A single social defeat induces short-lasting behavioral sensitization to amphetamine

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Abstract

Repeated, intermittent exposure to psychostimulants or stressors results in long-lasting, progressive sensitization of the behavioral effects of a subsequent amphetamine (AMPH) challenge. Although behavioral sensitization has also been observed following a single drug pretreatment, the sensitizing potential of a single exposure to stress is not clear. Both drug- and stress-induced sensitization depend on an enhanced dopaminergic neurotransmission in the mesolimbic DA system. Apart from responding to rewards, this system is also involved in responding towards aversive social stimuli. Therefore, social stressors may be particularly effective in inducing cross-sensitization to stimulant drugs. We examined the time course of sensitization to the locomotor effects of the stimulant, AMPH, following a single social stressor: a social defeat. Wistar rats were exposed in a resident–intruder paradigm to an unfamiliar dominant male conspecific (Wild-Type Groningen), resulting in defeat. The locomotor effects of a subsequent AMPH challenge (0.25 or 1.0 mg/kg) were evaluated 3, 14, and 21 days later by scoring horizontal movement in an open field. AMPH had significantly larger locomotor-activating effects in animals that had been defeated 3 days earlier compared to nondefeated controls. However, this sensitized response was no longer present 14 or 21 days after defeat. Therefore, we conclude that social defeat induces short-lasting cross-sensitization to the locomotor effects of AMPH in rats, but is not sufficient for long-term sensitization. The transient enhancement of responses to dopaminergic drugs may be indicative of a temporary role of dopamine in the cascade of physiological and behavioral changes following social defeat.

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1. Introduction

Repeated, intermittent administration of amphetamine (AMPH) and related stimulants results in a long-lasting, progressive increase in the behavioral response to a subsequent challenge with the drug [1–3]. This phenomenon is termed “behavioral sensitization” and has been linked to a persistent hyperactivity of the mesolimbic dopamine (DA) system after repeated activation. The induction of the processes leading to sensitization appears to depend on an action of stimulant drugs in the ventral tegmental area (VTA), specifically on somatodendritically released DA from the VTA dopaminergic neurons. The expression of sensitization is related to an increase of DA release in the nucleus accumbens (NAc) in response to further psychostimulant administration [1,2,4-5]. The mesolimbic DA system is thought to be involved in motivation, and responding to rewards and sensitization of this system has been associated with an increased vulnerability to drug abuse [6-10]. Disturbances of the dopaminergic system may also be involved in the development of psychotic and depressive disorders [3,5,11,12].

Besides responding to rewards, the mesolimbic DA system is also involved in responding towards aversive stimuli. Indeed, it has been shown that exposure to stressors, such as tail pinch, restraint, electric shocks, or food restriction, induces augmented locomotor responses to stimulants as well [1,13-16]. This phenomenon was first
described by Antelman et al. [13] and is referred to as stress-induced cross-sensitization. Cross-sensitization to psychostimulants induced by previous stress may be one of the factors influencing individual variation in susceptibility to drug abuse [6,12,17].

Most reports have employed repeated stressors to induce cross-sensitization. Studies examining single exposures to stressors have produced contradictory results [12,18–21]. In general, stressors, such as restraint or foot shocks, administered several times result in sensitization to psychostimulant drugs, whereas single stress exposure has no effect [18,19] or may even promote hyposensitivity [12]. This is somewhat surprising, since a single high stimulant dose does induce a slowly developing, but long-lasting sensitization to subsequent psychostimulants [22,23].

Because of the specific involvement of the mesolimbic DA system in species-specific defensive behavior to aggressive conspecifics [12,24–25], social stressors may be particularly effective in inducing cross-sensitization to stimulant drugs. Social defeat by a dominant rat is a very potent, natural stressor that elicits a very strong acute stress response and induces long-term changes in several behavioral and physiological parameters [26–34]. Some of these, such as enhanced responding to novelty, may be related to changes in DA functioning. Therefore, this stressor may be sufficiently salient to induce sensitization even after a single presentation. Recently, it has been reported that a single social defeat experience induces behavioral sensitization to cocaine in mice [35]. However, it appears that the time course of this single defeat-induced sensitization is much shorter than that generally reported after pharmacologically induced sensitization or sensitization following repeated stress.

