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# **Anatomische "Dyskonnektion" des präfrontalen Kortex: ein Tiermodell zur Schizophrenie**

Quantitative Tracer- und immunhistochemische Studien  
zu Glutamat und Serotonin bei *Meriones unguiculatus*

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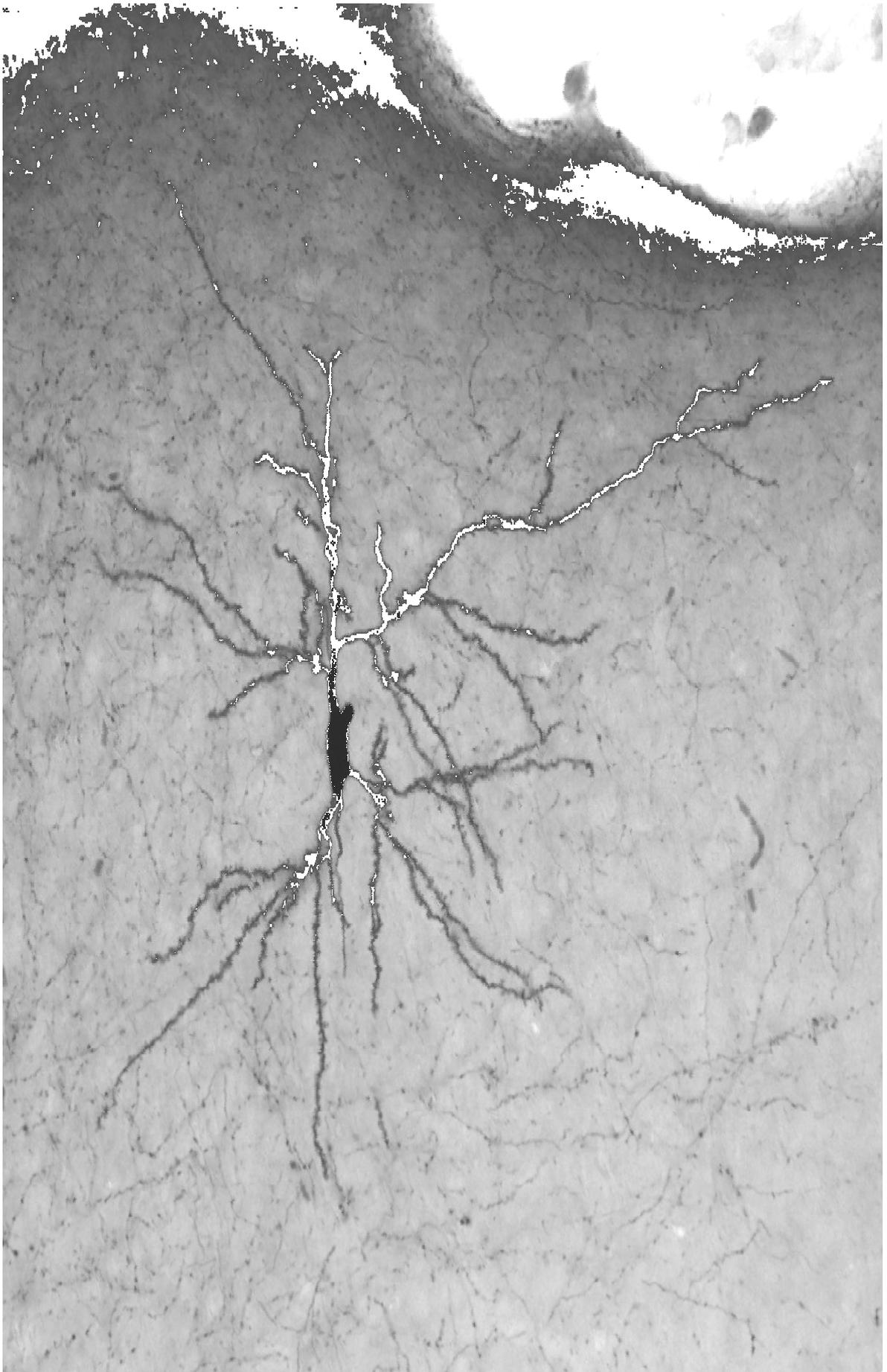


Foto: mit Biocitin markierte Pyramidenzelle in der Lamina III des frontalen Kortex (Fr2), farblich verändert.

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# 1. Zusammenfassung

Eine Balance verschiedener Transmitter-Interaktionen ist unabdingbar, um im Gehirn homöostatische Zustände zu erreichen. Für die Schizophrenie wurde erst in den letzten Jahren erkannt, dass die Ursachen der Störung in einer *Transmitterimbalance* und einer funktionellen *Diskonnektion* zwischen verschiedenen Hirnarealen zu suchen ist.

Das in unserer Arbeitsgruppe entwickelte nicht-invasive Tiermodell bezieht sich auf (1) eine restriktive Isolationsaufzucht und (2) eine einmalige frühkindliche Intoxikation mit Methamphetamin. Diese zwei Interventionen verursachen eine Störung der *Transmitterreifung*. Durch mehrere Untersuchungen konnten wir bereits eine Imbalance des *dopaminergen* Systems nachweisen. Die vorliegende Studie befasst sich mit den Auswirkungen der zwei nicht-invasiven Interventionen auf das *glutamaterge* und *serotonerge* System. Störungen der Interaktionen des präfrontalen Kortex (PFC) mit anderen kortikalen Arealen (FC, PC und IC) konnten in Form einer "Dyskonnektion" glutamaterger U-Fasersysteme gezeigt werden.

Die in drei publizierten Arbeiten erwirtschafteten Befunde werden in der vorliegenden Schrift zusammenfassend vorgestellt und diskutiert:

- (1) Jeweils eine der beiden Interventionen führt zur *gleichmäßigen Reduzierung* der glutamatergen Efferenzen des PFC in die untersuchten Kolumnen des Frontalkortex (FC), Parietalkortex (PC) und insulären Kortex (IC).
- (2) Gemeinsam veranlassen die beiden Interventionen *isolierte Aufzuchtbedingung* und *Methamphetamin-Intoxikation* eine "Dyskonnektion" des präfrontalen Kortex zum FC, PC und IC. Darin kommt eine Imbalance der präfrontalen U-Fasersysteme zwischen Projektionen der Lamina III-Pyramiden und der Laminae V/VI-Pyramiden zum Ausdruck.
- (3) Die serotonergen Faserdichtenmessungen bestätigen erneut die hohe Plastizität dieses Transmitters. Im Gegensatz zum präfrontalen und entorhinalen Kortex, in denen ebenfalls adaptive Faserveränderungen festzustellen sind, reguliert sich die serotonerge Faserdichte trotz der einzelnen Interventionen oder der Kombination beider Interventionen im FC, PC und IC auf einen "Standardlevel" ein.
- (4) Eine zusätzliche Untersuchung zeigt, dass dieser "Standardlevel" einer annualen Rhythmik unterliegt und im Winter höher ist als im Sommer.

Die Befunde lassen folgende Interpretation zu:

- zu (1) Die glutamaterge Faserdichte kann sich nicht plastisch an die Interventionen anpassen, sondern reagiert mit dauerhaften Strukturveränderungen. Eine gleichmäßige Reduzierung der Faserdichten nach jeweils einer Intervention spiegelt eine angepasste Entwicklung wider, die sich möglicherweise als eine höhere *Vulnerabilität* des ganzen Systems und somit als ein erster Schritt in die Psychose darstellt.
- zu (2) Die "Dyskonnektion" lässt sich gleichermaßen für mehrere unimodale Kortexareale feststellen und belegt somit, dass in Erweiterung des Modells zum Diskonnektionssyndrom von Mesulam (Mesulam, 2000) mehrere präfrontal-gesteuerte Befindlichkeitskontexte in der Schizophrenie gestört sein könnten.
- zu (3) Das serotonerge System besitzt eine hohe plastische Fähigkeit, mit der die induzierten Störungen präfrontaler und limbischer Areale derart kompensiert werden, dass sich auf Strukturebene im FC, PC und IC keine Faserdichteveränderungen ausbilden.
- zu (4) Diese hohe plastische Fähigkeit zeigt sich auch in der annualen Rhythmik. Die serotonerge Faserdichte ändert sich zwar nach äußeren jahreszeitlichen Einflüssen, diese sind jedoch nicht adaptiv an veränderte Transmitterdichten von Dopamin und Glutamat angepasst. Mögliche daraus resultierende Folgen für psychotische Störungen wie z.B. Winterdepressionen oder affektive Störungen werden in der Literatur zurzeit kontrovers diskutiert.

Die aufgrund von vielen Untersuchungen mit bildgebenden Verfahren postulierte Grundlage der schizophrenen Störung konnte mit Hilfe der Tracermethode hier erstmalig dokumentiert werden: die Anatomie der "Dyskonnektion".

## 2. Einleitung

Das *Vulnerabilitäts-Stress-Modell* (siehe Kapitel 3.1) bietet ein derzeit gutes und anerkanntes Modell zur Ätiologie der schizophrenen Störung. Eine neuronale Fehlentwicklung durch schädigende Faktoren während der Reifung der Nervennetze verursacht eine Prädisposition zur Schizophrenie, und erst weitere Faktoren wie z.B. erheblicher Stress lassen die schizophrene Störung über die Manifestationsschwelle treten, was einen Ausbruch der Symptome erzeugt. Vor dem Hintergrund dieses Tiermodells wurde aus unserer Arbeitsgruppe dazu in jüngster Zeit ein Review verfasst (Lehmann und Teuchert-Noodt, 2005). Vulnerabilität und Stress werden somit als zentrale komplementäre ätiopathogenetische Faktoren bei der Krankheitsmanifestation aufgefasst (siehe Abb. 2). Die Symptome der Schizophrenie lassen darauf schließen, dass der *präfrontale Kortex* (PFC) als höchstes assoziatives Integrationszentrum maßgeblich betroffen ist (Weinberger, 1987; Weinberger et al., 1988; Goldman-Rakic, 1994a, 1994b; Goldman-Rakic und Selemon, 1997). Drei Transmittersysteme standen in den vergangenen 50 Jahren im Fokus der verursachenden Faktoren: so entstanden die Dopaminhypothese (seit: Van Rossum, 1966), die Serotoninhypothese (seit: Woolley und Shaw, 1954) und die Glutamathypothese (seit: Luby et al., 1962). Mittlerweile wird deutlich, dass ein reines Zuviel oder Zuwenig eines einzelnen Transmitters das Krankheitsbild nicht erklärt. Vielmehr geht man heute von einem Ungleichgewicht zwischen den sich gegenseitig beeinflussenden Transmittersystemen bzw. einem Ungleichgewicht durch Fehlverschaltung zwischen einzelnen funktionellen Hirnarealen aus, um Hauptaspekte der schizophrenen Störung zu erklären.

Das seit nunmehr 20 Jahren in unserem Labor etablierte Tiermodell zur Psychoseforschung hat es ermöglicht, mit Hilfe quantitativer Bildanalyseverfahren die Transmitter Dopamin (DA), Serotonin (5-HT), GABA und Acetylcholin (ACh) in verschiedenen Arealen des Gehirns und speziell im *limbo-präfrontalen System* zu analysieren (Busche et al., 2005, 2004, 2002; Dawirs und Teuchert-Noodt, 2001; Dawirs et al., 1994, 1993; Lehmann et al., 2004, 2003, 2002; Lesting et al., 2005; Neddens et al., 2004, 2003, 2002, 2001; Nossoll et al., 1997; Winterfeld et al., 1998). Die gewonnenen Daten belegen ganz wesentliche Aspekte der aus der klinischen Forschung stammenden Erkenntnisse zur Schizophrenie. Bildgebende Verfahren können bislang allerdings ein wesentliches Problem der schizophrenen Störung nicht klären, und das betrifft die Frage, ob bei schizophrenen Patienten eine Überaktivierung des PFC (=Hyperfrontalität) oder

eine reduzierte Funktion des PFC (=Hypofrontalität) vorliegt (Callicott et al., 2003), bzw. ob ein hyper- oder ein hypoglutamaterger Level im PFC die Störung unterhält (Tsapakis und Travis, 2002). Deswegen war es ein Ziel meiner Arbeit, die schon vorliegenden Daten der Transmitterdichten in unserem Tiermodell durch Untersuchungen zu Glutamat und Serotonin zu vervollständigen. Der Schwerpunkt meiner Studien lag dabei auf Glutamat. Über Tracerstudien sollten die *U-Fasersysteme* mit Ursprung im Präfrontalkortex zum Frontalkortex (FC), Parietalkortex (PC) und insulären Kortex (IC) untersucht und quantitativ bewertet werden.

Unserem Tiermodell liegen folgende Kriterien zugrunde: der "erste Hit" dieses "*Two-Hit-Modells*", die Schädigung der neuronalen Entwicklung, wird durch eine *pharmakologische nicht-invasive Intervention* am postnatalen Tag 14 (P14) erzeugt. Die dadurch verursachte Schädigung bewirkt eine *suppressive Reifung* der gesamten dopaminergen Projektion in den präfrontalen Kortex (Dawirs et al., 1994) und Nucleus accumbens (Neddens et al., 2002) und dadurch bedingte adaptive Gleichgewichtsstörungen weiterer Transmitter. Der "zweite Hit" wird durch *massiven chronischen Stress* verursacht, den wir durch eine *isolierte Aufzuchtbedingung* erzeugen. Diese allein bewirkt auch eine *suppressive Reifung* von Dopamin in den präfrontalen Kortex (Winterfeld et al., 1998) und führt zu adaptiven Veränderungen sowie weiteren Strukturanpassungen in Schaltkreisen des limbo-präfrontalen Systems (Teuchert-Noodt, 2000). Auch andere Studien zum sogenannten "*social isolation syndrom*" belegen den Einfluss von Haltung und damit verbundenem Stress auf die Transmitterumsätze insbesondere von Dopamin und Serotonin (Hall, 1998; Heidbreder et al., 2000; Lapiz et al., 2003). Vor dem Hintergrund der beiden Störgrößen, (1) der Fehlsteuerung in der postnatalen Entwicklung durch pharmakologische Intervention, und (2) dem chronischen Stress durch Isolationsaufzucht, bietet unser Tiermodell ideale Voraussetzungen zur Schizophrenieforschung, und es war zu hoffen, dass die offenen Probleme des Glutamatstoffwechsels daran geklärt werden könnten.

## ***2.1 Zur Schizophrenie als einer Transmitterstörung***

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Der Psychiater Emil Kraepelin fasste schon 1896 unter den Namen "*Dementia praecox*" verschiedene Krankheitskomponenten, die "*Dementia hebephrenia*", "*Dementia catatonica*" und "*Dementia paranoides*", zu einer Krankheitseinheit zusammen, die von Eugen Bleuler

1911 in *Schizophrenie* umbenannt wurde und heute noch so bezeichnet wird. Ursachen dieser Störung sind pathologische Prozesse, die sich insbesondere im *limbischen* und *präfrontalen Kortex* abspielen (Beckmann et al., 1988). Die für die Diagnose der einzelnen Schizophrenietypen (WHO, 1992; APA, 1991) heute eingesetzte Klassifizierung der Symptome zeigt deutlich, dass die Krankheit Schizophrenie keine nosologische Einheit darstellt, sondern vielmehr als Syndrom betrachtet werden sollte, das klinisch drei Subsyndrome kognitiver Funktionsstörungen umfasst: psychomotorische Verarmung, Desorganisation und Derealisation von Denken. Das heterogene Symptombild wird danach in zwei Hauptklassen unterteilt: die produktiven, Plus- oder Positivsymptome und die Defekt-, Minus- oder Negativsymptome (Andreasen, 1983, 1984). Zu den Positivsymptomen zählen alle Arten von *Halluzinationen* (Wahrnehmungen ohne adäquaten Sinnesreiz von außen), *inhaltliche Denkstörungen* (Wahnvorstellungen und Paranoia), *positive formale Denkstörungen* (Inkohärenz, Desorganisation oder Verlust des Kommunikationsziels durch Paralogik, Konkretismus, Neologismen, Schizophasie, etc.) und *bizarres Verhalten* durch so genannte Ich-Störungen (Autismus, Depersonalisation, Derealisation, Gedankeneingebung oder Gedankenentzug). Die Negativsymptome umfassen *Affektverflachung und Affektstarrheit* (Verminderung bzw. Verlust des Gefühls- und Gemütslebens), *Alogie und Paralogie* (kommunikative Störung durch Sprachverarmung und Verlängerung der Antwortlatenz, bzw. durch krankhaft falschen Gebrauch von Wörtern), *Abulie und Apathie* (Mangel an Willen, Initiative oder Energie, bzw. eine krankhafte Gleichgültigkeit, Teilnahmslosigkeit, Nicht-Ansprechbarkeit), *Anhedonie und Asozialität* (Unfähigkeit, Vergnügen oder Freude zu empfinden, bzw. eingeschränkte oder fehlende Konfliktfähigkeit und dadurch Mangel an sozialen Interaktionen) sowie *Aufmerksamkeitsdefizite* (Verlust der selektiven und inhibitorischen Funktion der Aufmerksamkeit) (Andreasen et al., 1995).

Als ursächlich für diese Symptome verantwortlich wurden verschiedene Neurotransmitter genannt, wie aus einer Vielzahl klinischer Beobachtungen zur pharmakologischen Wirkung von Drogen abgeleitet werden konnte. Auch wenn die frühen Monotransmitterhypothesen nur bestimmte Aspekte der schizophrenen Störung berücksichtigt haben und sie folglich in vielen Zusammenhängen unzureichend waren, möchte ich die wichtigsten von ihnen kurz aufführen, da sie doch die Grundlagen der darauf aufbauenden Betrachtungen zu den diskreten Störungen von *Transmittergleichgewichten* bilden:

Die *Serotonin-Hypothese* beruht auf Studien über die Auswirkungen psychedelischer Halluzinogene aus der Klasse der Indol-Derivate (z.B. LSD, "*lysergic acid diethylamide*") und Phenethylamine (z.B. Mescaline) auf die Serotonin-Rezeptoren. Die Effekte von Halluzinogenen auf komplexe Prozesse wie Kognition, Perzeption und der allgemeinen Grundstimmung lassen eine Einbindung des Kortex in ihrem Wirkungskreis vermuten. Der direkte postsynaptische Effekt von Serotonin im Kortex ist unterschiedlich. Er kann depolarisierend oder hyperpolarisierend sein oder aber auch keine direkte Wirkung zeigen. Dies hängt vom Gleichgewicht exzitatorischer 5-HT<sub>2</sub> oder inhibitorischer 5-HT<sub>1A</sub>-Rezeptoren ab, die unterschiedlich stark in den Lamina V-Pyramiden angesprochen werden (Aghajanian und Marek, 1997; Tanaka und North, 1993). Man vermutete auch ursprünglich, dass eine "Geistesstörung" durch mangelhafte Serotonin-Aktivität hervorgerufen werde (Woolley und Shaw, 1954). So soll LSD eine inhibitorische Wirkung auf die tonische Feuerrate serotonerger Neurone haben (Aghajanian et al., 1968), was über den 5-HT<sub>1A</sub>-Autorezeptor vermittelt wird. Später ging man jedoch von einer Überaktivität der 5-HT<sub>2</sub>-Rezeptorsubtypen (Glennon et al., 1984) als mögliche Ursache für schizophrene Symptome aus, nachdem atypische Antipsychotika (z.B. Clozapine) gefunden wurden. Die Wirkung von Indol-Derivaten und Phenethylaminen wird über die 5-HT<sub>2A</sub>-Rezeptoren vermittelt. In beiden Fällen wird über eine Stimulation der 5-HT<sub>2A</sub>-Rezeptoren die *glutamaterge* Transmission im PFC erhöht, was sich durch eine deutliche Erhöhung der EPSP-Frequenz in der Region der apikalen Dendriten von Lamina V-Pyramiden zeigt (Aghajanian und Marek, 1997). Eine Hemmung der 5-HT<sub>2A</sub>-Rezeptoren, wie sie z.B. durch Clozapine hervorgerufen wird, führt zu einer Disinhibition der *dopaminergen* Transmission.

Die *Serotonin-Dopamin-Interaktion* ist sehr vielschichtig, da sie über mehrere 5-HT-Rezeptorsubtypen vermittelt wird und verschiedene Aspekte dopaminergener Funktionen anspricht. Anatomisch findet man nicht nur eine serotonerge Innervation direkt an dopaminergen Neuronen in der *Area ventralis tegmentalis* (VTA) und *Substantia nigra* (SN) (Van Bockstaele et al., 1994), sondern auch serotonerge Projektionen zu dopaminergen Terminationsfeldern im *Caudatus putamen*, *Amygdala*, *Nucleus accumbens* und *medialen Präfrontalkortex* (Lavoie und Parent, 1990; Steinbusch et al., 1981; Van der Kooy und Hattori, 1980; Imai et al., 1986). Das serotonerge System inhibiert also nicht nur die dopaminergen Funktionen direkt am Ursprung der dopaminergen Projektionen in der VTA und SN, sondern auch an deren Terminationsfeldern im Frontalhirn (Jacobs und Azmitia, 1992; Kelland et al., 1990). Läsionen der medialen Raphe zeigen eine Erhöhung

der kortikalen dopaminergen Funktion (Herve et al., 1981). Ebenso kann durch Serotonin-Antagonisten der dopaminerge Turnover im Kortex erhöht werden (Imperato und Di Chiara, 1988). Sowohl mechanische bzw. chemische Läsionen der Raphe-SN-Projektionen (Giambalvo und Snodgrass, 1978), als auch 5-HT<sub>1A</sub>-Agonisten, die diese Verbindung funktionell inhibieren (Arborelius et al., 1993) bzw. 5-HT<sub>2A</sub>-Antagonisten, die den Effekt des Raphe-SN-Systems antagonisieren (Ugedo et al., 1989), führen zu einer biochemischen und funktionellen Disinhibition des dopaminergen Systems.

Die ursprünglich formulierte *Dopamin-Hypothese* der Schizophrenie basiert auf den pharmakologischen Beobachtungen, dass (1) direkte oder indirekte Dopaminagonisten (z.B. Amphetamine, Cocain, L-Dopa) Positivsymptome der Schizophrenie verursachen bzw. diese verschlimmern (Carlsson, 1978, 1988; Snyder, 1974; Matthysse, 1973; Heinz et al., 1995) und (2) Neuroleptika, die als Dopaminrezeptor-Blocker wirken, therapeutisch zum Teil wirksam sind (Carlsson et al., 2001; Seeman, 1987). So wurde in den 60er Jahren die Dopamin-Hypothese als Hyperaktivität der dopaminergen Transmission oder als zunehmende Sensitivität der Dopaminrezeptoren postuliert (Van Rossum, 1966; Matthysse, 1973; Meltzer und Stahl, 1976). Seeman zeigte in PET-Studien Änderungen der dopaminvermittelten Transmission bei Schizophrenen, die auf eine erhöhte Besetzung von D<sub>2</sub>-Rezeptoren durch endogenes Dopamin zurückzuführen waren (Seeman, 1987). Die Hyperdopaminergie allein reicht jedoch nicht aus, um alle Aspekte der Krankheit zu erklären. So besitzen Dopaminrezeptor-Antagonisten nur eingeschränkt die Fähigkeit, Negativ-Symptome der Schizophrenie zu lindern. Auch setzt die therapeutische Wirkung dieser Neuroleptika erst nach Tagen bis Wochen ein, was darauf schließen lässt, dass ihr Mechanismus auf indirekte Weise wirkt und eher neuronale Adaptation als bloße D<sub>2</sub>-Rezeptor-Blockade voraussetzt (Konradi et al., 1993).

Es wird angenommen, dass Dopamin-Agonisten nicht alle Symptome sondern nur die Positivsymptomatik der Krankheit hervorrufen (Carlsson, 1978; Snyder et al., 1974; Matthysse, 1973). Außerdem wird angenommen, dass eine dopaminerge Dysfunktion in erster Linie zwar zur Positivsymptomatik führt, eine Dopamin-Hypofunktion aber auch Negativsymptome der Schizophrenie auslösen könne (MacKay et al., 1980; Berman und Weinberger, 1990). In jüngerer Zeit spricht man daher nicht mehr von einer allgemeinen Hyperdopaminergie, sondern von einem dopaminergen Ungleichgewicht zwischen mesolimbischen und mesokortikalen Gebieten. So sprechen sich einige Autoren für eine Hypoaktivität der DA-Transmission im frontalen/mesokortikalen Bereich, verbunden mit einer Hyperaktivität im subkortikalen/mesolimbischen Bereich aus (Weinberger, 1987;

Robbins, 1990; Davis et al., 1991; Lynch, 1992). Die Hypoaktivität der dopaminergen Transmission im präfrontalen Kortex sollte somit die Negativ-Symptomatik erklären. Eine damit verbundene Disinhibition efferenter Neurone im PFC führe zu einer Hyperaktivität dopaminergen Transmission im mesolimbischen System und somit zur Positivsymptomatik. Diese Vorstellung wird durch Befunde an unserem Tiermodell unterstützt: Mitarbeiter unsere Arbeitsgruppe zeigen deutlich eine *Imbalance des dopaminergen Systems* (Abb. 1) aufgrund einer überhöhten dopaminergen Faserdichte in mesolimbischen Arealen (z.B. im basolateralen Kern der Amygdala (AMY) und im ventralen lateralen Entorhinalkortex (LEC)), sowie einer verminderten dopaminergen Faserdichte im präfrontalen Kortex und Nucleus accumbens (NAC) (Busche et al., 2004; Polascheck, 2004; Winterfeld et al., 1998; Neddens et al., 2001, 2002; Dawirs et al., 1994; Zusammenfassung siehe Busche, 2004).

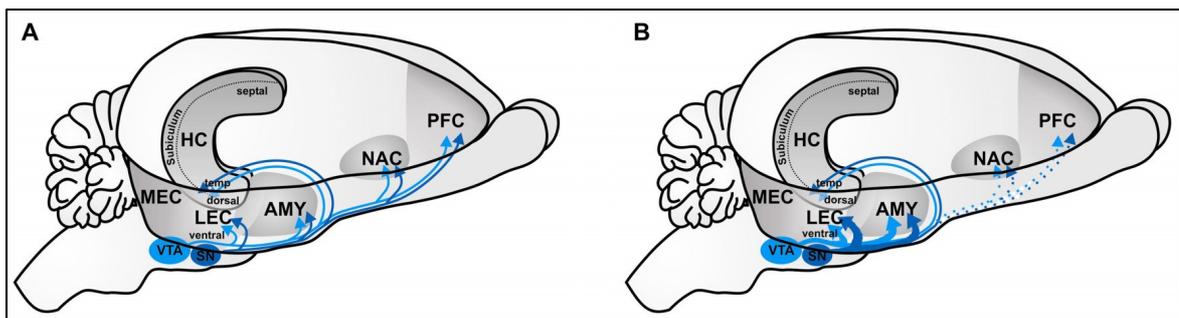


Abb. 1: (A) Darstellung der dopaminergen Innervation und (B) Entstehung einer Imbalance des dopaminergen Systems im limbo-präfrontalen System am Beispiel unseres Tiermodells. (Abbildung entnommen aus Busche, 2004).

Unabhängig von diesen strukturellen und physiologischen Belegen zur dopaminergen Imbalance gibt es andererseits die Vorstellung, dass eine generell verminderte dopaminerge Grundaktivität eine Überempfindlichkeit gegenüber transienten Dopaminexzessen bedingt (Grace, 1992).

Carlsson postuliert, dass eine reduzierte striatale Inhibition auf den Thalamus, hervorgerufen durch einen erhöhten dopaminergen (oder einen verminderten glutamatergen) Tonus, zu einer Erhöhung der Erregung und der psychomotorischen Aktivität und zu einem verstärkten sensorischen Input führt, der dem Kortex übermittelt wird (Carlsson, 1995). Wenn dabei eine bestimmte Reizschwelle überschritten wird, ist die integrative Kapazität des Kortex insuffizient und dies führt zur Positiv-Symptomatik der Schizophrenie. Nachweise einer frontalen Hypoaktivität im Ruhezustand bei

Schizophrenen fanden mehrere Forschergruppen (Buchsbaum et al., 1982; Brodie et al., 1984; Farkas et al., 1984; Kishimoto et al., 1987; Vita et al., 1995). Die meisten dieser strukturell- und funktionell-bildgebenden Untersuchungen konnten Störungen in frontalen und temporo-limbischen Hirnarealen nachweisen. Eine entsprechend erhöhte subkortikale Dopaminfreisetzung wurde nicht nur durch Weinberger gefordert (Weinberger, 1987), sondern auch von Grace, der zwar eine tonisch verminderte aber phasisch erhöhte striatale Dopamin-Freisetzung postuliert (Grace, 1991). Als Ursache einer Dysfunktion in den frontalen und temporo-limbischen Hirnarealen wird mittlerweile nicht nur eine dopaminerge sondern auch eine glutamaterge kortikale Fehlregulation vermutet (Breier et al., 1998; Kegeles et al., 1999; Carlsson, 1995). Als Erweiterung der Dopamin-Hypothese wurde auch eine Beteiligung serotonerger und GABAerger Systeme postuliert (Kornhuber und Weller, 1994).

Das *glutamaterge System* ist **d a s** exzitatorische System im Gehirn. Da über die exzitatorische Transmission ebenso die neuronale Inhibition stimuliert wird, moduliert Glutamat die Feuerrate beinahe jedes Neurons im Zentralnervensystem (Tamminga, 1998). Dieser Transmitter erfüllt außerdem eine Vielzahl physiologischer Funktionen bei Gedächtnis und Lernen, Entwicklung und motorischen Funktionen (Parsons et al., 1998; Krystal et al., 1999). Weiterhin reguliert Glutamat mit neurotropher Wirkung den Ausbau nichtglutamaterger Systeme und fördert autoregativ die Entwicklung des eigenen Systems oder nachgeschalteter glutamaterger Systeme (Kornhuber und Weller, 1994). Es lässt sich nach Meinung dieser Autoren eine Hypothese für die Entwicklung glutamaterger Schaltkreise formulieren, deren Störung an verschiedenen Stellen zu veränderten Transmittergleichgewichten führen könnte und mit Vorstellungen über eine embryonale oder frühkindliche Hirnentwicklungsstörung gut vereinbar ist.

