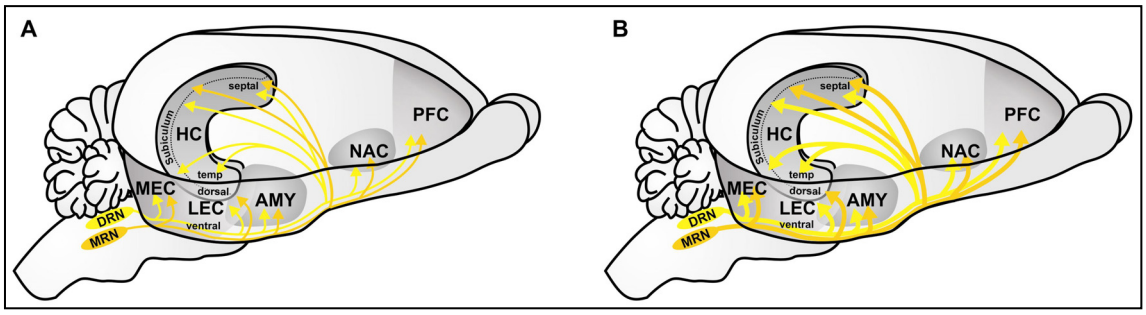

Abb.6: Epigenetisch induzierte Veränderungen des 5-HTergen Systems im limbo-präfrontalen System
(**A**) 5-HTerge Fasern innervieren sehr gleichmäßig kortikale und subkortikale Areale des limbo-präfrontalen Systems. (**B**) Die restriktive Aufzucht oder die einmalige frühkindliche Methamphetamin-Intoxikation führen zu einer Erhöhung der 5HTergen Faserdichten in den verschiedenen Arealen des limbo-präfrontalen Systems.

(1) Sowohl die *Isolationsaufzucht* als auch die einmalige *MA-Intoxikation* bei Tieren aus semi-natürlicher Aufzucht führen generell zu einer *Erhöhung* der 5HTergen Faserdichten.
(2) Die MA-Intoxikation in *Kombination* mit der isolierten Aufzucht *senkt* dagegen (mit wenigen Ausnahmen) die 5HTerge Faserdichte wieder auf das Niveau der Gehegeaufzuchten ab.

Hier stellt sich die Frage, welche Faktoren zu der generellen Hyperinnervation 5HTerger Fasern führen können.

Befunde zu einer 5HTergen Hyperinnervation im HC nach Implantation fötaler 5HTerger Neurone (Azmitia et al., 1981) ließen vermuten, dass von dem Terminationsgebiet spezifische Nervenwachstumsfaktoren ausgeschüttet werden (siehe Jacobs und Azmitia, 1992). Einige Zeit später konnte dann nachgewiesen werden, dass 5HTerge Neurone in der Lage sind, ihr eigenes Axonwachstum über den sehr spezifischen Wachstumsfaktor S-100$_{ß}$ steuern zu können (Azmitia et al., 1990; Ueda et al., 1994). Infolge einer Aktivierung von 5HT$_{1A}$ Rezeptoren auf Astrogliazellen kommt es zu einer erhöhten Ausschüttung von S-100$_{ß}$ (Whitaker-Azmitia et al., 1990). Dabei ist der Anstieg von S-100$_{ß}$ positiv korreliert mit einer erhöhten Faserdichte (Haring et al., 1993). Erst kürzlich ist gezeigt worden, dass auch der Nervenwachstumsfaktor brain-derived neurotrophic factor (BDNF) eine Rolle für das regenerative Sprouten 5HTerger Neurone spielt (Mamounas et al., 2000). Dabei nimmt 5HT selbst regulatorischen Einfluss auf die Expression der BDNF mRNA (Vaidya et al., 1997; Zetterström et al., 1999). Inwieweit diese Wachstumsfaktoren für die 5HTerge Hyperinnervation in unserem Tiermodell ursächlich sein könnte, bleibt noch durch weitere Arbeiten zu klären.

Die Interaktion mit dem DAergen System spielt ebenfalls eine wichtige Rolle für das 5HTerge Reifungsgeschehen. So wurde eine 5HTerge Hyperinnervation im Striatum und in der Substantia nigra nach der Zerstörung DAerger Fasern beobachtet (Berger et al., 1985; Towle et al., 1989; Zhou et al., 1991). Auch in unserem Tiermodell zeigt sich ein 5HTerger Faserüberschuss bei gleichzeitiger verminderter DAerger Faserdichte im dorsalen Striatum und NAC und wird von den Autoren als heterotypes Sprouten 5HTerger Fasern interpretiert (Lehmann et al., 2003). In den von

mir untersuchten Regionen der HF erscheint ein heterotypes Sprouten 5HTerger Axone auf Grund eines DAergen Faserverlustes als Erklärungsansatz sehr unwahrscheinlich, da in keiner der ausgewerteten Regionen der HF eine Verminderung der DAergen Innervationsdichte detektiert werden konnte (Busche et al., 2004). Jedoch ist ein Sprouting-Effekt im 5HTergen System bedingt durch Veränderungen anderer Transmitter – beispielsweise GABA oder Noradrenalin – in der HF nicht auszuschließen.

Die Befunde an unserem Tiermodell zeigen, dass insbesondere die assoziativen Kortexareale – der PFC, EC und GD – Veränderungen der 5HTergen Faserdichten aufweisen, während primär sensorische und motorische Kortexareale – der frontale und parietale Kortex – von den experimentellen Parametern nicht betroffen sind (Neddens et al., 2003). Wie eingangs erwähnt, reifen die primär sensorischen und motorischen Kortexareale sehr früh, weshalb sie möglicherweise von der restriktiven Aufzucht und der MA-Intoxikation nicht mehr beeinflusst werden. Die Vulnerabilität 5HTerger Fasern scheint demnach eng mit der zeitlichen Reifung der einzelnen Hirnareale zusammen zu hängen. Die 5HTerge Hyperinnervation, die sich insbesondere in den Regionen des limbo-präfrontalen Systems zeigt, ist wahrscheinlich Ausdruck komplexer aktivitäts-gesteuerter Veränderungen innerhalb dieses Systems, die durch die experimentellen Parameter hervorgerufen werden. Demzufolge sollten neben dem 5HTergen und DAergen (s.o.) System vermutlich auch noch weitere Transmittersysteme betroffen sein, was sich durch unsere aktuellen Studien auch zu bestätigen scheint:

Neben DA und 5HT spielen gerade in der HF noch speziell andere Neuromodulatoren eine wichtige Rolle in der Steuerung von Strukturplastizität. Insbesondere Acetylcholin interagiert physiologisch und funktionell sehr eng mit 5HT im HC (referiert in Cassel und Jeltsch, 1995; Steckler und Sahgal, 1995; Vizi und Kiss, 1998; Gulyás et al., 1999) und ist ähnlich stark wie 5HT im GD repräsentiert (Frotscher und Leranth, 1985; Kása, 1986). Die quantitative Untersuchung der acetylcholinergen Faserdichten im GD an unserem Tiermodell zeigt den 5HTergen Veränderungen nahezu entsprechende, durch die gleichen experimentellen Parameter induzierte, Resultate: Acetylcholinerge Faserdichten sind ebenfalls bei isoliert aufgewachsenen Tieren und MA-behandelten Gehegeaufzuchten erhöht (Bagorda A, in Vorbereitung; Busche et al., submitted). Ebenso wurden erhöhte acetylcholinerge Faserdichten im LEC bei Tieren aus Isolationsaufzucht gefunden (Lehmann et al., in press).

3.4. Zur Bedeutung von Dopamin und Serotonin für die Zellproliferation im Gyrus dentatus

Beide monoaminergen Transmitter, DA und 5HT, wirken während der Gehirnentwicklung als morphogene Substanzen (siehe Lauder, 1988¸ 1993). Dieses ist insbesondere für die postnatale Reifungsphase des GD von Bedeutung, der zeitlebens in einem unreifen Zustand verbleibt: Der GD

ist neben der subventrikulären Zone eines der wenigen bisher bekannten Areale, in dem noch im adulten Gehirn von Nagern (Altman und Das, 1965; Kaplan und Bell, 1984; Altman und Bayer, 1990) und des Menschen (Eriksson et al., 1998) neue Neurone gebildet werden und in besonders hohem Maße Synaptogenese (s.o.) stattfindet. Es ist gut belegt, dass die anhaltende Neurogenese und Synaptogenese im GD aktivitäts-abhängig ist und über das NMDA-Rezeptorsystem reguliert wird, wobei NMDA-Rezeptor Agonisten die mitotische Aktivität senken (Cameron et al., 1995) und Antagonisten die mitotische Aktivität entsprechend anheben (Gould et al., 1994).

An unserem Tiermodell konnte schon vor einigen Jahren in umfassenden Studien gezeigt werden, dass sowohl die Isolationsaufzucht als auch die einmalige frühkindliche MA-Intoxikation die mitogene Aktivität im GD dauerhaft beeinflussen (Hildebrandt, 1999; Hildebrandt et al., 1999; Keller et al., 2000). Weiterhin konnte eine unmittelbare Beteiligung von DA an der Regulation der Zellproliferation einerseits durch die Gabe von Haloperidol (DA D_2-Rezeptor-Blocker) und andererseits durch die MA-Applikation (DA agonisierende Wirkung) nachgewiesen werden (Dawirs et al., 1998; Hildebrandt et al., 1999; Teuchert-Noodt et al., 2000). 5HT nimmt ebenfalls Einfluss auf die mitogene Aktivität im GD (Brezun und Dazuta, 1999, 2000). Zusammengefasst geht aus all diesen Befunden hervor, dass DA eine absenkende und 5HT eine anhebende Wirkung auf die Zellproliferationsrate hat, die über die direkte Innervation des GD (im Fall von 5HT) als auch über die Modulation des glutamatergen Perforant Path verwirklicht werden könnte. Die an unserem Tiermodell gezeigte anders gereifte DAerge und 5HTerge Innervation in der HF sollte sich demzufolge chronisch auf die mitogene Aktivität im GD auswirken.

Dieses unterstützend ist die Erhöhung der 5HTergen Faserdichten im GD und LEC von restriktiv aufgewachsenen Tieren und MA-behandelten Gehegeaufzuchten (Busche et al., 2002; Neddens et al., 2003, submitted) positiv korreliert mit unseren Befunden zu einer erhöhten Zellproliferation in dem gleichen Tiermodell (Hildebrandt, 1999; Keller et al., 2000). Dagegen geht diese positive Korrelation bei den MA-behandelten restriktiv aufgewachsenen Tieren verloren, insofern als eine abgesenkte Zellproliferationsrate und eine erhöhte 5HTerge Faserdichte im septalen GD und linken LEC bei diesen Tieren auftreten. Diese Resultate zeigen deutlich, dass sich die Regulation der mitogenen Aktivität im GD komplexer darstellen muss, d.h. nicht allein über den lokalen hippocampalen Circuit erklärt werden kann. Vielmehr scheint der entscheidende Faktor für die Regulation der Neurogenese die neuronale Aktivität im GD zu sein, auf die das gesamte limbo-präfrontale System modulierend Einfluss nehmen kann.

Über die entorhinale Pforte können weiter entfernt gelegene Areale des limbo-präfrontalen Systems – insbesondere der PFC – die Aktivitäten des GD modulieren. Beispielsweise realisiert sich der DAerge Einfluss auf die Zellproliferation vermutlich stark über den PFC und EC, die beide hohe DAerge Faserdichten aufweisen im Gegensatz zum GD, der selbst nur sehr spärlich DAerg innerviert ist. Restriktiv aufgewachsene Gerbils „leiden“ unter einer abgesenkten Aktivität aus dem PFC auf Grund der vermindert gereiften präfrontalen DA Innervation (Winterfeld et al., 1998), was

zu einem Anstieg der Zellproliferation beitragen könnte. Die zusätzliche MA-Intoxikation der Käfigaufzuchten führt zwar zu einer noch stärkeren Absenkung der präfrontalen Aktivität (Dawirs et al., 1994), aber gleichzeitig zu einer Zunahme der DAergen Innervation in caudal limbischen Gebieten (Busche et al., 2004). Die hohe lokale Präsenz von DA in eben diesen Regionen stellt möglicherweise eine Erklärung für die Absenkung der Zellproliferationsrate im GD bei MA-behandelten restriktiv aufgewachsenen Gerbils dar.

4. Adaptive Veränderungen im limbo-präfrontalen System

Die beiden Neurotransmitter DA und 5HT gelten als diejenigen Neuromodulatoren des Gehirns, die in kognitive und emotionale Funktionen entscheidend eingebunden sind. Allerdings sind DA und 5HT durch ganz unterschiedliche Innervationmuster im Gehirn gekennzeichnet: Dopamin ist in den einzelnen Arealen des limbo-präfrontalen Systems durch sehr spezifische Innervationsmuster vertreten, während 5HT diffus das gesamte Gehirn innerviert. Das deutet an, dass DA möglicherweise eine kritische Komponente im Interaktionsgeschehen des limbo-präfrontalen Systems darstellt. Unsere Befunde zeigen, dass DA und 5HT auf die restriktive Isolationsaufzucht und die frühkindliche MA-Intoxikation sehr unterschiedlich reagieren. Während die Effekte auf 5HT markanter bei Tieren aus isolierter Aufzucht und bei MA-behandelten Gehegeaufzuchten sind, reagiert die DAerge Innervation stärker auf die Kombination von MA-Intoxikation und isolierter Aufzucht.

Es ist wahrscheinlich, dass neben den von uns gefundenen strukturellen Veränderungen der DAergen und 5HTergen Innervation auch physiologische Eigenschaften dieser Transmitter (Ausschüttung, Synthese, Abbau, Rezeptordichte und -aktivität) betroffen sind. Auf Grund der teilweise sehr engen anatomischen und funktionellen Wechselwirkungen zwischen DA und 5HT (siehe Kapur und Remington, 1996) – insbesondere auf der Ebene des Hirnstamms (Herve et al., 1987; Peyron et al., 1995; Mylecharane, 1996; Gervais und Rouillard, 2000) – wäre es somit denkbar, dass sich **alle** experimentellen Parameter sowohl direkt als auch indirekt auf beide monoaminergen Systeme auswirken. In welcher Weise sich diese strukturellen Veränderungen der Faserdichten auf den Transmittergehalt und die Rezeptordichten niederschlagen, ist Gegenstand laufender Untersuchungen an unserem Tiermodell.

Als Interpretation aller Teilergebnisse bleibt an dieser Stelle folgendes festzuhalten: Sowohl die chronische Beeinträchtigung während der Entwicklung (in Form der restriktiven Isolationsaufzucht) als auch ein einmaliges frühkindliches traumatisches Erlebnis (in Form der MA-Intoxikation) lösen äußerst komplexe adaptive Prozesse in den verschiedenen Transmittersystemen des limbo-präfrontalen Systems aus. Die an unserem Tiermodell erhobenen Befunde stimmen gut überein mit aktuellen Vorstellungen in der klinischen Forschung zur Schizophrenie, denen zufolge sich Störungen in der postnatalen Reifungsphase nicht auf einzelne

Neurotransmitter oder Systeme beschränken. So war beispielsweise über lange Jahre die Dopamin-Hypothese die entscheidende Theorie für diese Erkrankung auf Grund der Wirkung der Neuroleptika auf die DA-Rezeptoren (Creese et al., 1976; Seeman et al., 1976). In den letzten Jahren hat man erkannt, dass auch andere Transmittersysteme Veränderungen aufweisen, die mit dem Auftreten schizophrener Symptome im Zusammenhang stehen: entsprechend gibt es nun eine **5HT-Hypothese** (siehe Roth und Meltzer, 1995; Liebermann et al., 1998), eine **Glutamat-Hypothese (**Tamminga, 1998) und eine **GABA-Hypothese** (Simpson et al., 1989; Benes et al., 1992). Die Vielzahl der Befunde zu Veränderungen innerhalb der Transmittersysteme hat dazu beigetragen, die Schizophrenie seit einigen Jahren auch als komplexe Entwicklungsstörung zu verstehen. Die Erkenntnisse aus der Experimentalforschung zu den spezifischen strukturellen Veränderungen in den verschiedenen Transmittersystemen sollten für das klinische Verständnis hilfreich sein.

Als Ursachen psychischer Erkrankungen werden Störungen insbesondere des PFC, der HF, des NAC und der Amygdala genannt. So wird im Allgemeinen von einer Hypoaktivität des PFC und einer Hyperaktivität limbischer Gebiete gesprochen, die sich im Laufe der Kindesentwicklung herausbilden und im Erwachsenenalter schizophrene Symptome hervorbringen können. Es stellt sich die Frage, welche Umstände dazu beitragen, dass sich während der Entwicklung dieses Ungleichgewicht der Aktivitäten formen und manifestieren kann. Einen ganz entscheidenden Faktor für die Induktion dieser Imbalance scheint die prolongierte Reifung des PFC darzustellen. Die betroffenen Menschen erkranken überwiegend zwischen dem 18. und 35. Lebensjahr an einer Schizophrenie, also etwa ab dem Zeitpunkt, zu dem der PFC als ausgereift gilt. Das folgende Modell soll eine Möglichkeit aufzeigen, was während des Entwicklungsprozesses geschehen könnte, damit es zu der oben genannten Imbalance kommt:

Zu Beginn einer „normalen“ Entwicklung spielt die emotionale Bindung zu den Eltern eine entscheidend wichtige Rolle für das Kind. Die Emotionalität reift somit sehr früh in der Entwicklung eines Kindes und im Gehirn stellen die HF, die Amygdala und die monoaminergen Kerne des Hirnstammes, die zusammen den kleinen limbischen Circuit (Abb. 7A) bilden, entscheidende Zentren für die Emotionalität dar. Diese anfangs starke Emotionalität wird mit zunehmender Entwicklung den präfrontalen Leistungen wie Kognition und Reflexion unterstellt, und der kleine limbische Circuit gerät mit dem Heranwachsen sukzessiv unter die Kontrolle des PFC (= großer limbischer Circuit) (Abb. 7B). Wird die Entwicklung des PFC irreversibel gestört, beispielsweise auf Grund der verminderten Reifung der mesopräfrontalen DA Bahn, kann die präfrontale Kontrolle über die kleine limbische Schleife nur in geschwächter Form aktiviert werden (Abb. 7C). Als Folge davon könnte sich im adulten Lebewesen eine Imbalance im limbo-präfrontalen System manifestieren mit einer Überbetonung des kleinen limbischen Circuits. Möglicherweise könnte es sogar zu einer Diskonnektion des PFC von den limbischen Gebieten kommen.

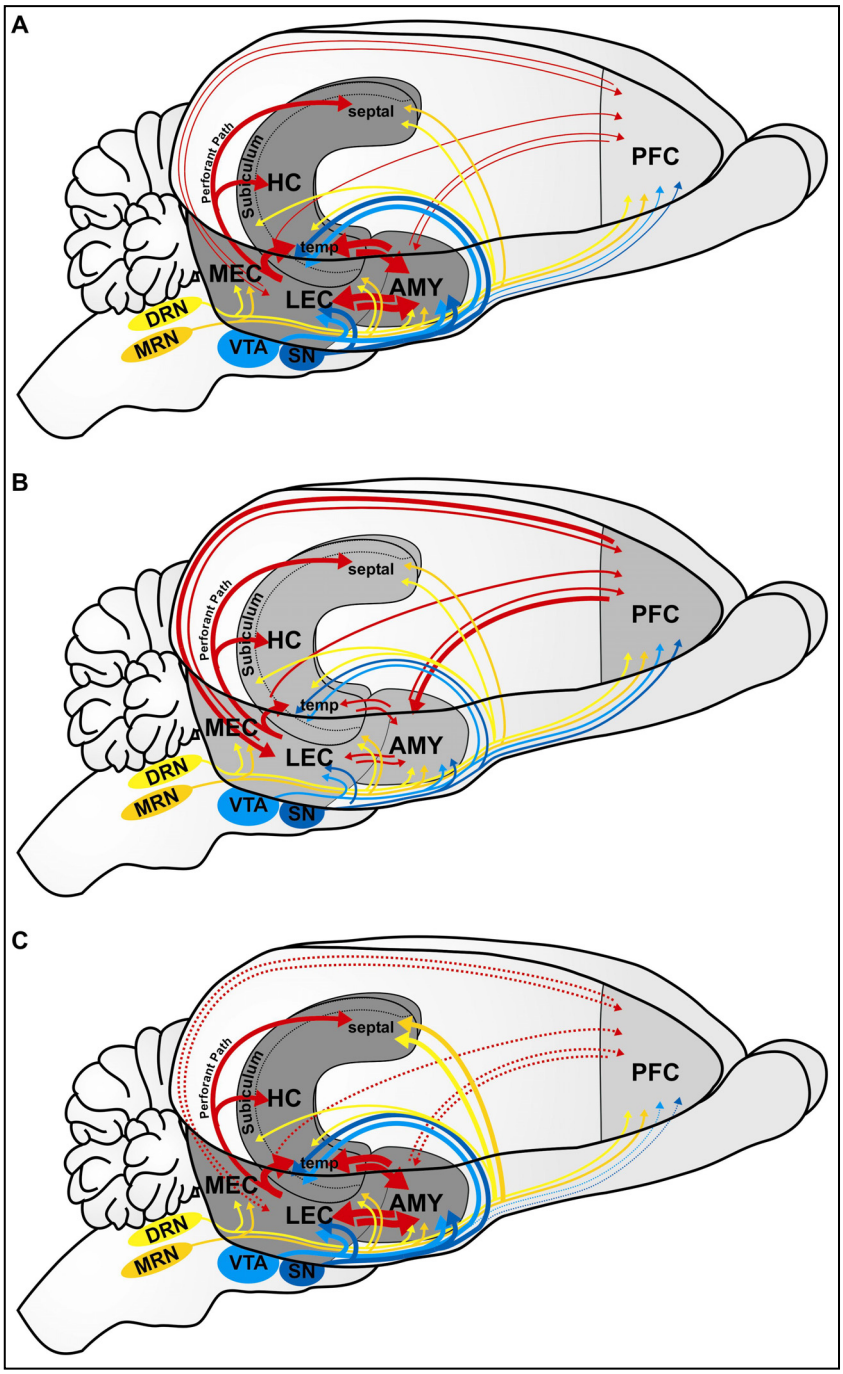

Abb.7: Modellvorstellung zur Entwicklung einer Imbalance im limbo-präfrontalen System von Nagern

Zu Beginn der postnatalen Entwicklung (**A**) ist die Emotionalität beim Kind besonders stark ausgeprägt. Entsprechend sind die limbischen Hirnareale, die die Emotionalität repräsentieren, schon weit entwickelt und zeichnen sich durch eine transient überschießende Dopamin Innervation aus, die über der adulten Faserdichte liegt. (**B**) Mit zunehmender Reifung des PFC, die insbesondere durch das Einwachsen dopaminerger Fasern bestimmt ist, gewinnt der PFC sukzessiv die Kontrolle über die limbischen Areale und die Emotionalität weicht mehr und mehr der Kognition und Reflexion. Gleichzeitig verringert sich aktivitäts-abhängig die transiente dopaminerge Hyperinnervation in den caudal limbischen Gebieten und erreicht die adulte Faserdichte. (**C**) Kann sich der PFC auf Grund einer verminderten Reifung der mesopräfrontalen Dopamin Bahn nicht richtig entwickeln, kommt es vermutlich zu einer gestörten Kontrolle des PFC über die limbischen Areale und möglicherweise sogar zu einer Diskonnektion des PFC von den limbischen Gebieten. Durch die fehlende präfrontale Kontrolle bleiben vermutlich sowohl die transiente dopaminerge Hyperinnervation als auch die Verstärkung des limbische Circuits bestehen. Diese Veränderungen stellen möglicherweise das strukturelle Korrelat für die Symptomatik psychischer Störungen dar. Insbesondere in der klinischen Forschung zur Schizophrenie werden eine frontale Hypoaktivität und eine gleichzeitige Hyperaktivität in limbischen Gebieten als mögliche Ursachen diskutiert.

