Mechanisms underlying the long-term behavioral effects of traumatic experience in rats: The role of serotonin/noradrenaline balance and NMDA receptors

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Abstract

Traumatic stressors induce long-lasting changes in behavior. It is believed that all three glutamatergic, serotonergic and noradrenergic neurotransmission play a role in the development of such behavioral changes, but their relative importance and relationship is poorly understood. We have shown previously that a single exposure of rats to electric shocks induces social avoidance for about 10 days. Here we assessed social avoidance 24 h after shock exposure in rats with chemically lesioned serotonergic and noradrenergic neurons. The effects of the NMDA receptor blocker MK-801 were also studied. When the serotonin/noradrenaline balance was shifted towards serotonergic dominance via chemical lesions, the behavioral dysfunction was markedly attenuated. The disruption of serotonergic neurotransmission (that lead to noradrenergic dominance) significantly increased the behavioral deficit. Shock responding was not secondary to lesion-induced differences in social behavior. Noteworthy, the brain noradrenaline/serotonin ratio correlated negatively with shock-induced social avoidance, suggesting that the ratio rather than absolute levels are important in this respect. In line with this assumption, double lesions had minor effects on social avoidance, suggesting that these monoaminergic systems modulate, but do not mediate the behavioral deficit. The blockade of NMDA receptors abolished the development of stress-induced social avoidance both when applied before shocks and when applied before behavioral testing. We confirmed that the long-term behavioral effects of traumatic experience result from glutamatergic activation, the effects of which are mediated by NMDA receptors. The development of the behavioral deficit is modulated by the balance between serotonergic and noradrenergic neurotransmission, possibly via effects on shock-induced glutamatergic activation.

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1. Introduction

Post-traumatic stress disorder is a difficult to treat, yet common disorder, which is associated with significant morbidity, mortality and societal burden. The first-line choices for monotherapy are the selective serotonin reuptake inhibitors suggesting that the enhancement of serotonergic neurotransmission is protective in this disorder [32]. Recent evidence suggests that the inhibition of noradrenergic neurotransmission by adrenergic blockers (especially α1 and β blockers) may also be useful for mitigating post-traumatic stress disorder symptoms or perhaps even preventing the development of the disorder [13,44,45,54]. These data suggest that serotonergic and noradrenergic neurotransmission play opposite roles in post-traumatic stress disorder. Yet, the interactions between the two neurotransmitter systems are poorly understood.

Behavioral dysfunctions induced by unpredictable and uncontrollable aversive events were suggested to resemble post-traumatic stress disorder symptoms and thus may model this disorder in the laboratory. Such behavioral dysfunctions include fear responses to contexts or cues associated earlier with aversive experiences (e.g. electric shocks) [16,22,29], and long-term increases in anxiety-like behavior induced by
a single exposure to a predator, or electric shocks [4,55,25]. Long-term behavioral (sometimes anxiety-like) changes were noticed after social defeat as well [40,24]. Conditioned fear and the behavioral consequences of predator exposure were explicitly viewed as laboratory models of post-traumatic stress disorder [16,22,29,4], whereas other responses were interpreted in more general terms, namely as manifestations of stress-induced anxiety. Nevertheless, the subjects of the models shortly reviewed above were exposed to severe stressors, which resulted in long-term behavioral changes. This resembles key aspects of post-traumatic stress disorder. Investigating the mechanisms that underlie such changes would shed light on how the long-term consequences of traumatic stress exposure develop. Especially so as understanding the neurobiology of the trauma response in general may be useful in designing prevention and treatment strategies for posttraumatic stress disorder [10].

Here we report on the behavioral effects of traumatic stressors in animals with experimentally inhibited noradrenergic and serotonergic neurotransmission. Chemical lesions by the serotonin neurotoxin 5,7-dihydroxytryptamine (5,7-DHT), and/or the selective norepinephrine neurotoxin N-(2-chloroethyl)-N-ethyl-2-bromobenzylamine–HCl (DSP-4) are widely used for the study of the involvement of serotonergic and noradrenergic neurotransmission in various phenomena including behavior (see e.g. [48,38,60,57]). We employed this approach here. Electric shocks were applied as traumatic stressors to intact and sham operated rats, as well as to rats submitted to single (5,7-DHT or DSP-4) or double (5,7-DHT + DSP-4) chemical lesions. Behavioral consequences were evaluated by the recently validated social avoidance test ([35]; see Section 4). In contrast to single lesions, double lesions had minimal effects on the development of the shock-induced behavioral deficit. To assess the mechanism of this monoamine-independent effect, we also studied the effects of lesions on the shock-induced social deficits were due to lesion-induced differences in the social behavior of lesioned rats. Earlier findings showed, however, that the social avoidance test is insensitive to drug effects in unshocked rats. For example, benzodiazepines that abolished the shock-induced behavioral deficit at low doses, did not change the behavior of unshocked rats [24,25,43]. Preliminary experiments showed that the treatments applied here are also without effect in unshocked rats, despite the fact that they readily affected the shock-induced behavioral deficit. Therefore, the social behavior of lesioned rats was assessed in the social interaction test, which is sensitive to effects on social interactions in rats that are not exposed to shocks [14,15].