Glutamat wirkt an drei Klassen ionotroper Rezeptoren (NMDA-, AMPA- und Kainat-Rezeptoren) sowie einem metabotropen (G-Protein gekoppelten) Rezeptor. Es wird postuliert, dass eine Dysfunktion eines dieser Rezeptorsysteme im glutamatergen System Symptome der Schizophrenie hervorrufen könnte: so zeigten Untersuchungen, dass Psychotomimetika wie Phencyclidine (PCP, "*angel dust*"), MK-801 und Ketamine, die als uncompetitive NMDA-Rezeptor-Blocker direkt am NMDA-Rezeptor wirken, einige Symptome sowohl aus der Positiv- (z.B. Veränderungen des Ich-Empfindens) als auch aus der Negativ-Symptomatik (z.B. kognitive Defizite) verursachten, und Medikamente, welche die NMDA-Rezeptorfunktion erhöhten, reduzierten die Negativsymptomatik bei chronisch schizophrenen Patienten (Javitt und Zukin, 1991; Coyle, 1996; Tamminga, 1998;

Krystal et al., 1999; Schroeder et al., 2000). Die psychotomimetische Wirkung nicht-kompetitiver NMDA-Antagonisten liefert ein indirektes Argument für eine Unterfunktion des glutamatergen Systems. Es wird daher allgemein angenommen, dass eine Hypofunktion des glutamatergen Systems, insbesondere der NMDA-Rezeptoren, bei schizophrenen Patienten vorhanden ist. Schizophrenie, so formuliert sich also die Glutamat-Hypothese, sei das Ergebnis einer Reduktion der glutamatergen Funktion in Form von verminderter glutamaterger Rezeptorfunktion, herabgesetzter Anzahl glutamaterger Rezeptoren oder verminderter extrazellulärer Glutamat-Konzentrationen (Kim et al., 1980; Kornhuber et al., 1984).

Das *glutamaterge System* interagiert eng mit dem *dopaminergen System*. So fanden Vasiliadis und Mitarbeiter im Neostriatum der Ratte heraus, dass Aktivierung oder Inhibition von NMDA-Rezeptoren die dopaminerge neuronale Aktivität und infolgedessen dopaminerge D<sub>1</sub>- und D<sub>2</sub>-Rezeptorbindungen verändern (Vasiliadis et al., 1999). Zheng und Mitarbeiter halten ebenfalls für möglich, dass kortikale Dopamin- und NMDA-Rezeptor vermittelte Transmissionen in enger Wechselbeziehung stehen, und die Fehlfunktion der einen Transmission zu Änderungen der anderen führt (Zheng et al., 1999). Es wurde in mehreren Arbeiten gezeigt, dass sowohl Dopamin als auch Glutamat sich *präsynaptisch* gegenseitig beeinflussen, um die Ausschüttung des jeweils anderen Transmitters zu modulieren (Jedema und Moghaddam, 1994; Jones et al., 1993; Reid et al., 1997). Dopamin moduliert aber auch *postsynaptisch* die NMDA-Rezeptor vermittelte glutamaterge Transmission im medialen PFC, und zwar konzentrationsabhängig: während geringe Konzentrationen von DA über D<sub>1</sub>-Rezeptoren gesteuert die NMDA-Antwort erhöht, wird sie über D<sub>2</sub>-Rezeptoren bei hoher DA-Konzentration erniedrigt (Zheng et al., 1999). Ähnliche Resultate wurden auch im menschlichen Neokortex ermittelt (Cepeda et al., 1992; Cepeda und Levine, 1998). Präsynaptische D<sub>2</sub>-Rezeptoren auf kortikostriatalen und limbischen glutamatergen Terminalien verhindern eine negative Regulation der Glutamat-Ausschüttung (Kerkerian et al., 1987; Maura et al., 1988). Eine neuroleptische Blockade dieser präsynaptischen Rezeptoren erhöht somit den exzitatorischen glutamatergen Input zum Caudatus/Putamen und zu dopaminerg innervierten Vorderhirnregionen.

Glutamaterge Efferenzen wiederum stehen in reziproken Interaktionen mit dopaminergen Prozessen in ihren Terminationsfeldern. So wurde gezeigt, dass sowohl die Glutamat- als auch die Dopamin-Freisetzung im PFC infolge von Stress erhöht ist (Moghaddam, 1993). Weiterhin kann durch eine lokale pharmakologische Blockade von

AMPA/KA-Rezeptoren im PFC die Stress-induzierte erhöhte Ausschüttung von Dopamin verhindert werden, was stark darauf hinweist, dass Glutamat die Dopaminausschüttung moduliert (Jedema und Moghaddam, 1994). Demgegenüber kann Dopamin über den postsynaptischen D<sub>1</sub>-Rezeptor eine *transsynaptische feed-forward-Hemmung* der glutamatergen Ausschüttung verursachen, die wiederum durch NMDA-Antagonisten geblockt wird (Harvey und Lacey, 1997).

Die vielen Studien zeigen, dass es ein komplexes dynamisches Gleichgewicht zwischen dopaminerger und glutamaterger Transmission gibt, in dem Glutamat zu erhöhter Dopaminausschüttung und Dopamin zu erniedrigter Glutamatausschüttung beisteuert. Bei der schizophrenen Psychose werden die Dopamin- und Glutamathypothese zu einem *Neurotransmitter-Imbalance-Syndrom* verknüpft (Carlsson und Carlsson, 1990a). Bei diesem Modell tragen die dopaminerge Hyperaktivität im subkortikalen Bereich und die glutamaterge Hypoaktivität in kortikalen Gebieten in einem Feedback-Prozess über "*Arousal-Modulation*" dazu bei, dass die striatale Kontrolle über die thalamische Filterfunktion sensorischer Inputs aus der Außenwelt reduziert wird. Hierüber soll eine kortikale "*Informationsüberflutung*" ausgelöst werden. Diese Vorstellungen werden durch unser Tiermodell insofern vervollständigt, als gezeigt werden konnte, dass der "*Two-Hit-Effekt*" eine Verstärkung der Dopaminfaserdichte in limbischen Arealen und eine Verminderung der Dopaminfaserdichte in präfrontalen Arealen verursacht (Busche et al., 2004; Neddens et al., 2001; Dawirs et al., 1994).

### **3. Zu den hier vorliegenden Arbeiten**

#### **3.1 Zum Hintergrund von Tiermodellen in der Schizophrenieforschung**

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Die grundlegende Annahme für die Entwicklung von Tiermodellen zur Schizophrenie ist, dass die normale Hirnentwicklung insbesondere in kritischen Phasen unterbrochen und somit gestört wird. Die daraus resultierenden Läsionen verursachen im Zusammenspiel mit natürlichen Reifungsprozessen des Gehirns gerade auch im späten Jugend- oder frühen Erwachsenenalter die typischen Symptome der Krankheit. Damit lehnen sich Tiermodelle prinzipiell an die Erkenntnisse zur natürlichen Entstehung von Psychosen an.

Als mögliche Ursachen einer Störung zählt eine Fülle von genetischen und nicht-genetischen Faktoren, die einen Einfluss auf die Entwicklung des auch lange nach der Geburt noch reifenden Gehirns nehmen. Das betrifft z.B. virale Infektionen oder Unterversorgung während der Schwangerschaft, frühkindlicher Stress durch Geburtskomplikationen, Drogenmissbrauch, hormonelle Veränderungen oder durch genetische Fehlfunktionen bedingte Missbildungen (Stöber et al., 2002, 1993; Murray et al., 1986; Jones und Cannon, 1998; Susser und Lin, 1992; Dalman et al., 1999; Van Oss und Selten, 1998; Andreasson et al., 1987). Weinberger, der selber ein sehr wertvolles Tiermodell entwickelt hat, postuliert zerebrale Strukturveränderungen als Grundlage der Schizophrenie (Weinberger, 1987). Die Schädigung findet schon im zweiten Schwangerschaftsdrittel statt, der Ausbruch der Krankheit erfolgt jedoch erst im jungen Erwachsenenalter, wenn der normale Reifungsprozess der Strukturen abgeschlossen ist und sich Funktionsdefizite herausgebildet haben. Eine frühe Störung der zerebralen Entwicklung vermutet auch Walker, der in seiner Arbeit eine zumindest verzögerte Reifung des neuronalen Motorsystems bei später erkrankten Schizophrenen darstellt (Walker, 1994). Eine früh erworbene Störung des *temporolimbisch-präfrontalen Kortex* finden auch andere Autoren (O'Connell et al., 1997; Weinberger et al., 1992). Tierversuche belegen, dass eine Störung dieses kortikalen Netzwerkes zur Enthemmung der subkortikalen DA-Freisetzung führt (Heinz et al., 1999; Lipska et al., 1993; Saunders et al., 1998).

So genannte *Umweltmodelle* werden im Tiermodell bevorzugt eingesetzt, um z.B. eine verschlechterte "*prepulse inhibition*" in der Schizophrenie zu untersuchen (Weiss und Feldon, 2001). Solche Modelle basieren (1) auf spezifischen zerebralen Läsionen, die

physikalisch herbeigeführt wurden, oder (2) auf Stress, der in Verbindung mit einer genetischen Prädisposition die Schizophrenie erzeugt.

Mittlerweile geht man von der Annahme aus, dass die Ursachen von schizophrenen Erkrankungen multikausal sind, und durch ein Zusammenwirken von biologischen und psychosozialen Faktoren ausgelöst und unterhalten werden. Diese unterschiedlichen konstitutionellen und umwelt- bzw. milieubedingten Faktoren werden im *Vulnerabilitäts-Stress-Modell* (auch Diathese-Stress-Modell) vereint. Zubin und Spring schlagen in ihrem Vulnerabilitätsmodell vor, dass jeder Mensch mit einem gewissen Grad an Vulnerabilität ausgestattet ist, was unter besonderen Umständen zu einer schizophrenen Episode führen kann (Zubin und Spring, 1977; siehe auch Abb. 2). Dieses Vulnerabilitätsmodell wurde schließlich weiterentwickelt zum Vulnerabilitäts-Stress-Modell (Nuechterlein und Dawson, 1984).

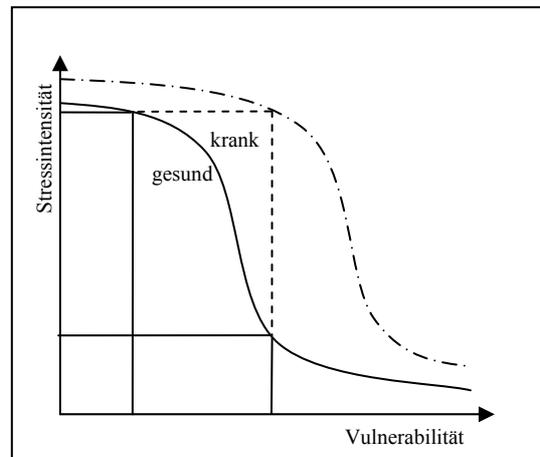


Abb. 2: Funktionaler Zusammenhang zwischen Vulnerabilität, Stressintensität und der Manifestationsschwelle zur schizophrenen Störung (Zeichnung aus Gaebel, 2003).

Gaebel spricht von einer angeborenen und/oder erworbenen, also multifaktoriell vermittelten Krankheitsdisposition, die erst durch das Hinzutreten zusätzlicher Faktoren die Störung über die Manifestationsschwelle treten lässt (Gaebel, 2003). Als Beispiel solcher Faktoren werden neben individuellen Belastungen und Konflikten aus dem psychosozialen Umfeld auch biologische Faktoren genannt. Die Verknüpfung zwischen biologischer Vulnerabilität und dem Ausbruch der Krankheit wird im Diathese-Stress-Modell als variable Funktion des Levels der HPA-Aktivität (*hypothalamic-pituitary-adrenal*) gedeutet. Die über die HPA-Achse vermittelte physiologische Stressantwort sei bei schizophrenen Patienten dysfunktional und führe zur Überaktivierung der subkortikalen dopaminergen Transmission (Walker und Diforio, 1997).

Das Vulnerabilitäts-Stress-Modell oder das "Two-Hit-Modell" (Mednick et al., 1998) vereinen somit das Modell einer neuronalen Entwicklungsstörung und das Umweltmodell in sich, wobei auch genetische Modelle der Schizophrenie (Bayer et al., 1999) Beachtung finden.

Vor diesem Hintergrund erhält unser seit Jahren im Einsatz befindliches Tiermodell seinen besonderen Wert. Es beruht auf zwei nicht-invasiven Interventionen, die das gesamte Reifungsgeschehen von Transmittern im Gehirn erheblich stören. Dieses Tiermodell wird nun im folgenden Kapitel genauer ausgeführt.

### 3.2 Unser Tiermodell

Das in dieser Arbeit verwendete Tiermodell wird bei uns seit langem als Standard-Modell eingesetzt. Es liegen uns viele Daten zu Transmitterdichten in den verschiedenen Regionen des *limbo-präfrontalen Systems*, zu Neuro- und Synaptogenese im hippocampalen Dentatus und zum Verhalten der Tiere vor, die uns eine genaue Vorstellung über die Auswirkungen der einzelnen Interventionen geben.

(1) Die restriktive Isolationsaufzucht als Intervention (siehe Abb. 3): allen Tieren gemeinsam ist, dass sie unter natürlichen Tag/Nacht Bedingungen gehalten werden und Futter *at libitum* erhalten. Als Kontrolltiere verwenden wir Gerbils, die unter sog. angereicherten Aufzuchtbedingungen (*enriched rearing condition* = ER) geboren und groß geworden sind. Diese ER-Bedingungen erzeugen wir in einem 1 m<sup>2</sup> großem Gehege mit Versteck- und Spielmöglichkeiten sowie der Möglichkeit zu sozialen Kontakten im

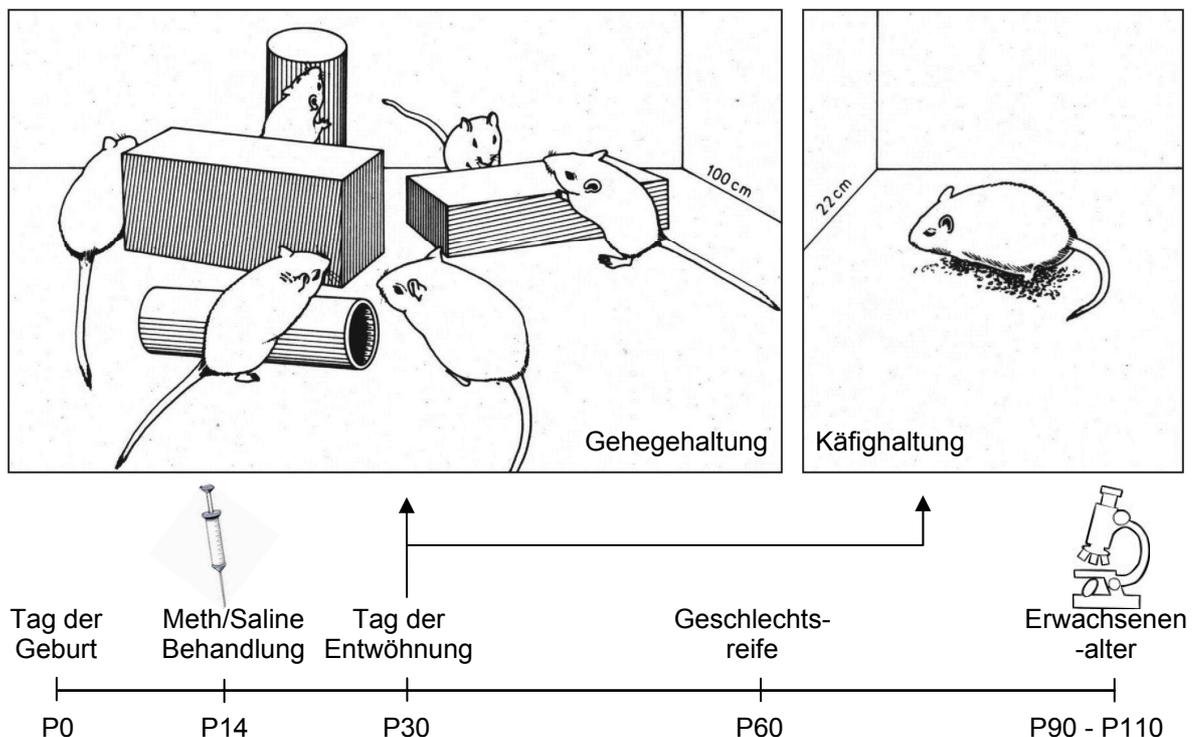


Abbildung 3: Zeittafel über Behandlung und Aufzuchtbedingungen.

Geschwisterverband (aus praktischen Gründen nur mit männlichen Geschwistertieren). Eine Intervention in Form von chronischem Stress erfahren Tiere, die unter reizarmen Aufzuchtbedingungen (*impoverished rearing condition* = IR) geboren und groß geworden sind. Die Zucht erfolgt hier in Standard-Makrolon®-Käfigen vom Typ IV (Fläche etwa 0,12 m<sup>2</sup>). Nach der Entwöhnung am postnatalen Tag 30 (P30) werden die Jungtiere vereinzelt in Standard-Makrolon®-Käfige vom Typ III (Fläche etwa 0,06 m<sup>2</sup>) gesetzt. Sie wachsen dort ohne direkten sozialen Kontakt zu anderen Tieren sowie ohne Versteck- und Spielmöglichkeiten auf.

(2) Jeweils eine Hälfte der Tiere aus beiden Aufzuchten werden einer weiteren Intervention in Form eines nicht-invasiven chemischen Stressors ausgesetzt (siehe Abb. 3). Den jungen Tieren werden am postnatalen Tag P14 einmalig eine hohe Dosis Methamphetamin (MA; 50 mg/kg, i.p.) appliziert, einer Kontrollgruppe wird Saline verabreicht. Auf diese Weise erhalten wir vier Tiergruppen: als reine Kontrollgruppe dienen uns Gehege-Saline-Tiere (ER), jeweils nur eine Intervention erhalten also Gehege-Methamphetamin-Tiere (ER-MA) und Käfig-Saline-Tiere (IR), und beide Interventionen erfahren Käfig-Methamphetamin-Tiere (IR-MA). Die autotoxische Wirkung von Methamphetamin ist auf biochemischer Ebene vielfach beschrieben worden (Seiden und Sabol, 1996). Dass bereits eine einmalige MA-Applikation selektiv dopaminerge Termination im Stirnhirn zerstört, wurde Anfang der 90er Jahre beschrieben (Dawirs et al., 1994).

Die Tracer- und immunhistochemischen Untersuchungen der Tiere erfolgen im Erwachsenenalter ab P90 (Tracerstudie) bis P110 (immunhistochemische Studien). Eine genauere Darstellung der einzelnen Färbemethoden ist in den jeweiligen Publikationen zu finden (Bagorda et al., 2005; Neddens et al., 2003, 2004).

### **3.3 Der Kortex unter besonderer Berücksichtigung des Präfrontalkortex (PFC)**

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Der präfrontale Kortex ist die Struktur mit den meisten Verbindungen zu anderen *heteromodalen, unimodalen, paralimbischen* und *limbischen* Strukturen im Neokortex und ist somit in der Lage, vorhandene Netzwerke zu aktivieren oder zu unterdrücken, und Interaktionen zwischen Netzwerken zu instrumentalisieren (Mesulam, 2000). Er erhält *Afferenzen* vom Hirnstamm, Hypothalamus, dem limbischen System (Hippocampus und Amygdala), dem Thalamus (anterioren und mediodorsalem Nucleus), und anderen

neokortikalen Gebieten wie dem parietalen, temporalen und occipitalen Kortex. Während Hirnstamm, Hypothalamus, Hippocampus und Amygdala dem PFC Informationen aus dem "inneren Milieu" zutragen, ist der Input aus dem posterioren Kortex involviert in sensorisch-motorische Integrationsleistungen auf höchster Ebene. Mesulam bezeichnet den präfrontalen Kortex deshalb als Integrationseinheit für Informationen der äußeren Umwelt und des inneren Zustandes (Mesulam, 2000). Mit all den oben genannten Strukturen ist der PFC wiederum reziprok durch seine *Efferenzen* verknüpft, die ich in dieser Arbeit mit Hilfe eines anterograden Tracers markieren konnte. Eine detaillierte Darstellung der Verbindungen soll folgen, weil sie dem Verständnis der Befunde zu den gruppenspezifischen Veränderungen dienen.

Über 70% aller kortikalen Neurone sind glutamaterge *Pyramidenzellen*. Die apikalen und basalen Dendriten von Pyramidenzellen besitzen kurze *Spines*, die die hauptsächlich Verbindungsstellen des überwiegend *exzitatorischen* synaptischen Inputs

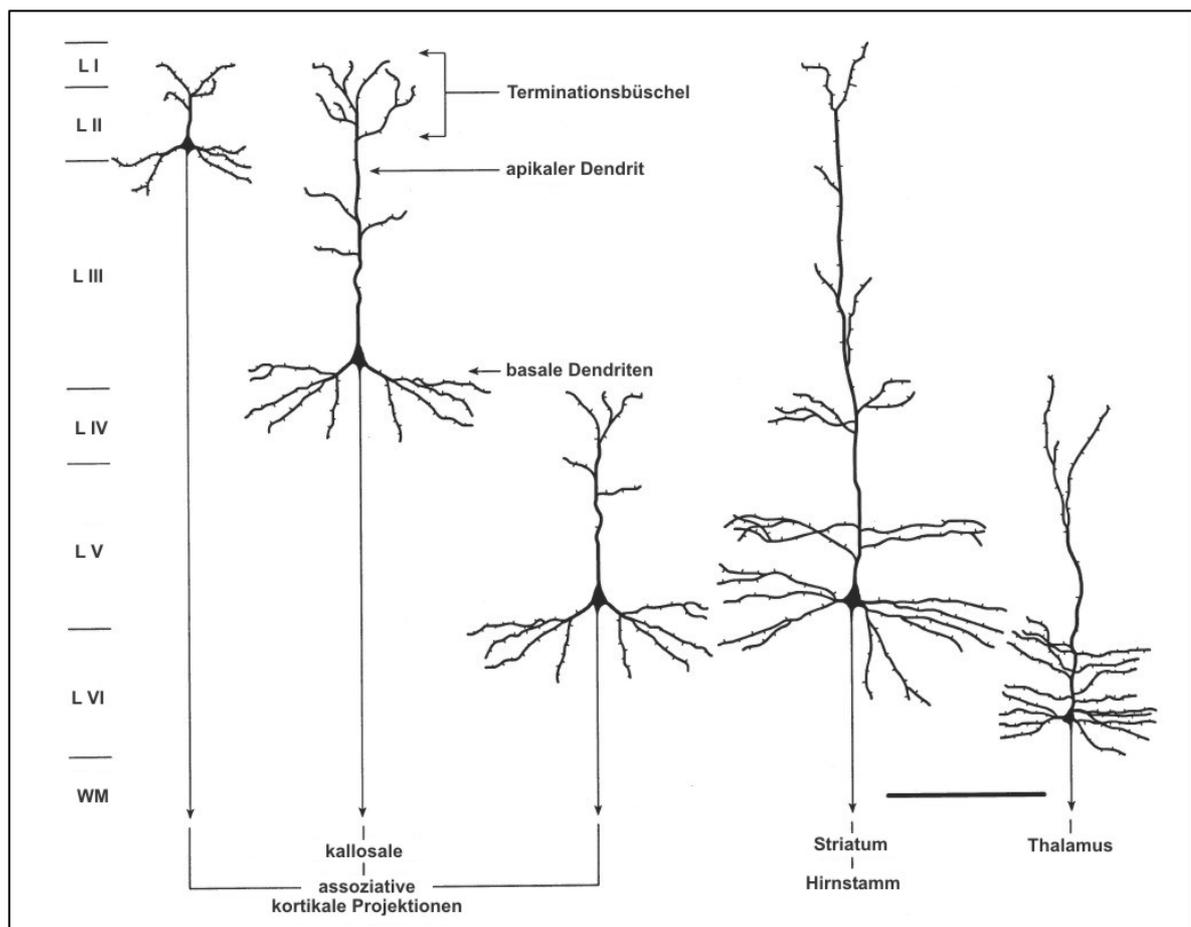


Abb. 4: Übersicht der charakteristischen morphologischen Eigenschaften von Pyramidenzellen in verschiedenen kortikalen Laminae mit Angabe ihrer bevorzugten Projektionsziele (verändert nach Jones, 1984).

der Zelle sind. Der *inhibitorische* Input einer Pyramide geht hauptsächlich über die dendritischen Schäfte, den Zellkörper bzw. das Initialsegment des Axons (De Felipe und Farinas, 1992). Ein kurzer Überblick soll das laminäre Verteilungsmuster der kortikalen Verbindungen verdeutlichen (Abb. 4).

Es gibt einige grundlegende Projektionssysteme, die anhand ihrer laminären Lage bestimmt werden. Kurze kortikokortikale Fasern, die innerhalb der gleichen Hemisphäre bleiben (= *Assoziationsfasern*), entspringen im allgemeinen von Pyramiden der Lamina II und oberen Lamina III während lange kortikokortikale Assoziationsbündel sowie die kommissuralen oder callosalen Projektionen (= *interhemisphärische Fasern*) hauptsächlich von der unteren Lamina III, und zu einem geringeren Teil aus den infragranulären Laminae V und VI stammen. Die kortiko-subkortikalen Projektionen zum Striatum, Thalamus oder in den Hirnstamm stammen hauptsächlich von den Pyramidenzellen der tieferen Schichten Laminae V und VI (Jones, 1984; Selemon und Goldman-Rakic, 1988; Barbas und Pandya, 1989) und nur einige wenige aus Lamina III. Das Innervationsmuster intrinsischer Axonkollaterale der Pyramidenzellen neigt dazu, über die unterschiedlichen Laminae hin zu variieren (Levitt et al., 1993). So besitzen die Pyramiden der Lamina II sowohl lokale

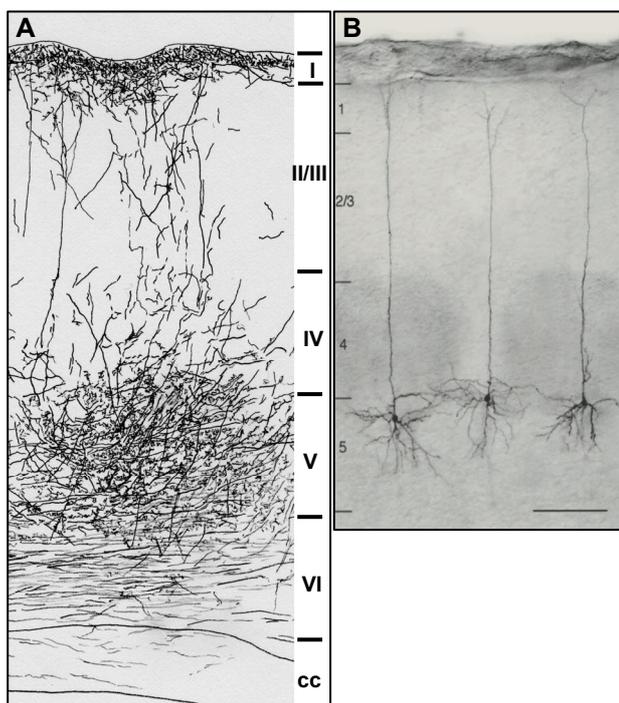


Abb. 5: **(A)** Camera lucida Zeichnung einer tracermarkierten Kolumne des präfrontalen U-Fasersystems im parietalen Kortex. **(B)** Lamina V-Pyramiden im "Barrelfield" des parietalen Kortex. (Balken: 200 $\mu$ m; Foto entnommen aus Lübke et al., 2000).

Kollaterale, die in der Nähe der Zellsoma verbleiben, als auch horizontale Axonprojektionen, die über eine beachtliche Distanz durch die *graue Substanz* entsendet werden und schließlich in einzelnen axonalen Terminationsklustern in den oberflächlichen Schichten des Kortex enden. Auch die Pyramiden der Laminae V und VI entsenden intrinsische horizontale Axonkollaterale, die jedoch weniger ausladend sind und auch nicht in deutlich abgegrenzten *Klustern* enden. Die Pyramidenzellen der Lamina IV haben überwiegend vertikal orientierte Axone, die interlaminäre Verbindungen sind. Abbildung 5 zeigt in (A) die *Camera lucida* Zeichnung eine durch

präfrontale Projektionen gebildete aufsteigende Kolumne im parietalen Kortex mit Terminationskluster in der Lamina V. Aus (B) wird ersichtlich, dass dieses Terminationsfeld im Bereich der basalen Dendriten von Lamina V-Pyramiden im Barrelcortex liegt, die präfrontalen Efferenzen also hier im parietalen Kortex auf Pyramiden der Lamina V verschalten.

