Dopaminergic sensitization of rats with and without early prefrontal lesions: implications for the pathogenesis of schizophrenia

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Abstract

To study whether an early lesion of prefrontal cortex (PFC) would influence mesolimbic dopaminergic sensitization induced by intermittent electrical stimulation (IES) of the ventral tegmental area (VTA) or change social interactions in animals exposed to both electrical sensitization and prefrontal lesions, we examined the behaviour of rats with or without early prefrontal lesions following repeated electrical stimulation of the VTA. Additionally, we wanted to study the influence of immobilization stress on rats exposed to a combination of prefrontal lesion and daily restraint in Plexiglas tubes prior to IES. Neither early lesion of PFC nor repeated restraint influenced development of sensitization. However, the combination of early prefrontal lesion and IES resulted in changes in social interactions neither seen following IES nor in lesioned rats. The changes were most pronounced in the group exposed to both IES, prefrontal lesions and restraint. Furthermore, repeated restraint caused a significant increase in the threshold current for provocation of the behavioural response related to VTA stimulation (head stereotypes/sniffing). The implications of the findings for sensitization of the mesolimbic dopaminergic system as a model for development of schizophrenia are discussed.

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Introduction

Perturbation of neurotransmitter function has for many years been in focus in schizophrenia research. The hope of finding a single biochemical defect as the cause of psychosis has been abandoned in favour of attempts to integrate present knowledge of transmitter disturbances and neuropathology. Both clinical and experimental studies are in agreement with a primary or early cortical defect involving the glutamatergic and/or the dopaminergic system, and a later developed intermittent hyperactivity of the dopaminergic system superimposed on a basal hypodopaminergic state (Breier et al., 1993; Carlsson 1988; Carlsson and Carlsson 1990; Davis et al., 1991; Deakin et al., 1994; Glenthøj, 1995; Glenthøj and Hemmingsen, 1997, 1999; Glenthøj et al., 1993, 1998; Grace, 1991; Harrison et al., 1991; Kerwin et al., 1998; Kim et al., 1980; Krystal et al., 1994; Lodge and Anis, 1982; Olney and Farber, 1995; Weinberger, 1987, 1995; Weissmann et al., 1991). A consistent clinical finding is, that the sensitivity to low doses of psychostimulants is higher in schizophrenics compared to controls (Lieberman et al., 1984, 1987, 1990). These drugs have as a common pharmacological property potentiation of presynaptic dopamine (DA) release and inhibition of DA reuptake. They induce thought disorder and other psychotic symptoms in patients in small, normally harmless doses (Janowsky et al., 1973; Levy et al., 1993; Lieberman et al., 1990). Accordingly, several authors have proposed, that behavioural sensitization involving the mesolimbic dopaminergic system is a valid model for development of schizophrenic symptoms (Akiyama et al., 1994; Glenthøj, 1995; Glenthøj et al., 1993, 1998; Haracz, 1982; Hemmingsen et al., 1995; Lieberman et al., 1990; Sato et al., 1992) and pointed out the resemblance with pharmacological sensitization of animals by intermittent treatment with DA agonists (Glenthøj, 1995; Glenthøj et al., 1993; Robinson and Becker, 1986; Sato, 1992).

The exact pathogenetic mechanisms of pharmacological and behavioural sensitization are still under debate,
but phasic increases in neuronal firing of mesolimbic dopaminergic cells are believed to be involved in the episodic dopaminergic hyperactivity in animals demonstrating dopaminergic sensitization (Glenthøj, 1995; Glenthøj et al., 1993; Grace, 1991; Kalivas and Weber, 1988). Recent findings, however, suggest that exposure to high doses of amphetamine (Amph) results in a rapid sensitization of the stereotypy response which requires activation of DA receptors (Kuczynski and Segal, 1999a,b). As the stereotypy response could be temporally dissociated from the extracellular DA response, enhanced DA receptor mechanisms might be of importance, at least for stereotyped movements following high doses of Amph. Low doses of Amph and infusion of DA into the nucleus accumbens cause increases in locomotion (Clark et al., 1988; Costall and Naylor, 1974, 1977). Increases in locomotor activity, sniffing and head stereotypies are linked to the limbic system, whereas other stereotyped behaviours are related to the nigro-striatal system (for discussion, see Glenthøj, 1995). It is not as yet known whether DA receptor enhancement is of importance for limbic DA sensitization.