Rats were sham operated or underwent chemical lesions (sample size: 12–14 per group). Following 1 week recovery, rats were submitted to the social interaction test. One day later, the brains of rats were sampled, and hippocampal serotonin and noradrenaline levels were assessed to check for the efficacy of chemical lesions. 5,7-DHT and DSP-4 decreased serotonin and noradrenaline levels, respectively, by about 80% in all but one rat, in which the double lesion lowered neither serotonin nor noradrenaline levels. Therefore, this rat and its counterpart in the social interaction test were excluded.

Experiment 2 assessed the effects of MK-801 on shock-induced social avoidance.

Experiment 2a—Intact rats were either left undisturbed in their home cage, or received electric shocks. Shocked rats were injected intraperitoneally with 0 (vehicle), 0.05, and 0.1 mg/kg MK-801, 30 min prior to shock exposure. Unshocked rats were treated with vehicle (saline). One day later, rats were assessed in the social avoidance test. Sample size was 8 per group.

Experiment 2b—Intact rats were either left undisturbed in their home cage, or received electric shocks. One day later, they were treated with vehicle, or 0.1 mg/kg MK-801 (the dose that proved effective in experiment 2). 30 min after injections, rats were assessed in the social avoidance test. Sample size was nine per group.

2. Methods

2.1. Animals and housing

Male Sprague–Dawley rats (Charles-River Laboratories, Hungary) weighing approximately 270–300 g were used. Rats were maintained in a 12:12h reversed day-night schedule (lights on at 06:00) under standard laboratory conditions (temperature: 21 ± 2°C; humidity: 60 ± 10%). Standard laboratory food (Charles-River Laboratories, Hungary) and tap water were freely available. Animals were isolated 7 days before stress application and housed individually. Cage size was 20 cm × 40 cm × 25 (height) cm. All studies were carried out in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC) and were reviewed and approved by the Animal Welfare Committee of EGIS Pharmaceuticals Ltd.

2.2. Experimental design

Experiment 1a—Rats were either sham operated or underwent chemical lesions. Intact rats were also used as comparison. Following 1 week recovery, rats were either left undisturbed in their home-cage, or submitted to electric shocks for 5 min. The following groups were formed: (1) intact rats, not shocked; (2) intact rats, shocked; (3) sham operated, not shocked; (4) sham operated, shocked; (5) rats with serotonergic lesions (5-HTx), shocked; (6) rats with noradrenergic lesions (NAx), shocked; (7) shocked rats with double lesions (xx). One day after shock exposure, rats were tested in the social avoidance paradigm, and next day in the open field test. One day later, the brains of rats were sampled, and hippocampal serotonin and noradrenaline levels were assessed to check for the efficacy of chemical lesions (sample size: 10–12 per group).

Experiment 1b was performed for two reasons. Firstly, we assessed whether the surgery per se or chemical lesions affected the sensitivity of rats towards shocks. Secondly, we assessed locomotor behavior one day after shocks. In experiment 1a, rats were tested in the social avoidance and open field tests 24 and 48 h after shocks, respectively (see above). To rule out that a short lasting effect of shocks on locomotion compromised the results of the social avoidance test, we assessed locomotion 24 h after shocks in experiment 1b. Rats were treated as in experiment 1a. The behavior of rats was videotaped during shock exposure. One day later, rats were exposed to the open field test. Sample size in this experiment was eight per group.

Experiment 1c assessed the effects of chemical lesions per se on social behavior. One can hypothesize that the effects of lesions on the shock-induced social deficits were due to lesion-induced differences in the social behavior of lesioned rats. Earlier findings showed, however, that the social avoidance test is insensitive to drug effects in unshocked rats. For example, benzodiazepines that abolished the shock-induced behavioral deficit at low doses, did not change the behavior of unshocked rats [24,25,43]. Preliminary experiments showed that the treatments applied here are also without effect in unshocked rats, despite the fact that they readily affected the shock-induced behavioral deficit. Therefore, the social behavior of lesioned rats was assessed in the social interaction test, which is sensitive to effects on social interactions in rats that are not exposed to shocks [14,15].

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infused over 6 min (flow rate: 1.7 μl per min). Sham operated controls were administered vehicle (saline + ascorbic acid).