Untersucht wurden in der vorliegenden Präfrontalstudie ausgewählte Kortexareale, die sich aufgrund ihrer darzustellenden Verbindungen mit dem medialen PFC klar abgrenzen lassen. Für die Quantifizierungen wurde aus der rostro-caudalen Schnittfolge bevorzugt die Schnittebene 2,5 mm anterior zum Bregma ausgewählt. Abb. 6 zeigt eine Übersicht über die Lage dieser Schnittebene im Gehirn an, Abb. 7 die entsprechenden Kortexareale dieser Schnittebene. Die einzelnen Untersuchungsfenster für die Quantifizierungen wurden in die Areale

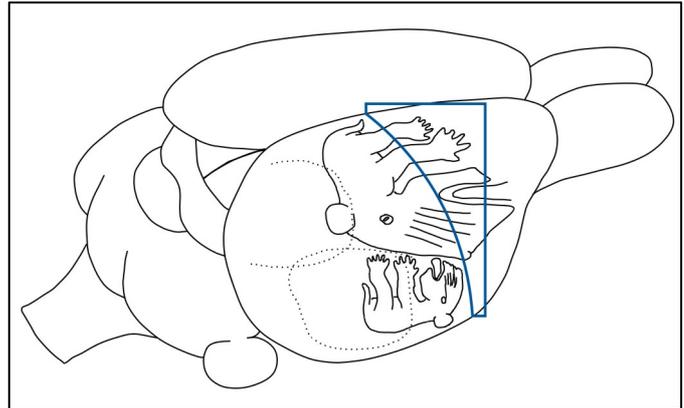


Abb. 6: Darstellung des Gehirns einer Wüstenrennmaus mit eingezeichneten "sensorischen Rattunkulus" (verändert nach Woolsey), und Höhe der Schnittebene der Auswertung (in blau).

dieser Schnittebene. Die einzelnen Untersuchungsfenster für die Quantifizierungen wurden in die Areale des frontalen Kortex (FC, hier Fr2), des parietalen Kortex (PC, hier Par1) und des insulären Kortex (IC, hier AId) gelegt. Der Fr2 zählt als *shoulder region* mit zum medialen PFC und vereint neben präfrontalen auch prämotorische Aspekte in sich. Auf Höhe der Schnittebene findet sich im Par1 das typische *barrel field*, hier ist der sensorische Aspekt maßgeblich. Der AId repräsentiert das caudale Ende des orbitalen PFC und grenzt somit unmittelbar an den entorhinalen Kortex. Die Untersuchungsfenster beinhalten dabei jeweils den Ausschnitt einer einzelnen Lamina.

Mit Hilfe der computerunterstützten Bilderkennung konnten die in der Lamina vorhandenen Fasern mittels eines *Valleys-Operator*, der einen Graustufen-Unterschied benachbarter Bildpixel auswertet, erfasst, und die Faserdichte innerhalb des Auswertfensters gemessen werden. Aufgrund der jeweils besseren Faserdarstellung erfolgte die Auswertung der glutamatergen Faserdichte (Tracerstudie) hierbei im *Dunkelfeld*, die der serotonergen Faserdichte (immunhistochemische Studie) im *Hellfeld*. Vor der durch eine Mitarbeiterin in einer Blindstudie durchgeführten Quantifizierung der Tracerstudie wurden *Camera lucida* Zeichnungen angefertigt, die es ermöglichten, die

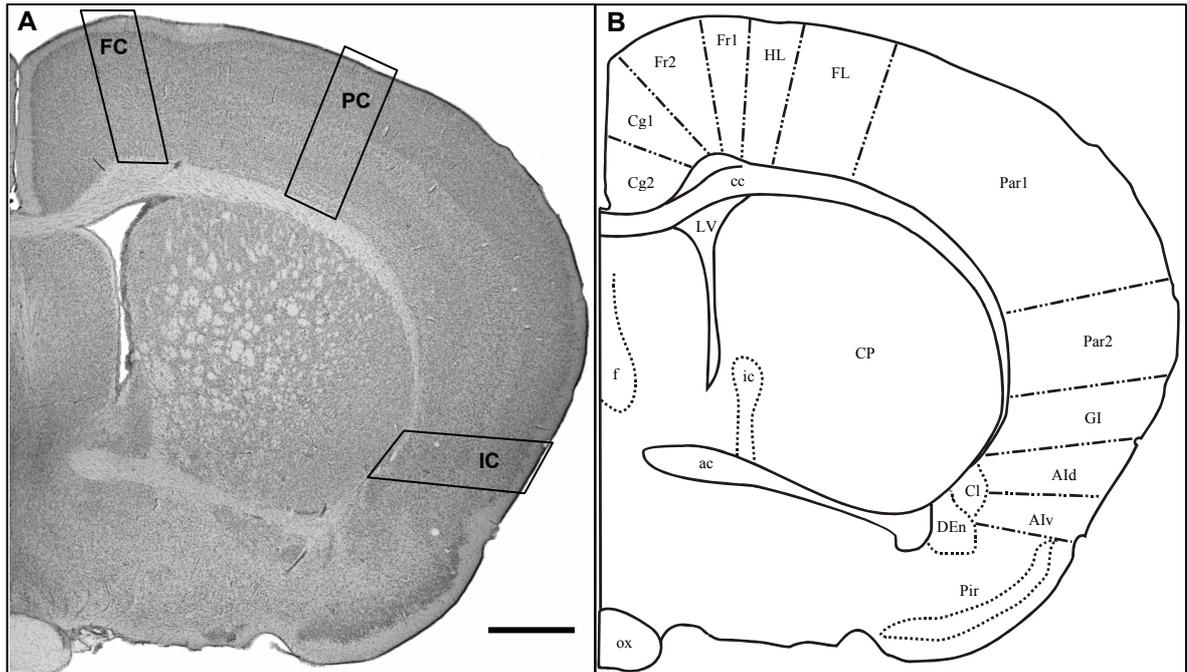


Abb. 7: Übersicht der untersuchten Schnittebene: (A) Nissl-Färbung, Balken: 1000 $\mu$ m, die eingezeichneten Vierecke geben die Positionen der ausgewerteten Bereiche im FC, PC und IC an. (B) Kortexareale nach Paxinos und Watson, 1986; Zilles, 1985.

Legende:

ac	anterior commissure	Fr1	frontal cortex, area 1
Ald	agranular insular cortex, dorsal	Fr2	frontal cortex, area 2
Alv	agranular insular cortex, ventral	GI	granular insular cortex
cc	corpus callosum	HL	hindlimp area of cortex
Cg1	cingulate cortex, area 1	ic	internal capsule
Cg2	cingulate cortex, area 2	LV	lateral ventricle
Cl	claustrum	ox	optic chiasm
CPu	caudate putamen	Par1	parietal cortex, area 1
DEn	dorsal endopiriform nucleus	Par2	parietal cortex, area 2
f	fornix	Pir	piriform cortex
FL	forelimb area of cortex		

einzelnen Injektionen in ihrer Lage sowie in ihrer Tiefe klar zu definieren, um so die einzelnen Fälle in die jeweiligen Gruppen einzuordnen.

### 3.4 Zu den Transmitterbefunden

#### Glutamat

Die qualitative Bewertung der pyramidalen *U-Fasersysteme* wurden mit Hilfe von *Camera lucida* Zeichnungen durchgeführt. Aus den Zeichnungen zu fast 100 Fällen wurden insgesamt 47 Fälle ausgewählt, die sich auf die 4 Versuchsgruppen gleichmäßig aufteilen und dabei die flachen und tiefen Injektionen berücksichtigen. Diese ausgewählten Fälle waren auf die Schnittebenen 4,5 bis 5 mm anterior zum Bregma eingegrenzt. Jeweils zwei dieser Fälle pro Gruppe und Injektionstiefe sind exemplarisch dieser Arbeit als Dokumente angefügt.

An den gezeichneten Reproduktionen der PFC-Efferenzen lassen sich die Faserzüge im Einzelnen verfolgen. Die Injektion des Tracers erfolgte auf der Schnittebene 4,5 bis 5 mm anterior zum Bregma in die "shoulder region" des medialen präfrontalen Kortex, im Grenzbereich zwischen Fr2 und Cg1 (Abb. 8). Der *anterograde* Tracer Biocitin zeigt nahe der Injektion auch einen über kurze Distanz vorhandenen retrograden Transport, so dass die Somata der markierten Pyramidenzellen deutlich zu erkennen sind (siehe Fotos in der Veröffentlichung Bagorda et al., 2005). Anhand dieser Markierungen lassen sich flache und tiefe Injektionen gut voneinander unterscheiden. Nahe der Injektion sind sehr viele Fasern dargestellt, dies liegt daran, dass neben den weit projizierenden Pyramidenzellen auch Interneurone angefärbt werden, deren kurze Fasern aber lokal in diesem Areal verbleiben und bei einer Auswertung auf weiter caudal gelegenen Schnittebenen nicht mit erfasst werden.

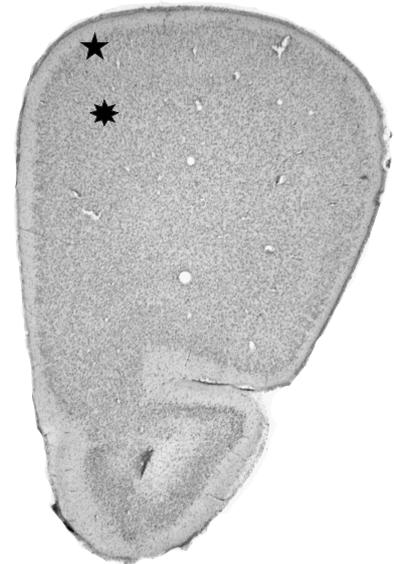


Abb. 8: Schnittebene auf Injektionshöhe; Nisslfärbung  
 ★ = flache Injektionsposition  
 \* = tiefe Injektionsposition.

Oft zeigt sich unterhalb der angefärbten Pyramiden und etwas caudal zur Injektionsebene ein sich verdichtendes Band von Projektionsfasern, das sich in Richtung des sichtbar werdenden *Corpus callosum* orientiert. Das Faserband tritt zum Teil in das *Corpus callosum* ein, teilt sich dort auf, um innerhalb des *Corpus callosums* ipsilateral

weiterzuziehen, und sich mit einigen Faserteilen zusätzlich nach medial zu wenden, um über das *Corpus callosum* auf die kontralaterale Hirnhälfte zu wechseln.

Neben diesem dichten und aus dicken Fasern bestehenden Band ziehen in einem lockeren Geflecht viele dünner wirkende Fasern von der Injektionsstelle über die Lamina VI nach lateral bis in den Bereich des insulären Kortex. Dabei steigen immer wieder in den einzelnen Arealen des Kortex Faserzüge aus der Lamina VI auf, die in zum Teil sehr prominenten *Kolumnen* bis zur Oberfläche ziehen. Dabei bilden diese aufsteigenden Fasern gerade in der oberen Lamina V zum Teil deutliche Terminationsfelder aus (siehe dazu Abb. 5). Aufgrund der Form ihres Verlaufes bezeichnet man diese Projektionen auch als U-Fasersystem. Die Kolumnen erscheinen an den Grenzen zu benachbarten Kortexarealen. So kann man gerade auf der Schnittebene, die zur Auswertung herangezogen wurde, mit geschultem Auge drei sehr dicht beieinander stehende Kolumnen zwischen Fr2/Fr1 (=ausgewertete FC-Kolumne), Fr1/HL und HL/FL unterscheiden (Übersicht der Kortexareale und Abkürzungen siehe Abb. 7), deutlicher abgrenzbar ist die Kolumne zwischen Par1/FL (=ausgewertete PC-Kolumne) und schwächer ausgebildet ist sie zwischen Par1/Par2. Über die Lamina VI und über das *Corpus callosum* erreicht das U-Fasersystem auch den IC. Hier ordnen sich die Fasern in der Lamina VI zum Teil streng parallel an und richten sich vertikal zur Oberfläche aus. Diese strenge Ausrichtung verliert sich aber oberhalb der Lamina VI, die Fasern erscheinen dadurch zwischen Lamina VI und V oft wie abgeschnitten. Ab der Lamina V zieht die Kolumne mit Terminationsgebieten in Lamina V bis III und auch in I bis zur Oberfläche. Diese IC-Kolumne ist jedoch nicht immer so deutlich ausgeprägt wie in den anderen Kortexarealen.

Ein weiterer Faserzug zieht von der Injektion über die Lamina I nach lateral und caudal. Im Bereich der oben angesprochenen Kolumnen sind in der Lamina I ebenfalls Terminationskluster und einzelne in die Kolumne absteigende Fasern zu erkennen, die bis in die obere Lamina V reichen.

Das grundlegende Muster der präfrontalen Efferenzen unterscheidet sich zwischen flachen und tiefen Injektionen und zwischen den einzelnen untersuchten Tiergruppen grundsätzlich nicht. Nur die jeweilige Dichte dieser Faserzüge zeigen Variationen, die im Folgenden dargelegt sind.

Die 47 Fälle wurden nach der zeichnerischen Vorbewertung einer quantitativen Analyse unterzogen. Denn nunmehr war es möglich, flache gegenüber tiefen Injektionen abzugrenzen, und sich auf 3 prominente *U-Faser-Kolumnen* zu fokussieren, die im frontalen Kortex (FC), parietalen Kortex (PC) und insulären Kortex (IC) gelegen sind. Insgesamt beruht die Datenerhebung für die 8 Gruppen (4 Gruppen für jeweils flache und tiefe Injektionen) auf ca. 6 Tieren pro Gruppe.

In den gemessenen Fenstern zur Faserdichtebestimmung finden sich nach dem *post-hoc* Test zahlreiche signifikante Unterschiede, die in den Abb. 9 bis Abb. 11 zusammenfassend dargestellt werden:

- Im Frontalkortex (FC; Abb. 9) sind hauptsächlich die Projektionen der tiefen Laminae V/VI-Pyramiden betroffen. Hier lässt sich gegenüber den Kontrolltieren (ER) eine verminderte Faserdichte in den Gruppen mit jeweils einer Intervention (ER-MA und IR) feststellen. In der IR-MA-Gruppe ist die Faserdichte gegenüber der ER-MA und IR-Gruppe stark erhöht, übersteigt zum Teil tendentiell (aber nicht signifikant) die Faserdichte der ER-Gruppe. Dies gilt für fast alle gemessenen Laminae mit einzelnen Ausnahmen, bei denen das Signifikanzniveau jedoch nur knapp verfehlt wurde. Die Projektionen der

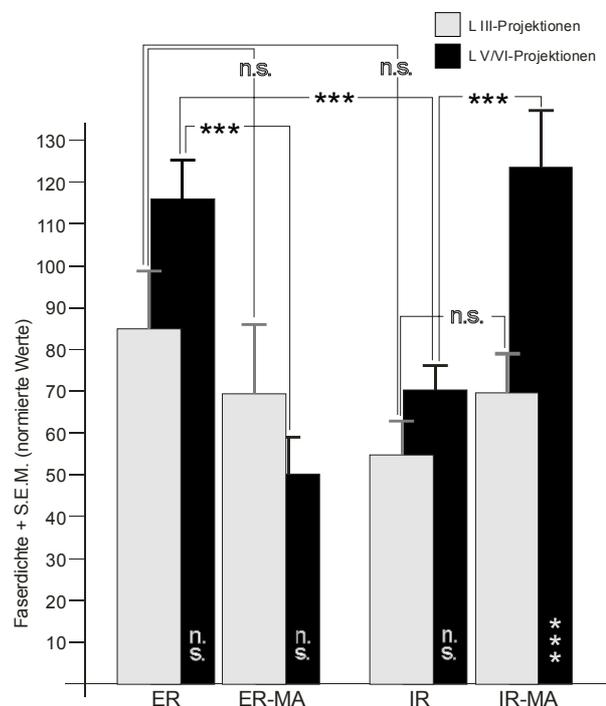


Abb. 9: Befunde aus dem Frontalkortex. In dieser und den beiden folgenden Abbildungen sind die einzelnen Laminae zusammengerechnet und in einer einzelnen Säule dargestellt worden. Für eine genauere Betrachtung der einzelnen Laminae siehe Bagorda et al (2005).

\*\*\*:  $p < 0,001$ ; \*\*:  $p < 0,01$ ; \*:  $p < 0,05$ .

Lamina III-Pyramiden scheinen durch die Interventionen nicht betroffen zu sein, hier findet sich nur in Lamina VI eine schwach signifikante Reduzierung der Faserdichte in der IR-Gruppe gegenüber den ER-Kontrollen.

- Im Parietalkortex (PC; Abb. 10) sind sowohl die oberflächlichen Projektionen (Lamina III-Pyramiden) als auch die tiefen Projektionen (Laminae V/VI-Pyramiden) beeinträchtigt. Man findet für beide Injektionstiefen und in fast allen untersuchten

Laminae eine Reduzierung der Faserdichten in den ER-MA und IR-Gruppen gegenüber den ER-Kontrollen, für die Lamina III-Projektionen gilt diese Reduzierung nach dem post-hoc-Test auch für die IR-MA-Gruppe, nur die Dichte der Laminae V/VI-Projektionen scheinen wie im FC auf dem Niveau der ER-Kontrollgruppe zu bleiben, im Untersuchungsfenster Lamina VI ist diese sogar signifikant erhöht. Im PC treten zusätzlich hochsignifikante Unterschiede innerhalb der IR-MA-Gruppe zwischen der Innervationsdichte von flachen und tiefen Projektionen auf, während sie in den übrigen Gruppen tendenziell gleich bleiben.

- Im insulären Kortex (IC; Abb. 11) sind hauptsächlich die flachen Lamina III-Projektionen betroffen. Hier findet sich in allen behandelten Gruppen eine größtenteils hochsignifikante Reduzierung der Faserdichte gegenüber den ER-Kontrollen wieder. Die Faserdichte der tiefen Laminae V/VI-Projektionen schwanken mit nicht signifikantem Unterschied um den Wert der Faserdichte der ER-Kontrollen, wobei auch hier eine tendenzielle Erhöhung bei der IR-MA-Gruppe zu erkennen ist. Diese wird sogar im Untersuchungsfenster Lamina VI signifikant zu den anderen drei Gruppen. Wieder ist ein signifikanter Unterschied zwischen der Faserdichte von Lamina III- und Laminae V/VI-Projektionen

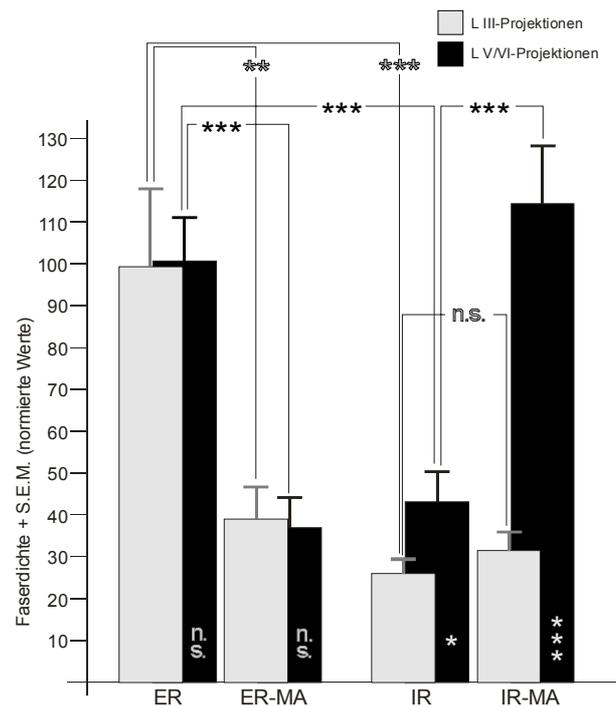


Abb. 10: Befunde aus dem Parietalkortex.

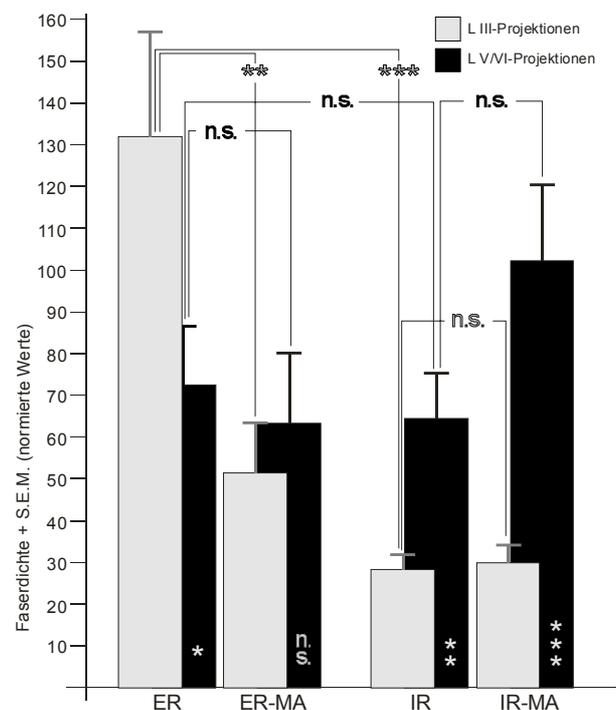


Abb. 11: Befunde aus dem insulären Kortex.

innerhalb der IR-MA-Gruppe zu finden (Lamina I, II, V und VI), wenn auch für die einzeln betrachteten Laminae nicht so stark wie im PC beschrieben. In den anderen drei Gruppen scheint der Unterschied zwischen Lamina III- und Laminae V/VI-Projektionen zwar deutlicher hervorzutreten, als im FC und PC, kann jedoch aufgrund einer hohen Standardabweichung das statistische Signifikanzniveau in der Betrachtung der einzelnen Laminae bis auf wenige Ausnahmen nicht erreichen.

Zusammenfassend ist festzuhalten: die Befunde zu den Faserdichten glutamaterger Pyramidenefferenzen in unimodale kortikale Regionen zeigen klar auf, dass sich Glutamat nicht etwa plastisch an die Interventionen anpassen kann, sondern mit dauerhaften Strukturveränderungen reagiert. Ein ganz anderes Resultat lieferten die Serotoninstudien (Neddens et al., 2003, 2004), deren Befunde folgend dargelegt sind.

## **Serotonin**

Die in den beiden Arbeiten (Neddens et al., 2003, 2004) eingegangenen immunhistochemischen Untersuchungen zur serotonergen Innervationsdichte beziehen sich auf Tiergruppen, die dem gleichen Ansatz unseres Tiermodells unterliegen wie schon die zuvor beschriebene Tracerstudie. In der ersten Arbeit (Neddens et al., 2003) wurden jeweils ca. 15 Tiere pro Gruppe (insgesamt 56 Tiere) untersucht. Der zweiten Arbeit (Neddens et al., 2004) lagen jeweils ca. 11 Tiere pro Gruppe (insgesamt 42 Tiere) zur Datenerhebung zugrunde. Die statistischen Analysen wurden in beiden Untersuchungen mittels MANOVA und dem post-hoc-Test nach Neuman-Keuls für multiple Vergleiche durchgeführt und ergaben folgende Befunde:

- Während die Faserdichten in subkortikalen und limbischen Arealen durch die einzelnen Interventionen in der Regel erhöht wurden (Ergebnisse der Co-Autoren; Neddens et al., 2003), konnte in den von mir untersuchten kortikalen Regionen FC, PC und IC keine signifikanten Unterschiede zwischen den vier einzelnen Gruppen festgestellt werden (bis auf eine schwach signifikante Reduzierung bei IR-MA gegenüber den tendenziell höheren Innervationsdichten der IR-Gruppe in den Untersuchungsfenstern der Laminae III und IV des IC).
- Grundsätzlich kann eine Asymmetrie in allen Tiergruppen zwischen linker und rechter Hirnhälfte bezüglich der serotonergen Innervationsdichte in fast allen untersuchten Regionen festgestellt werden (außer PC), wobei generell die rechte Hirnhälfte eine

deutlich höhere Faserdichte besitzt, mit Ausnahme im entorhinalen Kortex (EC) mit linksseitig höherer Dichte. Diese Asymmetrie wird aber in den kortikalen Regionen FC, PC und IC durch die unterschiedlichen Interventionen nicht signifikant verändert. Signifikante Unterschiede fanden die Co-Autoren (Neddens et al., 2004) nur im PFC bei der ER-MA-Gruppe und im EC bei der IR-MA-Gruppe in Form einer jeweiligen Verstärkung dieser Asymmetrie.

Als zusätzliches Ergebnis zu den serotonergen Befunden, die jedoch bisher noch nicht veröffentlicht wurden, konnte ein Unterschied der Innervationsdichte zwischen ER-Tieren, die im Sommer untersucht wurden, und ER-Tieren aus Untersuchungen im Winter festgestellt werden (Abb. 12). Die statistische Berechnung mit ANOVA ergab einen hochsignifikanten Unterschied in den oberen Laminae (Lamina I:  $p=0,00063$ ; Lamina II:  $p=0,00011$ ), der in den unteren Laminae nicht so deutlich (Lamina V schwach signifikant mit  $p=0,0216$ ) bis gar nicht (Lamina VI hat das Signifikanzniveau mit  $p=0,0568$  knapp verfehlt) darstellbar ist. Grundsätzlich zeigen "Wintertiere" eine höhere serotonerge Faserinnervation des parietalen Kortex als "Sommertiere".

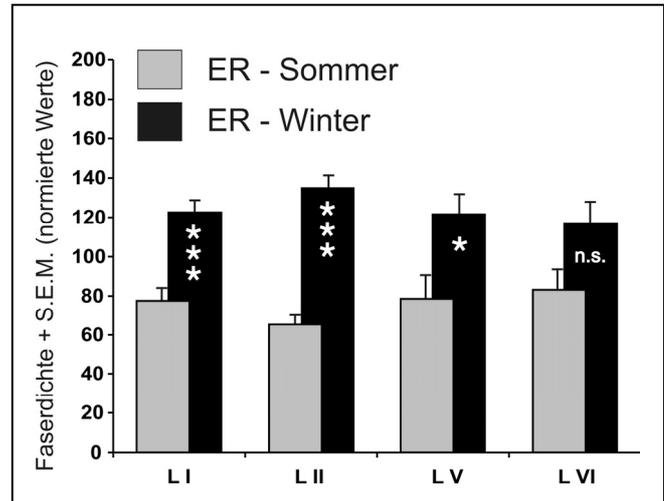


Abb. 12: Graphische Darstellung der Auswertung zur jahreszeitlichen Schwankung (Sommer vs. Winter) der serotonergen Innervationsdichte im parietalen Kortex (PC) von Kontrolltieren (ER).  $n$  (Sommer) = 5;  $n$  (Winter) = 7; \*\*\* =  $p < 0,001$ ; \*\* =  $p < 0,01$ ; \* =  $p < 0,05$ .

Zusammenfassend ist also festzustellen, dass Serotonin – anders als Glutamat – mit hoch plastischen Fähigkeiten auf die beiden Interventionen reagiert, d.h. Störgrößen auf Strukturebene kompensiert. Umso überraschender war der Befund, dass es andererseits jahreszeitliche Anpassungen von serotonergen Faserdichten im Kortex gibt.

Eine ganzheitliche Bewertung der quantitativen Analysen beider Transmitter, Glutamat und Serotonin, soll folgen.

#### 4. Zur Bewertung der adaptiven Transmitterveränderungen im Kortex

Für die glutamatergen Pyramidenefferenzen aus dem PFC gilt, dass die jeweiligen Interventionen (Isolationsaufzucht bzw. Methamphetamin-Intoxikation) eine verminderte glutamaterge Efferenz des PFC zu den untersuchten Kortexarealen FC, PC und IC reifen lässt. Dieses unterstützt die einleitend angesprochenen Vorstellungen, dass eine Glutamat-Hypofunktion bei Schizophrenen und eine dadurch bedingte Hypofrontalität zu beobachten ist. In dieses Geschehen sollte sich auch die kortikostriale Projektion in einer Rückkopplungsschleife integrieren (Carlsson, 1988; Carlsson und Carlsson, 1989, 1990b). Diese zieht vom frontalen Kortex über das Striatum zum Thalamus und zurück zum Kortex (Abb. 13). Sie soll den Kortex vor einem Überangebot an sensorischer Information schützen, die im Thalamus gefiltert wird. Die hypothetische

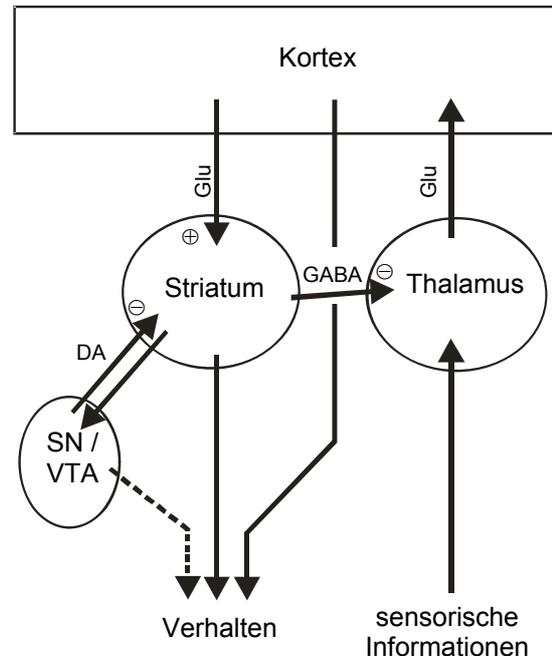


Abb. 13: Darstellung einer kortiko-striato-thalamischen Rückkopplungsschleife (nach Carlsson, 1988).

Unterfunktion der kortikostriatalen Projektion würde den thalamischen Filter öffnen, den Informationsfluss zum Kortex erhöhen und dadurch zu psychotischem Erleben führen.

Diese Fehlfunktion des *sensory gating* wurde auch in postmortem Studien (Pakkenberg, 1990), MR-Studien (Andreasen et al., 1990, 1994) und PET-Studien (Buchsbaum et al., 1996) gezeigt. Von einigen Autoren wird diese Hypofunktion im Zusammenhang mit einer "*kognitiven Dysmetrie*" und somit einer funktionellen *Diskonnektion* in Form einer Unterbrechung des Informationsflusses diskutiert (Schlösser et al., 2005; Andreasen et al., 1998, 1999; Friston, 1998). Aus unseren Befunden kann nunmehr abgeleitet werden, dass eine eigentliche Ursache für diese frontostriatale *Disfunktion* im Kontrollverlust aus dem PFC aufgrund reduzierter *U-Faserungen* in den FC beruht.