Diese Theorie wird vor allem durch zwei Befunde unserer Arbeitsgruppe gestützt: (1) Anterograde Tracerstudien geben uns klare Hinweise auf eine verminderte Ausbildung der Projektionen aus dem PFC in cinguläre, parietale und insuläre Kortizes bei Tieren aus Isolationsaufzucht und MA-behandelten Gerbils aus restriktiver Aufzucht (Bagorda F, in Vorbereitung). (2) Unsere Befunde zum mesokortikolimbischen DA-System in den MA-behandelten restriktiv aufgewachsenen Tieren zeigen eine Hypoinnervation im PFC und eine Hyperinnervation in der HF und der Amygdala. Die lokalen Veränderungen der Transmitter-systeme in den limbischen Regionen und der Verlust der präfrontalen Kontrolle über die limbischen Gebiete deuten daraufhin, dass es zu einer Überbetonung „basaler" Funktionen wie der Emotionalität kommen könnte. Zu unseren Befunden passend postuliert Bogerts (2002), dass „Struktur- und Funktionsstörungen in temporolimbischen Arealen zu einer Dissoziation zwischen höheren, kognitiven Prozessen und elementaren, emotionalen Reaktionsformen führen. In dieser Entkopplung von Kognition und Emotion liegt eine der Grundstörungen schizophrener Erkrankungen".

Da sowohl 5HT als auch Acetylcholin im Gegensatz zu DA sehr gleichförmig auf die experimentellen Interventionen reagieren, kann man weiterhin vermuten, dass DA eine Schlüsselrolle in diesem System zukommt, insofern als: (1) DA sich durch die sehr spezifische Innervation des limbo-präfrontalen Systems auszeichnet. (2) Die mesopräfrontale Projektion sehr langsam reift und erst im jungen Erwachsenenalter voll entwickelt ist. Über DA wird möglicherweise die Kaskade der adaptiven Veränderungen anderer Transmitter im limbo-präfrontalen System gesteuert.

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Developmentally induced imbalance of dopaminergic fibre densities in limbic brain regions of gerbils (*Meriones unguiculatus*)

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Summary. It is well established that epigenetic factors influence the maturation of neurotransmitter systems. Social isolation as well as an early intervention with methamphetamine (MA) lead to a diminished maturation of dopaminergic (DA) fibres in cortical and striatal areas in the brain of Mongolian gerbils. The aim of this study was to prove whether isolated rearing (IR) and the application of a single dose of MA on postnatal day 14 affect the maturation of DA fibres in caudal limbic areas. Therefore the DA fibre densities were quantified in the dorsolateral and ventrolateral entorhinal cortex (EC), the ventral subiculum (SUB) and in three amygdala nuclei – the basolateral (BLA), the lateral (LA) and the central (CA) nucleus. Our results showed that IR and an early MA application led to an increase of DA fibre densities in various caudal limbic areas. Whereas the BLA was affected by both IR and MA, the LA and the medial left CA were only influenced by MA in IR animals. The DA fibre surplus in the ventrolateral EC was significant in MA treated ER and IR animals in the left and right hemisphere, respectively. The SUB and the dorsolateral EC remained unaffected by both epigenetic factors. Altogether, the BLA seems to be the area which responds most sensitively to IR and MA. Previous studies in our laboratory showed a suppressive maturation of DA fibres in the prefrontal cortex (PFC) and nucleus accumbens (NAC) induced by the same set of epigenetic factors. Thus, due to the close functional connection between the PFC and limbic areas, it could be assumed that the suppressive maturation of prefrontal DA fibres implicates an enhancement of DA innervation densities in caudal limbic areas. Imbalances in the morphology and physiology of the different DA projections are suggested here to be crucial in the aetiology of schizophrenia.

Keywords: Dopamine, caudal limbic areas, fibre overshoot.

Introduction

There is increasing evidence that the regulation of corticolimbic functions by the neurotransmitter dopamine (DA) is essential for psychobiological adaptation in development. Psychotic disorders appear to be at least partly due to a complex imbalance within the DA system. Human studies have shown that low DA activity in the cortex coincides with high activity in subcortical limbic regions of schizophrenic patients (revs. Davis et al., 1991; Jentsch et al., 2000; Sesack and Carr, 2002; Meyer-Lindenberg et al., 2002). These observations are consistent with others coming from animal lesion and pharmacological studies which suggest that hypodopaminergic activities of the prefrontal cortex (PFC) may lead to hyperdopaminergic transmissions in the dorsal and ventral striatum and this inverse activity pattern probably induces behavioural impairment (rev. Nieoullon, 2002). Obviously, DA transmission in the mesocortical, mesostriatal and mesolimbic projections is controlled in a complex and interdependent way, which has important implications for the enormous spectrum of psychotic disorders (rev. Le Moal and Simon, 1991). However, the regional characteristics of DA maladaptations producing psychiatric disorders are by no means understood. Experimental interventions manipulating the postnatal DA maturation can offer valuable insights.

Dopaminergic fibres of the mesocorticolimbic projection originate in a rather small midbrain area, the VTA (Fallon et al., 1978; Swanson, 1982), but discretely target multiple subregions of the dispersely organised corticolimbic circuitry (Björklund and Lindvall, 1984; Descarries et al., 1987; Yoshida et al., 1988). Remarkably, each DA projection field is characterised by its own time sequence pattern of maturation in postnatal life. Principally, the maturation of the mesocorticolimbic DA projection progresses from caudal to rostral areas. In rodents and primates, the DA fibre densities of caudal limbic areas, namely the hippocampus (HC), the amygdala and the entorhinal cortex (EC), peak early in development and decline slightly before acquiring the adult pattern (Verney et al., 1985; Erickson et al., 1998). In the nucleus accumbens (NAC) of rats the number of varicosities of DA fibres increases strongly from postnatal day (PD) 8 to PD 20 and the adult DA innervation pattern is reached on PD 28 (Voorn et al., 1988). For the rat's dorsal and ventral striatum it was shown that the DA-transporter densities increase till puberty (Tarazi et al., 1998; Moll et al., 2000) and decrease steadily in further development (Moll et al., 2000). In contrast, the DA fibres of the orbital PFC still mature up to sexual maturity and portions of the medial PFC innervation obtain adult patterns at young adulthood (Kalsbeek et al., 1988; Dawirs et al., 1993). Therefore, developmentally induced maladaptation during transmitter maturation may become effective in quite different stages in the various targets of DA fibres.

In the concerted action of neuronal networks the maturation of transmitters is activity-dependent. Environmental and/or pharmacologically induced interventions in postnatal life lead to longlasting alterations of transmitter functions (revs. White et al., 1996; Hall, 1998; Lapiz et al., 2003; Steketee, 2003). Therefore, individual portions of the mesocorticolimbic DA projection may be susceptible to different disturbances during selective stages of postnatal maturation. Two

experimental challenges have been intensively investigated: First, isolated rearing (IR) compared with enriched rearing (ER) has been shown to interfere with the anatomical maturation (Winterfeld et al., 1998; Neddens et al., 2001; Lehmann et al., 2002) and function (Jones et al., 1992; Heidbreder et al., 2000) of DA in prefrontal, striatal and amygdaloid regions. Second, the application of a single dose of methamphetamine (MA) on PD 14, which we know to induce acute and selective autotoxic effects on the DA targets in the maturing PFC of gerbils (Teuchert-Noodt and Dawirs, 1991) also affects the DA innervation in the adult PFC (Dawirs et al., 1994) and NAC (Neddens et al., 2002). In all rostral brain areas where DA fibre densities were studied, a decline of DA fibres was detected in IR and MA treated animals (Dawirs et al., 1994; Winterfeld et al., 1998; Neddens et al., 2001, 2002; Lehmann et al., 2002).

The aim of the present study was to complement former studies and investigate further adaptive changes of the DA balance within the corticolimbic system. Since the immunohistochemical approach permits us to quantify the DA fibre densities in multiple brain regions simultaneously, we made use of the same set of afore mentioned interventions and focussed on DA innervation patterns in limbic terminal fields. We quantified the DA fibre densities in young adult gerbils (PD 90) in the dorso- and ventrolateral EC, the three DA innervated nuclei of the amygdala, which are the basolateral (BLA), central (CA) and lateral (LA) nucleus, and in the ventral subiculum (SUB) with software for image analysis.

Material and methods

Animals and rearing conditions

All experimental procedures were approved by the appropriate committee for animal care in accordance with the European Communities Council. For this study 34 male gerbils were used. Sixteen of them were bred in standard makrolon cages (type IV) under impoverished condition while 18 of them were bred in semi-naturally structured compounds (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), while the other group grew up under enriched rearing conditions (ER, kept as a group of siblings in semi-naturally structured compounds containing branches and hiding opportunities), both for further 60 days. On PD14 a total of sixteen pups received a single injection of methamphetamine hydrochloride (50 mg/kg; i.p.), nine gerbils of the ER group and seven gerbils of the IR group. The remaining eighteen gerbils, nine of either rearing group, were sham-treated by a single injection of saline. Under all sets of conditions food and water were provided *ad libitum*. All gerbils were kept on natural day/night cycles during summer season.

Immunohistochemistry

Preparation of tissue: Animals were transcardically perfused under deep chloralhydrate anaesthesia (1.7 g/kg, i.p.). The perfusion was performed with 60 ml cold 0.05 M phosphate buffer (pH 6.2), containing 1% sodium metabisulfite, followed by 500 ml 5% glutaraldehyde with 1% sodium metabisulfite in 0.1 M phosphate buffer (pH 7.5), and finally by wash buffer containing 0.05 M tris buffered saline (TBS) with 1% sodium metabisulfite (pH 7.5). Immediately after perfusion, the brains were dissected and 50 µm thick frontal sections cut with a vibratome (Leica VT 1000 S, Nussloch, Germany) and subsequently collected in wash buffer at 4°C.

General procedure: The first steps of the protocol were also performed in wash buffer with gentle agitation of the slices. The immunohistochemical procedure used (1) a 30 min preincubation in 10% normal goat serum and 0.4% Triton X100, (2) rabbit anti-dopamine serum (DiaSorin,

Stillwater, MN) diluted 1:600 with 1% normal goat serum and 0.4% Triton X100 for 40 h. The next steps were performed in 0.05 M Tris-HCl (pH 7.5) and were each followed by a 30 min washing in Tris-HCl. (3) A 30 min incubation in biotinylated goat anti-rabbit serum (Sigma) diluted 1:20 with 1% normal goat serum, (4) ExtrAvidin-Peroxidase (Sigma) diluted 1:20 for 30 min. (5) The staining solution contained 0.05% 3,3′-diaminobenzidine with 0.01% H_2O_2. After 4 min of staining, the sections were washed, mounted on glass slides, dried at room temperature

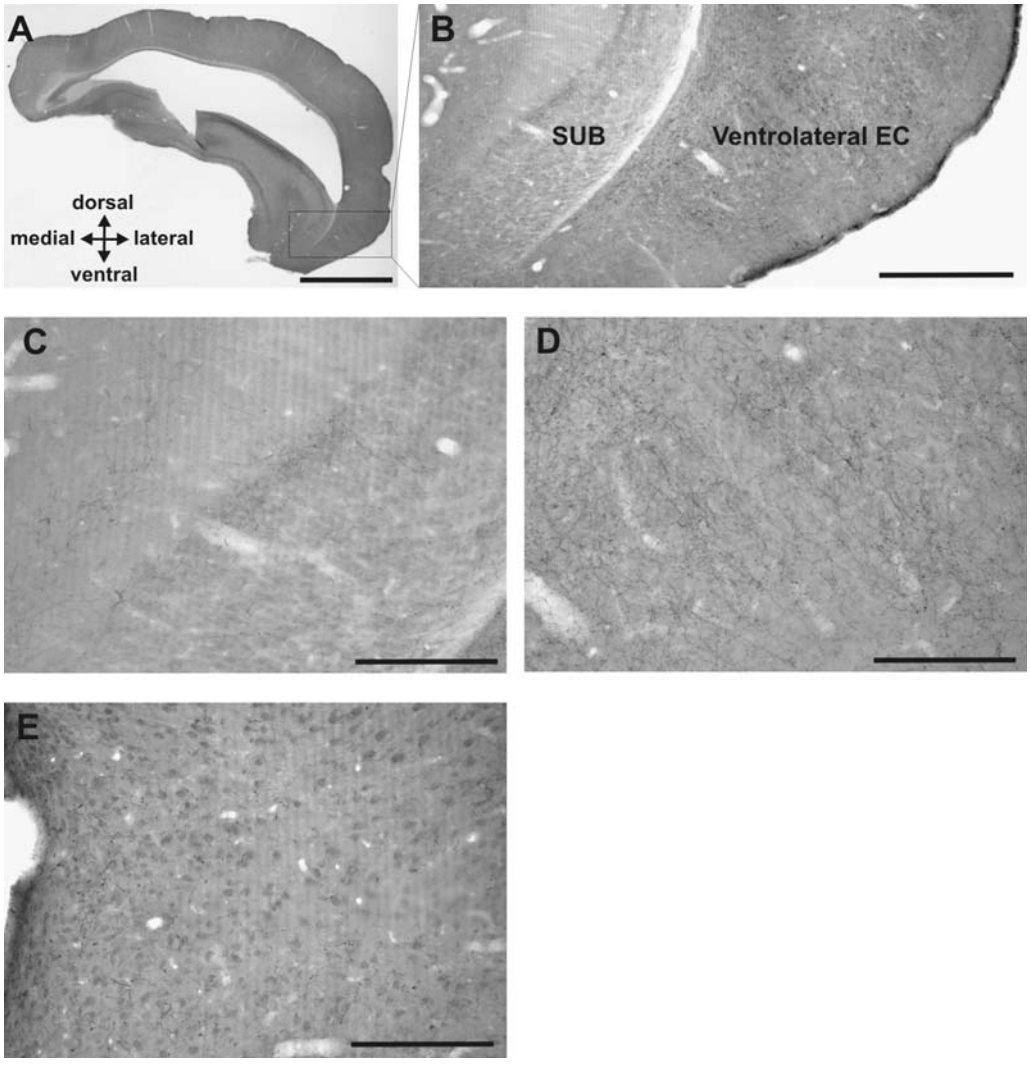

Fig. 1. A Brightfield photomicrograph of a representative coronar section at the level of the entorhinal cortex (EC). The area of the rectangle is magnified in (**B**) and comprises the ventral subiculum (SUB) and the ventrolateral EC. Within the cellular layer of the SUB DA fibres are only found in a restricted area at the border to the CA1 region of the hippocampus. In the molecular layer of the SUB the fibres run tangentially to the cellular layer (**C**). The DA fibres of the ventrolateral EC are arranged in clusters (**D**) and show rostrally a dense fibre network which thins out to caudal levels. On the other hand the dorsolateral EC, which is located dorsally to the ventrolateral EC, is less DAergic innervated and DA fibres appear densest in the deeper layers (L III–VI). In the superficial layers DA fibres are only found sporadically (**E**). Scale bars: 2 mm (**A**), 500 μm (**B**) and 200 μm (**C–E**)

overnight, dehydrated, mounted in DePeX and coverslipped. Control sections were treated by the same procedure but omitting the rabbit-anti-dopamine serum and showed no specific staining.

Quantification of DA innervation

The brain was serially cut across the entire rostro-caudal extent of the amygdala and the EC. For quantification every other slice of the right and left hemisphere was used. In the EC, the ventral SUB (Fig. 1) and the amygdala (Fig. 2) different test fields were defined. The measurements in

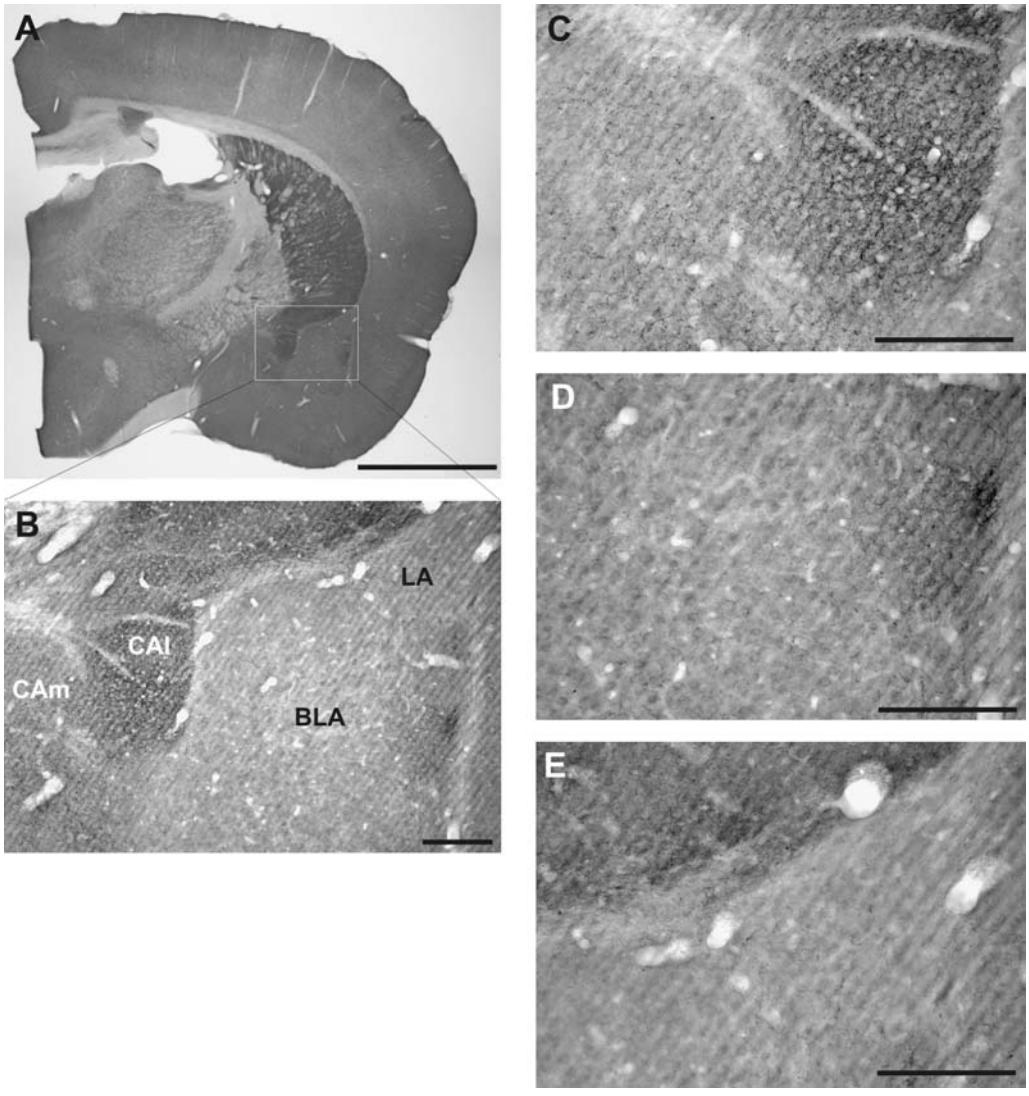

Fig. 2. Brightfield photomicrograph of a representative coronar section at the level of the amygdala (**A**). The rectangle shows the analysed amygdaloid nuclei, which are highlighted in (**B**). The DA innervation patterns of the amygdaloid nuclei are generally different. The lateral part of the central nucleus (CAl) is densely innervated by DA fibres whereas the medial part of this nucleus (CAm) is rather moderately innervated (**C**). The basolateral nucleus (BLA; **D**) shows a sparse DAergic innervation, which is densest in its lateral part near the capsula externa. (**E**) The lowest fibre density appears in the lateral nucleus (LA). Scale bars: 2 mm (**A**) and 200 μm (**B**–**E**)

the EC comprised the dorsolateral (layers III–VI) and ventrolateral part, which correspond to the DLEA and VLEA, respectively, defined by Krettek and Price (1977). The testfields in the SUB laid in the ventral part at the border to the CA1 area. The molecular layer (SUBml) and cellular layer (SUBcl) were separately analysed. Images were taken at 125-fold magnification of 8 consecutive slices. For the central amygdaloid nucleus (CA) images were taken of its medial (CAm) and lateral (CAl) part in 6 consecutive slices at 400-fold magnification with oil immersion. In the basolateral nucleus (BLA) two testfields were placed and images were taken in 7 consecutive slices at 200-fold magnification. For each image all detectable DA fibre fragments were visualised by the use of a brightfield microscope (Polyvar, Reichert-Jung, Vienna, Austria) and a digital camera for microscopy (ProgRes 3008, Jenoptik, Jena, Germany). The fibres were detected using the valleys function, which depicts the grey value difference of adjacent pixels and transform the result into a binary image (KS300, Jenoptik, Jena, Germany). The DA fibre densities were calculated as the percentage area of the fibres within each testfield.