Dosage was based on earlier findings and was evaluated in preliminary experiments. Usually, i.c.v. doses of 5,7-DHT are in the 80−100 μg range [48,57]. Our dose (300 μg) was in-between these extremes, and inhibited serotonergic neurotransmission by at least 80% in preliminary experiments. In contrast to 5,7-DHT, DSP-4 is usually injected intraperitoneally (25−100 mg/kg). We did not want to vary the route of administration as compared to 5,7-DHT; therefore, we tested several i.c.v. doses in preliminary experiments, and chose 400 μg i.c.v. that proved equivalent with 50 mg/kg i.p., and inhibited noradrenaline release by at least 80%. Our dose was similar to that used by Chan et al. [12] (250 μg i.c.v.) and Kumar and Karanth [33] (100−400 μg i.c.v.).

To lower unspecific damage, rats lesioned with 5,7-DHT were pretreated with desipramine−HCl (20 mg/kg), whereas rats treated with DSP-4 were pretreated with fluoxetine−HCl (10 mg/kg) to protect noradrenergic and serotonergic neurons, respectively. Such pretreatments (administered intraperitoneally, 30 min prior to surgery) are currently used to, and were found efficient in, preventing non-specific damage by inhibiting the uptake of neurotoxins at non-desired sites [30,52]. However, the serotonergic and noradrenergic neurons reciprocally influence each other by direct mutual connections between the raphe and locus coeruleus [8,31]. Therefore, it was expected that serotonergic lesions would affect noradrenergic neurotransmission and the other way round. Nevertheless, the specific effect is usually stronger, and lesions shift the serotonin/noradrenaline balance in the required direction. Although usually not investigated, the reciprocal connections between the raphe and locus coeruleus probably lead to similar interactions in the case of pharmacological treatments as well.

2.4. Shock exposure

Electric shocks were delivered 1 day before the social avoidance test, via the grid floor of a transparent Plexiglas box (25 cm × 25 cm × 25 cm). An alternating current of 100 V and 3 mA was applied. Shock duration was 0.01 s. Shocks were delivered in trains lasting 1 s with an inter-shock interval of 0.02 s. Two trains per min were delivered over 12 min. Behavior was videotaped, and later analysed by means of a computer-based event recorder. The following behaviors were assessed: grooming (washing with forepaws and scratching with hindpaws); exploration (walking and/or sniffing the floor, cage walls, or sniffing in the air), resting (no locomotion or sniffing; small postural changes allowed), freezing (no movements other than respiration), escape jumps. Behavior during shock application (1 s every 30 s) was not assessed because the shortness of shocks prevented a correct evaluation of behavior. In principle, all rats vigorously vocalized, jumped, and ran across the cage during current application.

2.5. Behavioral testing

2.5.1. The social avoidance test

The test was performed in two plastic cages connected by a sliding door. The subject was placed in the smaller cage (surface: 15 cm × 50 cm) for a 3 min habituation period. The larger cage (surface: 40 cm × 40 cm) was divided into two equal compartments by a transparent, perforated plastic wall. The distant compartment contained a large unfamiliar male. After the habituation period, the sliding door was removed, and the subject was allowed to explore the cage for 5 min. The cages were open in the upper part; their walls were 40 cm high. The experimental room was lit by dim red light. Behavior was video-recorded from above and later analysed. Two variables were recorded: the number and duration of visits made to the compartment containing the opponent ("opponent entries" and "%opponent time", respectively).

Locomotor activity was measured in a photobeam system (Biochemical Laboratory Service Inc., Hungary). Rats were placed into the corner of a Plexiglas chamber (57 cm × 20 cm × 28 cm) and were allowed to explore it for 15 min. Horizontal activity was recorded as a consecutive break of two photobeams, spaced at 190 mm from the walls and each other, and placed 40 mm above floor.

The social interaction test was performed as described in File and Hyde [14] and modified by Guy and Gardner [23] and File and Johnston [15]. Briefly, lesioned rats were familiarized with the test apparatus after one week recovery from surgery, by being placed individually twice into the test arena for 10 min on the two days that preceded testing. One day after habituation, pairs of rats were placed into the test arena for 10 min. The members of pairs were submitted to the same type of surgery. The social interaction test arena was a plastic box of 40 cm × 60 cm × 60 cm with wood shaving bedding. Boxes were lit by white light. The front wall of the box was made of transparent plastic. Six boxes are used in parallel. Fresh bedding was used for each session. Behavior was video recorded through the transparent front wall, and was later analyzed by an experimenter blind to the treatments. The following behavioral variables were considered: exploration/walking, grooming, social interactions (sniffing partner, following, allogrooming), agonistic interactions (aggressive grooming, wrestling, defensive upright, immobility, flight) and dominance-related behaviors (dominant posture, submissive posture).