The aim of the present study was to determine whether a single exposure to a defeat by a dominant rat induces cross-sensitization to the locomotor effects of AMPH. To establish the time course of this sensitization, we measured the locomotor response to AMPH 3 days, 2 weeks, and 3 weeks after defeat by scoring horizontal movement in an open field.

2. Materials and methods

2.1. Animals and housing conditions

All procedures in this study were approved by the Committee on Animal Bioethics of the University of Groningen, The Netherlands. Male Wistar rats, 3 months old, were group-housed until the start of the experiment, after which they were housed individually in clear Plexiglas cages (40×23×15 cm) on a layer of wood shavings. The light/dark cycle was fixed at 12/12 h (lights on at 0700 h) and temperature was maintained at 21 °C. Food and water were available ad libitum. All experimental procedures were conducted between 1000 and 1600 h. Due to the number of animals, the experiment was conducted in four series.

2.2. Social defeat

Rats were removed from their groups, weighed, transferred to a separate test room, and subjected to a social defeat. The social defeat consisted of placing the experimental rat (intruder) in the cage of an aggressive male conspecific (resident). Resident rats were of a wild-type strain and housed in large cages (80×55×40 cm) with a female to stimulate territorial aggression. They were trained on a regular basis by confronting them with naive male intruders, and only animals with attack latencies shorter than 2 min were used. One hour before the start of the defeat, females were removed from the resident’s cage.

The total social defeat procedure lasted 1 h, during which rats were attacked for a standard period of 15 min. Subsequently, animals were removed from the cage, placed in a protective wire mesh cage (30×15×15 cm), and returned to the resident’s cage for the remainder of the hour. During this period, rats were protected from further attacks and injury, but remained in full auditory, olfactory, and visual contact with the resident. This period of close proximity of the resident is known to be highly stressful for the intruder rat [36].

After the defeat procedure, animals were returned to their room and remained housed individually. Individual housing increases the behavioral and physiological effects of social defeat [29,34]. Control animals were also removed from their group, placed in a different position in the room for a period similar to social defeat, and housed individually for the rest of the experimental period as well.

2.3. Determination of locomotor activity

To determine the time course of cross-sensitization by the social stressor, challenges with AMPH were performed 3 days, 2 weeks, or 3 weeks after defeat. Separate groups of rats were used for each time point and dosage, so each animal was challenged only once. Locomotor responses to AMPH were determined in an open field arena. Since several authors have shown a reduction in open field activity after a social defeat [28,29,33], we decided to include a separate saline-injected group for each AMPH-injected group to be able to adjust for changes in basal activity in the defeated animals.

Animals were injected with 0.25 or 1.0 mg/kg d-AMPH, s.c., or saline and transferred to a separate test room. Twenty minutes after injection, locomotor activity was determined in a large circular arena with a diameter of 120 cm, surrounded by a 30-cm-high wall (open field). Animals were placed in the center of the arena at
the beginning of the test. Locomotor behavior was recorded during 10 min with a videocamera and automatically analyzed (Ethovision; Noldus Information Technology, Wageningen, The Netherlands).

2.4. Statistical analysis

Results are presented as mean±S.E.M. Statistical analysis was performed using the SPSS software package (version 11.0) and a probability level of \( p < 0.05 \) was considered significant.

To assess the effect of social defeat on body weight gain, defeat and control groups were compared by using analysis of variance (ANOVA) for repeated measurements with days as within-subjects factor and defeat (control and defeat) as between-subject variables. Because animals were sacrificed immediately following AMPH challenge, which decreased the number of animals per group over the course of the experiment, each period was tested separately. This resulted in three time periods: 0–3 days, 3–14 days, and 14–21 days. For post-hoc analysis of the various time points, we used pairwise comparisons (least significant difference or LSD) based on estimated marginal means.

