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ORIGINAL INVESTIGATION

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Isolation rearing enhances the locomotor stimulant properties of intra-perifornical sulpiride, but impairs the acquisition of a conditioned place preference

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Abstract Previous data indicated that infusions of the D_2/D_3 dopamine receptor antagonist sulpiride within the perifornical region of the lateral hypothalamus may engage neural circuitry relevant to activation of the mesoaccumbens dopamine projection. The present work examined this proposition further. Experiment 1 examined the ability of intra-perifornical sulpiride to induce a conditioned place preference, using an unbiased conditioning procedure. Thus, bilateral guide cannulae were implanted to gain access to the perifornical region of the lateral hypothalamus. Following recovery, animals were subjected to an initial exposure to the place preference apparatus. The apparatus consisted of three distinctive compartments, the central compartment allowing access to the two outermost compartments. Initial exposure indicated equal preference for each. Then, in alternating sessions, animals received infusions of sulpiride (5, 10 or 20 μg) before being placed in one of the two outermost compartments, and infusions of vehicle before being placed in the alternate compartment. Compartment-drug pairings were counterbalanced across animals. Four drug, and four saline sessions were completed, each being separated by at least 2 full days. On the final test day, animals were allowed free access to compartments, and the time spent in each was compared with that of initial exposure. Results showed that intra-perifornical sulpiride increased activity during drug-conditioning sessions in an incremental fashion, and supported dose-dependently the acquisition of a conditioned place preference. Experiment 2 examined the effects of isolation rearing upon the locomotor stimulant properties of intra-perifornical sulpiride, and the acquisition of a conditioned place preference. Rats were raised from weaning either alone (isolation-reared) or in groups of five (socially-reared controls) until 4 months of age. Consistent with previous reports of the effects of isolation rearing upon psychomotor stimulant responsivity, here isolates were found to be more responsive to the locomotor stimulant properties of intra-perifornical sulpiride, but were less responsive to the ability of intra-perifornical sulpiride to support the acquisition of a conditioned place preference. These data were suggested to provide further support for the proposition that blockade of dopamine receptors of the D_2 family within the perifornical region of the lateral hypothalamus results in the activation of the mesoaccumbens dopamine projection, via the ventral tegmental area.

Key words Perifornical region of the lateral hypothalamus · Activity · Conditioned place preference · Isolation rearing · Mesoaccumbens dopamine projection

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Introduction

Contemporary research on the role of brain dopaminergic systems in rewarded behaviour has focused in the main upon innervation of the nucleus accumbens by the "mesoaccumbens" dopamine projection (see Koob and Bloom 1988; Robbins and Everitt 1996 for reviews). Previously, however, the lateral hypothalamus had been considered to hold a pivotal position in brain mechanisms of reward (e.g. Olds 1962). Thus, electrical stimulation of this brain area was shown to evoke a variety of behaviours critical for survival and biological adaptation, including eating, drinking, locomotion, copulation and aggression (Valenstein 1969; Hoebel 1976). A number of factors may have contributed to a decline of interest in hypothalamic reward mechanisms, the potential involvement of fibres of passage being one such factor. Nonetheless, lateral

hypothalamic neurons have been shown to be responsive to arbitrary stimuli associated with primary rewards (Ono et al. 1981), and these responses could be blocked following procainisation of the amygdala (Ono et al. 1995). Also, lesion of the lateral hypothalamus intended to spare fibres of passage enhanced the acquisition of schedule-induced polydipsia (Winn et al. 1992), a behaviour dependent upon the mesoaccumbens dopamine projection (Robbins and Koob 1980).

Infusions with the dopamine D_2-receptor family antagonist sulpiride into the perifornical region of the lateral hypothalamus induce a range of behaviours, and recent work has yielded an intriguing configuration of data consistent with an indirect action upon the mesoaccumbens dopamine projection. Thus, intra-perifornical sulpiride formerly has been shown to enhance feeding, drinking and locomotor activity (Parada et al. 1988). Notably, doses known to increase locomotor activity have been found significantly to augment dopamine efflux within the nucleus accumbens (Parada et al. 1995). As a consequence, recent work has investigated the extent to which these data may reflect an action upon the mesoaccumbens dopamine projection. Recent data suggest an action via the region of origin of this projection, within the ventral tegmental area. Thus, repeated intra-perifornical region infusions with sulpiride were found to increase locomotor activity in an incremental fashion (Morutto and Phillips 1997a),and cross-sensitisation with a systemic challenge of *d*-amphetamine was also demonstrated. Further, co-administration of sulpiride in the perifornical region, with the NMDA receptor antagonist AP5 into the ventral tegmental area produced a potent, and synergistic behavioural response (Morutto and Phillips 1997b). Hence, blockade of dopamine D_2/D_3 receptors within the perifornical region of the lateral hypothalamus appears to induce a range of behaviours associated previously with activity specifically of the mesoaccumbens dopamine projection.