Data analysis

For the comparison mean values were calculated from the single testfield data over the rostro-caudal extent of the respective brain area for each animal. Mean values for each animal group were computed as arithmetic means $\pm$ standard deviation (S.D.) and compared by t-test (two-tailed) with preceding F-test. Region- and group-specific effects were additionally tested by a three-way multivariate analysis of variance (MANOVA) computed with Statistica 6 (StatSoft, Tulsa, USA). The levels of significance were set at $p<0.05$ (*), $p<0.01$ (**), $p<0.001$ (***).

Results

The DA innervation pattern in gerbils' amygdala and caudal limbic areas is similar to that described for the rat (Freedman and Cassell, 1994; Asan, 1998; Gasbarri et al., 1997). In the amygdala the lateral part of the CA shows the densest DA fibre network (Fig. 2C), the medial CA (Fig. 2C) and the ventrolateral EC (Fig. 1D) have moderate to high fibre densities, moderate fibre densities are found in the BLA (Fig. 2D), the dorsolateral EC (Fig. 1E) and ventral subiculum (Fig. 1C) and the lowest fibre density appears in the LA (Fig. 2E). The fibre density of the ventrolateral EC thins out from rostral to caudal and at more caudal levels the DA fibres are arranged in several clusters (Fig. 1D). In the dorsolateral EC DA fibres are mainly visible in the deeper layers (III–VI; Fig. 1E). The ventral subiculum shows a striking dopaminergic innervation pattern because within the cellular layer only a narrow stripe at the border to CA1 is innervated by DA fibres (Fig. 1C). In the molecular layer the fibres run tangentially and have their highest density even at this narrow stripe.

Influence of rearing conditions

The BLA is the only analysed structure which is significantly affected by rearing conditions. Impoverished reared animals show an increase of DA fibre densities in the BLA of 17% and 23% in the left and right hemisphere, respectively (Fig. 3C–D).

Influence of methamphetamine

In principle the MA treatment of animals leads to an increase of DA fibre densities in various caudal limbic areas. Its effect is generally more pronounced in IR than in

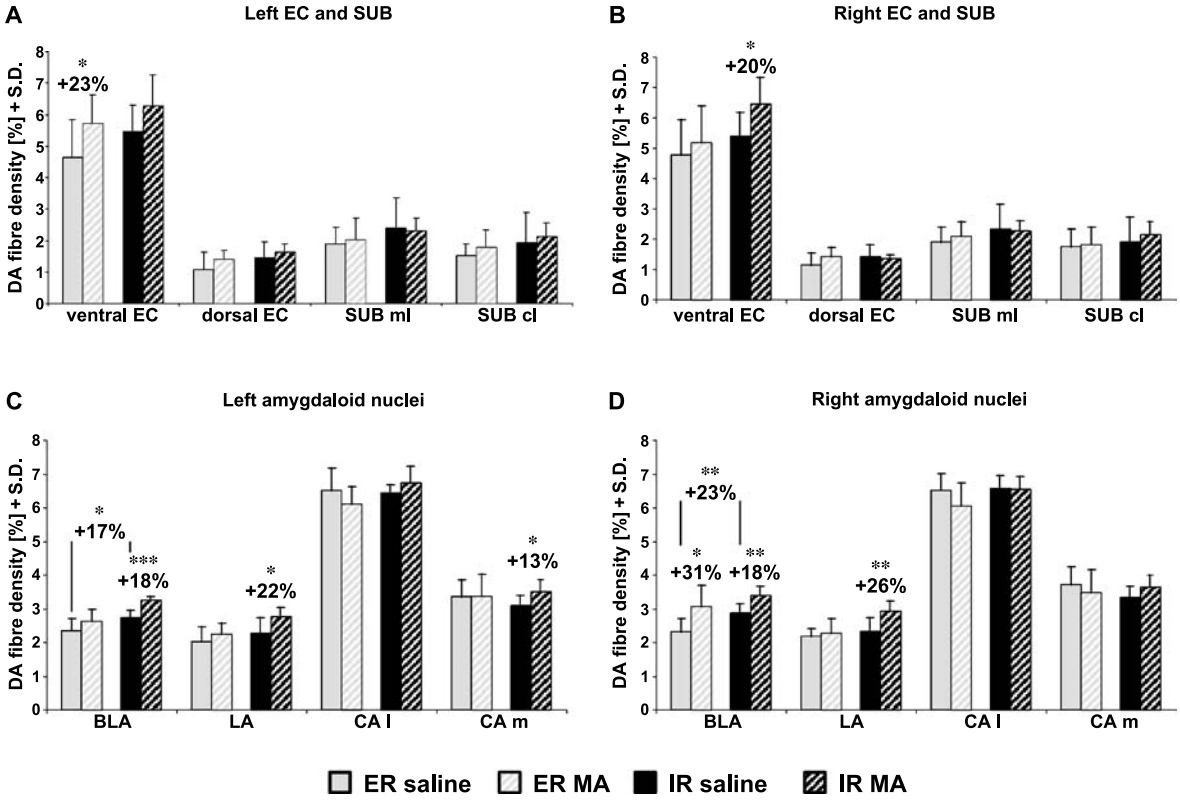

Fig. 3. Dopamine (DA) fibre densities in the analysed areas of gerbils from enriched (ER) and impoverished rearing (IR) conditions treated with either methamphetamine (MA) or saline given by means ± standard deviation (S.D.). **A**, **B** show the results of the left and right hemisphere, respectively of the entorhinal cortex (EC) and the subiculum (SUB). The DA fibre densities in the different amygdaloid nuclei, basolateral (BLA), lateral (LA) and central (CA), of the left and right hemisphere are given in (**C**, **D**), respectively. $p < 0.05$ (*), $p < 0.01$ (**), $p < 0.001$ (***)

ER animals (MANOVA: ER: saline vs. MA, $F = 5.6955$, $p = 0.0174^{*}$; IR: saline vs. MA, $F = 19.9829$, $p < 0.0001^{***}$). Methamphetamine treatment of IR animals leads to a fibre overshoot of 20% in the ventrolateral EC of the right hemisphere. In the amygdala a fibre surplus of 18% in the BLA of each hemisphere and of 22% and 26% in the LA of the left and right hemisphere, respectively, is detected. The CAm shows an overshoot of DA fibres of 13% in the left hemisphere (Fig. 3A–D). The MA application of ER animals increases the DA fibre densities of 23% in the ventrolateral EC of the left hemisphere and of 31% in the BLA of the right hemisphere (Fig. 3A, D). The dorsolateral EC, the SUB and the lateral part of the CA remain unaffected by both epigenetic factors (Fig. 3A–D).

Although the results revealed some hemisphere-specific experimental effects, no significant asymmetry of the DA fibre density could be detected (MANOVA: $F = 0.8721$; $p = 0.3509$).

Discussion

Evaluations of DA fibre densities in the present study have shown that both interventions, IR conditions and the postnatal MA treatment, do exert region-

specific effects in distinct limbic areas. In the densely aggregated DA clusters of the ventrolateral EC, the early MA application enhances the DA fibre densities in both ER (left hemisphere) and IR animals (right hemisphere). In contrast, the less innervated dorsolateral EC and SUB remain unaffected by any treatment. In the amygdala the MA challenge in IR animals leads to an enhancement of DA fibres in the LA and CAm. The DA fibre density of the BLA is affected by both experimental variables, which suggests this nucleus to be the most susceptible amygdaloid structure. Remarkably, the lateral CA, which is completely filled by DA terminations, shows no adaptive changes at all. Thus, the susceptibility of DA fibres is not correlated to the absolute fibre density of the area concerned. On the whole, IR animals reacted more sensitively to the early MA treatment as compared to ER animals.

The presented data on a DA fibre surplus in the EC and BLA, including recent ones that concern suppressive DA fibre maturation in the PFC, NAC and caudatus-putamen of this animal model (Dawirs et al., 1994; Winterfeld et al., 1998; Neddens et al., 2001, 2002; Lehmann et al., 2002), show that the DA fibre maturation of the different parts of the mesocorticolimbic system could be influenced in an inverse manner by epigenetic factors. Our suggestion is that the main subdivisions of the mesocorticolimbic DA projection, the mesocortical, the mesostriatal and mesolimbic one, closely interact in function and dysfunction and presumably interact in a hierarchical fashion. Using invasive 6-hydroxydopamine intoxication it has been demonstrated in adult rats that the pharmacologically lesioned PFC affects the DA turnover in NAC particularly under stress (Pycock et al., 1980; Martin-Iversen et al., 1986; Deutch et al., 1990; Rosin et al., 1992; King et al., 1997). The authors independently suggested that the activity efflux from the PFC to the ventral striatum generally influences the DA activity in this subordinated area. This idea is affirmed by our studies of DA fibre maturation of the NAC. The MA treatment at PD14 produced significant deficits of DA fibre densities up to adulthood in the core of IR gerbils and in both core and shell of the ER group (Neddens et al., 2002). The authors argued that presumably the MA intervention affected the mesostriatal DA projection just during a most critical period of maturation. Although it is well known that monoamine systems of rats are affected by a single high dose of MA due to the production of neurotoxic 6-hydroxydopamine and several other physiological mechanisms (rev. Seiden and Sabol, 1996; Seiden and Vosmer, 1984; Fukumura et al., 1998), it has been demonstrated that in gerbils the MA intoxication on PD14 selectively damaged prefrontal axon terminals (Teuchert-Noodt and Dawirs, 1991) and led to a suppressive maturation of prefrontal DA fibres up to adulthood (Dawirs et al., 1994). Therefore, a maladaptation of DA fibre densities in the NAC may be brought about by the weakened control coming from an underdeveloping PFC.

Le Moal and Simon (1991) proposed that the DA regulation in the anatomical and functional interdependent mesocorticolimbic circuitry might be ''organised in a hierarchial manner, with the PFC acting as the highest instance''. Thus, the PFC might be in a position to control, strengthen or weaken and even disturb the function not only of the corticostriatal but even of the whole limbic circuitry. For instance, Rosenkranz and colleagues could

demonstrate that the output neurons of the BLA are under the regulatory control of prefrontal efferents which are presynaptically modulated by DA (Rosenkranz and Grace, 2001, 2002; Grace and Rosenkranz, 2002). Thus, the PFC could exert its main influence on the activity of far distant mesolimbic areas by direct projections to the limbic termination fields. Within the amygdala complex the PFC has strong reciprocal connections particularly to the BLA and to a lesser extent to the LA (McDonald et al., 1996; Pitkanen, 2000). Mediated by distinct projections of the BLA, the ventrolateral EC and the CAm participate in the PFC activity flow (Krettek and Price, 1977, 1978; Pikkaraninen et al., 1999). Based on the paradigm that processes of maturation are activity-dependent, the DA fibre maturation should be dependent on extrinsic and intrinsic activities and presumably particularly on the activity of the prefrontal efferents to the termination fields. Likewise the PFC could indirectly affect DA maturation of caudal limbic structures via reciprocal connection to the VTA (Sesack and Pickel, 1992; rev. Kalivas, 1993). However, the prefrontal influence via the VTA seems not very likely to us, since the VTA can be regarded as an anatomical and functional continuum and DA neurons are equipped with intense compensatory mechanisms (rev. Le Moal and Simon, 1991). Moreover, there is no proof that prefrontal projections to the VTA generally terminate on DA neurons which project to the amygdala and entorhinal cortex. Therefore, our interpretation for the results would be that malfunctional efferents from the PFC, which are impaired by the suppressive prefrontal DA maturation (Dawirs et al., 1994; Winterfeld et al., 1998; Neddens et al., 2001), may cause selective maladaptations of DA fibre densities in specific limbic target areas. This interpretation is supported by the result that alterations of DA innervation were found in those brain regions which are closely interconnected with the PFC. Thus, selective effects on the limbic DA target fields support the idea of correspondingly selective dependences on intrinsic and/or extrinsic activities which function in an interdependent manner and modulate large regions of the brain. In other words, the ventrolateral EC, the BLA, the LA and the medial part of the CA are crucial points in respect of vulnerability of DA fibre maturation of the mesocorticolimbic system, whereas other amygdaloid and entorhinal areas seem to be less influenced by these intrinsic activity disturbances.

There are two versions of how the enhancement of DA fibres in caudal limbic areas may adjust to suppressive ones in the prefrontal cortex. First, following the pruning paradigm, the disconnection of mesocortical from mesolimbic DA projections in early postnatal life may produce a fibre overshoot sprouting nearby the VTA, i.e. in limbic areas. This mechanism has been demonstrated by systemic neurotoxic lesions of serotonergic and noradrenergic pathways of neonatal rats, which produced subsequent hyperinnervations in areas proximal to the brainstem nuclei, and hypoinnervations in distal aminergic target fields (Jonsson and Hallman, 1982; Fischer et al., 1995). Other literature reports that a partial lesion of the medial PFC of rat pups induced the mesocortical DA projection to evade into unlesioned frontal cortical fields (de Brabander et al., 1991, 1992). Furthermore, lesion-induced regenerative DA fibre sprouting has been proven for the mesostriatal DA projection (Mitsumoto et al., 1998; Bezard et al., 2000). However, no pathologically induced sprouting

effects have yet been shown for DA projections into caudal limbic areas. In primates and rats the normal process of DA maturation is characterised by a transient early postnatal fibre surplus in caudal limbic target fields (Verney et al., 1985; Erickson et al., 1998). The following decline of DA fibres continues into adolescence. Remarkably, this regressive process is correlated with the prolonged DA fibre maturation in the PFC (Kalsbeek et al., 1988; Dawirs et al., 1993). Therefore, the second possible version might be that the normal decline of the transient DA fibre surplus in limbic areas is suppressed by the epigenetically induced disconnection from the prefrontal activity control.

The present study displays the BLA as the most sensitive caudal limbic structure affected by the developmentally induced disturbances. This observation gets support from recent comparable investigations showing that the serotonergic innervation pattern of the BLA is also severely affected by the same set of interventions (Lehmann et al., 2003). Of all areas investigated in this study, the BLA has the strongest reciprocal connection with the PFC (Pitkanen, 2000). The late maturation of this connectivity has recently been shown and was proposed to influence the development and integration of normal or abnormal emotional behaviour during adolescence (Cunningham et al., 2002). These findings may help to explain why an apparently moderate chronic disturbance during development, namely IR condition, is sufficient to selectively affect the monoaminergic maturation of the BLA. On the other hand the early single MA intoxication represents an acute severe impairment in a critical period of DA maturation. In combination with social deprivation the single application is apparently sufficient to strongly disturb the balance of the mesocorticolimbic system. As a consequence not only the BLA is affected but also other interconnected caudal limbic areas. Thus, the effect of the MA intoxication seems to depend on the complexity of the social environment. This result strongly demands to think about postnatally acquired individual predispositions in view of clinical psychopharmacology.

On the whole, we come to the conclusion that during postnatal development the mesocorticolimbic DA projection forms an integrated whole, in which the role of retarded PFC maturation patterns might be to strike the right balance for cortical and limbic integrative functions. Consequently, the disturbance of this balance in early childhood may lead into another balance, which is a pathological one. The coincidence of two severe non-invasive interventions has shown that a pathological imbalance can produce multiple features of psychiatric diseases in dependence on individual predisposition.

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Differential Influence of Rearing Conditions and Methamphetamine on Serotonin Fibre Maturation in the Dentate Gyrus of Gerbils *(Meriones unguiculatus)*

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Key Words
Serotonin · Hippocampus · Methamphetamine · Rearing · Plasticity

Abstract
Environmental experience and drugs are two parameters that affect the maturation of neurotransmitter systems. The influence of impoverished rearing (IR) versus enriched rearing (ER) was compared in conjunction with postnatal methamphetamine (MA) treatment. The densities of immunostained 5-HT fibres were quantified in septal and temporal regions of the hippocampal dentate gyrus (DG) in young adult gerbils. In the IR group, 5-HT fibre densities were significantly increased in the molecular, granular and polymorphic layers of the DG in the temporal plane. After postnatal MA treatment, the 5-HT fibre density in the ER group reached a level equivalent to that of the IR group in nearly all respects. Under IR conditions, the pharmacological intervention significantly increased the maturation of fibre densities in septal layers only in the right hemisphere with no significant alterations in the left hemisphere and in temporal regions of either hemisphere. According to our previous studies on hippocampal neurogenesis, adaptations of 5-HT fibre densities partly proved to be positively correlated to cell proliferation rates for each of the specific conditions. Thus, the induced MA sensitivity, caused by pharmacological intervention at day 14, was manifested as direct interaction of 5-HT fibre maturation and cell proliferation in dependence of environmental factors. Both IR and MA together give us a better understanding of raphe-hippocampal plasticity and offer new perspectives for pharmacological studies on the 5-HT participation in mental disorders.

Introduction

The unique property of the raphe serotonin system [5-hydroxytryptamine (5-HT)] to act as a morphogenetic agent in the embryonic brain which remains conserved in adult life [for a review, see ref. 1, 2] has attracted hippocampal plasticity research in the past decades. Lesioning 5-HT fibres projecting to the hippocampus produced homotypical collateral sprouting of uninjured 5-HT raphe neurons to denervated hippocampal targets [3, 4]. Overshoot collateral sprouting of 5-HT fibres and hyperinnervation was provoked when foetal 5-HT neurons were transplanted to limbic areas [5, 6]. The implication of these findings is that the physiological status of 5-HT does influence functional versus malfunctional adaptations of limbic structures and this may imply mental defects. The

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basic mechanisms of the raphe-hippocampal plasticity, however, have not been understood.

More light has been shed on the integration of the raphe 5-HT system into hippocampal plasticity, since recently a germinative layer has been detected in the adult dentate gyrus (DG) of mammals [7, 8] including humans [9]. It has been demonstrated that on the one hand, 5-HT depletions reduce the neurogenesis in the adult DG of rats [10], and on the other hand that implanted foetal 5-HT raphe neurons are accepted for stimulating cell production in the host germinative dentate zone [11]. The assumption of a direct transmitter-regulated neurogenesis was affirmed by treating animals with steroid hormones [12] and NMDA receptor agonists/antagonists [13]. Pharmacological interventions with haloperidol and methamphetamine (MA) also documented the role of other aminergic transmitters, like dopamine, on controlling cell proliferation rates in the hippocampal DG [14–16]. Additionally, an environmental challenge by stress caused by isolated housing has been shown to decrease cell proliferation in the DG of rats [17]. In isolated rearings (social privation), however, an increase in cell proliferation persisted even in adults [18]. Therefore, environmental experience as well as drug influences are to provide proof for the susceptibility of 5-HT fibres to non-invasive systemic interventions and thus test the role of serotonin in the postnatal development of hippocampal plasticity.

Both isolated rearing conditions and amphetamine intoxication have independently been tested in experimental studies on mental disorders, in which the afferent raphe 5-HT projections and the hippocampal system have been the focus of attention. Isolated rearing and housing of rats has been found to decrease the 5-HT release and turnover in hippocampal terminal fields [19] in connection with the perturbation of postsynaptic 5-HT processes [20; for a recent review, see ref. 21], thus reducing serotoninergic functions as expressed in an animal model of anxiety [22] and depression [23]. With regard to the application of substituted amphetamines, a complex sequence of events induces neurotoxicity by the increase in free radicals in aminergic terminals [for a recent review, see ref. 24]. This also damages 5-HT projections in high brain areas including hippocampal regions [25, 26] and develops the so-called 'behavioural 5-HT syndrome' [27], which is quite similar to behavioural patterns observed after social privation. Remarkably, features of both types of intervention in animals are on a par with clinical findings in human depression [for a recent review, see ref. 28] with corresponding physiological impairment of the raphe-hippocampal interaction.

Altogether, the hippocampal DG provides an adequate model to explore the role for raphe 5-HT projections in hippocampal plasticity under defined non-invasive conditions, such as (1) the environment model, i.e. impoverished rearing (IR) with social privation versus semi-natural rearing in social groups [enriched rearing (ER)], and (2) the pharmacological perturbation of the 5-HT raphe system by the treatment of gerbils with a toxic dose of MA at postnatal day 14 (P14). Just the combination of both non-invasive interventions has been attempted to sound the depth of the raphe-hippocampal plasticity. This requires a clear depiction of developmental stages. For the MA treatment, we chose P14, since MA effects induced at this age may be critical for progress in maturation of the 5-HT axon terminal fields in the hippocampal DG. As the septal DG is innervated by the median raphe projection and the temporal plane predominantly by dorsal raphe projection fibres [2, 29], immunostained fibre densities were quantified separately in the septal and temporal infrapyramidal blades. Taking into account the typical lamination of 5-HT fibre patterns in the hippocampus, fibre densities were evaluated in the molecular, granular and polymorphic layers.

