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Pharmacologically induced neural plasticity in the prefrontal cortex of adult gerbils (*Meriones unguiculatus*)

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Abstract

Using a selective antibody serum against glutaraldehyde-conjugated γ-aminobutyric acid (GABA), GABAergic neurons were identified in the medial prefrontal cortex of young adult gerbils (*Meriones unguiculatus*) following a single non-invasive dose of methamphetamine (25 mg/kg i.p.) applied at the age of 90 days. GABA-immunoreactive profiles were electron microscopically counted in a defined test field (0.875 mm^2) covering the prefrontal prelimbic area after a single dose of either methamphetamine or saline. Within 30 days following the drug challenge the density of GABAergic innervation significantly increased by about 20%. Several lines of previous investigation indicate that a single dose of methamphetamine is an appropriate stimulus to cause selective autotoxic destruction of certain prefrontal dopamine fibres due to drug-induced hyperactivation. There is further indication of postsynaptic and transneuronal neuroplasticity since the densities of dendritic spines on prefrontal pyramidal cells went through a significant sequence of post-drug gain and loss. These structural dynamics resemble typical alterations seen after classical mechanical or chemical lesioning in other regions of the brain. The present results on drug-induced reactive neuroplasticity are discussed together with the current understanding of stimulus-induced adaptive reorganization in the mammalian central nervous system. © 1997 Elsevier Science B.V.

Keywords: Methamphetamine; Cortex, prefrontal; Synaptogenesis, reactive; GABA (γ-aminobutyric acid), innervation; (Gerbil)

1. Introduction

In the nervous system synaptic plasticity is most probably an ongoing natural process (Cotman and Nieto-Sampedro, 1984; Hatton, 1985; Frank, 1987; Represa et al., 1989). In this concept, it appears that a continuous remodelling of synaptic contacts and neuronal circuits is an inherent property of the developing and mature brain, the biological significance of which seems to be to ascertain adaptive interaction between the brain and environment (Jones, 1988; Petit, 1988; Mattson, 1988; Vaughn, 1989; Mattson and Kater, 1989; Calverley and Jones, 1990; Teuchert-Noodt et al., 1991; Dawirs et al., 1992; Teuchert-Noodt and Dawirs, 1996). Against this background we are especially interested in the development and function of the mammalian prefrontal cortex including the mesoprefrontal dopamine system which is a necessary prerequisite for normal functioning of the prefrontal cortex (Simon and Le Moal, 1984; Stam et al., 1989; Dawirs et al., 1993a,b, 1994).

Recently, we have introduced a non-invasive pharmacological model for stress-induced reactive synaptogenesis in juvenile and adult gerbils. A single dose of methamphetamine (i.p.) was found to be a stimulus strong enough to initiate synaptogenesis selectively in the prefrontal cortex, most probably brought about by the drug-induced autotoxic destruction of specific subpopulations of dopaminergic afferent fibres (Teuchert-Noodt and Dawirs, 1991; Dawirs et al., 1991, 1993a, 1994). The question remains whether a single pharmacological impact by methamphetamine, which results both in presynaptic dopaminergic deafferentation and transient proliferation of postsynaptic contact sites, may serve as a trigger of functional changes by means of compensatory neuroplasticity. There is an indication from previous studies that a single methamphetamine challenge might result in altered properties of a selectively reorganized afferent fibre spectrum of prefrontal pyramidal cells (Dawirs et al., 1993a). Further, several lines of investigation indicate that methamphetamine-induced loss of dopamine fibres is permanent in the prefrontal cortex (e.g. Seiden and Ricaurte, 1987;

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Dawirs et al., 1993a). Therefore, other local transmitter systems might compensate for the lost dopaminergic inhibition by substitution in order to re-establish the transiently lost input-output balance of the deafferentated prefrontal pyramidal cells.