Mesoaccumbens dopamine function has been linked with *d*-amphetamine-induced conditioned locomotor activity (Gold et al. 1988), and to conditioned place preference (Reicher and Holman 1977; Phillips et al. 1982; Spyraki et al. 1982; White et al. 1991; De Fonseca et al. 1995). Activation of the nucleus accumbens appears to be crucial to the establishment of a conditioned place preference when psychomotor stimulants are used (e.g. Carr and White 1983). Hence, given that intra-hypothalamic sulpiride has been observed previously to increase dopamine outflow within the nucleus accumbens (Parada et al. 1995), it was expected that similar infusions might induce a conditioned place preference.

The action of sulpiride in the perifornical region of the lateral hypothalamus is probably mediated by dopamine D_2-family receptors, rather than the D_1-family (Pizzi et al. 1986). Neuroanatomical links from this region to the cell body and terminal regions of the mesoaccumbens projection have been described previously (Phillipson 1978; Saper et al. 1979; Roberts 1980; Kirouac and Ganguly 1995; Maldonado-Irizarry et al. 1995), although further investigation is required to provide a full neurochemical characterisation of these projections. At any rate, the results of blockade of dopamine D_2 receptors in the perifornical region of the lateral hypothalamus are suggestive of a subsequent influence upon the mesoaccumbens dopamine projection.

Developmental factors can modify the sensitivity to the rewarding effects of drugs of abuse. Isolation rearing causes radical changes in the development of brain functions and behavioural responses to various stimuli. These changes can be brought about by a continuous period of social isolation beginning at weaning, and have been widely investigated with particular attention to neurochemical changes in some well described neurotransmitter systems (see Robbins and Everitt 1996). Specifically, several studies have implicated the particular involvement of the mesocorticolimbic dopamine system in the isolation syndrome. Hence, isolation rearing has been shown to enhance the overflow of dopamine within the nucleus accumbens in response to a systemic challenge with *d*-amphetamine (Jones et al. 1992). Consistent with these data, isolates repeatedly have been reported to be more sensitive to the locomotor activating properties of *d*-amphetamine and cocaine (e.g. Jones et al. 1990; Boyle et al. 1991; Phillips et al. 1994a).

By contrast, isolation rearing in the main has been found to impair, or to block the ability of a wide range of drugs of abuse to induce a conditioned place preference (Schenk et al. 1983, 1985, 1986; Wongwitdecha and Marsden 1995, 1996). Given also that the ability of psychomotor stimulant drugs to induce a conditioned place preference is recognised to depend specifically upon the mesoaccumbens dopamine projection (Reicher and Holman 1977; Phillips et al. 1982; Spyraki et al. 1982; Carr and White 1983; White et al. 1991), isolation rearing might be expected to impair the ability of intra-perifornical sulpiride to support a conditioned place preference. These data, in addition, would provide further evidence for a functionally significant relationship between dopaminergic systems within the lateral hypothalamus, and the mesoaccumbens dopamine projection itself.

Previous work has suggested that intra-perifornical infusions with sulpiride may activate indirectly the mesoaccumbens dopamine projection, via the ventral tegmental area (see above). The present work extends the findings of previous investigations, by examining the ability of intra-perifornical sulpiride to support the acquisition of a conditioned place preference. The generality of these findings is then scrutinised with respect to the characteristic effects of isolation-rearing upon locomotor activity and drug-supported conditioned place preference.

Materials and methods

Subjects

A total of 72 male Lister hooded rats was used (Charles Rivers, Margate, Kent, UK); 32 in experiment 1 and 40 in experiment 2. For experiment 1, animals were acquired at an adult age, were pair-housed and weighed 290–360 g at the time of surgery, while in experiment 2 the animals were obtained at 21 days of age and housed either in isolation or in groups of five for the duration of the experiment. Singly housed rats were placed in a cage measuring $33 \times 20 \times 10$ cm, while the social group cages were $53 \times 34 \times 9$ cm. Housing conditions were maintained at $22 \pm 2°C$, $55 \pm 10\%$ humidity and a 12 h:12 h light:dark cycle (lights on 0800 hours). Experiments were carried out between 1000 and 1600 hours. Food and water were available ad libitum.

The procedures described in the present study were subject to UK Home Office approval, Project Licence PPL 50/01257.