In diesem Sinn stellt sich auch Lewis eine differenziertere Betrachtung der Zusammenhänge vor, die unseren Befunden bereits nahe kommen: da die Efferenzen des

dorsolateralen PFC von Pyramidenzellen stammen, die sich in unterschiedlichen Laminae befinden, könnten laminaspezifische Abnormalitäten im PFC schizophrener Patienten Hinweise über die durch die Störung betroffenen Verbindungen geben (Lewis, 2002). Eben dafür gab es aber bislang keine konkreten Belege. In der vorliegenden Tracerstudie (Bagorda et al., 2005) wurde nunmehr zwischen Projektionen der Lamina III und Projektionen der Laminae V/VI unterschieden. Hierbei zeigte sich in Abhängigkeit der jeweiligen Zielgebiete, dass die Projektionen des PFC durch die entsprechende Intervention (Isolationsaufzucht oder Methamphetamin-Intoxikation) nicht überall gleichmäßig betroffen sind: Lamina III-Projektionen waren im IC und PC, Laminae V/VI-Projektionen im PC und FC betroffen. Hierbei führten die einzelnen Interventionen in der Regel zu einer Reduzierung der glutamatergen Faserdichte.

Interessanterweise hat die kombinierte Intervention aus Isolationsaufzucht und Methamphetamin-Intoxikation nicht den gleichen oder gar verstärkten Effekt auf die Faserdichten: während Fasern aus Lamina III-Projektionen weiterhin in den untersuchten Arealen reduziert werden, erfahren die Laminae V/VI-Projektionen eine exzessive Reifung auf das Niveau der Gehegetiere. Die Interpretation dieses Befundes könnte sein, dass eine in der Entwicklung des Gehirns laufende natürliche Reduzierung (*Pruning*) präfrontaler Efferenzen, die nach dem Einwachsen von z.B. thalamokortikalen Verbindungen einsetzt (Van Eden und Uylings, 1985; Van Eden, 1986; Van Eden et al., 1990), nicht stattgefunden hat, oder dass ein verstärktes *Sprouting* der glutamatergen Efferenzen, ähnlich wie bei der Regeneration von Läsionen im serotonergen System (Azmitia et al., 1978; Zhou und Azmitia, 1984; Gage et al., 1983), dem Effekt der Reduzierung entgegenwirkt. Eine laufende Entwicklungsstudie aus unserer Arbeitsgruppe soll diese offene Frage noch klären.

Welche Ursachen sich auch immer mit den unterschiedlichen Effekten nach einfacher bzw. nach doppelter Belastung der Tiere verbinden: wichtig scheint mir die Feststellung, dass es zwei voneinander deutlich abweichende pathologische Phänomene gibt, welche die Schizophrenie hinterlegen können. Die gleichmäßige Reduzierung der Faserdichten von Pyramiden der flachen und tiefen Laminae durch jeweils eine Intervention spiegelt nur eine an die äußeren Umstände angebundene angepasste Entwicklung wider. Möglicherweise stellt sich das insgesamt als eine höhere Vulnerabilität des ganzen Systems dar. Dies sollte ein erster Schritt auf dem Weg in die psychische Störung sein.

Erst in einem zweiten Schritt wird eine "Dyskonnektion" ausgelöst, die also eine erhebliche *Imbalance der Glutamininnervation* aus dem Stirnhirn in neokortikale Regionen erzeugt. Wie in der Einleitung schon erwähnt, konnten z.B. im LSD-Modell schizophrene Symptome erzeugt und eine Erhöhung der Aktivität von Lamina V-Pyramiden festgestellt werden (siehe unter Kapitel 2.1: *Serotonin-Hypothese*). Unser Tiermodell zeigt ein Ungleichgewicht zwischen Lamina V/VI- und Lamina III-Pyramiden in Form einer deutlich stärkeren Ausprägung der Lamina V/VI-Projektionen gegenüber den Lamina III-Projektionen. Um die eingangs gestellte Frage nach einer *hyperglutamatergen* (LSD-Modell) oder einer *hypoglutamatergen* Funktion (PCP-Modell, siehe Kapitel 2.1: Glutamat-Hypothese) als Ursache schizophrener Störungen zu beantworten: beide Aspekte sind richtig, wenn man sie in einem Lamina-abhängigen Gleichgewichtssystem zwischen

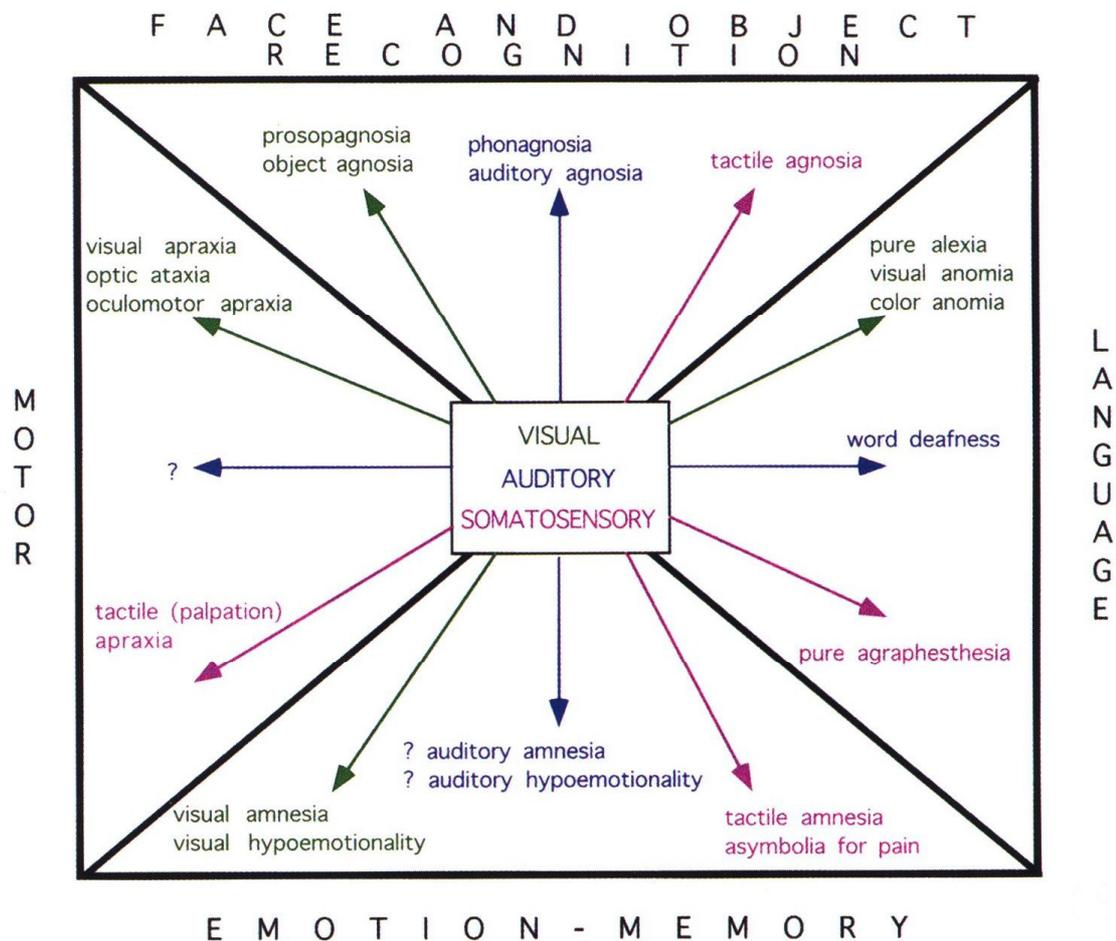


Abb. 14: Übersicht über mögliche Diskonnektions-Syndrome, die nach einer Diskonnektion unimodaler sensori-fugaler Verbindungen in den visuellen (grün), auditorischen (blau), und somatosensorischen (rot) Modalitäten auftreten können (aus Mesulam, 2000).

infragranulären und supragranulären Pyramidenzellen betrachtet, in der eine *Imbalance* zugunsten der tieferen Lamina V/VI-Pyramiden entsteht.

Bemerkenswert ist weiterhin, dass diese "Dyskonnektion" gleiche Auswirkungen auf den frontalen, parietalen sowie den insulären Kortex hat. Somit sollte sich eine frontostriäre Funktionsstörung wie durch die "*kognitive Dysmetrie*" gezeigt (Andreasen et al., 1998, 1999; Friston, 1999, Schlösser et al. 2005), auch auf parietale, sowie auf insulär- limbische Schaltkreise übertragen lassen. Das kann nur bedeuten, auch andere Befindlichkeitskontexte, die aus dem Präfrontalhirn gesteuert werden, können bei der Schizophrenie aktuell gestört werden. Denn letztendlich konvergieren alle Sinneseindrücke und Informationen nach dem kaskadenförmigen Durchlaufen von primären und sekundären (=unimodale) Sinnesfeldern und der Weiterverarbeitung in heteromodalen tertiären Assoziationsarealen des Präfrontal-, Frontal-, Parietal- und Temporalhirns in den supramodalen limbischen Assoziationsfeldern Hippocampus und Amygdala (Mesulam, 2000). Hier erfolgt das "*sensory gating*", das Ausfiltern von unwichtigen Informationen, in Zusammenarbeit mit dem vorgeschalteten Kortex. Mesulam zählt in seinem Buch mehrere *Diskonnektions-Syndrome* auf, die aufgrund einer *Diskonnektion* unimodaler sensorifugaler Verbindungen visueller, auditorischer oder somatosensorischer Modalität auftreten: er nennt neben motorischen (Apraxie/Ataxie), emotionalen (Hypoemotionalität) und gedächtnisbildenden (Amnesie) Aspekten auch solche, die Sprache (Alexie/Anomie) und Gesichts- bzw. Objekterkennung (Agnosie) beinhalten (siehe Abb. 14; aus Mesulam, 2000).

Eine zentrale Bedeutung im Geschehen der "Dyskonnektion" kommt den Pyramiden des PFC zu. So forderten bereits Levin und Mitarbeiter, dass nur das Studium der PFC-Schaltkreise während der Reifung Einblicke in die Qualität disfunktionaler Entwicklungsprozesse geben könne (Levin et al., 1991).

Lewis und die Arbeitsgruppe um Levitt stellen sich folgendes Modell vor: PFC-Pyramidenzellen der Laminae II und III senden ihre intrinsischen Axonkollaterale, deren Terminationsgebiete in streifenförmigen Feldern organisiert sind, horizontal durch die supragranulären Laminae (II und III). Auch die Pyramidenzellen selbst, die diese intrinsischen Axonkollaterale abgeben, sind in solchen Streifen organisiert (Levitt et al., 1993; Lewis, 1997). Die Projektionen zwischen diesen untereinander verbundenen Streifen sind reziprok, und die meisten dieser Axonterminalien binden an dendritischen Spines anderer Pyramidenzellen (Pucak et al., 1996). Diese monosynaptischen Verbindungen zwischen den Streifen könnten das anatomische Substrat bieten für die *anhaltende*

*neuronale Aktivität* von PFC-Pyramiden der Lamina II und III während der Verzögerungsphase im "*delayed response Test*", und – da dieser Test auch in der Schizophrenieforschung eingesetzt wird – ein Hinweis auf die Einbindung dieser supragranulären Pyramidenzellen in der Pathophysiologie der Schizophrenie sein (Lewis und Anderson, 1995).

Ein weiterer Aspekt für die Verwicklung der Lamina II/III-Pyramiden in die Schizophrenie liegt auch darin, dass die kritischen Umstrukturierungen des synaptischen Inputs dieser Neurone während der Adoleszenzphase stattfindet: die synaptische Dichte des Kortex erhöht sich während der frühen postnatalen Entwicklung und nimmt dann während der Adoleszenzphase wieder ab (Huttenlocher, 1979), wobei die supragranulären Pyramidenzellen (Lamina II und III) stärker betroffen sind als die der infragranulären Schichten (Lamina V und VI) (Bourgeois et al., 1994). Diese substanzielle Reduzierung des exzitatorischen Inputs der supragranulären Pyramiden des PFC während der Pubertät kann als Konsequenz eines *Prunings* intrinsischer Axonkollaterale angesehen werden, die die oben beschriebenen räumlich getrennten streifenförmigen Neuronenkluster verbinden (Lewis, 1997), und die eine selektive Vulnerabilität in Zusammenhang mit dem Beginn einer schizophrenen Symptomatik nach der Reifung des PFC ermöglicht (Woo et al., 1997; Lewis, 1997).

Eine Entwicklungsstudie, die derzeit an unserem Tiermodell durchgeführt wird, könnte die aufgeworfenen Fragen klären:

- Werden intrinsische Axonkollaterale früh in der Entwicklung des Gehirns transient angelegt und findet ein frühkindliches *Pruning* statt?
- Gibt es kritische Zeitfenster in der Reifung und Umstrukturierung der Projektionen, die durch schädigende Faktoren gestört wird?
- Findet diese Umstrukturierung Lamina-abhängig statt und kommt es dadurch bedingt bei Störungen während der Reifung zu Lamina-spezifischen Schädigungen?

Das Vorhandensein der "Dyskonnektion" ist durch die vorliegende Studie anatomisch gezeigt worden, die genaue Entwicklung zu dieser "Dyskonnektion" hin muss jedoch erst durch die - dieser Arbeit folgende - Entwicklungsstudie erarbeitet werden.

In unserem Tiermodell wird weiterhin erkennbar, dass sich - ganz anders als Glutamat oder Dopamin (siehe Einleitung), die mit einer starren Strukturveränderung reagieren - das Serotoninsystem auf die eingesetzten Interventionen flexibel anpasst.

In der Serotoninarbeit (Neddens et al., 2003) konnten wir im medial präfrontalen Kortex (Cg3) und im Entorhinalkortex (Ent) zwar signifikante Unterschiede zwischen den einzelnen Gruppen unseres Tiermodells feststellen, die auch in der Literatur in ähnlicher Weise referiert werden (Berger et al., 1985; Towle et al., 1989). Für die von mir untersuchten Areale im FC, PC und IC wurden jedoch keine signifikanten Unterschiede ermittelt. Wie schon für limbische Strukturen nachgewiesen (Azmitia et al., 1978; Zhou und Azmitia, 1984), scheinen auch die von mir untersuchten kortikalen Strukturen eine hohe Regenerations- oder Anpassungsfähigkeit durch *sprouten* serotonerger Axonkollaterale zu besitzen. In *unimodalen* Kortextfeldern reguliert sich Serotonin nach meinen Befunden immer auf einen Standardwert ein, egal welche Intervention zur Wirkung kommt, sogar wenn beide Interventionen gemeinsam eingesetzt werden. Damit bestätigt unser nicht-invasives Modell die umfangreiche Literatur zu den plastischen Fähigkeiten speziell von Serotonin im Gehirn (Fischer et al., 1995; Sotelo, 1991; Wilson und Molliver, 1991a, 1991b). Die Frage bleibt offen, welche Faktoren diese enorme Anpassungsfähigkeit veranlassen?

Das hohe Anpassungsvermögen von Serotonin zeigt sich in meinen Studien auch darin, dass das serotonerge System sich plastisch adaptiv jahreszeitlichen Schwankungen unterzieht, der oben beschriebene Standardwert also einer annualen Rhythmik unterliegt. "Wintertiere" weisen dabei eine höhere Faserdichte als "Sommertiere" auf. Dieses Ergebnis ist deshalb interessant, weil die saisonale Schwankung des serotonergen Systems auch in der Psychiatrie, besonders bei affektiven Störungen und Winterdepressionen (Fossey und Shapiro, 1992; Neumeister et al., 2001; Khait et al., 2002; Schwartz et al., 1999; Castrogiovanni et al., 1998) diskutiert wird. Auch wenn man die unter dem Begriff "*season-of-birth*"-Effekt bekannte Korrelation zwischen höherer Schizophrenieanfälligkeit und der Geburt in Wintermonaten (Franzek und Beckmann, 1992; Davies et al., 2003; Brown und Susser, 1996), oder auch die Korrelation zwischen Jahreszeit und Ausbruch schizophrener Symptome (Owens und McGorry, 2003) nicht direkt mit der saisonalen Schwankung des serotonergen Systems in Zusammenhang bringen konnte, halte ich den störenden Einfluss einer serotonergen Hyperaktivität im Winter auf die pränatale und frühe postnatale Reifung anderer Transmittersysteme als durchaus denkbar. Hinweise hierzu gibt schon das Modell einer inversen trophischen Interaktion zwischen Serotonin und Dopamin, das Cunningham und Mitarbeiter erstellt haben (Cunningham et al., 2005).

Fazit der hier vorgetragenen Betrachtungen ist: die einerseits hohe Plastizität von Serotonin im pathologischen Geschehen einer limbo-präfrontalen "Dyskonnektion" korreliert mit einer andererseits unflexiblen Reaktivität von Glutamat und Dopamin.

Insbesondere Tiermodelle, die primär auf *glutamaterge* Anormalitäten basieren, rücken in jüngster Zeit ins Zentrum der Interesse der Schizophrenieforschung, zeigen sie doch wichtige heuristische Eigenschaften hinsichtlich neuer potentieller Therapieansätze (Lipska und Weinberger, 2000). Die derzeit verwendeten Modelle z.B. mit Zugang über NMDA-Antagonisten berücksichtigen jedoch nicht die *Entwicklung* des Gehirns zur schizophrenen Störung. Hier kann mit unserem Tiermodell, in dem neben Glutamat auch Dopamin und Serotonin sowie der Aspekt der Balance zwischen diesen Transmittern untersucht ist, gerade dieser wichtigen Punkt der Genese berücksichtigt, und die Ergebnisse in der Forschung zur pharmakologischen Therapie oder gar Prävention eingesetzt werden.

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## 6. Publikationen

### 6.1 Bagorda et al. (2005)

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Autoren: F. Bagorda, G. Teuchert-Noodt, K. Lehmann

Titel: Isolation rearing or methamphetamine traumatization induce a "dysconnection" of prefrontal efferents in gerbils: implications for schizophrenia.

Zeitschrift: Journal of Neural Transmission (2005): *in press*<sup>1</sup>

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Zusammenfassung: A miswiring of prefrontal efferents is generally discussed by the name of "dysconnection" as the anatomical substrate of schizophrenia. Since direct histological confirmation of this hypothesis can hardly be obtained in humans, we used an animal model of schizophrenia to trace prefrontal efferents to distal cortical fields. Mongolian gerbils were intoxicated with a single high dose of methamphetamine on postnatal day 14 and reared in isolation after weaning (day 30). Controls received a saline injection and/or were reared under enriched conditions. Upon reaching adulthood (day 90), biocytin was injected into the medial prefrontal cortex into either deep or superficial laminae. The density of passing fibres and terminal fields in the frontal, parietal and insular cortices was assessed by digital image analysis. Isolation rearing or methamphetamine treatment alone reduced the projections from lamina V/VI to the frontal and from lamina III to the insular cortex, and from both laminae to the parietal cortex. In contrast, isolation rearing of methamphetamine-intoxicated gerbils significantly increased the projections from the deep laminae to the frontal and parietal cortices, compared to isolation-reared controls, with no difference in the efferents from outer laminae. These results are the first to demonstrate a miswiring of prefrontal efferents in response to adverse systemic influences. They might give a hint at the anatomical basis of "dysconnection" in schizophrenia.

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<sup>1</sup> Zum Zeitpunkt des Druckes der vorliegenden Arbeit lag die Publikation Bagorda et al. (2005) nur in der Online-Version vor, es konnten daher außer der DOI noch keine weiteren Angaben über Band, Heft und Seitenzahl gemacht werden.

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**Isolation rearing or methamphetamine traumatization  
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implications for schizophrenia**

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**Summary.** A miswiring of prefrontal efferents is generally discussed by the name of “dysconnection” as the anatomical substrate of schizophrenia. Since direct histological confirmation of this hypothesis can hardly be obtained in humans, we used an animal model of schizophrenia to trace prefrontal efferents to distal cortical fields. Mongolian gerbils were intoxicated with a single high dose of methamphetamine on postnatal day 14 and reared in isolation after weaning (day 30). Controls received a saline injection and/or were reared under enriched conditions. Upon reaching adulthood (day 90), biocytin was injected into the medial prefrontal cortex into either deep or superficial laminae. The density of passing fibres and terminal fields in the frontal, parietal and insular cortices was assessed by digital image analysis. Isolation rearing or methamphetamine treatment alone reduced the projections from lamina V/VI to the frontal and from lamina III to the insular cortex, and from both laminae to the parietal cortex. In contrast, isolation rearing of methamphetamine-intoxicated gerbils significantly increased the projections from the deep laminae to the frontal and parietal cortices, compared to isolation-reared controls, with no difference in the efferents from superficial laminae. These results are the first to demonstrate a miswiring of prefrontal efferents in response to adverse systemic influences. They might give a hint at the anatomical basis of “dysconnection” in schizophrenia.

**Keywords:** Glutamate, prefrontal cortex, two-hit-model, microcircuit, macrocircuit.

### **Introduction**

Although there is little doubt on the idea that a so-called “dysconnection” of the prefrontal cortex (PFC) from temporolimbic cortical fields and subcortical areas is a central anatomical correlate of schizophrenia (Feinberg, 1982/83; Weinberger and Lipska, 1995), the exact nature of this “dysconnection” is

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remarkably unclear. Evidence so far suggests that a disturbance during cortex development creates an aberrant layering in both prefrontal (PFC) and entorhinal (EC) cortices (Jakob and Beckmann, 1986; Benes et al., 1991; Akbarian et al., 1993a, b), which is accompanied by altered dendritic morphology of PFC pyramidal cells and a reduction in somatic inhibition by a subclass of GABAergic neurons (Glantz and Lewis, 2000; Kalus et al., 2000; Black et al., 2004). It is assumed that these cytoarchitectural abnormalities result in a faulty, “noisy” miswiring of prefrontal-temporolimbic connections (Weinberger and Lipska, 1995), providing the vulnerability for the later outbreak of a psychotic illness. If further harmful events during or after adolescence then impair the complete maturation of the PFC’s dopamine (DA) innervation (Akil et al., 1999), the resulting imbalances in PFC microcircuits unmask the pathology of the PFC-EC macrocircuits, which then becomes apparent in psychotic symptoms (Winterer and Weinberger, 2004). The central assumption in this compelling picture, i.e. the aberrant connectivity of PFC efferents, has so far only been inferred from analogy to metachromatic leukodystrophy (Hyde et al., 1992), and has been supported by both functional and diffusion tensor imaging (Meyer-Lindenberg et al., 2002; Hubl et al., 2004; Wang et al., 2004). Methodological limitations to date prevent the clarification of how exactly PFC pyramidal axons are miswired in schizophrenic patients.

To overcome these limitations, a variety of animal models of schizophrenia have been designed. Neonatal ibotenic acid lesion of the rat’s ventral hippocampus induces an aberrant maturation of PFC projections and results in behavioural disturbances that mimic those found in schizophrenic patients (see Lipska, 2004 for review). Responsivity to stress and DA agonists, PFC pyramidal cell morphology and firing patterns and a series of molecular parameters are also altered in this model in a similar way as in human schizophrenics (Lipska, 2004). Aiming at a two-hit-model of trauma-induced schizophrenia (see Read et al., 2001 for review) without invasive lesion, we designed and extensively studied an animal model which employs early postnatal trauma by methamphetamine (MA) intoxication, which lesions PFC DA terminals by oxidative stress (Teuchert-Noodt and Dawirs, 1991; Seiden and Sabol, 1996), as a developmental disturbance, and isolation rearing (IR) as an intervention that suppresses the maturation of PFC DA fibres during adolescence (Winterfeld et al., 1998; Neddens et al., 2001). In this model, we have previously been able to demonstrate changes in cortical and subcortical dopamine, serotonin and acetylcholine innervation (Dawirs et al., 1993, 1994; Neddens et al., 2003; Lehmann et al., 2002, 2003, 2004; Busche et al., 2005) and shifts in the lateralisation of transmitter systems (Neddens et al., 2004), all of which occur in a similar form in human schizophrenics. They result in impairments of working memory and prefrontal control, social deficits and an increased response to stress (Dawirs et al., 1996; Polascheck, 2004). Furthermore, the PFC pyramidal cells’ dendritic morphology is altered (Blaesing et al., 2001), and dendritic GABAergic inhibition is increased (Nossoll et al., 1997), suggesting an aberrant reorganisation of prefrontal microcircuits (Teuchert-Noodt, 2000; Dawirs and Teuchert-Noodt, 2001).

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Thus observing both local abnormalities in the PFC and disruptions in transmitter balances between the PFC and temporolimbic and striatal fields (Neddens et al., 2002; Busche et al., 2002, 2004), we were led to ask how the direct projections of the PFC might be altered by early MA traumatization and IR in gerbils. In order to answer this question, we injected biocytin into both superficial and deep laminae of the PFC and measured the fibre densities in the terminal fields in the frontal, parietal and insular cortex. We here report substantial abnormalities in the layering of prefrontal efferents in MA-treated IR gerbils, which is to our knowledge the first anatomical demonstration of prefrontal “dysconnection” by postnatal systemic influences.

## Materials and methods

### *Animals and rearing conditions*

Male gerbils were bred in our facilities either in standard cages or in semi-naturally structured compounds (for details, see Winterfeld et al., 1998). On postnatal day (PD) 14, some of the pups from each condition were injected with a single dose of 50 mg/kg methamphetamine hydrochloride (MA, Sigma, M-8750); the others received a saline injection. Applied at this age, methamphetamine exclusively lesions prefrontal DA fibres, whereas its effect shifts to striatal regions at later ages (Teuchert-Noodt and Dawirs, 1991). At weaning (PD30), in total 47 gerbils, saline or MA-treated, were assigned to either isolated (IR) or enriched (ER) rearing conditions for 60 days. IR animals were kept singly in standard makrolon cages, ER animals lived in sibling groups in compounds similar to the ones they were born in. Under both sets of conditions there was a bedding of wood shavings, and food and water were provided *ad libitum*. All gerbils were kept on natural day/night cycles.

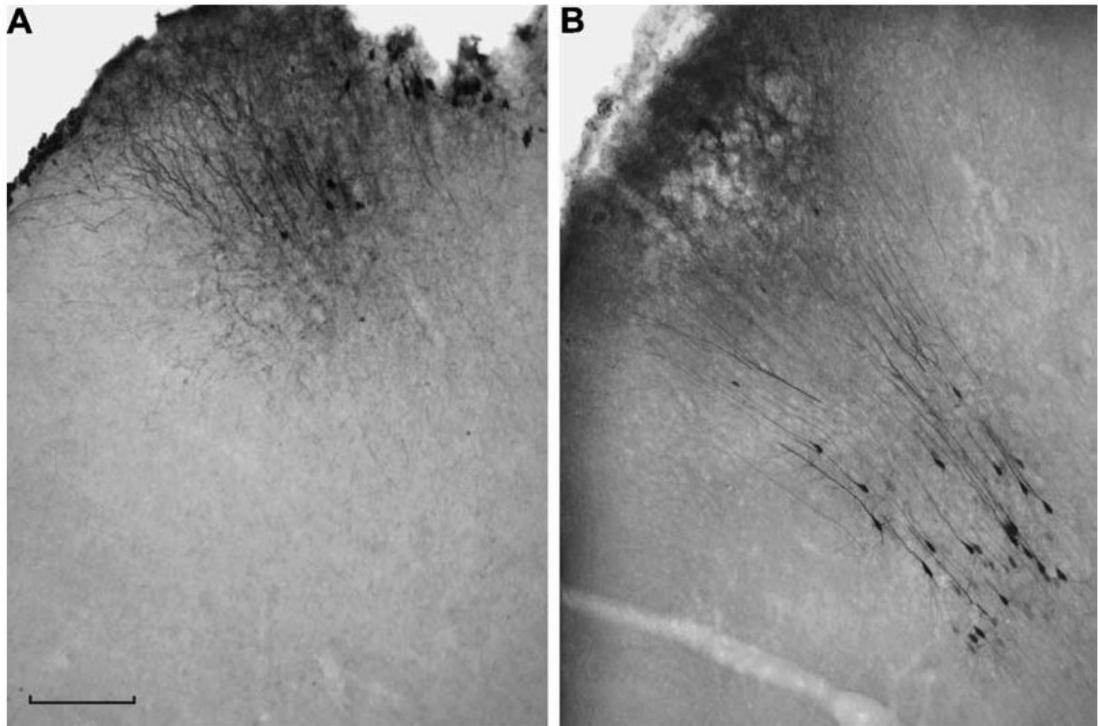
All experimental procedures were approved by the Bezirksregierung Detmold in 1999 (no. 23.0503.1.1.IV/99).

### *Biocytin fibre tracing*

On postnatal day (PD) 90, the animals were anaesthetised with diethylether, fixed into a stereotaxic frame, and a hole was drilled into the skull at the level of the prefrontal cortex. 3 µl of 0.5% biocytin solution in PB were injected at midline into the shoulder subfield which encompasses the medial precentral (Fr2) and the dorsal-anterior cingulate (Cg1) cortex at 4.5 mm anterior to bregma (Thiessen and Yahr, 1977). This field has reciprocal connections with motor, mixed somatosensory motor and somatosensory association cortices and occipital cortical fields (Van Eden et al., 1992; Reep et al., 1990; Donoghue and Parham, 1983). Both superficial injections aiming at pyramidal cells in lamina III and deep injections targeting lamina V/VI-pyramids were performed (Fig. 1). The skull was closed with histoacryl.

After 24 h, the animals were put down by diethylether and immediately perfused with 200 ml 0.1 M phosphate buffer (PB, pH7.4), followed by 4% paraformaldehyde and 0.5% glutaraldehyde in 0.1 M PB (pH7.4). The brains were removed and kept in fixative for one week in order to increase background staining. Frontal sections of 60 µm thickness were cut on a frigidomobile (Reichert-Jung) and every other section was collected in 0.01 M PB (pH7.4). The slices were treated with 1% sodium borohydride (Sigma) for 20 min, washed three times and incubated overnight with ExtrAvidin-Peroxidase (Sigma) diluted 1:125 in 0.01 M PB containing 1% bovine serum albumin and 0.5% triton X-100 (Sigma). The following day, the sections were washed twice in PB, twice in 0.05 M Tris-buffer (pH7.6) and stained by DAB reaction. After five last washes in PB, the sections were mounted on coated glass slides, dried overnight and coverslipped in DePeX.

