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

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

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

Introduction

Although there is little doubt on the idea that a so-called "dysconnection" of the prefrontal cortex (PFC) from temporolimbic cortical fields and subcortical areas is a central anatomical correlate of schizophrenia (Feinberg, 1982/83; Weinberger and Lipska, 1995), the exact nature of this "dysconnection" is remarkably unclear. Evidence so far suggests that a disturbance during cortex development creates an aberrant layering in both prefrontal (PFC) and entorhinal (EC) cortices (Jakob and Beckmann, 1986; Benes et al., 1991; Akbarian et al., 1993a, b), which is accompanied by altered dendritic morphology of PFC pyramidal cells and a reduction in somatic inhibition by a subclass of GABAergic neurons (Glantz and Lewis, 2000; Kalus et al., 2000; Black et al., 2004). It is assumed that

these cytoarchitectural abnormalities result in a faulty, ''noisy'' miswiring of prefrontal-temporolimbic connections (Weinberger and Lipska, 1995), providing the vulnerability for the later outbreak of a psychotic illness. If further harmful events during or after adolescence then impair the complete maturation of the PFC's dopamine (DA) innervation (Akil et al., 1999), the resulting imbalances in PFC microcircuits unmask the pathology of the PFC-EC macrocircuits, which then becomes apparent in psychotic symptoms (Winterer and Weinberger, 2004). The central assumption in this compelling picture, i.e. the aberrant connectivity of PFC efferents, has so far only been inferred from analogy to metachromatic leukodystrophy (Hyde et al., 1992), and has been supported by both functional and diffusion tensor imaging (Meyer-Lindenberg et al., 2002; Hubl et al., 2004; Wang et al., 2004). Methodological limitations to date prevent the clarification of how exactly PFC pyramidal axons are miswired in schizophrenic patients.

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

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

Materials and methods

Animals and rearing conditions

Male gerbils were bred in our facilities either in standard cages or in semi-naturally structured compounds (for details, see Winterfeld et al., 1998). On postnatal