Since GABAergic interneurons seem to be significantly important in lesion-induced synaptic reorganization of the hippocampus (Goldowitz et al., 1982; Davenport et al., 1990), in the present study we investigated whether a single dose of methamphetamine applied to young adult gerbils results in compensatory sprouting of prefrontal GABAergic fibre profiles. For that purpose, we examined GABAergic fibre profiles in the medial prefrontal cortex using a specific antibody against glutaraldehyde-conjugated GABA. GABA-immunoreactive fibre profiles were electron-microscopically detected and quantified in adult gerbils 30 days following either a single dose of methamphetamine or saline. The present results on drug-induced synaptogenesis are discussed with respect to the principal mechanisms of structural adaptation of neuronal circuits to normal or aberrant stimuli.

2. Materials and methods

2.1. Preparation of tissue

Male gerbils (*Meriones unguiculatus*) were kept under natural day/night cycles in home cages (30 × 40 cm) with 2 animals each. Food and water were provided ad libitum. At the age of postnatal day 90, 6 gerbils received a single dose of methamphetamine (25 mg/kg i.p.). For this, appropriate amounts of methamphetamine hydrochloride (Sigma, Deisenhofen, Germany) were diluted in 0.5 ml saline immediately before injection. Six control animals received equivalent volumes of saline. The animals were subsequently housed individually. At the age of postnatal day 120, the gerbils were transcardically perfused under deep chloralhydrate anaesthesia (1.7 g/kg i.p.). The blood was washed out with approximately 60 ml cold oxygen-enriched 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 7.6). Finally, the fixative was quickly washed out with 0.05 M Tris buffered saline containing 1% sodium metabisulfite (pH 7.5; wash buffer). Immediately after perfusion, the brains were dissected and 40-μm thick frontal sections performed with a vibratome (Model G, Campden Instruments, London, UK) which were collected in wash buffer at 4°C.

2.2. Immunocytochemical procedure

The peroxidase-antiperoxidase (PAP) method (Sternberger, 1979) was the chosen immunocytochemical procedure which was performed free-floating using the following incubations: (1) pre-incubation in 10% normal goat serum (Sigma) in wash buffer (0.5 h, 4°C) to reduce non-specific background staining; (2) rabbit antiserum against glutaraldehyde-conjugated γ-aminobutyric acid (GABA) (Incstar, Stillwater, MN, USA) diluted 1:5000 in wash buffer containing 1% normal goat serum (40 h, 4°C). This highly specific and sensitive antibody against GABA was first obtained by Storm-Mathisen et al. (1983) and characterized by Seguela et al. (1984). Specificity controls included radioimmunological assays and incubations with amino-acid-covered Sepharose beads to exclude cross reaction; (3) three 10-min washes in wash buffer; (4) goat anti rabbit serum (Sigma) diluted 1:50 in Tris-buffered saline containing 1% normal goat serum (1 h, 37°C); (5) three 10-min washes; (6) peroxidase-antiperoxidase (Sigma) in the same dilution buffer as for goat anti rabbit serum, 1:500 (1 h, 37°C); (7) three 10-min washes; (8) 0.05% 3,3-diaminobenzidine (Sigma), and 0.01% H_2O_2 in Tris-buffered saline (15 min, room temperature); (9) three 10-min washes; (10) for light microscopy, sections were dried on glass slides overnight, dehydrated, mounted in DePeX (Serva; Heidelberg, Germany) and coverslipped; (11) for electron microscopy, sections were incubated in 2% OsO_4 (1 h, room temperature) after the three 10-min

