Adaptive Reifung von Dopamin und Serotonin im Nucleus accumbens, der integrativen Schnittebene zwischen Emotion und Bewegung:

Isolationsaufzucht und Methamphetamin-Intoxikation als Induktoren einer gestörten Reifung bei *Meriones unguiculatus*.

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Inhaltsverzeichnis

Z u s a m m e n f a s s u n g

Arbeiten an unserem Tiermodell (*Meriones unguiculatus*) zu den neurobiologischen Grundlagen der Psychose zeigen massive Veränderungen in der Reifung der Monoamine Dopamin (DA) und Serotonin (5-HT) in limbischen (Busche et al., 2004) und präfrontalen Gehirnarealen. Diese entwicklungsbedingten Veränderungen sind Folge einer chronischen Schädigung durch (1) restriktive Isolationsaufzucht (IR) und (2) einmalige frühkindliche Methamphetamin-Behandlung (MA-Intoxikation) am postnatalen Tag (P) 14. Ziel der vorliegenden Arbeit war es, den Einfluss der beiden nicht-invasiven experimentellen Induktoren - getrennt, beziehungsweise in Kombination - auf die postnatale DA und 5-HT Reifung in *Core* und *Shell* des Nucleus accumbens (NAC), dem so genannten „*limbic-motor interface*“, quantitativ zu untersuchen. Darüber hinaus wurde die Reifung der DAergen Innervation von *Core* und *Shell* analysiert, um mögliche kritische Phasen in der Entwicklung des NAC zu detektieren.

- *Core* und *Shell* des NAC weisen unterschiedliche Reifungsverläufe der DAergen Innervation auf. Die DAerge Faserdichte des *Core* fällt zwischen P 14 und 30 ab, danach steigt sie stetig bis P 180 an und bleibt auf stabilem Niveau bis P 720. Im *Shell* erfolgt ein leichter Anstieg der DAergen Faserdichte bis P 70, dann folgt ein hoch signifikanter Anstieg bis P 90 mit anschließender Angleichung an die *Core* Werte (Lesting et al., 2005a, submitted).
- IR führt zu einer signifikanten exzessiven Reifung der DAergen Faserdichte in *Core* und *Shell* bis P 90. Stattdessen hat die einmalige MA-Intoxikation bei restriktiv aufgewachsenen Tieren eine suppressive Reifung der DAergen Faserdichte in *Core* und *Shell* der linken und im *Core* der rechten Hemisphäre zur Folge. Keine Wirkung zeigt die MA-Intoxikation bei semi-natürlich aufgewachsenen Tieren (Lesting et al., 2005b).
- Die 5-HTerge Faserdichte des NAC wird durch IR nicht beeinflusst (Lehmann et al., 2003; Lesting et al., 2005b). Die einmalige MA-Intoxikation zeigt eine auf die rechte Hemisphäre beschränkte Anhebung der 5-HTergen Innervation in *Core* und *Shell* für beide Aufzuchtbedingungen (semi-natürlich und restriktiv). Einen signifikanten Lateralisierungseffekt zeigt die Kombination von IR und einmaliger MA-Intoxikation in *Core* und *Shell* des NAC (Lesting et al., 2005b).

Die unterschiedliche Reifung der DAergen Innervation von *Core* und *Shell* spiegelt die zeitlich differenzierte funktionelle Einbindung der accumbalen Subregionen in motorische und limbische Reifungsgeschehnisse wider. Darüber hinaus zeigt die Reifungsstudie, dass die zwei nicht-invasiven Induktoren unseres Tiermodells in der erkannten kritischen Reifungsphase (P14-70) auf unterschiedliche Weise auf den NAC einwirken. Damit unterstützt und ergänzt die Pathologie der Transmitterreifung im NAC die bereits in kortikalen und limbischen Regionen erhobenen Daten zu

einem „Zwei-Stufen-Modell“ auf dem Weg in die Psychose: Im ersten Schritt wird durch die chronische Deprivation lediglich die DAerge Faserdichte angehoben. Erst durch die zusätzliche pharmakologische Traumatisierung wird eine funktionale Diskonnektion zwischen DA und 5-HT in *Core* und *Shell* des NAC ausgelöst.

E i n l e i t u n g

Schwerwiegende psychiatrische Störungen, wie z.B. Schizophrenie, stehen im engen Zusammenhang mit Defekten der Integration von senso-motorischen und motivational-emotionalen Funktionen. Für die Integration dieser komplexen Teilleistungen spielt der Nucleus accumbens (NAC) eine zentrale Rolle, was sich aus der topographischen Lage des Kerns im Gehirn von Säugetieren und dem Menschen heraus erklärt (Heimer, 2003; Meredith et al., 1996). Als ventraler Teil des Caudatus-Putamen-Komplexes (ventrales Striatum) liegt er an der Basis des Vorderhirns und ist sowohl in motorische als auch limbische Schaltkreise eingebunden. Diese Funktionsaspekte finden sich innerhalb des NAC in der motorischen Subregion *Core* und der limbischen Subregion *Shell* wieder (Heimer et al., 1991; Zahm und Brog, 1992). Den NAC deswegen als „limbic-motor interface“ zu bezeichnen, trifft exakt den integrativen Charakter dieses Kernkomplexes (Mogenson et al., 1980). Auf subkortikaler Ebene gibt es kaum ein anderes Zentrum, das derart unmittelbar in psychiatrische Erkrankungen einbezogen ist, wie der NAC.

Wie in den letzten Jahren gezeigt, sind Serotonin (5-HT) und besonders Dopamin (DA) essentiell an der motivationalen senso-motorischen Integrationbildung im NAC beteiligt (Banjaw et al., 2005; Swerdlow und Geyer, 1998; Weiner, 2003). Es wird angenommen, dass sich auf accumbaler Ebene 5-HT und DA wechselseitig beeinflussen (Winstanley et al., 2005). Einschränkungen der Funktionalität des NAC und den damit einhergehenden Verhaltensauffälligkeiten werden - ebenso wie in anderen limbokortikalen Regionen - auf Fehlfunktionen eben dieser beiden Transmitter zurückgeführt (Heidbreder et al., 2000; Jones et al., 1992; Moore et al., 1999; Pralong et al., 2002; Swerdlow und Geyer, 1998; Winstanley et al., 2005). So erhält der NAC im Rahmen von Erklärungsmodellen zur Psychose sowohl in der biologischen Hirnforschung als auch in der medizinischen Forschung einen hohen Stellenwert (Grace, 2000; Lieberman et al., 1998; Risterucci et al., 2005; Weiner, 2003). Die auf den NAC konvergierenden Afferenzen aus limbokortikalen Arealen, wie dem präfrontalen Kortex (PFC), der Amygdala und dem Hippokampus (Berendse et al., 1992; Groenewegen et al., 1987; Groenewegen, 1999) müssen deswegen bei der Bewertung der NAC Pathologie unbedingt berücksichtigt werden. Eine fehlerhafte senso-motorische Integration der limbokortikalen Eingänge im NAC äußert sich z.B. in dem Defizit, irrelevante sensorische Stimuli zu ignorieren (*latent inhibition*) und dementsprechend ein Fehlverhalten zu initiieren (Weiner, 2003; Weiner und Feldon, 1997).

Obwohl sich die Symptome einer Psychose erst postpubertär manifestieren, werden die Ursachen einer Erkrankung neben der genetischen Prädisposition in einer schweren Störung der kindlichen Hirnreifung gesehen (Weinberger und Lipska, 1995). Pharmakologisch- oder Umwelt-induzierte Störgrößen können massiv die aktivitätsgesteuerten plastischen Prozesse der postnatalen Hirnreifung beeinflussen. Insbesondere das accumbale monoaminerge System reagiert sehr vulnerabel auf pharmakologische und Umwelt-induzierte Störungen während der Reifung

(Alquicer et al., 2004; Bennay et al., 2004; Brake et al., 2000a; Brake et al., 2004; Miura et al., 2002a). Schädigungen der Reifung der monoaminergen Innervation des NAC führen zu einer fehlerhaften sensomotorischen Integration im Adultstadium (Heidbreder et al., 2000). Der Zeitpunkt der Störung ist dabei von großer Bedeutung, da es in der prä- und postnatalen Hirnreifung zeitlich und räumlich definierte kritische Phasen gibt, in denen sich neuronale Nervennetze in einem äußerst instabilen und damit formbaren Zustand befinden (Lehmann und Teuchert-Noodt, 2005; Teuchert-Noodt und Lehmann, 2003). Den beiden Monoaminen DA und 5-HT kommt in diesem Reifungsgeschehen eine entscheidende Bedeutung zu, da sie nicht nur als Neurotransmitter wirken, sondern während der Reifung auch als morphogene Substanzen die Strukturierung von Nervennetzen unmittelbar beeinflussen (Lauder, 1988; Mattson, 1988).

Ein Schwerpunkt unserer Forschung liegt darauf, die Auswirkungen nicht invasiver Induktoren auf die aktivitätsgesteuerte Reifung des Säugergehirns zu untersuchen. Bisherige Ergebnisse geben deutliche Hinweise, dass durch die frühkindliche MA-Intoxikation und IR, ein strukturelles Korrelat zur Psychose induziert werden kann. Diese Störungen führen zu einer Hypofunktionalität des PFC und einer regionalen Hyperfunktion in limbischen Regionen; Eigenschaften, die entscheidende Merkmale der Psychose darstellen (Busche, 2004; Lehmann und Teuchert-Noodt, 2005; Polascheck, 2004). Diesem Tiermodell habe ich nunmehr den NAC als subkortikales Areal hinzugefügt. Die Reifung der accumbalen DAergen und 5-HTergen Innervation wurden unter dem Einfluss der beiden Interventionen, einer chronischen Beeinträchtigung der Versuchstiere über die Adoleszenz (IR) und einer akuten Traumatisierung (einmalige, nicht invasive MA-Intoxikation) untersucht. Da alle vorausgegangenen Studien an diesem Modell gezeigt haben, dass es für die Reifung der Transmitter in limbischen und kortikalen Hirnregionen einen Unterschied macht, ob eine oder beide Interventionen zum Einsatz kommen, wurde dieses einschließlich der Frage nach Lateralisierungseffekten berücksichtigt.

1. Zum Nucleus Accumbens

1.1 Lage und Verbindungen des Nucleus accumbens

Der NAC ist ein im rostroventralen Bereich des basalen Vorderhirns gelegenes Areal des Striatums [(ventrales Striatum) Abb.1A]. Er integriert über vielfältige Afferenzen die Aktivitäten aus dem präfrontalen Kortex, Amygdala, Hippokampus, Thalamus und dem Hirnstamm, in welchem DAerge, 5HTerge und noradrenerge afferente Systeme ihren Ursprung nehmen (Berendse et al., 1992; Berendse und Groenewegen, 1990; Brog et al., 1993; Van Bockstaele et al., 1993). Efferenzen werden vom NAC vornehmlich über vier anatomische überwiegend GABAerge Bahnen weitergeleitet (Pennartz et al., 1994). Über diese Bahnen nimmt der NAC rückwirkend Einfluss auf die aminergen Afferenzen aus dem Hirnstamm. Zusätzlich steuert er extrapyramidale, viszerale, limbische und kortikale Regionen an. In dieser multifunktionalen Einbindung gilt der NAC als die subkortikale Schnittstelle senso-motorischer und limbisch-assoziativer Funktionen (Mogenson et al., 1980): Er vermittelt in Prozessen der Aufmerksamkeits-, Belohnungs- und Motivationsbildung und ist an der Regulierung von autonomen und lokomotorischen Aktivitäten zentral beteiligt (Brog et al., 1993; Zahm, 2000). Diese genannten Teilaspekte werden von diskreten Subregionen des NAC vertreten, die man heute anatomisch und funktionell sehr gut gegeneinander abgrenzen kann. Ein Grossteil pharmakologischer und verhaltensbezogener Studien zum NAC hat sich mit diesem Kernkomplex als Ganzem befasst, und damit sind vermutlich viele Teilaspekte übersehen worden, die zum Verständnis motorischer und limbischer Funktionen/Disfunktionen von Bedeutung sind. Daher wurden in meinen Untersuchungen die accumbalen Subregionen immer getrennt bewertet, deren Charakterisierung hier folgen soll.

1.2 Subregionen des Nucleus accumbens

Der NAC wird aufgrund einer regional differenzierten Ausstattung mit Neurotransmittern, Neuropeptiden und Transmitterrezeptoren in die drei Subareale *Core*, *Shell* und *Rostraler Pol* unterteilt (Brog et al., 1993; Heimer et al., 1991; Zaborszky et al., 1985; Zahm und Brog, 1992). Die *Core*-Region umschließt die Commissura anterior und ist von der *Shell*-Region medial, ventral und lateral umgeben (Abb.1B). Der *Rostrale Pol* wird als Gebiet betrachtet, in dem *Core* und *Shell* noch nicht voneinander getrennt sind. Einer der besten immunhistochemischen Marker, um *Core* und *Shell* zu unterscheiden, ist das kalziumbindende Protein Calbindin. Für *Meriones unguiculatus* konnten wir zeigen, dass dieses Protein im *Core* stark angereichert wird, wohingegen es im *Shell* kaum nachzuweisen ist (Abb.1C).

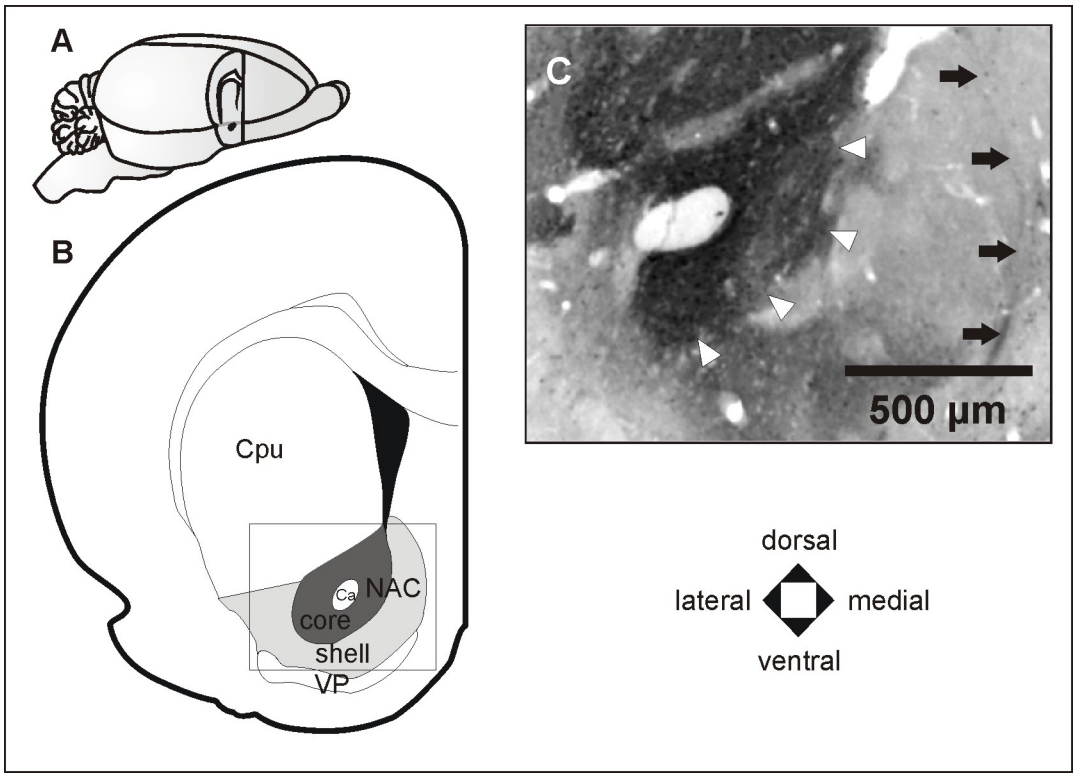

Abb. 1: Der Nucleus accumbens
(A) Lage des NAC. (B) Lage und Abgrenzung der Subregionen *Core* und *Shell*. Der Rahmen in (B) markiert die Lage des Photos in (C). (C) zeigt einen für Calbindin immunhistochemisch angefärbten Frontalschnitt. Die lateralen (weiße Pfeilspitzen) und medialen (schwarze Pfeile) Grenzen des *Shell* sind dargestellt.

Ca	Commissura anterior
Cpu	Caudatus-Putamen-Komplex
VP	ventrale Pallidum

Innerhalb der *Core*- und *Shell*-Region lassen sich funktionelle Zellgruppen (*neuronal ensembles*) unterscheiden, die auf differenzierte Ein- und Ausgangsverschaltungen schließen lassen (Meredith, 1999; Pennartz et al., 1994). Besonders deutlich treten diese Zellgruppen im *Shell* hervor, in dem sich der dorsale caudomediale Abschnitt, auch „septaler Pol“ oder „*cone*“ genannt, anhand hoher Zelldichte und spezifischer Immunreaktivität von den lateralen Gebieten abgrenzen lässt (Voorn et al., 1986; Voorn et al., 1989). Die Neurone des NAC bestehen zu 90-95 % aus den so genannten „*medium-sized spiny*“ Projektionsneuronen, deren Transmitter GABA ist. Eine heterogene Population von GABAergen und acetylcholinergen Interneuronen beläuft sich auf etwa 5%. Sowohl die Zelldichte als auch die Spinedichte sind im *Core* größer als im *Shell* (Meredith et al., 1993; Meredith, 1999).

Hinsichtlich der afferenten und efferenten Verbindungen zeigen sich deutliche Unterschiede der accumbalen Subareale *Core* und *Shell* (Brog et al., 1993; Heimer et al., 1991; Zahm und Brog, 1992). Glutamaterge Afferenzen aus dem dorsalen präfrontalen Kortex (PFC) (Berendse et al., 1992; Zahm und Brog, 1992), der basolateralen Amygdala (Groenewegen, 1982) und dem dorsalen Subiculum (Groenewegen et al., 1987) projizieren ins *Core*. Glutamaterge Afferenzen aus dem ventralen PFC (Berendse et al., 1992; Zahm und Brog, 1992), der basolateralen Amygdala

(Groenewegen, 1982), dem ventralen Subiculum und dem entorhinalen Kortex projizieren ins *Shell* [Abb.2 (Groenewegen, 1982)].

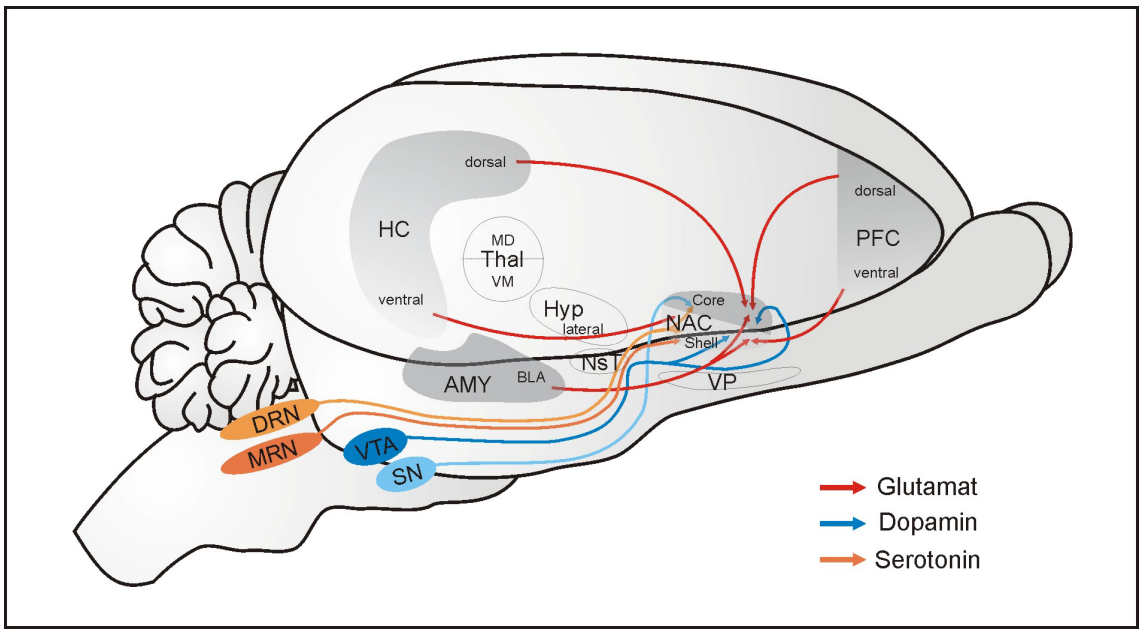

Abb. 2: Limbokortikale und monoaminerge Afferenzen von *Core* und *Shell* des NAC.
Dargestellt sind die glutamatergen Afferenzen aus dem dorsalen Hippokampus, dem dorsalen präfrontalen Kortex und der basolateralen Amygdala des *Core*. Monoaminerge Afferenzen des *Core* nehmen ihren Ursprung aus der Substantia nigra pars compacta (DA), der VTA (DA) und der dorsalen Raphe (5-HT). Das Shell erhält glutamaterge Afferenzen aus dem ventralen Hippokampus, der basolateralen Amygdala und dem ventralen präfrontalen Kortex. Die monoaminergen Afferenzen projizieren aus der VTA (DA) und der medialen und dorsalen Raphe (5-HT). Afferenzen aus dem Thalamus, dem Hirnstamm und dem ventralen Pallidum sind aus Gründen der Übersicht nicht dargestellt.

AMY	Amygdala	NsT	Nucleus subthalamicus
BLA	basolaterale Amygdala	PFC	präfrontaler Kortex
DRN	Nucleus raphe dorsalis	SN	Substantia nigra
HC	Hippokampus	Thal	Thalamus
Hyp	Hypothalamus	VM	Thalamus, ventromedial
MD	Thalamus, mediodorsal	VP	ventrale Pallidum
MRN	Nucleus raphe medialis	VTA	Ventrale tegmentale Area

Bezüglich der Efferenzen innerviert das *Core* fast ausschließlich typische Ausgangsareale der Basalganglien, wie das laterale ventrale Pallidum, den subthalamischen Kern und die Substantia nigra (SN) (Abb.3A). Demgegenüber projiziert das *Shell* verstärkt in subkortikale limbische Areale, wie lateralen Hypothalamus, VTA, ventromediales ventrales Pallidum und autonome Kerngebiete des Hirnstamms [Abb.3B (Zahm und Brog, 1992)].

Es zeigt sich also eine eindeutige topische Zuordnung von *Core* und *Shell*, die Verbindungen mit unterschiedlichen Anteilen gleicher Hirnareale aufweisen. Diese vielfältigen Ein- und Ausgänge geben Hinweis darauf, dass *Core* und *Shell* in eigenständige Funktionskreise eingebunden sind.

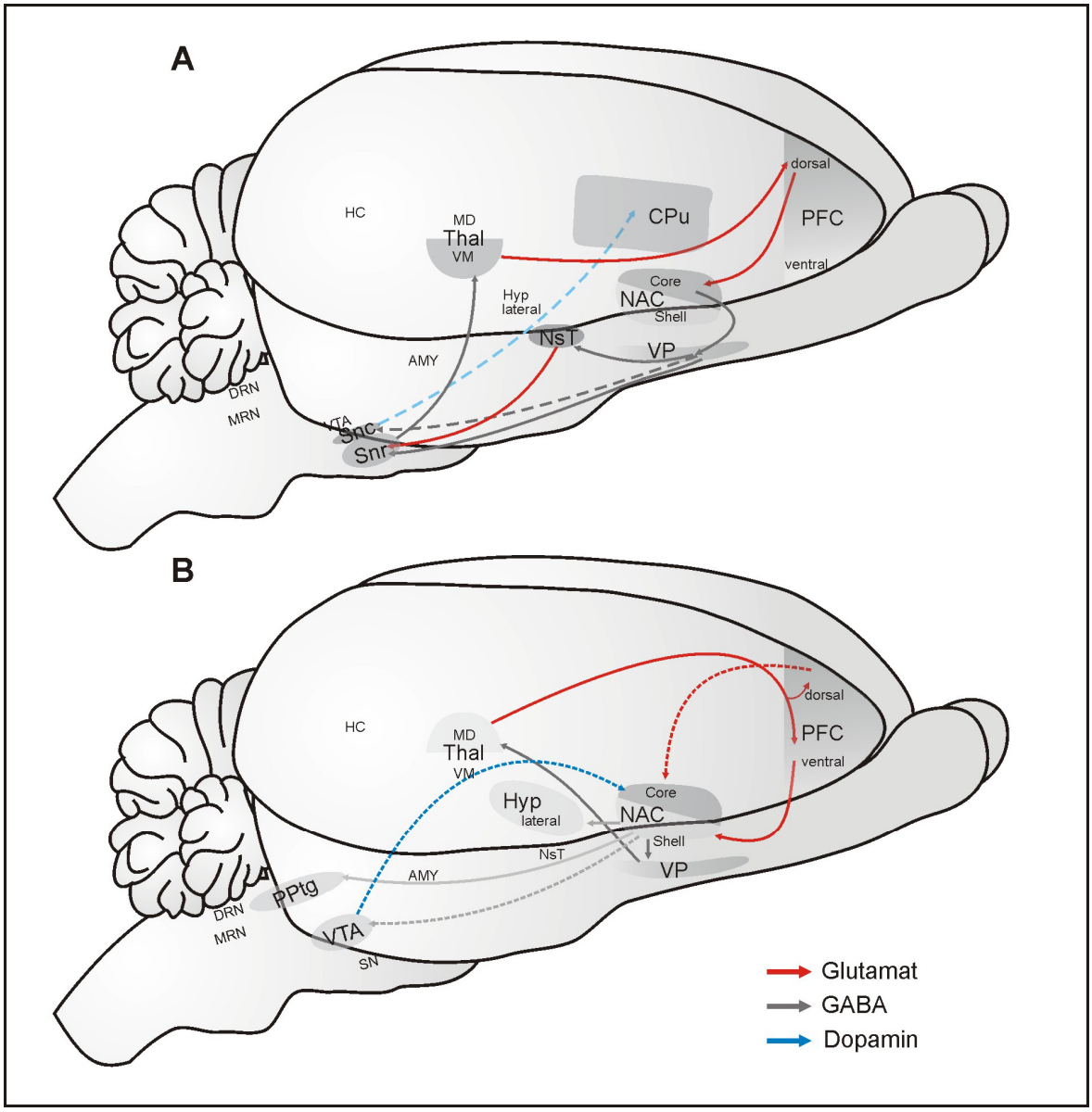

Abb. 3: Schaltkreise von *Core* und *Shell*
(A) Schaltkreise des *Core*. Durchgezogene Pfeile stellen den geschlossenen Schaltkreis vom PFC über das Core zurück zum PFC dar. Gestrichelte Pfeile markieren die Verbindungen zum Caudatus-Putamen-Komplex (siehe Text). (B) Schaltkreise des *Shell*. Durchgezogene Pfeile kennzeichnen die Verbindungen des *Shell* in viszero- (Hyp) und lokomotorische Areale (PPtg). Darüber hinaus ist der geschlossene Schaltkreis zum PFC dargestellt. Gestrichelte Pfeile kennzeichnen die Verbindungen, über die das *Shell* die Aktivität des Core moduliert [siehe Text (verändert nach Zahm, 2000)]. PPtg: Nucleus tegmenti pedunculopontinis (weitere Abkürzungen siehe Abb.1 und Abb.2)

Die Projektionen aus dem dorsalen PFC, die funktionell einen motorischen Gehalt haben (Berendse et al., 1992; Groenewegen et al., 1996; McFarland et al., 2004; Uylings et al., 2003), werden im *Core* mit den Eingängen aus der Amygdala und der Hippokampusformation integriert. GABAerge Projektionsneurone des *Core* terminieren im ventralen Pallidum; von dort bestehen direkte und indirekte Verbindungen zur SN (pars reticulata), weiter zum ventromedialen Thalamus und zurück in den dorsalen PFC, womit der „motorische“ Kreislauf des *Core* geschlossen ist (Zahm, 2000). Zusätzlich nimmt das *Core* durch die direkte und indirekte Projektion in die SN (pars compacta) Einfluss auf das DAerge System des dorsalen Striatums, welches motorische

Bewegungsabläufe verarbeitet [Abb. 3A (Brog et al., 1993; Heimer et al., 1991)]. Das *Core* wird aufgrund dieser spezifischen Verbindungen dem motorischen Funktionskreis zugeordnet.