Apparatus

Experiments were carried out in eight chambers. Each chamber consisted of a wooden shell, sound-insulated with a middle layer of polystyrene (5 cm depth) and electrical fan, which also provided adequate ventilation. Each chamber was lined internally with white PVC, and was fitted with three removable compartments of equal size (60 cm long × 19 cm wide × 29 cm high). Access to each compartment was gained via arched doorways (6.5 cm wide × 10.5 cm high). Archways were blocked during conditioning training. The compartments were assembled in coloured PVC, the two outermost being either white or black, the middle compartment grey. The white compartment was fitted with a wire grid floor, and the black compartment with a rubber floor. The front wall was fitted with the appropriate sections of coloured PVC, and was hinged for access. Each chamber was fitted with ten infra-red emitters and detectors, connected to a 16 channel infra-red control box (ENV-256I; Med. Associates, St Albans, Vt., USA). Four emitters and detectors were positioned on the side walls. Two of these were placed 4 cm above the box floor, and the second pair situated 13 cm from the floor. These sets were situated 20 and 40 cm from the front door of the chamber. There were six emitters and detectors positioned from the front door of the chamber to the back wall. These were located at the same heights as previously described, but at 11, 30 and 49 cm from the side walls. Each compartment was perforated in the areas corresponding to the photobeams fitted in the surrounding chamber, resulting in two photocells per compartment on the front and back walls, determining in which compartment the animal was located during the pre-exposure and the testing phases, while, during conditioning training, locomotor activity was measured additionally by four photocells located on the side walls. Illumination within the three compartments was provided by three ceiling lights operated at 15 W. The apparatus was controlled, and the data collected, using an appropriate software platform (Med. Associates) installed on a standard IBM compatible 386 PC. Data were collected in consecutive 5-min periods, and totals for 20 min or 40 min.

Drugs

(−)-Sulpiride (Sigma Chemical Co., Poole, UK) was dissolved in 0.5–1 µl glacial acetic acid and 100 µl sterile phosphate buffered saline, before being made up to appropriate volume (pH 7.4). Phosphate buffered saline also served as vehicle.

Surgery

General anaesthesia was induced with an injection IP of a solution containing 2,2,2-tribromoethanol in sterile phosphate buffered saline (volume injected: 10 ml/kg, prepared as described previously: Phillips et al. 1994b).

Animals were implanted with bilateral stainless-steel guide cannulae (22 gauge: Plastics One, Roanoke, Va., USA). In both experiments cannulae were aimed at the perifornical region of the lateral hypothalamus. With the incisor bar set at −3.6 mm, the stereotaxic coordinates used were: AP −2.8 mm from bregma, L ± 3.3 mm from the midline, V −7.7 mm , at an angle of 11.2° (coordinates were computed from the stereotaxic atlas of Paxinos and Watson 1986). After surgery, guide cannulae were occluded with tight fitting screw-in obdurators (Plastics One) and the animals returned to their home cages, where they were allowed to recover for at least 7 days.

Infusions

Intracerebral infusions were made bilaterally (Model A, 3.33RPM motor; Razel Scientific Instruments, Stamford, Conn., USA). Rats were hand-held while 29 gauge infusion cannulae (Plastics One) were inserted into the surgically implanted guide cannulae. The infusion cannulae were attached to the pump microsyringes (Hamilton 801RNE; Scientific Laboratory Supplies Ltd, Hessle, East Yorkshire, UK) by polyethylene tubing filled with HPLC grade water. Drug solutions were backloaded within the cannulae and tubing to prevent contamination of the microsyringes. Infusion cannulae projected from guide cannulae by 1 mm. The volume infused was 0.5 µl over 25 s, and infusion cannulae remained in place for a further 1-min period.

Procedure

Place preference conditioning

Pre-exposure. Drug-naive animals were placed in the central compartment, and permitted free access to the three compartments for 20 min. Group preferences were evenly distributed across compartments.

Conditioning. Arched side passages were blocked, isolating each compartment. The central section was not in use during this phase. Allocation of compartment (black or white) to infusion condition was randomised. For each subject, sessions alternated between drug- and vehicle-compartment pairings. Half of the subjects were exposed to the drug-compartment condition, and half to the vehicle-compartment condition on any one day. Session length was 40 min in experiment 1, 20 min in experiment 2. Drug infusions took place at least 72 h apart. A total of four drug and four vehicle infusions was carried out. Locomotor activity was recorded during each session.

Post-conditioning place preference. On the test day, animals were placed in the middle compartment and given free access to the two outermost compartments. Time spent within each compartment was monitored.

Pharmacology

In experiment 1, groups were infused with sulpiride (5, 10, or 20 µg). In experiment 2, isolates and socially reared controls were infused with sulpiride (5 or 10 µg).

Histology

Animals were overdosed with sodium pentobarbitone (Rhone Merieux, Ireland). Brains were removed and blocked from fresh,

then sectioned at 25 μm intervals (Bright OTF Cryostat; Bright Instrument Company Ltd, Huntingdon, Cambs, UK). Sections were mounted on glass slides and stained with cresyl violet. The accuracy of cannula placements, and the effects of intracerebral infusions upon brain tissue, were then assessed using the atlas of Paxinos and Watson (1986).

Statistical analysis

Data were subjected to parametric analyses of variance, "Group" representing the sole independent factor in these experiments. For place preference conditioning, the time spent in each compartment during pre-exposure was subtracted from the time spent during the final test session.

Statistically significant main effects ($P < 0.05$) were analysed further. Within-factor comparisons for factors containing two levels were made using simple main effects parametric analyses of variance (Winer 1971), then were completed post hoc using the Newman-Keuls (N-K) test (Winer 1971).