An additional technique used to induce sensitization is kindling. Kindling refers to the development of permanent excitability changes following repeated electrical stimulation of a given brain site with an initially subthreshold current (Goddard et al., 1969; Post and Ballenger, 1981; Racine, 1972; Wada et al., 1974). Like behavioural and pharmacological sensitization, kindling involves gradual development of behavioural changes (see Glenthøj, 1995). All three conditions are examples of neuronal plasticity responses, and all depend on an intermittent and not continuous influence on the involved receptors.

To study whether intermittent electrical stimulation (IES) of the ventral tegmental area (VTA) would lead to behavioural sensitization by analogy with DA agonist sensitization, we have previously studied the behaviour of rats that were either electrically stimulated or sham-stimulated in the VTA once daily for 70 d (Glenthøj et al., 1993). The study demonstrated behavioural sensitization following IES of the VTA. Furthermore, between stimulation periods sensitized animals showed a reduced social interaction. The latter finding is compatible with the pattern of isolation in schizophrenics, whereas the observed sensitization of the mesolimbic dopaminergic system is comparable with the increased sensitivity to low doses of psychostimulants in schizophrenia patients.

The behavioural response to electrical VTA stimulation resembles the response to pharmacological stimulation of the mesolimbic dopaminergic system (Clark et al., 1988; Costall and Naylor, 1974, 1977; Pijnenburg et al., 1975). It results in stereotyped sniffing and head stereotypies by the use of low current intensities and forward locomotion by the use of higher current intensities. The behavioural response is dependent on the exact placement of electrodes within the VTA (Glenthøj et al., 1993). It is of short duration and aimless and invariant in nature.

Prefrontal dysfunction has been found to have an effect on phasic increases in neuronal firing of DA cells and on dopaminergic sensitization (Overton and Clark, 1997; Pierce et al., 1998; Svensson and Tung, 1989; Wolf et al., 1995). Moreover, stress is known to activate monoaminergic pathways and induce cross-sensitization as well as cross-tolerance with psychostimulants (Robinson and Becker, 1986). Several studies have documented the importance of environmental stressors for dopaminergic sensitization (Glenthøj and Hemmingsen, 1991). In the present study we observed the behaviour of rats with or without early prefrontal lesion following repeated electrical stimulation of the VTA. The purpose was to test whether an early prefrontal defect would influence mesolimbic dopaminergic sensitization induced by IES of the VTA, or change social interactions in animals exposed to both electrical sensitization and prefrontal lesion. Additionally, we wanted to study the influence of immobilization stress on rats exposed to a combination of prefrontal lesion and daily restraint in Plexiglas tubes prior to sensitization. Finally, we wanted to examine the utility of a shorter-lasting interstimulus interval compared to Glenthøj et al. (1993).

**Methods**

**Subjects**

Experiments were carried out with male Wistar Rats (Møllegaard, Denmark) 3 wk old at the beginning of the study. All procedures were performed according to ‘Guidelines from the Department of Justice’s Committee for Animal Experiments’. The laboratory has permission to perform animal studies. Rats were randomly assigned into the following six groups: group 1, sham stimulated (SHAM); group 2, VTA stimulated (VTA); group 3, prefrontal sham stimulated (PFC SHAM); group 4, prefrontal VTA stimulated (PFC VTA); group 5, prefrontal restraint VTA stimulated (PFC RES VTA); group 6, restraint sham stimulated (RES SHAM).

Rats were kept individually in clear Plexiglas cages in a room with an automatically controlled light–dark cycle (lights on 06:00 to 18:00 hours). They had free access to a standard laboratory diet and water. The number of animals was chosen with consideration given to an expected drop-out connected to surgery/non-working electrodes. One animal from group 1 was taken out of the study due to generalized seizures/electrode failure. In group 2 one rat died, one left the study due to electrode failure. In group 3 one rat died, one left the study due to electrode failure. In group 4 one rat died, one left the study due to electrode failure. In group 5 one rat died, one left the study due to electrode failure. In group 6 one rat died, one left the study due to electrode failure.
failure and one was excluded due to electrode placement outside the VTA. In group 3 four rats died, two rats left the study due to electrode failure and one rat was excluded due to electrode placement outside the VTA. In group 4 four rats died left the study due to electrode problems before stimulation, one rat died following the first stimulation session and one rat died during IES. In group 5 six rats died before the study and one rat was excluded due to electrode placement outside the VTA. In group 6 two rats were excluded due to electrode placement outside the VTA. The cause of deaths was failure of restraint of headplugs in lesioned animals or non-functioning electrodes.