Demgegenüber wird das *Shell* dem limbischen Funktionskreis zugeordnet. Es unterhält prominente Efferenzen in den lateralen Hypothalamus, die VTA und den mediodorsalen Thalamus (über das ventrale Pallidum) und steht in enger funktioneller Verbindung zur *extended Amygdala* (Alheid und Heimer, 1996; Heimer et al., 1997). Darüber greift diese accumbale Region in die Regulation neuroendokriner Funktionen, in zielgerichtetes Handeln, Schmerzverarbeitung, Defensivverhalten und Lokomotion ein (Groenewegen et al., 1996; Heimer et al., 1991). Ein weiterer hoch komplexer Regelkreis sei genannt: Die afferenten emotional gefärbten Eingänge aus dem ventralen PFC (Berendse et al., 1992; Groenewegen et al., 1996; McFarland et al., 2004; Uylings et al., 2003) werden im *Shell* unter Abgleich der amygdaloiden und hippokampalen Projektionen integriert und die prominenten Projektionen des *Shell* zum ventralen Pallidum werden über den mediodorsalen Thalamus zurück zum ventralen PFC gesendet (geschlossener Schaltkreis).

Ferner sind Wechselbezüge zwischen den accumbalen Regionen gegeben, in die externe Schaltkreise einbezogen werden. So projiziert der mediodorsale Thalamus in den dorsalen PFC (offener Schaltkreis), welcher massiv das *Core* und damit den motorischen Kreislauf innerviert (Zahm, 2000). In ähnlicher Weise kann das *Shell* über die Projektionen ins ventrale Pallidum die DAerge Innervation des *Core* über die VTA modulieren. Von daher wird generell angenommen, dass emotional/motivational gefärbte Ereignisse im *Shell* den Aktivitätslevel im *Core* und im dorsalen Striatum modulieren [Abb.3B (Otake und Nakamura, 2000; Zahm, 2000)].

Nach diesen allgemeinen Hintergrundinformationen ist es nunmehr wichtig, auf die zur Untersuchung anstehenden Transmitter, DA und 5-HT, einzugehen, die die dargelegten hoch komplexen Strukturbezüge von *Core* und *Shell* aus dem Hirnstamm regulativ und integrativ ansteuern.

1.3 Die DAerge Innervation des Nucleus accumbens

Die dichte mesencephale DA-Innervation des NAC ist essentiell an der Filterung biologisch relevanter sensorischer Informationen aus den kortiko-limbischen Arealen beteiligt (Horvitz, 2002) und eng mit Belohnungs-assoziiertem Verhalten, lokomotorischer Aktivität, Stressreaktionen, Neuheitsdetektierung und assoziativen Lernprozessen verbunden (Cabib et al., 2002; Di Chiara et al., 2004; Kelley, 1999; Koch et al., 1996; Koch et al., 2000; Lillrank et al., 1999; Meredith, 1999; Pierce und Kalivas, 1995).

Am Nagergehirn konnte gezeigt werden, dass *Core* und *Shell* deutlich verschiedene DAerge Afferenzen aus den Kerngebieten des Mittelhirns erhalten und sich dementsprechend die Innervationsmuster gegeneinander abgrenzen lassen (Brog et al., 1993; Neddens et al., 2002; Voorn et al., 1986). Die SN pars compacta und die laterale VTA projizieren ins *Core*, wohingegen das mediale *Shell* ausschließlich DAerge Afferenzen aus der medialen VTA erhält (Brog et al., 1993).

Pharmakologische Studien zur physiologischen Beeinflussung des DA-Systems (Bassareo und Di Chiara, 1999; Li et al., 2004; Murphy et al., 2000; Weiner, 2003; Weiner und Feldon, 1997) und zur Verhaltenspharmakologie (Bassareo und Di Chiara, 1999; Li et al., 2004; Murphy et al., 2000; Weiner, 2003; Weiner und Feldon, 1997) bestätigen ein selektives Wirkungsspektrum von DA auf *Core* und *Shell*. So kann man allgemein festhalten, dass die DA Innervation des *Shell* im engen Zusammenhang mit dem assoziativen operanten Erlernen natürlicher appetitiver und aversiver Verstärker und der Initiierung einer lokomotorischen Reaktion bezüglich des Reizes steht. Hingegen korreliert das anschließende Erlernen einer angepassten instrumentellen motorischen Antwort mit der DAergen Innervation des *Core* (Di Chiara, 2002; Kelley, 1999; Phillips et al., 2003).

1.4 Die 5-HTerge Innervation des Nucleus accumbens

Ähnlich wie DA, hat die 5-HT Innervation des NAC modulierenden Einfluss auf glutamaterge emotional/motivational gefärbte Eingänge aus dem Hippokampus (Hippokampus), der Amygdala (Amygdala) und dem PFC in den NAC. Neben einer direkten Innervierung exzitatorischer Eingänge (Van Bockstaele und Pickel, 1993) nimmt 5-HT eine entscheidende Rolle in der Modulation accumbaler DAerger Afferenzen ein (Van Bockstaele und Pickel, 1993), die in Abhängigkeit spezifischer 5-HT Rezeptoren gehemmt oder verstärkt werden können (Chen et al., 1991; De Deurwaerdere und Spampinato, 1999). Diese axo-axonischen Terminalien nehmen 75% der 5-HTergen Innervation des NAC ein (Van Bockstaele und Pickel, 1993). Axo-dendritische Synapsen terminieren vorwiegend an proximalen Dendritenabschnitten der „*medium spiny neurons*“ und können so starken Einfluss auf die Generierung von Aktionspotentialen am Soma nehmen (Van Bockstaele und Pickel, 1993).

Die Zellkörper der accumbalen 5-HTergen Neurone liegen in zwei großen Kerngebieten des Hirnstamms, der dorsalen und der medialen Raphe. Diese beiden Nuklei unterscheiden sich bezüglich der Morphologie ihrer Axone. Fasern aus der dorsalen Raphe sind glatt und wenig verzweigt, im Gegensatz zu den gewundenen und dicht mit Varikositäten besetzten Fasern der medialen Raphe (Kosofsky und Molliver, 1987; Mamounas et al., 1991). Aufgrund dieser Charakteristika nimmt man an, dass das *Core* vorwiegend aus der dorsalen Raphe und das *Shell* aus der medialen und dorsalen Raphe innerviert wird (Brown und Molliver, 2000).

1.5 Lateralisierung im Nucleus accumbens

Die cerebrale Lateralisierung ist nicht mehr, wie vor Jahrzehnten angenommen, ein ausschließlich menschliches Charakteristikum. Anatomische und verhaltensbezogene Asymmetrien konnten auch in Nagern (Denenberg, 1983; Glick und Ross, 1981; Hiscock und Kingsbourne, 1995; Zilles et al., 1996) und Primaten (Hiscock und Kingsbourne, 1995; Zilles et al., 1996) nachgewiesen werden. Zusätzlich zeigen neurochemische Studien am Nagergehirn kortikale und

subkortikale monoaminerge Asymmetrien (Rosen et al., 1984), die DAerge und 5-HTerge Konzentrationen und Umsätze im NAC einschließen (Besson und Louilot, 1995; Rosen et al., 1984; Schwarting et al., 1998; Sullivan und Szechtman, 1995; Thiel und Schwarting, 2001). Solche Hemisphären-spezifischen Unterschiede können stark durch pharmakologische Interventionen (Andersen et al., 2002), pränatalen (Alonso et al., 1994; Alonso et al., 1997) und perinatalen (Brake et al., 2000b) Stress und frühkindliches *handling* (Denenberg et al., 1978; Denenberg et al., 1980; Tang und Verstynen, 2002; Verstynen et al., 2001) in der Entwicklung beeinflusst werden. Einige dieser experimentellen Parameter werden als Tiermodell menschlicher affektiver Störungen diskutiert, in denen der NAC eine entscheidende Rolle spielt (Andersen et al., 2002).

1.6 Der Nucleus accumbens als integrative Schaltstelle im limbopräfrontalen System

Das limbopräfrontale System schließt den PFC, limbische Areale wie die Hippokampusformation, Amygdala und die im Hirnstamm lokalisierten monoaminergen Kerngebiete ein. Diese Areale stehen sowohl anatomisch, als auch funktionell in enger Wechselwirkung zueinander und sind an der Verarbeitung motivationaler, emotionaler und lernspezifischer Gedächtnisleistungen beteiligt (Rosenkranz und Grace, 2002; Rosenkranz und Grace, 2003). Wie schon erwähnt, besteht die Rolle des NAC in diesem Schaltsystem darin, biologisch relevante sensorische Informationen aus den kortikolimbischen Arealen in adaptiertes, motorisches Verhalten umzusetzen und zusätzlich auf autonomer Ebene weiterzuverarbeiten.

Die kortikolimbischen glutamatergen Afferenzen konvergieren jeweils auf definierten Zellgruppen des NAC (Pennartz et al., 1994). Bei gleichzeitiger Aktivität zweier Afferenzen können Aktionspotentiale in den *medium spiny neurons*, den GABAergen Projektionsneuronen des NAC, ausgelöst werden (O'Donnell, 1999). Dies geschieht durch eine tonische Vordepolarisierung der Projektionsneurone (z.B. aus dem Hippokampus), gefolgt von einem phasischen Signal (z.B. aus dem PFC), welches sich dem tonisch aktivierten Membranpotential aufaddiert (Grace, 2000; O'Donnell, 1999). Ein solcher physiologischer Vorgang, „*ensemble coding*“, konnte z.B. für konvergierende Eingänge aus dem PFC und dem Hippokampus (French und Totterdell, 2002; O'Donnell und Grace, 1995), dem PFC und der Amygdala (Finch, 1996) und der Amygdala und dem Hippokampus (French und Totterdell, 2003) nachgewiesen werden.

Auf funktioneller Ebene bedeutet dies, dass die Eingänge aus dem PFC, welche motorische Handlungspläne und zielgerichtetes Verhalten vermitteln, mit sensorischer kontextspezifischer Information aus dem Hippokampus und affektiver Information aus der Amygdala abgeglichen werden (Grace, 2000). Hat ein Stimulus z.B. einen hohen affektiven Wert, so wird der aktuelle Kontext spezifische Planungsentwurf aus dem hippokampalen und präfrontalen System gedrosselt und das Verhalten emotional bestimmt. Dementsprechend wird das ursprüngliche Verhalten geändert und ein besser angepasstes ausgelöst, was man als eine im NAC verankerte „*switching-Funktion“* bezeichnet (Weiner und Feldon, 1997).

Durch die DAerge Innervation des NAC innerhalb dieses Funktionskreises wird die Aktivität der aktuell erregten *ensembles* verstärkt, und aktuell inaktive *ensembles* werden gehemmt, was einer selektiven Signalfilterung gleichkommt (O'Donnell, 1999). DA kann diesen selektiven Filtermechanismus über eine phasische und eine tonische Ausschüttung bewerkstelligen. Der tonische DA Spiegel spricht die hoch affinen präsynaptischen D2 Rezeptoren an und moduliert die präfrontalen Afferenzen. Eine erhöhte tonische DA Ausschüttung hemmt diese (Goto und Grace, 2005). Die phasische DA Ausschüttung aktiviert postsynaptische D1 Rezeptoren, die bei Ansprache die hippokampalen und vermutlich auch die amygdaloiden Eingänge verstärken (Charara und Grace, 2003; Goto und Grace, 2005).

2. *Zwei Induktoren als Störfaktoren in der Transmitterreifung*

Ein Schwerpunkt unserer Forschung liegt darauf, die bekanntlich aktivitätsabhängige strukturelle Reifung der monoaminergen Transmittersysteme DA und 5-HT im limbopräfrontalen und motorischen System des Säugergehirns (*Meriones unguiculatus*) vor dem Hintergrund umweltbezogener Einflussfaktoren darzustellen. Unsere Arbeitsgruppe bedient sich zweier „nicht invasiver“ Methoden, um schädigende Einflüsse auf die systemische Reifung des Gehirns männlicher Wüstenrennmäuse zu untersuchen und eine Traumatisierung der Tiere zu induzieren (siehe Lehmann und Teuchert-Noodt, 2005). Die quantitative Bewertung von adaptiven Veränderungen in der Transmitterreifung erfolgt, so auch in meinen Studien, im jung erwachsenen Alter [P 90 (Abb. 4)].

Das Tiermodell beschreibt ein so genanntes Zwei-Stufen-Modell (*Two-Hit-Modell*). Die MA-Intoxikation ist eine direkte akute Störung auf neuronaler Ebene und kann als frühkindliche Traumatisierung angesehen werden. Die restriktive Isolationsaufzucht (IR) ist eine chronische umweltbezogene, soziale Deprivation während der Adoleszenz. Das heißt die beiden nicht invasiven Induktoren setzen zu unterschiedlichen Reifungsphasen ein und unterscheiden sich bezüglich ihres Wirkungsspektrums. Jede Störung für sich nimmt Einfluss auf die Reifung der monoaminergen Innervation in kortikalen und limbischen Arealen. Die Kombination beider Induktoren („Two-Hit“), also eine frühkindliche Traumatisierung (erste Hit) gefolgt von einer chronischen Deprivation (zweite Hit), führt zu noch deutlicher ausgeprägten Veränderungen, indem die Effekte der IR verstärkt werden. Eine Kopplung solcher Störungen wird auch in der Grundlagenforschung der Schizophrenie postuliert. So spricht Gaebel von einer angeborenen bzw. erworbenen Prädisposition, die erst durch zusätzliche störende Faktoren während der Entwicklung zum späteren Krankheitsbild führen (Gaebel, 2003).

A. restriktive Isolationsaufzucht:

Gerbils werden in Standard-Makrolonkäfigen (Typ 4) geboren und am postnatalen Tag 30 nach ihrer Entwöhnung in Standard-Makrolonkäfigen (Typ 3) vereinzelt. Im Vergleich zu den Gerbils der Kontrollgruppe, welche im Gehege geboren werden und nach ihrer Entwöhnung im Geschwisterverband in mit Spiel- und Versteckmöglichkeiten angereicherten Gehegen (1x1m) aufwachsen, stellt die IR eine chronisch soziale Deprivation dar. Diese Deprivation führt zu signifikanten Veränderungen DAerger und 5-HTerger Faserdichten in mehreren Gehirnarealen erwachsener Gerbils (Lehmann et al., 2002; Lehmann et al., 2003; Neddens et al., 2001; Neddens et al., 2003; Winterfeld et al., 1998) und darüber hinaus zu einer Veränderung der präfrontalen Efferenzen in kortikale Terminationsgebiete (Bagorda et al., 2005). Die anatomischen und strukturellen Veränderungen spiegeln sich funktionell in mehreren gestörten Verhaltensweisen wider, wie z.B. erhöhter lokomotorischer Aktivität, erhöhter Ängstlichkeit, Defiziten in der Furchtbewältigung und Schwächung des Arbeitsgedächtnisses (Polascheck, 2004; Winterfeld et al., 1998).

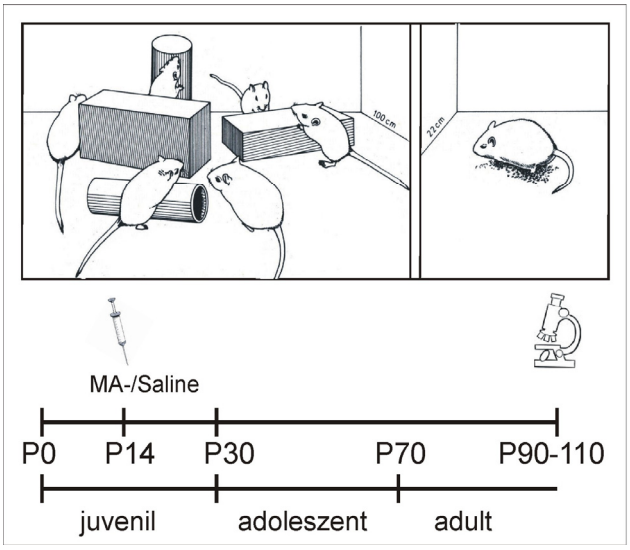

Abb. 4: Tiermodell
Gerbils aus angereicherter und IR erhalten am P 14 eine einmalige Dosis MA (50mg/kg i.p.) oder Saline. Im jung erwachsenen Alter (P90-110) werden die Tiere immunhistochemisch aufgearbeitet.

B. Methamphetamin-Intoxikation:

MA wirkt selektiv neurotoxisch auf DA und 5-HT im ZNS (Seiden et al., 1988). DA und 5-HT werden durch MA verstärkt ausgeschüttet, und der Reuptake in die Präsynapse wird gehemmt. Dadurch kommt es zu einer unphysiologisch hohen Anreicherung der Monoamine im synaptischen Spalt und infolge dessen zur nicht-enzymatischen Bildung von 6-Hydroxydopamin bzw. 5,7-Dihydroxitryptamin (Seiden und Vosmer, 1984). Diese werden von der Präsynapse wieder aufgenommen und bewirken dort degenerative Prozesse durch die Bildung freier Radikale (Ricaurte et al., 1980).

Am P 14 verabreicht, führt MA zu Veränderungen in der Reifung von DAergen und 5-HTergen Faserdichten in Abhängigkeit der jeweiligen Aufzuchtbedingungen. Isoliert aufgewachsene und MA behandelte Gerbils zeigen eine supprimierte Reifung der DA Faserdichte im PFC (Dawirs et al., 1994), wohingegen Gehegeaufzuchten keine Veränderungen aufweisen (Neddens et al., 2002). Die MA-Behandlung isoliert aufgewachsener Tiere zeigt keine

Veränderungen im 5-HTergen System. Dagegen kommt es zu einer signifikanten Erhöhung der 5-HTergen Faserdichte nach Gehegeaufzucht (Neddens et al., 2003). Im medialen Caudatus-Putamen Komplex führt die Kombination von MA-Intoxikation und Isolation zu einer Erhöhung der 5-HT Innervation (Lehmann et al., 2003).

Aufgrund dieser induzierten Effekte auf die Reifung der Transmitter in diversen Gehirnarealen, ergab sich die dringende Frage, ob auch der NAC von diesen nicht-invasiven Interventionen betroffen ist. Wie aus eingangs dargelegten Erkenntnissen zur Funktionsanatomie des NAC zu entnehmen ist, war eine getrennte Bewertung der beiden Subregionen *Core* und *Shell* unbedingt zu berücksichtigen. Das wiederum setzte eine Entwicklungsstudie voraus, denn bisher ist auf struktureller Ebene nicht bekannt, ob beide Subregionen eine ähnliche oder voneinander abweichende Reifung beschreiten. Aus diesem ganzheitlichen Ansatz ergeben sich die Untersuchungen, welche den beiliegenden Publikationen zugrunde gelegt waren.

Fragestellungen:

- Gibt es für DA unterschiedliche Reifungsverläufe in den accumbalen Subregionen *Core* und *Shell*?
- Lassen sich kritische Phasen, anhand der postnatalen Reifung des DAergen Systems, in der Entwicklung des NAC detektieren?
- Inwieweit wirken sich die Induktoren, IR und MA-Intoxikation, auf die Reifung der DAergen und 5-HTergen Faserdichte in *Core* und *Shell* des NAC aus?
- Gibt es Hemisphären-spezifische Einflüsse der Versuchsparameter auf die Reifung des NAC, d.h. gibt es Lateralisierungseffekte?

Aus diesem Fragenkatalog werden die publizierten Befunde im Folgenden zusammenfassend vorgestellt. Die bereits für viele Regionen des limbopräfrontalen Systems erhobenen Daten werden durch meine Arbeiten also ergänzt**.** Damit wird ein Baustein beigelegt, der die Tauglichkeit unseres Tiermodells für die Psychoseforschung weiterhin überprüfen soll.

3. Z u d e n B e f u n d e n

3.1 Zur Reifung der DAergen Innervation des Nucleus accumbens

(Lesting et al., 2005a, submitted)

Die Ergebnisse zur Reifung der DAergen Faserdichte des NAC zeigen*, dass erst im Erwachsenenalter zwischen Tag 90 und 130 ein stabiles Niveau der DAergen Faserdichte erreicht wird. Darüber hinaus zeigen sich deutliche Unterschiede in der DAergen Reifung der accumbalen Subareale Core und Shell. Die moderate DAerge Faserdichte des Core am postnatalen Tag (P) 14 verringert sich deutlich zum Tag 30. Danach steigt sie stetig an und bleibt auf einem stabilen Niveau bis zum Tag 720. Die Reifung der DAergen Faserdichte des Shell zeigt keine signifikanten Veränderungen bis zum Tag 70, gefolgt von einem rapiden, signifikanten Anstieg bis zum Tag 90, um sich dann rasch auf einem Niveau einzupendeln, das bis zum Tag 720 stabil erhalten bleibt* (Lesting et al., 2005a, submitted)[1].

Die DAerge Innervation von *Core* und *Shell* reift ab Tag 14 parallel zu den accumbalen Projektionsneuronen (*medium spiny neurons*). So konnte an der Ratte gezeigt werden, dass die Projektionsneurone erst zum Ende der dritten postnatalen Woche adulte physiologische Charakteristika angenommen haben (Belleau und Warren, 2000), und dass die Reifung und Synapsenbildung in enger Wechselwirkung zur DAergen und glutamatergen Innervation steht (Antonopoulos et al., 2002; Spencer et al., 1998). Das heißt, die Reifung und funktionelle Integration des NAC wird juvenil im Wechselspiel von reifenden afferenten Eingängen und reifenden accumbalen Neuronen gewährleistet (Belleau und Warren, 2000).

Die hier erstmalig auf struktureller Ebene aufgezeigten unterschiedlichen Reifungsverläufe von Core und Shell während der Adoleszenz lassen sich vermutlich mit den verschiedenen Ursprüngen der mesencephalen DAergen Projektionsneurone der accumbalen Subareale in Zusammenhang bringen. So wird das für diese Arbeit ausgewertete mediale *Shell* fast ausschließlich aus der VTA innerviert, wohingegen das *Core* neben der VTA zusätzliche Afferenzen aus der SN erhält (Brog et al., 1993). Die SN ist funktionell dem motorischen und die VTA dem limbischen System zuzuordnen (Pralong et al., 2002). Die Neurogenese (Altman und Bayer, 1981) sowie die physiologische Reifung der beiden mesencephalen Kerngebiete zeigt einen unterschiedlichen Verlauf (Wang und Pitts, 1994). Beachtenswert ist ferner, dass während der embryonalen Entwicklung die Projektionen beider DAergen Kerne im NAC ein undifferenziertes Terminationsmuster aufweisen. Erst mit fortschreitender Reifung werden falsch terminierende Kollaterale eliminiert und eine differenzierte accumbale DAerge Innervation angelegt (Hu et al., 2004). Die in meiner Arbeit gezeigte vorübergehende Absenkung der Faserdichte im *Core* zwischen P 14-30 könnte diese Reorganisation der unterschiedlichen Ursprünge der DAergen Innervation auf struktureller Ebene repräsentieren.