Results

Histological examination verified that all infusion sites intended for the lateral hypothalamus were within the perifornical region, adjacent to the fornix, and were well within 0.5 mm of each other in the rostro-caudal dimension (see Fig. 1). This placement corresponds to that used in previous studies (Parada et al. 1995).

Experiment 1

Training: effects of repeated infusions of sulpiride or vehicle on activity

Effects upon activity were most evident at the highest dose of sulpiride (20 μg; see Fig. 2). Thus, while the first infusion did not affect activity by comparison with vehicle [$F(1,4) = 5.0$, *NS*], subsequent infusions did significantly increase levels of activity [session, days 1 versus.2–4: $F(3,12) = 4.2$, $P < 0.05$], giving rise to a drug-session interaction [$F(3,12) = 13.9$ $P < 0.001$]. Hence, repeated sulpiride infusions prior to entering the place preference chamber induced a gradual increase of locomotor activity. The medium dose of sulpiride (10 μg) corresponded with the effects reported

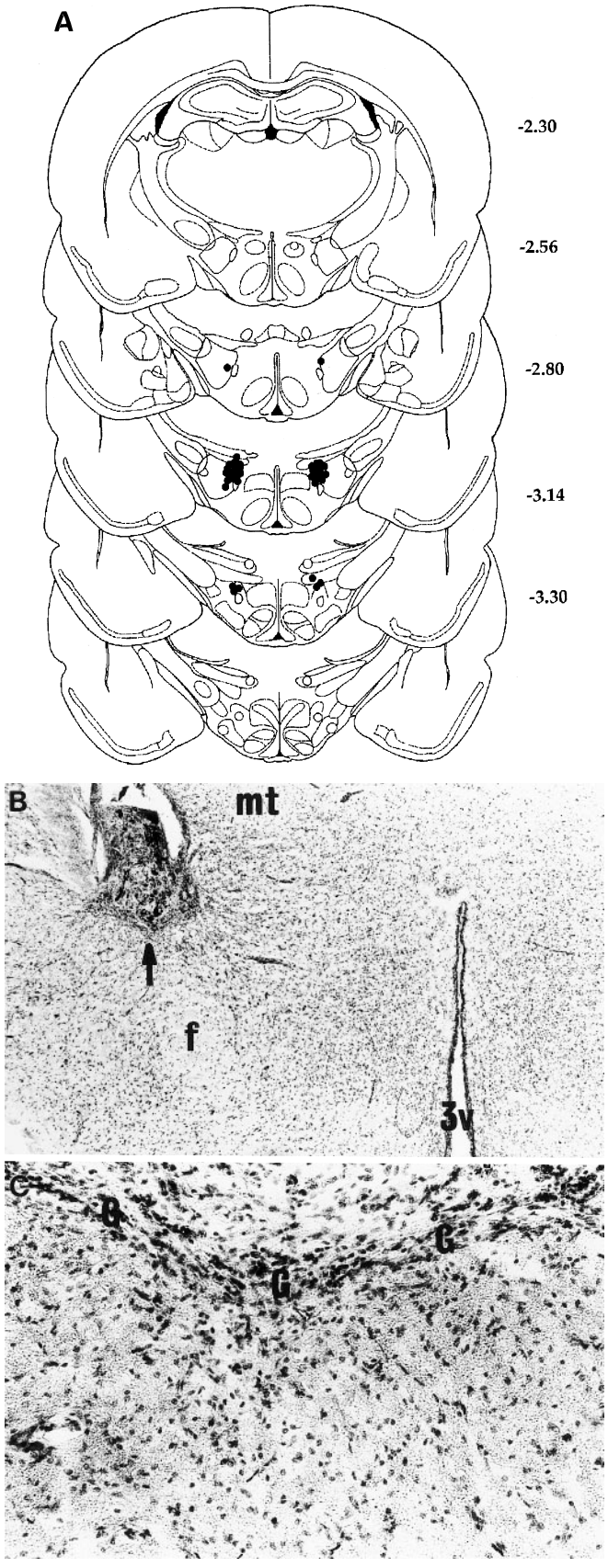

Fig. 1A–C Infusion sites within the perifornical region of the lateral hypothalamus. **A** Coronal sections through the rat brain, based upon the atlas of Paxinos and Watson (1986). *Numbers* adjacent to each section represent distances from bregma (mm) in the anterior-posterior plane. Infusion sites shown as *filled circles*. **B** And **C** Photomicrographs of infusion site of one animal. **B** Low power magnification showing base of infusion site (*arrowhead*) adjacent to fornix (*f*). *3v*, third ventricle; *mt*, mammillothalamic tract. **C** High power magnification of ventral tip of infusion site, corresponding to location indicated by arrowhead in **B**. A restricted band of necrosis (*G*) can be seen, which marks the most ventral tip of the infusion site. The surrounding brain appears to be involved only minimally in this pathology associated with repeated infusions