Materials and Methods

Animals and Rearing Conditions

Fifty-four male gerbils were used in this study. Twenty-six were bred in our facilities in standard cages and 28 in semi-naturally structured compounds [for details, see ref. 30]. On P14, some of the pups from each condition received a single injection of either MA (50 mg/kg, i.p.) or saline. At weaning (P30), the gerbils that were born under standard conditions were assigned to IR conditions for further 90 days. Ten of these animals were treated with MA. The other animals, 13 of which received an MA application, were assigned to ER conditions. IR animals were kept singly in standard makrolon cages, ER animals lived in sibling groups in compounds similar to those they were born in. Under both sets of conditions food and water were provided ad libitum. All gerbils were kept on natural day/night cycles.

Immunohistochemistry

Preparation of Tissue. On P120, animals were transcardially perfused under deep chloral hydrate anaesthesia (1.7 g/kg, i.p.). The perfusion was performed with 100 ml 0.1 *M* phosphate buffer (room temperature, pH 7.2), followed by 500 ml of 4% paraformaldehyde in 0.1 *M* phosphate buffer (pH 7.4). Immediately after perfusion, the brains were removed and postfixed for 2 h at 4°C. Horizontal sections of 20 μm of the hippocampus were taken on a frigocut (Reichert-Jung Modell 2700, Austria) and every other section was collected in ice-cold 0.1 *M* phosphate-buffered saline (PBS, pH 7.4).

Immunohistochemical Procedure. For immunostaining, the slices were rinsed 3 × 10 min in PBS, incubated for 10 min with 1% H_2O_2 to reduce background staining, and rinsed again thrice in PBS for

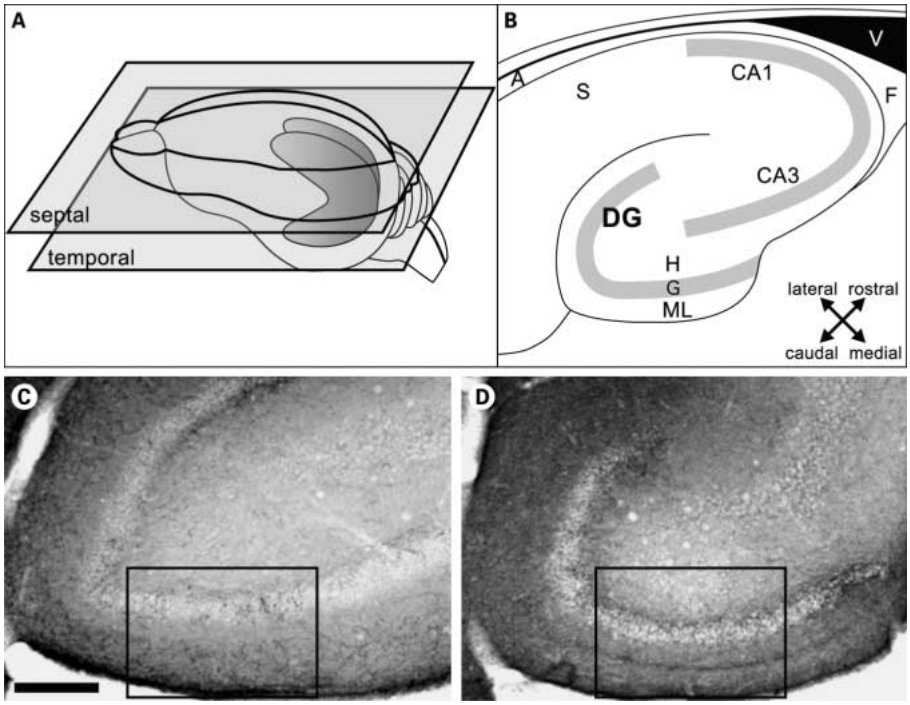

Fig. 1. Schematic illustration of the septal and temporal plane (**A**) and of the hippocampal formation (**B**). Brightfield pictures of the serotoninergic innervation of the septal (**C**) and the temporal (**D**) DG. The rectangles symbolize the position of the testfield in the infrapyramidal blade, in which the 5-HT fibres were quantified. A = Alveus; F = fimbria; G = granular layer; H = hilus; ML = molecular layer; S = subiculum; V = ventricle. Scale bar = 500 μm (**C**, **D**).

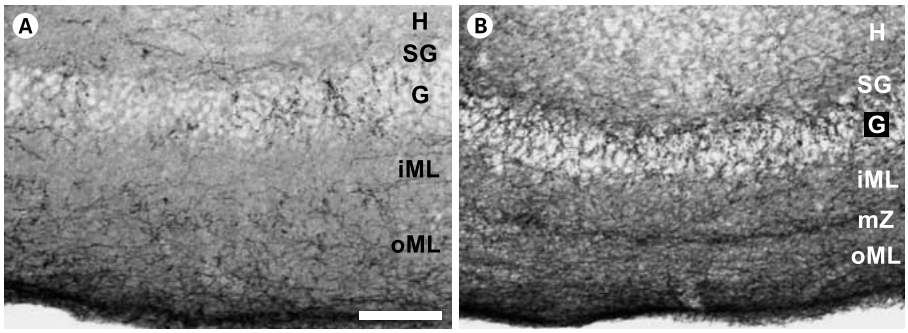

Fig. 2. Brightfield pictures of the serotoninergic innervation pattern. The laminar distribution of the serotoninergic fibres in the infrapyramidal blade are shown [septal (**A**), temporal (**B**)]. Highest fibre densities appeared in the outer molecular layer, the intermediate zone and the subgranular layer. G = Granular layer; H = hilus; iML = inner molecular layer; mZ = intermediate zone; oML = outer molecular layer; SG = subgranular layer. Scale bar = 200 μm.

10 min. Following 30 min of preincubation in 10% normal goat serum (NGS) in PBS containing 0.3% Triton X-100, the slices were incubated in rabbit anti-serotonin serum (Diasorin), diluted 1:20,000 in PBS with 1% NGS and 0.3% Triton X-100, for 18 h at 4°C. For the next procedure, 0.05 *M* Tris-buffered saline (pH 7.6) was used. The slices were incubated first in biotinylated goat anti-rabbit serum (Sigma) and then in Extravidin peroxidase (Sigma) diluted 1:20 with 1% NGS for 30 min each. Both steps were followed by a 3 × 10 min rinse in Tris-buffered saline. The slices were stained in 0.05% 3,3-diaminobenzidine (Sigma) and 0.01% H_2O_2 for 4 min and then rinsed again four times. Finally, they were mounted on adhesive coated glass slides, dried overnight, dehydrated and coverslipped with Depex (Serva, Heidelberg, Germany).

Quantification and Data Analysis

Four and 3 consecutive sections derived from the septal and temporal plane, respectively, were selected in each animal and all detectable 5-HT fibre fragments were visualized in standard test fields (1,992 × 1,450 pixel; 0.881 mm^2) of the infrapyramidal blade of the DG (fig. 1). Standard test fields cover all layers of the DG, hilus, subgranular and granular layer, inner and outer molecular layer, and intermediate zone. One standard test field was evaluated in each section, with fibre fragments visualized using a brightfield microscope (Polyvar, Reichert-Jung, Austria) and a digital camera for microscopy (ProgRes 3008, Jenoptik, Jena, Germany) at 200-fold magnification. The fibres were detected in each subarea using 'valleys' function, which depicts the grey value difference of adjacent pixels. The percentage area of the fibres within each subarea was calculated by a software for image analysis (KS 300, Jenoptik). Results are given as relative measures describing individual mean densities of 5-HT immunoreactive fibres derived from 27 to 64 standard test fields in both the left and right DG of both MA- and saline-treated animals from both ER and IR conditions (for n = number of animals, see legends of fig. 3–5). Mean values were computed as arithmetic means ± SD and compared by t test (two-tailed) with preceeding F test [31].

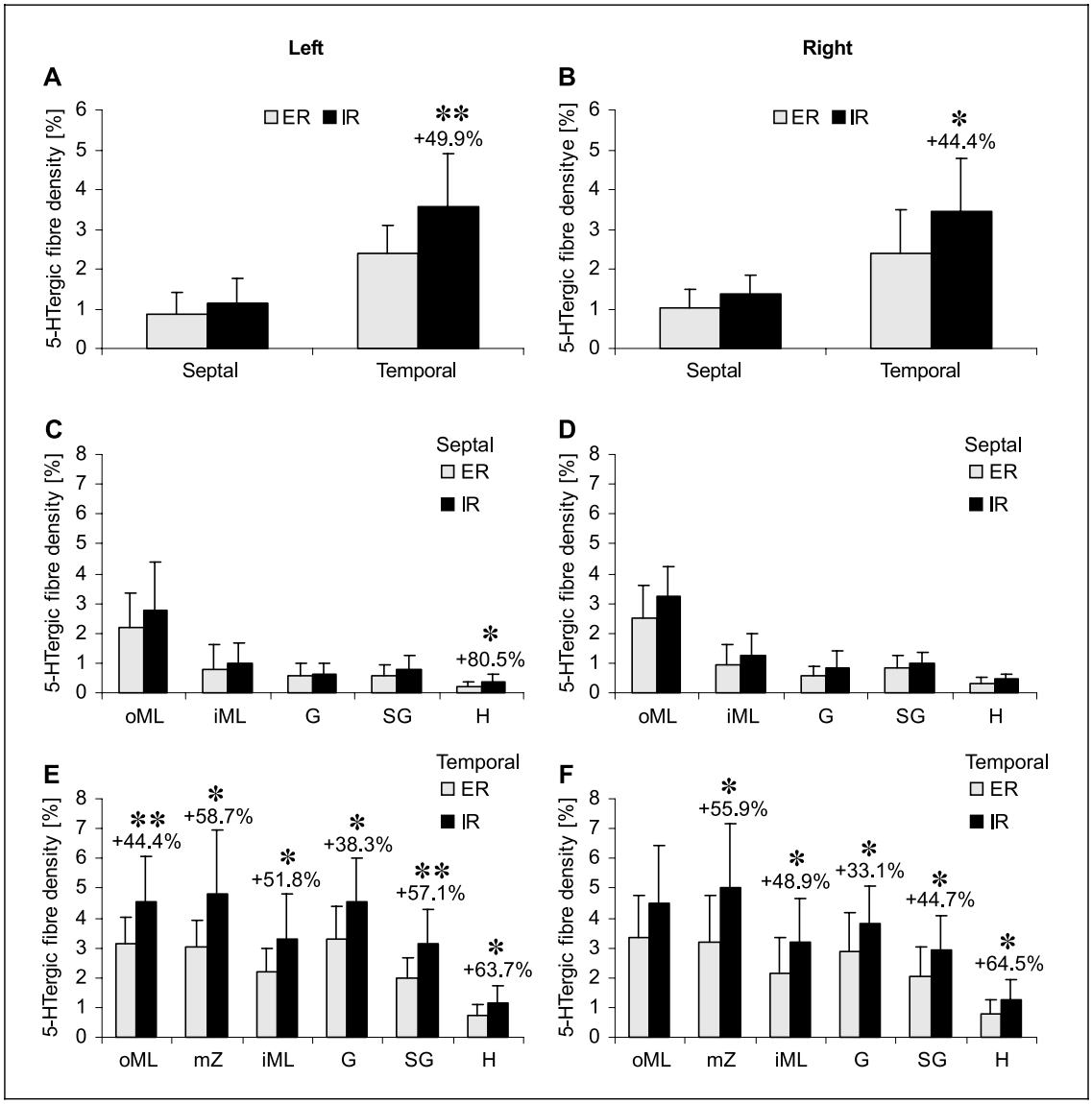

Fig. 3. Serotoninergic fibre densities in the DG of gerbils from ER and IR conditions given by means ± SD. **A**, **B** overall 5-HT fibre densities in the septal and temporal infrapyramidal blade of the left and right DG, respectively. The results of the dentate subareas are illustrated for the septal (**C**, **D**) and the temporal plane (**E**, **F**). G = Granular layer; H = hilus; iML = inner molecular layer; mZ = intermediate zone; oML = outer molecular layer; SG = subgranular layer. n = 15 (ER, left and right DG); n = 13 (IR, left DG); n = 16 (IR, right DG). * $p < 0.05$, ** $p < 0.01$.

Results

Figure 2 shows the distribution of 5-HT immunoreactive fibres in the right hemisphere of a saline-treated male gerbil raised under restricted conditions. As a qualitative result, densities of 5-HT immunoreactive fibres appeared to be clearly higher in the temporal plane of the DG throughout all layers investigated when compared with its septal plane (fig. 2A, B). In the temporal plane, 5-HT immunoreactive fibres expressed highest densities in the subgranular layer, the outer molecular layer, and an intermediate zone at the border between the inner and outer molecular layer (fig. 2B).

As a quantitative result, figure 3 represents some significant influences of postnatal rearing conditions on 5-HT immunoreactivity in layers of the adult DG. General-

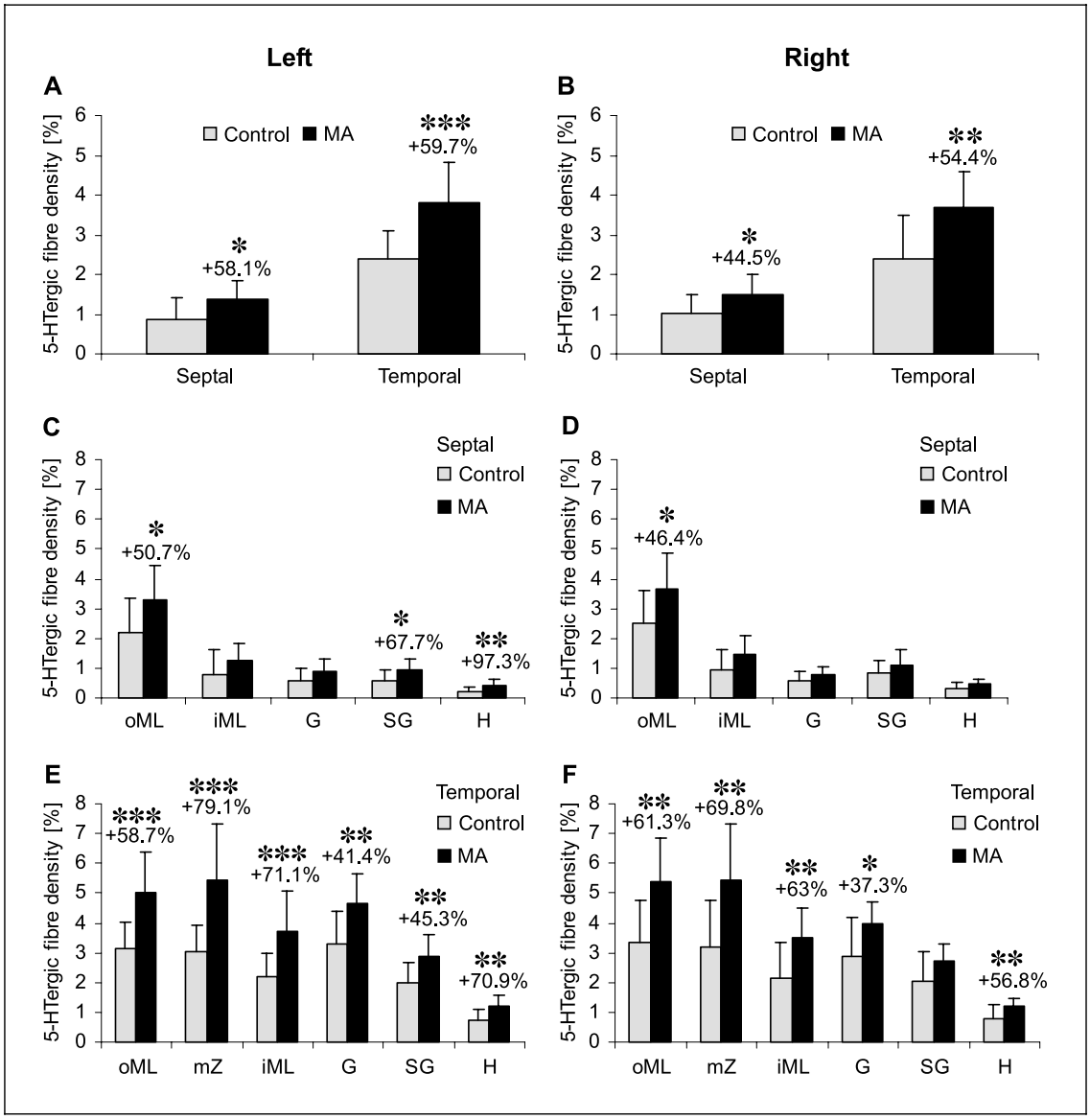

Fig. 4. Serotoninergic fibre densities in the DG of MA-intoxicated animals from ER conditions given by means ± SD. **A**, **B** total 5-HT fibre densities in the septal and temporal infrapyramidal blade of the left and the right DG, respectively. The results of the dentate subareas are shown for the septal (**C**, **D**) and the temporal plane (**E**, **F**). G = Granular layer; H = hilus; iML = inner molecular layer; mZ = intermediate zone; oML = outer molecular layer; SG = subgranular layer. n = 15 (ER control, left and right DG); n = 12 (ER MA, right DG); n = 13 (ER MA, left DG). * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

ly, it has been found that 5-HT immunoreactivity was significantly affected in the temporal plane of the DG of both hemispheres, whereas the septal plane was unaffected by differential rearing in the left as well as in the right hemisphere (fig. 3A, B). More specifically, postnatal IR conditions clearly caused an overshoot production of 5-HT immunoreactive fibres by about 33–65% throughout almost all layers of the DG at the temporal plane in both hemispheres investigated, but with one exception, i.e. the outer molecular layer in the right hemisphere showing no difference in fibre density between ER and IR conditions (fig. 3E, F). On the contrary, the left hemispheric hilus was found to be the only septal layer which showed some significant effect of differential rearing on

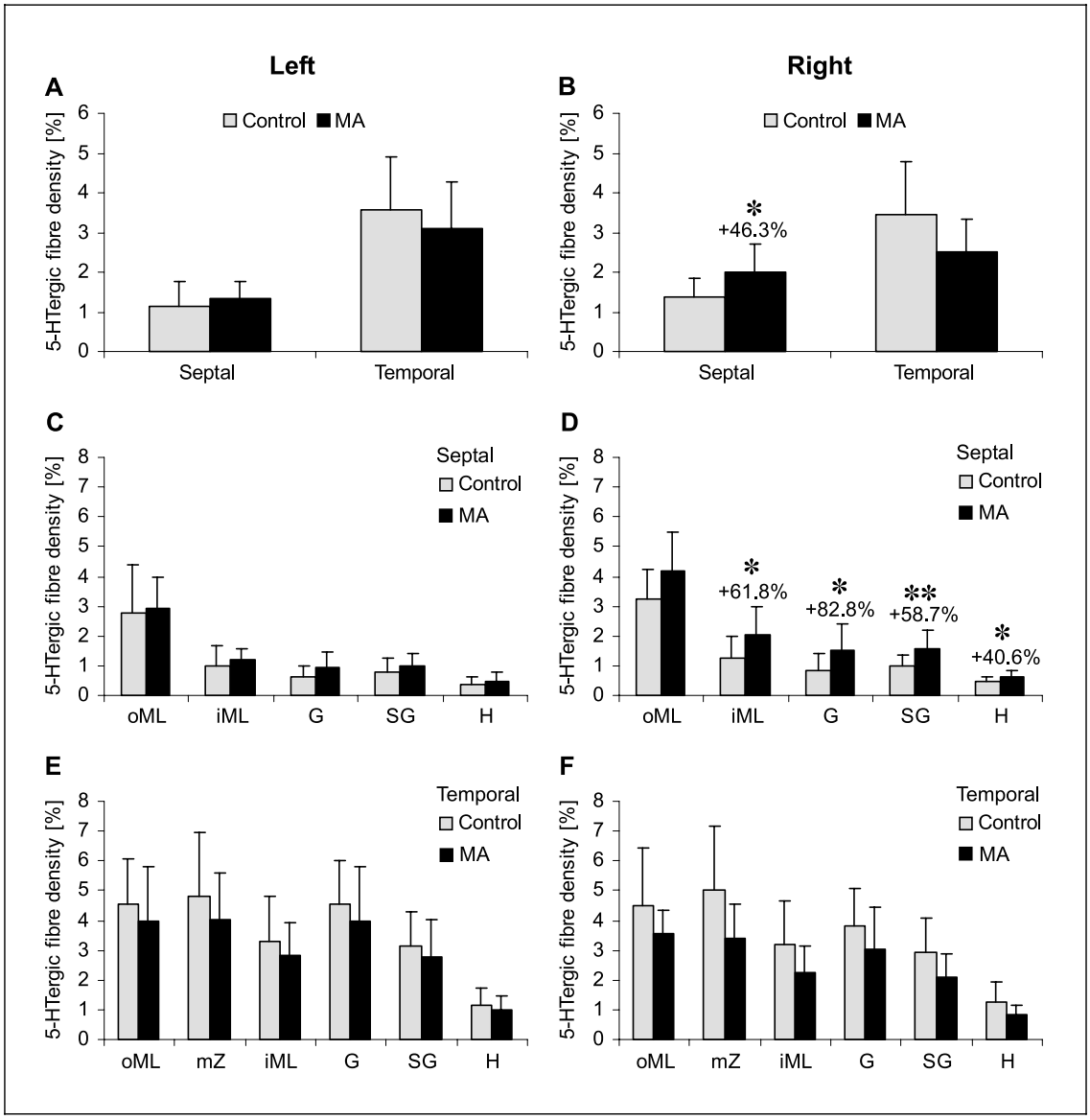

Fig. 5. Serotoninergic fibre densities in the DG of MA-intoxicated IR animals given by means ± SD. **A**, **B** total 5-HT innervation in the septal and the temporal infrapyramidal blade of the left and right DG, respectively. The results of the dentate subareas are presented for the septal (**C**, **D**) and the temporal plane (**E**, **F**). G = Granular layer; H = hilus; iML = inner molecular layer; mZ = intermediate zone; oML = outer molecular layer; SG = subgranular layer. n = 16 (IR control, right DG); n = 13 (IR control, left DG); n = 9 (IR MA, right DG); n = 10 (IR MA, left DG). * $p < 0.05$; ** $p < 0.01$.