**Surgery**

**Prefrontal lesions**

The entire anteromedial (prefrontal) cortex was removed by subpial aspiration under clean but not sterile conditions. Ablations were performed under visual guidance with the aid of an operation microscope.

**Electrode implantation**

Rats were anaesthetized with Equithesine i.p. and mounted in a stereotactic apparatus. Bipolar stainless steel electrodes (Rodes Medical Instruments) were implanted bilaterally according to the ‘Stereotactic Atlas’ of George Pazinox and Charles Watson in the VTA containing the mesolimbic and mesocortical DA cell bodies (A10 DA region) (0.48 mm posterior to bregma, 0.1 mm lateral to midline and 0.83 mm below dura).

**Electrical stimulation**

Electrical stimulation was performed no less than 15 d after electrode implantation, beginning between 08:00 and 09:00 hours for all rats. Rats were bilaterally electrically stimulated in the VTA for 2 s four times daily with the threshold current plus 10 % (2 s trains of 1 ms pulses at 60 Hz) (70 stimulations). On the first and last day of IES rats were only stimulated once. Stimulations were separated by at least 90 min. Rats were connected to the stimulating-recording equipment and allowed to adapt to the test cage (Plexiglas, 46 cm × 51 cm, with 2 blue sides) until they were calm. After stimulation they were left in the test cage for at least 30 s before removal. At every stimulation session ‘sham-stimulated’ rats were connected to the stimulating-recording equipment and allowed to stay in the test cage for as long as stimulated rats.

In a previous study (Glenthøj et al., 1993) we found that rats exposed to IES of the VTA responded with head stereotypies and/or sniffing when stimulated with a low current intensity. At a higher current intensity they responded with forward locomotion. In order to establish threshold currents animals in groups 2 (VTA), 4 (PFC VTA) and 5 (PFC RES VTA) were initially stimulated bilaterally in the VTA in two sessions with increasing current intensity from 5 µA until they demonstrated head stereotypies and/or sniffing (the ‘low current response’). Moreover, both during the first and second stimulation session, and following IES, threshold values for the lowest current that elicited invariant and immediate forward locomotion (at least two steps) (the ‘high current response’) were determined. None of the animals were stimulated with these higher current intensities at IES.

**Behavioural observations**

The behavioural observations consisted of the ‘social group formation’ test and the ‘male/male interaction’ test. For both tests a detailed account of apparatus as well as behavioural procedure have been given elsewhere (Glenthøj et al., 1993). Consequently, only short accounts of the test procedures involved will be given.

**Social group formation**

Eight animals from the same experimental group were studied in an open-field area allowing the animals 1 h of undisturbed activity. From the videotaped sessions the animals’ activities were analysed. The analysis focused on six 1-min periods (after 10, 20, 30, 40, 50 and 60 min of the session). The 1-min periods were scored independently and analysed statistically as individual samples. The following measures of social interactions were obtained for each of these periods: (1) the total duration of periods in which at least one of the corner areas (defined as a corner quadrant in the open field) contained three or more rats, (2) the total duration of periods in which three or more rats could be found outside corner areas, and (3) the total duration of periods in which ‘total social isolation’ (defined as complete absence of bodily contact between all animals) occurred.

**Male/male interaction**

Two rats from the same experimental group were put in an observation cage and allowed a 10-min session of undisturbed activity. The rats’ behaviour in the cage was...
videotaped and two parameters were obtained: (1) the total duration of interactions and (2) the latency from the beginning of the session until the first interaction. An interaction would be registered if at least one of the animals demonstrated a behaviour oriented towards any part of the body of the other rat. Such a behaviour could be sniffing, biting, grooming, manipulation or any other type of social behaviour, whereas seemingly accidental body contacts such as stepping on the tail of the other rat would not be considered an interaction.