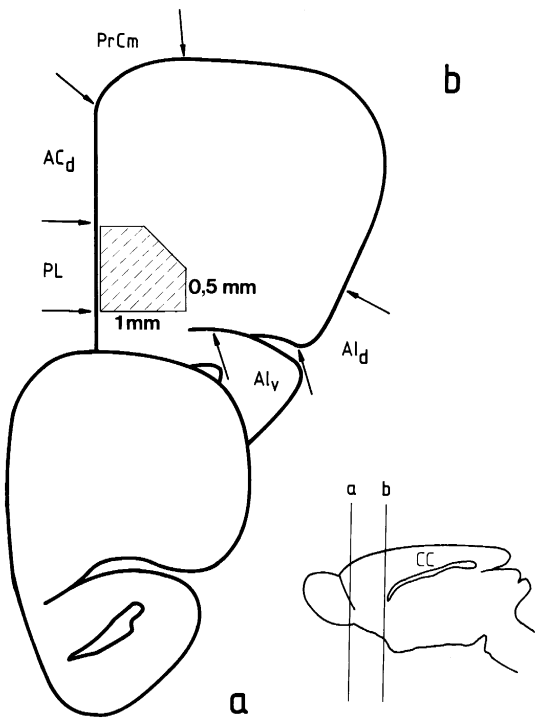

Fig. 1. That part of the prelimbic area (PL) investigated is shown between frontal section planes (a) and (b); medial precentral cortex (PrCm); dorsal anterior cingulate cortex (AC_d); ventral and dorsal agranular insular cortex (AI_v, AI_d); corpus callosum (CC); striped field in (b) mark location and size of ultra-thin cut parts.

Table 1
Number of GABA-immunoreactive profiles in the prelimbic area of 5 adult gerbils (S1–S5) 30 days after a single dose of saline

#	Saline									
	S1		S2		S3		S4		S5	
	$\bar{x} \pm$ S.D.	n	$\bar{x} \pm$ S.D.	n	$\bar{x} \pm$ S.D.	n	$\bar{x} \pm$ S.D.	n	$\bar{x} \pm$ S.D.	n
1	56.6 ± 7.6	40	43.0 ± 8.2	47	60.8 ± 10.4	37	97.6 ± 9.1	30	45.3 ± 8.3	44
2	42.5 ± 8.1	43	102 ± 10.1	43	58.7 ± 10.2	39	58.6 ± 10.4	38	45.4 ± 6.8	36
3	51.8 ± 9.4	46	95.8 ± 9.7	41	61.2 ± 8.4	43	52.0 ± 8.8	39	79.6 ± 8.5	41
4	80.0 ± 12.2	45	44.0 ± 8.6	44	59.0 ± 9.3	32	102.4 ± 9.6	42	73.9 ± 8.4	37
5	100.2 ± 13.2	35	98.1 ± 10.6	36	86.5 ± 6.9	40	110.2 ± 10.2	32	75.5 ± 7.2	38
6	88.9 ± 9.6	42	96.3 ± 9.7	39	46.7 ± 8.4	33	62.6 ± 10.0	45	46.5 ± 6.5	40
7	97.7 ± 11.6	41			68.2 ± 7.5	41	59.6 ± 8.3	38	82.5 ± 7.4	33
8	61.1 ± 9.3	35			81.7 ± 10.1	44	67.8 ± 8.0	40		
9	63 ± 11.2	38			81.6 ± 13.0	36	105.2 ± 9.1	31		
10							58.9 ± 7.2	35		
11							46.6 ± 7.0	32		
12							51.7 ± 8.1	37		
$\bar{x}_1 \pm$ S.D.	71.3 ± 21.0		79.9 ± 28.3		67.2 ± 13.4		72.8 ± 23.8		64.1 ± 17.4	
$\bar{x}_2 \pm$ S.D.					71.0 ± 6.0					

Mean number ($\bar{x}$) ± standard deviation (S.D.) is given per square hole of the copper grids covered by ultra-thin sections of the prelimbic area trimmed down to test fields of 0.875 mm^2; entire test fields were evaluated; number of sections (test fields) per individual (#); number of square holes which could be evaluated in a single test field (n); mean number in single test fields ($\bar{x} \pm$ S.D.); mean number in individuals ($\bar{x}_1 \pm$ S.D.); mean number in saline-treated controls ($\bar{x}_2 \pm$ S.D.).

washes (9); (12) the slices were finally washed, dehydrated, and flat-embedded in Epon (Serva).

Control sections were treated as described above, but the primary antibody was omitted or pretreated with GABA conjugated to bovine serum albumin with glutaraldehyde, the same antigen used to generate the antibody. No fibres and no cell somata or any other structure were immunolabelled in the prefrontal cortex or elsewhere in the brain either.