5-HT immunoreactivity, with IR conditions causing 5-HT fibre densities being about 80% higher when compared with ER conditions (fig. 3C, D).

Figure 4 represents some significant influence of a single early MA challenge on postnatal development of 5-HT immunoreactivity in layers of the DG under ER conditions. Generally, it has been found that 5-HT immunoreactivity was significantly affected in both the temporal and septal plane of the DG of either hemisphere (fig. 4A, B). More specifically, an early MA challenge clearly caused an overshoot production of 5-HT immunoreactive fibres by about 37–79% through almost all layers of the DG at the temporal plane in both hemispheres investigated, but with one exception, i.e. the subgranular layer in

the right hemisphere showing no difference in fibre densities between MA- and saline-treated animals (fig. 4E, F). On the contrary, in the septal plane, only the left hilus and subgranular layer as well as the outer molecular layer in both hemispheres revealed some significant MA-induced overshoot production of 5-HT immunoreactive fibres by about 46–97%, whereas the remaining majority of layers were not significantly affected (fig. 4C, D).

Figure 5 represents some significant influence of a single early MA challenge on postnatal development of 5-HT immunoreactivity in layers of the DG under IR conditions. Generally, it has been found that 5-HT immunoreactivity was significantly affected only in the septal plane of the right hemisphere, whereas both the left septal plane and the temporal plane in either hemisphere were unaffected by the early MA treatment (fig. 5A, B). More specifically, an early MA challenge clearly caused an overshoot production of 5-HT immunoreactive fibres by about 41–83% through almost all layers of the DG at the right septal plane, but with one exception, i.e. the outer molecular layer showing no difference in fibre densities between MA- and saline-treated animals (fig. 5D). On the contrary, not any layer investigated in the DG of neither the left septal plane nor the temporal planes of both hemispheres were significantly affected by the early postnatal MA treatment (fig. 5C, E, F).

Discussion

The data presented testify that environmental experiences and drugs are two variables that selectively interfere with the maturation of serotoninergic (5-HT) fibre densities in the hippocampal DG. IR in social isolation produced a surplus of 5-HT fibre maturation only in layers of the temporal DG as compared with the ER group. The ER group adapted by manifesting increased fibre densities when treated with a single dose of MA postnatally. When MA-treated animals were reared under IR conditions, the 5-HT fibre density increased only at the right septal plane, while at the temporal plane, no alterations were registered. Summarizing, it appears that in temporal regions of the DG, postnatal maturation of 5-HT immunoreactive fibres is more sensitive against both IR conditions and early MA treatment when compared with septal regions.

The result of similar surplus values of 5-HT fibres under both IR and MA-intoxicated ER conditions warrants scrutiny. In the literature, it has been sufficiently demonstrated that IR conditions affect the raphe 5-HT system [21], thus causing specific behavioural disturbances [22, 23]. However, the literature also indicates that the molecular layers of the hippocampal DG may be spared from an underdeveloped 5-HT structure and function resulting from IR conditions [32]. As to pharmacological manipulations, an amphetamine-toxic effect on the serotoninergic innervation of the hippocampus was nearly recovered 40 days after treatment [33]. 5-HT projection fibres have been proved to produce reactive homotypic sprouting of axon collaterals in degraded target fields within a few weeks after neurotoxic lesion of the cingulum bundle [4, 34]. Reports on the selective hyperinnervation of the DG by foetal 5-HT neuron transplants into limbic structures [5, 6] further support that this brain area must be regarded separately.

From recent literature, evidence increases that *reactive plasticity* in the hippocampal tissue has its parallel in a natural plasticity emerging from the DG in the postnatal and also in the adult mammalian brain (see Introduction). Keeping this in mind, the unique function of the hippocampal DG is seemingly supported by two independent anatomical substrates with neurotrophic function: one of them is the raphe 5-HT projection equipped with the ability to increase transmitter turnover rate and adaptive fibre sprouting in response to increased neuronal activity [for a review, see ref. 2]; the other one is the germinative subgranular layer, which remains a source of morphogenetic activity in the DG, supporting structural and functional plasticity for life [for a review, see ref. 35, 36]. To analyse the connection between the two variables under the circumstance of non-invasive deprivations of the raphe 5-HT system is currently of prime interest.

The 5-HT fibre surplus production in both the IR group and the MA-intoxicated ER group occurred against the background of an anatomical maturation that is considerably delayed in comparison with most other brain areas [37, 38]. The MA intoxication induced at P14 may be extremely critical in two respects. In rodents, the local circuitry just starts to acquire adult patterns [8], and serotoninergic fibres first show a laminar segregation similar to that in the adult [39]. Serotonin depletion during this critical period of synaptogenesis showed to result in a permanent loss of spines on granule cell dendrites of adult rats and in learning deficits [40–42]. Similar effects can be assumed to occur from the MA intoxication during the critical period of postnatal maturation of 5-HT target fields in the DG. Whether a short environmental stress during this critical period would also be sufficient to perturb the raphe-hippocampal projection is worthy of further investigation. Another aspect of the prolonged ontogeny of dentate circuitry is that the hippocampal formation

is innervated by separate 5-HT pathways, some of which are still growing 3 months postnatally. One of the latest is the ventral route of the dorsal raphe projection, which contributes importantly to the innervation of the temporal DG [for a review, see ref. 2]. Therefore, the special surplus values of 5-HT in the temporal plane of the IR group as compared with the ER group might be interpreted as a disturbance of synaptogenesis emerging from the prolonged transmitter maturation throughout adolescence.

Although the time schedule of intervention was quite different in the IR and the MA-intoxicated ER group, they both showed at least similar trends of overshoot sprouting. The weakly affected 5-HT fibres in the septal DG rise from the median raphe projection and the deeply affected fibres in the temporal DG from the dorsal raphe projection. Some publications emphasize that the dorsal raphe axons are extremely vulnerable to neurotoxic amphetamine derivatives, while median raphe axons appear to be more resistant to neurotoxic drug effects [43]. On the other hand, lesion studies with median raphe-hippocampal projections documented a homotypic compensatory sprouting response in the hilus after 6 weeks, which proved to be transient when tested after 10 weeks after lesion [44]. The author speculates that the hilar region by itself controls the course of reinnervation. This idea gets recent support by hippocampal plasticity research.

Considerable evidence suggests that both the septal and temporal DG differ by their plastic potential expressed by neurogenesis and synaptogenesis [for a recent review, see ref. 36]. Our studies in the past have revealed that the septal DG is characterized by high mitotic activity in connection with synaptic turnover rates which are highest in those molecular layers that contain intrinsic circuits (i.e. polymorphic subgranular layer and inner molecular layer). On the other hand, the temporal DG is characterized by comparatively low levels of neurogenesis and synaptogenesis [45, 46]. Quite the same cellular and synaptic dynamics over the longitudinal gradient occurred in gerbils from IR and ER conditions [18]. On the whole, weak 5-HT fibre densities at the septal plane correlate with high rates of cell production and synaptic remodelling. Towards the temporal plane, the situation inverts into the opposite. This suggests that the challenge to the median raphe 5-HT becomes largely compensated by an increased ontogenetic plasticity in the septal DG. On the other hand, the lower morphogenetic capacity at the temporal plane may not be resistant enough to early interventions. Thus, the unique plasticity of the raphe-hippocampal axis can be explained as a distinct trophic interaction between raphe 5-HT activity and dentate neurogenesis. Principally, the connection between both trophic structures could neither be disrupted by IR conditions alone nor by MA-intoxicated ER conditions. This interpretation is supported by our recent quantitative evaluations of cell proliferation in the DG of gerbils applying exactly the same experimental set of interventions as in the present study: interventions as applied here produced a permanent increase in the mitotic activity following a septotemporal gradient. A positive correlation of the adaptation of cell production rates and 5-HT fibre densities becomes apparent for both the IR and MA-intoxicated ER groups [16, 47]. This relation does also affect the ER group, which organizes relatively low fibre densities and likewise moderate values of neuro- and synaptogenesis when compared with the IR group [18].

The differential overshoots of 5-HT fibres in response to both IR and MA-intoxicated ER interventions probably occurred in connection with changes in serotoninergic mechanisms. This demands changes in general pre- and postsynaptic receptor functions. One mechanism by which 5-HT influences both the morphogenesis of its own fibres and the dentate plasticity is the release of the serotoninergic neurotrophic factor S-100$_\beta$ from astroglial cells, mediated by the 5-HT1A receptor [41]. It has been shown that S-100$_\beta$ stimulates synaptogenesis [48] and is involved in the lesion-induced reorganization of serotoninergic fibres in the adult rat DG [44; for a review, see ref. 49]. Furthermore, 5-HT1A receptors mediate cell proliferation in the DG by serotonin activation [50]. However, the density of the 5-HT1A receptor is reported to remain essentially constant throughout the septotemporal axis [51]. It remains to be shown whether the applied interventions are associated with selective changes in these and still other parameters that support serotonin function. Independent of intrinsic mediators of the 5-HT activity flow, causal factors for the persisting 5-HT fibre surplus should no longer be discussed in the limited context of local circuitry. The significance of extrinsic factors has been illustrated in the combination of isolated rearing and drug challenge and will be addressed below.

The serotoninergic interaction with the dentate neurogenesis has to be seen in the regulatory potential of the glutamatergic perforant path, whose target fields in the DG are densely endowed with NMDA receptors. Recent evidence suggests that 5-HT release in the hippocampus is modulated via the NMDA receptor system [52]. The distribution of the NMDA receptors in the molecular layer of the DG has been shown to follow a gradient over the longitudinal axis with decreasing densities from the septal to

the temporal pole [53]. Thus, the NMDA receptor gradient corresponds to the one of the mitotic activity [46]. Probably the MA intoxication and the social deprivation together produced a disconnection apparently of the whole limbic circuitry.

Anatomical [for a review, see ref. 54], electrophysiological [55] and behavioural [56] studies support different functional roles for the septal and temporal hippocampal system. The topographic organization of the entorhinal-to-dentate projection suggests that the septal DG is strongly integrated in multisensory cortical circuits and the temporal DG in subcortical ones with the amygdala and striatum as relay centres [57–60]. Therefore, the activity afflux from cortical and brainstem circuits takes differential influence via the glutamatergic perforant path on both the septal and the temporal DG, respectively. One main input to the septal DG comes from prefrontal cortical areas. We previously reported that the maturation of dopamine fibres was severely suppressed in subareas of the prefrontal cortex in connection with behavioural deficits when MA-intoxicated gerbils were additionally deprived by IR conditions [61]. Thus, functional distortion of the prefrontal projection to the DG might interfere with the control of dentate neuroplasticity. Pharmacological studies with the MA-intoxicated IR group gave us further evidence of a dopaminergic regulatory influence on neurogenesis in the DG [14, 16].

In conclusion, both IR conditions and a single early MA challenge result in compensatory overshoot sprouting of serotonergic fibres in the hippocampal DG with adaptive values for the dentate gyrus plasticity as expressed by neurogenesis along the septotemporal axis. It might further be concluded that, with respect to this response, septal regions may be more resistant, especially under IR conditions, probably due to generally higher rates of neurogenesis and synaptogenesis in the septal plane of the DG. Additionally, it appears that rearing under IR conditions partly protects 5-HT immunoreactive fibres from responding to an early MA challenge, which at least could be seen in the left septal plane of the DG. Finally, we may conclude that further investigations of combining various environmental and pharmacological interventions, differentially interfering with specific aspects of brain maturation, might be specifically useful for the development of future therapeutic strategies against mental disorders in humans.

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Research report

Epigenetic factors differentially influence postnatal maturation of serotonin (5-HT) innervation in cerebral cortex of gerbils: interaction of rearing conditions and early methamphetamine challenge

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Abstract

The effects of disjunctive environmental deprivation combined with a single methamphetamine (MA) challenge on postnatal maturation of the serotonin (5-HT) innervation pattern in cerebral cortex of gerbils were studied. Gerbils were assigned to either enriched (ER) or impoverished (IR) environmental rearing conditions. On postnatal day 110, 5-HT was immunostained. The 5-HT innervation pattern of the brain was qualitatively evaluated and provided in graphic form. The densities of 5-HT fibres were quantified in areas of prefrontal, insular, frontal, parietal, and entorhinal cortices of the right hemisphere using digital image analysis. The early MA challenge led to an overshoot of the fibre density in medial and orbital prefrontal cortex and entorhinal cortex of ER animals. IR animals mostly resisted MA effects except of a restraint of the innervation of the insular cortex. In comparison to enriched rearing, restricted rearing caused overshoot maturation of 5-HT innervation in insular and entorhinal cortices. The present data provide evidence for a region-specific postnatal vulnerability of the maturing 5-HT innervation, namely in association cortices. In contrast, both sensory and motor cortices showed no significant changes at all. The results are discussed in context with previously presented findings of alterations of the cortical dopamine innervation depending on both epigenetic factors. In conclusion, both experimental variables together give new insight into raphe-cortical plasticity that may contribute to a better understanding of the role of 5-HT fibre systems in structural maturation of the cortex. Postnatal environment may be involved in individual vulnerability of a variety of mental disorders during adolescence and aging.

Theme: Neurotransmitters, Modulators, Transporters, and Receptors
Topic: Serotonin

Keywords: Development; Environment; Epigenetics; Immunohistochemistry; Monoamines; Neuroplasticity

1. Introduction

Epigenetic factors are known to influence the maturation of neurotransmitter systems in the postnatal period. A steadily increasing number of authors attach importance to epigenetics when discussing the aetiology of a variety of mental disorders [7,8]. The appearance of monoamine systems in early development suggests that they may play a key role in morphogenesis of the mammalian CNS. Moreover, it has been shown that during postnatal development, namely of the serotonin (5-HT) innervation, critical periods exist [19,27], in which crucial events may determine the way of the subsequent maturation of the brain. Early aberrant stimuli could possibly lead to dysfunctional activities of dopamine (DA) and 5-HT neurons in adult life [42,56,72]. Apparently the 5-HT projections from the dorsal and medial raphe nuclei exert a unique ability to recover from even strong damage [4,59,74,75], but it seems questionable that the functional properties of the neural network can be completely restored. Thus, the fundamental mechanisms of postnatal activity dependent structural and functional development of the brain and their mutually lifelong impact on CNS functions urgently need to be clarified in more detail.

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We are interested in the reaction of the maturing cortical 5-HT innervation on epigenetic factors since our laboratory has provided evidence for the ability of both a single systemical application of methamphetamine (MA) and differential rearing conditions to significantly affect the maturation of the DA innervation in prefrontal cortex (PFC) and nucleus accumbens [21,48,49,70]. In accordance with the well-known functional interaction of the DA and the 5-HT fibre systems [17,46,47,53,64], it has been shown that both transmitters contribute to the adaptive maturation of neuronal networks [18,29]. In this context, alterations of the prefrontal DA/5-HT ratio and of the GABAergic network have been found to correlate with cognitive disturbances [5,6,56]. The question has to be answered which mechanisms naturally ensure the maturation of a balanced transmitter activity and to what extent they may be influenced by complexity of the environment or pharmacological stimuli during early postnatal development. Another aspect that seems to be crucial for understanding activity dependent processes of self-organisation in neuronal networks is the existence of well-defined critical periods that may more or less clearly differ between cortical regions. Because of growing evidence for strong interactions among different functional systems, especially during postnatal maturation [8,45], the evaluation of the impact of epigenetic factors should be extended beyond regionally restricted effects.

One might reasonably assume that the environment, namely its complexity comprising the option of social interaction, should be crucial for the postnatal maturation of both morphology and function of various transmitter systems. Besides previously described physiological effects [33,34], we expect epigenetic factors to significantly affect the maturation of the cortical 5-HT innervation even on a morphologically detectable level, in addition to recently reported effects in subcortical areas [41]. Both incidence and extent of putative changes of the 5-HT innervation pattern are supposed to be region-specific but to some degree functionally interdependent events. To test this hypothesis, animals from both enriched (ER) and impoverished (IR) rearing conditions received a single injection of either MA (50 mg/kg, i.p.) or saline during maturation on postnatal day 14 (P14). 5-HT was immunostained in brain slices of male young adult gerbils and the fibre density was determined throughout different layers of the prefrontal, insular, frontal, parietal, and entorhinal cortices of the right hemisphere using both qualitative evaluation and a quantitative digital image analysis technique.

2. Materials and methods

2.1. Animals

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 56 male pups (weight 58–74 g; age 105–114 days) were used in this study, 54 of which were also used for 5-HT quantification in dentate gyrus [14]. All animals had free access to food and water and were kept on natural day/night cycles during summer season.

2.2. Breeding and rearing conditions

Twenty-six gerbils were bred in standard cages (Macrolon® type 4) without any content except of sawdust, whereas 30 animals were bred in semi-naturally structured compounds (width 100 × 100 cm, height 50 cm) furnished with wooden boards and houses, plastic tubes, and stones distributed on sawdust ground. At weaning (P30), the male gerbils that were born in standard cages were assigned to IR conditions. IR animals were reared individually in standard cages (Macrolon® type 3). Male ER animals grew up in groups of siblings (three to five individuals) in compounds similar to those they were born in. Both experimental groups persisted for approximately further 80 days.

2.3. Systemic administration of methamphetamine

On P14, a total of 26 pups received a single systemic injection of MA hydrochloride (Sigma; 50 mg/kg, i.p.). Fifteen and eleven of which were obtained from semi-natural breeding and standard breeding and were subsequently assigned to ER conditions and IR conditions, respectively. Thirty pups, fifteen from each breeding condition, were sham-treated by an i.p. injection of saline (Table 1).

2.4. Preparation of tissue

Animals were transcardically perfused under deep chloralhydrate anaesthesia (1.7 g/kg, i.p.). The perfusion was performed with 100 ml of 0.1 M phosphate buffer (room temperature, pH 7.2), followed by 500 ml of freshly

Table 1
Experimental design

	No. of animals (*n*)	Age at perfusion (days)	Injection, i.p., at P14	No. of sections (*n*)
ER saline	15	108–114	saline	507
ER MA	15	106–111	MA, 50 mg/kg	515
IR saline	15	105–112	saline	499
IR MA	11	108–114	MA, 50 mg/kg	380

Abbreviations are defined in the text.

prepared 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4). Immediately after perfusion, the brains were removed and postfixed for 2 h at 4 °C. To avoid deviations due to probably lateralised cortical 5-HT innervation densities, only right hemispheres were used for quantification. The hemisphere was divided at the rostral edge of the hippocampal formation. Coronal sections (20 μm thick) of the anterior part were taken on a frigocut (Reichert-Jung, Vienna, Austria) and every third section was collected in ice-cold 0.1 M phosphate-buffered saline (PBS, pH 7.4). The posterior part of the hemisphere, including hippocampus and entorhinal cortex, was cut in a horizontal plane but apart from that treated identically.