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

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

Biocytin fibre tracing

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

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

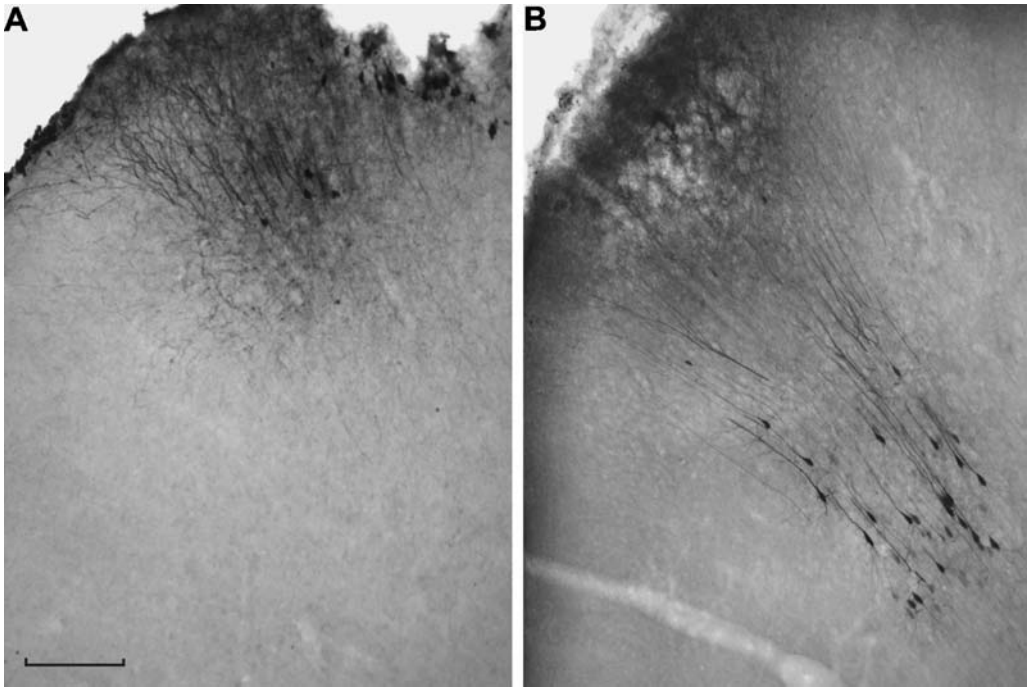


Fig. 1. Prefrontal pyramidal cells filled by an injection of biocytin into the superficial (**A**) or deep (**B**) layers of the medial prefrontal cortex (Cg1/Fr2-region). Scale bar is 200 µm

washes in PB, the sections were mounted on coated glass slides, dried overnight and coverslipped in DePeX.

Qualitative evaluation of projection patterns

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

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

Computerised assessment of terminal field densities

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

The sections were viewed in dark field at 125× magnification on a microscope (Polyvar, Reichert-Jung). Pictures were taken by a digital camera (ProgRes 3008 mF, Jenoptik, Jena) and processed by a software for image analysis (KS300, Zeiss, Jenoptik, Jena). Three separate pictures were taken of superficial, middle and deep laminae, converted to black-and-white pictures and inverted. Background was suppressed by high-pass filtering. Fibres were recognised by a function (''valleys operator'') that detects local brightness minima and represents them as lines of one pixel width. The area of these lines was calculated as percentage of the measurement window, which was the same size for all laminae. In FC and PC, laminae I, II, V and VI were evaluated separately. In IC, all six cortical laminae were investigated.

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

Statistical analysis

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

Results

Qualitative analysis

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

The non-parametric comparison of striatal projections was only done for MA-intoxicated and control IR animals out of technical reasons. Both the Kolmogorov-Smirnov and the Wilcoxon-test confirmed that projections from superficial laminae were significantly