2.3. Quantification of GABAergic innervation

Only right hemispheres were evaluated since it cannot be excluded so far that primarily affected dopamine inner-

Table 2
Number of GABA-immunoreactive fibre profiles in the prelimbic area of 6 adult gerbils (M1–M6) 30 days after a single dose of methamphetamine

#	Methamphetamine											
	M1		M2		M3		M4		M5		M6	
	$\bar{x} \pm$ S.D.	n	$\bar{x} \pm$ S.D.	n	$\bar{x} \pm$ S.D.	n	$\bar{x} \pm$ S.D.	n	$\bar{x} \pm$ S.D.	n	$\bar{x} \pm$ S.D.	n
1	113.1 ± 9.9	33	91.6 ± 8.1	36	83.9 ± 5.0	35	64.2 ± 6.6	37	77.1 ± 6.2	38	74.5 ± 7.6	38
2	82.3 ± 8.5	37	101.9 ± 12.0	42	82.9 ± 5.9	38	66.3 ± 5.6	40	103.7 ± 7.2	34	74.8 ± 7.0	33
3	59.8 ± 7.2	32	86.7 ± 9.1	35	89.4 ± 7.3	40	74.2 ± 6.3	41	102.5 ± 6.0	37	85.7 ± 6.9	35
4	58.4 ± 6.4	39	79.8 ± 7.8	38	80.6 ± 4.6	37	114.1 ± 10.2	35	106.6 ± 6.9	36	107.1 ± 7.7	36
5	64.3 ± 5.3	35	75.9 ± 8.2	34	61.8 ± 7.1	38	102.5 ± 7.1	36	86.3 ± 7.2	41	86.1 ± 6.5	37
6	104.2 ± 8.7	36	104.1 ± 10.2	36	50.9 ± 4.1	36	77.6 ± 7.4	39	83.9 ± 6.6	37	106.1 ± 8.2	40
7	101.4 ± 6.7	30			86.1 ± 5.6	34	113.4 ± 8.4	42	74.3 ± 7.4	40	105.9 ± 6.5	33
8	79.8 ± 5.2	45			97.3 ± 6.6	35	94.6 ± 7.9	38	68.7 ± 4.9	38	83.9 ± 7.8	37
9	99.8 ± 7.1	37			66.6 ± 5.0	39	69.3 ± 7.6	37			102.1 ± 5.7	38
10					51.9 ± 4.1	36	73.5 ± 7.5	37			76.9 ± 5.8	39
11					82.7 ± 5.9	36	67.2 ± 5.8	32			77.1 ± 7.2	36
12					54.0 ± 6.7	37					83.7 ± 5.8	41
13											83.5 ± 6.0	38
14											73.4 ± 5.5	34
$\bar{x}_1 \pm$ S.D.	84.8 ± 20.8		90.0 ± 11.5		74.0 ± 16.1		83.4 ± 19.2		87.9 ± 14.6		87.2 ± 12.7	
$\bar{x}_2 \pm$ S.D.							84.5 ± 5.7					

Mean number ($\bar{x}$) ± standard deviation (S.D.) is given per square hole of the copper grids covered by ultra-thin sections of the prelimbic area trimmed down to test fields of 0.875 mm^2; entire test fields were evaluated; number of sections (test fields) per individual (#); number of square holes which could be evaluated in a single test field (n); mean number in single test fields ($\bar{x} \pm$ S.D.); mean number in individuals ($\bar{x}_1 \pm$ S.D.); mean number in saline-treated controls ($\bar{x}_2 \pm$ S.D.).