**Stimulation with DA agonists**

Two weeks after IES all rats were challenged first with apomorphine (Apo) and 2 d later with Amph. After stimulation with Apo rats were scored for stereotypies after 20, 40 and 60 min. Following Amph stimulation rating took place after 20, 40, 60, 80, 100, 120 and 180 min.

Stereotypies were scored 0–2, with 0 representing not present, 1 representing discontinuously present, and 2 representing continuously present. The following items were observed: 1, stereotyped sniffing; 2, head stereotypies; 3, stereotyped movements of forelimbs; 4, stereotyped movements of trunk; 5, stereotyped circling; 6, stereotyped forward locomotion; 7, stereotyped mouth movements (licking, biting or gnawing); 8, other stereotyped movements.

**Drugs**

Commercial solutions of d-amphetamine-sulphate and apomorphine HCl were used. The following doses were given s.c.: 0.2 mg/kg Apo and 2 mg/kg Amph.

**General remarks**

Rating of rats challenged with DA agonists and establishment of threshold currents for rats were all made blindly by B.Y.G.

**Histology**

The rats were perfusion-fixed through the left ventricle of the heart with 4% buffered paraformaldehyde. After 1–2 h the electrodes were removed from the skull. Subsequently, the brains were removed and further fixed in 4% buffered paraformaldehyde. After 24 h the brains were cut in coronal sections around the lesioned area and the canals after the electrodes. Serial sections, 4 µm thick, were cut through the blocks to determine the histological picture and the exact positions of the electrode tips. The sections were stained with H & E and Klüver Barrera for myelin.

**Procedures (Figure 1)**

Eighty-seven rats were randomly assigned into the following groups:

- **Group 1** (SHAM). Fourteen control rats 'sham-stimulated' every time the other groups were electrically stimulated.
- **Group 2** (VTA). Fifteen rats stimulated bilaterally in the VTA with the lowest current (added 10%) that elicited head stereotypies and/or sniffing (low current response) at the beginning of the study.
- **Group 3** (PFC SHAM). Fifteen control rats with early prefrontal lesions sham-stimulated as group 1.
- **Group 4** (PFC VTA). Fourteen rats with early prefrontal lesions stimulated as group 2.
- **Group 5** (PFC RES VTA). Fifteen rats with early prefrontal lesions exposed to restraint in Plexiglas tubes for 20 min, Monday–Friday, during the 4 wk before electrode implantation. Rats were stimulated as groups 2 and 4.
Group 6 (RES SHAM). Fourteen control rats exposed to daily restraint in Plexiglas tubes for 20 min, Monday–Friday, during the 4 wk before electrode implantation. Rats were sham-stimulated as groups 1 and 3.

Prefrontal lesions (PFC SHAM, PFC VTA and PFC RES VTA rats) were performed when rats were 3 wk old. Restraint in Plexiglas tubes (PFC RES VTA and RES SHAM rats) took place at age 6–10 wk. Bipolar stainless-steel electrodes were implanted when rats were 10 wk old. Threshold currents for IES [the low current response (head stereotypies and/or sniffing)] and for the high current response (forward locomotion) were established between age 3 and 4 months (VTA, PFC VTA and PFC RES VTA rats). All rats had the same age when surgery was carried out. They were randomly assigned to groups and no between-group age differences existed. The disparity of age from the time of establishment of threshold currents was the result of different dates of births and the fact, that it takes about 1 month to perform surgery on 87 rats. Subsequently IES (with the threshold current for the low current response) and sham stimulation took place. After 70 stimulations, threshold currents for both the low current response and the high current response were determined blindly for all groups. One week later, when rats were approx. 4–5 months old, all groups were challenged with DA agonists after which behavioural observations were performed.

**Statistical analysis**

Comparison of threshold current intensities and behavioural observation: between-treatment group differences were analysed by means of non-parametric test procedures (Mann–Whitney, Kruskal–Wallis). Also within group differences across stimulations were tested by means of non-parametric procedures (Wilcoxon test, Friedman). Analysis of behavioural items (DA stimulation) was performed by testing in contingency tables with treatment and score values as entries (Fisher’s exact test). Two-tailed tests were used throughout with the level of significance equal to 5 % (if not described otherwise).