Locomotor data for AMPH-injected groups are expressed relative to their saline-injected controls. For calculations of individual locomotor responses, we determined the percentage of locomotor activity by dividing the activity of each AMPH-injected animal by the average of their saline-injected control group. Since no time effect of the control treatment was expected, the number of animals in these groups was reduced. After ANOVA had shown no differences between the control animals tested on the three different time points, animals were pooled and taken as a single group. The effect of social defeat on locomotor activity was analyzed by ANOVA with group (control, defeat day 3, defeat day 14, and defeat day 21) as between-subject variable. Post-hoc analysis was performed by multiple comparisons (LSD).

3. Results

All animals were attacked during the social defeat procedure within 2 min after being placed in the territory of the dominant rat. Social defeat resulted in a submissive posture in all intruders.

Fig. 1 show the long-term effects of social defeat on body weight. Because there were small differences in body weight between the eight groups, body weight was expressed as delta increase relative to the weight just before the defeat. Social defeat initially resulted in a small weight loss and suppressed growth for the first 3 days. Analysis with ANOVA for repeated measurements revealed an overall effect of defeat (\( F_{1,125}=19.005, \ p<0.001 \)) and an interaction between time and defeat (\( F_{3,375}=11.470, \ p<0.001 \)). During the second period (3–14 days), animals resumed their normal growth rate, yet body weight was still suppressed compared to controls (overall effect of defeat (\( F_{1,81}=12.388, \ p=0.001 \)), but no interaction effect of time\times\text{defeat (\( F_{3,243}=1.241, \ p=0.296 \)). During the last 7 days, defeated animals caught up with their control counterparts and, by 18 days, weight gain was no longer different from controls (significant interaction effect of time\times\text{defeat (\( F_{2,80}=4.507, \ p=0.014 \)), but no overall effect of defeat (\( F_{1,40}=0.428, \ p=0.517 \))}.

Responses to the low and high doses of AMPH are shown in Table 1 and Fig. 2. Both the 0.25- and 1.0-mg/kg doses of AMPH increased locomotor activity (Table 1). For the nonstressed controls (Fig. 2, panel A), AMPH-injected animals covered a larger distance in the

Table 1

<table>
<thead>
<tr>
<th>Group</th>
<th>0.25 mg/kg AMPH</th>
<th>1.0 mg/kg AMPH</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Saline (m)</td>
<td>AMPH (m)</td>
</tr>
<tr>
<td>Control</td>
<td>46.9±2.0</td>
<td>62.7±2.4*</td>
</tr>
<tr>
<td>Defeat day 3</td>
<td>37.2±4.4</td>
<td>63.2±2.5*</td>
</tr>
<tr>
<td>Defeat day 14</td>
<td>48.9±4.8</td>
<td>57.8±4.0*</td>
</tr>
<tr>
<td>Defeat day 21</td>
<td>49.7±2.4</td>
<td>57.9±2.4*</td>
</tr>
</tbody>
</table>

* \( p<0.05 \) vs. equivalent saline-injected group.

** \( p<0.05 \) vs. control.

Fig. 1. Effects of social defeat on body growth. Data are presented as delta increases in body weight compared to body weight shortly preceding social defeat (mean±S.E.M). Compilation of all groups.
10-min test compared to their saline-injected counterparts ($F_{1,13}=24.962$, $p<0.001$ and $F_{1,17}=10.871$, $p=0.004$ for the 0.25- and 1.0-mg/kg groups, respectively). Although the increase in traveled distance in the 1.0-mg/kg dose group appeared slightly larger, the dose effect was nonsignificant. A high dose of AMPH may cause stereotyped behavior as well as locomotion, and this would result in lower locomotor activity. Direct observation, however, revealed no clear stereotyped behavior.