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**Fig. 1.** Prefrontal pyramidal cells filled by an injection of biocytin into the superficial (A) or deep (B) layers of the medial prefrontal cortex (Cg1/Fr2-region). Scale bar is 200  $\mu$ m

### *Qualitative evaluation of projection patterns*

In order to gather an overview of the projection patterns and get a general impression of putative changes, camera lucida drawings were made of three sections per animal. The rostrocaudal positions at which these sections were chosen are: a) the commissura anterior (CA) approaches the lateral ventricle (3.5 mm anterior to bregma), b) the CA crosses (2.5 mm anterior to bregma) and c) ca. 100  $\mu$ m rostral to the septal pole of the hippocampus (0.5 mm anterior to bregma).

Furthermore, projections to the striatum which elude computerised quantitative assessment due to the optical problems caused by myelinated fibre bundles, were viewed in a dark-field microscope, ranked according to their density in IR and MA-intoxicated IR gerbils and analysed by non-parametric tests (Wilcoxon, Kolmogoroff-Smirnov).

### *Computerised assessment of terminal field densities*

Prefrontal projections enter their receptive fields in distinct columns spanning the whole depth of the cortex. For evaluation, we chose three characteristic columns which reliably appear at the rostrocaudal level of the commissura anterior crossing (2.5 mm anterior to bregma), i.e. in the frontal, parietal and insular cortices. In the frontal cortex, prefrontal and premotor aspects are intermingled, the parietal cortex contains the barrel field, and the insular cortex represents the caudal end of the orbital PFC, as it borders on the EC. Three neighbouring sections at this level were evaluated.

The sections were viewed in dark field at 125 $\times$  magnification on a microscope (Polyvar, Reichert-Jung). Pictures were taken by a digital camera (ProgRes 3008 mF, Jenoptik, Jena) and processed by a software for image analysis (KS300, Zeiss, Jenoptik, Jena). Three separate pictures were taken of superficial, middle and deep laminae, converted to black-and-white pictures and inverted. Background was suppressed by high-pass filtering. Fibres were recognised by a function ("valleys operator") that detects local brightness minima and represents them as lines

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of one pixel width. The area of these lines was calculated as percentage of the measurement window, which was the same size for all laminae. In FC and PC, laminae I, II, V and VI were evaluated separately. In IC, all six cortical laminae were investigated.

All measurements (both qualitative and quantitative) were done by investigators who were blind to the respective treatment of each specimen.

#### *Statistical analysis*

We compared the fibre densities in each lamina of a projection area among the groups for a given injection depth, and within each group between the injection depths. Comparisons between groups were done by two-way analysis of variance (ANOVA), using two main factors of treatment and rearing and a repeated-measurements factor of layers. Subsequently, single comparisons were performed by LSD-post hoc-testing. Differences within a group and layer between injection depths were evaluated by t-test after F-test. For representational reasons, all obtained densities are given both as original values (% fibres of total area) and as normalized values. For this, the combined mean of superficial and deep injections of the ER control group in each measurement window was defined as 100%. Data are represented as means  $\pm$  standard error of means (S.E.M.). The levels of significance were set at  $p < 0.05$  (\*),  $p < 0.01$  (\*\*) and  $p < 0.001$  (\*\*\*).

## Results

### *Qualitative analysis*

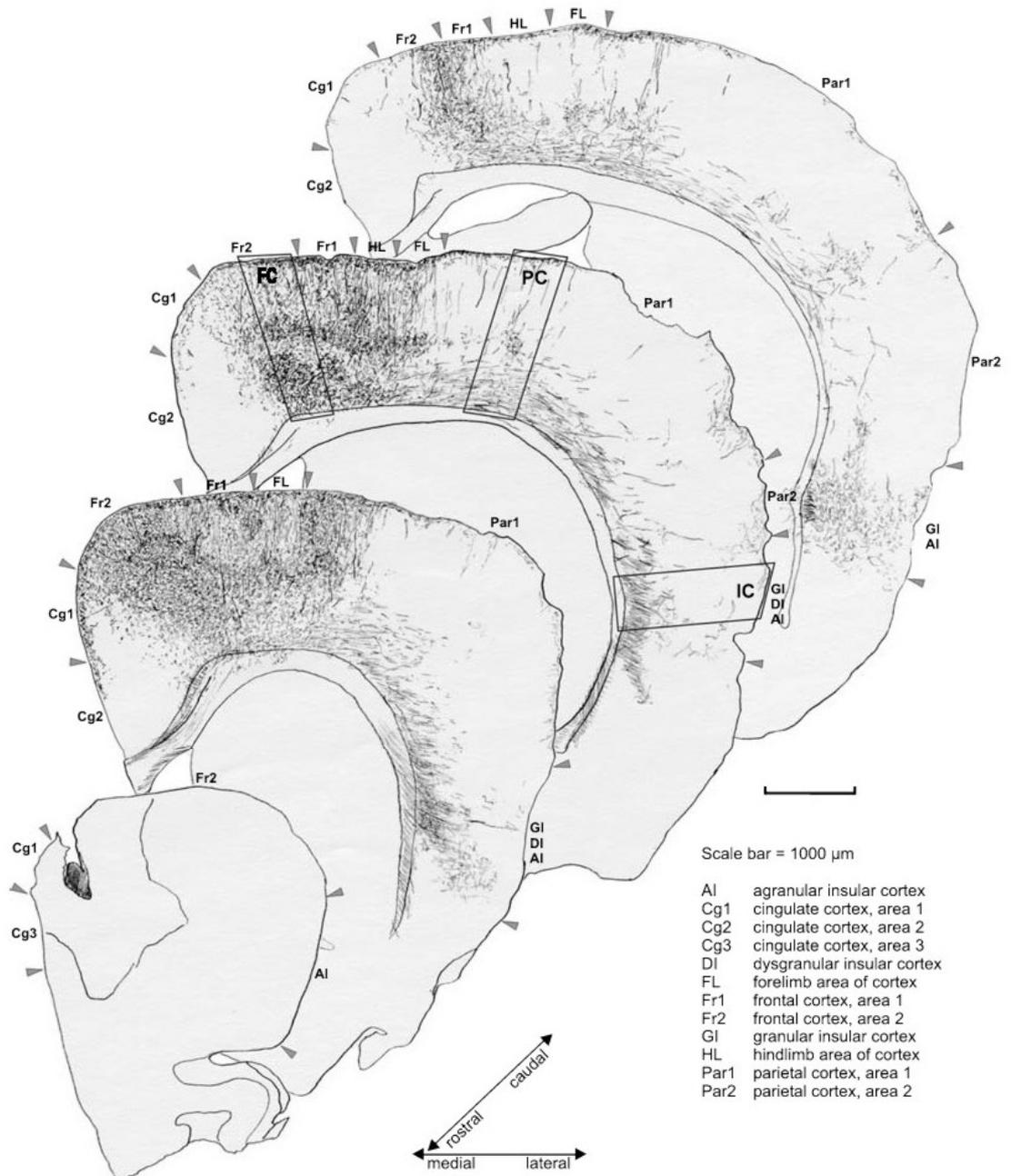
The camera lucida drawings shown in Fig. 2 give a representative impression of the typical cortical termination patterns (subcortical fibres not shown) after a deep injection. Superficial injections result in a similar, but less heavily stained picture. Fibres descend from the PFC towards the corpus callosum (targeting contralateral and distal cortical fields), towards the caudate-putamen and deeper subcortical areas, and towards caudal and lateral cortical fields. These latter projections, which reach frontal, parietal and insular cortices, are very dense in the vicinity of the PFC and become more sparse in the distance. They preferentially surface from lamina VI upwards, but some also descend from lamina I, forming well-defined columns which are segregated into fibres of passage in laminae II/III and VI, and dense, intertwined terminal fields in laminae I, upper V and VI (Fig. 2).

The non-parametric comparison of striatal projections was only done for MA-intoxicated and control IR animals out of technical reasons. Both the Kolmogorov-Smirnov and the Wilcoxon-test confirmed that projections from superficial laminae were significantly ( $p < 0.05$ ) weaker in MA-treated than in saline-treated IR animals (data not shown).

### *Quantitative analysis*

In the frontal cortex (FC, Fig. 3), ANOVA did not show any effects of MA treatment or rearing on the projection densities from either superficial or deep injections. Post-hoc testing, however, revealed a significant reduced projection from shallow injections to layer VI by IR in saline-treated animals, and a large number of effects in the comparison of deep injections: In saline-treated animals, IR reduced the terminal density in layers I and V ( $p = 0.057$  for layer VI), whereas IR effected an increase in MA-treated gerbils in all layers. Within the ER groups, MA-intoxicated animals had markedly reduced terminal densities in

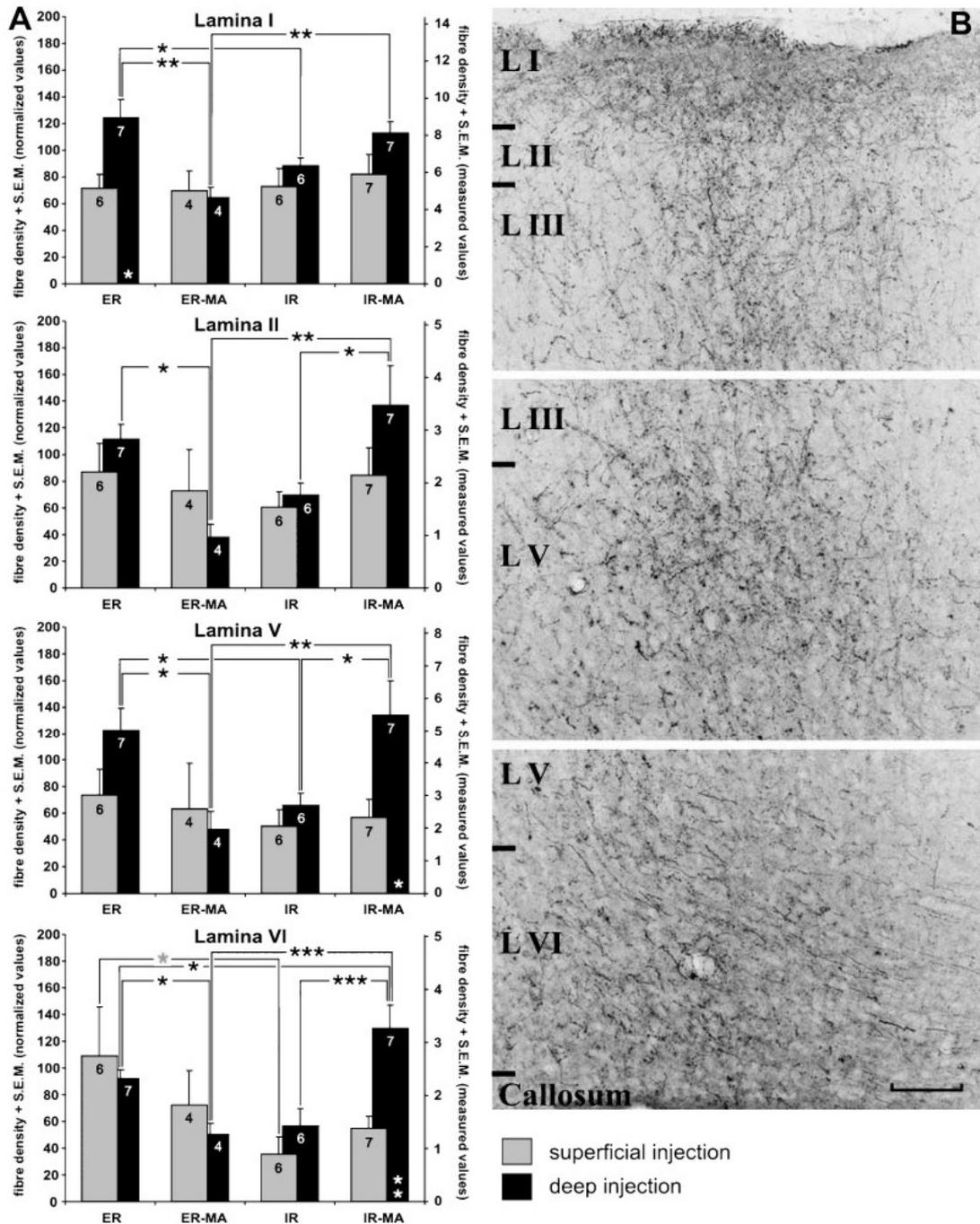
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**Fig. 2.** Camera lucida drawings of selected frontal slices through an exemplary gerbil brain with efferents of the medial prefrontal cortex (Cg1/Fr2-region) stained by a deep biocytin injection. The most rostral section indicates the position and depth of the injection. The boxes in the third section depict the columns in the frontal cortex (FC), parietal cortex (PC) and insular cortex (IC) in which the fibre densities were assessed

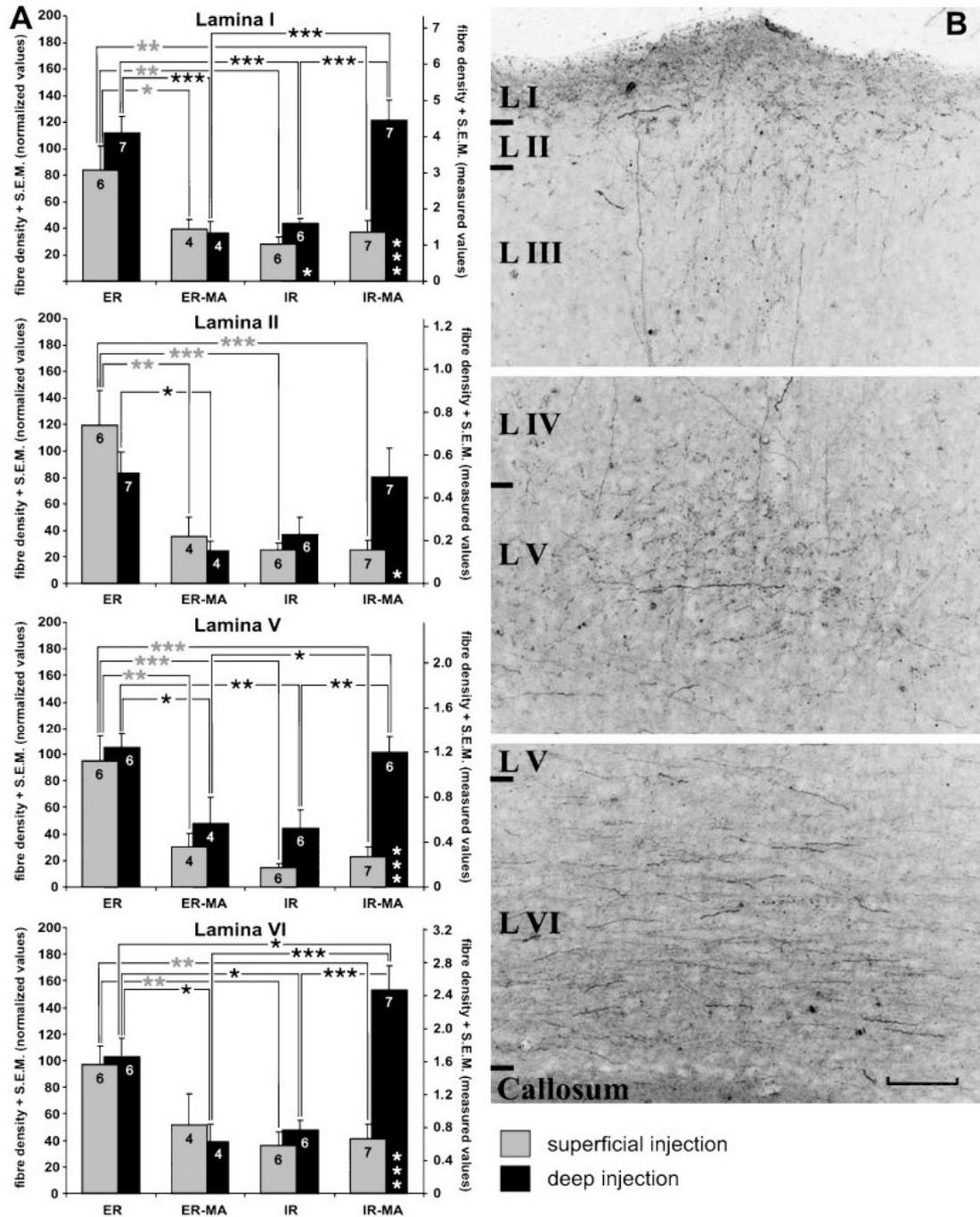
all layers, whereas in the IR groups, MA-treated animals had higher terminal densities in layers II–VI ( $p = 0.095$  for layer I) than saline-injected controls. In this way, IR-MA animals maintained roughly the level of ER animals. In the deep FC laminae of IR-MA animals, efferents from prefrontal laminae V/VI-pyramids were also significantly denser than those from lamina III.

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**Fig. 3.** A Densities of terminal fields and passing fibres in the frontal cortex stained by biocytin injections into superficial (grey columns and asterisks) or deep (black columns and asterisks) layers of the medial prefrontal cortex. The left ordinate gives normalized values (mean of deep and shallow ER injections taken as 100%), the right ordinate gives the original fibre densities. Data are given as means ± S.E.M. Sample sizes are indicated as numbers in the columns. Asterisks in the columns indicate differences between deep and superficial injections within a group. \*:  $p < 0.05$ , \*\*:  $p < 0.01$ , \*\*\*:  $p < 0.001$ . B Representative section through a biocytin-stained fibre column in the frontal cortex of an ER animal. Scale bar is 100  $\mu\text{m}$

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**Fig. 4.** A Densities of terminal fields and passing fibres in the parietal cortex stained by biocytin injections into superficial (grey columns and asterisks) or deep (black columns and asterisks) layers of the medial prefrontal cortex. The left ordinate gives normalized values (mean of deep and shallow ER injections taken as 100%), the right ordinate indicates the original fibre densities. Data are given as means  $\pm$  S.E.M. Sample sizes are indicated as numbers in the columns. Asterisks in the columns indicate differences between deep and superficial injections within a group. \*:  $p < 0.05$ , \*\*:  $p < 0.01$ , \*\*\*:  $p < 0.001$ . B Representative section through a biocytin-stained fibre column in the parietal cortex of an ER animal. Scale bar is 100  $\mu$ m

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In the parietal cortex (PC, Fig. 4), there were effects of MA intoxication and rearing on efferents from superficial but not from deep layers, as assessed by ANOVA, but highly significant interactions of both main factors in both injection depths. Post-hoc testing nevertheless confirmed several differences observed among the shallow injections to be significant. IR reduced the projection density throughout all layers in saline-treated animals, but had no effect in MA-intoxicated animals. Likewise, MA-treated ER animals had fewer terminal fibres in all layers of the PC than the respective controls, whereas no effect could be found in IR animals. Projections from deep prefrontal laminae to the PC were reduced by IR in the saline-treated animals in layers I, V and VI ( $p = 0.053$  in layer II), but increased in the MA-treated animals in the same laminae. MA intoxication led to a reduced maturation of projections to all PC laminae in the ER animals, but to denser terminations in IR animals, again in layers I, V and VI. Consequently, in IR-MA gerbils deep pyramidal cells projected with a higher density to PC than lamina III-pyramids.

In the insular cortex (IC, Fig. 5), ANOVA showed a significant effect of rearing for the shallow injections, with no effect for the deep injections. For the superficial prefrontal injections, post-hoc testing showed that IR reduced the terminal density in all layers in saline-treated animals, but had no effect in MA-treated animals. Similarly, MA-intoxicated animals had less projecting fibres in layers I–IV of the IC than controls after ER, but were not different from controls after IR. For deep injections, only projections to layer VI were denser in IR-MA animals than in all other groups. Whereas deep lamina efferents were weaker than those from outer laminae in ER animals ( $p < 0.05$  in L IV,  $p = 0.07$  in L III,  $p = 0.08$  in LVI), they were significantly stronger in IR-MA gerbils in most laminae ( $p = 0.07$  in L IV).

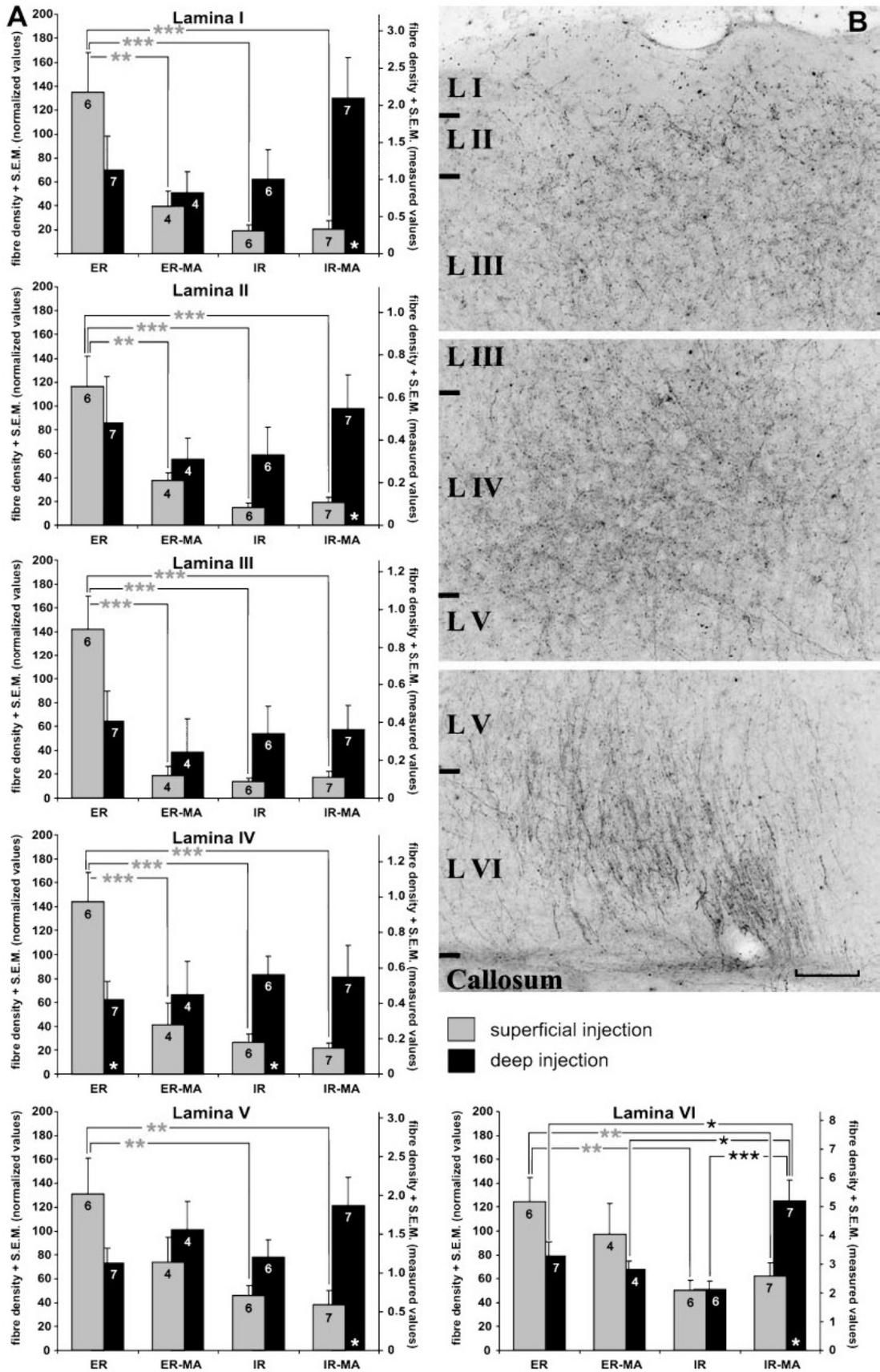
## Discussion

In this study, we present the first direct evidence of an anatomical “dysconnection” of prefrontal efferents to cortical and subcortical fields. Early traumatisa-tion by methamphetamine (MA) and isolated rearing (IR) each led to reduced projections from superficial laminae to the parietal (PC) and insular cortex (IC), and from deep laminae to the frontal cortex (FC) and PC. The combination of both interventions, in contrast, had no further effect on efferents from outer laminae, but prevents (or reverses) the pruning of projections from deep laminae. The effect is an imbalance between the prefrontal connections arising from outer vs. inner laminae.

### *Dysfunctional reorganisation of prefrontocortical efferents*

Obviously, the effects of rearing environment on prefrontal cortex (PFC) efferents differ from and depend on the effects of an early MA intoxication. Only in saline-injected animals, the terminal outgrowth of associative fibres to distal areas (PC, IC) from superficial laminae was enhanced by ER – which is complementary to the finding of higher dendritic branching, spine density and synapse area in occipital fields of ER rodents as compared to IR animals (Greenough and Volkmar, 1973; Globus et al., 1973; Møllgaard et al., 1971).

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The lack of a rearing effect on fibres projecting to the FC is also in line with the failure to detect higher dendritic branching in this region (Greenough et al., 1973). It seems that MA intoxication at PD 14 creates a PFC maladaptation that does not allow layer III-pyramidal cells to benefit from environmental stimulation.

The situation is by at least one degree more complex for prefrontal efferents from deeper laminae. Their axonal arbors to FC and PC likewise profit from ER in saline-injected animals – but in MA-intoxicated gerbils, ER reduces the terminal density in these areas. It seems plausible that ipsilaterally projecting PFC efferents undergo a similar reorganisation in the second and third postnatal week as it has been described for interhemispheric visual connections (Ivy and Killackey, 1981): These projections originally derive from pyramidal cells in upper and lower lamina V. During the third postnatal week in rats, the efferents from lower lamina V are pruned, while new axons sprout from lamina III. MA intoxication on PD 14 presumably disrupts this process in its most critical phase, preventing both the withdrawal of layer V efferents and the outgrowth of projecting fibres from layer III-pyramids. One could speculate that the normally pruned connections are dysfunctional; they would be retained in IR-MA-intoxicated animals, but replaced by functionally relevant synapses later in saline-injected ER gerbils. What remains mysterious is the finding that MA-injected ER gerbils also have weak efferents from deep PFC layers. In these animals, the stimulating environment possibly allows to reduce dysfunctional connections, but the lasting reorganisation within the PFC prevents a fully functional involvement of associative projections.

“Dysconnection” of micro- and macrocircuits

It now seems that puberty, which is also the period during which IR exerts its most devastating effects (Einon and Morgan, 1977), is the critical phase for the establishment of prefrontal control over the dopaminergic system (Weinberger and Lipska, 1995). This control is exerted in the nucleus accumbens (Grace, 1993) as well as the amygdala (Rosenkranz and Grace, 2002) and keeps the dopaminergic fibres from firing when an informed representation of the situation declares rapid action unnecessary. In a reciprocal way, the PFC itself is dependent on a proper maturation of its dopaminergic afferents, such that a reduced DA activity or fibre density leads to impairments in working memory (Dawirs et al., 1996; Winterfeld et al., 1998) and to the outbreak of psychotic symptoms (Weinberger, 1987; Winterer and Weinberger, 2004). Our previous research has shown that the lack of DA is compensated by a sprouting of

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**Fig. 5. A** Densities of terminal fields and passing fibres in the insular cortex stained by biocytin injections into superficial (grey columns and asterisks) or deep (black columns and asterisks) layers of the medial prefrontal cortex. The left ordinate gives normalized values (mean of deep and shallow ER injections taken as 100%), the right ordinate indicates the original fibre densities. Data are given as means  $\pm$  S.E.M. Sample sizes are indicated as numbers in the columns. Asterisks in the columns indicate differences between deep and superficial injections within a group. \*:  $p < 0.05$ , \*\*:  $p < 0.01$ , \*\*\*:  $p < 0.001$ . **B** Representative section through a biocytin-stained fibre column in the insular cortex of an ER animal. Scale bar is 100  $\mu$ m

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GABAergic synapses (Nossoll et al., 1997) and an addition of dendritic spines on pyramidal cells (Blaesing et al., 2001). In this way, dysfunctional microcircuits are formed in the PFC, which then radiate their detrimental influence through macrocircuits to the whole brain (Teuchert-Noodt, 2000; Winterer and Weinberger, 2004).

Again, the maladaptation of macrocircuits thus caused becomes first apparent in the DA projections: Fibres to caudal cortical fields (i.e. entorhinal cortex and amygdala), which go through an overgrowth during their early development, are not pruned in IR and/or MA-intoxicated gerbils (Busche et al., 2004). It is likely that this aberrant maturation is a consequence of the disturbed interplay of prefrontal and caudal “limbic” areas. The dopaminergic imbalance, in turn, affects cholinergic and serotonergic fibre systems in these areas (Neddens et al., 2003, 2004; Lehmann et al., 2004). Eventually, caudal and subcortical areas can be considered to work out of sync with the PFC, thus manifesting a “dysconnection” not only in the literal sense – as shown in the present results –, but also in a wider sense.

#### *Implications for PFC function and activity*

The PFC is the highest integrated control centre for capacities like volition, avoidance strategies and spatio-temporal planning. Concomitant functional impairments of the anatomical abnormalities discussed above may therefore be expected to emerge from those brain areas that the PFC usually controls or competes with: Emotional disturbances arise from an overactive DA transmission in the nucleus accumbens and amygdala (cp. Grace, 1993; Rosenkranz and Grace, 2001), manifesting themselves as increased locomotion, fearfulness and resistance to extinction in IR gerbils (Polascheck, 2004), and being typical as well in schizophrenic patients. Lack of control over basic motor loops may be one factor in the causation of stereotypic behaviour (Whishaw et al., 1992), again found in these animals (Lehmann, 2001) and schizophrenics. As for a noisy crosstalk with sensory fields, which is hypothesized to contribute to hallucinations (Feinberg, 1982/83; Weinberger and Lipska, 1995; Hubl et al., 2004), there is no way to test it in animals, but our data suggest that these long projections are “dysconnected” as well. We therefore consider the treatment paradigm presented here a valid animal model of at least some important aspects of trauma-induced schizophrenia.