[1] Sämtliche kursiv gedruckten Sätze beinhalten Ergebnisse meiner Publikationen.

Eine selektive Betroffenheit der DA-Reifung im *Core* wird durch weitere Daten unterstützt. So verläuft die DA-Reifungskurve im *Core* genau entgegengesetzt zur Entwicklung von DA Rezeptordichten. Ratten zeigen einen Überschuss der accumbalen DA Rezeptordichten zum Zeitpunkt der Entwöhnung, gefolgt von einer adoleszenten Abnahme [*pruning* (Tarazi und Baldessarini, 2000)]. Darin könnte ein Kompensationsmechanismus gegenüber der Faserreifung zum Ausdruck kommen (Coulter et al., 1996). So wird z.B. eine selektive Zerstörung nigrostriärer DA Fasern durch eine Anhebung der DA-Rezeptordichten im dorsalen Striatum kompensiert (Gnanalingham et al., 1993). Weitere Hinweise sprechen für eine selektive Empfindlichkeit vom *Core* während der Adoleszenz, für die nunmehr der Einbruch in der DA-Faserdichtezunahme (P 14-30) verantwortlich gemacht werden kann: DAerge Neurone der SN lassen sich anhand der höheren DA-Transporter (DAT) Dichte von denen der VTA unterscheiden (Blanchard et al., 1994), wobei das *Core* eine deutlich höhere DAT Expression als das *Shell* aufweist (Nirenberg et al., 1997). Die Konzentration des DAT im dorsalen Striatum, welches fast ausschließlich aus der SN innerviert wird, zeigt signifikante Veränderungen in der Entwöhnungs- und Adoleszenz-Phase (Moll et al., 2000). Auch dies lässt also die Vermutung zu, dass sich die kritisch-dynamischen Veränderungen des DAergen Systems zum Zeitpunkt der Entwöhnung und des Heranwachsens auf von der SN innervierte Areale, wie dorsales Striatum und *Core* des NAC, fokussieren.

Die im *Core* beobachteten Reorganisationsprozesse lassen auf eine erhöhte Vulnerabilität gegenüber äußeren Einflüssen in dieser kritischen Phase schließen: Das *Core* spielt eine Schlüsselrolle in der Kontrolle impulsiven Wahlverhaltens, welches bei ADHS Patienten grundlegend gestört ist (Cardinal et al., 2004; Cardinal und Cheung, 2005). Da diese Störung verstärkt im kindlichen und jugendlichen Alter auftritt (Lehmkuhl und Döpfner, 2003), bieten die vorliegenden Daten ein strukturelles Korrelat für die erhöhte Vulnerabilität der funktionalen Einbindung des accumbalen *Core* in diesem Lebensabschnitt.

Im Gegensatz zu den unterschiedlichen Reifungsverläufen der accumbalen Subareale in der Pubertät zeigen die Befunde, dass Core und Shell danach einen einheitlichen signifikanten Anstieg der DAergen Innervation aufweisen. Hier stellt sich die Frage nach einer Instanz, die zu diesem Zeitpunkt sowohl *Core* assoziierte motorische als auch *Shell* assoziierte emotionale Funktionssysteme gleichermaßen beeinflusst. Der PFC, dessen funktionale Integrität sehr prolongiert reift (Kalsbeek et al., 1988), könnte vermutlich eine solche Schlüsselstellung einnehmen. Der PFC spielt eine übergeordnete Rolle in der Kontrolle motorischer und limbischer Systeme und seine postnatale Reifung nimmt bekanntlich direkten Einfluss auf DA in limbischen und motorischen Arealen (Bennay et al., 2004; Busche et al., 2004; Carlson et al., 1996; Deckel et al., 1996). Ergebnisse unserer Arbeitsgruppe konnten ganz konkrete Anhaltspunkte dafür geben: Die Reifung der DAergen Innervation des PFC steigt stetig bis ins junge Erwachsenenalter an (Dawirs et al., 1993). Parallel dazu sinkt die DA-abhängige Neurogenese im hippokampalen Gyrus dentatus stetig ab (Dawirs et al., 2000). Diese direkten Abhängigkeiten von Strukturprozessen im

limbischen System zur reifenden DA-Innervation des PFC wurden bereits seinerzeit aus der Fülle der an unserem Modell gesammelten Daten abgeleitet (Teuchert-Noodt, 2000). Die Vermutung liegt also nahe, dass die Umstrukturierungen in *Core* und *Shell* des NAC gleichermaßen engstens mit der präfrontalen Reifung verbunden sind. Eine suppressive DA-Reifung im PFC nach IR (Neddens et al., 2001; Winterfeld et al., 1998) sollte erwartungsgemäß auf accumbale Reifungsgeschehnisse Auswirkungen haben (zu diesen Befunden s.u.).

Die vorliegende Reifungsstudie zeigt keinen strukturellen Alterungseffekt der DAergen Innervation in den accumbalen Subregionen. Dieses Ergebnis bestärkt sich aus Studien an der Ratte, wo gezeigt wurde, dass die Speicherung und Synthese von DA im Alter nicht verändert wird, obwohl sich die funktionelle Wirkungsweise der accumbalen DAergen Afferenzen abwandelt (Hebert und Gerhardt, 1998). Eine alterungsbedingte accumbale Abnahme Kalium-induzierten DA-Ausstoßes konnte mit *in vivo* durchgeführten elektrochemischen Ableitungen gemessen werden. Demgegenüber zeigte die HPLC Analyse keine Veränderungen des DA-Gehalts, der DAergen Metaboliten und des DAergen Umsatzes (Friedemann und Gerhardt, 1992). Ferner zeigten Miura und Mitarbeiter (2002) alterungsbedingte Unterschiede der monoaminergen Umsatzraten, wohingegen die Synthese unverändert blieb (Miura et al., 2002b). Aus den zitierten Befunden und dem Ergebnis der vorliegenden Studie kann man festhalten, dass Alterungseffekte nicht direkt das accumbale DAerge System auf struktureller und biosynthetischer Ebene beeinflussen. Von daher muss die Ursache der sich veränderten physiologischen Eigenschaften des accumbalen DA-Systems im Alter einen anderen Ursprung haben. Wiederum könnte der Einfluss des PFC eine kritische Größe sein. So konnte eine alterungsbedingte Abnahme der Glutamat induzierten DA Ausschüttung im NAC nachgewiesen werden (Segovia et al., 1999; Segovia und Mora, 2005). Dringend erforderlich wäre es deswegen, eine Glutamat-Tracerstudie, wie sie im Kortex von uns z. Zt. durchgeführt wird, auf den NAC auszudehnen, sowie die DA Innervationsstudien an noch älteren Tieren durchzuführen. Im nigrostriären System unterschiedlicher Spezies konnten bereits alterungsbedingte Abnahmen der DA-Innervation gezeigt werden (Stark und Pakkenberg, 2004).

3.2 Einfluss der zwei Induktoren auf die DAerge Innervation des Nucleus accumbens

(Lesting et al., 2005b)

Diese Studie bezieht sich auf gereifte Faserdichten, die am Tag 90 der Tiere nach restriktiver/semi-natürlicher Aufzucht und nach frühkindlicher MA-Intoxikation beider Aufzuchten gemessen wurden. Zentrales Thema war es, einen Seitenvergleich für DA vorzunehmen. *Für DA war der Befund, dass kein Hemisphären-spezifischer Effekt auftrat, aber beide Hemisphären dennoch von den Interventionen betroffen waren. IR bedingt eine signifikant erhöhte DAerge Faserdichte in Core und Shell der linken und rechten Hemisphäre. Die einmalige juvenile MA-Intoxikation zeigt keine Veränderungen der DAergen Faserdichten in Core und Shell bei semi-natürlich aufgewachsenen Gerbils. Demgegenüber führt MA bei isoliert aufgewachsenen Tieren zu einer signifikanten Verringerung der DA Innervation des NAC. Dieser Effekt betrifft, bis auf eine nicht signifikante Absenkung im Shell der rechten Hemisphäre, beide accumbalen Subregionen in der rechten und linken Hemisphäre* (Lesting et al., 2005b).

Die Befunde zur restriktiven Isolationsaufzucht gehen mit physiologischen Veränderungen des accumbalen DAergen Systems bei Ratten einher. Soziale Isolation erhöht den basalen DA Gehalt im NAC (Hall et al., 1998b; Jones et al., 1992), verändert den DAergen Umsatz bei Neuheitsstress (Miura et al., 2002a) und erhöht die DA Ausschüttung nach Amphetamin- (Lapiz et al., 2003) und Kokaingabe (Howes et al., 2000). Allgemein zeigen Tiere aus Isolationsaufzucht eine höhere motorische Aktivität (Hall et al., 1997; Hall et al., 1998a; Heidbreder et al., 2000), höhere Ängstlichkeit (Hall et al., 1998a), erhöhte Sensitivität gegenüber Belohnungsreizen (Harmer und Phillips, 1998; Jones et al., 1991; Lapiz et al., 2003) und Aufmerksamkeitsdefizite (Heidbreder et al., 2000). Diese Verhaltensauffälligkeiten werden auch unter dem Begriff „*social isolation syndrom*“ zusammengefasst (Heidbreder et al., 2000) und werden als Tiermodell für psychische Erkrankungen, wie z.B Schizophrenie und Depression, diskutiert (Heidbreder et al., 2000; Lapiz et al., 2003). Die erhöhte lokomotorische Aktivität bei Ratten in einer neuen Umgebung nach isolierter Aufzucht (Hall et al., 1997; Sahakian et al., 1977) wird unter anderem auf eine Hyperfunktion der mesoaccumbalen DA-Projektion zurückgeführt (Hall et al., 1999; Lapiz et al., 2003; Wilkinson et al., 1994). Gerbils aus unserer IR zeigen das gleiche „hyperaktive“ Verhaltensmuster im *open field* Test (Polascheck, 2004; Winterfeld et al., 1998). Die in meinen Untersuchungen festgestellte erhöhte accumbale DA-Faserdichte der isolierten Tieraufzucht bildet das strukturelle Korrelat zu diesem Verhaltensmuster (Lesting et al., 2005b).

Für unser Tiermodell gilt weiterhin, *dass die signifikant erhöhte DAerge Faserdichte im NAC nach IR mit einer erhöhten DAergen Faserdichte in der basolateralen Amygdala* (Busche et al., 2004) und einer suppressiven Reifung der DA-Innervation im PFC nach IR (Neddens et al., 2001; Winterfeld et al., 1998) einhergeht. Aufgrund der übergeordneten Funktionen des PFC und der späten Reifung der mesopräfrontalen DA Innervation (Dawirs et al., 1993; Kalsbeek et al., 1988) vermuten wir, dass die Stabilisierung subkortikaler DA-Systeme mit der Reifung der

mesopräfrontalen DA-Bahn unmittelbar in Verbindung steht [s.o. (Busche et al., 2004; Lesting et al., 2005a, submitted; Lesting et al., 2005b)]. Die supprimierte DA-Reifung in den PFC könnte gleichzeitig eine unkontrollierte exzessive DA-Reifung in subkortikalen Arealen stabilisieren. Da die vorliegende Reifungsstudie (Lesting et al., 2005a, submitted) an Tieren aus restriktiver Isolationsaufzucht durchgeführt wurde, stellt sich an dieser Stelle natürlich auch die Frage, ob die signifikante Anhebung der DAergen Faserdichte im NAC zwischen dem P 70-90 ein grundlegendes Phänomen in der Reifung des NAC darstellt oder aber auf IR zurückzuführen ist.

Eine Hypothese des Isolationssyndroms besagt, dass die DAerge präsynaptische Hyperfunktion im NAC mit einer kortikalen Hypofunktion und einer damit verbundenen dysfunktionalen glutamatergen Innervation des NAC in Zusammenhang steht (Lapiz et al., 2003). Unser Tiermodell belegt diese Vorstellung mit den quantitativen Transmitterdaten. So konnten wir zeigen, dass sich glutamaterge Projektionen vom PFC in sensorische Kortexareale und ins ventrale Striatum unter IR deutlich verringern (Bagorda et al., 2005; Lehmann, 2001). Dies wird durch Ergebnisse anderer Arbeitsgruppen untermauert, die nachweisen konnten, dass gezielte neonatale Läsionen des PFC einen direkten Einfluss auf das accumbale DA-System in adulten Ratten nehmen (Bennay et al., 2004; Brake et al., 2000a; Flores et al., 1996) und zu einer Demyelinisierung des NAC führen (Schneider und Koch, 2005).

Gleiche adaptive Änderungen der Faserdichten wurden an unserem Tiermodell auch für die MA-Wirkung gezeigt: Die mesolimbischen DA-Projektionen sind zum Zeitpunkt der MA-Intoxikation am P 14 besonders vulnerabel gegen pharmakologische Eingriffe (Teuchert-Noodt und Dawirs, 1991) und das sollte speziell auch das *Core* betreffen, wie oben ausgeführt. Dementsprechend wird dem accumbalen DAergen System des *Core* auch von anderer Seite eine erhöhte Vulnerabilität gegenüber pharmakologischen Intoxikationen zugeschrieben (Lancia et al., 2004). Gezielte Läsionen des medialen Vorderhirnbündels mit 6-OHDA führen zu einer stärker ausgeprägten Verringerung der DAergen Innervation im *Core* verglichen mit dem *Shell* (Tan et al., 2000). Hohe Dosen von MA lösen verstärkt degenerative Prozesse der DAergen Innervation des *Core* aus (Broening et al., 1997; Brown und Molliver, 2000). Solche spezifischen degenerativen Prozesse werden auf eine höhere Vulnerabilität der DAergen Neurone der SN pars compacta gegenüber denen der VTA zurückgeführt (Gilad und Reis, 1979). Außerdem wird bezüglich der MA-Intoxikation dem DA-Transporter (DAT) eine entscheidende Rolle zugesprochen. Amphetamine wirken auf den DAT, was zum einen den Transport von DA aus dem synaptischen Spalt verringert und zum anderen über reversen Transport eine erhöhte DA-Ausschüttung induziert (Sulzer et al., 1995). Dadurch kommt es zur neurotoxischen Anreicherung von 6-OHDA (Seiden und Vosmer, 1984), welches wiederum verstärkt über DAT aufgenommen wird (Nirenberg et al., 1997) und die neurotoxischen Prozesse potenziert. Da die DAT Dichte im *Core* höher ist als im *Shell* (Nirenberg et al., 1997) und das *Core* prominente Eingänge aus der SN (pars compacta) erhält (Brog et al., 1993), dürfte sich die hohe Störanfälligkeit daraus erklären.

Das Shell weist nach vorliegenden Daten ebenfalls eine Absenkung der DAergen Faserdichte nach MA-Intoxikation bei Tieren aus IR auf, wenn auch nicht ganz so ausgeprägt wie im Core (Lesting et al., 2005b). Das möchte sich daraus erklären, dass die neurotoxische Wirkung von MA nicht ausschließlich an die Funktion des DAT gekoppelt ist. Dabei sollten auch weitere Zellbestandteile, die mit dem DA-Stoffwechsel assoziiert sind, wie beispielsweise das DA-metabolisierende Enzym Monoaminooxidase (Seiden und Vosmer, 1984), durch MA gestört werden.

Die Tiere aus semi-natürlicher Aufzucht können den akuten MA-Stressor durch die sozialen Kontakte, die Möglichkeit zur Exploration etc. offensichtlich kompensieren. Eventuell gelingt das durch reaktives Auswachsen von DAergen Fasen, die nicht durch die MA-Intoxikation zerstört wurden (Vos et al., 1996). Das belegt am Beispiel des NAC einmal mehr, wie stark die aktivitätsgesteuerte DA-Reifung von Aktivitäten aus der Umwelt abhängig ist. Konkret heißt das, dass die unterschiedlichen Aufzuchtbedingungen zum Zeitpunkt der MA-Intoxikation noch keinen entscheidenden Einfluss auf die Hirnreifung haben (z.B. ist die motorische Aktivität noch stark eingeschränkt und die Augen sind noch geschlossen), aber die adoleszente Reifung essentiell an die Umwelt gekoppelt ist. Speziell die Adoleszenz, die bei Nagern den Zeitraum zwischen P 28-60 umfasst (Smith, 2003) [bei Gerbils eventuell über den P 60 hinaus (Ulibarri und Yahr, 1993)], wird auch von anderen Autoren als kritisches Zeitfenster angesehen (Andersen, 2002; Andersen et al., 2000; Moll et al., 2000; Tarazi und Baldessarini, 2000; Teicher et al., 1995), in dem massive Reorganisationsprozesse ablaufen sollten.

Im Vergleich der Daten zur DAergen Faserdichte der einzelnen Versuchsgruppen fällt auf, dass die durch IR angehobene Faserdichte durch die zusätzliche MA-Intoxikation auf das Niveau der Kontrolltiere (Saline behandelt und semi-natürlich aufgewachsen) abgesenkt wird. Diese „Wiederherstellung" dürfte aber im ganzheitlichen Funktionsgeschehen funktionell keineswegs der Ausgangssituation von Kontrolltieren entsprechen. Denn gleichzeitig manifestieren sich ja tatsächlich Anhebungen bzw. Absenkungen DAerger Faserdichten in anderen Arealen des *limbischen Systems [basolaterale Amygdala, entorhinaler Kortex* (Busche et al., 2004)] und im PFC (Dawirs et al., 1994; Dawirs und Teuchert-Noodt, 2001; Neddens et al., 2001; Winterfeld et al., 1998). Da all diese Areale in enger funktionaler und physiologischer Verbindung zum NAC stehen (Grace, 2000; Mogenson et al., 1980; Schmajuk et al., 2001) und wechselseitig die Aktivitäten der accumbalen DAergen Projektionsneurone des Mittelhirns steuern (Louilot et al., 1985; Louilot et al., 1989; Louilot und Le Moal, 1994), sollte auch das DA-System des NAC in dem adaptiven Geschehen gestört sein.

3.3 Einfluss der zwei Induktoren auf die 5-HTerge Innervation des Nucleus accumbens

(Lehmann et al., 2003; Lesting et al., 2005b)

Für 5-HT war ein Hemisphären-spezifischer Befund gegeben, mit dem sich dieser Transmitter überraschenderweise deutlich von DA abgrenzte. Wiederum müssen die beiden Interventionen getrennt bewertet werden. Die restriktive Isolationsaufzucht bewirkt keine Veränderungen der 5-HTergen Faserdichte, weder im Core noch im Shell (Lehmann et al., 2003; Lesting et al., 2005b). Sozial isolierte Ratten zeigen allerdings veränderte physiologische Charakteristika des accumbalen 5-HTergen System (Heidbreder et al., 2000; Jones et al., 1992). Da eine Faserdichtemessung keine direkten Aussagen zur Physiologie der Neurone treffen kann, sind Unterschiede bezüglich der Konzentration von Transmittern und deren Metabolite in unserem Tiermodell nicht auszuschließen. In einer momentan laufenden HPLC Studie wird dieses untersucht.

Die MA-Intoxikation führt sowohl bei isoliert als auch bei semi–natürlich aufgewachsenen Gerbils zu einem signifikanten Anstieg der 5-HTergen Faserdichte in Core und Shell der rechten Hemisphäre (Lesting et al., 2005b). *Die nur im Core signifikante Anhebung der accumbalen 5-HTergen Innervation in einer Vorstudie* (Lehmann et al., 2003) *lässt sich aus den unterschiedlichen Zahlen von Versuchstieren in beiden Studien ableiten. Auch das Shell zeigt in jener Studie eine tendenzielle Anhebung.* So kann man prinzipiell von einer Erhöhung der Faserdichte in beiden accumbalen Subregionen ausgehen. Zur Erklärung dieser Befunde bieten sich folgende Überlegungen an:

Zum Zeitpunkt der MA-Intoxikation ist die 5-HTerge Innervation des NAC von Nagern noch nicht voll ausgereift (Lidov und Molliver, 1982) und damit sehr vulnerabel gegenüber pharmakologischen Eingriffen. Doch im Gegensatz zu DA ist 5-HT viel schneller in der Lage, mechanische als auch pharmakologische Zerstörungen der 5-HTergen Projektionen durch Aussprossen noch intakter Axone zu kompensieren (Zhou et al., 1995; Zhou und Azmitia, 1984). 5-HTerge Neurone können ihr eigenes Wachstum über den spezifischen Wachstumsfaktor S-100$_{ß}$, ausgeschüttet von Astrogliazellen, steuern (Azmitia et al., 1990; Whitaker-Azmitia et al., 1990). Ein Anstieg von S-100$_{ß}$ korreliert dabei positiv mit einer erhöhten 5-HTergen Faserdichte (Haring et al., 1993).

Allerdings erklärt sich damit noch nicht die beobachtete Hyperinnervation 5-HTerger Fasern im NAC. Heterotypisches Sprouten von 5-HTergen Fasern infolge einer Degeneration DAerger Fasern bietet eine weitere mögliche Erklärung. Im Gegensatz zu 5-HT kann DA die juvenile MA-Intoxikation nur eingeschränkt kompensieren, was eine Verringerung der DAergen Faserdichte zur Folge hat (s.o). Kompensatorisch sollte 5-HT verstärkt reifen, was mehrere Studien zeigen konnten. Adulte und neonatale 6-OHDA Läsionen des DAergen Systems führen zu einer Hyperinnervation von 5-HT in den betroffenen Arealen, was als heterotypes Sprouting diskutiert wird (Descarries et al., 1992; Kostrzewa et al., 1998; Yamazoe et al., 2001). Unsere

Daten zur 5-HT Hyperinnervation verschiedener Areale legen eine ähnliche Interpretation nahe (Lehmann et al., 2003; Lesting et al., 2005b).

Zusammenfassend kann man sagen, dass die Hyperinnervation durch MA-Intoxikation vermutlich auf der enormen Regenerationsfähigkeit des 5-HTergen Systems und auf dem heterotypen Sprouten der 5-HTergen Fasern als Reaktion auf eine Zerstörung DAerger Fasern beruht.

3.4 Einfluss der zwei Induktoren auf die Lateralisierung von DA und 5-HT

(Lesting et al., 2005b)

Im Vergleich beider Aufzuchtbedingungen zeigen meine Befunde keine accumbalen Asymmetrien in der DAergen und 5-HTergen Faserdichte. Dieses Ergebnis stimmt mit symmetrischen accumbalen Konzentrationsverteilungen von DA und 5-HT bei sozial und isoliert aufgewachsenen Ratten überein (Heidbreder et al., 2000; Jones et al., 1992). Daraus lässt sich ableiten, dass IR zu keinen substantiellen asymmetrischen Verschiebungen der accumbalen DAergen und 5-HTergen Innervation führt.

Für DA gilt weiterhin: *Im Vergleich der Tiergruppen mit MA-Intoxikation zeigten die Tiere ebenfalls keine spezifischen Effekte in der accumbalen DAergen Faserdichte der rechten und linken Hemisphäre und keinen Lateralisierungseffekt.* Dieses Ergebnis entspricht auf den ersten Blick nicht unseren Erwartungen, da in Studien an der Ratte deutliche asymmetrische Effekte bezüglich der DAergen Konzentration nach juveniler pharmakologischer Intervention gezeigt wurden (Andersen et al., 2002; Bortolozzi et al., 2003). Doch sind physiologische Asymmetrien des accumbalen DAergen Systems auch in unserem Tiermodell zu erwarten, *was auf die deutliche Hemisphären spezifische Wirkung von MA auf die 5-HTerge Faserdichte des NAC zurückzuführen ist*: 20 % der accumbalen 5-HTergen Innervation terminiert präsynaptisch auf DAerge Afferenzen des NAC (Van Bockstaele und Pickel, 1993) und moduliert dadurch die tonische (De Deurwaerdere und Spampinato, 1999) und phasische (Chen et al., 1991) DA Ausschüttung.

Für 5-HT gilt: *Die deutlich, nur auf die rechte Hemisphäre beschränkte Wirkung der MA-Intoxikation auf die 5-HTerge Faserdichte des NAC* lässt sich möglicherweise auf asymmetrische physiologische und funktionelle Eigenschaften der accumbalen 5-HT Afferenzen zurückführen. So konnte an der Ratte eine Hemisphären-spezifische positive und negative Korrelation zwischen der 5-HTergen Konzentration im NAC und der Größe einer PFC Läsion nachgewiesen werden (Deckel et al., 1996) und zusätzlich eine negative Korrelation zwischen der Aufenthaltsdauer im geschlossenen Arm des elevated plus maze und einem erhöhten 5-HT-Gehalt im linken ventralen Striatum (Schwarting et al., 1998). Dennoch bleibt der grundlegende Mechanismus der asymmetrischen Wirkung einer einmaligen MA-Intoxikation am P 14 spekulativ. So könnte generell die juvenile Reifung der accumbalen 5-HTergen Innervation asymmetrisch verlaufen. Darüber hinaus könnte sich die Konzentration des 5-HT Transporters, welcher maßgeblich an der

neurotoxischen Wirkung von MA beteiligt ist (Brown und Molliver, 2000), am P 14 zwischen den Hemisphären unterscheiden. Es bedarf weiterer Studien zur Entwicklung des 5-HTergen Systems, unter Berücksichtigung der Lateralisierung, um den genauen Mechanismus weiter zu entschlüsseln.