2.5. Histochemistry

For immunostaining of 5-HT neurons, the sections were rinsed 3 × 10 min in PBS, incubated for 10 min with 1% H_2O_2 to reduce background staining, and rinsed again thrice in PBS for 10 min. Following 30 min of preincubation in 10% normal goat serum (NGS) in PBS containing 0.3% Triton X-100, the sections were incubated in rabbit anti-serotonin serum (DiaSorin, Stillwater, USA), diluted 1:20,000 in PBS with 1% NGS and 0.3% Triton X-100 for 18 h at 4 °C. For the next procedures, 0.05 M Tris-buffered saline (TBS, pH 7.6) was used. The sections were first incubated in biotinylated goat anti-rabbit serum (Sig-

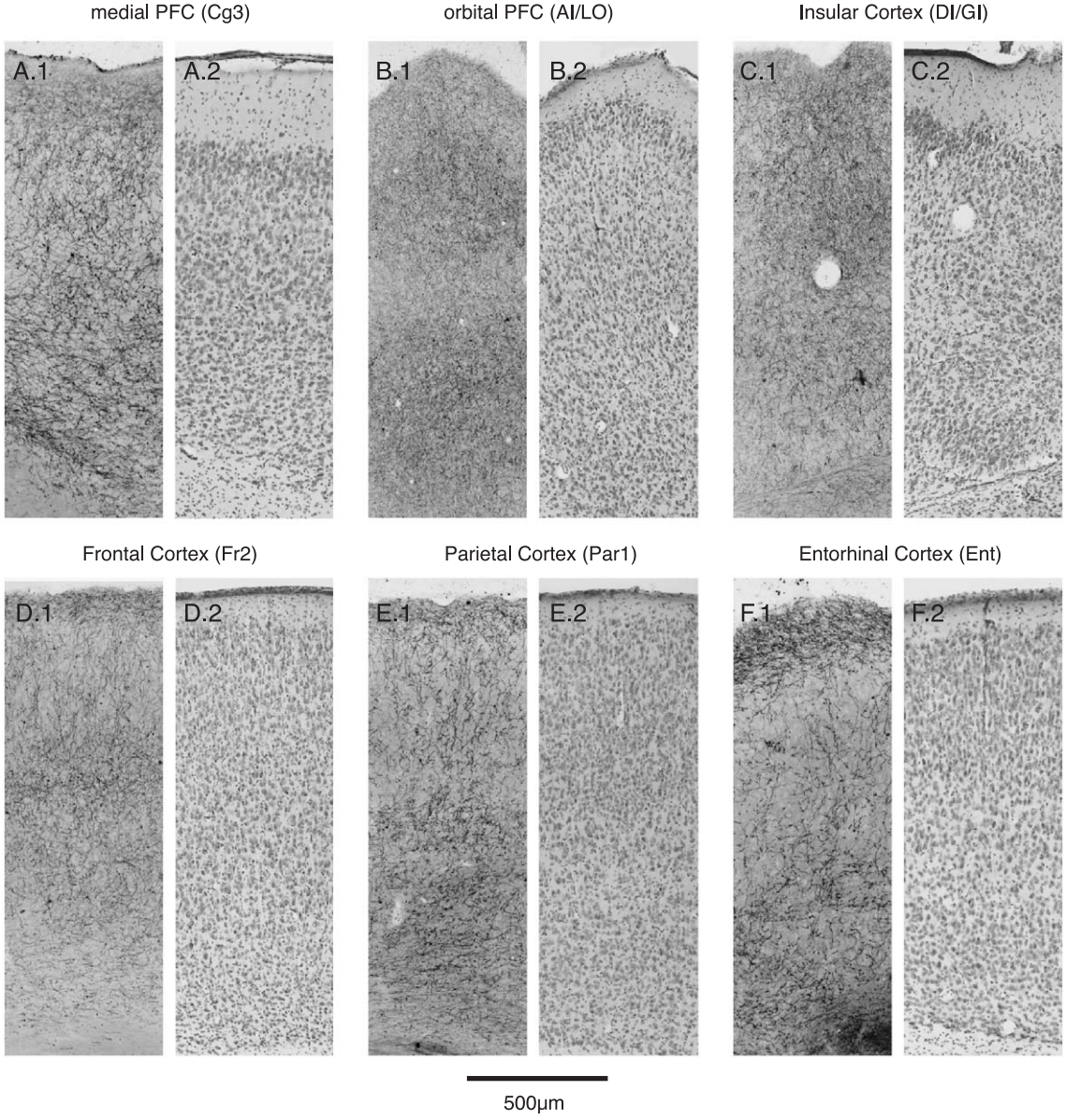

Fig. 1. Representative photomicrographs of 5-HT-immunoreactive fibres (A.1–F.1) of each of the quantified regions of the cortex taken from an animal of the ER saline group. Note the differential innervation pattern and density of 5-HT fibres in the respective cortical regions. Generally, layers I/II are innervated by a dense plexus of thin fibres, whereas deep layers are innervated by fewer but thicker fibres. However, note the dense innervation of thin fibres in orbital and insular cortices. Brightfield images of Nissl-stained sections (A.2–F.2) illustrate the position and delimitation of single cortical layers of *Meriones*.

ma) and then in ExtrAvidin–Peroxidase (Sigma) diluted 1:20 in TBS with 1% normal goat serum for 30 min each. Both steps were followed by a 3 × 10 min rinse in TBS. Staining procedure was performed in 0.05% 3,3-diaminobenzidine and 0.01% H_2O_2 in TBS for 4 min. The sections were then rinsed again four times. Finally, they were mounted on adhesive-coated glass slides, dried overnight, dehydrated with ethanol, cleared with xylene, and coverslipped with DePeX (Serva, Heidelberg, Germany). On additional sections, Nissl staining was used to confirm the laminar boundaries and the distribution of 5-HT fibres in single cortical layers (Fig. 1).

2.6. Data collection

Through the entire extent of the forebrain of the right hemisphere, the local densities of 5-HT-immunoreactive fibres were subjectively estimated in all animals of the ER saline group and the typical innervation pattern was graphically attributed to one of four categories (Fig. 2).

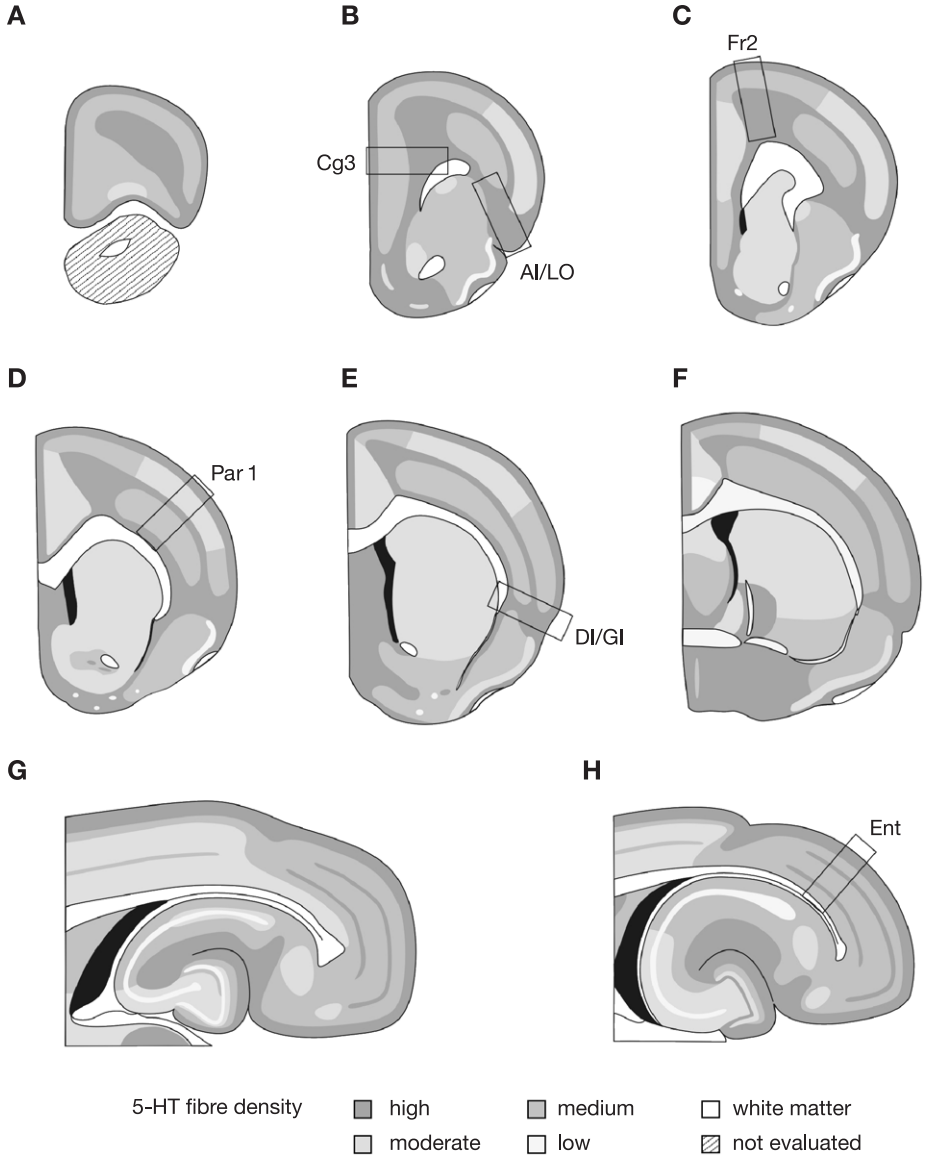

Fig. 2. Schematic drawings of the 5-HT innervation pattern in brain sections of a male gerbil of the ER saline group. Sections A–F were cut in a coronal plane, whereas G–H were cut in a horizontal plane. The identification of cortical areas follows the nomenclature of Paxinos and Watson [52]. The outline drafts were redrawn also according to the atlas of Paxinos and Watson, with appropriate changes made where necessary to meet the anatomy of the gerbil brain. The innervation pattern of 5-HT-immunoreactive fibres was subjectively estimated and assigned to four classes of density. Note the distinct organised innervation pattern of different cortical regions, e.g. prefrontal vs. parietal cortex or neocortex vs. allocortex. The highest fibre densities in the forebrain were found in ventral pallidum (B–E), in hypothalamus (F), around the hippocampal fissure (G–H), and in the basolateral nucleus of the amydala complex (not shown). Rectangles outline the areas in which the photographs of Fig. 1 were taken and in which the density of the 5-HT innervation was quantified by the use of image analysis.

For quantification of fibre densities, brain sections were chosen in cortical areas of interest by means of anatomical characteristics according to brain atlases of the rat [52] and the mouse [65]. The identification of cortical areas follows the nomenclature of the atlas of the rat [52]. The average number of analysed sections was 34 per animal, with a range of five up to eight sections in single cortical areas. In the defined cortical area of each section, all detectable fibre fragments were visualised in standard test fields (1992×1450 pixel; 0.22 mm^2) using a brightfield microscope (Polyvar, Reichert-Jung) and a digital camera for microscopy (ProgRes 3008, Jenoptik, Jena, Germany) at 200-fold magnification. Test fields were placed at the cortical surface, comprising layers I–III, and at the boundary of the white matter, mostly comprising layers V–VI. 5-HT fibres were quantified by a software for image analysis (KS300, Jenoptik). Immunoreactive fibres of different diameter were standardised to identical thickness and visualised using a combination of Gauss filter and Gerig operator that depicts differences of grey values of adjacent pixels and transforms the result into binary images. Measurements were taken of each cortical layer separately and the 5-HT fibre density was computed as percentage of the evaluated test area.

2.7. Data analysis

The measurements of the layers were computed as follows: (1) Arithmetic means by-case and by-group of all layers ± S.E.M. for each cortical area (Fig. 3). 2. Arithmetic means by-case and by-group of single layers ± S.E.M. for each cortical area (Fig. 4). Statistical analysis revealed laminar-, region-, and group-specific effects by the use of three-way multivariate analysis of variance (MANOVA) and by a post hoc analysis with Newman–Keuls test for multiple comparisons [61], both computed with Statistica 6 (StatSoft, Tulsa, USA). The levels of significance were set at $*p < 0.05$, $**p < 0.01$, and $***p < 0.001$. The measurement of 5-HT fibre density in a single cortical area, comprising all experimental groups by means of paired controls, was exclusively done by a single rater. The whole study, however, outlines data from six cortical areas that were collected by a total of four raters. To reduce probable inter-rater bias in an overview on all regional effects, the average fibre density across all layers of the ER saline group was defined to be 100 in every cortical area, from which the proportional values of single layers of the ER saline group and the other experimental groups of the same cortical area were calculated (Table 2; Figs. 3 and 4). Original mean values are additionally provided in Table 2 (column 2).

3. Results

3.1. Qualitative evaluation

The innervation pattern of 5-HT-immunoreactive fibres in the gerbil forebrain was found to be region-specific

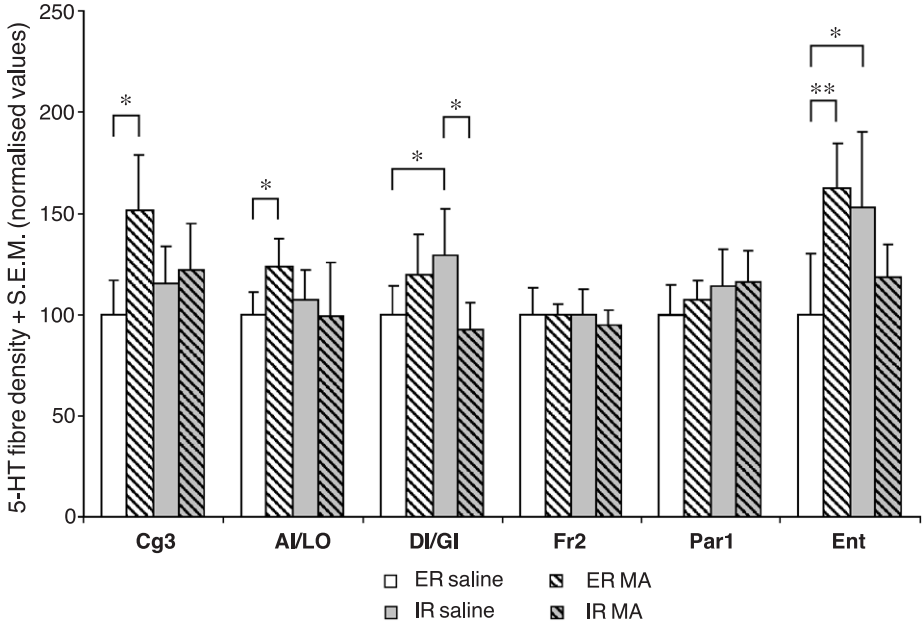

Fig. 3. Group-specific differences in cortical 5-HT fibre density across all regions and all layers, revealed by Manova. IR: Sal/MA, $F = 4.1742$, $p = 0.0422$*; ER: Sal/MA, $F = 21.7203$, $p < 0.0001$***; Sal: ER/IR, $F = 13.6738$, $p = 0.0003$***; MA: ER/IR, $F = 8.7538$, $p = 0.0034$**. Differences in regional innervation patterns were found to be highly significant throughout all experimental groups. ER, IR, Sal, MA: mPFC/oPFC/InsC/FR2/ParC/EntC, $F = 11.5264$, $p < 0.0001$***. Significant region-specific changes in response to environmental rearing conditions and early MA treatment were found, using post hoc analysis (Newman–Keuls test), in medial PFC (Cg3), orbital PFC (AI/LO), insular cortex (DI/GI), and entorhinal cortex (Ent), whereas no effect was detected in both frontal motor cortex (Fr2) and parietal somatosensory cortex (Par1). Significance values: $*p < 0.05$, $**p < 0.01$.

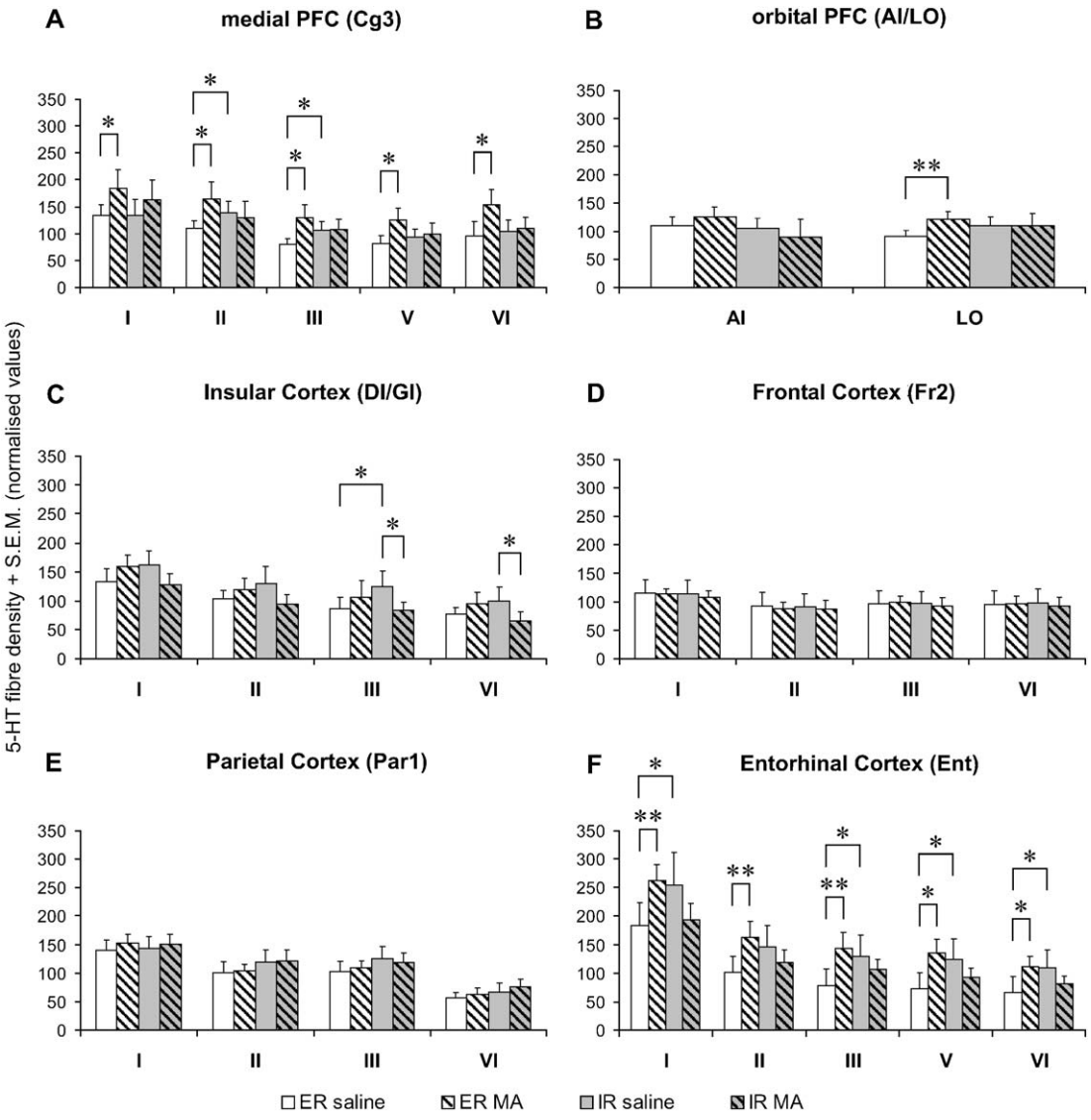

Fig. 4. Analysis of layer-specific differences in response to experimental variables using post hoc analysis (Newman–Keuls test). Consistent significant effects on laminar innervation pattern were found in medial PFC and entorhinal cortex, whereas insular cortex showed only sparse effects. Again, frontal and parietal cortices showed no reactions at all. Significance values: $*p<0.05$, $**p<0.01$.

(Figs. 1 and 2). Generally, the fibre density appeared to be higher in frontal and caudal association cortices compared with somatosensory and motor cortices. Throughout all cortical areas, layer I showed a dense fibre plexus, whereas the innervation pattern of the other layers varied between different areas. Mostly, the fibre density decreased across layers II–VI. This gradient, however, was interrupted by a band of more dense innervation in layer IV of somatosensory cortex and upper layer V of motor and association cortices. In dorsolateral cortical regions rostral to the anterior commissure, a plexus of high fibre density was found close to the white matter in deep layer VI (Fig. 2C–E). The 5-HT innervation of the PFC was consistently of high density with less difference between single layers (Fig. 2A and B). The somata of principal neurons in three-layer allocortex, namely piriform cortex and hippocampal formation, showed only sparse innervation, whereas in its molecular layers, a clearly higher fibre density was found (Fig. 2B–D,G–H). In all cortical regions of *Meriones*, both of the two types of 5-HT fibres of different morphology were found that were originally characterised in the rat brain [36].

Representative photographs of the differential 5-HT innervation densities and patterns of the six cortical areas that were subsequently studied in more detail are provided in Fig. 1, taken from a male gerbil of the ER saline group.

3.2. Overall quantification

Quantitative data were obtained from a total of 1901 sections that derived from 56 gerbils of four experimental

Table 2
Mean cortical 5-HT fibre density ± S.E.M.

	Total mean		Single layer mean (normalised)				
	Original	Normalised	I	II	III	V	VI
ER saline							
Cg3	5.15 ± 0.87	100.0 ± 16.9	134.8 ± 18.9	109.6 ± 14.5	79.4 ± 11.1	81.2 ± 15.8	95.0 ± 27.7
AI/LO	5.31 ± 0.58	100.0 ± 10.9	AI: 109.2 ± 15.6	LO: 90.8 ± 9.7	–	–	–
DI/GI	3.99 ± 0.56	100.0 ± 14.2	133.3 ± 22.5	103.5 ± 14.0	86.2 ± 20.5	–	77.0 ± 10.6
Fr2	4.05 ± 0.54	100.0 ± 13.3	115.3 ± 13.5	92.0 ± 14.2	96.7 ± 13.3	–	96.0 ± 14.0
Par1	4.01 ± 0.59	100.0 ± 14.6	139.2 ± 18.1	101.1 ± 18.3	102.9 ± 17.5	–	56.8 ± 9.3
Ent	4.36 ± 1.31	100.0 ± 30.0	183.2 ± 39.7	100.8 ± 29.0	78.6 ± 27.6	72.2 ± 29.2	65.2 ± 29.1
ER MA							
Cg3	7.82 ± 1.42	151.5 ± 27.5	183.6 ± 35.5	164.3 ± 32.3	130.3 ± 24.0	125.1 ± 22.6	154.2 ± 28.5
AI/LO	6.54 ± 0.77	123.2 ± 14.5	AI: 125.5 ± 17.3	LO: 121.0 ± 13.6	–	–	–
DI/GI	4.79 ± 0.78	120.2 ± 19.6	159.7 ± 19.9	119.4 ± 18.8	107.0 ± 27.6	–	94.6 ± 19.5
Fr2	4.04 ± 0.21	99.9 ± 5.2	114.0 ± 4.5	88.9 ± 6.1	99.9 ± 5.4	–	97.1 ± 7.1
Par1	4.31 ± 0.39	107.4 ± 9.7	152.5 ± 15.1	104.3 ± 10.2	109.6 ± 10.9	–	63.1 ± 10.5
Ent	7.10 ± 0.96	162.6 ± 21.9	261.2 ± 28.5	162.1 ± 28.1	143.6 ± 27.5	135.7 ± 23.9	110.4 ± 19.1
IR saline							
Cg3	5.94 ± 0.97	115.1 ± 18.8	133.7 ± 28.6	139.4 ± 19.4	105.6 ± 17.0	93.1 ± 16.3	103.9 ± 21.7
AI/LO	5.69 ± 0.79	107.2 ± 14.8	AI: 105.4 ± 17.0	LO: 109.1 ± 15.9	–	–	–
DI/GI	5.15 ± 0.92	129.2 ± 23.0	161.8 ± 24.4	130.3 ± 30.0	125.3 ± 25.8	–	99.3 ± 24.5
Fr2	4.04 ± 0.52	99.8 ± 12.9	113.6 ± 14.1	90.7 ± 13.0	97.0 ± 11.9	–	98.0 ± 13.7
Par1	4.57 ± 0.73	113.9 ± 18.2	143.0 ± 20.1	120.2 ± 21.3	125.4 ± 20.7	–	67.0 ± 14.9
Ent	6.66 ± 1.64	152.6 ± 37.5	255.1 ± 57.2	146.3 ± 36.8	129.1 ± 37.3	123.7 ± 35.8	108.9 ± 32.4
IR MA							
Cg3	6.29 ± 1.18	121.8 ± 22.9	162.5 ± 36.5	130.1 ± 29.6	106.7 ± 20.1	100.2 ± 18.4	109.7 ± 20.8
AI/LO	5.27 ± 1.38	99.3 ± 26.1	AI: 89.7 ± 31.5	LO: 108.9 ± 21.8	–	–	–
DI/GI	3.70 ± 0.52	92.8 ± 13.0	127.0 ± 19.5	93.9 ± 16.4	84.2 ± 13.4	–	66.0 ± 15.3
Fr2	3.85 ± 0.29	95.2 ± 7.2	108.4 ± 6.0	87.4 ± 8.4	93.1 ± 7.8	–	92.0 ± 9.3
Par1	4.65 ± 0.62	116.0 ± 15.3	150.4 ± 18.1	120.3 ± 20.9	117.8 ± 17.5	–	75.5 ± 13.4
Ent	5.17 ± 0.71	118.5 ± 16.2	193.0 ± 29.7	118.4 ± 22.5	107.4 ± 16.0	93.1 ± 15.6	80.8 ± 13.6

Abbreviations are defined in the text.

groups (Table 1). Both original and normalised mean values ± S.E.M. are provided in Table 2.