2.6. The assessment of chemical lesion efficacy

5,7-DHT and DSP-4 leave the cell bodies intact, but destroy axon terminals [30,18,52]. A partial destruction of cell bodies occurs usually half a year after treatment [19]. Therefore, the degree of the damage cannot be evaluated by histological techniques. Usually, the efficacy of treatments is evaluated by assessing the brain levels of noradrenaline and serotonin. It was shown earlier that the monoamine content of different brain regions undergoes parallel changes after chemical lesions with DSP-4 and 5,7-DHT [20,52,57]. Therefore, the efficacy of chemical lesions was assessed here by measuring the hippocampal levels of serotonin and noradrenaline. We mention that the hippocampus is rich in both serotonergic and noradrenergic terminals, for which it can be considered a good representative site for assessing the efficacy of chemical lesions. The dopamine content of the nucleus accumbens was also assessed.

2.6.1. The assessment of noradrenaline and serotonin in the hippocampus

After brain sampling, the hippocampus was quickly removed and stored at −70 °C until analysis. Biochemical assessments were done by the method of Adams and Mansden [5]. Briefly, the brain was homogenized in perchloric acid that contained antioxidant and EDTA. Homogenates were centrifuged and the supernatant was assessed for serotonin and noradrenaline content by means of reverse phase high-performance liquid chromatography with electrochemical detection. We used a Beckman System Gold HPLC (column: C-18 ESA Cathecolamine HR-80) and ESA Coulochrom II electrochemical detector. The mobile phase consisted of NaH2PO4, octanesulphonic acid (as ion-pair reagent), EDTA (pH 3.1) and acetonitrile. The working electrode potential was set to +250 mV. Monoamine content was expressed as ng/g wet weight.

2.6.2. The assessment of dopamine and its metabolites (DOPAC, HVA) in nucleus accumbens

Tissues were homogenized in 0.2N perchloric acid (for deproteinization), containing 0.4 mM Na2S2O5 as an antioxidant and 0.5 mM EDTA. Homogenates were centrifuged at 20,000 rpm (47,900 × g) for 15 min at 4 °C. 50 μl of supernatant were used for analysis of dopamine and its metabolites the 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) without any further purification, by means of high-performance liquid chromatography (HPLC) with electrochemical detection. A Beckman System Gold HPLC was used. The HPLC system consisted of an autosampler (Beckman 502), a pump (Beckman 126), a reversed phase column (C-18 ESA Cathecolamine HR-80, 3 μm particle size) with a precolumn (C-18 BST Rutin, 10 μm particle size), a conditioning cell (ESA, Model 5021), an analytical cell (ESA, Model 5011), an electrochemical detector (ESA Coulochrom II) and an analog interface (Beckman 406). The mobile phase consisted of 75 mM NaH2PO4, H2O, 1.4 mM octane-1-sulphonic acid Na, 50 μM EDTA-Na2 and 5% acetonitrile, was adjusted to pH 3.1 with phosphoric acid, then filtered on 0.22 μm Millipore filter and degassed. The flow rate was 1 ml/min and the working electrode potential was set to +320 mV. Contents of dopamine and its metabolites were quantified by comparing the peak area obtained with those produced by known concentration of standards and were expressed as ng/g tissue.

2.7. Drugs

Equithesin was prepared from chloral-hydrate (4.2 g), magnesium-sulfate-7-hydrate (2.12 g), nembutal (pentobarbital-Na) (16.2 ml), 1,2-propyleneglycol
(40.0 ml), ethanol 96% (10 ml) and distilled water (30 ml). These compounds were purchased from Sigma–Aldrich Ltd. (Hungary), and Reanal Ltd. (Hungary). The NMDA blocker MK-801, the selective serotonin neurotoxin 5,7-dihydroxytriptamine (5,7-DHT) and the selective noradrenaline neurotoxin N-(2-chloroethyl)-N-ethyl-2-bromobenzylamine–HCl (DSP-4) were purchased from Sigma–Aldrich Ltd. (Hungary). Fluoxetine–HCl and desipramine–HCl were synthesized in EGIS Pharmaceuticals Plc. (Hungary). The compounds used in HPLC assessments were purchased from Sigma-Aldrich Ltd. (Hungary).

2.8. Statistics

Treatment effects were assessed by one-factor ANOVA, and Fisher LSD post hoc analysis. The relationship between the efficacy of chemical lesions (i.e. hippocampal noradrenaline and serotonin levels) and behavior was assessed by multiple regression analysis and the Pearson correlation test.