#### *“Dysconnection” and the glutamate hypothesis of schizophrenia*

In human schizophrenics, a disruption of the natural process of cortical layering occurring during the second trimester of pregnancy is usually assumed to produce “dysconnection”, speculated not to consist in a separation, a “disconnection”, of fibre tracts, but in aberrant layering and connectivity (Jakob and Beckmann, 1986; Weinberger and Lipska, 1995; Kalus et al., 1999). This notion has received support from a number of recent DTI studies which have detected reduced fractional anisotropy in the white matter of schizophrenics, meaning that fibres are less clearly directed than in healthy controls (Buchsbaum et al.,

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1998; Hubl et al., 2004; Wang et al., 2004). It seems conceivable that random connections are kept that should have been pruned during development (Changeux and Danchin, 1976).

How these anatomical data combine with the pharmacological and imaging findings that have, in recent years, inspired the glutamate hypothesis of schizophrenia (Carlsson and Carlsson, 1990; Carlsson et al., 1997; Tamminga, 1998; Goff and Coyle, 2001), is an open question. Following the first observation that the glutamate content in the cerebroventricular fluid of schizophrenics is much lower than in controls (Kim et al., 1980), studies in the last decade have established that the glutamate transmission is impaired especially in the anterior cingulate and hippocampal cortices of schizophrenic patients, with receptor densities being raised (Toru et al., 1988; Simpson et al., 1992; Dracheva et al., 2001). Reduced excitation in these areas could result in a lack of glutamatergic transmission in their mutual projection field (i.e. the ventral striatum, O'Donnell and Grace, 1998), as is also found in the present study, and, eventually, in an imbalance of glutamate and dopamine in this area (Carlsson and Carlsson, 1990; Grace, 1993).

The shift of projection patterns we observe in the present study is more complex than a simple reduction of transmission. It seems plausible, however, that improper wiring of connections impairs their activity. Alternatively, only the projections from superficial layers may be those relevant for schizophrenia in the human brain, since they are the ones that form ipsilateral intrinsic and associative cortical connections (Melchitzky et al., 1998), whereas infragranular pyramidal cells preferentially project to contralateral and subcortical targets (Jones et al., 1975). These associative projections to the parietal and insular cortices are significantly reduced in gerbils by IR and MA intoxication, such that, physiologically, a lack of glutamatergic activation in their target fields might ensue.

### Conclusion

We here present the first direct evidence for an anatomical “dysconnection” in what we take to be an animal model of schizophrenia. The abnormalities found need not correspond exactly to those of each and every schizophrenic patient, but possibly represent a valid model for patients with a history of childhood trauma (Read et al., 2001), and provide helpful insights for the study of “dysconnection” in general. They lend substantial support to the intriguing, but weakly founded glutamate hypothesis of schizophrenia that has been much discussed in recent years. At the same time, the data we present demonstrate how this hypothesis and the classical dopamine hypothesis need not only not be rivals, but can be smoothly and elegantly reconciled on the anatomical as well as the functional level. The most urging problem now is to investigate in this model when and how the anatomical “dysconnection” develops: In contrast to human patients, the animal model allows to study this process in its course, rendering valuable insights into the mechanisms of cortical reorganisation. Pursuing this track, we may one day understand how schizophrenia develops.

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Titel: 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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Zusammenfassung: 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.

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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.

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### 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 develop-

ment, 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 transcardially 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  $3 \times 10$  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-

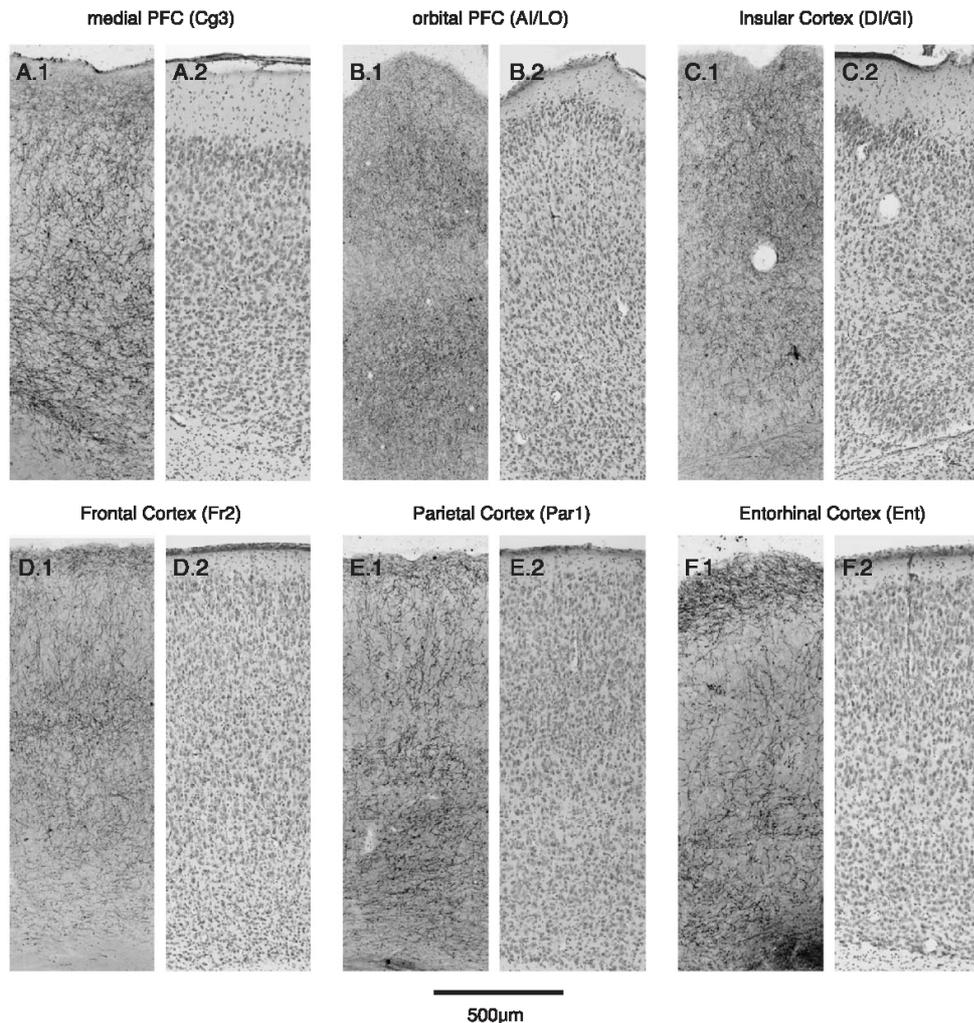


Fig. 1. Representative photomicrographs of 5-HT-immunoreactive fibres (A.1–F.1) of each of the quantified regions of the cortex taken from an animal of the ER saline group. Note the differential innervation pattern and density of 5-HT fibres in the respective cortical regions. Generally, layers I/II 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  $3 \times 10$  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 cover-slipped 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).

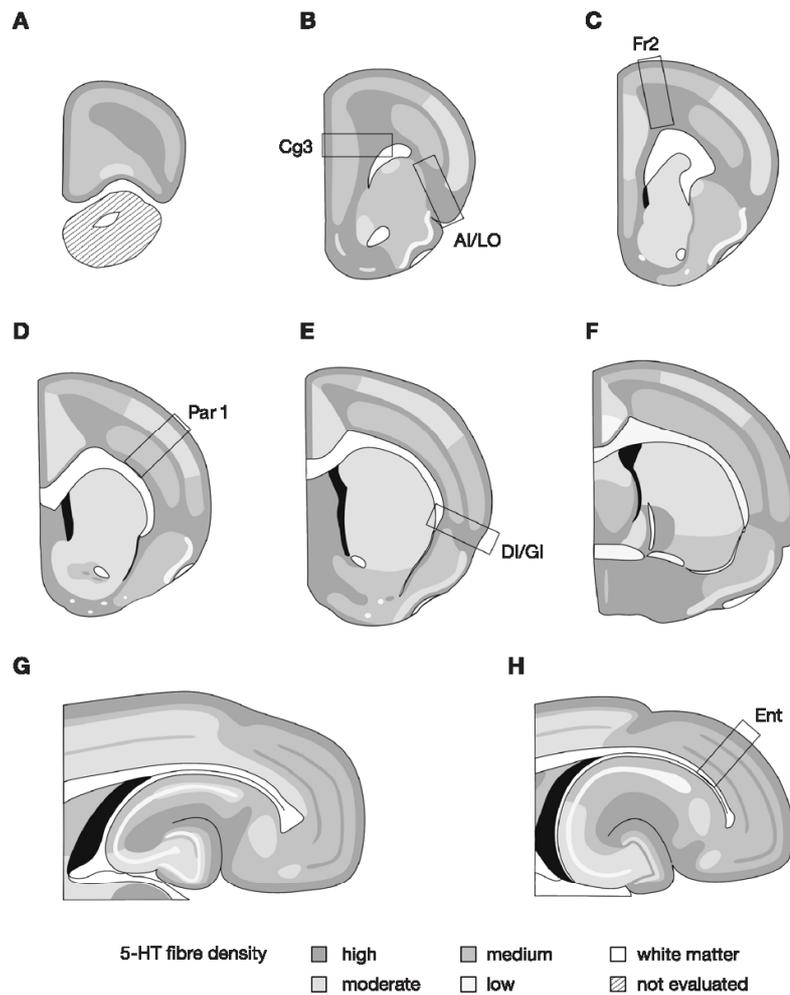


Fig. 2. Schematic drawings of the 5-HT innervation pattern in brain sections of a male gerbil of the ER saline group. Sections A–F were cut in a coronal plane, whereas G–H were cut in a horizontal plane. The identification of cortical areas follows the nomenclature of Paxinos and Watson [52]. The outline 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 amygdala 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.

Fehlerkorrektur: Das Rechteckfenster für den Par1 ist irrtümlicherweise in Schnittebene D eingezeichnet worden. Richtig ist jedoch Schnittebene F.

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 ( $1992 \times 1450$  pixel;  $0.22 \text{ mm}^2$ ) 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  $\pm$  S.E.M. for each cortical area (Fig. 3). 2. Arithmetic means by-case and by-group of single layers  $\pm$  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

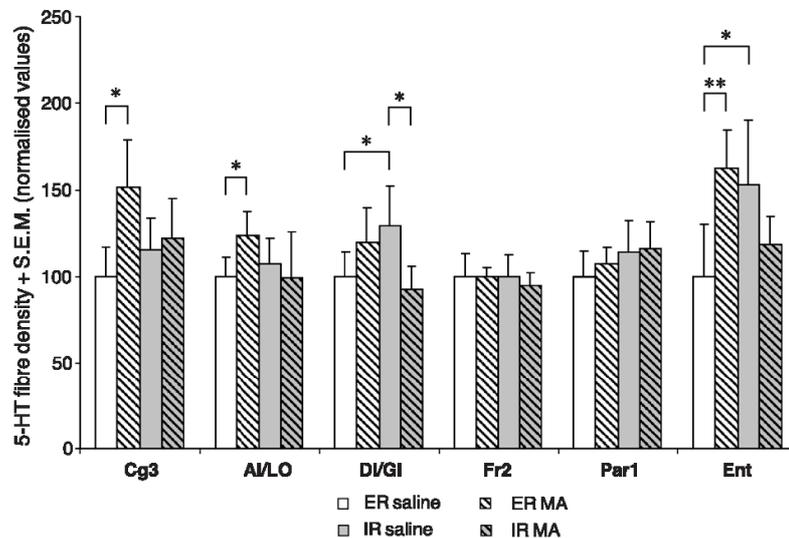


Fig. 3. Group-specific differences in cortical 5-HT fibre density across all regions and all layers, revealed by Manova. IR: Sal/MA,  $F=4.1742$ ,  $p=0.0422*$ ; ER: Sal/MA,  $F=21.7203$ ,  $p<0.0001***$ ; Sal: ER/IR,  $F=13.6738$ ,  $p=0.0003***$ ; MA: ER/IR,  $F=8.7538$ ,  $p=0.0034**$ . Differences 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:  $*p < 0.05$ ,  $**p < 0.01$ .

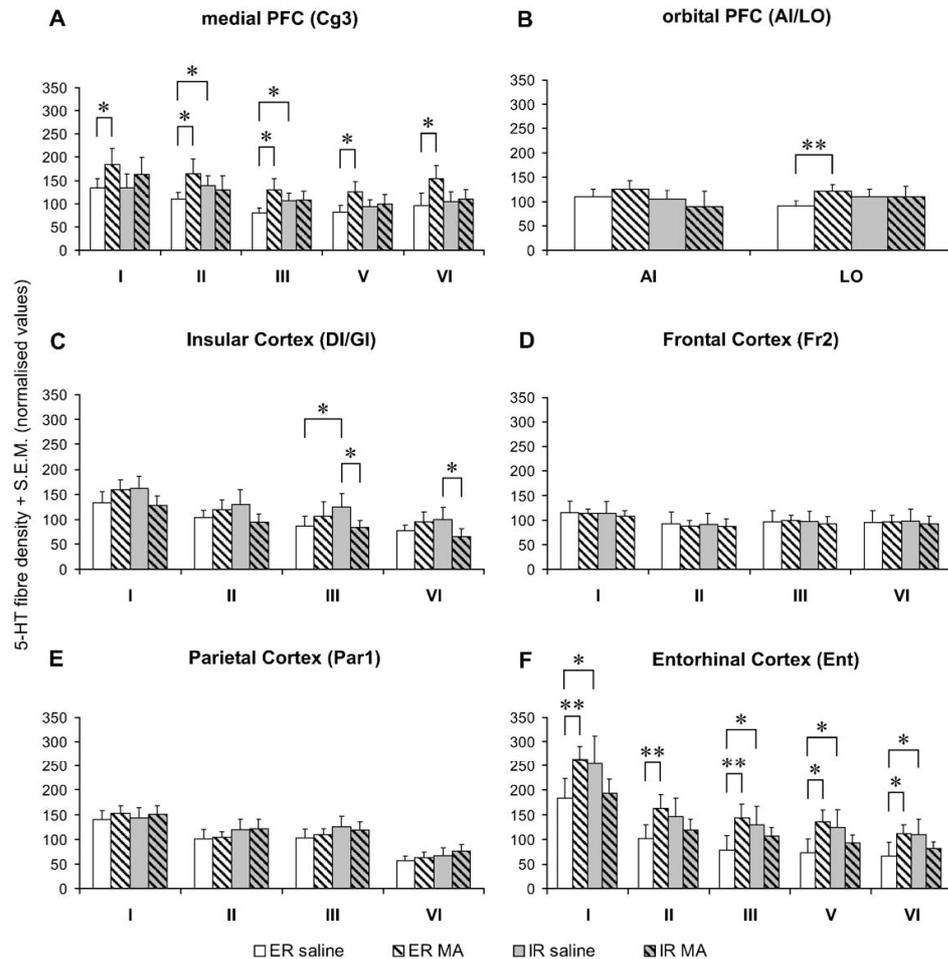


Fig. 4. Analysis of layer-specific differences in response to experimental variables using post hoc analysis (Newman–Keuls test). Consistent significant effects on laminar innervation pattern were found in medial PFC and entorhinal cortex, whereas insular cortex showed only sparse effects. Again, frontal 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  $\pm$  S.E.M.

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

Abbreviations are defined in the text.

groups (Table 1). Both original and normalised mean values  $\pm$  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<sub>2</sub> 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 adaptation 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<sub>1</sub> 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, exclu-

sively 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 maladaptation 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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Zusammenfassung: Long-term effects of postnatal differential rearing conditions and/or early methamphetamine (MA) 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 MA (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 MA challenge with subsequent restricted rearing tended to reverse the effects of MA on 5-HT cerebral asymmetry in some of the cortical areas investigated; and (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.

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Research report

## Postnatal maturation of cortical serotonin lateral asymmetry in gerbils 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 (MA) 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 MA (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 MA challenge with subsequent restricted rearing tended to reverse the effects of MA on 5-HT cerebral asymmetry in some of the cortical areas investigated; and (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.

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### 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]. In addition, 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, depression, and schizophrenia [18,27,29,31,51,58,59]. However, cerebral asymmetries are characteristics that 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

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species [6,23,34,57] 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, yet only few data are available describing the probable role of serotonergic (5-HT) transmission in lateral asymmetry (e.g. Refs. [1,7]).

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 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 from semi-natural rearing (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

### 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 42 male pups (weight 58–77 g; age 104–114 days) 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.

### 2.2. 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 100×100 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.

### 2.3. 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.

### 2.4. Preparation of tissue

Animals were transcardially perfused under deep chloral hydrate 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 prepared 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4). Immediately after perfusion, brains were removed and post-fixed for 2 h 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.

### 2.5. Immunohistochemistry

For immunostaining of 5-HT fibres the sections were rinsed 3×10 min in phosphate-buffered saline (PBS), incubated for 10 min with 1% H<sub>2</sub>O<sub>2</sub> 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

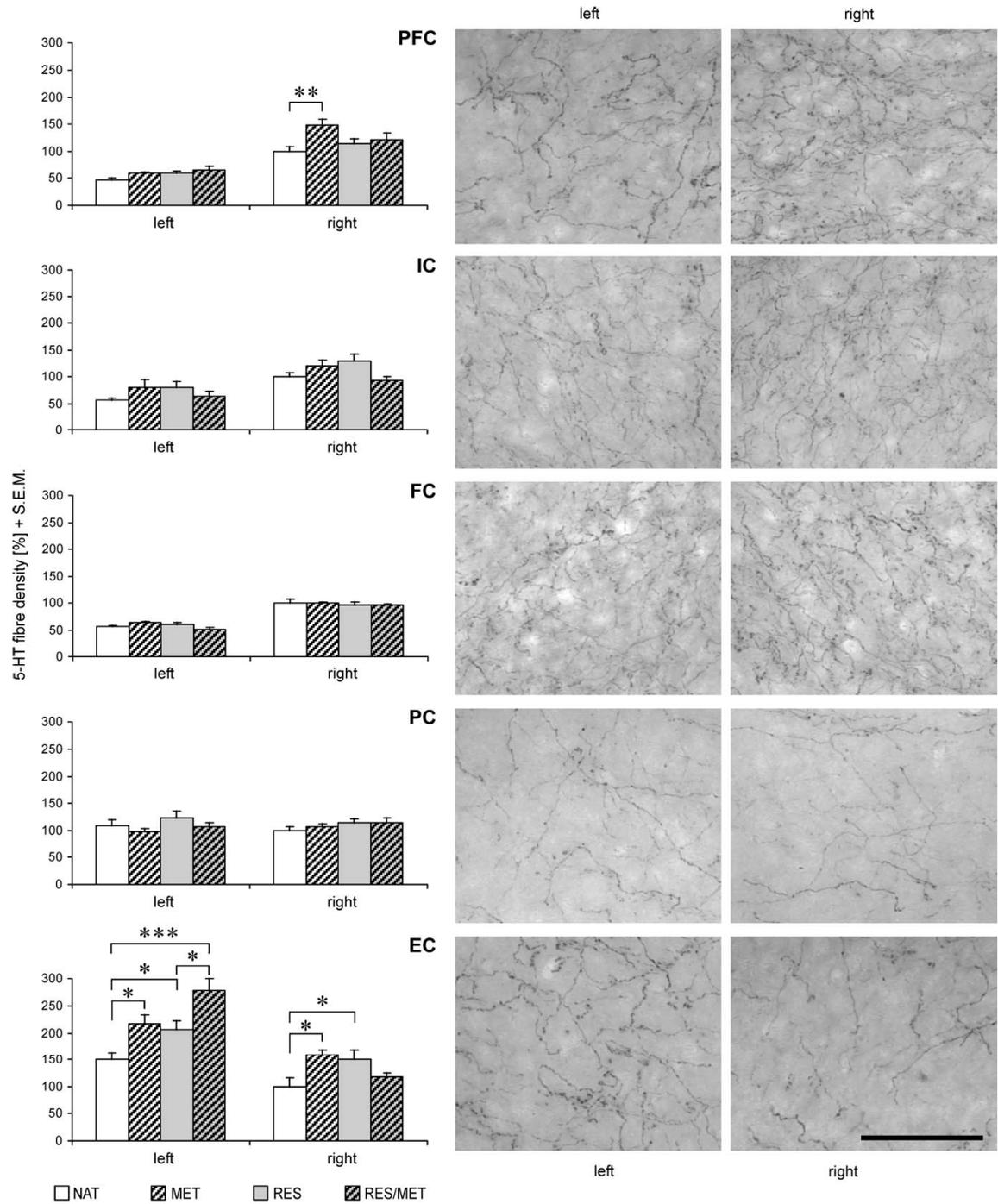


Fig. 1. Standardised 5-HT innervation density  $\pm$  S.E.M. is presented in five cortical areas of both cerebral hemispheres. Significant region-specific changes in response to environmental rearing conditions and early methamphetamine treatment were found in the right hemisphere of the prefrontal cortex (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). Abbreviations: NAT, saline-treated semi-naturally reared; MET, MA-treated semi-naturally reared; RES, saline-treated restrictively reared. 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=50  $\mu$ m.

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 3×10 min rinse in TBS. Staining procedure was performed in 0.05% 3,3-diaminobenzidine and 0.01% H<sub>2</sub>O<sub>2</sub> 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.

### 2.6. 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 [48] 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 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 pixels; 0.22 mm<sup>2</sup>) using a bright-field microscope (Polyvar, Reichert-Jung) 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). 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.

### 2.7. 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 four-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. A single rater exclusively did the measurement of 5-HT fibre density in a single cortical area, comprising all experimental groups by means of paired controls. 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.

Table 1  
Comparison of left vs. right 5-HT fibre density±S.E.M. in five areas of cerebral cortex

		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
		<i>p</i> =0.0003***	<i>p</i> =0.0002***	<i>p</i> =0.0002***	<i>p</i> =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
		<i>p</i> =0.0002***	<i>p</i> =0.0015**	<i>p</i> =0.0035**	<i>p</i> =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
		<i>p</i> =0.0002***	<i>p</i> =0.0002***	<i>p</i> =0.0002***	<i>p</i> =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
		<i>p</i> =0.2235 n.s.	<i>p</i> =0.1485 n.s.	<i>p</i> =0.3169 n.s.	<i>p</i> =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
		<i>p</i> =0.0002***	<i>p</i> =0.0123*	<i>p</i> =0.0062**	<i>p</i> =0.0002***

ANOVA and post-hoc Newman–Keuls test, significant values: \**p*<0.05, \*\**p*<0.01, \*\*\**p*<0.001. Abbreviations: NAT, saline-treated semi-naturally reared; MET, MA-treated semi-naturally reared; RES, saline-treated restrictively reared; RES/MET, MA-treated restrictively reared; PFC, prefrontal cortex; EC, entorhinal cortex; IC, insular cortex; FC, frontal cortex; PC, parietal cortex.

Compared with NAT animals, which serve as controls, significant alterations only occur in right PFC and in right and 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%

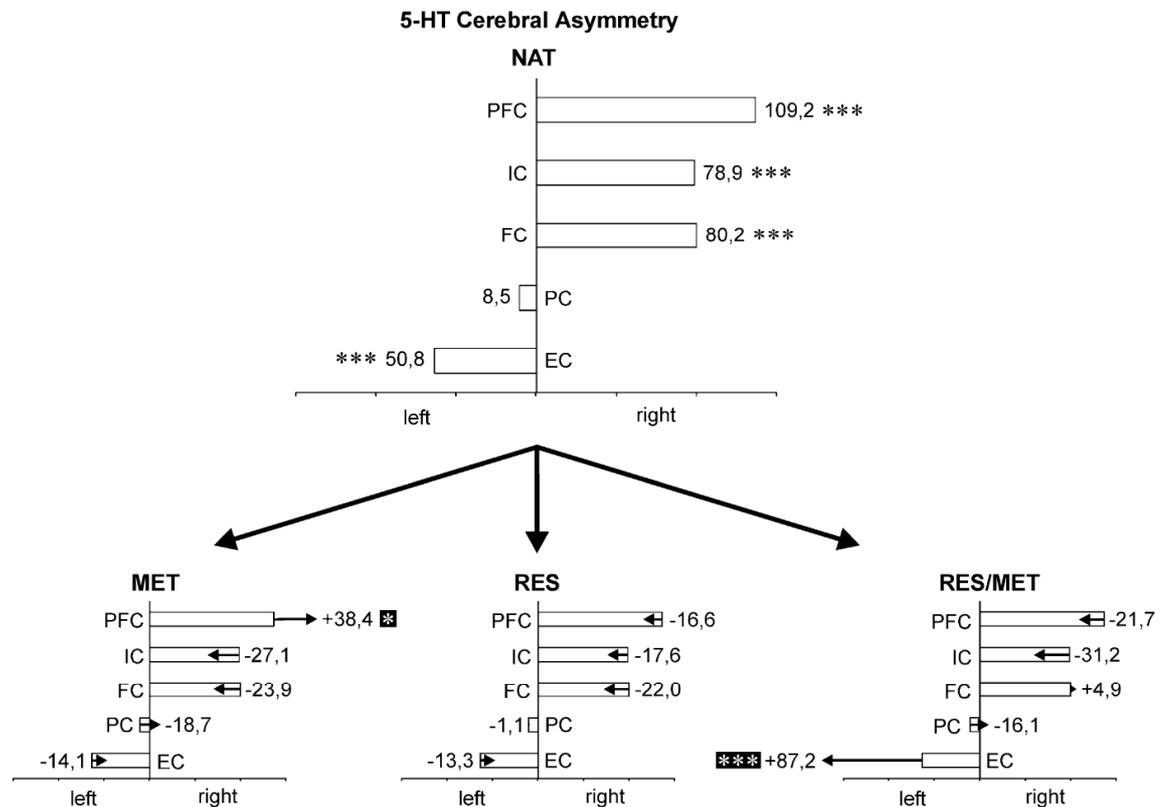


Fig. 2. The extent and significance (black asterisks) of 5-HT asymmetry in five cortical areas of NAT animals (saline-treated semi-naturally reared) is shown by means of percent over-plus of the more densely innervated hemisphere. In frontal areas like prefrontal cortex (PFC), insular cortex (IC), and frontal cortex (FC) 5-HT innervation is denser in the right hemisphere. Parietal cortex (PC) shows no significant asymmetry, whereas 5-HT innervation of the entorhinal cortex (EC) 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: \* $p<0.05$ , \*\* $p<0.01$ , \*\*\* $p<0.001$ .

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.

#### 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]. In addition, there is considerable evidence to argue that lateralised brain functions are already present early in life 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]), somatosensory 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 focused on lateralised characteristics of some 5-HT properties in the human frontal cortex: In post-mortem 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<sub>2A</sub> binding being significantly reduced in the left frontal cortex when compared with normal volunteers [3]. 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 sites, 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 hemisphere [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, 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<sub>1A</sub> 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 [7]. From these results, we may assume that 5-HT<sub>1A</sub> 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 septal plane of 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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## **6.4 weitere Publikationen**

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**Keller A, Bagorda F, Hildebrandt K, Teuchert-Noodt G (2000):** Effects of enriched and of restricted rearing on both neurogenesis and synaptogenesis in the hippocampal dentate gyrus of adult gerbils (*Meriones unguiculatus*).  
*Neurol Psychiat Brain Res* 8: 101-108.

**Hoffmann K, Bagorda F, Stevenson AFG, Teuchert-Noodt G (2001):** Electromagnetic exposure effects the hippocampal dentate cell proliferation in gerbils (*Meriones unguiculatus*).  
*Ind J Exp Biol* 39: 1220-1226.

**Teuchert-Noodt G, Bagorda F (2002):** Drogeninduzierte Destabilisierung psychokognitiver Leistungen als Gefahr für den Straßenverkehr. Wie sich Ecstasy und andere Drogen in das Gehirn einschreiben, und die Frage nach den Chancen einer Therapie.  
*Blutalkohol* 39: 21-31.

**Teuchert-Noodt G, Bagorda F, Schroeder U, Polascheck D, Grund T (2003):** "Lernen durch Lehren: Physiologische Grundlagen des Lernens".  
*Film auf DVD, erschienen im IWF (Göttingen) unter der Mediennummer C 12425.*

**Lehmann K, Grund T, Bagorda A, Bagorda F, Busche A, Teuchert-Noodt G (2005):** Post-weaning handling partly protects dopamine projections and hippocampal cell proliferation from the adverse effect of isolated rearing.  
*Exp Neurol* (submitted).

## **6.5 Posterbeiträge**

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- Bagorda F, Lehmann K, Schroeder U, Teuchert-Noodt G:** The maturation of overlapping serotonergic and prefrontofugal afferents is affected by pharmacological and environmental interventions in gerbils.  
28<sup>th</sup> Göttinger Neurobiology Conference, 2001, Poster-Nr. 855, und  
7<sup>th</sup> World Congress of Biological Psychiatry, Berlin, 2001, Poster-Nr. 236S
- Keller A, Bagorda F, Hildebrandt K, Teuchert-Noodt G:** Effects of enriched and of restricted rearing on both neurogenesis and synaptogenesis in the hippocampal dentate gyrus of adult gerbils (*Meriones unguiculatus*).  
28<sup>th</sup> Göttinger Neurobiology Conference, 2001, Poster-Nr. 631
- Neddens J, Busche A, Bagorda F, Teuchert-Noodt G:** An early methamphetamine intoxication exerts region-specific morphogenetic effects on the maturation of the cortical serotonin (5-HT) innervation: Interaction with environmental experience.  
29<sup>th</sup> Göttinger Neurobiology Conference, 2003, Poster-Nr. 939
- Bagorda F, Witte V, Teuchert-Noodt G:** Pattern changes of prefrontal efferent terminal fields in the neocortex in an animal model of psychosis.  
6. Drei-Länder-Symposium für Biologische Psychiatrie, Bern, 2004, Poster-Nr. A2

## 7. Anhang

### 7.1 *Camera lucida* Zeichnungen

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Abb. 15 – 31 zeigen *Camera lucida* Zeichnungen ausgewählter Fallbeispiele der Tracerstudie (jeweils zwei pro Gruppe und Injektionstiefe). Sie dienen der qualitativen Beurteilung der untersuchten glutamatergen Projektionsmuster präfrontaler Pyramidenzellen in den Kortex. Subkortikale Gebiete wurden hierbei nicht berücksichtigt.