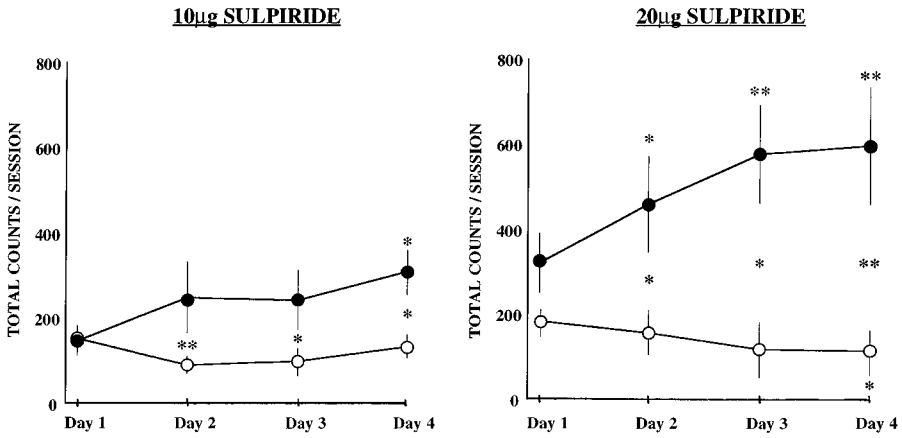

Fig. 2 Place conditioning with sulpiride induced an incremental increase of locomotor activity. *Left hand panel*: total photobeam breaks over 40 min session: rats were exposed to the conditioning compartments under 10 μg sulpiride (drug side ●) or vehicle (vehicle side ○). *Right hand panel*: total photobeam breaks over 40-min session: rats received 20 μg sulpiride (drug side ●) or vehicle (vehicle side ○). Values represent means, with 1 SEM. *Stars* represent the statistical significance of comparisons indicated; $*P<0.05$; $**P<0.01$

above, although in smaller magnitude. Hence, while the first infusion with sulpiride did not affect activity by comparison with vehicle [$F(1,7) = 0.0371$, *NS*], subsequent infusions again significantly increased locomotor activity [session 2, sulpiride versus vehicle $F(1,7) = 29.2171$ $P = 0.001$; session 3, sulpiride versus vehicle $F(1,7) = 9.2561$ $P < 0.05$; session 4, sulpiride versus vehicle $F(1,7) = 10.9727$, $P < 0.05$], giving rise to a drug-session interaction [$F(3,21) = 4.0756$, $P < 0.05$]. Hence, repeated sulpiride infusions prior to entering the place preference chamber again induced a gradual increase of locomotor activity. Thus, both doses of sulpiride elevated locomotor activity in an incremental fashion. Due to software failure, data for the lowest dose could not be gathered in sufficient quantity to convey a meaningful picture of the drug effect. However, a recent study (Morutto and Phillips 1997b) contains equivalent activity data for the 5 μg dose, derived from the same apparatus.

Assessment of conditioned place preference

Intra-perifornical sulpiride induced a conditioned place preference [Fig. 3; drug versus middle versus vehicle $F(2,58) = 3.771$, $P < 0.05$] through a shift in time allocation from the vehicle-paired compartment to the sulpiride-paired compartment (Newman-Keuls, drug versus vehicle $P < 0.05$). However, the ability of sulpiride to induce a conditioned place preference was most evident at the lowest dose tested (5 μg), and declined at higher doses (drug versus vehicle compartment: 5 μg $P < 0.01$, 10 μg $P < 0.05$, 20 μg *NS*). Hence, at the range of doses tested, there appeared to be an inverse relationship between intra-perifornical sulpiride and the establishment of a conditioned place preference.

Experiment 2

Training: effects of isolation-rearing upon the locomotor stimulant properties of intra-perifornical sulpiride

Mean locomotor scores across the four training sessions are shown in Fig. 4. Vehicle scores for the two doses within each rearing group did not differ, and hence were combined.

Overall, activity levels of isolates did not differ from control animals during vehicle sessions [$F(1,37) = 3.3$,