Erst die Kombination von IR und MA-Intoxikation zeigt einen signifikanten Lateralisierungseffekt der 5-HTergen Faserdichte in Core und Shell des NAC zu Gunsten einer höheren Innervation in der rechten Hemisphäre (Abb.4). Von der kombinierten Intervention sind auch Areale betroffen, die in enger funktioneller Verbindung zum NAC stehen. So konnten wir zeigen, dass die hoch signifikante präfrontale Lateralisierung der 5-HTergen Faserdichte semi-natürlich aufgewachsener Gerbils durch die Kombination von Isolation und MA stark reduziert wird, wohingegen die Asymmetrie des entorhinalen Kortex signifikant verstärkt wird (Neddens et al., 2004). Außerdem konnte ein Hemisphären-spezifischer Effekt für die 5-HT Faserdichten im Gyrus dentatus des Hippokampus festgestellt werden [Abb. 5 (Busche et al., 2002)].

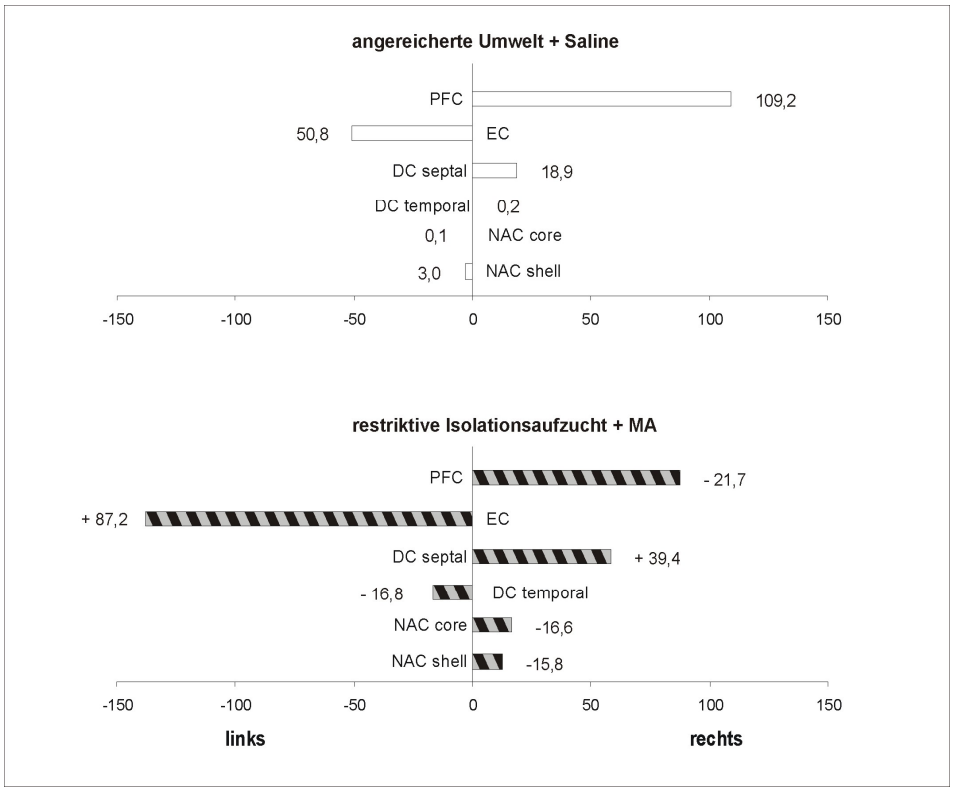

Abb. 5: Lateralisierung von 5-HT im limbopräfrontalen System.
Werte der angereicherten Umwelt bezeichnen die prozentuale Lateralisierung der 5-HTergen Faserdichten. Werte der Kopplung restriktiver Isolationsaufzucht mit MA-Intoxikation sind die prozentualen Verschiebungen zu den Kontrolltieren.
Zusammengefasste Daten aus: (Busche et al., 2002; Lesting et al., 2005b; Neddens et al., 2004)

Diese Befunde geben Anlass zu der Vermutung, dass die Kombination von MA-Intoxikation und IR eine reifungsbedingte Verlagerung der Lateralisierung von allo- (Hippokampus, EC) und subkortikalen (NAC) Arealen auf neokortikale (PFC) Areale unterbindet. Solches möchte man aus zahlreichen Entwicklungsstudien zur Lateralisierung verschiedener Hirnareale ableiten: So besagt ein sog. „*bottom up*“ Modell, dass kortikale Asymmetrien

Ausweitungen früher gereifter allo- und subkortikaler Asymmetrien sind (Hiscock und Kingsbourne, 1995; Tang, 2003; Trevarthen, 1996) und sich dieser Prozess im ausgereiften Gehirn umkehrt. Die Reifung kortikaler Strukturen würde allo- und subkortikale Areale unter ihre Kontrolle nehmen. Das führt dazu, dass speziell der PFC, als höchste Instanz einer hierarchisch organisierten Kontrolle (Le Moal und Simon, 1991), asymmetrischen Einfluss auf subkortikale Bereiche nimmt. Dann gilt weiterhin: Wird die Reifung des PFC, wie in unserem Fall durch die Kombination von Isolation und MA, stark beeinträchtigt (Dawirs et al., 1994), geht die Kontrolle auf früher gereifte subkortikale Bereiche verloren und führt zu Fehlentwicklungen im Allokortex mit ausgeprägten Asymmetrien.

4. B e w e r t u n g

Adaptive Veränderungen accumbaler Afferenzen aus dem monoaminergen und limbopräfrontalen System vor dem Hintergrund des „Two-Hit-Modells“ der Psychose

Der NAC ist als „*limbic-motor interface“* ein entscheidendes Integrationszentrum innerhalb des limbopräfrontalen Systems, welches die Verbindung zur Motorik herstellt (Mogenson et al., 1980). Störungen der DAergen und 5-HTergen Reifung im NAC, wie sie in der vorliegenden Arbeit nach IR und frühkindlicher MA-Intoxikation gefunden wurden, müssen aufgrund der systemischen Einbettung immer mit Blick auf das gesamte limbopräfrontale System interpretiert werden. Vorangegangene Arbeiten hatten bereits dramatische Veränderungen der aminergen Faserdichten in limbischen und kortikalen Gebieten aufzeigen können (Busche et al., 2004; Busche et al., 2002; Dawirs et al., 1993; Neddens et al., 2004; Neddens et al., 2001; Neddens et al., 2003; Winterfeld et al., 1998).

Wie eingangs erwähnt geht man davon aus, dass pathologische Veränderungen der afferenten Transmitter (DA, 5-HT und Glutamat) speziell von diesem Kern gesteuertes Verhalten beeinträchtigen. So beschreibt Grace die Rolle des NAC in einem Modell zur Schizophrenie folgendermaßen: Im pathologischen Zustand werden die glutamatergen Eingänge aus PFC und Hippokampus verringert und die aus der Amygdala verstärkt. Dies führt dazu, dass ein angepasstes Verhalten gegenüber kontextspezifischen (Hippokampus) und zielgerichteten (PFC) sensorischen Stimuli verloren geht, die Verarbeitung nur noch auf emotionaler Ebene (Amygdala) stattfindet und sich in einem erhöhten impulsiven Verhalten ausdrückt (Grace, 2000). Neben Glutamat wird auch die monoaminerge Innervation des NAC im Modell von Grace als essentiell angesehen. So sind Integrationsprozesse, die sich anhand der Verhaltensexperimente der *prepulse inhibition* und der latenten Inhibition messen lassen, sehr anfällig gegen Veränderungen des accumbalen monoaminergen Systems und werden ebenfalls im engem Zusammenhang mit Symptomen der Schizophrenie diskutiert (Gray et al., 1997; Murphy et al., 2000; Weiner, 2003).

Überträgt man diese Ausführungen auf die erhobenen Befunde der vorliegenden und vorangegangenen Studien auf das „Two-Hit-Modell“ der Psychose unserer Arbeitsgruppe, so zeichnen sich deutliche Übereinstimmungen ab. Auf die systemische Einbindung des NAC bezogen, lassen sich diese Veränderungen des DAergen und 5-HTergen Systems folgendermaßen zusammenfassen:

- Das DAerge System zeigt bei IR-Tieren eine Anhebung der Faserdichte im NAC und in limbischen Arealen und eine Absenkung der Faserdichte im PFC.
- IR hat keinen 5-HT-Effekt auf den NAC, führt aber zu einer Anhebung der 5-HTergen Faserdichte in den meisten limbischen Arealen.
- Die MA-Intoxikation zeigt keinen DA-Effekt im NAC und PFC semi-natürlich aufgewachsener Tiere und führt zu leichten Anhebungen in limbischen Arealen.

- Wenn sich Effekte im 5-HT-System nach der MA-Intoxikation einstellen, dann zeigt sich das in beiden Tieraufzuchten und in allen untersuchten Regionen als Anstieg der Faserdichte.
- Die Kombination von IR und MA-Intoxikation bewirkt eine Absenkung der DAergen Faserdichte im NAC. Die supressive Reifung des PFC wird verstärkt und andererseits wird die exzessive Reifung limbischer Areale potenziert.
- Das DAerge System weist hinsichtlich der Innervation keine Lateralisierung auf, während das 5-HTerge System, insbesondere bei der Kombination von IR und MA-Intoxikation, starke Lateralisierungseffekte zeigt.

Diese Befunde zeigen deutlich, dass es zu massiven Veränderungen der Monoamine im gesamten limbopräfrontalen System kommt, die letztlich auch die Aktivitäten der limbischen Areale beeinflussen sollten. Generell kann man festhalten, dass auf DA bezogen die MA-Intoxikation weitestgehend durch eine semi-natürliche Aufzucht in der Adoleszenz kompensiert werden kann. Denn eine soziale Einbindung etc. der Tiere bietet offensichtlich genügend Reize an, um die aktivitätsgesteuerte Reifung der mesopräfrontalen und mesoaccumbalen DA-Bahn hinreichend zu stimulieren. Ein Anstieg der 5-HTergen Innervation, hervorgerufen durch eine Hyperinnervation infolge der MA-Intoxikation, sollte aufgrund der engen Wechselwirkung der Transmitter das DAerge System indirekt auch betreffen (Ferre et al., 1994; Mendlin et al., 1999; Winstanley et al., 2005), doch wirkt sich das nicht strukturell aus.

IR-Bedingungen führen zu massiven Veränderungen der monoaminergen Innervation. Zudem sind die glutamatergen Efferenzen des PFC in kortikale und subkortikale Regionen durch IR stark beeinträchtigt (Bagorda et al., 2005; Lehmann, 2001). Zusätzlich konnten wir nachweisen, dass auch die hippokampalen Aktivitäten entscheidend beeinflusst werden. So weisen restriktiv aufgewachsene Gerbils eine erhöhte Neurogeneserate auf (Hildebrandt et al., 1999; Keller A et al., 2000). Alle Befunde zusammengenommen zeigen, dass die durch IR hervorgerufenen Störungen der Reifung dem von Grace beschriebenen Modell der Schizophrenie sehr nahe kommen, was in Abbildung 6 zusammenfassend festgehalten wird. Der Einfluss des PFC auf den NAC (Abb. 6A) wird zurückgenommen, die Information aus dem Hippokampus ist entscheidend gestört (gestrichelte Linien in Abb.6B) und die DAerge und 5-HTerge Innervation der basolateralen Amygdala ist erhöht. Das sollte zu einer Disinhibierung glutamaterger Efferenzen in den NAC führen [verstärkte Linien in Abb.6B (Polascheck, 2004)]. Meinen am NAC gewonnenen Befunden zufolge, werden diese Effekte zusätzlich durch die erhöhte DAerge Innervation des NAC verstärkt; hier bleibt natürlich ungeklärt, ob beide, die tonische und phasische, oder nur eine dieser Aktivitäten erhöht wird. Jedenfalls sollte damit eine weitere Fehlsteuerung der Aktivitäten aus dem PFC über D2 Rezeptoren und der amygdaloiden Afferenzen über D1 Rezeptoren einhergehen (Goto und Grace, 2005).

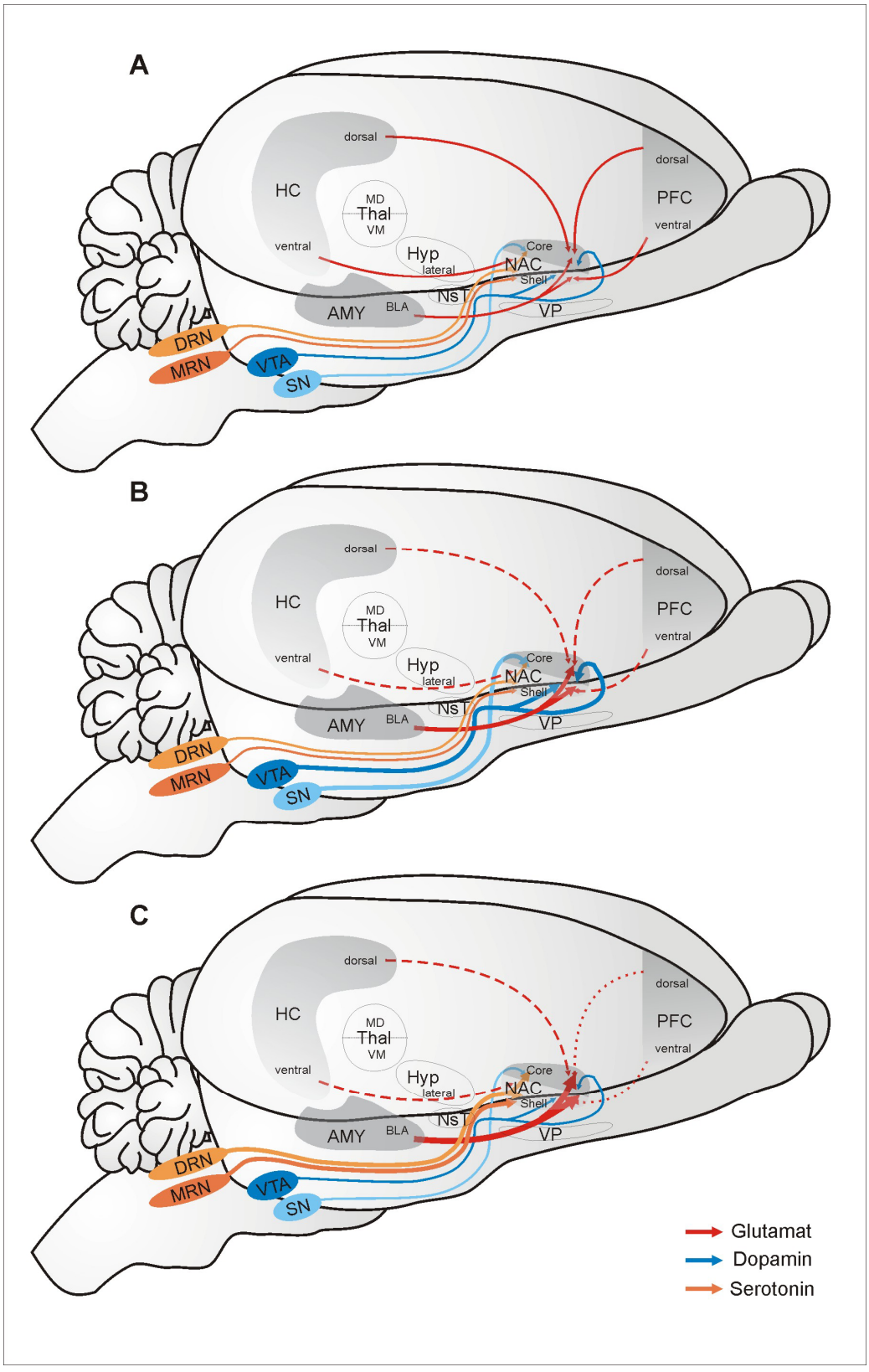

Abb.6: Veränderung der glutamatergen und monoaminergen Innervationen des NAC nach IR und der Kombination mit MA-Intoxikation

(A) Nicht pathologischer Zustand nach Aufzucht in angereicherter Umwelt und Saline Behandlung. (B) Pathologischer Zustand nach IR. (C) Verstärkter pathologischer Zustand nach Kombination von MA-Intoxikation und IR (siehe Text). Gestrichelte Pfeile kennzeichnen eine Abnahme der accumbalen Afferenzen. Eine weitere Verringerung wird durch gepunktete Pfeile angezeigt. Verstärkte Pfeile kennzeichnen eine erhöhte accumbale Innervation (Abkürzungen siehe Abb. 2)
Grafik abgewandelt nach Grace (2000)

Durch die Kombination der MA-Intoxikation mit IR (*Two-Hit-Modell*) wird die pathologische Situation auch für den NAC erheblich verstärkt (Abb. 6C). Die durch IR induzierte accumbale Hyperinnervation wird durch die MA-Intoxikation abgesenkt und die 5-HTerge Innervation wird angehoben. Das heißt, die normalerweise fein justierte Interaktion der beiden Transmitter, DA und 5-HT, erfährt auf anatomischer accumbaler Ebene eine massive Umstrukturierung. Nicht nur das, sondern das gesamte limbopräfrontale System läuft sozusagen „aus dem Ruder" und gelangt in einen nahezu unkontrollierten Zustand, den man als „funktionale Diskonnektion" beschreiben kann (Lipska, 2004; Lipska und Weinberger, 2002). Entscheidend ist wohl, dass die natürliche PFC-Kontrolle - über kortikale, limbische, motorische und subkortikale Areale - verloren geht und diese Areale nunmehr unkontrolliert agieren (Bagorda et al., 2005; Busche, 2004; Busche et al., 2004; Lehmann, 2001). Selbst der PFC ist, wie uns das Tiermodell gezeigt hat, nach dieser doppelten Belastung während der Gehirnreifung ganz erheblich destrukturiert: Die DAerge Faserdichte ist massiv abgesenkt (Dawirs et al., 1994) und die GABAerge Innervation angehoben (Nossoll et al., 1997). Diese lokale Imbalance der Transmitter ist gekoppelt mit einer anatomischen „Dyskonnektion" der glutamatergen kortiko-kortikalen und kortiko-striatalen Efferenzen (Bagorda et al., 2005; Lehmann, 2001). Weitere Befunde dieses Modells haben gezeigt, dass die DAerge Innervation der basolateralen Amygdala zusätzlich angehoben wird (Busche et al., 2004), die Zellproliferationsrate im hippokampalen Dentatus massiv abgesenkt wird (Hildebrandt, 1999) und gleichzeitig die 5-HTerge Innervation im Dentatus und entorhinalen Kortex signifikant angehoben wird (Busche et al., 2002). In dieses Szenario ist der NAC zentral eingebunden. Man kann also erwarten, dass die gefundenen pathologischen Veränderungen nicht die Einzigen sind.

Bemerkenswert ist die funktionelle Einbindung von *Core* und *Shell* in dem ganzen Geschehen der Traumatisierung durch doppelte Belastung. Zwar führen die nicht-invasiven Interventionen zu weitestgehend gleichförmigen Veränderungen der DAerge und 5-HTergen Innervation von *Core* und *Shell*, doch kann man auf funktioneller Ebene Unterschiede erwarten. Wie ich oben ausgeführt habe, sind diese beiden Regionen in verschiedenen funktionellen Kontexten eingebunden. So sollten die motorisch zuzuordnende Funktion des Core und die limbisch zuzuordnende Funktion des Shell gestört sein. Inwieweit die Induktoren unseres Tiermodells Ihre Wirkung in den accumbalen Subregionen ausprägen, bedarf dringend zusätzlicher Studien auf anatomischer und verhaltensbezogener Ebene. So würde z.B. eine retrograde Tracerstudie, bezogen auf Core und Shell des NAC, genauere Auskunft über das Ausmaß der funktionellen Fehlverschaltungen aus limbokortikalen Arealen in unserem Tiermodell geben. Auf der Verhaltensebene würde die latente Inhibition einen geeigneten Versuchsansatz darstellen, da Core und Shell innerhalb dieses Verhaltens deutlich abgrenzbare Funktionen zugesprochen werden (Jeanblanc et al., 2002; Murphy et al., 2000; Schmajuk et al., 2001; Weiner, 2003; Weiner und

Feldon, 1997). Zusätzlich könnten gezielte Gaben von DA- und 5-HT-Rezeptor Agonisten und Antagonisten in diesem Versuchsansatz, das Ausmaß des accumbalen Diskonnektion entschlüsseln.

Die beobachtete deutliche Lateralisierung der 5-HTergen Innervation im NAC und die Änderungen der Lateralisierung im PFC, entorhinalen Kortex und dem Hippokampus bezüglich der Kombination von MA-Intoxikation und IR, unterstützen zusätzlich unser Two-Hit-Modell auf dem Weg in die Psychose. So beschreiben zum Beispiel Louilot und LeMoal eine asymmetrische Beeinflussung des accumbalen DAergen Systems durch den entorhinalen Kortex und den Hippokampus in einem Tiermodell der Schizophrenie (Louilot und Le Moal, 1994). Da, wie schon erwähnt, das DAerge System maßgeblich von 5-HT moduliert wird, ist auch in unserem Modell eine asymmetrische Wirkung beider Transmittersysteme zu erwarten. Darüber hinaus werden Veränderungen der Lateralisierung des 5-HTergen und DAergen Systems als Ursache der Depression und Angststörung des Menschen diskutiert (Andersen et al., 2002; Andersen und Teicher, 1999).

Unser Tiermodell stimmt in weiten Teilen mit den medizinischen Hypothesen zur Psychose überein, die jedenfalls auch von einer funktionellen Diskonnektion präfrontaler, limbischer und subkortikaler Systeme ausgehen (Grace, 2000; Lillrank et al., 1995; Moore et al., 1999; Weinberger et al., 1992; Weinberger, 1987). Die anhand von Tiermodellen gewonnenen tiefen Einblicke in das neuronale Geschehen der Psychose erlauben es, immer gezieltere – insbesondere pharmakologische - Therapien zu entwickeln, die auf die unterschiedliche Betroffenheit von limbischen, subkortikalen und präfrontalen Arealen bei der Psychose abgestimmt sind.

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Ontogeny of the dopamine innervation in the nucleus accumbens of gerbils

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Abstract

The postnatal maturation of immunohistochemically stained dopamine (DA) fibres was quantitatively examined in the core and shell subareas of the nucleus accumbens (NAC) of gerbils. Animals of different ages, ranging from juvenile [postnatal day (PD) 14, 30)], to adolescent (PD70), adult (PD90, PD180, PD360) and ageing (PD540, PD720) were analysed. The timescale of the maturation of the accumbal DA innervation was regionally different, probably due to the different origin of DA fibres in the mesencephalon. Both the accumbal core, with DA afferents arising from the lateral ventral tegmental area (VTA) and the substantia nigra pars compacta, as well as the accumbal shell, with DA afferents arising from the medial VTA, show moderate DA fibre densities at PD14. The core displayed a marked decrease of the DA fibre density up to PD30 and a subsequent significant increase between PD70-90 and PD90-180, whereas the shell solely showed an augmentation of the DA innervation between PD70-90. Our data suggest that the different maturation of the DA innervation in core and shell might reflect differences in the development of motor and limbic functions, mediated by the nigrostriate and the mesolimbic system, respectively.

1. Introduction

The nucleus accumbens (NAC) is considered a "limbic-motor interface" [41] which integrates various cortical and subcortical inputs to select and adapt context-related

motivational behaviours [30]. Based on the regional distribution of neurotransmitters, neuropeptides and receptor densities, the NAC has been divided into two subareas, the inner core and the outer shell [34;36;39;67;68]. Afferent and efferent connections differ between these subdivisions and reveal functional heterogeneity. The accumbal core represents the ventral extension of the caudate-putamen complex and is closely linked to the motor system, whereas the accumbal shell is more allied to the limbic circuitry [22;27;31].

The dense mesencephalic dopamine (DA) innervation of the NAC gates information arising from the amygdala, prefrontal cortex, hippocampus, and other limbic and motor regions [32]. Both the innervation pattern and the origin of DA fibres in the midbrain differ between the accumbal subareas of rodents [11;44;61]. The substantia nigra (SN) pars compacta and the lateral ventral tegmental area (VTA) innervate the core, and afferents arising from the medial VTA innervate the shell [12]. Several pharmacological [22;49-51] and pharmaco-behavioural [5;38;43;64;65] studies additionally proved a functional dichotomy of the mesoaccumbal DA projections.

Disturbances of the DA innervation of the NAC have been found and discussed in a wide range of mental disorders [2;17;26;29;64] and drug addiction [23], suggesting that the core and shell might be differentially involved. For example, Cardinal and co-workers proposed the NAC core as being a critical site for the processing of impulsive choices, a feature of attention-deficit/hyperactivity disorder (ADHD) [17]. As an animal model for schizophrenia, Weiner and Feldon assigned different roles of the accumbal subdivisions in latent inhibition (LI), with the shell being necessary for the expression and the core for the disruption of LI [65]. Neurodevelopmental interference is thought to account for aberrations in the accumbal system. Accordingly, interventions like prenatal stress [8],

neonatal lesion [7] or different rearing conditions [10] induce region-specific changes in the accumbal DA system. For an understanding of the mechanisms which trigger the different impact of interventions during maturation, it is necessary to know about critical periods in the development of the accumbal DA system.

Several critical periods in the development of the accumbal DA system were already defined in the rat: Substantial reorganisation processes in the accumbal DA system occur during the juvenile period [3], preadolescence [56;62], adolescence [47;48] and ageing [24]. However, only a few studies have distinguished between the subdivisions of the NAC [3;19].

In the present study we quantified the postnatal development and maturation of the DA innervation separately in the core and shell subareas, covering most of the lifespan of gerbils to specify also age-related alterations in motor vs. limbic circuits of the NAC.