3. Results

3.1. The effects of chemical lesions on monoamine release

Sham operated and intact rats showed similar serotonin and noradrenaline levels in the hippocampus. In intact but not sham operated rats, shock exposure slightly reduced both serotonin and noradrenaline content. Chemical lesions brought about dramatic changes in hippocampal monoamine levels. 5,7-DHT decreased serotonin levels by about 95%, whereas DSP-4 decreased noradrenaline levels by about 80% (F(6,67) = 80.69; p < 0.0001, and F(6,67) = 51.99; p < 0.0001 for hippocampal serotonin and noradrenaline levels, respectively) (Fig. 1). A small decrease in nucleus accumbens dopamine levels was also noticed; however, the change was well below 20%. In addition, DOPAC and HVA levels did not change. Therefore, the insignificant changes noticed in dopamine neurotransmission were not considered when the effects of shocks were evaluated. Serotonergic lesions also decreased noradrenaline levels by about 30%. Noradrenergic lesions affected serotonin levels even more strongly (the decrease in serotonin release was around 65%). Despite these parallel changes in the two systems, the lesion of serotonergic neurons shifted monoamine balance towards a noradrenergic dominance, whereas the lesion of noradrenergic neurons shifted the balance towards a serotonergic dominance. This was shown by the 5-HT/norepinephrine ratio, which was 0.06 ± 0.01 in rats with serotonergic lesions, and 1.62 ± 0.41 in those with noradrenergic lesions. The difference between the two values was highly significant (p < 0.0001). Noteworthy, the 5-HT/norepinephrine ratio was 0.75 ± 0.07 in sham operated controls, a value that differed significantly from the values seen in rats with chemical lesions (p < 0.005 and p < 0.0006 compared to rats with serotonergic and noradrenergic lesions, respectively). Rats with double lesions also showed noradrenergic dominance, but at very low noradrenaline brain levels (20.7% of control).

3.2. The behavior of lesioned rats during shock delivery

As compared with unshocked controls, shock exposure abolished grooming, markedly reduced exploration, and dramatically increased resting and freezing (Table 1). Escape jumps were noticed only during the application of the current, but not in-between. Neither sham operation, nor chemical lesions affected this pattern of behavioral responses, demonstrating that the treatments did not affect the sensitivity of rats towards shocks.

3.3. Behavior in the social avoidance test

One day after shock exposure, both the time spent with, and the frequency of, opponent visits were reduced (F(6,67) = 9.45, p < 0.0001, and F(6,67) = 6.77, p < 0.0001 for opponent time and opponent visits, respectively). The effect was produced in both intact and sham operated rats (Fig. 2). The lesion of serotonergic neurons aggravated the shock-induced behavioral deficit, whereas the noradrenergic lesion abolished the behavioral response to shocks. Thus, serotonergic neurotransmission inhibited, whereas noradrenergic neurotransmission promoted the development of shock-induced social avoidance. Multiple regression and correlation analysis supported this assumption. Hippocampal noradrenaline and serotonin levels (i.e. the efficacy of chemical lesions) predicted significantly behavior in the social avoidance test (for time spent in opponent chamber: multiple R = 0.448; F(2,71) = 8.93; p < 0.001; for opponent entries: multiple R = 0.293; F(2,71) = 3.33; p < 0.04). The same analysis showed that serotonin levels correlate positively, whereas noradrenaline levels correlate negatively with the duration of opponent visits (beta for serotonin = 0.600, p < 0.0001; beta for noradrenaline = −0.220, p < 0.03). To further assess the interaction between the efficacy of chemical lesions and behavior, we calculated the noradrenaline-serotonin ratio, and assessed

Fig. 1. Effects of surgeries on brain serotonin and noradrenaline levels. Chemical lesions were efficient as serotonin and noradrenaline levels decreased by 95 and 80%, respectively. Despite some parallel changes, the balance between the two neurotransmitters shifted to the expected direction (see Section 3.1). Sh, sham operation; 5-HTx, serotonergic lesion; NAx, noradrenergic lesion; xx, double lesion; * significant difference from both shocked and unshocked control; ** significant difference between 5-HTx and NAx rats. N = 10–12 per group.
Table 1