**Results**

**Stimulation**

The mean current intensity needed to provoke head stereotypes and/or sniffing (the low current response) following stimulation in IES animals (VTA rats) was 25.42 µA (range 10–60 µA). The mean threshold value for control rats (SHAM rats) was 46.92 µA (range 20–90 µA). The difference between stimulated animals and control rats is significant (p < 0.013) and demonstrates electrical sensitization to IES of the VTA (Figure 2). Comparable sensitization was observed for rats with early prefrontal lesions. IES rats with prefrontal lesions (PFC VTA rats) had a mean threshold value for the low current response of 25.56 µA (range 10–40 µA) following IES opposed to 42.50 µA (range 10–70 µA) in the sham-stimulated group with prefrontal lesions (PFC SHAM rats) (p < 0.042) (Figure 2). The group with early prefrontal lesions that had been exposed to repeated restraint before IES (PFC RES VTA rats) differed significantly from the other stimulated groups both before and after IES. The mean threshold current after IES was 42.50 µA (range 30–60 µA) (Figures 2, 3). Also the non-
lesioned sham-stimulated group exposed to repeated restraint (RES SHAM rats) differed significantly from the other two control groups (SHAM rats and PFC SHAM rats) following IES. The mean threshold current for the low current response for RES SHAM rats was 50.83 \( \mu \text{A} \) (range 20–100 \( \mu \text{A} \)) (Figure 2).

Figure 3 demonstrates mean threshold currents for the low current response before (stimulation sessions 1 and 2) and after IES for all three stimulated groups. In both the VTA and the PFC RES VTA groups no significant within-group differences were observed between stimulation sessions 1 and 2. Rats in the PFC VTA group demonstrated a significant fall in threshold currents between the two first-stimulation sessions (from mean 71.11 to mean 54.44 \( \mu \text{A} \); \( p < 0.05 \)). For all stimulated groups comparable significant falls in threshold currents were observed between stimulation session 1 and after IES, i.e. neither prefrontal lesions nor restraint affected development of sensitization. Mean threshold currents fell from stimulation session 1 to after IES as follows. VTA rats, from 60.0 \( \mu \text{A} \) (range 30–130) to 25.42 \( \mu \text{A} \) (range 5–60) (\( p < 0.003 \)); PFC VTA rats, from 71.11 \( \mu \text{A} \) (range 20–140) to 25.56 \( \mu \text{A} \) (range 10–40) (\( p < 0.016 \)); PFC RES VTA rats, from 111.25 \( \mu \text{A} \) (range 30–200) to 42.50 \( \mu \text{A} \) (range 30–60) (\( p < 0.016 \)). Significant falls in threshold currents were also observed between stimulation session 2 and after IES. VTA rats, from 50.83 \( \mu \text{A} \) (range 30–100) to 25.42 \( \mu \text{A} \) (\( p < 0.004 \)); PFC VTA rats, from 54.44 \( \mu \text{A} \) (range 20–80) to 25.56 \( \mu \text{A} \) (\( p < 0.016 \)); PFC RES VTA rats, from 86.25 \( \mu \text{A} \) (range 20–130) to 42.50 \( \mu \text{A} \) (\( p < 0.016 \)).

The fall in threshold values in the group with combined prefrontal lesions and exposure to repeated restraint in tubes (PFC RES VTA rats) was equal to the falls in the other two stimulated groups. However, both at the first and the second stimulation session and following IES this group differed significantly from the two other stimulated groups (\( p < 0.03 \)).

Additionally, when all stimulated groups (VTA, PFC VTA and PFC RES VTA rats) were compared before and after IES, a highly significant fall in threshold currents for the low current response was observed (\( p < 0.0001 \)). A fall in threshold currents was also found between stimulation sessions 1 and 2 (\( p < 0.01 \)).

Rats were not IES with the high current response (forward locomotion). Sensitization to this response was only observed when all stimulated rats were scored together. Significant falls in threshold values were found both between stimulation sessions 1 and 2 (\( p < 0.01 \)), when stimulation session 1 was compared to values following IES (\( p < 0.0001 \)) and when session 2 was compared to values following IES (\( p < 0.002 \)). However, looking at the individual groups, at the different stimulations, neither within-group nor between-group differences were observed, except for the fact, that the group with combined exposure to prefrontal lesions and restraint in tubes before IES (PFC RES VTA rats) scored higher than the other stimulated groups. This is in parallel to what was observed for the low current response. Following IES significant differences in threshold currents for the high current response were only seen when VTA rats were compared with PFC RES VTA rats (\( p < 0.04 \)).