2. Materials and Methods

Animals

All experimental procedures were approved by the appropriate committee for animal care in accordance with the European Communities Council Directive. A total of 67 male Mongolian gerbils (*Meriones unguiculatus*) were used for this study. The animals were bred in standard cages (Macrolon® type 4) and, after weaning on postnatal day (PD) 30, were reared individually in standard cages (Macrolon® type 3). All gerbils were kept under natural day/night cycles with food and water being provided *ad libitum*. Eight experimental animal groups of different ages were investigated to cover convincing periods of the life

span of gerbils: PD14 (n=13), PD30 (n=7), PD70 (n=10), PD90 (n=10), PD180 (n=6), PD360 (n=10), PD540 (n=4), and PD720 (n=7).

Dopamine Immunohistochemistry

The animals were transcardially perfused under deep chloralhydrate anaesthesia (1.7g/kg, i.p.). The perfusion was performed with 60ml cold 0.05M phosphate buffer (pH 6.2) containing 1% sodium metabisulfite, followed by 200ml (on PD14) of fixative, containing 5% glutaraldehyde with 1% sodium metabisulfite in 0.1M phosphate buffer (pH 7.5). (The amount of fixative on later stages was: 300ml for PD30, 500ml for PD70, and 750ml for PD90-720.) Immediately after perfusion the brains were dissected and 50µm thick frontal sections of the right hemisphere were cut with a vibratome (Leica VT 1000S). Every third section was collected in wash buffer at 4°C. For immunostaining the slices were rinsed three times for 10min in wash buffer, followed by a 30min preincubation in 10% normal goat serum and 0.4% Triton X-100 (Sigma). Then the slices were incubated with the primary antibody (rabbit anti-dopamine, DiaSorin, Stillwater, MN) diluted 1:600 with 1% normal goat serum and 0.4% Triton X-100 for 40h. The following rinses, all three times for 10min, and dilutions were done with 0.05M Tris-HCL (pH 7.5). The slices were rinsed and incubated for 30min in biotinylated goat-anti-rabbit antibody (Sigma) diluted 1:20 with 1% normal goat serum, rinsed again and incubated with ExtraAvidin-Peroxidase (Sigma) diluted 1:20 for 30min. After another rinse the slices were stained in 0.05% 3.3-diaminobenzidine (DAB, Sigma) with 0.01% H_2O_2 for 4min. Then the slices were washed, mounted on glass slides, dried overnight, dehydrated with ethanol, cleared with xylene and coverslipped with DePeX (Serva, Heidelberg, Germany).

Quantification of DA and 5-HT Innervation

Accumbal DA fibre densities were measured in three consecutive coronal slices of the NAC. All detectable fibre fragments were visualised in standard test fields, two located dorsoventral in the medial shell and one in the core medial to the anterior commissure (Fig.1), using a bright-field microscope (Polyvar, Reichert- Jung, Vienna, Austria) and a digital camera for microscopy (ProgRes 3008mf, Jenoptik, Jena, Germany) at 400-fold magnification. Fibres were quantified by software for image analysis (KS300, Jenoptik, Jena, Germany). To detect the fibre density, not amount of fibres, immunoreactive fibres of different diameters were standardised to identical thickness and visualised using a valleys operator that depicts local differences of grey values of adjacent pixel, but not a general threshold, and transforms the results into binary images. DA fibre densities were computed as percentage of area of the evaluated test field. We additionally analysed the growth of the whole NAC in each individual at 20-fold magnification. Measurement of DA densities and the area of the NAC were exclusively done by a single rater, blind to the coding of the samples.

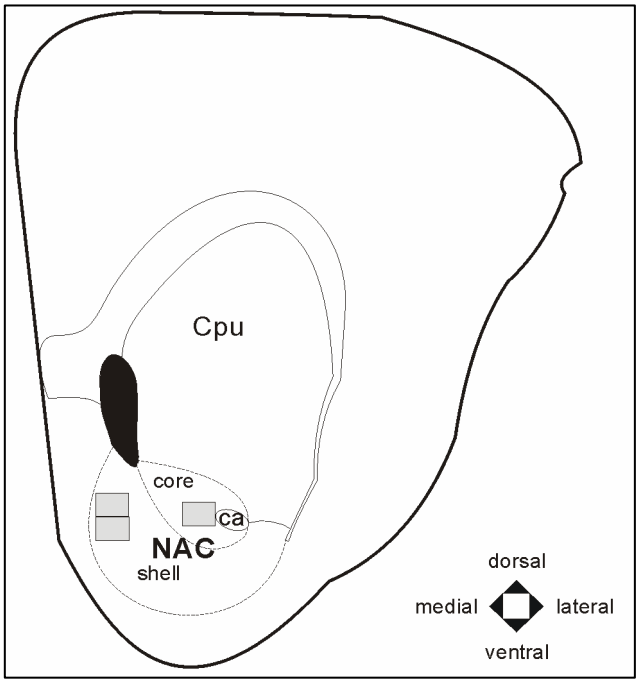

Fig. 1: Measurement windows in the NAC. Measurement windows for the DA fibre densities in the nucleus accumbens core and shell, indicated as grey squares in a coronal section through the gerbil brain.

Abbreviations:
Cpu caudate-putamen
ca commissura anterior

Data Analysis

To detect whether changes in the accumbal DA innervation might be due to an augmentation of the NAC volume and a simultaneous pausing of DA fibre sprouting, the fibre density in standard test fields was multiplied with the respective area of the NAC for each individual (Table 1). The data were computed as arithmetic means by-case and by-group ± S.D. for core and shell of the NAC. Statistical analysis checked the effects of age (8 levels) as an independent variable and region (2 levels) as dependent variables by use of 2-way analysis of variance (ANOVA) and by *post-hoc* analysis with LSD-test for multiple comparisons. Statistical analysis was computed with Statistica 5.5 (StatSoft, Tulsa, USA). The levels of significance were set at * $p<0.05$, ** $p<0.01$ and *** $p<0.001$.

3. Results

Qualitatively, the overall distribution pattern of accumbal DAergic fibres was similar in animals from all developmental stages, showing more immunoreactive fibres in the shell compared with the core (Fig. 2).

According to ANOVA, as main effects, age significantly alters the DA innervation of the NAC ($F= 28.74$, $p<0.001$), and DA fibre densities differ between core and shell ($F= 98.46$, $p<0.001$). According to the *post-hoc* analysis with LSD-test, the effects of age in the core and shell are as follows: The juvenile period (PD14-30), up to weaning, is characterised by a non significant decrease of DA fibre densities, followed by an increase up to PD70 in the core of the NAC, whereas no alterations occur in the shell. The late adolescent or early adult period (PD70-90) shows a significant increase of DA fibre densities in both the core ($p<0.01$) and the shell ($p<0.001$). In the early adult period (PD90-180) the augmentation of the DA innervation continues in the core ($p<0.01$) but not in the shell of the NAC. No significant effects were found during adulthood and ageing [PD180-720 (Fig.3)].

Table 1

Original data on fibre density and NAC area ± S.E.M.

	core		NAC	shell	
Age [d]	Fibre density [%] ± S.E.M.	Area x fibre density ± S.E.M.	Area of the NAC [mm^2] ± S.E.M.	Fibre density [%] ± S.E.M.	Area x fibre density ± S.E.M.
14	3.90 ± 0.24	4.34 ± 0.23	1.12 ± 0.03	4.53 ± 0.24	5.09 ± 0.30
30	2.16 ± 0.17	3.02 ± 0.31	1.43 ± 0.07	3.76 ± 0.46	5.39 ± 0.73
70	2.75 ± 0.32	4.41 ± 0.56	1.59 ± 0.05	3.64 ± 0.32	5.83 ± 0.59
90	3.70 ± 0.31	6.28 ± 0.52	1.70 ± 0.03	6.28 ± 0.17	10.67 ± 0.34
180	5.02 ± 0.45	8.72 ± 1.13	1.71 ± 0.08	6.29 ± 0.20	10.78 ± 0.61
360	5.31 ± 0.35	8.60 ± 0.50	1.63 ± 0.06	6.20 ± 0.34	10.04 ± 0.50
540	4.97 ± 0.36	8.11 ± 0.27	1.65 ± 0.08	5.81 ± 0.22	9.59 ± 0.73
720	5.49 ± 0.34	9.01 ± 0.57	1.65 ± 0.07	6.31 ± 0.31	10.31 ± 0.40

The table provides the original data on age-specific mean DA fibre densities ± S.E.M. in both the core (column 2) and the shell (column 5) of the NAC. Columns 3 and 6 comprise the age-specific product of fibre densities and the referring total area of the NAC (column 4) ± S.E.M.

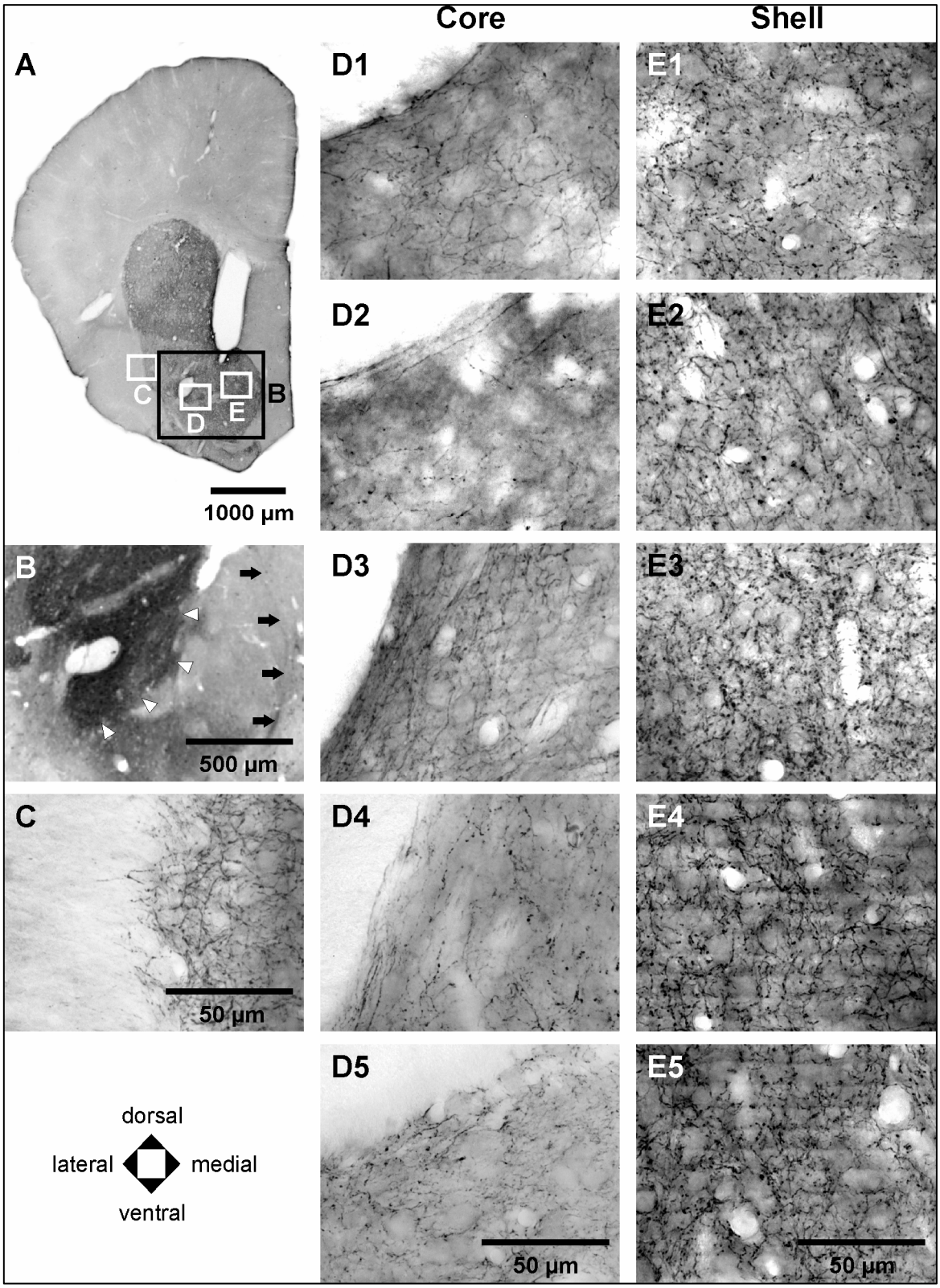

Fig. 2: DA fibres in the core and shell and delineation of the NAC. Brightfield photomicrograph of a representative coronar section at the level of the NAC, immunohistochemically stained for DA (**A**). Rectangles in (A) indicate the position of the photos in [B (black)] and [C-E (white)]. The delineation of the shell is marked in a frontal slice being immunostained for calbindin (**B**), indicating the medial (black arrows) and the lateral (white arrowheads) margins. (**C**) Illustrates the specificity of DA immunostaining at the border of insular cortex (left) and ventral striatum (right). **D1-5** (core) and **E1-5** (shell) show the representative accumbal subregions of different developmental stages (PD 14, 30, 70, 90, 180) respectively.

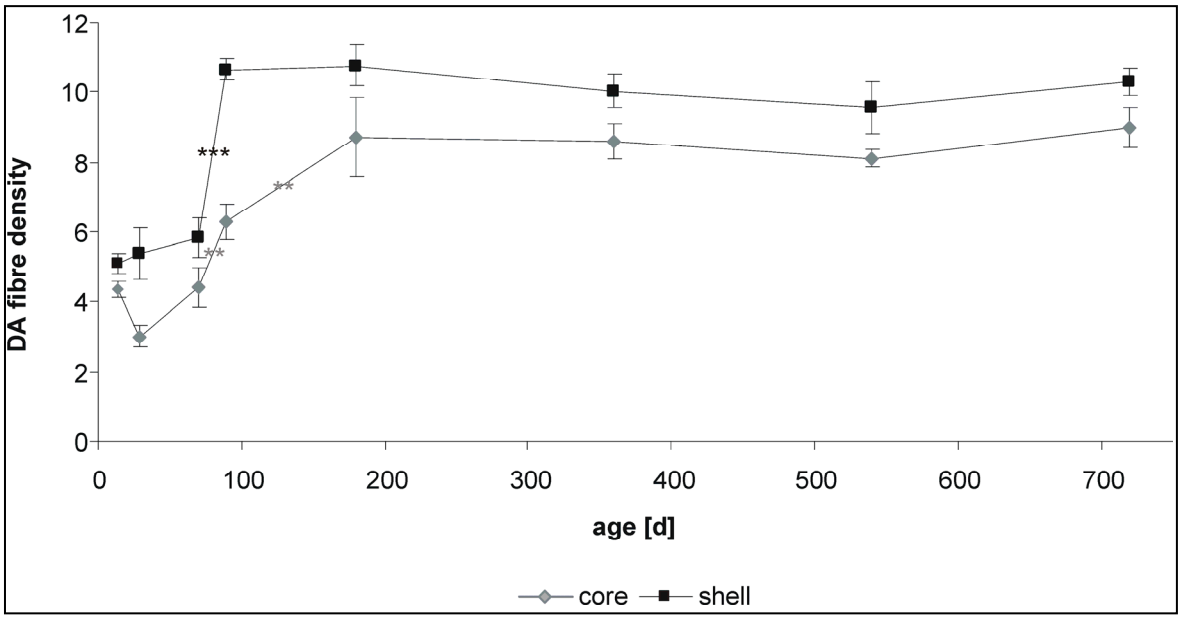

Fig. 3: Development of DA fibre densities. Development of DA fibre densities in the accumbal core and shell of gerbils ± S.E.M. at postnatal day (PD) 14, 30, 70, 90, 180, 360, 540 and 720. The accumbal core shows a significant increase of DA fibre densities between PD 70-90 and PD 90-120 and the accumbal shell between PD 70-90.
ANOVA and *post-hoc* LSD-test, significant values: $p < 0.05$ (*), $p < 0.01$ (**), $p < 0.001$ (***)

4. Discussion

The data presented show that the postnatal maturation of the DA innervation differs in the core and the shell subareas of the NAC of gerbils, whereas during adulthood and ageing the fibre densities are stable in both subareas. In the core the fibre density decreased between PD14-30, followed by a prolonged increase up to adulthood (PD180) and a final stabilisation that persisted until PD720. The fibre density in the shell, in contrast, remained steadily sparse until PD70, followed by a rapid increase up to PD90, and almost no further change occurred during adulthood and ageing up to PD720. What may be the meaning of these developmental differences between the subareas of the NAC, considering the functional distinctions of the core and the shell? In order to shed light on this we will follow the time course of postnatal development from infancy to ageing.

Our results of the juvenile maturation of the accumbal DA innervation are generally in line with recent studies which have reported on persistent variations of DA receptor densities

[56;58], DA varicosities [3], DA transporter densities [19;59], as well as the physiology of mesoaccumbal DA neurons [62] in the rat. These dynamics of the early juvenile, still immature, postnatal DA system of the NAC might parallel the initial functional integration of core and shell in local as well as in higher neuronal circuits of both motor and limbic systems. The medium spiny neurons, which are the principal output neurons of the NAC, do not acquire adult characteristics until the end of the third postnatal week [6]. Their glutamatergic input from diverse sources and their own firing are regulated by DA [32], which has been suggested to act as "state stabilizer" [46]. Thus, their development, maturation, and synapse formation may be closely linked to the DA innervation [3;55]. Accordingly, the anatomical substrate for the functional integration of core and shell is achieved during development through competing and cooperating interactions between the different inputs to the NAC [6]. Lesion studies of, for example, the neonatal PFC support this interrelation between accumbal glutamatergic and dopaminergic systems, with core and shell being differently affected [7].

Due to the different origin of DA fibres innervating core and shell, the altered course of DA development might represent structural reorganisation of DA afferents arising from different subpopulations of SN and VTA neurons, being allied to the motor and limbic circuitry, respectively [52]. Whereas the VTA mainly innervates the shell, the core gains its dopaminergic input from both the VTA and the SN pars compacta [13]. Neurogenesis [1] and physiological maturation [62] differ between these DA midbrain nuclei, with SN afferents developing earlier. Hu and co workers [33] have recently suggested that initial embryonic projections from midbrain DA neurons may target the NAC non-specifically. Later during development, the separate nigrostriatal and mesolimbic pathways differentiate by selectively eliminating misdirected collaterals. The attenuated fibre density in the

accumbal core between PD14-30, accordingly, might represent reorganisations of converging DA afferents arising from the different DAergic midbrain nuclei.

The temporary fall of the accumbal core fibre density at weaning, followed by a constant increase during adolescence, proves to be the inverted image of the dynamics of the DA receptor density. One recent study of the accumbal DA receptor maturation has indicated a pattern of overproduction around weaning, followed by pruning during adolescence [58]. However, another study suggests that this effect may occur exclusively in the striatum but not in the NAC [60]. Perhaps, the changes in the receptor density follow the dynamics of the fibre innervation which is the initial event in the establishing DA system [19], and compensate for the transient hypoinnervation. This phenomenon was shown in a lesion study with the neurotoxin 1-Methyl-4-Phenyl-1,2,3,6-Tetrahydropyridine (MPTP), where the selective depletion of DAergic fibres originating in the SN coincided with a compensating DA receptor increase in the caudate-putamen complex [25]. Generally, the dopaminergic SN neurons can be distinguished by their higher expression of the DA transporter mRNA compared to VTA neurons [9] and the DA transporter protein shows a higher density in the core of the NAC compared with the shell [45]. Moll and co-workers [42] described significant changes of the DA transporter physiology during the period around weaning and adolescence in the caudate-putamen. Thus, our results on the dynamic DA innervation during weaning in the core and the above cited DA receptor and transporter changes in the overlying caudate-putamen complex suggest that this critical period in particular concerns DA innervations arising from the SN of juvenile rodents.

In contrast to the early difference between the accumbal subareas during postnatal maturation of the DA fibres (PD14-70), the transfer from adolescence to adulthood (PD70-120) proceeds in a parallel significant augmentation of DA fibre densities. This raises the question if a higher-level instance may eventually synchronise the maturation of both the

motor associated core and the limbic associated shell during this period. Indeed, the prefrontal cortex (PFC), a multimodal association cortex being characterised by a rather late maturation [37], plays a major role in the control of limbic and motor functions and has been postulated to stabilise DA subsystems depending on its postnatal development [7;14;18;21;63;66]. In gerbils the severely retarded maturation of DA fibres in the medial PFC shows a steady increase up to early adulthood [20], and thus may initiate a prolonged restructuring of accumbal DA fibre densities in both subareas.

Our results describe no effect of ageing on DA fibre patterns in both accumbal subareas and thereby are in line with recent studies: Whereas in the rat age-induced alterations occurred in DAergic neuron functioning, the storage and synthesis of DA in the NAC was not altered [28]. An age related accumbal decrease in potassium evoked DA overflow was measured with in vivo electrochemical recordings, whereas no differences in DA level, DA metabolite and turnover rates were observed with HPLC analysis [24]. Moreover, ageing significantly altered monoamine turnover in the NAC, whereas biosynthesis was unchanged [40]. These physiological changes could be traced back to a decrease of glutamatergically induced DA release in the NAC, as described for the aged rat [53;54]. However, our results of a stable DA fibre density up to PD 720 do not exclude alterations in older animals, which were found in the nigrostriate system of different species [57].

Conclusion

In consideration of the different involvement of DA in the accumbal subareas, the varying development and maturation of the core and shell up to early adulthood might indicate differences in the maturation of the core allied motor and the shell allied limbic circuitry. Environmental variables (e.g. stress and pharmacological treatment) during the juvenile period and adolescence might differently affect the functional integration of the accumbal subareas, due to their region-specific critical phases. Thus, the time point of a challenge may be of great importance for the interpretation of the different involvement of core and shell in various neurodevelopmental disorders, namely schizophrenia [35;43;65] and ADHD [16;17], and also for the incidence of drug addiction [4;15;23].

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Research report

Hemisphere-specific effects on serotonin but not dopamine innervation in the nucleus accumbens of gerbils caused by isolated rearing and a single early methamphetamine challenge

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Abstract

The aim of this study was twofold: We examined whether serotonin (5-HT) and dopamine (DA) innervations of the nucleus accumbens are lateralised and whether the environment or the combination with an early pharmacological impact might interfere with the postnatal maturation of the monoaminergic innervation. Male gerbils were assigned to either enriched rearing (ER) or isolated rearing (IR). Animals from both rearing conditions additionally received a single dose of either methamphetamine [MA (50 mg/kg ip)] or saline on postnatal day 14. DA and 5-HT fibres of the adult animals (postnatal day 90–110) were immunocytochemically stained and fibre densities were quantified in nucleus accumbens core and shell of both the left and right hemisphere. Our data demonstrate that the DA and 5-HT innervation is not lateralised in saline-treated animals of both rearing conditions. IR increases the DA fibre density in both hemispheres of saline controls, whereas an additional MA treatment reverses this effect. In both ER and IR groups, MA provokes an excessive 5-HT fibre in growth of only the right hemisphere. The combination of IR with MA induces right-side asymmetries of the 5-HT fibre density in both the core and shell. From the data obtained, we conclude that the maturation of the monoaminergic innervation of the nucleus accumbens is vulnerable to postnatal stimuli. The subtle "innervation imbalance" observed in our studies is consistent with previously reported effects in other brain regions of this animal model and may be causative for behavioural disturbances.

Theme: Neurotransmitters, modulators, transporters and receptors
Topic: Serotonin

Keywords: Asymmetry; Environment; Immunohistochemistry; Laterality; Psychopathology

1. Introduction

Cerebral lateralisation is no longer thought to be an exclusively human feature. Behavioural and anatomical asymmetries have also been shown in rodents [21,26,32,69] and primates [32,69]. Neurochemical studies, most focussing on brain monoaminergic systems, indicate cortical and subcortical asymmetries in rodent brains [51]. Especially prenatal stress [2], perinatal distress [10], neonatal handling [22,23,56,62] and pharmacological interventions [4,15,16] alter the pattern of monoaminergic brain asymmetry during development. Some of these experimental parameters are discussed as an animal model for human affective disorders in which the nucleus accumbens (NAC) forms one of the key structures being affected [4].

The nucleus accumbens (NAC) is considered a functional interface between the limbic and the motor system [46] and plays a major role in motivational and reinforcement processes. The accumbal core represents the ventral extension of the caudate–putamen complex and is closely linked to the motor system, in contrast to the accumbal shell which is allied to the limbic circuitry [24,28,31,68]. Both core and shell receive afferent projections from DA-synthesising neurons of the ventral tegmental area and substantia nigra [11] and 5-HT-synthesising neurons of the raphe nucleus

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[61]. Morphology and innervation patterns of dopaminergic and serotonergic fibre systems differ between the accumbal subregions [12,37,48,63]. Also asymmetries in concentration and turnover of DA and/or 5-HT were found in the NAC of rodents [1,8,51,52,54,58]. The lateralisation of aminergic innervation patterns is well documented for the prefrontal cortex that stands in close interaction with the NAC in terms of physiological parameters such as stress and affective stimuli. Some studies therefore focussed on the lateralisation pattern in response to these intervening parameters [6] and were discussed in the light of human depression and anxiety [7].

In previous studies with gerbils we have shown that isolated rearing (IR) influences the development of cortical and subcortical DA and 5-HT innervation patterns [37, 36,47,67]. A single early systemic methamphetamine (MA) intoxication produced similar effects with most severe changes of transmitter maturation in limbic [13] and motoric [36] brain regions when combined with IR. Moreover we reported that these parameters interfere with the postnatal development of lateralised cortical 5-HT innervation patterns [49]. To expand our previous work we conducted the present study of 5-HT and DA fibre patterns in the NAC to investigate whether IR and/or MA-challenge interfere with possible asymmetries.