Based on previous experiments, we expected a decrease in open field locomotion following defeat [28,29,33]. However, in this study, social defeat did not result in a significant decrease in locomotion in the open field in saline-injected animals ($F_{3,21}=2.621$, $p=0.077$ and $F_{3,28}=0.880$, $p=0.463$ for the 0.25- and 1.0-mg/kg groups, respectively). When challenged 3 days following defeat, the locomotor responses to both the low and high doses of AMPH (Fig. 2, panel B) were increased compared to nondefeated rats. For both the 0.25- and 1.0-mg/kg dose, ANOVA revealed a significant effect of group ($F_{3,26}=14.020$, $p<0.001$ and $F_{3,32}=3.969$, $p=0.016$, respectively), with animals defeated 3 days earlier having a significantly higher AMPH-induced increase in distance moved compared to controls ($p=0.001$ and $p=0.031$). AMPH challenges 2 and 3 weeks after defeat produced similar levels of locomotor activity in defeated and control animals.

4. Discussion

The results demonstrate that a single exposure to the stress of a social defeat is sufficient to induce behavioral sensitization to a subsequent challenge with AMPH. The locomotor responses to both AMPH doses were significantly increased in animals that experienced a defeat by a dominant rat. However, this sensitizing effect of social defeat appears to be short-lasting, since sensitization was only present 3 days after defeat.

AMPH administration in control animals caused an increase in open field activity 20–30 min later. The locomotor-activating effects of AMPH are well established [2] and are clearly shown by the larger total distance moved in the AMPH-injected control groups as compared to their saline-injected control groups. There was no significant effect of dose: both the 0.25- and 1.0-mg/kg AMPH doses produced approximately the same increase in locomotion. Even though direct observation revealed no clear stereotyped behavior, it cannot be excluded that short bouts of stereotyped behavior did occur. Stereotyped behavior would have reduced the measured distance moved in some animals and may be responsible for the lack of difference between the two dosages and the larger variation seen in the 1.0-mg/kg dose group. The behavioral observations also clearly indicate that social defeat is inducing sensitization to AMPH. The locomotor responses to both dosages of the
A recent report showed that also a single pretreatment with a high dose of AMPH is sufficient to produce behavioral sensitization [22]. However, this AMPH-induced sensitization developed gradually and was most prominent 3 weeks after the initiating dose. Repeated exposure to sensitizing drugs causes sensitization that is long-lasting as well, and may even be permanent [1,2,5]. Most research works into stress-induced cross-sensitization employ repeated or chronic stressors, such as repeated restraint or chronic food restriction, and these appear to induce long-term sensitization to subsequent psychostimulants as well [1,13–16,37–39]. Studies examining the sensitizing potential of single stressors have produced contradictory results, however [12,18–21]. Some suggest that a single stressor is not sufficient to induce sensitization and that long-term sensitization requires several exposures to the same stressor [12,18,19]. On the other hand, a single but prolonged restraint session does produce a sensitized response to AMPH for up to 8 days [20,21].

The fact that also a single exposure to social defeat stress induces sensitization may be related to the nature and severity of this stressor. Social defeat is a natural stressor, which produces a very strong acute stress response as measured by the amount of corticosterone and catecholamines released [40,41]. After this acute response, long-lasting changes in behavior and physiology develop, which include changes in body growth, circadian rhythmicity, neuroendocrine functioning, and behavioral responses to novelty [26–34]. The severity of the social defeat is also clearly demonstrated by the prolonged suppression of body weight gain in the defeated animals in our study. This reduction in body weight gain is probably partly due to a reduction in food intake [33], but changes in thermal balance and energy expenditure may also play a role [28]. Whether sensitization develops could therefore be a function of both the magnitude of the inducing stressor and the number of stress exposures.

Still, the transient nature of sensitization following social defeat demonstrates that the time course of behavioral sensitization after such a single stressor is significantly shorter than sensitization induced by psychostimulants. Similar findings have been reported by Miczek et al. [35]. Mice that had been exposed to a single social defeat stress episode showed an increased locomotor response to a subsequent challenge with cocaine. However, locomotor sensitization was limited to 7 days after the stressor and no longer present 9 days following defeat. Possibly, repeated social defeat has longer-lasting effects analogous to the effects of other repeated stressors. One study employing four consecutive defeats reported behavioral sensitization to both AMPH and cocaine for up to 10 days following the last defeat session [42].