Generally, the 5-HT innervation pattern is specific to cortical areas. Statistical analysis revealed differences in regional laminar innervation pattern to be highly significant throughout all experimental groups (Fig. 3). Overall statistical analysis of the average fibre densities across all layers of the different cortical areas showed group-specific effects of both chronic environmental and single early pharmacological challenges on cortical 5-HT innervation pattern. The post hoc analysis revealed several region-specific effects: Early MA treatment of ER animals was found to cause regional overshoots of the 5-HT innervation of 51%, 23%, and 63% in the medial PFC (Cg3), orbital PFC (AI/LO), and entorhinal cortex (Ent), respectively. In contrast, MA restored the fibre density in insular cortex (DI/GI) of IR animals (28%) to ER control level. Significant effects of postnatal rearing conditions were found in DI/GI and Ent, where overshoots of the fibre densities of 29% and 53% were detected under IR conditions, respectively. In both frontal motor cortex (Fr2) and parietal somatosensory cortex (Par1), no significant alterations following MA treatment or differential environmental rearing could be detected.

3.3. Single layer quantification

Fig. 4 provides a more detailed analysis of the fibre density of single layers in the different cortical areas. In medial PFC (Fig. 4A) of ER animals, the single MA injection yielded 36–65% overshoots of the 5-HT fibre density that are significant throughout all layers. Compared with ER conditions, a fibre surplus of 26% and 34% was found in layers II and III, respectively, of IR animals. In the lateral orbital subdivision (LO) of the orbital PFC (Fig. 4B), MA treatment is correlated with a 33% overshoot of the fibre density in ER animals, whereas in the agranular insular subdivision (AI), no statistically relevant alteration was found. In layer III of the insular cortex (Fig. 4C), postnatal IR conditions produced an overshoot of 5-HT-immunoreactive fibres of 45% compared with ER conditions. The early MA challenge, however, restored adult 5-HT innervation densities in layers III and VI (− 33% each) of the IR group to a level similar to the ER saline group. The fibre density across all layers of both frontal cortex (Fig. 4D) and parietal cortex (Fig. 4E) was apparently unaffected by the experimental procedures. In contrast, following MA treatment, the entorhinal cortex (Fig. 4F) of ER animals uniformly shows

an overshoot of 5-HT fibres throughout all layers investigated, with a range of 43–89%. The highest values were found in pyramidal layers III and V (82% and 89%, respectively). Postnatal IR conditions clearly caused an overshoot production of 5-HT fibres across almost all layers that ranged from 39% to 72%, except layer II where statistical significance was missed.

4. Discussion

4.1. 5-HT innervation pattern in gerbils

Generally, the findings of the qualitative evaluation of the 5-HT fibre density in gerbils are similar to the previously described 5-HT innervation pattern of the rat [43,44,63]. Slight discrepancies occurring in several brain areas may be either species-related or due to methodical differences, e.g. depending on whether immunoreactivity of 5-HT–formaldehyde conjugate or of 5-HT transporter [68] was visualised. Additionally, the attribution of fibre densities into four classes may have been different as compared with the work of Steinbusch [63]. However, although 5-HT immunoreactivity was determined in sections of the whole forebrain of *Meriones*, the discussion will focus on innervation of the cortex.

In all cortical areas, the estimated local fibre density was found to be highest in layer I/II and in a narrow fibre plexus in upper layer V. The description revealed a laminar organisation of cortical innervation, especially in frontomedial, parietal, and entorhinal areas (Fig. 2). The influence of 5-HT on cortical neuron activity thus seems to be specific to single layers and probably to subclasses of cortical neurons. This result is in line with investigations of cortical 5-HT_2 receptor distribution [12] and physiological properties of cortical neurons responding to 5-HT application [1,2,38]. Prefrontal cortex, namely its orbital subdivision, was found to be the most densely innervated cortical region, which mirrors the results of different studies of either local receptor binding and 5-HT turnover [58] or distribution of 5-HT receptors [30,51,73] and 5-HT-related enzymes [54]. In sections comprising the barrelfield cortex, vertical columns of higher fibre density throughout cortical layers were found, which may correspond to the known size and position of individual barrels [71].

4.2. Regional specificity of experimental effects

The key finding of this study is that frontal and caudal association cortices differ from primary sensor and motor cortices in vulnerability of the maturing 5-HT innervation to both a long-term exposure of environmental factors and a single early systemic pharmacological challenge. Furthermore, distinct interaction of both experimental variables was detected.

Both frontal motor cortex and parietal somatosensory cortex did not show any adaption of the 5-HT fibre density, neither in response to an early MA challenge nor to environmental rearing conditions. Moreover, also in agranular insular cortex which was shown to have sensory, namely gustatory, properties [36], no alterations were found. On the contrary, pharmacological and environmental manipulations caused consistent reactions throughout almost all cortical layers of association cortices, namely medial PFC and entorhinal cortex.

The findings are in line with the idea of a postnatal sequence of maturational events that begins in primary sensory fields and ends in association cortices. We may speculate that according to the early maturation of sensory and motor cortices, our experimental design failed to influence the formation of the 5-HT innervation in these regions. The functional morphogenesis of association cortices, however, requires much longer periods. Particularly the maturation of the PFC continues beyond puberty in rats [66,67]. In gerbils, it was shown that the density of DA fibres in prefrontal areas still increases until early adulthood at P90, which was interpreted as being part of a prolonged maturation process of the PFC [20]. If these findings are representative, we may conclude that the vulnerability of local cortical 5-HT fibre populations to early disturbances might be related to an ongoing regional maturation of these cortical regions. In this respect, the recently demonstrated vulnerability of the 5-HT innervation of hippocampal dentate gyrus of gerbils [14] is in line with the present results, if we recognise its persistent immature status that is characterised by the unique property of lifelong production of new neurons [23].

We have to face on the other hand that alterations of the 5-HT innervation preferentially occur in neocortical areas which are also densely innervated by DA. Since we have provided evidence that, in medial PFC, both early MA treatment and restricted rearing cause a restraint of the maturation of the dopamine innervation, alter pyramidal cell morphology, increase GABAergic innervation, and affect behavioural and cognitive ontogeny of gerbils [10,21,22,48,50,70], an interrelation of these findings seems obvious. Studying long-term effects of in utero exposition to cocaine, similar findings were reported to occur in the cortex of rabbits [42]. The alterations were specific to DA-innervated cortical regions [62], namely PFC and anterior cingulate cortex. Experimental effects included altered pyramidal cell morphology and an increased number of GABA-immunoreactive cells and were correlated with a reduction of DA D_1 receptor–Gs protein coupling [25]. The similarity of the findings in both models is remarkable, considering probable species-dependent differences, pre- vs. early postnatal manipulations, and cocaine vs. MA or environmental impact. This issue raises the question whether both models may affect any fundamental mechanism that is sufficient to trigger this specific sequence of altered cortical development and maturation. The coincident

changes of the DAergic, GABAergic, and serotonergic networks that occur preferably in ''limbic'' cortical regions which are densely innervated by DA suggest that a destabilisation of the maturing DA fibres may be the primary effect of our experimental manipulations. Recent progress in the analysis of gene expression, epigenetic methylation pattern and protein interaction during development may probably help to elucidate the involved biochemical pathways.

More light has been shed on the important role of neurotransmitter interaction particularly during maturation of the brain [39,40]. Electrophysiological studies using locally applied 5-HT agonists and antagonists provided evidence for a 5-HT modulation of cortical DA terminals [17,53]. Additionally, a colocalisation of 5-HT and DA terminals on GABAergic cells could have been shown [64]. Basing on mechanisms of activity-dependent self-organisation, a subsequent distorted maturation of both DA and 5-HT fibre systems might cause a region-specific impairment of the cortical morphology and signal processing in the affected local circuits. The cortical efficiency may permanently be impaired in a region-specific manner [26], because a functional status that is coordinated with mesencephalic and thalamic activities probably cannot be sufficiently established.

4.3. Neuroplasticity of 5-HT projection systems

Wherever significant changes in response to experimental variables occurred in the present study, only overshoots of the 5-HT fibre density compared to the semi-natural ER saline group were found. It may be concluded that, in response to aberrant stimuli of very different quality, 5-HT neurons preferentially react with an overshoot production of axonal branches in different areas of the brain [11,14,24,59,74–76]. However, physiological changes of 5-HT fibres appear to be more heterogeneous, e.g. social isolation of rats enhances local 5-HT turnover in the ventral striatum [28] but attenuates 5-HT release in neocortex and hippocampus [10].

It could have been shown that neurotransmitters—including 5-HT—act as morphogenetic agents in embryonic brain [39,40]. Unlike other neurotransmitters, however, 5-HT retains this property for the most part conserved in adult life [32], which may be related to its double role as neurotransmitter and trophic factor [3]. 5-HT is believed to play a key role in structural brain development and adult neuroplasticity and is therefore termed an early organizer or differentiating factor [3]. In this respect, the region specifically altered cortical 5-HT fibre density and innervation pattern may indicate adaptive changes during maturation of other transmitter systems, probably DA, which were initially caused by external stimuli but subsequently mediated by 5-HT. The crucial role of the early 5-HT activity on cortical maturation in rats was recently demonstrated. Excessive 5-HT, due to a disruption of the MAO-A encoding gene, has been found to cause abnormal barrel formation that, exclusively in a narrow perinatally occurring critical period, could be restored to normal by an early intervention with parachlorophenylalanine, an inhibitor of 5-HT synthesis [15,16,69]. Our results show similar, albeit regionally restricted, effects of an early impact on 5-HT transmission: In both InsC and EntC, IR conditions increased adult fibre density whereas perinatally MA treatment, which causes transient damage to 5-HT fibres and terminals, restored adult 5-HT innervation of IR animals to normal. A similar effect was recently reported to occur in hippocampal dentate gyrus [14]. IR conditions may thus be thought to enhance 5-HT turnover in young gerbils, which is to be tested in further experiments.

4.4. The significance of rearing conditions

In Ent and in both medial and orbital parts of the PFC, overshoots of the 5-HT innervation following MA treatment of ER animals were found, whereas no alterations in IR animals occurred (Fig. 3). In DI/GI, a reverse impact of rearing conditions was detected. We may conclude that IR conditions affect the long-term adaptive changes that were provoked by a single early MA intoxication in these cortical areas. In addition, IR conditions alone produced an overshoot of 5-HT innervation in Ent, DI/GI, and layers II/III of Cg3. Thus, artificial environment seems to act as an aberrant stimulus leading to explicit physiological and, finally, to morphological changes during maturation of specific cortical areas. IR conditions are characterised by absence of most extrinsic stimuli that may foster the formation of sense-making neuronal networks. We believe the deficiency of multimodal sensory input to preferably impair associative areas that are involved in complex functions rather than primary sensory or motor cortices.

In a recent study, Kolb et al. [35] reported in fact that medial PFC differently reacts to environmental complexity compared with ParC and nucleus accumbens. However, in the latter regions, increases of dendritic arborisation were detected that did not occur in medial PFC, whereas dendritic spine density was increased in all regions. Given the fact that medial prefrontal pyramidal cells have proven to possess extensive plasticity in response to different manipulations [10,55], mPFC may have other ways to react to early environmental challenges, e.g. by an altered maturation of the 5-HT and DA innervation as our results suggest. Neurochemical adaptations such as altered turnover rates of both 5-HT and DA in response to environmental enrichment have already been shown in frontal cortex [9,31].

Finally, we like to comment that the ''impoverished rearing'' paradigm that is used by our laboratory is a combination of both postnatal environmental deprivation and postweaning social isolation. Therefore, the impact of IR conditions on changes in brain architecture should not be reduced to either environmental effects or a lack of social interaction, for it is seemingly a massive perturbation of activity-dependent postnatal maturation of the brain. What

we call "enriched" rearing conditions actually are normal conditions for gerbils. Any changes that we see reflect the normal state of the brain rather than some add-on effect, because we study brains that were designed by evolution to function in the wild. The present study was primarily designed to explore general features of adaptive developmental neuroplasticity rather than specific effects of social isolation or environmental complexity. However, in line with earlier statements [13,60], it becomes more and more obvious that artificial environment is a crucial factor when pharmacological and other effects are studied in animal models reared under home-cage conditions.

5. Conclusions

The present study, together with other investigations, provides evidence for a crucial role of early experience on the maturation of functional cortical networks and the seemingly important contribution of the 5-HT system. The extent of the MA-induced distortion of the 5-HT innervation is region-specific to frontal and caudal association cortices and coincides with environmental rearing conditions. Thus, investigations of early pharmacological impact on brain development should also consider probable environmental effects. Given the fact that the vast majority of pharmacological studies uses animals that were reared in standard cages, transfer of the findings to humans may result in misleading interpretation.

Psycho-motoric disturbances following MA intoxication or restricted rearing have been published by several groups. These behavioural deficits may—at least in part—be due to functional maladaption of the 5-HT innervation of specific cortical and subcortical brain regions. The present results, in conjunction with other investigations, underline that the brain organises itself in close relation to epigenetic environmental stimuli. Aberrant morphological development during early postnatal life, probably in conjunction with genetic factors, may thus predetermine individual risks for onset of a variety of neurological and psychiatric diseases during adolescence or aging.

6. Uncited references

[37]
[57]

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Postnatal Maturation of Cortical 5-HT Lateral Asymmetry is Vulnerable to Both Environmental and Pharmacological Epigenetic Challenges

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Abstract

Long-term effects of postnatal differential rearing conditions and/or early methamphetamine application on serotonin (5-HT) fibre density were investigated in several cortical areas of both hemispheres of gerbils. The aim of this study was twofold: (1) Is the 5-HT fibre innervation of the cerebral cortex lateralised, and (2) if so, do postnatal environmental conditions and/or an early drug challenge interfere with development of 5-HT cerebral asymmetries? For that purpose, male gerbils were reared either under semi-natural or restricted environmental and social conditions, under both conditions once (on postnatal day 14) being treated with either a single dose of methamphetamine (50 mg/kg, i.p.) or saline. On postnatal day 110, 5-HT fibres were immunohistochemically stained and innervation densities quantified in prefrontal cortex, insular cortex, frontal cortex, parietal cortex, and entorhinal cortex. It was found that (1) 5-HT innervation in the cerebral cortex was clearly lateralised; (2) direction and extent of this asymmetry were not uniformly distributed over the different areas investigated; (3) both early methamphetamine challenge and rearing condition differentially interfered with adult 5-HT cerebral asymmetry; (4) combining methamphetamine challenge with subsequent restricted rearing tended to reverse the effects of methamphetamine on 5-HT cerebral asymmetry in some of the cortical areas investigated; (5) significant responses in 5-HT cerebral asymmetry only occurred in prefrontal and entorhinal association cortices. The present findings suggest that the ontogenesis of cortical laterality is influenced by epigenetic factors and that disturbances of the postnatal maturation of lateralised functions may be associated with certain psychopathological behaviours.

1. Introduction

Today it is beyond dispute that in the human brain certain properties, such as handedness, language related functions, cognition, emotions and attention, are differently represented by its hemispheres and that they might feature specific anatomical asymmetries [28, 56, 74]. Also, there is growing evidence that altered or abnormal structural, neurochemical, and functional neuropsychophysiological cerebral asymmetries are involved in various forms of psychopathology, such as attention-deficit hyperactivity disorder (ADHD), depression, and schizophrenia [18, 27, 29, 31, 51, 58, 59]. However, cerebral asymmetries are characteristics which are no longer thought to be unique to the human brain. In fact, various forms of lateral cerebral asymmetries have been described in the rat and other non-human species [7, 23, 34, 57] and meanwhile some effort in trying to bridge data on human and animal cerebral laterality is in evidence [17].

It has been suggested that neurotransmission might play a major role in determining asymmetric behavioural responses in the rat [32]. In this respect, the dopaminergic (DA) system has been investigated most intensively and various forms of cerebral asymmetries have been described, comprising DA receptors, DA metabolism, and DA content in different brain areas [15, 26, 38, 43, 60, 64, 66, 67]. Thus, the participation of the DAergic system in the laterality of brain function seems to be well documented. However, as yet only few data are available describing the probable role of serotonergic (5-HT) transmission in lateral asymmetry (e.g. [1, 6]).

We have recently shown that epigenetic factors, such as rearing conditions and early methamphetamine (MA) challenge which induce several morphological reactions during postnatal brain maturation [9, 41, 44, 72] differentially interfere with postnatal development of 5-HT innervation in the cerebral cortex [42] and hippocampus [13] of gerbils. The latter revealed lateralised responses to an early dose of MA only under restricted rearing conditions in adult 5-HT fibre innervation density. The present study has been conducted to investigate whether cortical 5-HT innervation of the gerbil is lateralised and whether different postnatal rearing conditions and/or early MA challenge might differentially influence adult 5-HT innervation in the left and right hemisphere. For that purpose, animals reared under semi-natural (NAT) and restricted (RES) rearing conditions received a single dose of either MA or saline on postnatal day 14. The saline treated NAT animals served as control group compared with MA-treated animals (MET), and either saline-treated RES animals or MA-treated RES animals (RES/MET). 5-HT fibres were visualised immunohistochemically in male young adult gerbils and fibre densities were determined throughout selected areas of the prefrontal, insular, frontal, parietal, and entorhinal cortices of the left and right hemisphere using quantitative digital image analysis.

2. Materials and Methods

Animals

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 42 male pups (weight 58-77 g; age 104-114 d) were used in this study, 40 of which were also used for 5-HT quantification in dentate gyrus [13]. All animals had free access to food and water and were kept on natural day/night cycles during summer season.

Breeding and Rearing Conditions

Twenty-one gerbils were bred in standard cages (Macrolon® type 4) without any content except of sawdust, whereas 21 animals were bred in semi-naturally structured compounds (width 100 x 100 cm, height 50 cm) furnished with wooden boards and houses, plastic tubes, and stones distributed on sawdust ground. At weaning (P30) the male gerbils that were born in standard cages were assigned to RES conditions. RES animals were reared individually in standard cages (Macrolon® type 3). Male

NAT animals grew up in groups of siblings (3-5 individuals) in compounds similar to those they were born in. Both experimental groups persisted for approximately further 80 days.

Systemic Administration of Methamphetamine

On P14, a total of 20 pups received a single systemic injection of methamphetamine hydrochloride (Sigma; 50 mg/kg, i.p.). Ten and 10 of which were obtained from semi-natural breeding and standard breeding and were termed MET and RES/MET, respectively. Twenty-two pups, 11 from each breeding condition, were sham-treated by an i.p. injection of saline.

Preparation of Tissue

Animals were transcardically perfused under deep chloral hydrate anesthesia (1.7 g/kg, i.p.). The perfusion was performed with 100 ml of 0.1 M phosphate buffer (room temperature, pH 7.2), followed by 500 ml of freshly prepared 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4). Immediately after perfusion, the brains were removed and post-fixed for 2 hours at 4 °C. To detect probably lateralised cortical 5-HT innervation densities, right and left hemispheres were separated. Either hemisphere was divided at the rostral edge of the hippocampal formation. Coronal sections (20 µm thick) of the anterior part were taken on a frigocut (Reichert-Jung, Vienna, Austria) and every third section was collected in ice-cold 0.1 M phosphate-buffered saline (PBS, pH 7.4). The posterior part of either hemisphere, including hippocampus and entorhinal cortex, was cut in a horizontal plane but apart from that treated identically.

Immunohistochemistry

For immunostaining of 5-HT fibres the sections were rinsed 3x10 min in phosphate-buffered saline (PBS), incubated for 10 min with 1% H_2O_2 to reduce background staining, and rinsed again thrice in PBS for 10 min. Following 30 min of pre-incubation in 10% normal goat serum in PBS containing 0.3% Triton X100, the sections were incubated in polyclonal rabbit anti-serotonin serum (DiaSorin, Stillwater, USA), diluted 1:20,000 in PBS with 1% normal goat serum and 0.3% Triton X100 for 18 h at 4 °C. For the next procedures 0.05 M tris-buffered saline (TBS; pH 7.6) was used. The sections were first incubated in biotinylated goat anti-rabbit serum (Sigma) and then in ExtrAvidin-Peroxidase (Sigma) diluted 1:20 in TBS with 1% normal goat-serum for 30 min each. Both steps were followed by a 3x10 min rinse in TBS. Staining procedure was performed in 0.05% 3,3-diaminobenzidine and 0.01% H_2O_2 in TBS for 4 min. The sections were then rinsed again four times. Finally they were mounted on adhesive coated glass-slides, dried overnight, dehydrated with ethanol, cleared with xylene, and cover-slipped with DePeX (Serva, Heidelberg, Germany). On additional sections Nissl staining was used to confirm the cortical architecture.