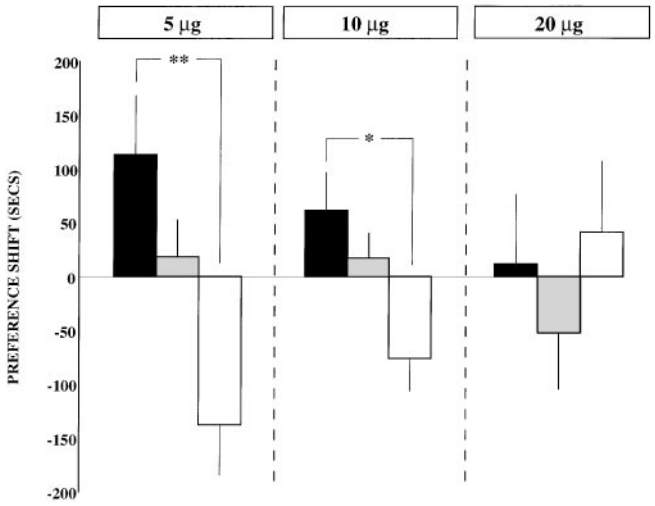

Fig. 3 Conditioned place preference. In this paradigm, conditioning increased the time spent in the drug-paired compartment dose-dependently. Difference between pre-exposure time and test time spent in each compartment, expressed in seconds: animals were exposed to the apparatus drug free, for 20 min. Values represent means, with 1 SEM. *Stars* represent the statistical significance of comparisons indicated; $*P < 0.05$; $**P < 0.01$. ■ Sulpiride; ▒ central; □ vehicle compartment

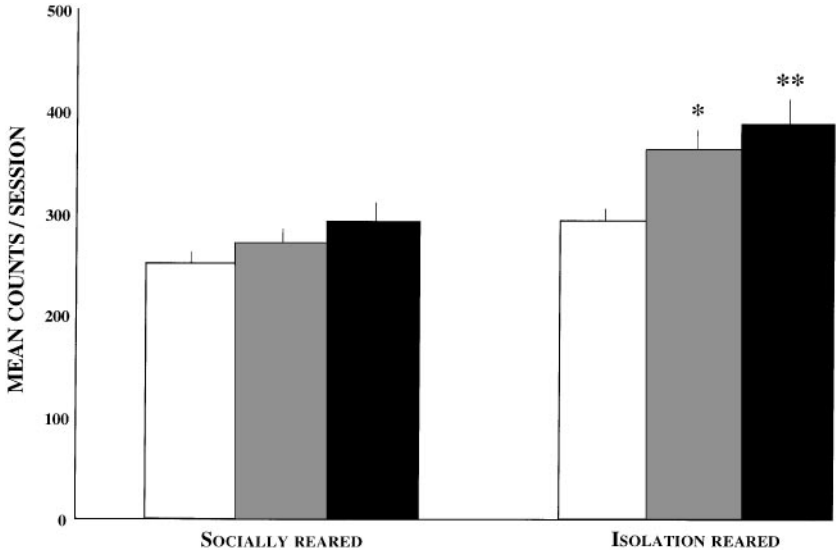

Fig. 4 Enhanced behavioural activation by isolates under vehicle and sulpiride. Average counts over 20-min session: socially reared (*Soc*) and isolation reared rats (*Iso*) were exposed to the conditioning compartments under vehicle □, 5 μg sulpiride (▩) or 10 μg sulpiride (■). Values represent means, with 1 SEM. *Stars* represent the statistical significance of comparisons indicated; * $P < 0.05$; ** $P = 0.01$

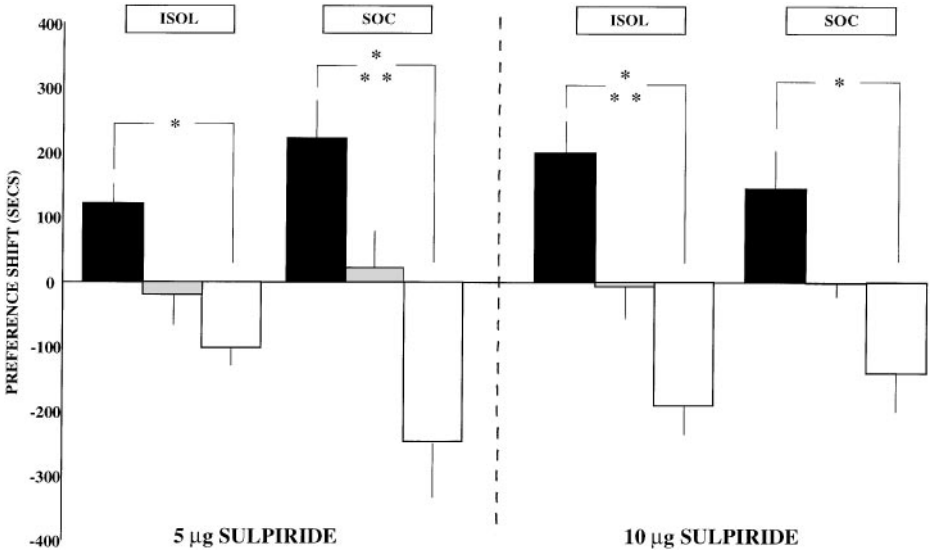

Fig. 5 Conditioned place preference. In this paradigm, conditioning increased the time spent in the drug-paired compartment dose-dependently: while the lower dose was more effective in the socially reared group (*Socially reared*), isolates (*Isolation reared*) appear less sensitive. Difference between pre-exposure time and test time spent in each compartment, expressed in seconds: animals were exposed to the apparatus drug free, for 20 min. Values represent means, with 1 SEM. *Stars* represent the statistical significance of comparisons indicated; * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

NS]. Intra-perifornical sulpiride did not affect rates of activity shown by socially reared controls during the shorter 20-min sessions [$F(1,18) = 1.51$, *NS,* Experiment 1: 40 min]. However, isolates exhibited a significant increase in activity following sulpiride infusions [$F(1,19) = 5.854$, $P < 0.05$]. Post hoc tests revealed significant effects of sulpiride at both doses tested: 5 μg ($P < 0.05$), 10 μg ($P = 0.01$). Hence, isolation-rearing enhanced locomotor stimulant properties of intra-perifornical sulpiride.