2. Materials and methods

2.1. Animals and rearing conditions

All experimental procedures were approved by the appropriate committee for animal care in accordance with the European Communities Council Directive. A total of 67 male Mongolian gerbils (*Meriones unguiculatus*) were used for this study, 29 for DA-immunohistochemistry and 38 for 5-HT-immunohistochemisty. The animals were bred either under enriched (ER) conditions in semi-naturally structured compounds (1 × 1 m, height 0.5 m), containing branches and hiding opportunities, or under isolated conditions (IR) in standard cages (Macrolon® type 4) without any content except of sawdust. On postnatal day (PD) 14 some of the pups from each breeding condition were injected with a single dose of methamphetamine (MA) hydrochloride (50 mg/kg ip); the others were sham treated by a single injection of saline. The injection of a neurotoxic dose of MA on PD 14 is used as a standard model to induce neuroplasticity during maturation of the monoaminergic fibre systems [57]. At weaning (PD 30), 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 (3–4 individuals) in compounds similar to those they were born in. Both experimental groups persisted for approximately further 60–80 days. This resulted in four experimental animal groups for each transmitter. For DA: 7 ER, 5 MA-treated ER (ER-MA), 10 IR and 7 MA-treated IR (IR-MA) animals. For 5-HT: 10 ER, 12 ER-MA, 9 IR and 7 IR-MA animals. All gerbils were kept under natural day/night cycles with food and water being provided ad libitum.

2.2. Immunohistochemistry

2.2.1. Dopamine immunohistochemistry

On PD 90 animals were transcardially perfused under deep chloral hydrate anaesthesia (1.7 g/kg ip). The perfusion was performed with 60 ml cold 0.05 M phosphate buffer (pH 6.2) containing 1% sodium metabisulfite, followed by 500 ml 5% glutaraldehyde with 1% sodium metabisulfite in 0.1 M phosphate buffer (pH 7.5) and finally by wash buffer containing 0.05 M Tris-buffered saline (TBS) with 1% sodium metabisulfite (pH 7.5). Immediately after perfusion, the brains were dissected and 50 μm thick frontal sections of each hemisphere were cut with a vibratome (Campden Instruments, London, UK). Every third section was collected in wash buffer at 4 °C. For immunostaining the slices were rinsed three times for 10 min in wash buffer, followed by a 30 min preincubation in 10% normal goat serum and 0.4% Triton X-100 (Sigma). Then the slices were incubated with the primary antibody (rabbit anti-dopamine, DiaSorin, Stillwater, MN) diluted 1:600 with 1% normal goat serum and 0.4% Triton X-100 for 40 h. The following rinses, all three times for 10 min, and dilutions were done with 0.05 M Tris–HCL (pH 7.5). The slices were rinsed and incubated for 30 min in biotinylated goat-anti-rabbit antibody (Sigma) diluted 1:20 with 1% normal goat serum, rinsed again and incubated with ExtraAvidin–Peroxidase (Sigma) diluted 1:20 for 30 min. After another rinse the slices were stained in 0.05% 3,3-diaminobenzidine (DAB, Sigma) with 0.01% H_2O_2 for 4 min. Then the slices were washed, mounted on glass slides, dried overnight, dehydrated with ethanol, cleared with xylene and coverslipped with DePeX (Serva, Heidelberg, Germany).

2.2.2. 5-HT immunohistochemistry

On PD 110 animals were transcardially perfused under deep chloral hydrate anaesthesia (1.7 g/kg ip). The perfusion was performed with 100 ml 0.1 M phosphate buffer (room temperature, pH 7.2), followed by 500 ml 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4). Immediately after perfusion, the brains were dissected and postfixed for 2 h at 4 °C. Then 20 μm thick frontal sections of each hemisphere were cut with a frigocut (Reichert-Jung, Vienna, Austria) and every third was collected in phosphate-buffered saline (PBS, pH 7.4) at 4 °C. For immunostaining the slices were rinsed three times for 10 min in PBS, incubated for 10 min with 1% H_2O_2 to reduce background staining, rinsed again three times for 10 min, followed by a 30 min preincubation in 10% normal goat serum in PBS with 0.3% Triton X-100 (Sigma). Then the slices were incubated with the primary antibody (rabbit-anti-serotonin serum, DiaSorin, Stillwater, MN) diluted 1:20,000 in PBS with 1% normal

goat serum and 0.3% Triton X-100 for 18 h. The next steps follow the DA protocol.

2.3. Quantification of DA and 5-HT innervation

Accumbal 5-HT and DA fibre densities were measured in six consecutive frontal slices. All detectable fibre fragments were visualised in standard test fields, four dorsoventral in the medial shell and two in the core medial to the commissura anterior (Figs. 1, 2), using a bright-field microscope (Polyvar, Reichert- Jung, Vienna, Austria) and a digital camera for microscopy (ProgRes 3008mf, Jenoptik, Jena, Germany) at 400-fold magnification. Fibres were quantified by software for image analysis (KS300, Jenoptik, Jena, Germany). To detect the fibre density, not amount of fibres, immunoreactive fibres of different diameters were standardised to identical thickness and visualised using a valleys operator that depicts local differences of grey values of adjacent pixel, but not a general threshold, and transforms the results into binary images. DA and 5-HT fibre densities were computed as percentage of the evaluated test field. Measurement of DA and 5-HT fibre densities was exclusively done by a single rater, blind to the coding of the samples.

2.4. Data analysis

The data were computed as arithmetic means by-case and by-group ± SD for core and shell of the NAC. Statistical analysis checked the effects of pharmacological interventions and rearing conditions (2 levels each) as independent variables and hemisphere (2 levels), region (2 levels) and section (6 levels) as dependent variables by use of five-way analysis of variance (ANOVA) and by post hoc analysis with LSD test for multiple comparisons. Statistical analysis was computed with Statistica 5.5 (StatSoft, Tulsa, USA). The levels of significance were set at $*P < 0.05$, $**P < 0.01$ and $***P < 0.001$.

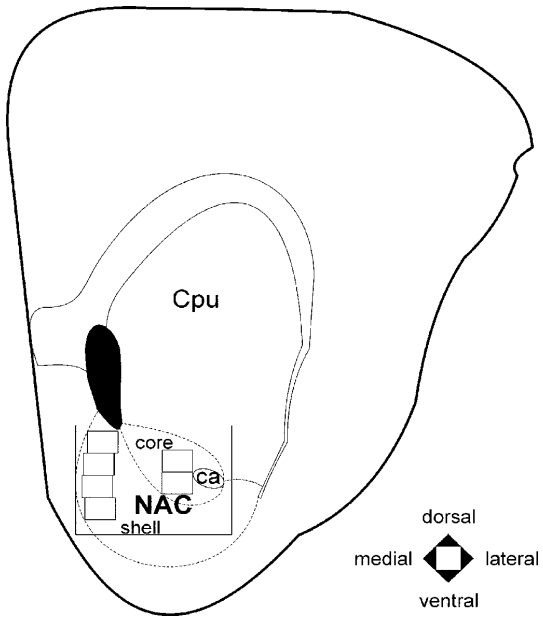

Fig. 1. Measurement windows for the DA and 5-HT fibre densities in the nucleus accumbens core and shell, indicated as grey squares in a coronal section through the gerbil brain. Rectangle shows position of photomicrographs A and B in Fig. 2. Abbreviations: Cpu, caudate–putamen; ca, commissura anterior.

3. Results

3.1. Dopaminergic fibre density in the nucleus accumbens

According to ANOVA, as main effects, rearing conditions ($F = 7.68$, $P < 0.05$) and MA treatment ($F = 4.66$, $P < 0.05$) significantly alter the DA innervation of the NAC. DA fibre densities differ between core and shell ($F = 407.03$, $P < 0.001$), whereas no asymmetric DA innervation pattern is found in the NAC. Following post hoc analysis with LSD-test, the effects of both experimental variables, IR and MA are as follows: IR causes a significant augmentation of DA fibre densities in the left (+40.60%, $P = 0.019$) and right (+35.15%, $P = 0.034$) core and the left (+11.43%, $P = 0.041$) and right shell (+18.01%, $P = 0.008$) compared with ER animals. MA treatment causes a significant decline of DA fibre densities in the left (−28.98%, $P = 0.019$) and right (−28.49%, $P = 0.022$) core, and the left shell (−18.01%, $P = 0.048$) of IR animals with no effect in ER animals. Taking ER saline animals as controls, the combination of both rearing conditions and MA treatment (IR-MA) causes no significant changes in DA patterns (all data shown in Fig. 3). According to ANOVA no significant lateralisation of the DA innervation occurs in the core and shell of all experimental groups (Fig. 4).

3.2. Serotonergic fibre density in the nucleus accumbens

According to ANOVA, as main effects, MA treatment ($F = 10.76$, $P < 0.01$) significantly alter the 5-HT innervation of the NAC, with no effect for rearing conditions. 5-HT fibre densities are clearly asymmetric in the overall NAC ($F = 9.71$, $P < 0.01$) as well as region-specific comparing core and shell ($F = 7.98$, $P < 0.01$). Following ANOVA and post hoc analysis with LSD test all significant effects occur in the right NAC (Fig. 3): IR causes no significant changes of 5-HT fibre densities compared with ER animals. MA treatment causes a significant increase of 5-HT innervation in both ER and IR animals of the right core (ER/ER-MA: +12.52%, $P = 0.029$; IR/IR-MA: +14.08%, $P = 0.001$) and the right shell (ER/ER-MA: +23.18%, $P = 0.03$; IR/IR-MA: +22.17%; $P = 0.005$). Taking ER animals as controls, the combination of both rearing conditions and MA treatment (IR-MA), significantly increases the 5-HT fibre innervation of the right core

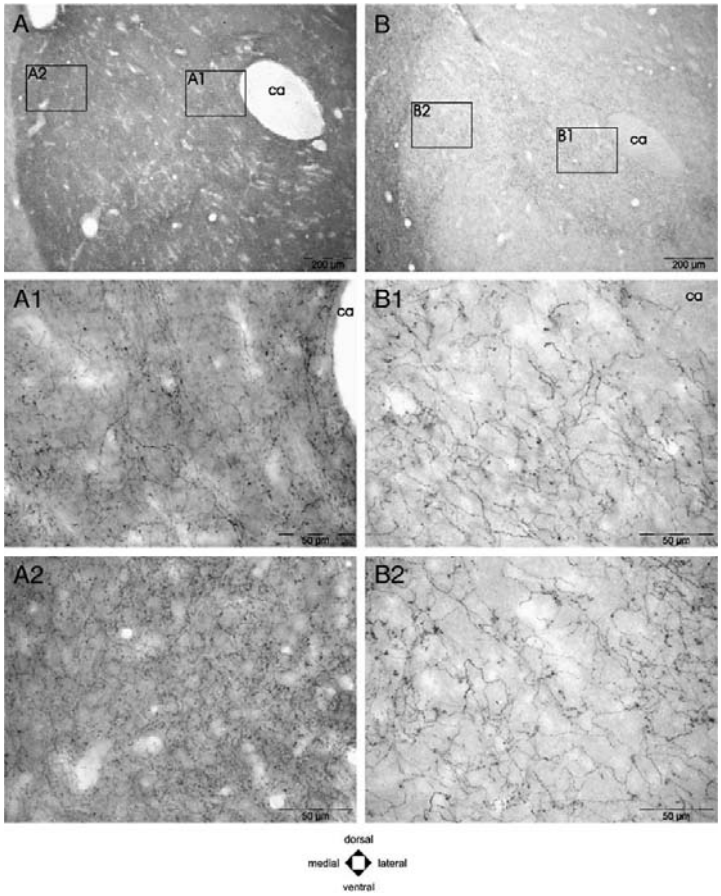

Fig. 2. Bright-field photomicrograph of representative coronar sections at the level of the nucleus accumbens of the dopaminergic (A) and serotonergic (B) innervation. Rectangles show the representative accumbal subregions core and shell, highlighted in A1/B1 (core) and A2/B2 (shell). Abbreviations: ca, commissura anterior.

(+21.10%, $P = 0.004$) and shell (+21.60%, $P = 0.009$). According to ANOVA no significant lateralisation of 5-HT innervation occurs in the core and shell of ER, ER-MA and IR animals. The combination of IR and MA results in a significant asymmetrical (right > left) 5-HT innervation of both core (+16.60%; $F = 9.68$, $P = 0.004$) and shell (+15.81%; $F = 6.79$, $P = 0.013$) (Fig. 4).

4. Discussion

We have studied the innervation of DA and 5-HT immunoreactive fibres in both the left and right NAC of adult male gerbils and the influence of isolated rearing (IR) and/or a single early challenge with MA on the postnatal maturation of these monoaminergic transmitters. Our data demonstrate that neither DA nor 5-HT innervation patterns in the NAC are generally asymmetric. IR produces a DA hyperinnervation that is reversed by an early MA challenge. MA treatment produces a 5-HT hyperinnervation selectively in the right hemisphere of ER animals and the combination of restricted environment and MA treatment induces a hemispherical asymmetry of the 5-HT innervation.

4.1. Environmental effects

The DA and 5-HT innervation of the NAC is not lateralised in both ER and IR saline-treated groups. This result is akin to those of other studies reporting on similar DA and 5-HT concentrations in both hemispheres of the NAC of socially and isolated reared rats [30,34]. In our experimental model IR conditions significantly increase the DA fibre density in both the left and the right hemisphere of the NAC subareas. This, to our knowledge, is the first study reporting on an environmentally induced adaptation of the DA fibre density in the NAC of the rodent brain. However, physiological alterations have already been shown: In rats, social isolation increases the basal DA levels in the NAC

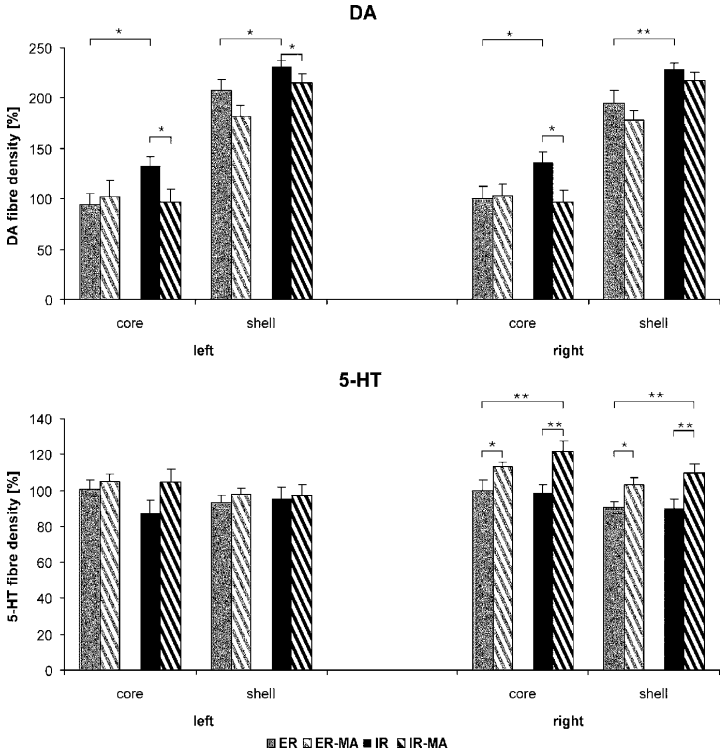

Fig. 3. Dopamine (DA) and serotonin (5-HT) fibre densities in the left and right accumbal core and shell of gerbils from enriched (ER) and isolated rearing (IR) conditions treated with either methamphetamine (MA) or saline given as standardised values (fibre density in the right core is standardised to 100%) ± SEM. (A) Bilateral significant increase of DA fibre densities in core and shell of the NAC. MA significantly reduces the DA fibre density of IR animals, except in the right shell. (B) MA significantly augments the 5-HT fibre density of ER and IR animals with all significant effects being restricted to the right side. ANOVA and post hoc LSD test, significant values: $P < 0.05$ (*), $P < 0.01$ (**), $P < 0.001$ (***).

[29,34], attenuates DA turnover elicited by novelty stress [44,45] and raises DA efflux induced by cocaine administration [33]. As to 5-HT, no region-specific nor hemisphere-specific effects could be detected in the NAC comparing ER and IR animals in the present study and thus confirm our previous results obtained in the right hemisphere of this animal model [37]. In contrast to these morphological data, isolated rats have a lower 5-HIAA/5-HT ratio [34] as well as a decreased basal turnover of 5-HT in the NAC [30] compared with socially reared rats. The different effects that the environment exerts on morphology and physiology of the DA and 5-HT fibres may indicate concomitant functional differences of the two transmitters in the NAC. This interpretation is supported by the respective response mechanism of both transmitters to pharmacological challenge in ER and IR groups.

4.2. MA effects

An early systemic challenge with MA has no effect on the adult DA fibre density in the NAC of gerbils from ER conditions but inverses the excessively increased DA fibre density in animals from IR conditions and thus apparently restores ER conditions; the pharmacological intervention did not induce any hemisphere-specific effect. On the other hand, recent studies on the DA physiology following juvenile pharmacological intervention showed striking evidence for lateralised effects in the NAC of rats [4,9]. However, concentrations of transmitters or their metabolites may not directly be predictive of the fibre density; i.e., the occurrence of concomitant asymmetric physiological reactions of the accumbal DA innervation cannot be ruled out in our experimental setup. For example, 5-HT, which is affected by MA in a lateralised fashion and innervates DAergic terminals with about 20% of its synapses in the NAC [60], might modulate tonic [20] and phasic [17] DA levels.

In a previous study we showed that MA intoxication leads to an excessive 5-HT innervation in the NAC of the right hemisphere [37]. This result is confirmed by the present re-evaluation. In contrast, the 5-HT fibres of the left NAC are less vulnerable to MA. The literature concerning asymmetric 5-HT concentration, turnover or utilization of the NAC is relatively sparse. Wallace Deckel and coworkers

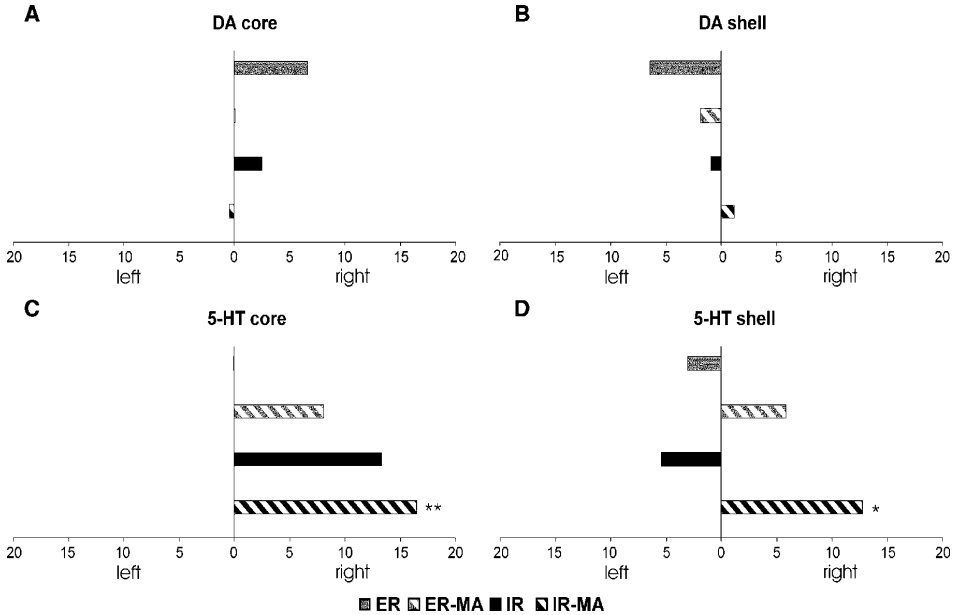

Fig. 4. The extent of either DA (A: core, B: shell) or 5-HT (C: core, D: shell) lateral asymmetry in the NAC is shown as means of percent over-plus of the more densely innervated hemisphere. The combination of isolated rearing and MA (IR MA) displays a right-sided asymmetry of 5-HT fibre densities in core and shell of the NAC. ANOVA, significant values: $P < 0.05$ (*), $P < 0.01$ (**), $P < 0.001$ (***).

noted a hemisphere-specific either negative or positive correlation between 5-HT levels in the NAC and the size of a prefrontal cortex lesion [19]. Rosen and coworkers found a higher concentration of 5-HT in the right and a higher turnover in the left NAC of albino rats [51]. Andersen and coworkers showed a shift of laterality in the NAC from the left to the right hemisphere of both 5-HT and DA content in response to clomipramine treatment [4]. Correspondingly, Schwarting and coworkers found a negative correlation between increased 5-HT tissue levels in the left ventral striatum and the amount of time that rats spent in the closed arm of the elevated plus maze [52]. Thus, lateralised effects of the accumbal 5-HT innervation may be due to different physiological properties of the 5-HT fibres in the respective hemisphere; however, the mechanism of this asymmetrical response still remains unanswered or speculative. The lateralised effect may be caused by an asymmetric maturation of the 5-HT projections at PD 14 of the NAC inducing a lateralised effect up to adulthood. Also, asymmetric concentrations of the 5-HT-transporter, which is known to participate in the toxic effect of MA [12], could be causative for this effect. Additionally, asymmetric concentrations of the growth factor S-100$_\beta$, which is released by astroglia after the destruction of 5-HT fibres, may induce lateralised heterotypic sprouting effects [5,65]. Further studies are needed to reveal the mechanisms underlying the asymmetric effects on 5-HT innervation of the NAC.

Generally, MA has been proven to damage monoaminergic terminals [50,53]. It might be surprising that treatment of juvenile gerbils with a neurotoxic dose of MA induces diminished densities of DA fibres and concomitant excessive 5-HT fibre densities in adult animals. This finding, as well as the restoration of "normal" DA fibre densities by the combination of IR and MA, may appear more reasonable if we consider the time course of activity-dependent interactions of limbo-cortical brain regions during the postnatal maturation.

4.3. Development of the limbo-cortical network

Though the causal mechanisms of interaction of environmental complexity and early MA treatment must remain speculative, the following interpretation seems reasonable: The end of the second postnatal week is supposed to be a critical phase for the maturation of the limbic motor system [66], which may be highly vulnerable to external stimuli at this early stage. The single MA treatment, due to neurotoxic effects on monoaminergic terminals [57], might induce some transient instability of the local circuitry in the NAC during this critical phase. Environmental stimuli are presumably strong enough to trigger a kind of neuronal re-modelling that may finally lead to the observed differences in the monoaminergic innervation pattern of the NAC. Remarkably, the impact of the environment is at least to some degree hemisphere-specific, probably indicating some currently unknown lateral asymmetry of NAC function. The numbers of DA fibre varicosities strongly increase from postnatal day (PD) 8 to PD 20 and the adult

innervation pattern is found at PD 28 in the rat NAC [64]. Probably, the MA-induced degeneration of maturing fibres in ER animals induces a reactive overshoot sprouting of surviving fibres, thus leading to an unchanged DA innervation of the NAC. In IR-MA animals the chronic exposition to impoverished environment seems to counterbalance this mechanism.

Obviously, the re-establishment of control fibre densities in the NAC by the combination of IR conditions and early MA treatment does not reflect the original state: The acute MA intoxication rather is a severe "stressor" in a critical period of DA maturation and leads to substantial DAergic fibre re-organisation in several limbic brain regions, namely the basolateral amygdala, entorhinal cortex [14] and prefrontal cortex [57]. All these structures stand in close functional and physiological interaction with the NAC [27,46]. Especially the interdependence of activities of the DAergic mesencephalic projections to the basolateral amygdala, the entorhinal and prefrontal cortices and the NAC [39–42] suggests that physiological and functional properties of neural networks in the NAC and other brain regions might be substantially different between ER and IR-MA animals. This is supported by our previous studies with gerbils: IR conditions also result in an excessive DA innervation in the basolateral amygdala [14]. This overshoot maturation in the subcortical DA terminal field of the mesolimbic projection is accompanied by a suppressive maturation in the medial and orbital prefrontal cortex [47,67]. The prefrontal cortex indeed plays a major role in the interactions of limbic regions at all [38]. Since the mesoprefrontal DA projection matures rather late in mammals [18,35], the postnatal stabilisation of other DAergic subsystems may depend on the retarded ontogeny of the prefrontal cortex. Therefore, the challenge of the prefrontal DA innervation by IR conditions may be the initial cause for an uncontrolled surplus maturation of DA in subcortical regions [14].

4.4. Development and laterality

Using the same animal model, we have recently investigated the postnatal development of the 5-HTergic system in various other brain regions. All effects, under the aspect of laterality, occurred in areas which are closely linked to the NAC: In IR animals, an early MA intoxication reduced the 5-HT innervation in the right hippocampal dentate gyrus [13] and right prefrontal cortex, but significantly increased it in the left entorhinal cortex. In contrast, no effect occurred in parietal somatosensory and frontal motor cortices [49]. These results indicate selective and lateralised effects of MA on the 5-HTergic system in predominantly associative limbo-cortical structures. Interestingly, asymmetries in 5-HT binding are associated with human depression [43] and shifts in laterality of 5-HT and DA may be involved in the ontogeny of depression [4] and possibly anxiety [3]. Furthermore, anxiety is coupled with 5-HTergic asymmetries in the NAC [52]. Our animal model may therefore suggest possible pathways by which aberrant external stimuli could contribute to the ontogeny of functional disturbances of the human brain in an experience-dependent way.