Abb. 15 gibt eine Übersicht der in der Quantifizierung eingegangenen Schnittebene aller hier ausgewählten Fallbeispiele wieder, sortiert nach Tiergruppen sowie Injektionstiefen.

Die Abb. 16 – 31 zeigen jeweils eine Folge aus vier Schnittebenen eines Fallbeispiels über die gesamte rechte Hemisphäre des Gehirns, in der auch die Injektion des Tracers erfolgt ist. Diese Ebenen sind anhand einiger Merkmale leicht zu erkennen:

- Die Injektionsebene liegt etwa 4,5 – 5 mm anterior zum Bregma. Das wenige  $\mu\text{m}$  caudal liegende Knie des *Corpus callosums* ist noch nicht zu erkennen.
- Die zweite Schnittebene liegt 3,5 mm anterior zum Bregma. Die Commissura anterior ist als tropfenförmige Struktur zu erkennen und liegt unmittelbar dem lateralen Ventrikel an.
- Die dritte Ebene ist die Schnittebene, in der die quantitative Auswertung stattfand. Diese Ebene liegt 2,5 mm anterior zum Bregma und zeichnet sich dadurch aus, dass die Commissura anterior die mediale Kante der rechten Hirnhemisphäre erreicht hat.
- Die vierte Ebene liegt 0,5 mm anterior zum Bregma und somit ca. 100  $\mu\text{m}$  rostral zum septalen Pol des Hippocampus.

Für fünf dieser Abbildungen (Abb. 17, 24, 28, 29 und 31) lagen zusätzlich Detailzeichnungen der Kolumne im PC vor. Diese sind in den jeweiligen Abbildungen als Inset eingesetzt worden.

Für weitere Einzelheiten, z.B. die Einteilung der Schnittebenen in die einzelnen Kortexareale etc., siehe Abb. 7 oder Fig. 2 der Veröffentlichung [Bagorda et al., 2005]. Fig. 2 der Veröffentlichung entspricht dem Fallbeispiel in Abb. 18.

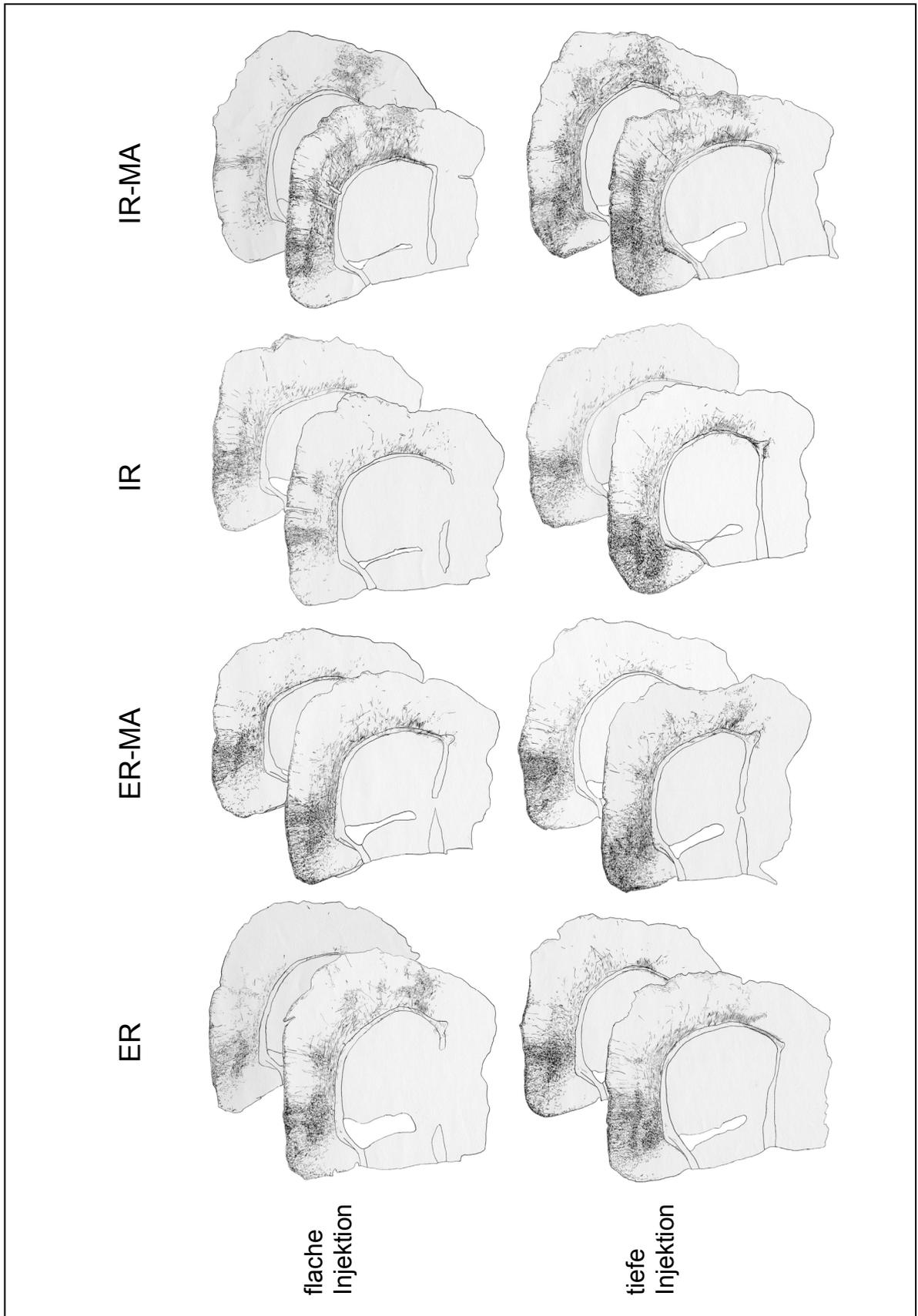


Abb. 15: Camera lucida Zeichnungen als qualitative Übersicht zur Tracerstudie. Aufgeführt sind die untersuchten Schnittebenen von jeweils zwei Fallbeispielen aus allen Tiergruppen und Injektionstiefen.

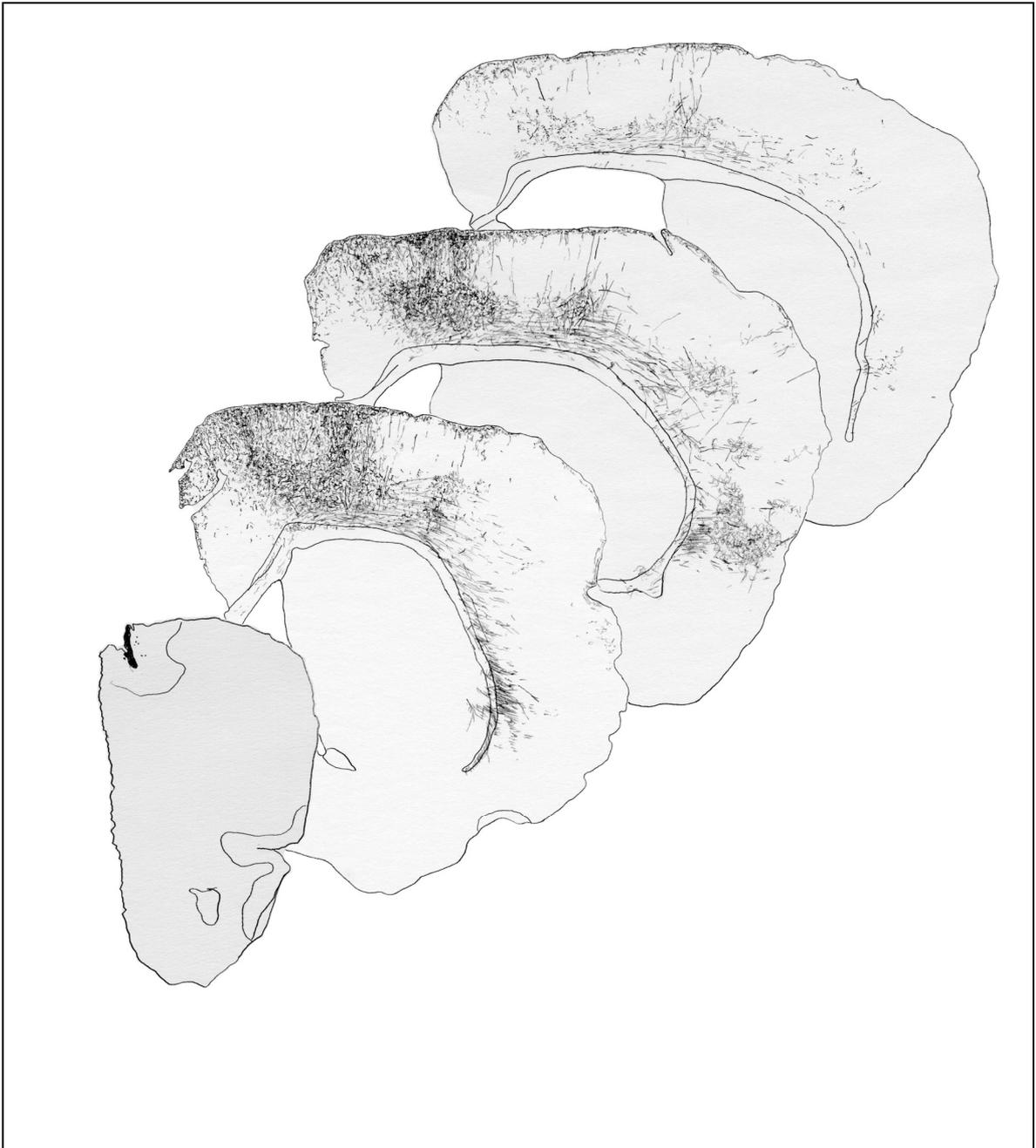


Abb. 16: Camera lucida Zeichnung eines ersten Fallbeispiels aus der Gruppe **ER – flache Injektion** (Fallnummer MC 171).  
Zu weiteren Hinweisen siehe Seite 52.

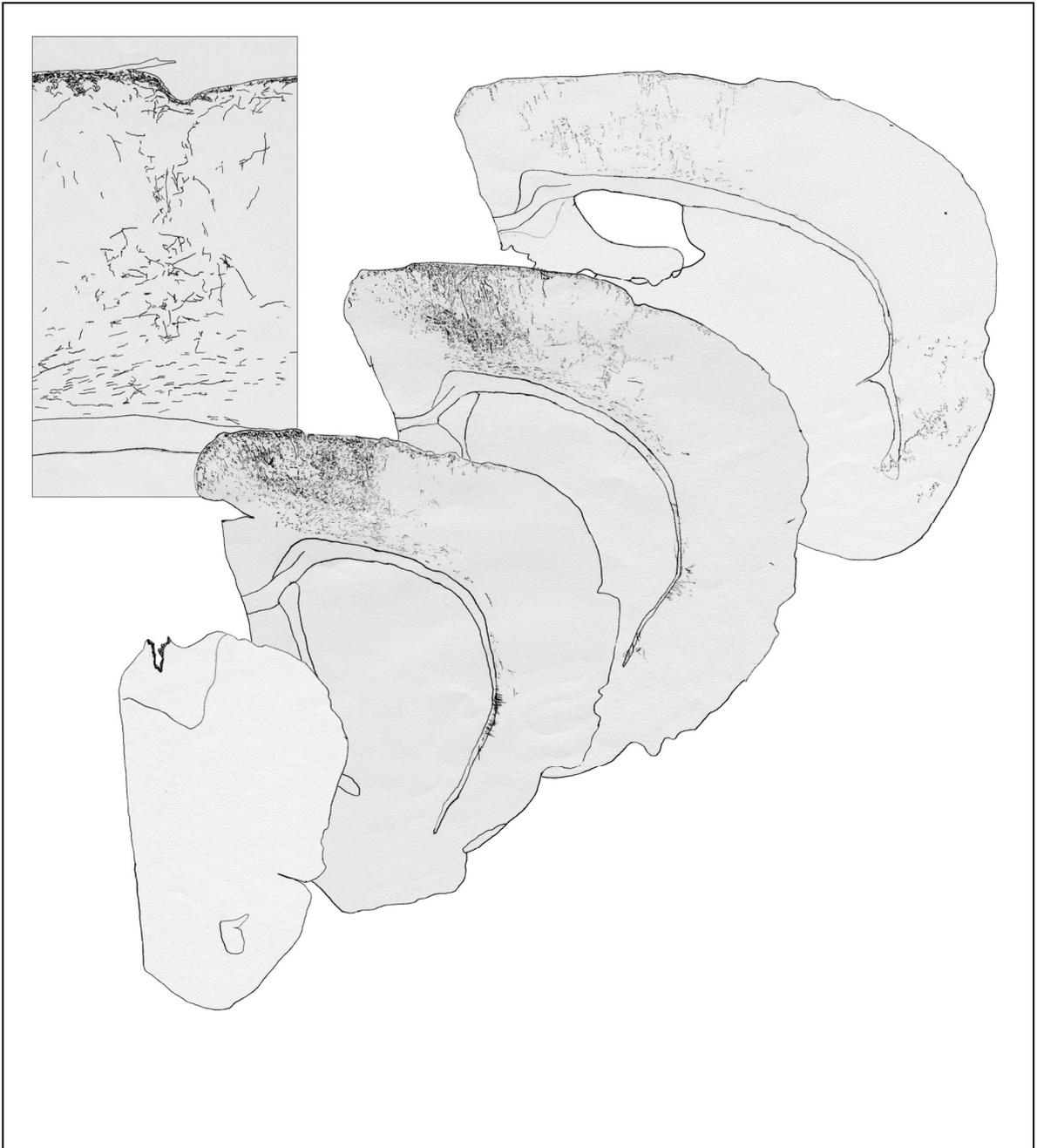


Abb. 17: Camera lucida Zeichnung eines zweiten Fallbeispiels aus der Gruppe **ER – flache Injektion** (Fallnummer MC 98).  
Zu weiteren Hinweisen siehe Seite 52.

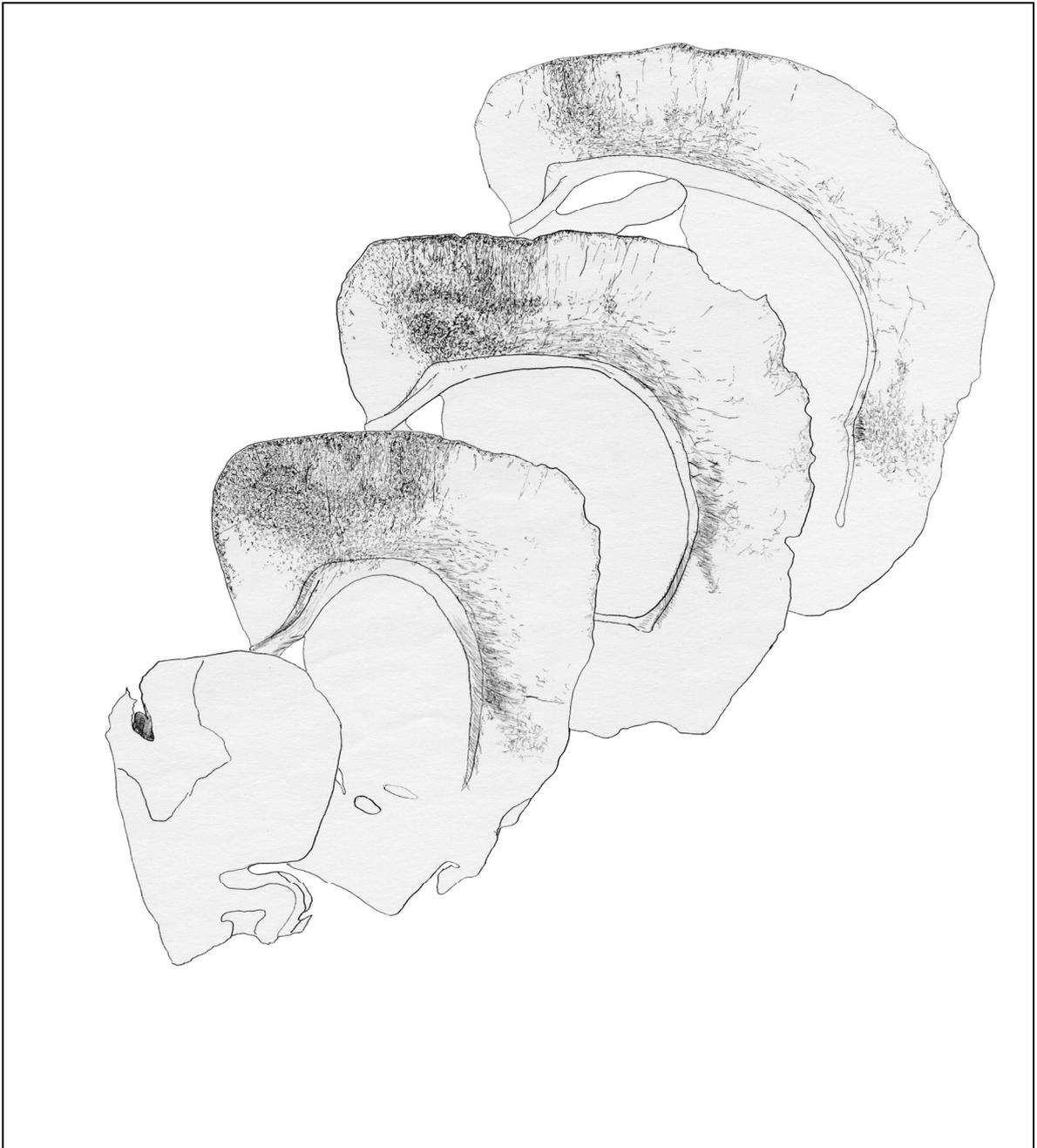


Abb. 18: Camera lucida Zeichnung eines ersten Fallbeispiels aus der Gruppe **ER – tiefe Injektion** (Fallnummer MC 167).  
Zu weiteren Hinweisen siehe Seite 52.



Abb. 19: Camera lucida Zeichnung eines zweiten Fallbeispiels aus der Gruppe **ER – tiefe Injektion** (Fallnummer MC 176).  
Zu weiteren Hinweisen siehe Seite 52.



Abb. 20: Camera lucida Zeichnung eines ersten Fallbeispiels aus der Gruppe **ER-MA – flache Injektion** (Fallnummer MC 154).  
Zu weiteren Hinweisen siehe Seite 52.



Abb. 21: Camera lucida Zeichnung eines zweiten Fallbeispiels aus der Gruppe **ER-MA – flache Injektion** (Fallnummer MC 157).  
Zu weiteren Hinweisen siehe Seite 52.

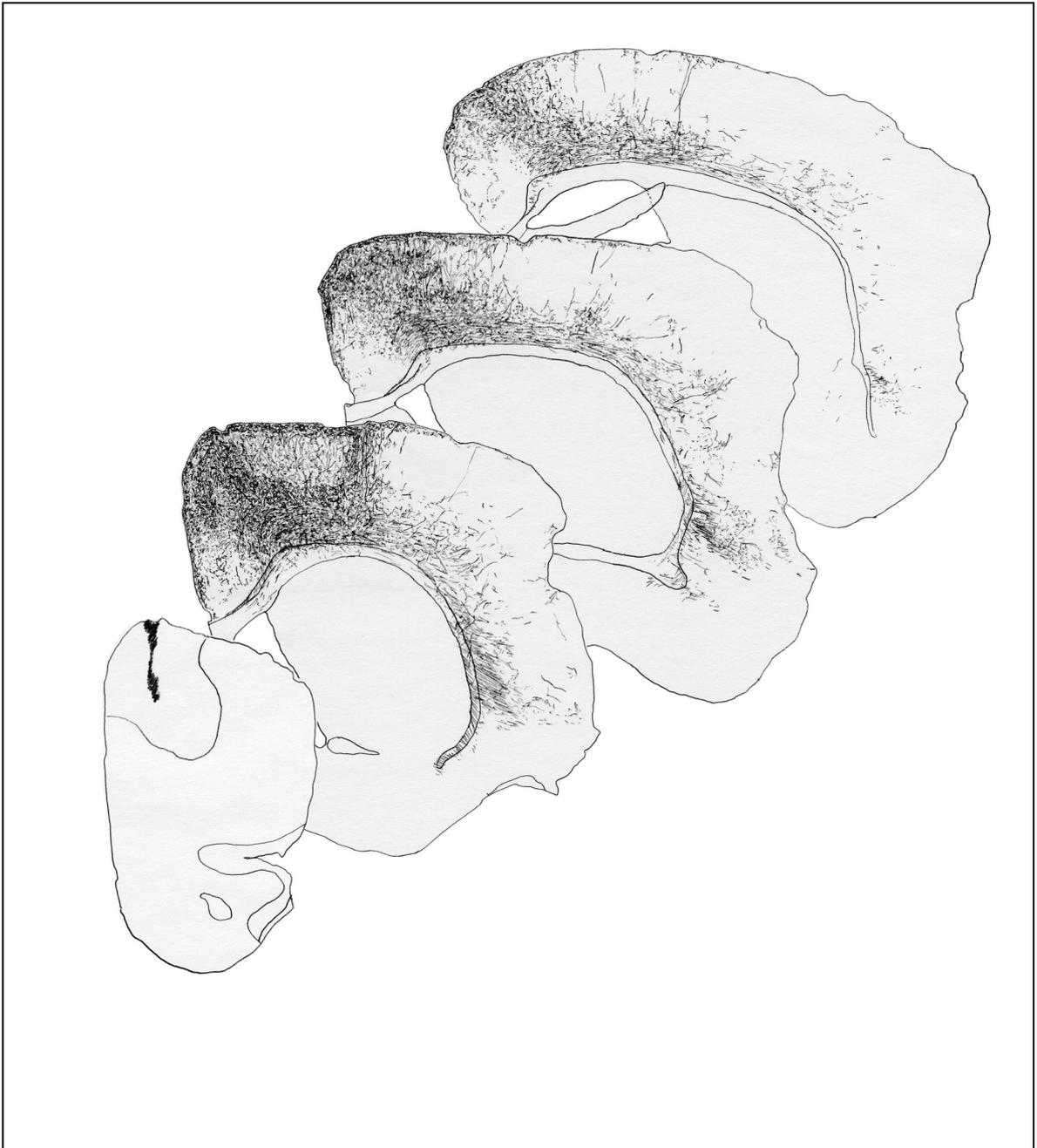


Abb. 22: Camera lucida Zeichnung eines ersten Fallbeispiels aus der Gruppe **ER-MA – tiefe Injektion** (Fallnummer MC 181).  
Zu weiteren Hinweisen siehe Seite 52.

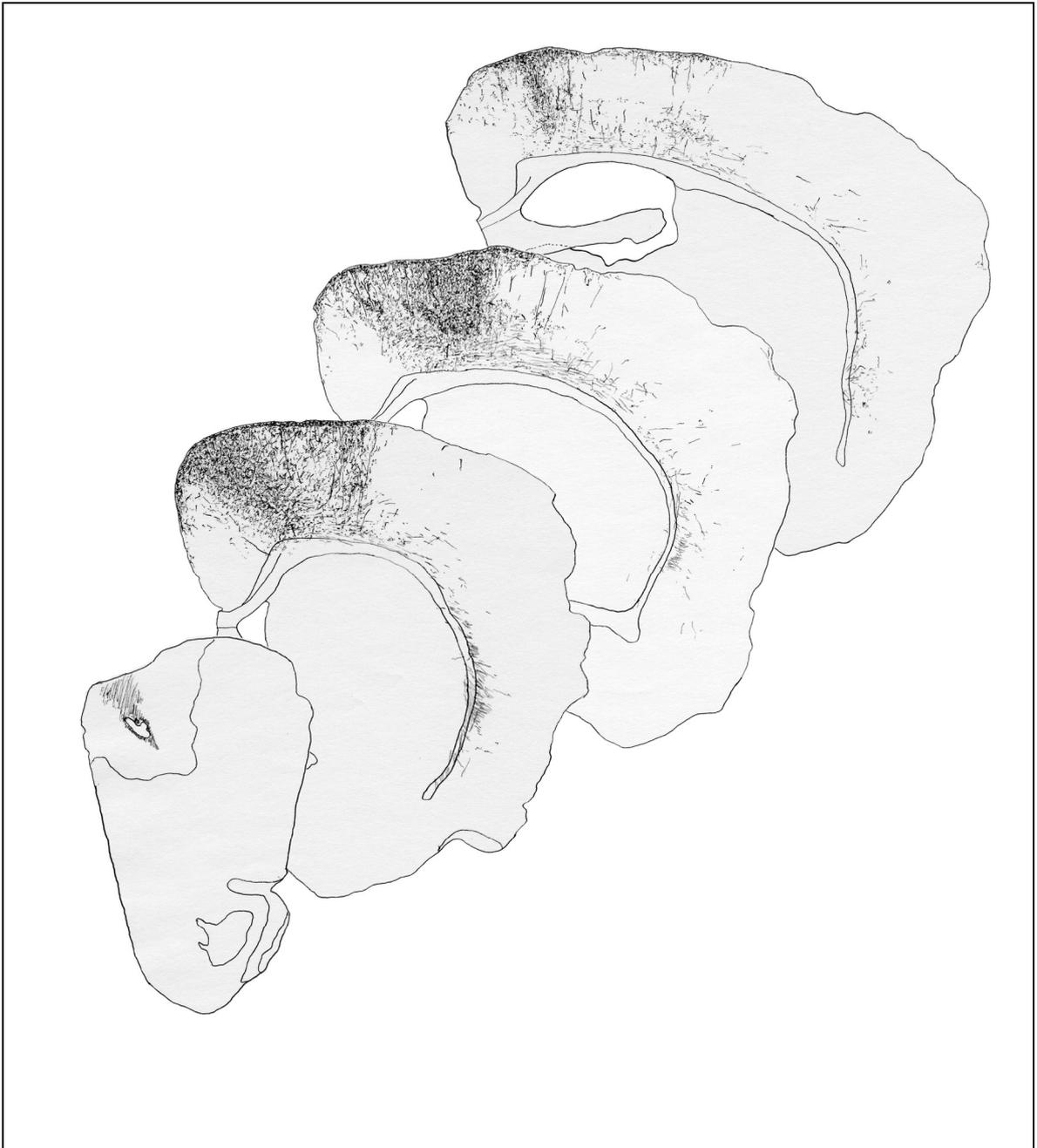


Abb. 23: Camera lucida Zeichnung eines zweiten Fallbeispiels aus der Gruppe **ER-MA – tiefe Injektion** (Fallnummer MC 182).  
Zu weiteren Hinweisen siehe Seite 52.

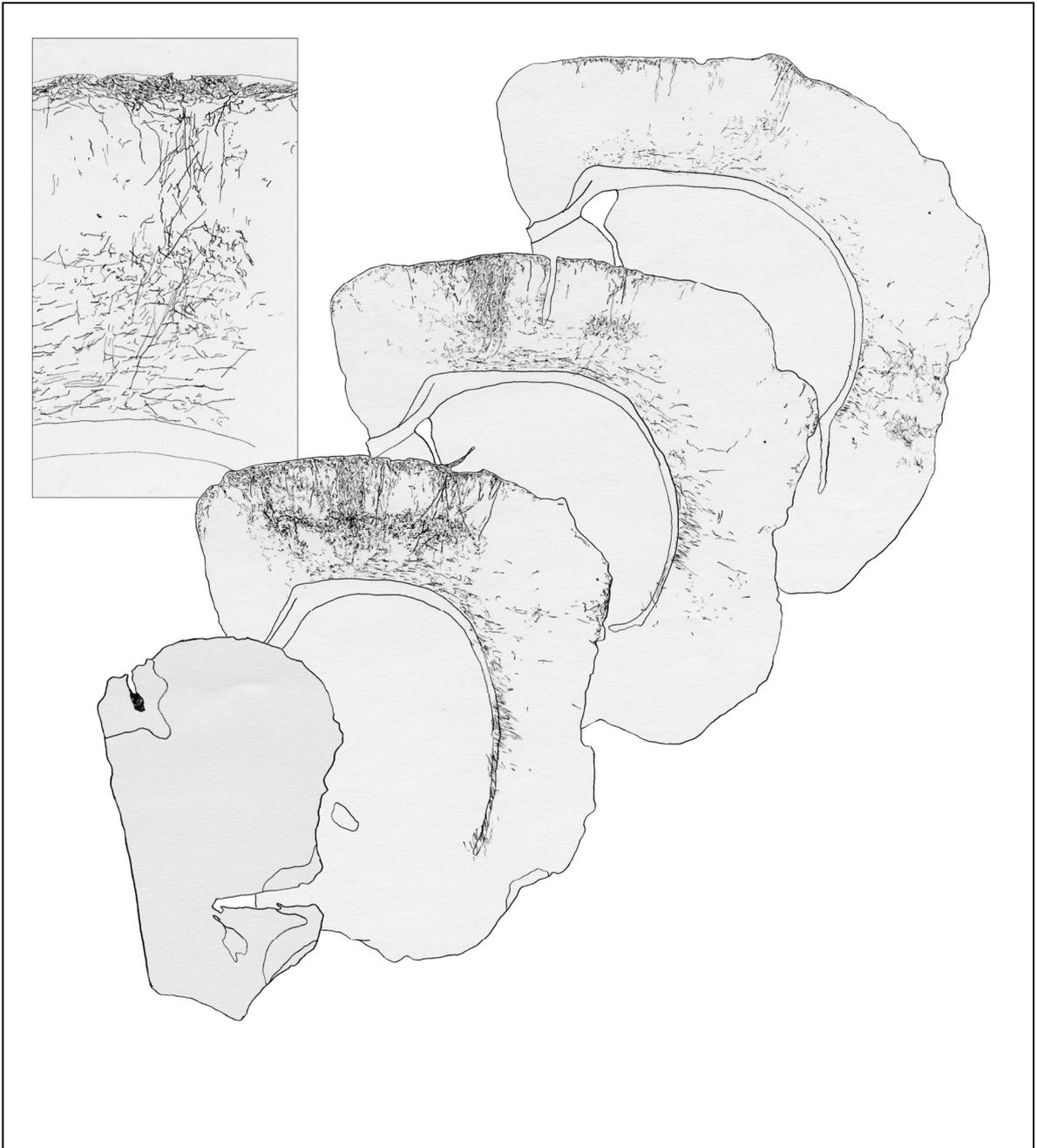


Abb. 24: Camera lucida Zeichnung eines ersten Fallbeispiels aus der Gruppe **IR – flache Injektion** (Fallnummer MC 124).  
Zu weiteren Hinweisen siehe Seite 52.