Effects of isolation rearing upon the ability of intra-perifornical sulpiride to establish a conditioned place preference

The two rearing conditions led to differing sensitivities to the ability of intra-perifornical sulpiride to induce a conditioned place preference [isolates versus group housed: $F(1,36) = 6.918$, $P < 0.05$]. In common with results reported in Experiment 1, socially reared animals exhibited an inverse relationship between the dose of intra-perifornical sulpiride, and the degree of conditioned place preference (Fig. 5; vehicle versus drug compartment, 5 μg: $F(1,19) = 24.332$ $P < 0.001$; 10 μg: $F(1,17) = 7.402$ $P < 0.05$). By contrast, isolates were relatively insensitive to the effects of the lowest dose of sulpiride [5 μg: $F(1,19) = 6.138$ $P < 0.05$], but instead showed the largest response to 10 μg sulpiride [10 μg: $F(1,17) = 15{,}575$, $P = 0.001$].

Discussion

The present work examined the role of dopamine receptors of the D_2 family within the perifornical region of the lateral hypothalamus in the modulation of

locomotor behaviour and the acquisition of a conditioned place preference. Repeated sulpiride infusions enhanced locomotor activity, which increased gradually across days. Furthermore, repeated drug-context pairings gave rise to a conditioned place preference when tested subsequently in the absence of sulpiride. Rearing in social isolation had a dual effect on the efficacy of intra-perifornical sulpiride. First, it enhanced further the locomotor stimulant effects of intra-perifornical sulpiride. Second, it produced a shift to the right in the ability of intra-perifornical sulpiride to induce a conditioned place preference.

The locomotor stimulant properties of sulpiride infusions within the perifornical region of the lateral hypothalamus are suggestive of an involvement with the mesoaccumbens dopamine projection. Consistent with this interpretation, previous studies have shown intra-perifornical region sulpiride significantly to increase levels of extracellular dopamine and its metabolites in the nucleus accumbens (Parada et al. 1995). Similarly, psychomotor stimulant drugs such as *d*-amphetamine and cocaine have been shown to increase locomotor activity by enhancing extracellular dopamine in the nucleus accumbens region (Bradberry and Roth 1989; Kuczenski and Segal 1989). Furthermore, *d*-amphetamine has been shown preferentially to increase extracellular dopamine in the nucleus accumbens by comparison with the dorsal striatum (Di Chiara and Imperato 1986, 1988; Di Chiara et al. 1993), while destruction of mesoaccumbens, but not mesostriatal dopamine terminals blocked the locomotor stimulant properties of *d*-amphetamine (Kelly et al. 1975). Hence, although the current data do not rule out a non-dopaminergic method of locomotor activation, they are at least suggestive of an indirect effect of intra-perifornical sulpiride via the mesoaccumbens projection.

Repeated intra-perifornical sulpiride gradually increased locomotor activity; this result is consistent with recent work adopting recurrent intra-hypothalamic infusions with sulpiride which showed this treatment to increase locomotor activity in an incremental fashion (Morutto and Phillips 1997a). In the same study, cross-sensitisation with a systemic challenge of *d*-amphetamine was also demonstrated. Repeated treatment with psychomotor stimulants is known subsequently to sensitise the locomotor response to a *d*-amphetamine challenge (Robinson et al. 1988). This is supported further by the finding that repeated injections with psychomotor stimulants induce a gradual increase of extracellular dopamine in the nucleus accumbens (e.g. Kalivas and Duffy 1993a,b; Wolf et al. 1993). Nevertheless, this sensitised response, known to occur following repeated psychomotor stimulants and impinging upon the mesoaccumbens dopamine projection, has been shown specifically to be dependent upon effects at the dopamine cell body level within the ventral tegmental area (Kalivas and Weber 1988; Vezina and Stewart 1990). Hence, increased sensitivity to repeated intra-perifornical sulpiride may depend upon an indirect action involving the region of origin of the mesoaccumbens dopamine projection within the ventral tegmental area. This proposition is additionally supported by recent data. Co-administration of sulpiride in the perifornical region, with the competitive NMDA receptor antagonist AP5 in the ventral tegmental area produced a synergistic effect upon locomotor activity, while impairing the establishment of a conditioned place preference (for discussion, see Morutto and Phillips 1997b). Taken together, these data suggest that the increased sensitivity to intra-hypothalamic sulpiride reported in the present work may depend upon an indirect action the mesoaccumbens dopamine projection, via the ventral tegmental area.