<table>
<thead>
<tr>
<th>Group</th>
<th>Shock</th>
<th>Grooming ± S.E.M.</th>
<th>Exploration ± S.E.M.</th>
<th>Freezing ± S.E.M.</th>
<th>Resting ± S.E.M.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intact</td>
<td></td>
<td>5.1± ± 1.2</td>
<td>88.4± ± 1.3</td>
<td>0.1± ± 0.1</td>
<td>0.5± ± 0.3</td>
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<tr>
<td>Sham</td>
<td>+</td>
<td>0.1± ± 0.1</td>
<td>18.8± ± 3.6</td>
<td>53.6± ± 5.1</td>
<td>10.9± ± 1.8</td>
</tr>
<tr>
<td>5-HT lesion</td>
<td></td>
<td>6.1± ± 1.8</td>
<td>80.4± ± 5.7</td>
<td>6.1± ± 5.7</td>
<td>1.2± ± 0.8</td>
</tr>
<tr>
<td>NA lesion</td>
<td>+</td>
<td>0.0± ± 0.0</td>
<td>16.5± ± 3.6</td>
<td>56.6± ± 7.2</td>
<td>11.8± ± 2.7</td>
</tr>
<tr>
<td>Double lesion</td>
<td>+</td>
<td>0.0± ± 0.0</td>
<td>12.6± ± 1.9</td>
<td>59.8± ± 5.3</td>
<td>11.1± ± 2.1</td>
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<tr>
<td>5-HT lesion + NA</td>
<td>+</td>
<td>0.0± ± 0.0</td>
<td>19.6± ± 2.8</td>
<td>52.5± ± 4.0</td>
<td>11.3± ± 0.3</td>
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<tr>
<td>5-HT lesion + NA</td>
<td></td>
<td>0.0± ± 0.0</td>
<td>19.3± ± 2.8</td>
<td>55.4± ± 4.1</td>
<td>15.2± ± 2.0</td>
</tr>
</tbody>
</table>

Data show the mean duration of behaviors (expressed as % time) ± the standard error of the mean (S.E.M.). Data labeled with different letters differed significantly in post hoc comparisons (p<0.01 at least). Shock exposure abolished grooming, markedly reduced exploration, and markedly increased both resting and freezing. Chemical lesions did not affect significantly this pattern of changes. Thus, lesion-induced behavioral dysfunctions are not due to changes in shock responsiveness.

3.4. Locomotor effects

Locomotor behavior was affected by surgery neither 24 h (F(6,50)=0.29; p>0.9) nor 48 h after treatments (F(6,67)=0.41; p<0.9) (Fig. 6). Thus, the shock-induced social deficit could not be explained by shock-induced changes in locomotion.

3.5. Behavior in the social interaction test

The social behavior of rats was not affected by either lesion as shown by the social interaction test (Table 2). Thus, the differences seen in the social avoidance test were not due to lesion-induced differences in social behaviors, but rather reflected a differential long-term response to shock exposure. We note that dominance-related behaviors (dominant and submissive postures) were scarce (their duration was less than 2% time). Resting, flight and immobility (freezing) were absent.
Table 2

The effect of lesions in the social interaction test

<table>
<thead>
<tr>
<th>Group</th>
<th>EXP ± S.E.M.</th>
<th>SOC ± S.E.M.</th>
<th>GRO ± S.E.M.</th>
<th>ATT ± S.E.M.</th>
<th>AGO ± S.E.M.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham</td>
<td>64.90 ± 5.16</td>
<td>9.03 ± 1.12</td>
<td>0.44 ± 0.21</td>
<td>0.29 ± 0.22</td>
<td>21.11 ± 5.47</td>
</tr>
<tr>
<td>5-HTx</td>
<td>65.57 ± 3.86</td>
<td>9.30 ± 0.81</td>
<td>1.52 ± 1.04</td>
<td>0.00 ± 0</td>
<td>20.59 ± 3.59</td>
</tr>
<tr>
<td>NAx</td>
<td>69.56 ± 2.66</td>
<td>10.01 ± 1.13</td>
<td>0.50 ± 0.25</td>
<td>0.45 ± 0.2</td>
<td>14.93 ± 3.22</td>
</tr>
<tr>
<td>xx</td>
<td>64.23 ± 4.14</td>
<td>9.60 ± 1.39</td>
<td>0.55 ± 0.20</td>
<td>0.62 ± 0.46</td>
<td>22.96 ± 4.88</td>
</tr>
<tr>
<td>H(3, 54)</td>
<td>1.24</td>
<td>0.54</td>
<td>2.15</td>
<td>5.11</td>
<td>2.16</td>
</tr>
<tr>
<td>p</td>
<td>0.7</td>
<td>0.9</td>
<td>0.5</td>
<td>0.16</td>
<td>0.5</td>
</tr>
</tbody>
</table>

Data show the mean duration of behaviors (expressed as %time) ± the standard error of the mean (S.E.M.). As attacks are very short in rats, the frequency was given for this variable. Sham, sham operated; 5-HTx, serotonergic lesion; NAx, noradrenergic lesion; xx, double lesion; H(3, 50), Kruskall–Wallis coefficient; p, statistical significance; EXP, exploration; SOC, social interactions; GRO, grooming; ATT, attack counts; AGO, agonistic interactions. No significant differences were noticed between groups.