We can only speculate regarding the mechanisms underlying the short-term increase in responding to AMPH following defeat in the present study. Even though there is a significant reduction in body growth in the social defeat groups, it is unlikely that changes in metabolism have caused the increase in locomotor response. An increase in metabolism may have pushed the peak of the locomotor response forward; however, during the period we measured (20–30 min following injection), also the response in the control animals should have been at its maximum. Furthermore, another study using repeated social defeat as a sensitization-inducing stressor found no earlier onset of the locomotor response to morphine but found instead a prolonged effect [19].

Behavioral sensitization is thought to involve a hyperactivity of the mesolimbic DA system [1,2,4,5]. In addition, glutamatergic, GABAergic, serotonergic, and opioid mechanisms have all been implicated in the development of sensitization to psychostimulant drugs [2,5,43–46]. Since repeated stress and psychostimulants appear to be interchangeable in inducing a sensitized response to subsequent psychostimulant administration [13], it has been suggested that behavioral sensitization is dependent on an action of the HPA axis [47]. Although some publications have questioned the role of the glucocorticoids in psychostimulant-induced sensitization [48,49], it is generally assumed that an activation of the HPA axis is necessary for stress-induced sensitization to subsequent stimulants and may at least play a facilitatory role in psychostimulant-induced sensitization as well [6,17,37,47,49–53]. During chronic or repeated stress, recurring increases in CORT and DA may result in sensitization of the mesolimbic DA system [6,17], and cross-sensitization between stress and psychostimulants is indeed accompanied by augmented NAc DA levels and DA release in response to AMPH [1,47,54].

It is not clear whether this extends to sensitization following a single social stressor, however. Social defeat induces a strong acute increase in CORT [40,41] and has also been shown to increase DA in the NAc [24,25,55]. This increase in DA was synchronous with high levels of orienting toward the resident and may therefore be related to the defensive response [25]. Strong activation of these responses during a single social defeat may be sufficient to sensitize the DA system for further stimuli, but the transient nature of the augmented response to AMPH following defeat indicates that this sensitization is not permanent. It has been suggested that short-term and long-term behavioral sensitization, although similar in its expression, are partly dependent on different mechanisms [1,5,49,50]. Apparently, social defeat is able to activate some of the mechanisms accompanying behavioral sensitization, but not sufficiently so to induce long-term effects.

Apart from inducing a sensitized DA response, social defeat may have indirectly caused an augmented locomotor
effect of AMPH via increased HPA axis activation in the novel environment of the open field. Social defeat induces long-lasting, temporal dynamic changes in HPA axis regulation [30,31] and may sensitize animals to subsequent stressors [26,27]. In addition to their potential role in long-term stress-induced sensitization, glucocorticoids have also an acute facilitory effect on AMPH-induced locomotion, probably by augmenting DA transmission in the shell of the NAc [17,47]. So, in addition to inducing long-term changes in DA transmission, glucocorticoids may also acutely affect DA release and DA-induced locomotion. Therefore, a sensitized glucocorticoid response to novelty could explain the enhanced locomotor response to AMPH of the defeated animals in the open field as well.

In conclusion, a single social defeat induces short-lasting cross-sensitization to the locomotor effects of AMPH. Transient changes in DA functioning may very well play a role in the cascade of physiological and behavioral changes observed following social defeat, specifically in altered responding to novelty and reward. The difference in the temporal dynamics between single stress-induced cross-sensitization and psychostimulant-induced sensitization suggests that the two phenomena may partly depend on different underlying mechanisms. Still, the short-term enhancement of responding to dopaminergic drugs indicates that a even single major stress experience may influence an individual’s susceptibility to drug abuse.

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References