The evidence presented in the current report shows that intra-perifornical infusions with sulpiride produced a conditioned place preference.At least consistent with this, sulpiride has been shown to be readily self-administered within the perifornical region (Parada et al. 1995). Comparable results have been obtained following systemic infusions of psychomotor stimulant drugs such as cocaine and *d*-amphetamine (Reicher and Holman 1977; Spyraki et al. 1982). Specifically, activation of dopaminergic receptors within the nucleus accumbens appears to be crucial for the establishment of a conditioned place preference (Carr and White 1983; White et al. 1991). There is good evidence for an efferent system from the lateral hypothalamus to the ventral tegmental area (Phillipson 1978; Saper et al., 1979; Roberts 1980). It may be that this system is responsible, at least in part, for the ability of intra-perifornical sulpiride to induce a conditioned place preference.

Animals reared in isolation displayed an enhanced response to the locomotor activating properties of sulpiride injections in the perifornical region of the lateral hypothalamus.Increased sensitivity to the psychomotor stimulant effects of compounds such as *d*-amphetamine and cocaine, as reported here with intra-hypothalamic sulpiride, represents one of the cardinal traits of the isolation syndrome. This effect has been accounted for previously in terms of isolation-induced sensitisation of the mesoaccumbens dopaminergic projection. Thus, a differential response to *d*-amphetamine by isolates has been shown in terms of enhanced hyperactivity in a novel environment (Sahakian et al. 1975; Garzon et al. 1979; Jones et al. 1990), and an elevated locomotor response to cocaine (Sahakian et al. 1975; Phillips et al. 1994b) or *d*-amphetamine (Jones et al. 1990). Accordingly, isolation rearing was found to enhance the degree of dopamine overflow which occurred within the ventral striatum in response to a systemic challenge with *d*-amphetamine (Jones et al. 1992; see also Robbins et al. 1996). Thus, isolation rearing produces changes in the sensitivity to psycho-

motor stimulant drugs via an enhanced responsivity of the mesoaccumbens dopamine projection. Given that isolation rearing also enhanced the locomotor stimulant properties of intra-perifornical sulpiride, data presented in the current report again are consistent with an action of intra-perifornical sulpiride upon the mesoaccumbens dopamine projection.

In contrast, isolation rearing was found to produce a shift to the right in the ability of intra-perifornical sulpiride to induce a conditioned place preference. This result is consistent with previous reports of the effects of isolation rearing upon drug-induced conditioned place preference. Thus, rearing in social isolation was shown to block the ability of cocaine (Schenk et al. 1986), *d*-amphetamine (Wongwitdecha and Marsden 1995) and morphine (Wongwitdecha and Marsden 1996) and to impair the ability of heroin (Schenk et al. 1983, 1985) to induce a conditioned place preference. Given that the acquisition of a conditioned place preference is considered to be dependent upon a functionally intact mesoaccumbens dopamine projection (see above, and Introduction), the consistency of the current result with previous reports of the effects of isolation rearing upon drug-induced conditioned place preference lends further support to the proposition that blockade of dopamine receptors of the D_2 family within the perifornical region engages neural circuitry relevant to activation of the mesoaccumbens dopamine projection.

Although drug diffusion to other areas, such as the ventral tegmental area, could be considered to represent a confound to these data, previous work has confirmed the specificity of this infusion site. Thus, sulpiride infusions either posterior or medial to the perifornical region were unable to induce an increase in extracellular dopamine within the nucleus accumbens (Parada et al. 1995). Control injections previously carried out in this laboratory (Morutto and Phillips 1997a) indicated that infusions aimed at 1 mm anterior or posterior to the perifornical area did not give rise to an enhancement in locomotor activity, a result which weighs against the results reported here being due to take-up by fibres of passage in the medial forebrain bundle. Hence, previous evidence, together with the observation that behavioural effects occur within 1 min following infusion, suggests strongly that the behavioural effects seen following dopamine D_2/D_3 receptor blockade in the perifornical region of the lateral hypothalamus may be specific to this region. The specific action of sulpiride in this brain region upon dopamine receptors has been previously shown. Thus, pre-treatment in the perifornical region with dopamine 15 min prior to sulpiride infusion completely antagonised the locomotor enhancing effects of sulpiride (Parada et al. 1990).

In summary, isolation rearing has been shown previously to enhance the locomotor response to psychomotor stimulants, but to reduce the abilities of the same compounds to induce a conditioned place preference. Both behavioural outcomes are considered to be dependent upon functions regulated by the mesoaccumbens dopamine projection. In the present report, intra-perifornical region sulpiride exhibited a comparable behavioural profile to that shown by psychostimulant drugs following a period of isolation rearing. Thus, dopaminergic blockade at the level of the perifornical region may lead to activation of the mesoaccumbens dopamine projection.

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