In brief IES of the VTA resulted in significant falls in intensities in threshold currents for the low current response (head stereotypies and/or sniffing) when stimulated rats were compared with sham-stimulated rats. Development of sensitization did not differ between lesioned and non-lesioned animals. The fall in threshold values for the low current response in PFC RES VTA rats was equal to the fall in the other two stimulated groups. However, this group differed significantly from the other stimulated groups both before and after IES, expressing a higher threshold for abnormal behaviour.

### Stimulation with DA agonists

#### Apomorphine

Following stimulation with 0.2 mg/kg Apo all rats developed intense stereotypies. No group differences were observed.

#### Amphetamine

IES rats (VTA, PFC VTA and PFC RES VTA rats scored together) demonstrated significantly more stereotyped sniffing (\( p < 0.00001 \)), head stereotypies (\( p < 0.0006 \)) and stereotyped movements of trunk (\( p < 0.04 \)) compared to sham-stimulated rats (SHAM, PFC SHAM and RES SHAM rats). Looking at the individual groups, differences were most pronounced when non-lesioned control rats (SHAM rats) were compared with non-lesioned IES rats (VTA rats) (Table 1) (sniffing, \( p < 0.0001 \)).

<table>
<thead>
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<th>Group</th>
<th>Score</th>
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<td>20</td>
<td>32</td>
<td>39</td>
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<tr>
<td>VTA</td>
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<td>3</td>
<td>16</td>
<td>65</td>
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\( p < 0.00001 \).

Number of observations of stereotyped sniffing in group 1 (SHAM rats) and group 2 (VTA rats) 20, 40, 60, 80, 100, 120 and 180 min after challenge with 2 mg/kg Amph (25 rats at 7 observations).
Electrical sensitization of the VTA

Figure 4. Behavioural performance of the six experimental groups during the social group formation test (see Methods section for procedural details). The three individual figures indicate: (a) the duration (group medians with ranges) of periods during which 3 or more rats were found within a given corner area; (b) periods during which 3 or more rats were found out of corner areas; (c) periods during which the animals demonstrated a total social isolation. SHAM rats (group 1) had been exposed to sham stimulation; VTA rats (group 2) to IES; PFC SHAM rats (group 3) to prefrontal lesions and sham stimulation; PFC VTA rats (group 4) to prefrontal lesions and IES; PFC RES VTA rats (group 5) to prefrontal lesions, repeated restraint and sham stimulation. Significant differences between the results obtained by various experimental groups are indicated by:

* significantly different ($p < 0.05$) from SHAM; ** significantly different ($p < 0.01$) from SHAM; × significantly different ($p < 0.05$) from PFC SHAM; ×× significantly different ($p < 0.01$) from PFC SHAM; + significantly different ($p < 0.05$) from PFC VTA; ++ significantly different ($p < 0.01$) from PFC VTA; $\$\$$ significantly different ($p < 0.01$) from RES SHAM; $$ significantly different ($p < 0.01$) from VTA.

Behavioural observations

While the Kruskal–Wallis non-parametric ANOVA indicated that no significant group differences were found within the male/male interaction test, the three parameters of the social group formation test turned out to yield significant group differences by the outcome of the Kruskal–Wallis non-parametric ANOVA for the parameter ‘3 or more rats out of corners’ the level of significance was $p < 0.01$, while the two other parameters of the same test demonstrated $p$ values of $< 0.0001$.

The subsequent analysis of individual group differences within the parameters of the social group formation test led to the results illustrated in Figure 4.

Histology

The prefrontal cortical lesions were verified according to the groups listed in the Methods section. The electrodes were placed in the VTA in most rats, but a few animals evenly dispersed through the groups showed misplacement and were, as stated in the Methods section, excluded from the analysis. In the needle tracks slight gliosis or fibrosis could be seen at the edge. Also very small dots of calcification could be seen, again evenly distributed in all
groups. In a few animals haemosiderin-containing macrophages could be seen indicating previously minor haemorrhage. In one animal an extensive fibrosis and inflammation was seen, and this animal was excluded from the experiment. In none of the animals did we see neuronal changes in these routinely stained sections.