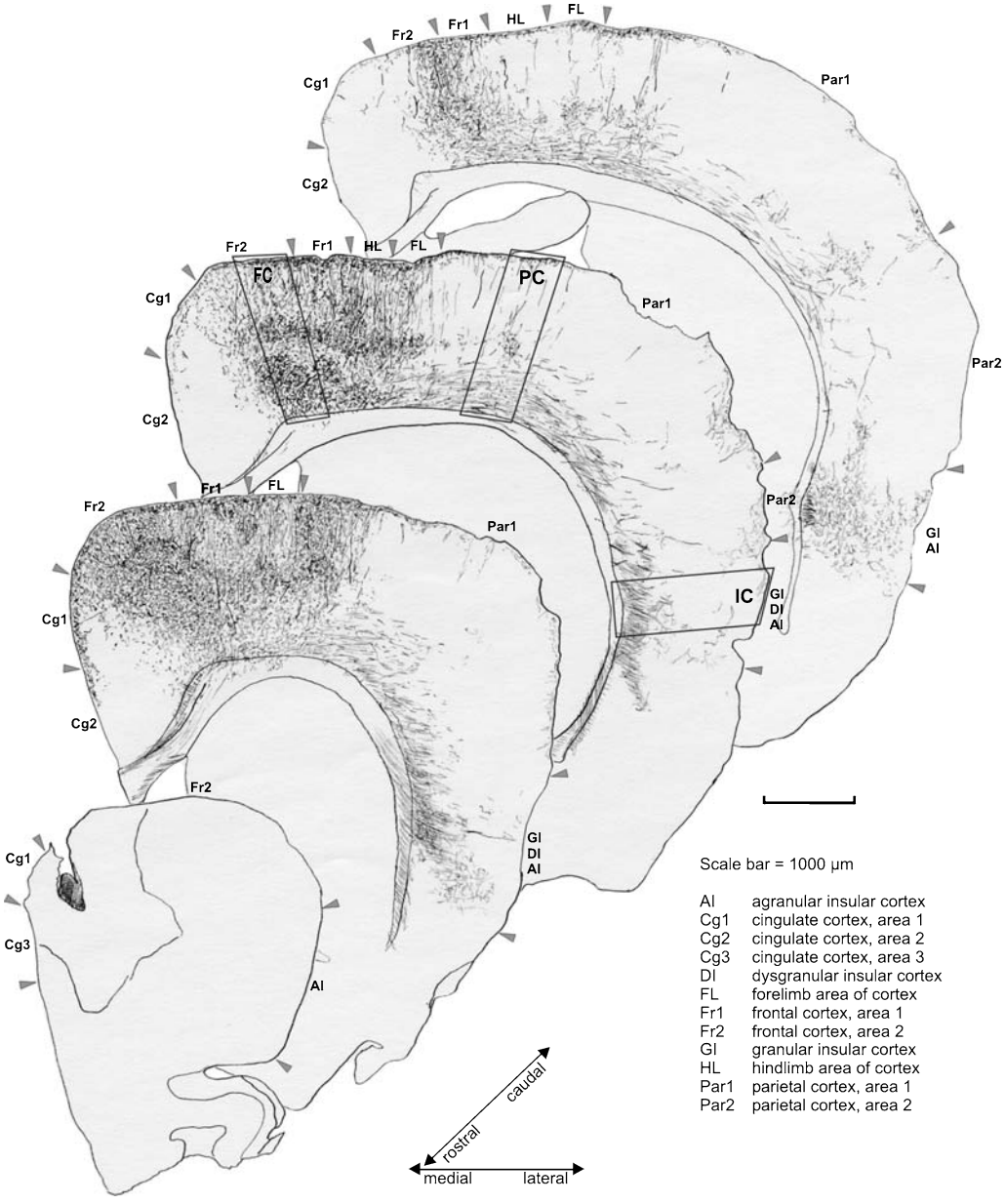


Fig. 2. Camera lucida drawings of selected frontal slices through an examplary gerbil brain with efferents of the medial prefrontal cortex (Cg1/Fr2-region) stained by a deep biocytin injection. The most rostral section indicates the position and depth of the injection. The boxes in the third section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

($p < 0.05$) weaker in MA-treated than in saline-treated IR animals (data not shown).

Quantitative analysis

In the frontal cortex (FC, Fig. 3), ANOVA did not show any effects of MA treatment or rearing on the projection densities from either superficial or deep injections. Post-hoc testing, however, revealed a significant reduced projection from shallow injections to layer VI by IR in saline-treated animals, and a large number of effects in the comparison of deep injections: In saline-treated animals,