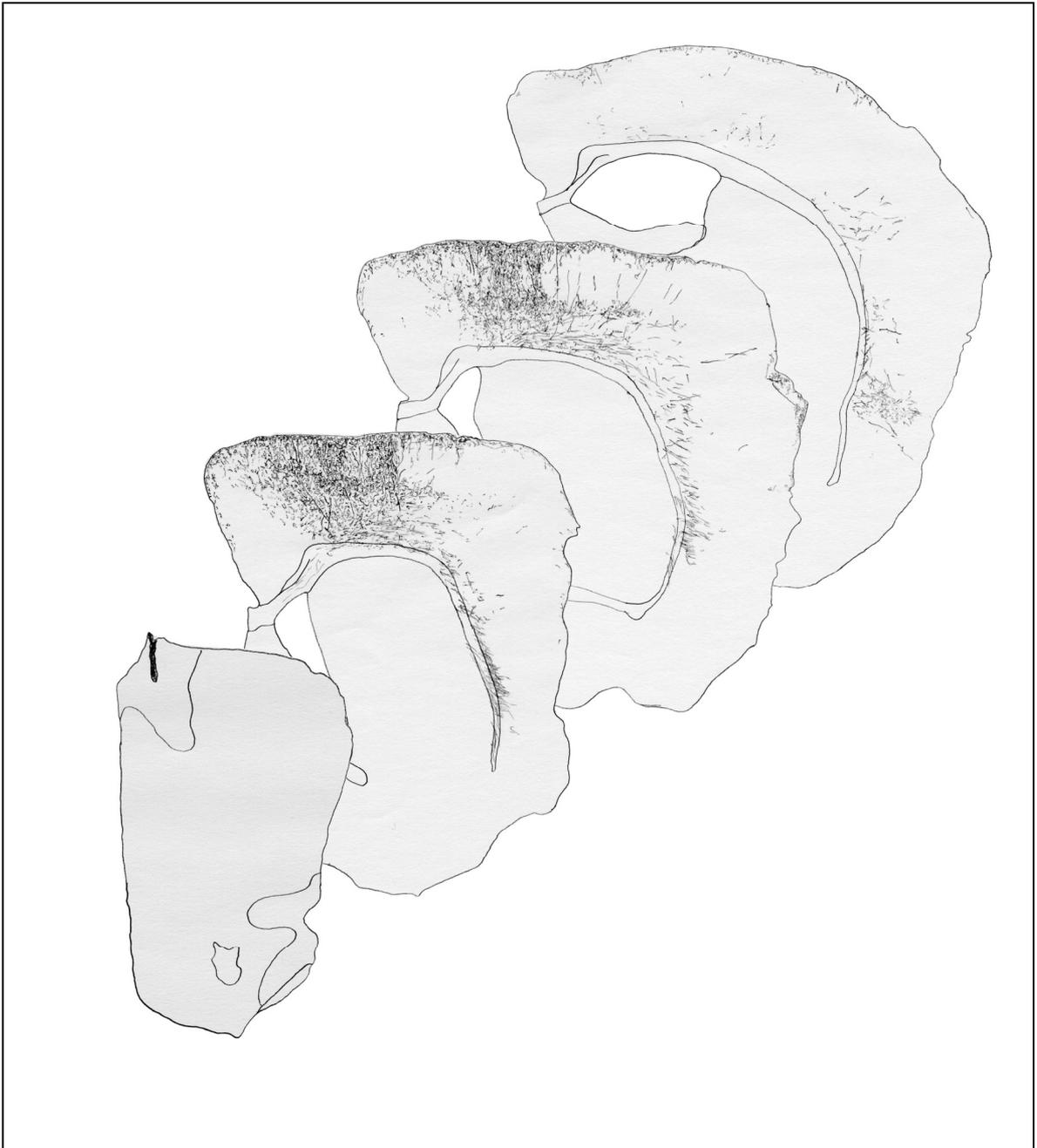


Abb. 25: Camera lucida Zeichnung eines zweiten Fallbeispiels aus der Gruppe **IR – flache Injektion** (Fallnummer MC 168).  
Zu weiteren Hinweisen siehe Seite 52.

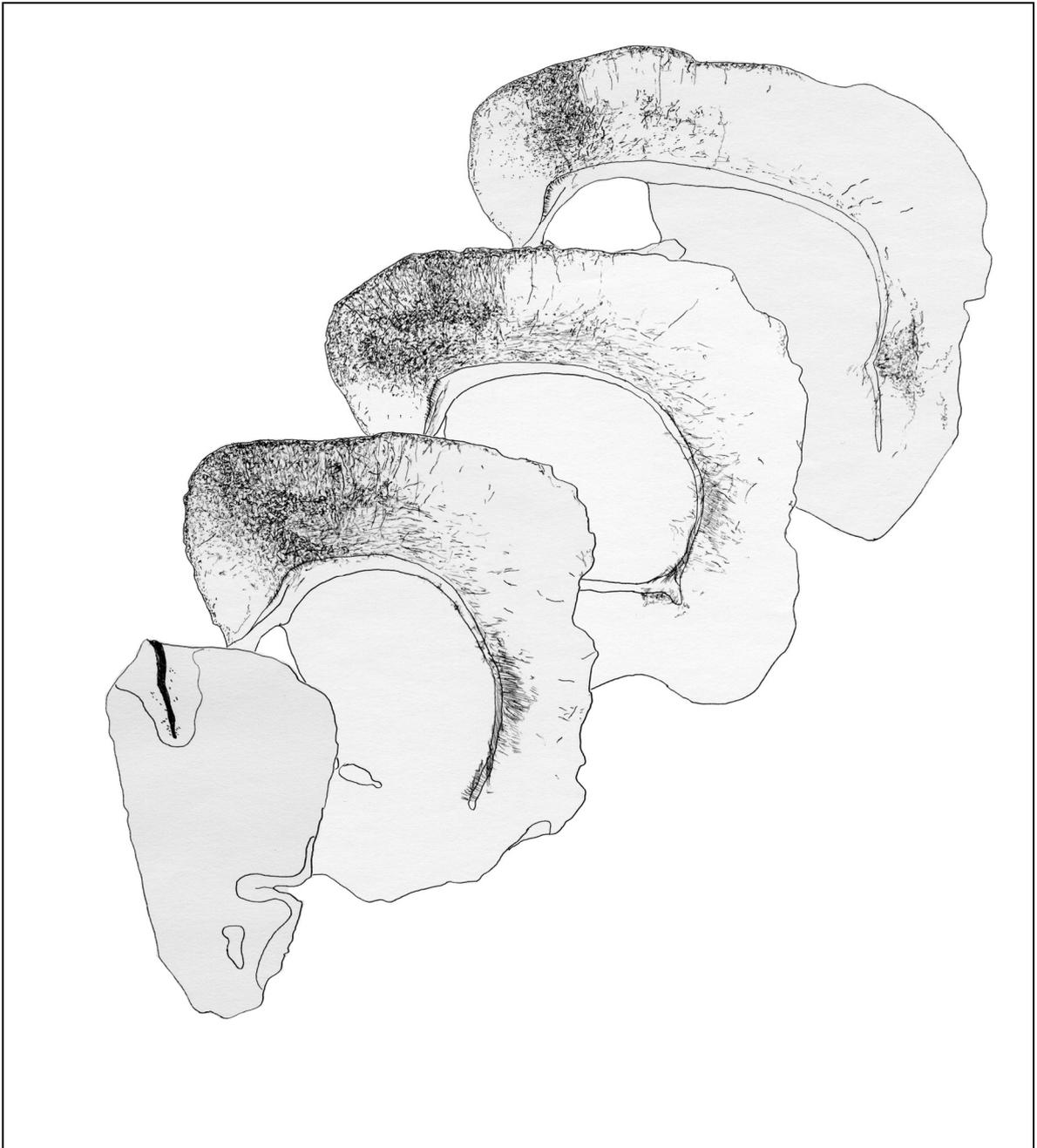


Abb. 26: Camera lucida Zeichnung eines ersten Fallbeispiels aus der Gruppe **IR – tiefe Injektion** (Fallnummer MC 180).  
Zu weiteren Hinweisen siehe Seite 52.

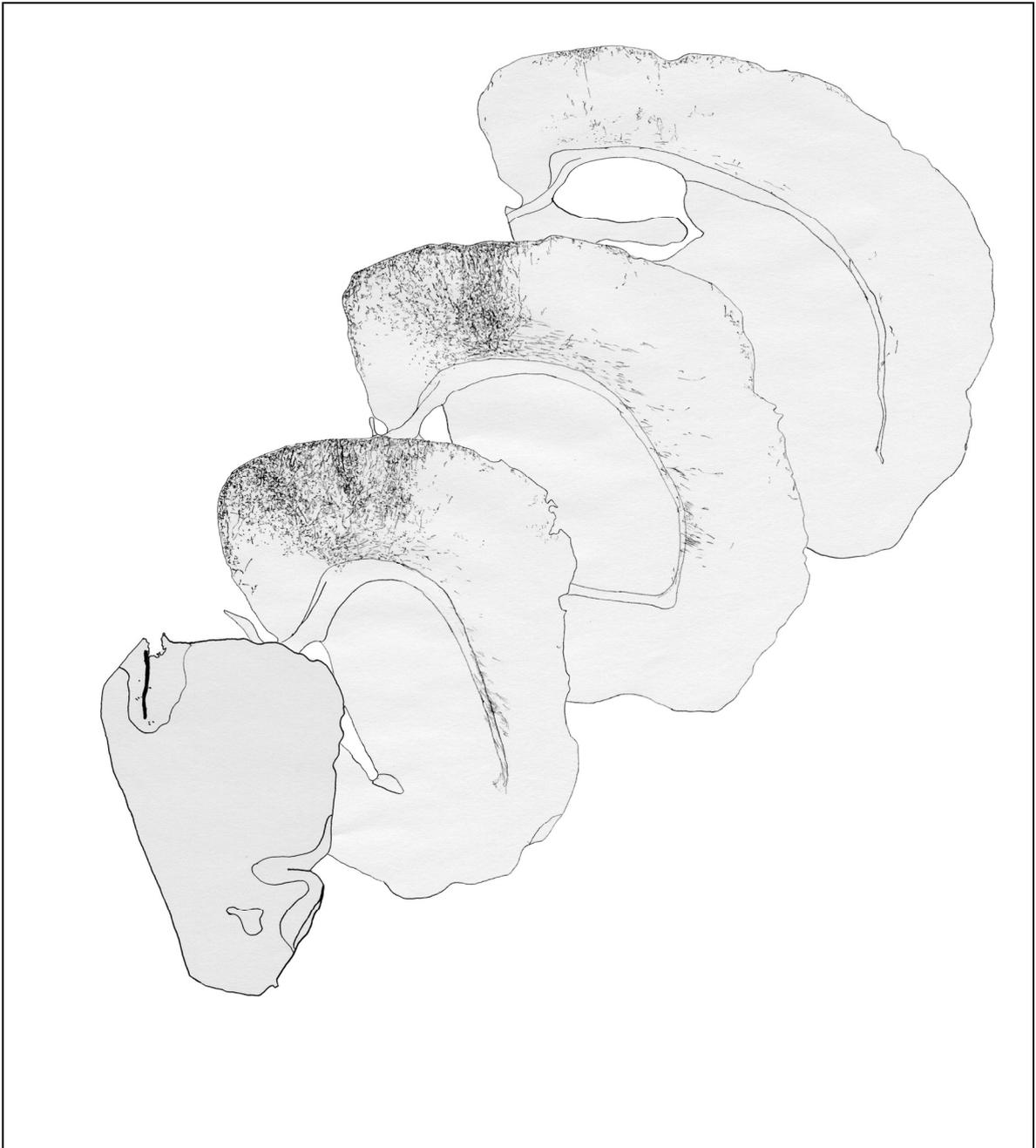


Abb. 27: Camera lucida Zeichnung eines zweiten Fallbeispiels aus der Gruppe **IR – tiefe Injektion** (Fallnummer MC 165).  
Zu weiteren Hinweisen siehe Seite 52.

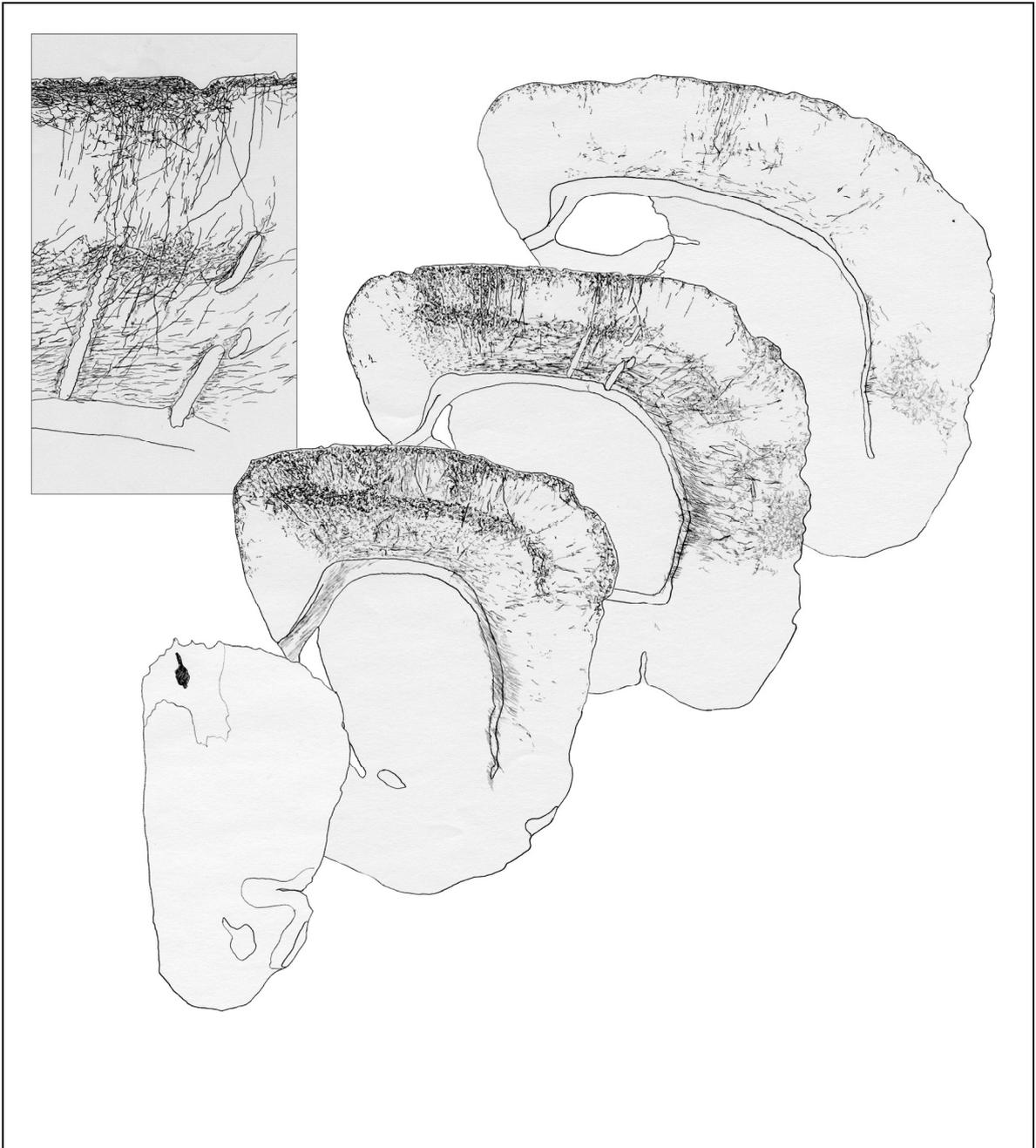


Abb. 28: Camera lucida Zeichnung eines ersten Fallbeispiels aus der Gruppe **IR-MA – flache Injektion** (Fallnummer MC 108).  
Zu weiteren Hinweisen siehe Seite 52.

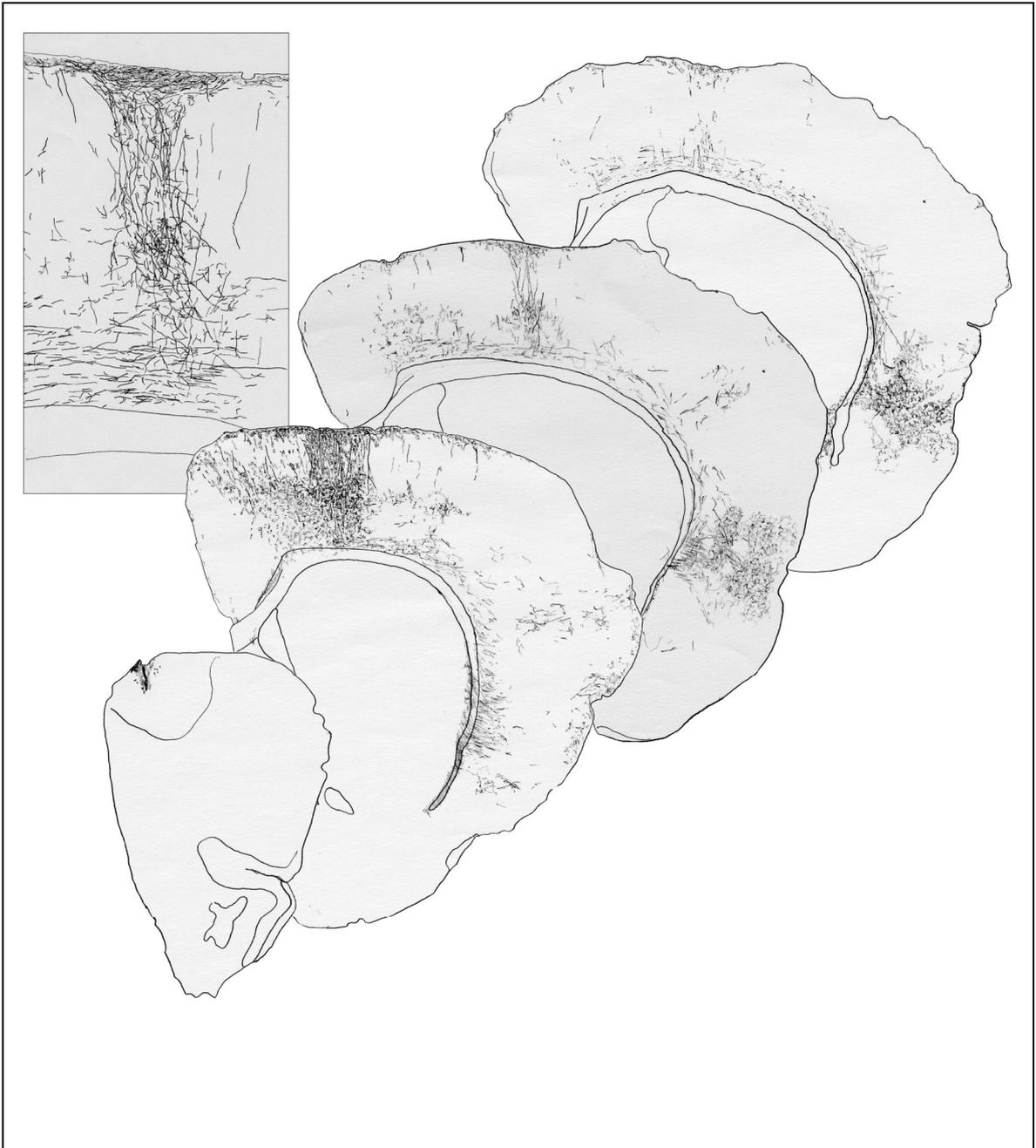


Abb. 29: Camera lucida Zeichnung eines zweiten Fallbeispiels aus der Gruppe **IR-MA – flache Injektion** (Fallnummer MC 95).  
Zu weiteren Hinweisen siehe Seite 52.

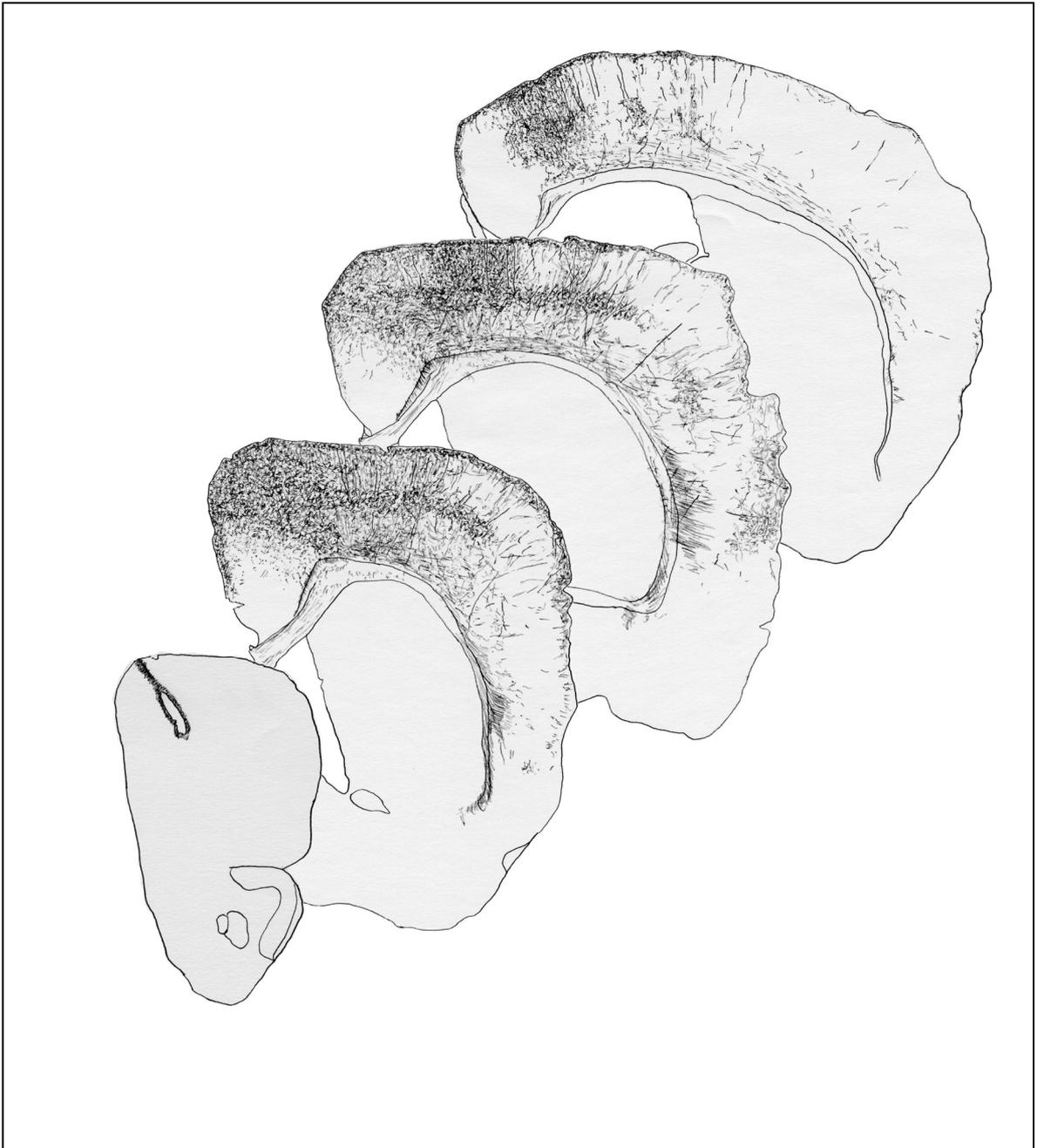


Abb. 30: Camera lucida Zeichnung eines ersten Fallbeispiels aus der Gruppe **IR-MA – tiefe Injektion** (Fallnummer MC 175).  
Zu weiteren Hinweisen siehe Seite 52.

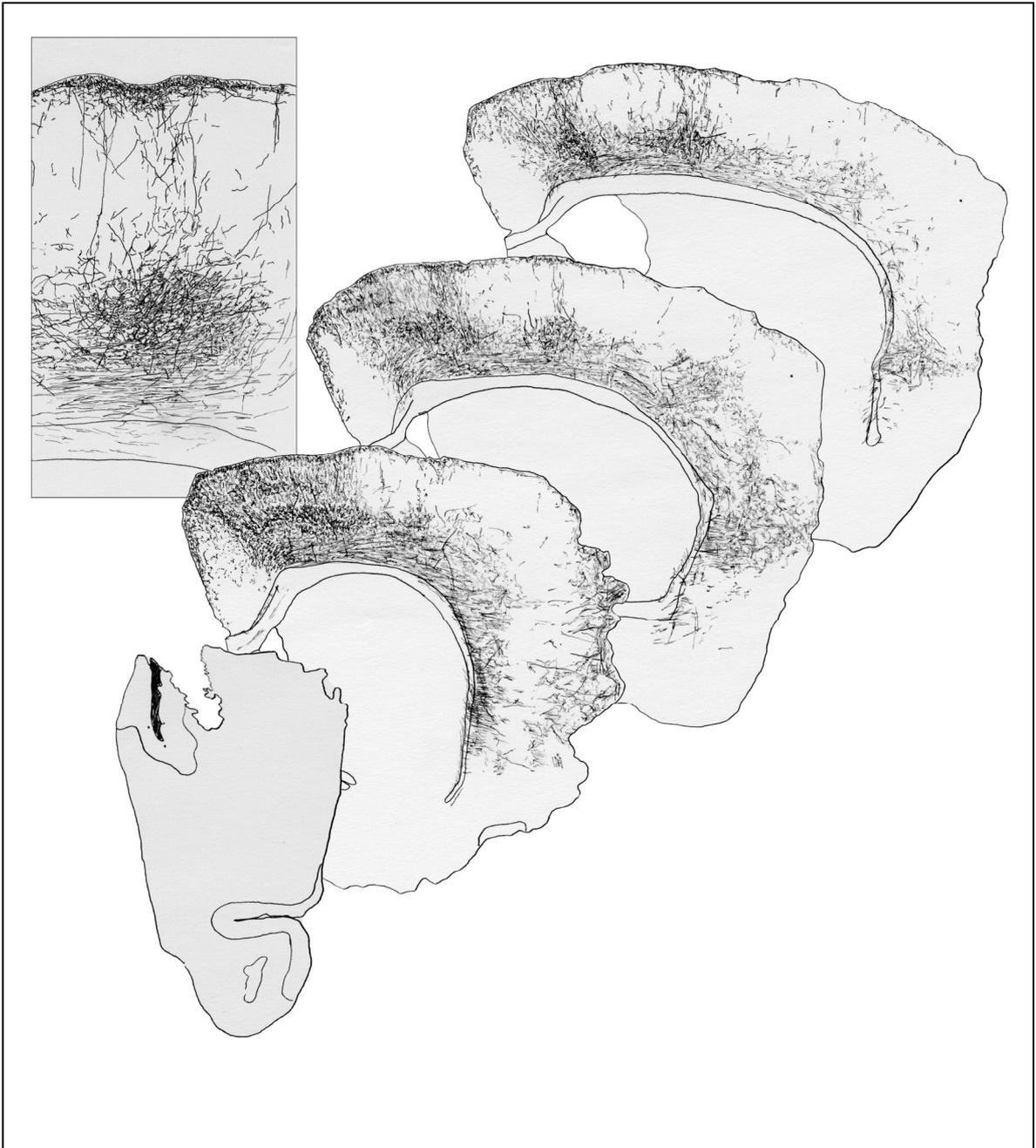


Abb. 31: Camera lucida Zeichnung eines zweiten Fallbeispiels aus der Gruppe **IR-MA – tiefe Injektion** (Fallnummer MC 109).  
Zu weiteren Hinweisen siehe Seite 52.

## **7.2 Danksagung**

---

Diese Schrift entstand in der Abteilung Neuroanatomie der Fakultät für Biologie an der Universität Bielefeld. Die Durchführung der Untersuchungen sowie der Abschluss dieser Arbeit wären ohne die Unterstützung vieler Personen nicht möglich gewesen.

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Ich danke auch dir - mein Sohn Samuel - denn durch dich hatte ich ein neues Ziel vor Augen. Dir sei diese Arbeit gewidmet.

### **7.3 Eidesstattliche Erklärung**

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Ich erkläre, dass ich die vorliegende Arbeit selbstständig angefertigt habe. Direkt oder indirekt übernommene Gedanken aus fremden Quellen sind als solche kenntlich gemacht.

Ferner erkläre ich, dass es sich um meinen ersten Promotionsversuch handelt.

Bielefeld, im Juli 2005

Francesco Bagorda