Discussion

The present study confirms, that it is possible to potentiate mesolimbic dopaminergic activity by IES of the VTA. In this study we used a shorter interstimulus interval compared to a previous study where rats were stimulated once daily for 70 d (Glenthøj et al., 1993). Using this faster sensitization regimen we observed sensitization of behaviours related to the mesolimbic dopaminergic system (sniffing and head stereotypies), comparable to what was observed in Glenthøj et al. (1993). Moreover, even though rats in the present study were not stimulated with the high current response (see Methods section, and Glenthøj et al., 1993), a significant fall in threshold currents for the high current response was observed when all stimulated rats were scored together. In agreement with what was found in the previous study (Glenthøj et al., 1993), sensitization was most pronounced for stereotyped sniffing and head stereotypies, the behaviours directly related to low current stimulation of the VTA.

The fact, that no significant differences in the development of electrical sensitization were observed between lesioned and non-lesioned animals contradicts that prefrontal damage is a condition for the development of dopaminergic sensitization, or indicate that the lesion should take place before the age of 3 wk. Wolf et al. (1995) found that prefrontal lesions prevented sensitization of post-stereotypy locomotion, but not stereotypy, following repeated Amph administration. In a later study, Li and Wolf (1997) concluded that intrinsic neurons of PFC, most likely those sending excitatory amino acid-containing projections to the VTA, are required for the development, but not the expression of behavioural sensitization to Amph. That excitatory prefrontal projections to the VTA influence development and perhaps expression of sensitization of the mesolimbic dopaminergic system is also supported by the finding that an endogenous excitatory drive from PFC seems to be a condition for phasic activation of DA cells in the VTA (Grenhoff, 1990; Grenhoff et al., 1988; Svensson and Tung, 1989). In contrast to Li and Wolf (1997), Pierce et al. (1998) observed disruption of the expression of behavioural sensitization to cocaine following lesions of the dorsal PFC. In the present study prefrontal lesions neither affected the development, nor the expression of sensitization. As we observed stereotyped behaviours, our results are in agreement with the observations of Wolf et al. (1995). However, our findings do not exclude an influence of PFC on sensitization under normal physiological conditions. The apparent discrepancy with Pierce et al. (1998) might be the result of the fact that we, in our study, activated the VTA directly, i.e. independent of excitatory prefrontal projections.

IES rats (VTA, PFC VTA and PFC RES VTA rats) demonstrated significantly more stereotyped sniffing, head stereotypies and stereotyped movements of trunk compared to sham-stimulated rats (SHAM, PFC SHAM and RES SHAM rats) when challenged with Amph. Looking at the individual groups, differences were most pronounced, when VTA rats were compared with SHAM rats. Differences between PFC VTA rats and PFC SHAM rats did not reach the level of significance. PFC RES VTA rats, however, developed more stereotyped behaviours than did PFC SHAM rats. These findings support the results on electrical VTA sensitization, contradicting a significant influence of prefrontal damage on DA sensitization, but again they might reflect the fact that sensitization was the result of direct activation of the VTA.

Using a longer lasting regimen of IES, we have previously described cross-sensitization with both Apo and Amph (Glenthøj et al., 1993). In the present study cross-sensitization was only observed between IES and challenge with Amph, but not Apo. Differences in the development of cross-sensitization between IES and Amp and Apo point to presynaptic potentiation as being of more importance compared to postsynaptic potentiation. The latter finding is in agreement with what has previously been found in DA agonist sensitization (see Glenthøj et al., 1993). Furthermore, using the longer-lasting regimen (Glenthøj et al., 1993), we demonstrated development of sensitization not only of sniffing and head stereotypies, but also of oral behaviours. Sniffing, head stereotypies, and other behaviours linked to the mesolimbic dopaminergic system, are known to show rapid sensitization, whereas oral behaviours demonstrate slow potentiation (Eichler and Antelman, 1979). The less conspicuous response to DA agonist challenge in the present study points to the longer interstimulus interval as the most powerful to produce dopaminergic sensitization.