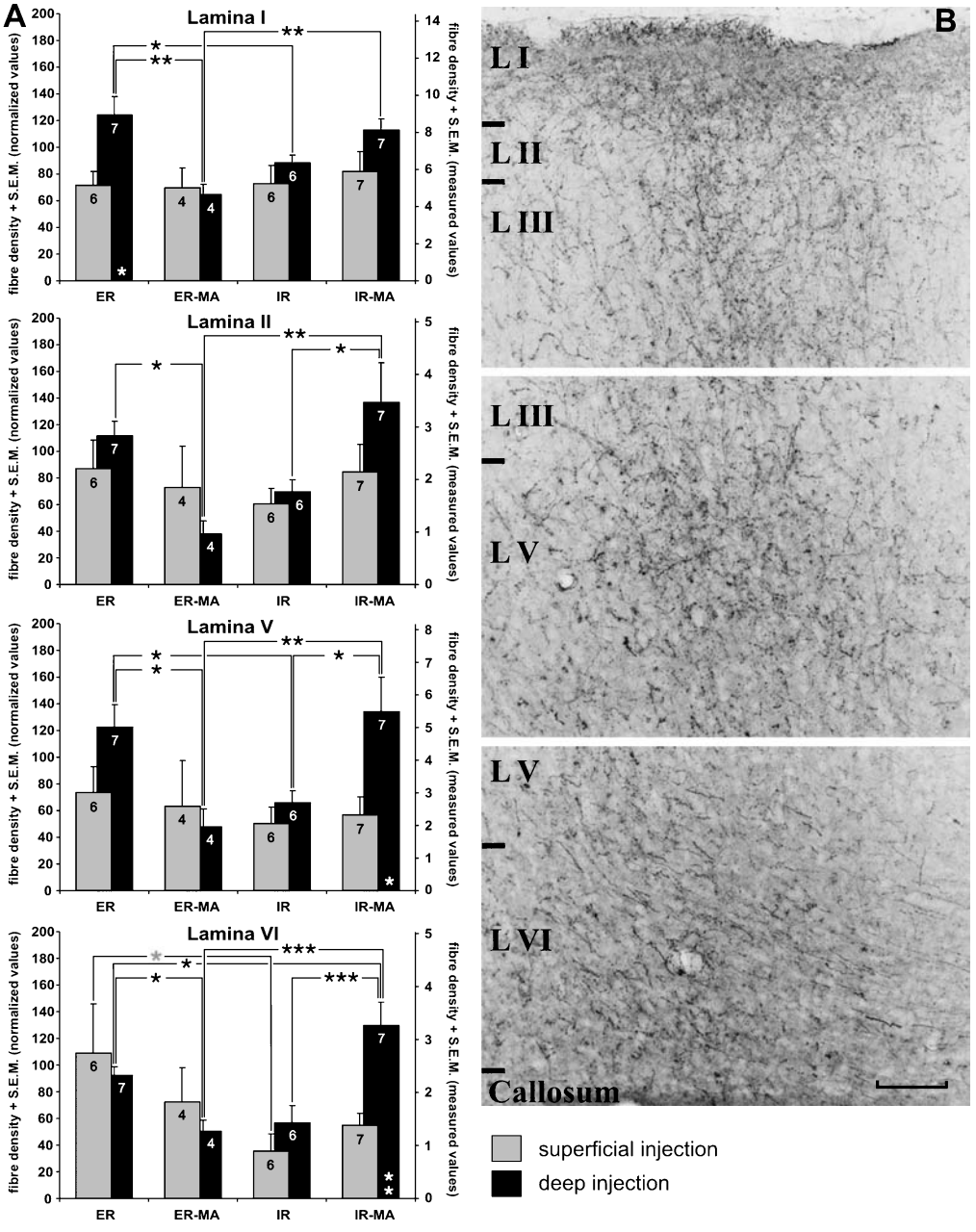


Fig. 3. **A** Densities of terminal fields and passing fibres in the frontal cortex stained by biocytin injections into superficial (grey columns and asterisks) or deep (black columns and asterisks) layers of the medial prefrontal cortex. The left ordinate gives normalized values (mean of deep and shallow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

IR reduced the terminal density in layers I and V ($p = 0.057$ for layer VI), whereas IR effected an increase in MA-treated gerbils in all layers. Within the ER groups, MA-intoxicated animals had markedly reduced terminal densities in all layers, whereas in the IR

