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Short communication

Isolation rearing prevents the reinforcing properties of amphetamine in a conditioned place preference paradigm

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Abstract

Social isolation has been demonstrated to alter individual reactivity to addictive drugs. The present experiments compared the effects of isolation rearing and socially rearing rats on the reinforcing properties of amphetamine. Male Lister hooded rats were housed from 21 days (weaning) either alone (isolation reared rats) or in groups of four rats per cage (socially reared rats). Six weeks later, the rats were tested for their sensitivity to *d*-amphetamine (1.5 and 5 mg/kg) using a conditioned place preference paradigm. The treatment quadrant was selected as that in which the rat spent least time during a preconditioning trial. After saline conditioning, the socially reared rats showed a significant ($P < 0.05$) aversion for the treatment quadrant relative to the opposite quadrant. Following amphetamine (1.5; 5 mg/kg) conditioning, socially reared rats spent significantly more time ($P < 0.05$) in the treatment quadrant relative to the opposite quadrant while isolation reared rats failed to display either the aversion effect of saline conditioning or amphetamine induced place preference. These results demonstrate isolation rearing prevents the reinforcing properties of amphetamine.

Keywords: Isolation rearing; Place preference; *d*-Amphetamine; (Rat)

1. Introduction

Social isolation in the early stages of life can modify the behavioural effect of drugs in later life. Rearing rats in social isolation from weaning has been demonstrated to enhance the locomotor response to *d*-amphetamine (Jones et al., 1990, 1992), and increase the stereotype responses produced by apomorphine and *d*-amphetamine (Einon and Sahakian, 1979). In addition, isolation rearing of rats has been shown to increase the rate of the self-administration of some drugs of abuse such as ethanol (Schenk et al., 1988) opiates (Alexander et al., 1981; Bozarth et al., 1989) and cocaine (Schenk et al., 1986). In contrast other studies have shown no effect of isolation rearing on the self-administration of either *d*-amphetamine (Schenk et al., 1988) or cocaine (Bozarth et al., 1989), while Phillips et al. (1994) found an impairment of cocaine self-administration into the nucleus accumbens. Furthermore, isolation reared rats are less sensitive to opiate-induced analgesia (Kostowski et al., 1977) and conditioned place preference (Schenk et al., 1985), and show less severe opiate withdrawal syndrome (Adler et al., 1975) than socially reared rats. Thus, the effect of isolation rearing on the reinforcing action of psychomotor stimulants remains unclear and the early social environment may be one of the critical factors that determines the behavioural sensitivity of an adult animal to psychomotor stimulant drugs. The present study examines the effect of isolation rearing on the response to amphetamine using a conditioned place preference paradigm to further clarify the effects of early isolation on the adult response to amphetamine in the rat.

2. Materials and methods

2.1. Animals

Male Lister hooded rats (Nottingham University, Medical School) were obtained at weaning (21 days postnatal). They were randomly divided into two groups

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and were housed either in groups of four per cage (socially reared rats), or singly (isolation reared rats). All cages were constructed of plastic and were lined with sawdust. The socially reared rats were housed in cages 52 × 32 × 20 cm high whereas the isolation reared rats were housed in cages 41 × 26 × 20 cm high. Both groups had food and water available ad libitum and were housed within the same room for 6 weeks before use in the place-preference trials. A constant dark-light cycle (on 06.00 h, off 20.00 h) was maintained in the animal house and temperature controlled at 22 ± 1° C.

2.2. Apparatus

For the place preference experiments, each rat was conditioned and tested in a circular open field arena 83 cm in diameter by 32 cm in height, of metal construction. Black lines divided the floor into four quadrants of equal size and identical floor and wall textures (Hasenohrl et al., 1989). Surrounding the arena were two screens, attached to which were a series of visual cues so rats could orient themselves. This uses the same well-established method of visual cues as used with the Morris water maze (Morris, 1984) for spatial memory. The positioning of these cues in relation to each quadrant was constant for the experiment. The arena was placed beneath a video camera so trials could be recorded for later analysis.

2.3. Procedures

All experiments were performed between 09.00 and 17.00 h. The procedure used was a modification of that described by Hasenohrl et al. (1989). Each experiment was carried out over 6 consecutive days. There were three phases of behavioural testing: phase I, the preconditioning phase or baseline trial (day 1); phase II, the conditioning phase or drug treatment (day 2–5); and phase III, the postconditioning phase or a test trial (day 6).

Phase I

The preconditioning phase or the baseline trial was used to determine a treatment quadrant for each rat. During this phase, socially or isolation reared rats were individually placed in the centre of the open field arena facing away from the experimenter and were allowed free access to all parts of the apparatus. A rat was determined to be in a particular quadrant when their two forepaws crossed the dividing lines. The time spent in each of the four quadrants was recorded over a 10 min period for 18 socially reared and 18 isolation reared rats. These rats were tested in an alternating schedule (socially, isolation, socially, isolation, etc.) in order to maintain consistency of circadian rhythms between the two groups. By this measure the normal preference for the four quadrants was determined for each rat. The quadrant in which each individual rat spent the least time in this baseline trial was selected as the subsequent treatment quadrant.