The combination of IR and MA has opposing effects on the asymmetry of 5-HT fibres in neocortex (e.g., prefrontal cortex) compared to allocortical areas (hippocampal formation, entorhinal cortex) and subcortical brain regions (NAC) [13,49]. The highly significant asymmetry of prefrontal 5-HT fibre densities of ER gerbils is reduced in MA-treated IR animals, whereas the asymmetry in the entorhinal cortex increases [49]. Likewise the combination of IR and MA treatment induces an imbalance of 5-HT fibre densities in the NAC that is absent in control animals (present study) and also impairs the 5-HT innervation of the hippocampus in a lateralised fashion. We assume that during development the combination of IR and MA treatment prevents a shift of lateralised innervation patterns, at least for 5-HT, from allo- and subcortical to neocortical brain regions. Likewise, Hiscock and Kingsbourne [32] supposed that infantile behavioural asymmetries can be traced back to a lateralised subcortical mechanism and that cortical asymmetries may be extrapolations of the subcortical asymmetries. Tang [55] described a theoretical "bottom-up" model, which centred experience-dependent neurochemical hippocampal asymmetries as a driving force for the development of cerebral lateralisation. Trevarthen [59] introduced an "Intrinsic Motive Formation", which emerges in the foetal human brain stem to regulate asymmetries in the functional development of the cerebral cortex. The influence of subcortical asymmetries on the development of cortical lateralisation reverses during brain maturation. "Top–down" regulations, from cortical to subcortical regions, are common in the mature brain. Especially the prefrontal cortex, acting as the highest instance in the hierarchically organised brain [38], displays lateralised influences on subcortical DA utilisation and its related behaviours [15]. These influences could be traced back to structural changes. A developmental reduction of lateralisation in sub- and allocortical areas [25] accompanies the experience-dependent shift of laterality to later mature and higher organised cortical areas. The disruption of this process by combining IR and MA treatment could result in an aberrantly developed brain, with its lateralisation being restricted to sub- and allocortical areas.

5. Conclusion

A single juvenile MA intoxication in combination with differing rearing conditions leads to a substantial reorganisation of DA and 5-HT fibre innervations in the NAC with lateralised effects. Since shifts in laterality of the accumbal monoaminergic system are discussed as an animal model for depression [4], the present experimental setup

could be viewed as an animal model for psychotic processes.

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

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

Keywords: Dopamine, caudal limbic areas, fibre overshoot.

Introduction

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

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

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

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

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

Material and methods

Animals and rearing conditions

All experimental procedures were approved by the appropriate committee for animal care in accordance with the European Communities Council. For this study 34 male gerbils were used. Sixteen of them were bred in standard makrolon cages (type IV) under impoverished condition while 18 of them were bred in semi-naturally structured compounds (1×1 m; enriched condition). At weaning (30 days), the gerbils that were born in cages were assigned to impoverished conditions (IR, animals kept alone in standard makrolon cages type III), while the other group grew up under enriched rearing conditions (ER, kept as a group of siblings in semi-naturally structured compounds containing branches and hiding opportunities), both for further 60 days. On PD14 a total of sixteen pups received a single injection of methamphetamine hydrochloride (50 mg/kg; i.p.), nine gerbils of the ER group and seven gerbils of the IR group. The remaining eighteen gerbils, nine of either rearing group, were sham-treated by a single injection of saline. Under all sets of conditions food and water were provided *ad libitum*. All gerbils were kept on natural day/night cycles during summer season.

Immunohistochemistry

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

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

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

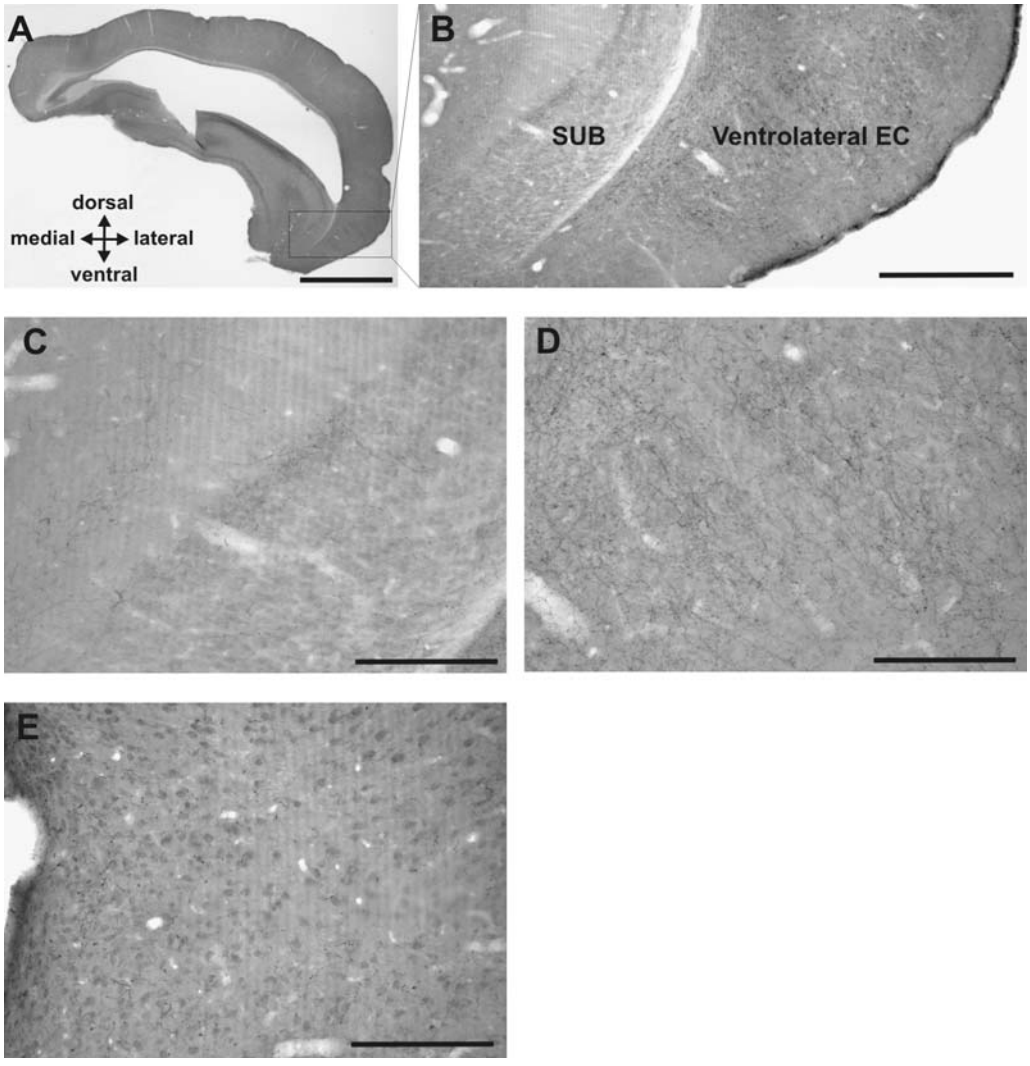

Fig. 1. A Brightfield photomicrograph of a representative coronar section at the level of the entorhinal cortex (EC). The area of the rectangle is magnified in (**B**) and comprises the ventral subiculum (SUB) and the ventrolateral EC. Within the cellular layer of the SUB DA fibres are only found in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

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

Quantification of DA innervation

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

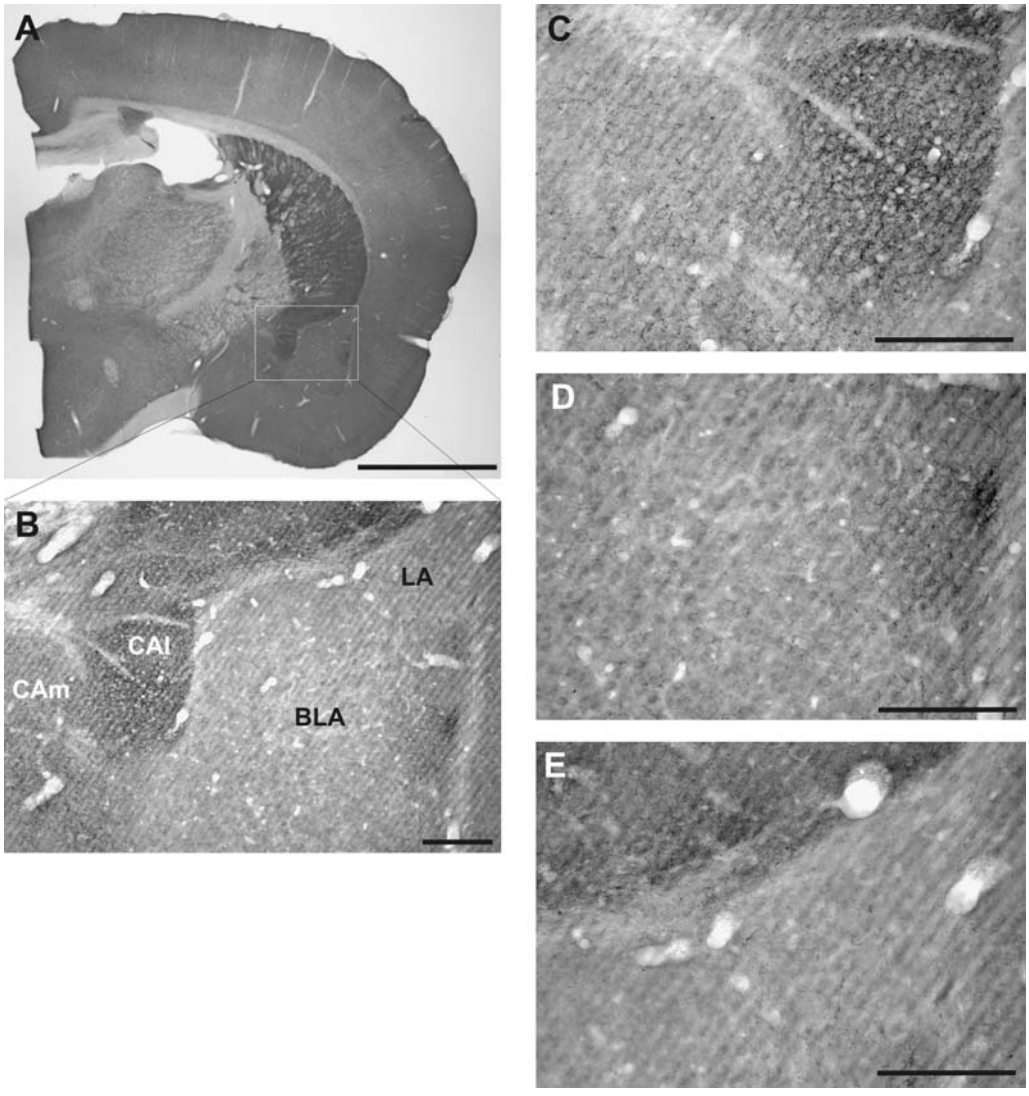

Fig. 2. Brightfield photomicrograph of a representative coronar section at the level of the amygdala (**A**). The rectangle shows the analysed amygdaloid nuclei, which are highlighted in (**B**). The DA innervation patterns of the amygdaloid nuclei are generally different. The lateral part of the central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

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

Data analysis

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

Results

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

Influence of rearing conditions

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

Influence of methamphetamine

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

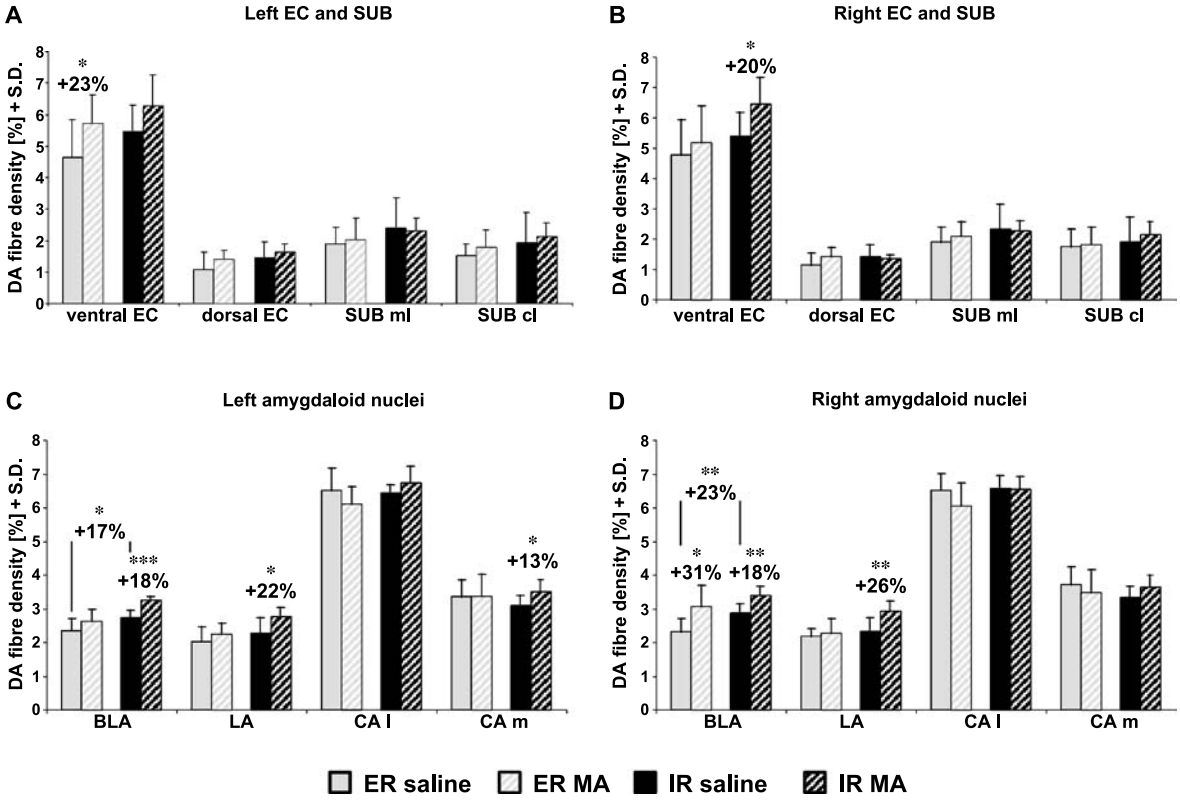

Fig. 3. Dopamine (DA) fibre densities in the analysed areas of gerbils from enriched (ER) and impoverished rearing (IR) conditions treated with either methamphetamine (MA) or saline given by means ± standard deviation (S.D.). **A**, **B** show the results of the left and right hemisphere, respectively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

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

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

Discussion

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

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

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

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

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

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

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

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

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

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Research report

Serotonin fibre densities in subcortical areas: differential effects of isolated rearing and methamphetamine

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Abstract

Serotoninergic neurons interact with dopaminergic cells on all levels and are physiologically affected by both isolated rearing (IR) and a single early methamphetamine (MA) injection. We therefore checked for anatomical effects of both interventions by immunohistochemically staining serotonin fibres and assessing fibre densities in the caudate-putamen (CPu), nucleus accumbens (NAc) and amygdala of Mongolian gerbils. IR led to significantly increased 5-HT fibre densities in the dorsal part of the CPu and in the central and basolateral amygdala. No effects were seen in the ventral CPu, in the NAc and in the lateral amygdala. The early MA injection resulted in a denser 5-HT innervation in the dorsomedial and ventromedial CPu, in the NAc shell of animals reared in an enriched environment and in the NAc core of both rearing conditions, leaving the lateral CPu and the amygdala unaffected. Thus, the single pharmacological versus the environmental challenge exerts an almost complementary effect on the 5-HT innervation in different areas of the brain, which demonstrates that systemic interactions, e.g. with dopaminergic and glutamatergic afferents, must be taken into account when the seemingly uniform 5-HT projections are investigated.

Theme: Development and regeneration

Topic: Neurotransmitter systems and channels

Keywords: Serotonin; Rearing condition; Methamphetamine; Caudate-putamen; Nucleus accumbens; Amygdala

1. Introduction

The subcortical brain nuclei represent key structures for most of an animal's emotional and motivational life. In this, they are heavily influenced by their often dense neuromodulatory input. Serotonin (5-HT) seems to be implicated in the mediation of reward in the nucleus accumbens [48], in the control of impulsivity in the caudate-putamen (CPu) [18,54] and in the regulation of fear in the amygdala [21,57]. Additionally, these three structures stand out by their dopamine (DA) innervation that subserves important behavioural functions, i.e. stress and reinforcement in the NAc [1,6,31], motor control in the CPu [2] and stress in the amygdala [20]. 5-HT and DA interact closely on the level of their cell somata as well as in their mutual projection fields [13,34,36,41,67]. This interaction is probably crucial for the postnatal maturation of the 5-HT projections [59,63], and its imbalance may play a role in the pathology of schizophrenia [25,33].

Environmental and pharmacological influences are capable of inducing such an imbalance in various parts of the rodent brain. Rearing rodents in isolation has become a classical model for an environmentally disturbed brain maturation (see Ref. [17] for review). A combination of maternal separation and isolation rearing (IR) leads to less DA fibres but more 5-HT fibres in the prefrontal cortex (PFC) of degus [5]. In previous studies, we showed that IR by itself results in a weaker DA innervation in the medial and orbital PFC [37,65] and in the CPu [30]. In the lateral orbital PFC, this is counteracted by a denser growth of 5-HT fibres [39]. These anatomical alterations go along with altered transmission of both 5-HT and DA in IR rodents [19,23,29,47,61].

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The DAergic fibre density of the PFC and the NAc can also be reduced by a single early injection of a high dose of methamphetamine (MA) [11,38], which suppresses the prolonged postnatal maturation of the DAergic projections [10]. Possibly in response to this thinning of DA fibres, there is a serotoninergic sprouting in the medial and the lateral orbital PFC of MA-treated gerbils reared in an enriched environment, whereas treated IR animals show no effect [39]. This is reminiscent of the consequences of a pathological or neurotoxin-induced loss of DA innervation which results in an enhanced 5-HT fibre density in the CPu [45,56]. The question arises whether a similar 5-HT sprouting can also be found in the subcortical areas of MA treated and of IR gerbils.

We approached this question by immunohistochemically staining 5-HT fibres in the brains of gerbils reared either in an enriched or an impoverished environment, some of which had received a single high dose of MA on postnatal day 14. The fibre densities were then quantitatively assessed in the NAc, CPu and lateral, basolateral and central amygdala using software for computer-aided image analysis.

2. Materials and methods

2.1. Animals and rearing conditions

Mongolian gerbils were chosen for this study, as they are considered to resemble their respective wild-type more than laboratory rats do [49]. Male gerbils were bred in our facilities either in standard makrolon cages or in large (1 m^2), semi-naturally structured compounds enriched with branches and hiding places (for details, see Ref. [65]). On postnatal day (PD) 14, some of the pups from each condition were injected i.p. with a single dose of 50 mg/kg methamphetamine hydrochloride (MA); the others received a saline injection. At weaning (PD30), at least 20 animals each, half of them MA-treated, were assigned to either impoverished (IR) or enriched (ER) rearing conditions for 80 days. IR animals were kept singly in standard makrolon cages, ER animals lived in sibling groups in compounds similar to those they were born in. Under both sets of conditions, there was a bedding of wood shavings, and food and water were provided at libitum. All gerbils were kept on natural day/night cycles.

2.2. Serotonin immunohistochemistry

On PD 110, the animals were transcardically perfused under deep choralhydrate anesthesia (1.7 g/kg, i.p.). The perfusion was carried out with 100 ml 0.1 M phosphate buffer (room temperature, pH 7.2), followed by 500 ml of 4% phosphate buffered paraformaldehyde (pH 7.4). Immediately after perfusion, the brains were removed and postfixed for 2 h at 4 °C. Frontal sections of 20 μm were taken on a frigocut and every third section collected in ice-cold phosphate-buffered saline (PBS, pH 7.4).

For immunostaining, the slices were rinsed three times for 10 min in PBS, incubated for 10 min with 1% H_2O_2 to reduce background staining, and rinsed again three times in PBS for 10 min. This was followed by a pre-incubation with 10% normal goat serum (NGS) in PBS containing 0.3% Triton X-100, after which the slices were incubated with the primary antibody (rabbit-anti-serotonin, Incstar), diluted 1:20 000 in PBS with 1% NGS and 0.3% Triton X-100, for 18 h at 4 °C. All following rinses and dilutions were done with Tris-buffered saline (TBS). The slices were rinsed three times for 10 min, then incubated with the biotinylated second antibody (goat-anti-rabbit, Sigma) diluted 1:20, rinsed again three times for 10 min and incubated with ExtrAvidin peroxidase (Sigma) diluted 1:20 in TBS containing 1% NGS. After another three rinses, the slices were stained in 0.05% 3,3-diaminobenzidine (DAB, Sigma) and 0.01% H_2O_2 for 4 min. The slices were then rinsed again four times and mounted on coated glass-slides, dried overnight, dehydrated and coverslipped with DePeX (Serra, Heidelberg, Germany).

2.3. Quantification of 5-HT innervation

To avoid deviations due to lateralized 5-HT innervation [9], only right hemispheres were used for quantification. All measurements were carried out using a light microscope

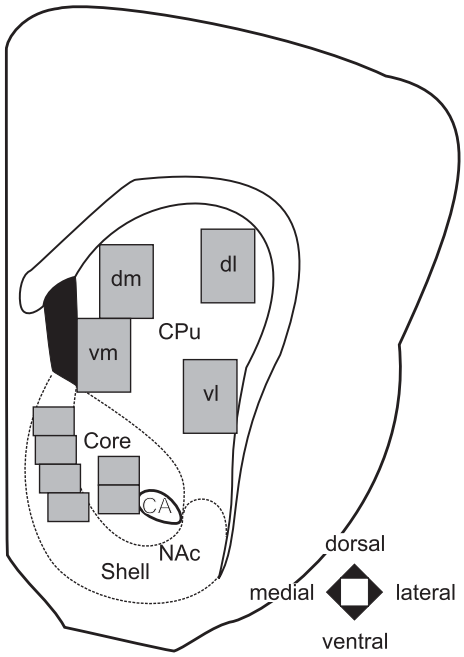

Fig. 1. Measurement windows in CPu and NAc. Measurement windows for the 5-HT fibre density in the caudate-putamen (CPu) and nucleus accumbens (NAc) core and shell, indicated as grey squares in a coronal section through the gerbil brain. CA, commissura anterior; dm, dorsomedial; dl, dorsolateral; vl, ventrolateral; vm, ventromedial.

(Polyvar, Reichert-Jung), a digital video camera (ProgRes 3008 mF, Jenoptik, Jena) and software for image analysis (KS300, Zeiss, Jenoptik, Jena).

In the NAc, the 5-HT fibre density was measured in six consecutive frontal slices. In each slice, four picture frames were defined in the medial shell in a vertical column ventral to the lateral ventricle, and two fields in the core medial to the commissura anterior (Fig. 1). Pictures were taken at a magnification of 400 ×. After blood vessels had been excluded from the reference area, fibres were automatically recognized by a function that detects local brightness minima and represents them as lines of one pixel width; the total area of these lines was computed as a percentage of the reference area.

In the CPu, beginning with the rostral pole, 12 positions were defined anatomically that, in the average gerbil brain, are each approximately 250 μm distant from the preceding one. The last position thus defined corresponds to −1.3 mm from bregma in the rat brain [43]. In each of the 12 selected slices, the 5-HT fibre density was determined in four regions of the CPu, i.e. in the dorsomedial, dorsolateral, ventrolateral and ventromedial corner (Fig. 1). Pictures were taken at a magnification of 200 × and a resolution of 768 × 580 square pixels. Fibres were detected by a similar routine as in the NAc and their area calculated as a percentage of the total image area.

In the amygdala, fibre densities were assessed in the central, lateral and basolateral part separately. The measurement in the central and lateral parts roughly followed the routine described for the CPu and NAc: exclusion of blood vessels from reference area, detection of fibres as local brightness minima, calculation of percentages. In the basolateral amygdala, however, serotoninergic fibres form a web that is too dense for the detection of single fibres. Therefore, the overall staining intensity was measured densitometrically as grey values ranging from 0 (white)

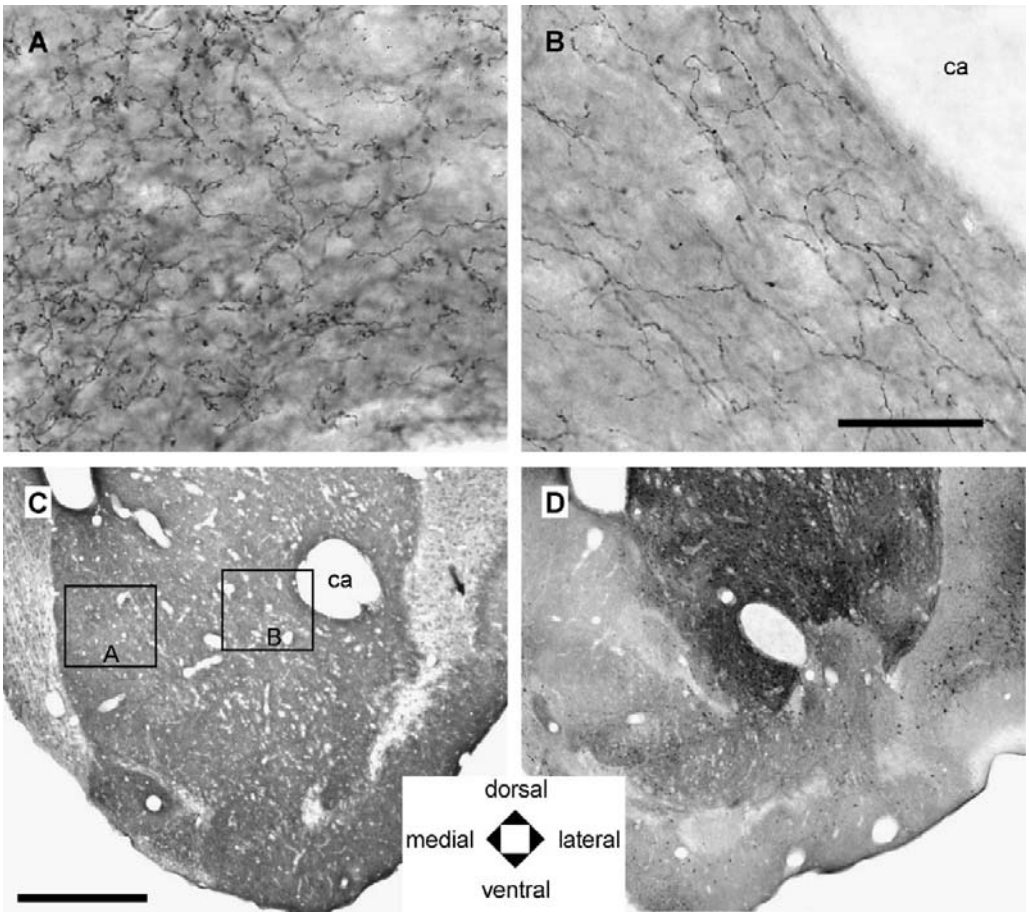

Fig. 2. 5-HT fibres in NAc core and shell. Representative pictures of the 5-HT innervation in the shell (A) and core (B) of the nucleus accumbens. Rectangles in (C) indicate the position of the photos. The delineation of core and shell is visible in (D) in a frontal slice immunostained for calbindin. CA, commissura anterior. Scale bars: 200 μm (A, B), 1000 μm (C, D).

to 255 (black). In order to compensate for variations in background staining, the acquisition brightness was automatically set to such a value that the background of the central amygdala (which contains especially few 5-HT fibres) maintained a fixed grey value in each slice.