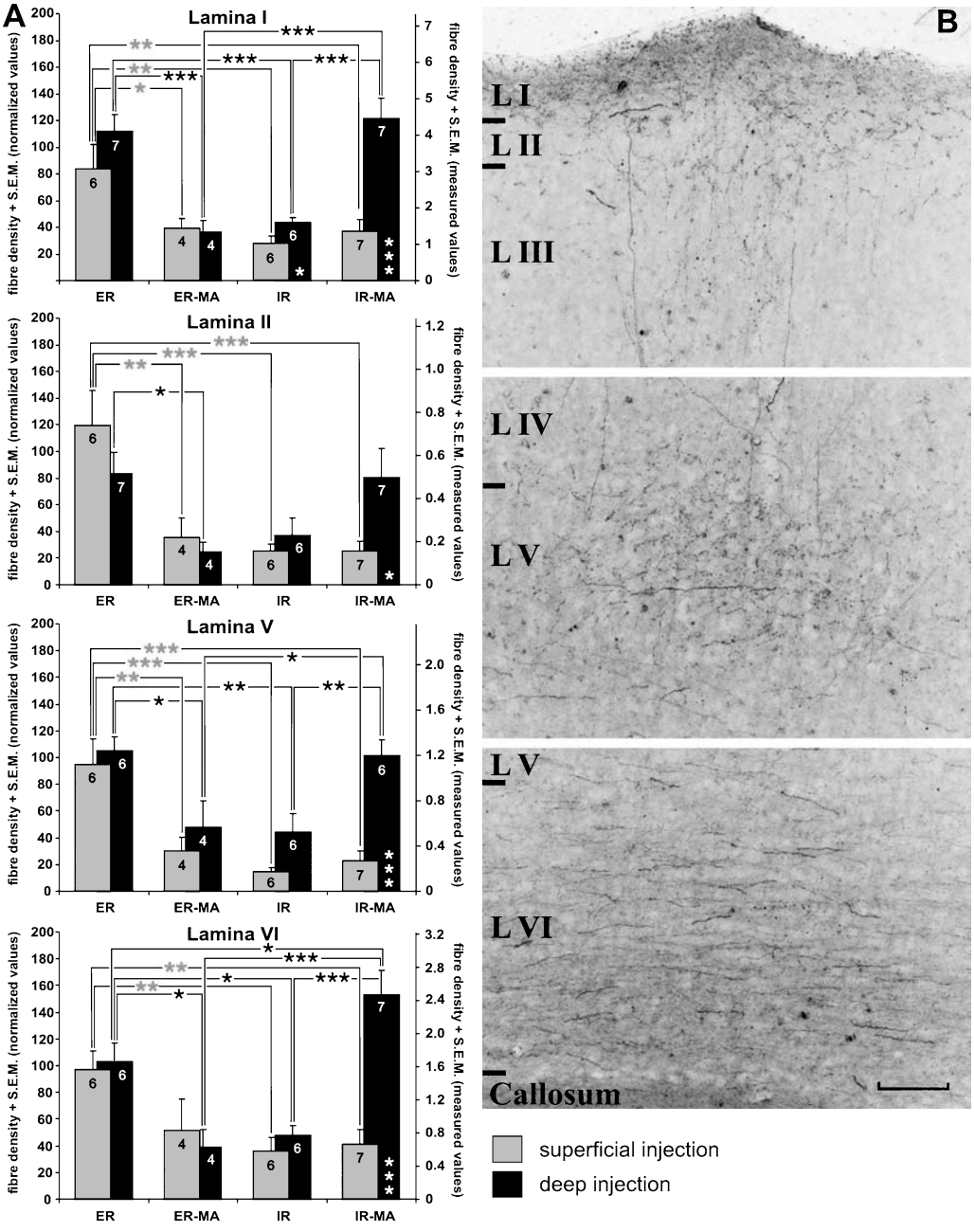
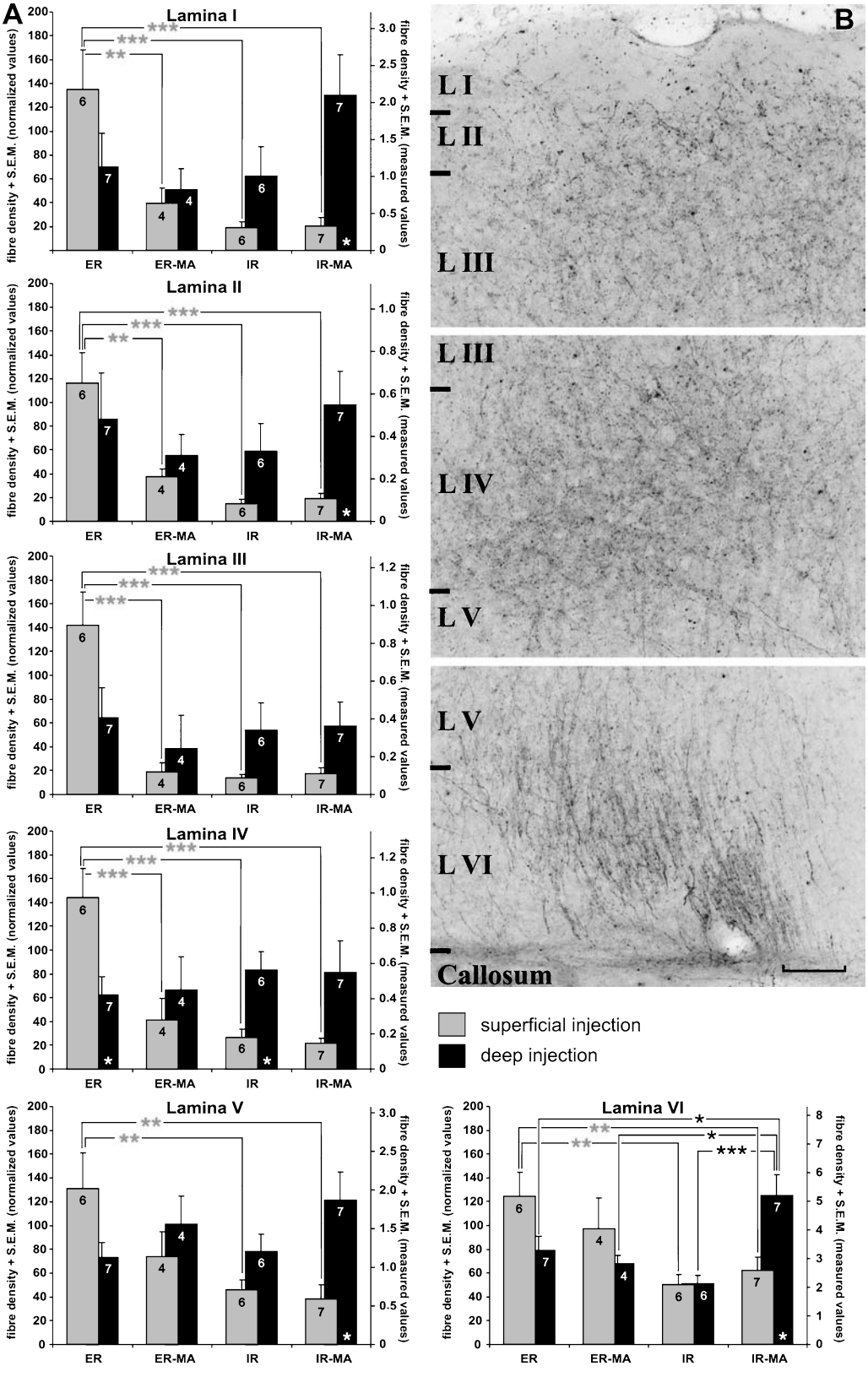