Fig. 4. The glutamate receptor blocker MK-801 abolished the effects of electric shocks on social avoidance when applied before shocks (i.e. 24 h before behavioral testing). Columns labeled with different letters showed statistically significant differences in post hoc comparisons. N = 8 per group.

3.6. The impact of NMDA blockade on the shock-induced behavioral deficit

When applied 30 min before shock exposure (i.e. 24 h before behavioral testing), the NMDA blocker MK-801 dose dependently inhibited the development of shock-induced social avoidance (Fig. 4) \( F(3,28) = 19.71, p < 0.0001 \), and \( F(3,28) = 11.65, p < 0.0001 \) for opponent time and entries, respectively. Moreover, the same antagonist prevented the expression of the behavioral deficit when applied 30 min before behavioral testing (i.e. 24 h after shock exposure) \( F(2, 24) = 11.71, p < 0.0003 \) and \( F(2,24) = 10.95, p < 0.0005 \, \) for opponent time and entries, respectively (Fig. 5).

4. Discussion

4.1. Main findings

Chemical lesions were successful, as the serotonin/noradrenaline balance shifted to the expected direction: serotonergic lesions induced strong noradrenergic dominance, whereas noradrenergic lesions induced serotonergic dominance. The noradrenergic dominance aggravated the shock-induced behavioral deficit, whereas the serotonergic dominance abolished it. These findings (also supported by correlation analysis) demonstrate that noradrenergic neurotransmission promotes, whereas serotonergic neurotransmission inhibits the development of stress-induced social avoidance. The differential response to shock exposure was not due to lesion-induced changes in social behavior, as lesions did not affect behavior in the social interaction test. Surprisingly, the behavioral deficit did develop in rats with double lesions, suggesting that the two monoaminergic systems rather modulate than induce the stress-induced behavioral deficit. NMDA blockade abolished the development of stress-induced social avoidance both when applied before shocks and when applied before behavioral testing. Based on these findings, we hypothesize that the lasting effects of traumatic experience on behavior are mediated by an NMDA-dependent mechanism.
and is modulated by the balance between serotonergic and noradrenergic neurotransmission.

4.2. The effect of chemical lesions on brain monoamine content

Chemical lesions have been used from the mid-1970s to elucidate the involvement of noradrenergic and serotonergic neurotransmissions in the control of various behaviors (see e.g. [11,34,51,59]). When appropriate precautions are taken (e.g. 5,7-DHT and DSP-4 treatments are combined with desipramine and fluoxetine, respectively), non-specific damage is insignificant [30,52]. Nevertheless, serotonergic and noradrenergic neurons reciprocally influence each other by direct mutual connections between the raphe and locus coeruleus [8,31]. More recently, it was demonstrated that the serotonergic system directly contributes to the regulation of extracellular norepinephrine concentration in the CNS [56]. This study showed that serotonergic varicosities can accumulate and release norepinephrine as a result of the heterologous uptake of transmitters. This remarkable finding clearly shows that there is a dynamic interaction between these two monoaminergic systems.

Not surprisingly, the selective destruction of one neuron type (serotonergic or noradrenergic) induces changes in the function of the other. In line with our findings, earlier studies showed that selective serotonergic lesions reduced noradrenaline release, whereas selective noradrenergic lesions reduced serotonin release in various brain regions [9,46,47]. The specific effect was, however, stronger with both lesions: serotonergic lesions decreased serotonin levels more strongly than noradrenaline levels, whereas noradrenergic lesions had a more pronounced effect on noradrenaline than on serotonergic levels. Therefore, selective serotonergic lesions result in a relative increase in noradrenaline release, whereas the selective noradrenergic lesion results in the relative increase of serotonergic release. In our sample, chemical lesions shifted serotonin/noradrenaline balance in the expected direction, and allowed the study of the effect of this balance on trauma-induced behavioral dysfunctions.

4.3. The social avoidance test

The behavioral endpoint of the experiments reported here was the social avoidance test developed earlier [24,25], and recently validated pharmacologically [35]. We showed that electric shocks induce social avoidance that lasts about 10 days. Shocked rats also avoided the exploration of empty cages, but the avoidant response was two times stronger when the cage contained a potential opponent confined behind a Plexiglas wall (the difference was, naturally, significant; [24]). Thus, the response studied here depended on a social context. Anxiolytic compounds (e.g. chlordiazepoxide, diazepam, buspirone, and fluoxetine) abolish this response; the anxiogenic compound m-chlorophenylpiperazine mirrors it in unshocked animals, whereas substances neutral to anxiety (e.g. haloperidol) do not affect social avoidance. Our findings were recently replicated by an independent group [43]. The test is similar to a certain degree to the conditioned fear test. However, it is able to evidence shock-induced behavioral deficits in a context different from the one where the shocks were applied, i.e. it shows a generalized-like anxiety.