Data collection

For quantification of fibre densities, brain sections were chosen in cortical areas of interest by means of anatomical characteristics according to brain atlases of the rat (Paxinos & Watson, 1986) and the mouse [70]. The identification of cortical areas followed the nomenclature of the atlas of the rat brain [48]. The average number of analysed sections was 34 per animal in each hemisphere, with a range of 5 up to 8 sections in single cortical areas. In the defined cortical area of each section all detectable fibre fragments were visualised in standard test fields (1992 x 1450 pixels; 0.22 mm^2) using a bright-field microscope (Polyvar, Reichert-Jung, Vienna, Austria) and a digital camera for microscopy (ProgRes 3008, Jenoptik, Jena, Germany) at 200-fold magnification. Equal numbers of test fields were placed at the cortical surface, comprising layers I-III, and at the boundary of the white matter, comprising layers V-VI. 5-HT fibres were quantified by software for image analysis (KS300, Jenoptik, Jena, Germany). To detect the fibre density, not amount of fibres, immunoreactive fibres of different diameter were standardised to identical thickness and visualised using a valleys operator that depicts local differences of grey values of adjacent pixels, but not a general threshold, and transforms the results into binary images. The 5-HT fibre density was subsequently computed as a percentage of the evaluated test area.

Data Analysis

The data were computed as arithmetic means by-case and by-group ± S.E.M. for each cortical area. Statistical analysis checked region-, hemisphere-, pharmacological-, and rearing-specific effects by the use of 4-way analysis of variance (ANOVA) and by a *post-hoc* analysis with Newman-Keuls test for multiple comparisons [63], both computed with Statistica 5.5 (StatSoft, Tulsa, USA). The levels of significance were set at * $p<0.05$, ** $p<0.01$, and *** $p<0.001$. The measurement of 5-HT fibre density in a single cortical area, comprising all experimental groups by means of paired controls, was exclusively done by a single rater. The whole study, however, outlines data from five cortical areas that were collected by a total of four raters. To reduce probable inter-rater bias in an overview on all regional effects, the average fibre density of the right hemisphere of the NAT group was defined to be 100 in every cortical area, from which the proportional values of the left hemisphere of the NAT group and both hemispheres of the other experimental groups of the same cortical area were calculated (Fig. 1, Table 1).

3. Results

Following ANOVA and the *post-hoc* analysis with Newman-Keuls test, the effects of both experimental variables, rearing condition and pharmacological impact, are region-specific and to some degree hemisphere-specific. Compared with NAT animals, which serve as controls, significant alterations only occur in right PFC and preferably in left EC, whereas IC, FC and PC show no effect (Fig. 1). 5-HT fibre densities are increased in MET animals in right PFC (+49%, $p=0.0092$), left EC (+44%, $p=0.0302$) and right EC (+58%, $p=0.0291$). RES animals show increased fibre densities in both left (+36%, $p=0.0333$) and right (+49 %, $p=0.0469$) EC. RES/MET

conditions cause an increase of the fibre density selectively in left EC, compared with both NAT conditions (+84%, p=0.0002) and RES conditions (+35%, p=0.0159). Fig. 1 additionally provides representative images, taken from a NAT animal, indicating the laterality of 5-HT fibre densities in all cortical areas except of PC. There has been recently given some qualitative evaluation and explanation of general features of the cerebral 5-HT innervation pattern of gerbils in more detail [42].

According to ANOVA, as a main effect, the overall 5-HT innervation of the cortex is clearly asymmetric in NAT animals (F=35.6353, p<0.0001). The *post-hoc* analysis revealed that 5-HT fibre density is significantly higher in the right hemisphere as compared to the left hemisphere in frontal areas, namely PFC, IC and FC, the extent being +109%, +79% and +80%, respectively. However, in PC no significant difference in 5-HT fibre density between both hemispheres occurs, whereas the EC is characterised by a significant left greater than right 5-HT cerebral asymmetry of +51% (Fig. 2 NAT, Table 1). The MET group show slightly reduced 5-HT asymmetries in all areas investigated, but a significant augmentation in the 5-HT right greater than left asymmetry in the PFC of +38% (p=0.0108). Analysis of RES animals generally reveals some slight but insignificant reduction in 5-HT cerebral asymmetry of all areas investigated. The combination of both environmental and pharmacological impact in RES/MET animals leads to a slightly but not significantly reduced 5-HT asymmetry in the PFC, as opposed to the above mentioned significant 38% increase in the MET group and further to non-significant reductions of 5-HT asymmetry in the IC and PC. These responses implement loss of lateral asymmetry in the IC (Table 1). However, in FC almost no alteration could be detected, whereas left greater than right cerebral asymmetry is significantly augmented in the EC (+87%, p<0.0001), as opposed to the above mentioned decrease in the MET group.

Table 1: Comparison of left vs. right cortical 5-HT fibre density ± S.E.M.

		NAT (n=11)	MET (n=10)	RES (n=11)	RES/MET (n=10)
PFC	left	47.80 ± 3.00	59.97 ± 2.34	59.40 ± 4.63	64.72 ± 8.08
	right	100 ± 8.21	148.51 ± 11.67	114.41 ± 8.96	121.39 ± 11.93
		p=0.0003 ***	p=0.0002 ***	p=0.0002 ***	p=0.0013 **
IC	left	55.90 ± 4.61	79.43 ± 14.81	80.58 ± 11.23	62.73 ± 10.23
	right	100 ± 6.89	120.56 ± 10.90	129.93 ± 12.78	92.67 ± 7.38
		p=0.0002 ***	p=0.0015 **	p=0.0035 **	p=0.1046 n.s.
FC	left	55.48 ± 3.03	63.67 ± 2.69	60.52 ± 3.51	51.58 ± 2.06
	right	100 ± 6.49	99.54 ± 2.18	95.78 ± 5.71	95.48 ± 3.43
		p=0.0002 ***	p=0.0002 ***	p=0.0002 ***	p=0.0002 ***
PC	left	108.51 ± 10.68	97.49 ± 5.00	122.24 ± 12.71	106.07 ± 7.39
	right	100 ± 7.34	107.47 ± 4.99	113.82 ± 7.75	114.09 ± 9.87
		p=0.2235 n.s.	p=0.1485 n.s.	p=0.3169 n.s.	p=0.1078 n.s.
EC	left	150.83 ± 12.50	216.69 ± 16.76	205.61 ± 17.81	277.99 ± 22.38
	right	100 ± 15.06	158.46 ± 10.12	149.49 ± 19.17	116.78 ± 7.78
		p=0.0002 ***	p=0.0123 *	p=0.0062 **	p=0.0002 ***

ANOVA and *post-hoc* Newman-Keuls test.

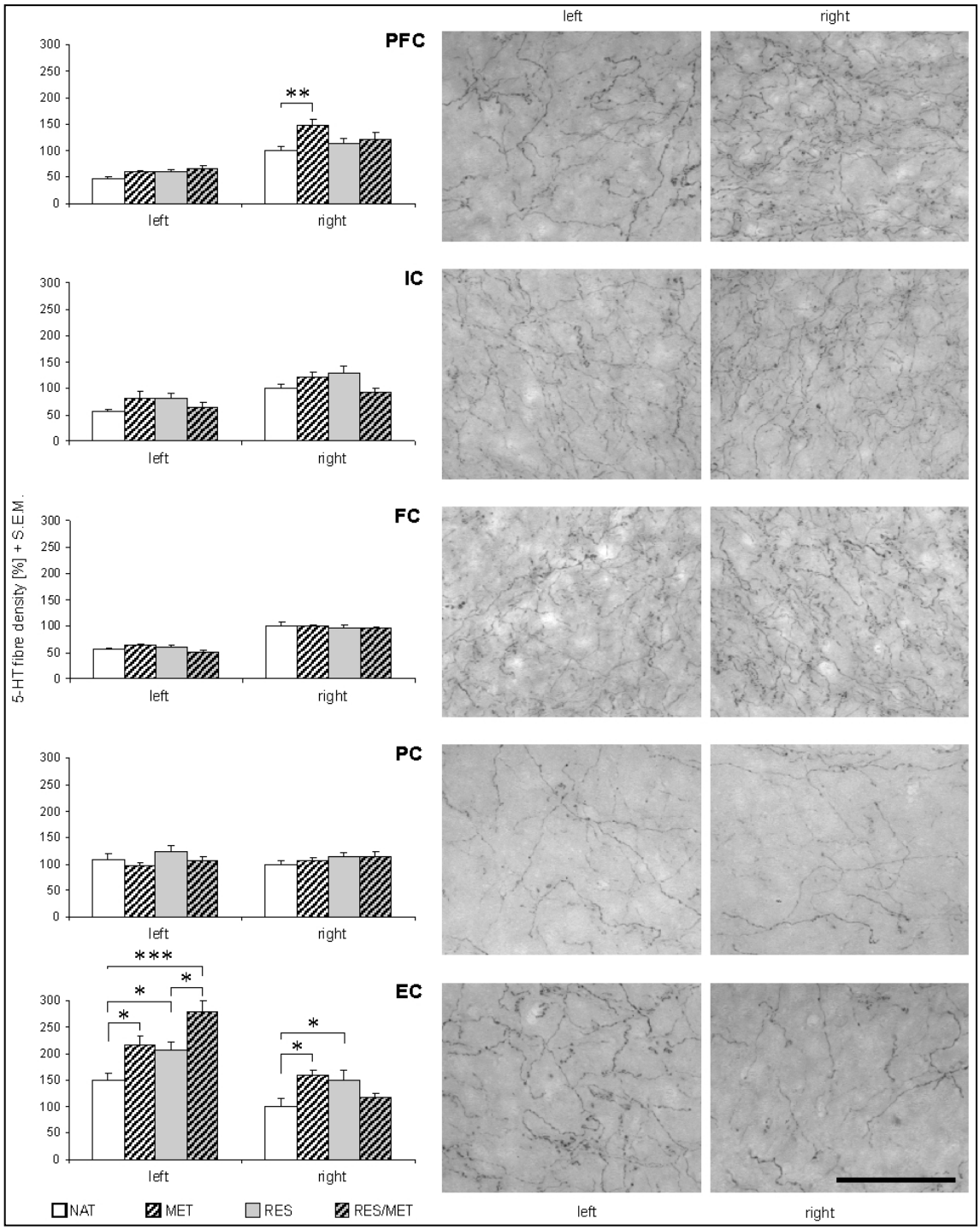

Fig.1. Standardised 5-HT innervation density ± S.E.M. is presented in five cortical areas of both cerebral hemispheres. Significant region-specific changes in response to environmental rearing conditions and early methamphetamine treatment were found in the right hemisphere of the prefrontal cortex (PFC) and in both hemispheres of the entorhinal cortex (EC). No effect was detected in insular cortex (IC), frontal cortex (FC), and parietal cortex (PC). ANOVA and *post-hoc* Newman-Keuls test; significance values: * $p<0.05$, ** $p<0.01$, *** $p<0.001$. Photomicrographs represent samples of 5-HT immunostained cortical fibres taken from a saline-treated animal reared under semi-natural conditions (NAT group). Scale bar = 50µm.

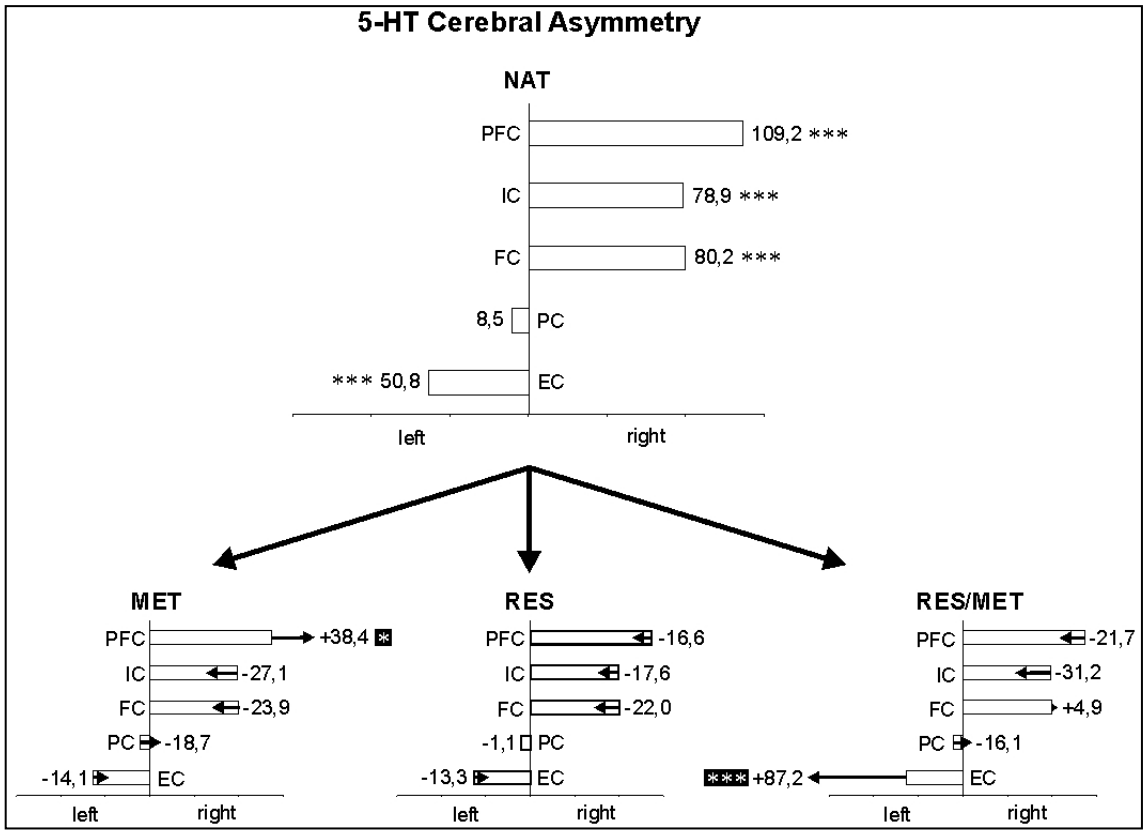

Fig.2. The extent and significance (black asterisks) of 5-HT asymmetry in five cortical areas of NAT animals (saline-treated semi-naturally reared) is shown by means of percent over-plus of the more densely innervated hemisphere. In frontal areas like prefrontal cortex (PFC), insular cortex (IC), and frontal cortex (FC) 5-HT innervation is denser in the right hemisphere. Parietal cortex (PC) shows no significant asymmetry, whereas entorhinal cortex (EC) 5-HT innervation is more pronounced in the left hemisphere (top). MET animals (MA-treated semi-naturally reared) show slightly reduced asymmetry in all areas except of a significant (white asterisk) increase of asymmetry in the PFC. RES animals (saline-treated restrictively reared) show a general but not significant reduced asymmetry in all areas except of PC. RES/MET animals (MA-treated restrictively reared) show reduced asymmetry in PFC, IC and PC. However, in FC almost no alteration was found, whereas the asymmetry of the 5-HT innervation is significantly increased in EC (white asterisks). ANOVA and *post-hoc* Newman-Keuls test, significance values: * $p<0.05$, ** $p<0.01$, *** $p<0.001$.

4. Discussion

In the present study, we investigated whether 5-HT fibre innervation is lateralised in different areas of the cerebral cortex and whether postnatal rearing conditions and/or an early MA drug challenge interfere with postnatal development of 5-HT cerebral asymmetry. From data presented in this study, we may conclude that (1) 5-HT fibre innervation in the cerebral cortex of male gerbils is lateralised; (2) as to different cortical areas, there is no uniform right greater than left asymmetry or vice versa; (3) postnatal development of 5-HT cerebral asymmetry interferes with both rearing conditions and early MA-challenging; (4) combining restricted rearing with early MA-challenge reverses some of the effects of the drug occurring under semi-natural rearing conditions; (5) significant responses in 5-HT cerebral asymmetry only occur in prefrontal and entorhinal association cortices.

Currently, it is well accepted that various structural and functional properties of the brain are lateralised, being more or less unequally distributed between the left and right hemispheres. Subsequent to Broca's first report on this issue [11] it had been argued for a long period of time

that cerebral asymmetries should be a phenomenon unique to the human brain. Originally, this was particularly stressed for language related functions [55] and hand preference [50], associated with conspicuous neuroanatomical asymmetries in the planum temporale and inferior frontal gyrus of the human brain [51]. In this context, the left hemisphere of the human brain is generally believed to be preferably concerned with some aspects of speech production and language, while the right hemisphere should be in charge of functions, such as emotion and spatial cognition [62]. Also, there is considerable evidence to argue that lateralised brain functions are already present early in live influencing postnatal development of experience-dependent characteristics of the hemispheres [69].

As yet, various forms of lateral cerebral asymmetry have been detected and analysed in studies both with human and non-human subjects, which consider areas investigated in the present study, comprising the prefrontal cortex (human: [45]; rat: [10, 14, 65]), insular cortex (human: [5, 39, 46]; rat: [4]; monkey: [73]), motor cortex (human: [74]; rat: [25]), somato-sensory cortex (human: [33, 40, 61]; rat: [19, 54, 68]), and entorhinal cortex (human: [30]; rat: [37]). Unfortunately, the 5-HT system has been widely left unconsidered in these investigations. However, a few recent studies have been focused on lateralised characteristics of some 5-HT properties in the human frontal cortex: In postmortem neurochemical investigations of the human brain a clear inter-hemispheric asymmetry has been found in the medio-frontal region, indicating higher 5-HT turnover rates in the right hemisphere (1). A relative increase and asymmetry of 5-HT receptors could be demonstrated in depressed patients using SPECT imaging, revealing a right greater than left asymmetry in the inferior frontal region [24]. A comparable left-right asymmetry has been found in the frontal cortex of patients with anorexia nervosa, with 5-HT_{2A} binding being significantly reduced in the left frontal cortex when compared with normal volunteers (Audenaert et al., 2003). Using PET imaging, decreased 5-HT synthesis has been found in either the left or right frontal cortex of autistic boys, with 5-HT synthesis being elevated in the contralateral dentate nucleus [16]. The number of imipramine binding sites, being closely associated with the 5-HT uptake sides, in the frontal cortex of healthy volunteers was significantly higher in the right hemisphere than in the left hemisphere. Inversely, in post-mortem brains of drug-free psychiatric subject homicide victims the number of imipramine binding sites was significantly higher in the left hemispere [22]. Therefore, it might be assumed that a disturbed or inverse asymmetry of some 5-HT mechanisms may play a role in psychiatric disorders. However, a further study could not confirm any hemispheric asymmetry of 5-HT uptake sites in post-mortem brains from suicide victims and controls [2].

In experimental animals, several alterations in 5-HT systems have been described after various forms of early drug challenging [12, 36, 49], stress [8, 35, 71], and social deprivation [42, 52]. From these studies, it might be concluded that both environmental conditions and drugs may significantly interfere with postnatal development of brain 5-HT system's structure and function. However, although the brain is known to be asymmetrically organised in animals as well as

humans, as yet there is only little evidence for lateralised 5-HT functions in experimental animals. For instance, it has been found that a systemic immune challenge in adult mice induced asymmetrical changes in brain activity, with increased 5-HT metabolism in the left hypothalamus and the left hippocampus [21]. Further, behavioural responses of the rat appeared to be lateralised to unilateral and bilateral injections of the selective 5-HT_{1A} receptor agonist 8-OH-DPAT into the hippocampal CA1 area, with a left biased reactive locomotion activity and a right biased learning and memory impairment in the shuttle box (6). From these results we may assume that 5-HT_{1A} receptors are differentially distributed in the hippocampal CA1 areas of the left and right hemispheres.

We have recently investigated postnatal development of 5-HT fibre innervation in the hippocampal dentate gyrus of gerbils reared under physically and socially restricted versus semi-naturally enriched environmental conditions [13]. As a major result, restricted rearing caused a significant augmentation of adult 5-HT fibre densities in certain layers of the dentate gyrus selectively in the temporal plane of either hemisphere. Under semi-natural rearing conditions, comparable 5-HT fibre densities could be detected in adults following a single early MA-challenge to young gerbils, whereas under restricted rearing conditions this effect was biased to the right hemisphere. Further, evidence has been provided that 5-HT fibres projecting to the rat medial PFC modulate cortico-cortical interhemispheric transmission by attenuating depolarising synaptic potentials [53]. Together with differentially developed 5-HT input from neurons in the medial raphe nucleus, the latter might be assumed a probable means in realising 5-HT induced lateralised behavioural responses. In previous studies we have found that both restricted rearing and an early MA-challenge may cause severe deficits in PFC-related behaviours, such as working memory and open field behaviour [20, 72]. In humans certain schizophrenic symptoms seem to be related to impairment of working memory [47]. Thus, the present results may provide a clue to probable associations between certain psychopathological behaviours and lateralised brain structure and function which should be investigated in more detail.

From the present data we may conclude that both rearing condition and early MA application differentially interfere with postnatal development of 5-HT innervation in different areas of the cerebral cortex of gerbils. Further, we may assume that epigenetic factors might be important candidates in controlling development of adult lateralised brain functions and behaviour. Notably, because of the significant role of 5-HT systems in various forms of psychopathology, there is an outstanding need for future research to further evaluate the part of 5-HT in orchestrating lateralised brain function in concert with other neurotransmitters.

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Eigene Arbeiten

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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, Juni 2004

(Andrea Busche)