2.4. Data analysis

Each sample usually consisted of 10 animals, but due to the imponderabilities of immunohistochemistry, fewer animals could be used for assessment in some areas. Mean values were calculated as arithmetic means ± S.D., and compared by *t*-test after a preceding *F*-test. For fibre densities in the CPu, the profiles along the rostrocaudal axis were analyzed by regression analysis and compared by bivariate analysis of variance (ANOVA) with repeated measurements.

3. Results

3.1. Serotoninergic fibre density in the nucleus accumbens

Immunocytochemistry with a serotonin antibody yields a specific and detailed staining in the gerbil brain, with fibres clearly visible upon a weak background (Figs. 2, 4 and 6). In the nucleus accumbens (NAc), a difference in the serotoninergic innervation between the core and shell subareas is easily visible. Fibres in the core are long, smooth, scarcely branched and possess few varicosities (Fig. 2B). In contrast, 5-HT fibres in the shell region appear shorter, frequently branched and carry many, often large varicosities (Fig. 2A). However, there is no detectable quantitative difference between the fibre densities in the two subareas.

In the comparison of each subarea among the groups, no difference can be found between the IR animals and the ER controls (Fig. 3). The early MA injection, however, renders a conspicuous effect: In both the ER and the IR group, MA intoxication led to significantly increased 5-HT fibre densities in the NAc core (12.5 and 18.5%, $P<0.05$). In the NAc shell, the 5-HT innervation was only denser by 14% ($P<0.05$) in ER-MA animals, with no effect in the IR group.

3.2. Serotoninergic fibre density in the caudate-putamen

The general pattern of 5-HT innervation in the caudate-putamen (CPu) of gerbils corresponds to the distribution in rats [36]. Even by qualitative microscopic inspection, it is apparent that the innervation density increases from the dorsal towards the ventral and from the lateral towards the medial parts of the CPu (Fig. 4). The rostrocaudal increase in fibre density that is well described in the literature [4], however, eludes detection.

The computer-aided analysis (Fig. 5) reveals that in the dorsomedial CPu, the 5-HT fibre density is higher by 26.5% in IR animals compared to ER animals ($P<0.01$). In the dorsolateral CPu, there is a difference of 20.7% ($P<0.1$). In the comparison of ER and IR animals, no difference in 5-HT innervation could be detected in the ventral quadrants of the CPu.

Infantile treatment with methamphetamine (MA) led to a higher 5-HT fibre density in all quadrants of the CPu in ER animals, with a significant hyperinnervation by 31.6% ($P<0.05$) in the dorsomedial CPu and by 34.6% ($P<0.01$) in the ventromedial CPu. The increase by 21.3% in the ventrolateral quadrant is only a trend ($P<0.1$). In contrast to the effects in ER animals, early MA application has no detectable effect in IR gerbils.

3.3. Serotoninergic fibre density in the amygdala

The serotoninergic innervation in the central amygdala is about the weakest in the whole brain (Fig. 6). The lateral amygdala contains notably more fibres, and the basolateral amygdala is so densely innervated that it can easily be recognized on the slides without the help of a lens. Among

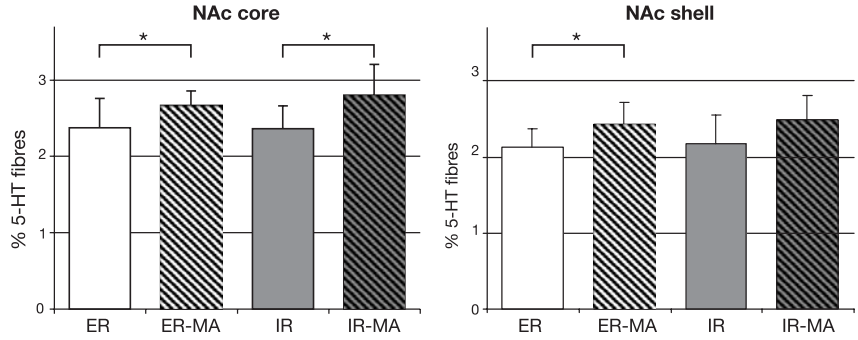

Fig. 3. 5-HT fibre densities in the NAc. 5-HT fibre density in the NAc core and shell compared among enriched-reared (ER) gerbils ($n=10$), methamphetamine-treated ER gerbils (ER-MA) ($n=12$), isolation-reared (IR) gerbils ($n=10$) and MA-treated IR gerbils (IR-MA) ($n=10$). In the NAc core, MA treatment increases the 5-HT fibre density significantly ($P<0.05$) in ER and IR animals, respectively. In the NAc shell, a significant increase ($P<0.05$) is seen in ER-MA animals compared to ER controls. Data are expressed as mean ± S.D.

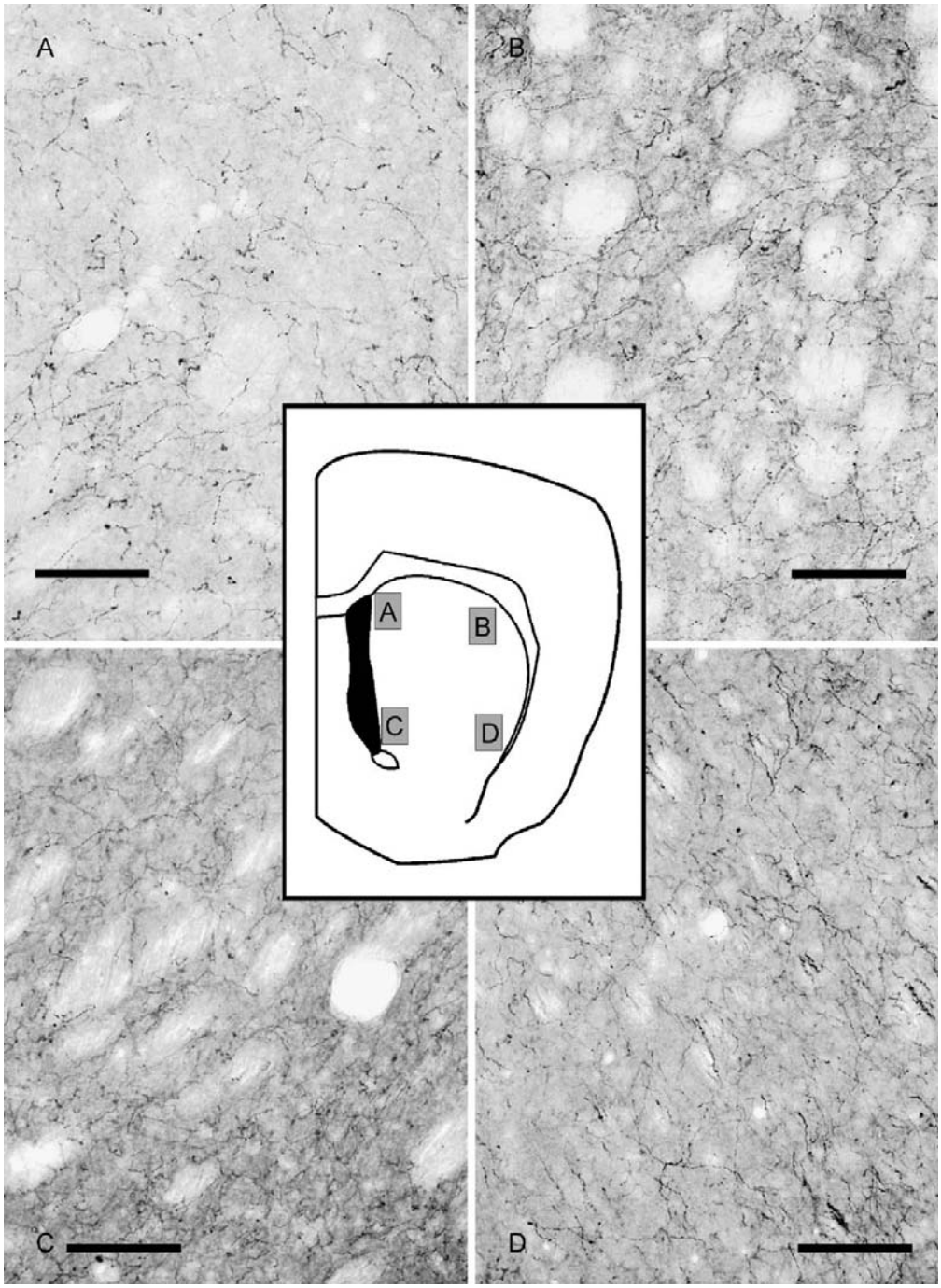

Fig. 4. 5-HT fibres in the CPu. Representative pictures of the 5-HT innervation in the dorsomedial (A), dorsolateral (B), ventromedial (C) and ventrolateral (D) measurement window within the CPu. The inset shows a schematic drawing of a frontal section through the gerbil brain, with the positions of (A–D) indicated as grey rectangles. Scale bar = 100 μm.

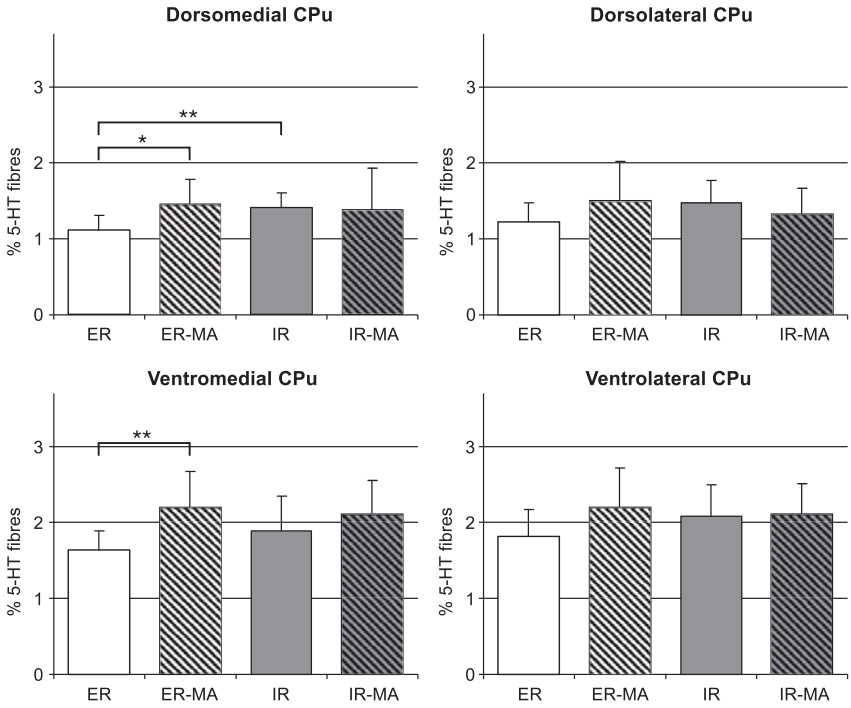

Fig. 5. 5-HT fibre densities in the CPu. 5-HT fibre density in the four measurement areas of the CPu compared between enriched-reared (ER) gerbils ($n=8$), methamphetamine-treated ER gerbils (ER-MA) ($n=10$), isolation-reared (IR) gerbils ($n=8$) and MA-treated IR gerbils (IR-MA) ($n=10$). In the dorsomedial CPu, ER-MA animals ($P<0.05$) and IR animals ($P<0.01$) have more 5-HT fibres than ER controls. In the dorsolateral quadrant, IR animals show a trend towards denser 5-HT innervation ($P<0.1$). In the ventromedial CPu, MA treatment significantly increases the 5-HT fibre density ($P<0.01$) in ER animals, in the ventrolateral CPu, a corresponding trend is seen ($P<0.1$). Data are expressed as mean ± S.D.

the groups (Fig. 7), IR, compared to ER, significantly increased the 5-HT fibre density by 30.5% ($P<0.01$) in the central and 10.7% ($P<0.05$) in the basolateral amygdala. No effect is observed in the lateral amygdala. MA treatment shows no effect on the 5-HT innervation in any part of the amygdala.

4. Discussion

In this study, we demonstrate that the 5-HT fibre density in subcortical brain nuclei, i.e. the caudate-putamen (CPu), nucleus accumbens (NAc) and amygdala, of adult gerbils is influenced by isolation rearing (IR) and by a single early challenge with methamphetamine (MA). In detail, IR increased the 5-HT fibre density in the dorsomedial and, as a trend, the dorsolateral parts of the CPu, leaving the ventral parts unaffected, and it led to a denser projection in the central and basolateral parts of the amygdala. There was no effect in the NAc. MA treatment, in turn, resulted in an enhanced 5-HT innervation of the medial CPu in ER animals, of the NAc core in both rearing conditions and the NAc shell in ER animals, showing no effect in the amygdala.

Thus, the regions in which 5-HT fibres are affected by IR or by early MA intoxication are almost totally complementary to each other. In none of the studied areas except for the dorsomedial CPu does the 5-HT projection react to both treatments, and the lateral amygdala is the only area that shows no effect of either challenge. Obviously, there are at least two independent mechanisms mediating the effects of IR and MA on 5-HT fibre outgrowth.

4.1. Isolation rearing shifts the balance between 5-HT and DA

In a previous study, we showed that IR reduces the DA fibre density of the CPu in gerbils [30]. The sprouting of 5-HT fibres in the same condition resembles a similar shift between dopamine (DA) and 5-HT in the prefrontal cortex after both maternal separation and IR or IR alone [5,37,39,65]. As this effect in the CPu is limited to its dorsomedial and dorsolateral parts, which receive only relatively sparse 5-HT innervation in the normal rodent (Figs. 4 and 5 [4,35]), this resembles a similar pattern observed both after neonatal 6-hydroxydopamine (6-OHDA) lesion and in the DA deficient CPu of the weaver mutant mouse [12,28,45,56]. The dynamic balance of 5-HT

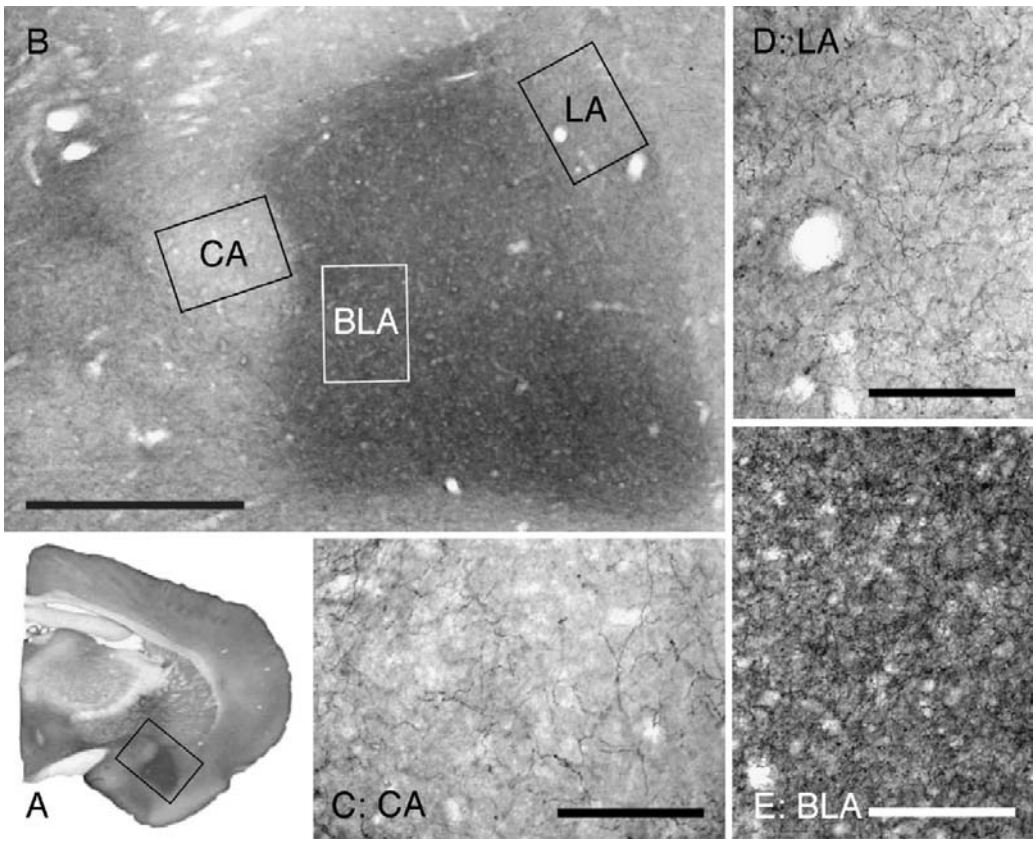

Fig. 6. 5-HT fibres in the amygdala. Representative pictures of the 5-HT innervation of the amygdala in a coronal slice through the gerbil brain (A). The rectangle in (A) indicates the position of (B). (B) gives an overview of the amygdaloid complex comprising the central (CA, magnified in C), basolateral (BLA, D) and lateral (LA, E) amygdala. Scale bars: 500 μm (B), 100 μm (C, D, E).

and DA in the striatum is based on close anatomical and physiological interaction, with DA tonically inhibiting 5-HT activity [13,26,53]. If a lower DA fibre density results in reduced tonical transmission [14], the disinhibited 5-HT fibres could promote their own outgrowth by enticing the emission of the growth factor S-100beta from the astroglia [3,63]. A similar mechanism may be the reason for the denser 5-HT innervation in the central and basolateral amygdala of IR gerbils compared to ER animals. While an immunohistochemical assessment of DA fibres in the amygdala in our laboratory is still under way, Heidbreder and co-workers [19] have demonstrated in a recent study that the DAergic phasic transmission is enhanced in the amygdala of IR rodents. Higher phasic DA transmission can be a consequence of reduced tonic transmission [14] and itself activates 5-HT transmission [34].

4.2. MA intoxication leads to heterotypic 5-HT sprouting

Although MA affects both 5-HT and DA and, in toxic doses, can destroy nerve endings of both transmitters [7,46], its toxic effects depend, among other factors, on the age of the experimental animals [52,58,60]. Given at the age of 14 days to rodents, it selectively damages DA terminals, leaving 5-HT fibres intact [58,62]. Like 6-OHDA or even ibotenic acid lesions (which do not affect aminergic fibres directly), this induces heterotypic sprouting of 5-HT fibres [45,69]. This becomes most clear in the NAc, where in a recent study we demonstrated that MA intoxication exerts an effect on the accumbal DA projection which is exactly complementary to the result shown in this study [38]: The DA fibre density is drastically diminished in both subareas in ER animals, but only in the NAc core in IR gerbils. Similarly, in the CPu, experimental neonatal lesion to the DA innervation by 6-OHDA causes 5-HT fibres to hyperinnervate the area to an extent that reaches twice or three times the density of the normal innervation [12,28,45]. As we did not yet investigate the effect of early MA intoxication on the DA fibres of the CPu, the reasons for the limitation of the 5-HT hyperinnervation to medial regions and ER animals must remain speculative. For the latter, it cannot be excluded that events

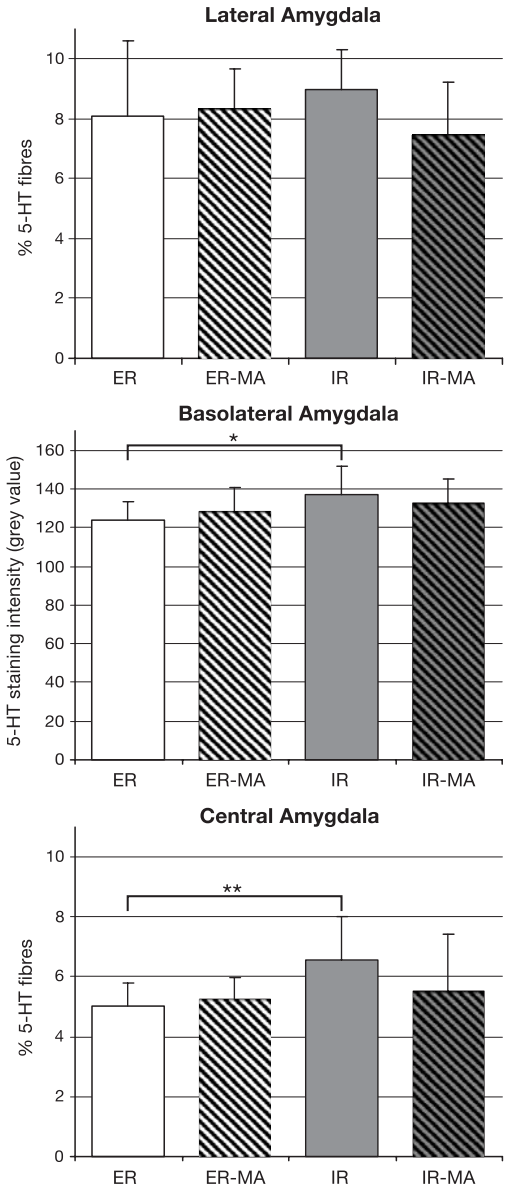

Fig. 7. 5-HT fibre densities in the amygdala. 5-HT fibre density in the amygdaloid complex compared among enriched-reared (ER) gerbils ($n=10$), methamphetamine-treated ER gerbils (ER-MA) ($n=17$), isolation-reared (IR) gerbils ($n=10$) and MA-treated IR gerbils (IR-MA) ($n=9$). IR animals have a significantly denser 5-HT innervation in the BLA ($P<0.05$) and CA ($P<0.01$). Data are expressed as mean ± S.D.

at fetal age and early childhood exert an early influence on the maturation and vulnerability of DA fibres. Lastly, from what was said above, the lack of an MA effect on the 5-HT innervation of the amygdala can easily be tracked back to the lack of DA transporter—which mediates MA toxicity—on DA fibres in the amygdala (own unpublished observations and Ref. [15]).

4.3. Developmental aspects

An additional developmental aspect should not be neglected. The time-courses of serotoninergic maturation differ markedly between the amygdala and the striatum: Whereas the 5-HT innervation of the amygdala resembles the adult situation by postnatal day 3, fibres only just start to ramify within the CPu and NAc in the third postnatal week in rodents [32]. A pharmacological insult at this age would therefore meet with a presumably stable neuroarchitecture in the amygdala, but with a dynamic, developing situation in the striatum, which would be more easily subject to change. In contrast, the influence of rearing conditions on the brain is a diffuse and chronic one, leading to alterations in cholinergic, dopaminergic, serotoninergic and noradrenergic function in various regions of the brain [23,30,40,47,50,65]. Thus, there is a multidimensional, complex interaction in time among numerous factors resulting in the brain abnormalities of adult IR animals. By such systemic mechanism, the chronic deprivation in IR can induce shifts of transmitter balances even in areas that are relatively invulnerable to single pharmacological challenges.

4.4. Functional consequences

Functionally, the typical way in which 5-HT neurones respond to hyperinnervation is by reducing their turnover [24]. Consequently, whereas the total 5-HT concentration in the brain is not changed by IR [23,27,40], the turnover is reduced in the NAc [23], the lateral hypothalamus [27] or even in the whole brain [61]. Depending on the brain area concerned, 5-HT hypoactivity has a variety of behavioural consequences. In the first place, it is considered the cause for hyperaggression in IR animals [61,64,66]. This effect may be mediated primarily in the amygdala, where 5-HT inhibits amygdala projection neurons directly and indirectly [8,44]. Second, in the motor areas of the brain, including the CPu, 5-HT is said to keep impulsivity in check [22,54], and indeed dorsal raphe-lesioned rats show increased impulsivity in a learning task [18]. A reduced 5-HT function in the CPu based on hyperinnervation might therefore contribute to the motor disturbances of IR and MA-treated rodents [28,68]. This suggestion is in line with the observation that 5-HT transmission is affected in most movement disorders [51]. In the NAc, finally, 5-HT transmission has been linked to reward [48] and the consumption of ethanol, sucrose and saccharin, which is increased in IR rats [16].

4.5. Conclusion

Since 5-HT and its interactions with DA have recently received increased attention in schizophrenia research [25,33], and IR has been repeatedly proposed as an animal

model of psychosis [23,42,55], the present results should be viewed as contributions to the scientific study of psychotic processes.

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Eidesstattliche Erklärung

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

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(Jörg Lesting)