Rats with prefrontal lesions that had been exposed to repeated restraint prior to IES did not differ from the other stimulated groups in their ability to develop sensitization. However, the finding, that threshold currents for this group were significantly higher at all stimulation sessions, including the first, was very surprising. Immobilization is a well-known stressor for rats (Tache et al., 1976). Numerous studies have demonstrated that the acute
The response of an animal to dopaminergic activation is strongly influenced by its past history of stress (see Glenthøj and Hemmingsen, 1991) and we would expect cross-sensitization between VTA stimulation and prior tube restraint. Instead, animals exposed to repeated restraint demonstrated significantly higher threshold values from the beginning of the study, indicating development of tolerance to dopaminergic activation. That the finding was not accidental, but related to restraint, is supported by the fact that non-lesioned, non-stimulated rats exposed to repeated restraint in tubes also had higher threshold values when all rats were compared blindly following IES.

Tolerance to DA agonist challenge is displayed following frequent high doses, while low and infrequent dose regimens are know to evoke sensitization (Robinson and Becker, 1986). The development of tolerance to restraint in our study might be the result of handling and predictable, circadian, long-lasting restraint (20 min) daily for a long period of time. This is supported by findings by Csernansky et al. (1984) of development of tolerance to sniffing induced by intermittent oscillation stress for 30 min. Our results are also in agreement with data suggesting that Amph-induced alterations in brain transcription factor gene expression can display tolerance and possibly cross-tolerance with stress caused by i.p. injections twice daily (Persico et al., 1993). Moreover, Campeau et al. (1991) have observed that repeated handling completely blocks the elevation of c-fos mRNA levels in amygdala induced by mild footshocks.

Additional studies, including a relevant control group (sham-stimulated, lesioned and exposed to restraint) and a stimulated group exposed to restraint, but not PFC lesions, are needed to further elucidate the findings. Also needed are studies including different modalities of stress exposure, presented at different intervals. Such studies might, in addition, explain the finding of the most disturbed social interactions following combined exposure to early prefrontal lesion, intermittent restraint and IES. The degree of DA sensitization in PFC RES VTA rats did not differ from the other IES groups, but the threshold currents for provocation of DA-related behaviours did. It might be speculated that the reduced ability to activation of the mesolimbic dopaminergic system in these rats was responsible for their reduced social interactions. However, also PFC VTA rats demonstrated significant changes in social group formation and the response of this group to VTA stimulation did not differ from the response of non-lesioned animals (which did not develop behavioural disturbances; see below).

The behavioural observations clearly demonstrated an influence of combined early prefrontal damage and mesolimbic dopaminergic sensitization on social inter-

actions, whereas prefrontal lesion did not in itself cause an increase in isolation; PFC SHAM rats actually spent more time together in a corner than did any other group (Figure 4). By application of a longer-lasting regimen of IES (Glenthøj et al., 1993) we found repeated electrical stimulation of the VTA area to be associated with significant behavioural changes in both the social group formation test and the male/male interaction test. In a rather marked contrast to those results, the presently studied regime of electrical stimulation of the VTA was, in itself, unable to modify the outcome of either test. Although alternative interpretations are possible, the most likely explanation for the discrepancies between the behavioural consequences of repeated electrical stimulation of the VTA area in Glenthøj et al. (1993) and the present study is that the pattern of stimulation employed within the two studies is clearly different. The presently studied ‘fast sensitization’ seems unable to elicit long-lasting behavioural symptoms similar to those previously described (Glenthøj et al., 1993). The pathogenetic mechanisms behind the fortifying effect of prefrontal lesions, IES and repeated restraint on social behaviours are as yet very speculative, but the combination of interventions might mutually potentiate failure in DA signalling/function (see also Glenthøj and Hemmingsen, 1999; Glenthøj et al., 1998).

In conclusion the study confirmed that sensitization of the behavioural response to electrical stimulation of the VTA is possible. The shorter-lasting stimulation regimen used in the present study was, however, not as efficient as a longer-lasting regimen used in a previous study. Prefrontal lesion did not in itself affect development or expression of sensitization, but behavioural observations stressed the significance of combined VTA sensitization and cortical damage for social interactions.

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