Phase II

The conditioning phase consisted of 4 consecutive days of conditioning sessions. On each day of this phase transparent Plexiglas barriers were inserted into the open field arena to restrict the animals to a particular quadrant. Rats were divided into six groups of six (three groups of socially reared and three groups of isolation reared rats). The groups of socially and isolation reared rats were treated with either saline (i.p.) or *d*-amphetamine sulphate (1.5 and 5 mg/kg, i.p.). These doses were chosen on the basis of previous place preference studies (Erb and Parker, 1994; Mithani et al., 1986). Ten minutes after injection, each rat was placed in the treatment quadrant for 15 min and then returned to their home cage. A 10 min period between injection and placement in the treatment quadrant was selected as this period was established as the time of onset of the behavioural effects of amphetamine.

Phase III

The postconditioning test was on day 6. The Plexiglas barriers were removed and neither amphetamine nor saline was injected. Each rat was placed into the centre of the open field arena with access to the entire arena, and the time spent in each of the four quadrants measured over a 10 min period. The observer of the test trial was blind to which quadrant each animal had been treated in.

2.4. Drugs

d-Amphetamine sulphate (Sigma) was dissolved in 0.9% saline and injected in a volume of 1 ml/kg.

2.5. Statistical analysis

Data are expressed as mean ± S.E.M. Analysis of data was performed using one-way analysis of variance (one-way ANOVA) followed by Dunnett's test to compare the time the animals spent in the four quadrants. The place preferences in socially and isolation reared rats were compared using two-factor ANOVA. The effect of different housing on the conditioned place preference response to saline and amphetamine administration was also analysed by two-factor ANOVA. In all statistical tests a value of $P < 0.05$ was considered to be significant.

3. Results

3.1. The effects of different housing on place preference before conditioning

Fig. 1 shows the amount of the time spent in the treatment quadrant, the opposite treatment quadrant, the right and the left quadrants before conditioning in socially and isolation reared rats.

The results from the preconditioning phase or baseline trial (Fig. 1) demonstrate that each socially and isolation reared rat spent least time for one quadrant but this was not the same quadrant for all rats and this quadrant was later selected as the treatment quadrant.

3.2. The effect of different housing on the conditioned place preference response to saline and amphetamine administration

Fig. 2 represents the amount of time spent in the treatment quadrant, the opposite quadrant, the right and the left quadrants after 4 days conditioning with saline or amphetamine (1.5 and 5 mg/kg, i.p.) in socially and isolation reared rats.

Effects of saline

Socially reared rats given saline still showed a significant ($P < 0.05$) aversion for the treatment quadrant relative to the opposite quadrant. Isolation reared rats did not display this aversion effect of saline treatment, with non-significant differences between the time spent in each quadrant following 4 days saline treatment.

Effects of amphetamine

The result showed that socially reared rats treated with amphetamine (1.5 and 5 mg/kg) spent more time in the treatment quadrant than in the opposite quadrant. Rats given amphetamine (1.5 mg/kg) showed a significant ($P < 0.05$) increase in their selection to the treatment quadrant relative to preconditioning and to saline controls. There was no significant difference between the time spent in the treatment quadrant and the other quadrants. However, the higher dose of amphetamine (5 mg/kg) resulted in a trend for the rats to select the treatment quadrant that was significant ($P < 0.05$) relative to the opposite quadrant and the left quadrant. In contrast amphetamine conditioning in the isolation reared rats did not cause an increase in selection of the treatment quadrant relative to saline controls.

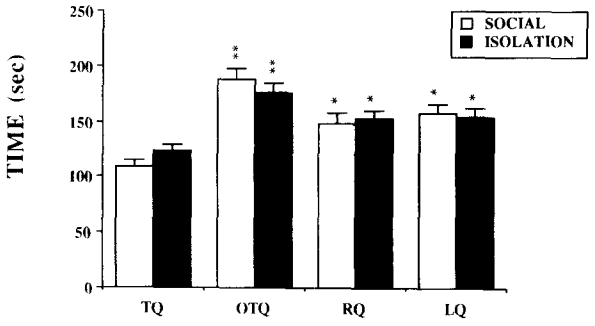

Fig. 1. Time spent on the treatment quadrant (TQ), the opposite treatment quadrant (OTQ), the right (RT) and the left (LT) quadrants before conditioning in the socially and isolation reared rats. Data represent mean ± S.E.M. of 18 rats in each group. $^{*}P < 0.05$ and $^{**}P < 0.01$ significantly different from the treatment quadrant.