vation is lateralized in the prefrontal cortex of gerbils (cf., Slopsema et al., 1982). The frontal cortex was serially cut with a vibratome over the entire rostro-caudal extent of the pregenual prefrontal cortex (for details see Dawirs et al., 1993b). To avoid sampling bias, every other consecutive section was selected in each animal. Following flat-embedding, each selected vibratome section was trimmed down to a test field of 0.875 mm^2 covering all laminae of the prelimbic area before ultra-thin sections were provided (Fig. 1). Thereby, quantification of GABAergic profiles would not be affected by laminar differences in the density of fibres. One intact ultra-thin section obtained from right below the cutting edge of each trimmed vibratome slice was selected for 'blind' quantification. Ultra-thin non-con-

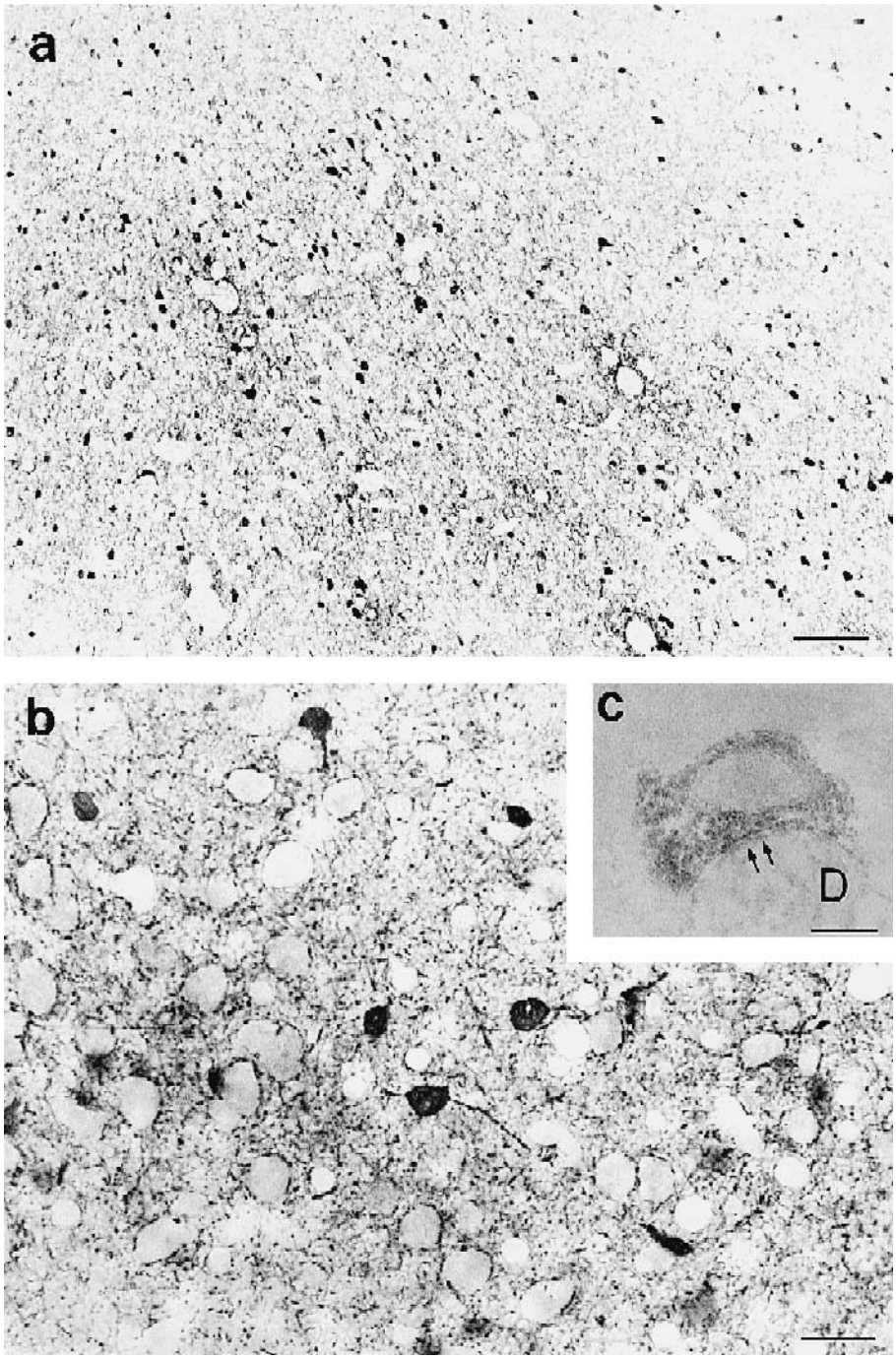

Fig. 2. Brightfield photomicrographs of GABA-immunoreactivity in the prelimbic area of the prefrontal cortex (a,b); GABAergic pericarya are diffusely distributed throughout the cortical depth (a), and an extensive net of GABAergic fibre-like structures is seen besides non-labelled neurons (b); Electron micrograph of a GABA-immunoreactive profile from non-contrasted ultra-thin sections (c); GABA-immunoreactivity was easily detected and quantified in non-contrasted sections (c); synaptic complex (double arrows); dendrite (D); scale 250 μm (a), 50 μm (b), 400 nm (c).

trasted sections were collected on copper grids (G 300 Cu), and all detectable GABA-immunoreactive fibre profiles were electron-microscopically identified and counted in the entire test field (TEM H-5001, Hitachi) (3000-fold magnification). All parts of the sections were evaluated which were undamaged and completely covered a square hole of the grid. In order to make counts comparable between different sections, results are given as the mean number of immunoreactive fibre profiles per square hole (approximately 80-μm^2). Ultra-thin sections derived from 6 to 14 intact vibratome slices over the whole rostro-caudal extent of the prefrontal cortex of each animal. A total of 43 and 60 ultra-thin sections were evaluated in 5 saline-treated controls and 6 methamphetamine-treated animals, respectively. All together, 1668 and 2216 holes were evaluated in ultra-thin sections of saline- and drug-treated animals respectively (Tables 1 and 2). Because all but two sections of one saline-treated control were accidentally lost this animal was left out of consideration. Mean values were computed as arithmetic means $\pm$ S.D., and compared by *t*-test with preceding *F*-test (Sachs, 1974).