Fig. 4. **A** Densities of terminal fields and passing fibres in the parietal cortex stained by biocytin injections into superficial (grey columns and asterisks) or deep (black columns and asterisks) layers of the medial prefrontal cortex. The left ordinate gives normalized values (mean of deep and shallow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


A
Lamina I
Lamina II
Lamina III
Lamina IV
Lamina V
Lamina VI
fibre density + S.E.M. (normalized values)
fibre density + S.E.M. (measured values)
ER
ER-MA
IR
IR-MA
B
L I
L II
L III
L IV
L V
L VI
Callosum
superficial injection
deep injection

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

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

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

Discussion

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

Dysfunctional reorganisation of prefrontocortical efferents

Obviously, the effects of rearing environment on prefrontal cortex (PFC) efferents differ from and depend on the effects of an early MA intoxication. Only in saline-injected

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

animals, the terminal outgrowth of associative fibres to distal areas (PC, IC) from superficial laminae was enhanced by ER – which is complementary to the finding of higher dendritic branching, spine density and synapse area in occipital fields of ER rodents as compared to IR animals (Greenough and Volkmar, 1973; Globus et al., 1973; Møllgaard et al., 1971). The lack of a rearing effect on fibres projecting to the FC is also in line with the failure to detect higher dendritic branching in this region (Greenough et al., 1973). It seems that MA intoxication at PD 14 creates a PFC maladaptation that does not allow layer III-pyramidal cells to benefit from environmental stimulation.