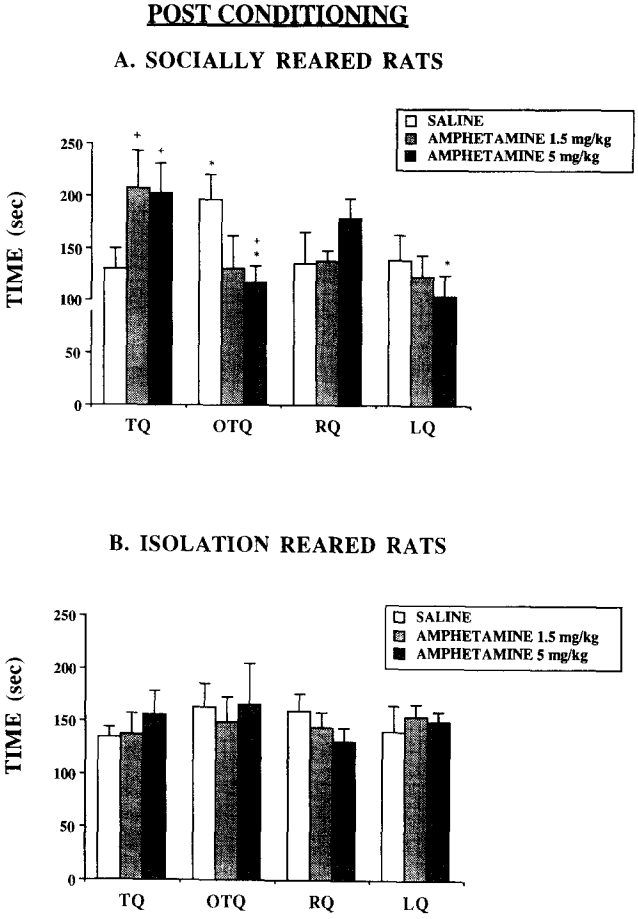

Fig. 2. Time spent on the treatment quadrant (TQ), the quadrant opposite the treatment one (OTQ), the right (RT) and the left (LT) quadrants after conditioning with saline, amphetamine (1.5 and 5 mg/kg) in (A) socially reared rats; (B) isolation reared rats. Data represent mean ± S.E.M. of six rats in each group. $^{*}P < 0.05$; $^{*}P < 0.01$ significantly different from the treatment quadrant. $^{+}P < 0.05$ significantly different from saline treated group in the same quadrant.

4. Discussion

The present experiments demonstrate differential effects of amphetamine in the conditioned place pref-

erence paradigm as a function of the initial preference in socially and isolation reared rats. In our apparatus, we observed that both socially and isolation reared rats initially spent less time in one quadrant with a preference for the opposite quadrant; this quadrant was selected as the treatment quadrant. After saline treatment, the socially reared rats showed significant aversion to the treatment quadrant relative to the opposite quadrant while the isolation reared rats showed no such aversion. This result suggests that social isolation at an early age impairs spontaneous behaviours resulting in a lack of aversion to the saline injection procedure.

Socially reared rats treated with amphetamine (1.5 and 5 mg/kg) showed a significant preference for the treatment quadrant relative to saline controls with respect to the same quadrant as well as the opposite quadrant. There was no difference in the responses to 1.5 and 5 mg/kg amphetamine which is in agreement with previous studies showing no significant increase in conditioning at higher doses of amphetamine (Erb and Parker, 1994; Laviola et al., 1994). Our data add to the growing number of studies employing conditioned placed preference as a procedure for identifying the rewarding properties of psychoactive drugs (Mucha and Iversen, 1984; Schenk et al., 1985; Erb and Parker, 1994). Rats housed in isolation immediately post-weaning however failed to show place preference to amphetamine. This finding is consistent with other studies using cocaine (Mucha and Iversen, 1984; Berry and Marsden, 1994), morphine (Alexander et al., 1981), and heroin (Schenk et al., 1985). The result suggests that the early social environment may influence specific neurochemical systems in the developing nervous system thus differentially affecting the mature rat's sensitivity to psychostimulant drugs. Neurochemical studies have shown that the activity of mesocortical-frontal dopamine neurons is decreased in isolation reared rats (Alexander et al., 1981) while other studies have shown increased presynaptic dopamine release in the accumbens in response to amphetamine in isolation reared rats (Jones et al., 1992). Evidence for a mesolimbic dopaminergic link in the rewarding effects of amphetamine has come from studies showing facilitation of brain stimulation reward by amphetamine (Jones et al., 1990; Phillips et al., 1994). There are limited data available on the effects of isolation rearing on postsynaptic mesolimbic dopaminergic function though there is evidence for reduced dopamine D_1 receptor responsiveness (Jones et al., 1992). The present results may suggest isolation reared rats demonstrate a dysfunction of their reward system with respect to amphetamine; this could be associated with direct changes in postsynaptic dopaminergic receptor function or changes secondary to alterations in other neurotransmitter systems such as 5-hydroxytryptamine (Wright et al., 1991).

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