3. Results

The rabbit antiserum used selectively labelled GABAergic cell somata and fibres which, like other cortical areas, reveal typical ubiquitous distribution in the prelimbic area of the prefrontal cortex (Fig. 2a). Dense GABAergic fibre networks were identified with numerous varicosities which frequently were in contact with non-labelled postsynaptic cell somata (Fig. 2b).

The intention of the present study was to quantify probable drug-induced changes in GABAergic innervation of the prelimbic area. An appropriate means to do so was to ultrastructurally identify GABA-immunoreactive profiles in test fields of defined size. GABA boutons were easily discerned in non-contrasted ultra-thin sections of the prelimbic area (Fig. 2c). The overall mean number of GABA-immunoreactive profiles (per square hole of collecting copper grids) in test fields of 0.875 mm^2 was about 71 and 85 in adult animals 30 days after a single dose of saline and methamphetamine, respectively (Tables 1 and 2). Thus, the present results indicate that a single methamphetamine challenge in 90 days old male gerbils was a stimulus significantly strong enough ($P < 0.01$) to cause sprouting of GABAergic fibres in the prefrontal prelimbic area, the result of which was a 20% increase in the density of GABAergic innervation during the subsequent 30 days.

4. Discussion

In the present study we investigated whether the density of GABAergic innervation of the prefrontal cortex is sensitive to a single dose of methamphetamine in adult gerbils. We found that 30 days after a single methamphetamine challenge in the prelimbic area of the medial prefrontal cortex the density of GABA-immunoreactive fibre profiles had significantly increased by about 20%. We have adequate reason to argue that GABAergic sprouting in the prefrontal cortex is part of a cascade of neuroplastic responses to a drug-induced acute increase of mesoprefrontal dopamine functions.