4.4. The impact of 5-HT, NA and glutamate on stress-induced behavioral deficits

The impact of serotonergic and noradrenergic neurotransmission on shock induced behavioral deficits is not entirely unexpected. In response to electric shocks, serotonin and noradrenaline release increase in extended brain regions including the amygdala, cerebral cortex, dorsal raphe, hypothalamus, midbrain, locus coeruleus, thalamus, ventral hippocampus, etc. [27,36,6,7,39,53,58]. Our data suggest that this response plays a role in the development of shock-induced behavioral deficits as the shift towards noradrenaline dominance promoted, whereas the shift towards serotonergic dominance inhibited shock-induced social deficits. This is in line with human findings. Both selective serotonin reuptake inhibitors (i.e. the enhancement of serotonergic neurotransmission) and α1 and β adrenoceptor blockade (i.e. the inhibition of noradrenergic neurotransmission) mitigate various symptoms of post-traumatic stress disorder [13,32,44,45,54]. Similar findings were obtained...
in laboratory studies. The enhancement of serotonergic neurotransmission by various means reduced the behavioral effects of traumatic experience in rats [28,49,26], whereas noradrenergic lesions reduced the freezing response to various aversive stimuli [42,37]). In addition, both α1 antagonists and α2 agonists (the latter inhibiting noradrenaline release by stimulating the presynaptic autoreceptor) inhibited conditioned fear responses [21,50]. Taken together, these earlier and our present findings show that noradrenergic neurotransmission promotes, whereas serotonergic neurotransmission inhibits the development of shock-induced behavioral deficits. In addition, our data suggest that the balance rather than the amplitude of monoamine output is important. When serotonergic neurotransmission was strongly inhibited, noradrenergic neurotransmission promoted shock-induced deficits even when it was reduced as compared with sham operated controls. Similarly, even a decreased serotonergic neurotransmission inhibited the shock-induced behavioral deficits when noradrenergic neurotransmission was inhibited even more strongly. The importance of the balance between the two systems is best illustrated by the fact that the behavioral outcome of shock exposure was similar in sham operated rats and those submitted to double lesions. These findings also show that serotonergic and noradrenergic neurotransmissions only modulate but do not induce shock-induced behavioral deficits.

Noteworthy, various serotonin receptors appear to play different roles in stress-induced behavioral deficits, despite the fact that serotonin neurotransmission in general is protective. The involvement of particular 5-HT receptors was recently studied by Adamec et al. [1,2]. Findings of both studies are consistent in that serotonin neurotransmission in general is protective. The selective serotonin reuptake inhibitor and 5-HT1A receptor antagonist vilazodone blocked the consolidation of stress effects on behavior, consistent with a protective function of 5-HT. In contrast, 5-HT2A antagonism also blocked stress effects on behavior suggesting an enabling function of this 5-HT receptor in the consolidation of stress effects on affect.

Earlier findings suggested that the main mechanism underlying stress-induced behavioral deficits is glutamatergic, and involves the NMDA receptor. It was shown for instance that the blockade of NMDA receptors in rats with MK-801, AP7, or CPP, given 30 min prior to exposure to a cat, prevented the increase in anxiety-like behavior assessed 1 week later [3]. The same NMDA blockers were without effect when administered 30 min after predator exposure, suggesting that NMDA receptors are involved in the initiation but not the maintenance of neural changes mediating lasting increases in anxiety following predator exposure. NMDA receptors were also implicated in conditioned fear responses or neural processes (e.g. long-term potentiation) that underlie lasting behavioral responses to shocks [17,41]. Noteworthy, both cat exposure and conditioned fear are believed to model symptoms of post-traumatic stress disorder [16,22,29,4]. In line with these earlier observations, shocks did not affect social avoidance when administered to rats with blocked NMDA receptors. Interestingly, the blockade of NMDA receptors inhibited not only the development, but also the expression of the shock-induced social deficit.

Based on present and earlier findings, we hypothesize that the lasting behavioral dysfunctions that result from traumatic experience derive from glutamatergic activation, the effects of which are mediated by NMDA receptors. The development of the behavioral deficit is modulated by the balance between serotonergic and noradrenergic neurotransmission, possibly via effects on shock-induced glutamatergic activation. Behavioral effects are diminished when the balance is shifted towards serotonin, and exacerbated when it is shifted towards noradrenaline. One can hypothesize that individual differences in this balance may underlie individual differences in the susceptibility to trauma-induced behavioral dysfunctions.

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References