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

"Dysconnection" of micro- and macrocircuits

It now seems that puberty, which is also the period during which IR exerts its most devastating effects (Einon and Morgan, 1977), is the critical phase for the establishment of prefrontal control over the dopaminergic system (Weinberger and Lipska, 1995). This control is exerted in the nucleus accumbens (Grace, 1993) as well as the amygdala (Rosenkranz and Grace, 2002) and keeps the dopaminergic fibres from firing when an informed representation of the situation declares rapid action unnecessary. In a reciprocal way, the PFC itself is dependent on a proper maturation of its dopaminergic afferents, such that a reduced DA activity or fibre density leads to impairments in working memory (Dawirs et al., 1996; Winterfeld et al., 1998) and to the outbreak of psychotic symptoms (Weinberger, 1987; Winterer and Weinberger, 2004). Our previous research has shown that the lack of DA is compensated by a sprouting of GABAergic synapses (Nossoll et al., 1997) and an addition of dendritic spines on pyramidal cells (Blaesing et al., 2001). In this way, dysfunctional microcircuits are formed in the PFC, which then radiate their detrimental influence through macrocircuits to the whole brain (Teuchert-Noodt, 2000; Winterer and Weinberger, 2004).

Again, the maladaptation of macrocircuits thus caused becomes first apparent in the DA projections: Fibres to caudal cortical fields (i.e. entorhinal cortex and amygdala), which go through an overgrowth during their early development, are not pruned in IR and/or MA-intoxicated gerbils (Busche et al., 2004). It is likely that this aberrant maturation is a consequence of the disturbed interplay of prefrontal and caudal ''limbic'' areas. The dopaminergic imbalance, in turn, affects

cholinergic and serotoninergic fibre systems in these areas (Neddens et al., 2003, 2004; Lehmann et al., 2004). Eventually, caudal and subcortical areas can be considered to work out of sync with the PFC, thus manifesting a "dysconnection" not only in the literal sense – as shown in the present results –, but also in a wider sense.

Implications for PFC function and activity

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

"Dysconnection" and the glutamate hypothesis of schizophrenia

In human schizophrenics, a disruption of the natural process of cortical layering occurring during the second trimester of pregnancy is usually assumed to produce "dysconnection", speculated not to consist in a separation, a "*dis*connection", of fibre tracts, but in aberrant layering and connectivity (Jakob and Beckmann, 1986; Weinberger and Lipska, 1995; Kalus et al., 1999). This notion has received support from a number of recent DTI studies which have detected reduced fractional anisotropy in the white matter of schizophrenics, meaning that fibres are less clearly directed than in healthy controls (Buchsbaum et al., 1998; Hubl et al., 2004; Wang et al., 2004). It seems conceivable that random connections are kept that should have been pruned during development (Changeux and Danchin, 1976).

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

The shift of projection patterns we observe in the present study is more complex than a simple reduction of transmission. It seems plausible, however, that improper wiring of connections impairs their activity. Alternatively, only the projections from

superficial layers may be those relevant for schizophrenia in the human brain, since they are the ones that form ipsilateral intrinsic and associative cortical connections (Melchitzky et al., 1998), whereas infragranular pyramidal cells preferentially project to contralateral and subcortical targets (Jones et al., 1975). These associative projections to the parietal and insular cortices are significantly reduced in gerbils by IR and MA intoxication, such that, physiologically, a lack of glutamatergic activation in their target fields might ensue.

Conclusion

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

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