It is now well established that under certain conditions even a single dose of methamphetamine is a stimulus strong enough to selectively induce partial dopaminergic deafferentation of prefrontal pyramidal cells (for recent review, see Dawirs et al., 1991, 1994; Teuchert-Noodt and Dawirs, 1991). Pharmacologically, in the mammalian brain methamphetamine has an agonistic effect on dopamine functions, mainly presynaptic release of dopamine and blockade of the degrading enzyme monoamine oxidase (Ricaurte et al., 1982). It has been shown that under conditions of large doses of methamphetamine which causes large effective concentrations of the drug in the nervous tissue and high acute concentrations of dopamine in the synaptic cleft, dopamine is frequently metabolized non-enzymatically to 2,4,5,-trihydroxy-phenethylamine (6-hydroxydopamine) (Seiden and Vosmer, 1984). This endogenously produced 6-hydroxydopamine enters the affected dopamine terminals by high affinity re-uptake, and as an effective monoaminergic neurotoxin causes autotoxic degeneration of 'methamphetamine-sensitive' subpopulation of mesoprefrontal dopamine fibres (for recent discussion see Teuchert-Noodt and Dawirs, 1991).

Besides presynaptic responses, there is evidence that a single dose of methamphetamine induces significant transneuronal effects on postsynaptic sites. After application of the drug prefrontal pyramidal cells frequently appear as 'dark neurons' (Wahnschaffe and Esslen, 1985). These affected cells show typical signs of degradation, mostly characterized by an increased portion of free ribosomes in the cytoplasm (Auer et al., 1985; Gallyas et al., 1990). There is an indication that these postsynaptic responses were actually caused by methamphetamine-induced deafferentation, since comparable states of degradation have been discussed with respect to lesion-induced deafferentation or excessive stimulation of postsynaptic neurons in other regions of the brain (Heimer and Kalil, 1978; Clarke and Nussbaumer, 1987; Gallyas et al., 1990). Nevertheless, after a single dose of methamphetamine these effects were reversible and symptoms completely disappeared at the latest 10 days following application of the drug (Wahnschaffe and Esslen, 1985). Therefore, there are sufficient grounds for the assumption that after drug-induced partial dopaminergic deafferentation the affected pyramidal cells were able to surmount the resulting acute destabilization of their input-output balance by reactive structural and functional compensation (Wolff and Wagner, 1983; Dammasch et al., 1986). Actually, during this period of post-lesion recovery the density of dendritic

spines dramatically increased on prefrontal pyramidal cells (Dawirs et al., 1991, 1993a). Nevertheless, spine density was only transiently increased. Following a rapid increase by more than 80% within 7 days after application of the drug, the density of dendritic spines returned to almost normal original values within the next 3 weeks (Dawirs et al., 1993a).

It can be noticed immediately that the dynamic characteristics of methamphetamine-induced acquisition and subsequent loss of dendritic spines were in good accordance with typical features of lesion-induced reactive collateral sprouting (cf. Cotman and Nieto-Sampedro, 1984). Additionally, several lines of investigation emphasize that dendritic spines are of particular importance for synaptic reorganization (Calverley and Jones, 1990; for recent review see Dawirs et al., 1993a). Thus, the question was whether or not a single pharmacological impact by methamphetamine, which results both in presynaptic dopaminergic deafferentation and transient proliferation of postsynaptic contact sites, may serve as a trigger for functional changes by compensatory synaptogenesis.

Our results allow us to state that GABAergic neurons are significantly involved in neuronal reorganization of the prefrontal cortex induced by a single dose of methamphetamine, and that novel GABA synapses might probably participate in the remodelling of the pyramidal cells' inhibitory afferent inputs (for recent discussion see Dawirs et al., 1993a). Nevertheless, as yet we may not state conclusively whether intrinsic or/and extrinsic GABAergic neurons (cf. Pirot et al., 1992) are involved. Beyond this, it cannot be excluded so far that further transmitter systems and projections have a part in methamphetamine-induced synaptogenesis. The answer to these questions must be left to future investigations. Finally, it is of major interest to further investigate how far typical prefrontal cortex functions, e.g. motor control, cognitive processes and emotion, are concerned by the drug-induced synaptogenesis described (cf. Dawirs et al., 1996).

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