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Increased sensitivity to amphetamine and reward-related stimuli following social isolation in rats: possible disruption of dopamine-dependent mechanisms of the nucleus accumbens

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Abstract. These experiments compared isolation-reared and socially-reared rats in two complementary paradigms for measuring responding to signals of reward, both undrugged and following either systemic or intra-accumbens *d*-amphetamine (AMPH). In experiment 1, locomotor activity conditioned to food presentation was measured in rats exposed to a restricted feeding schedule. The interaction between this conditioned activity, AMPH administration (0.5, 2.0, 3.5, 5.0 mg/kg IP) and motivational state was measured. In experiment 2, hungry rats were trained to associate a compound light/noise stimulus with sucrose reward and were then implanted with guide cannulae in the nucleus accumbens. In the test phase, responding on one of two novel levers produced the compound stimulus (conditioned reinforcer; CR). Responses on the other lever had no effect. Each rat received four counterbalanced intra-accumbens infusions of AMPH (0, 3, 10, 20 μg). In both experiments, isolated rats responded more with stimuli associated with reward and this differential rearing effect was further exaggerated by AMPH. The isolation-induced sensitivity to these stimuli and to AMPH was critically dependent on motivational variables. Thus, in experiment 1 there were no differences between the groups when sated or during extinction and in experiment 2 the increased responding was restricted to the lever providing CR. Measurements of the locomotor response to intra-accumbens AMPH (0, 3, 10 μg) also revealed that isolated rats were more sensitive to a low dose of the drug when tested food-deprived in a relatively novel environment. These results suggest that the experience of isolation-rearing interacts either directly or indirectly with dopamine-dependent mechanisms of the nucleus accumbens to enhance the effects of reward-related stimuli.

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Early social experience is well known to modify the behavioural effects of drugs in animals (Katz and Steinberg 1972; Valzelli 1977). In particular, rearing rats in social isolation enhances the stereotyped responses produced by psychomotor stimulant drugs, including *d*-amphetamine and apomorphine (Sahakian et al. 1975; Einon and Sahakian 1979; Chitkara et al. 1984), increases cocaine self-administration (Schenk et al. 1987), reduces the response to neuroleptic drugs such as α-flupenthixol (Sahakian and Robbins 1977) and decreases the sensitivity to barbiturate anaesthesia (Einon et al. 1976; Juraska et al. 1983). In addition, socially-deprived rats exhibit some behavioural disturbances resembling those produced by treatment with drugs such as amphetamine. For example, they are spontaneously hyperactive (Weinstock and Speiser 1973; Einon and Morgan 1978; Gentsch et al. 1988), display perseverative responses in operant situations (Morgan and Einon 1975; Jones, Marsden and Robbins, manuscript in preparation) and are impaired in the expression of schedule-induced behaviours (Jones et al. 1989). These similarities, as well as the exaggerated stereotyped response to dopamine agonists, suggest that rearing in isolation may produce some of its behavioural effects through central dopaminergic mechanisms. Preliminary evidence using in vivo dialysis suggests that isolates have increased extracellular levels of dopamine in response to *d*-amphetamine (Jones et al. 1988), as well as elevated striatal D_2 receptor binding (Guisado et al. 1980). Moreover, social isolation also affects measures of dopamine turnover in the frontal cortex (Blanc et al. 1980).

Many of the behavioural effects of amphetamine-like drugs depend upon dopamine-dependent mechanisms in the nucleus accumbens or ventral striatum. Thus, depletion of mesolimbic dopamine attenuates the hyperactivity (Kelly et al. 1975), the disruption of schedule-induced behaviour (Robbins et al. 1983), and the reinforcing effects (Lyness et al. 1979; Pettit et al. 1984) produced by psychomotor stimulants. In addition, intra-accumbens infusions of *d*-amphetamine produce a conditioned place preference (Carr and White 1983), enhance the conditioned reinforcing properties of environmental stimuli (Taylor and Robbins 1984) and serve as reinforcers themselves (Hoebel et al. 1983). Such observations have led to the hypothesis that mesolimbic dopamine mediates behavioural processes such as incentive motivation (Fibiger and Phillips 1986).

There has been relatively little examination of motivational effects of rearing rats in isolation. Morgan and Einon (1975) provided some evidence of enhanced motivation for food using a procedure thought to measure incentive-motivation, also sensitive to ventral striatal lesions (Neill et al. 1974). Consequently, in these experiments we examined the response of socially isolated animals to reward-related stimuli in two complementary paradigms: 1) locomotor activity conditioned to the presentation of food and 2) acquisition of new behaviour reinforced by stimuli formerly predictive of food (conditioned reinforcers; CR). In both experiments possible interactions between social-isolation and the effects of amphetamine on incentive-motivational processes were investigated: in experiment 2 the effects of intra-accumbens *d*-amphetamine in socially-reared and socially-isolated animals were compared directly, not only on responding with CR but also on locomotor activity.

Methods

Subjects

The 30 female Lister hooded rats used in these experiments were obtained at 21 days of age (Olac, Bicester, UK) and were divided into the two rearing condition groups counterbalanced by weight. Rats were housed either in isolation or in social groups of six rats per cage for the duration of the experiment. All cages were constructed of plastic with grid floors and an underhanging sawdust tray. Isolation-reared rats were housed singly in a cage, 45 cm × 28 cm × 20 cm high. The social-group cages were 56 cm × 38 cm × 18 cm high. Both groups had continuous access to food and water. All rats were housed in a colony room maintained at 21° C, on a 12-h light/dark cycle (lights-on 0700 hours) and could see, hear, and smell other rats. One week after being allocated to the appropriate rearing condition group all rats were given a 2-h test for locomotor activity in the photocell cages described below. Before entering these experiments some of these rats had been used to assess water consumption following social-isolation.

Experiment 1

In experiment 1 the effects of social isolation on the anticipatory locomotor response to signaled food presentation during a restricted feeding schedule were examined. Both the acquisition of the response and its reduction during conditions of extinction were determined. The interaction between this conditioned locomotor activity (Sheffield and Campbell 1954), amphetamine administration, and deprivation state was also investigated.

Apparatus. Tests for locomotor activity were conducted in a bank of 16 individual, wire photocell cages (40 cm × 25 cm × 18 cm high). Each cage was fitted with two parallel infrared photocell beams, 1 cm above the floor, and spaced equally along the long axis of the cage. Interruption of either beam resulted in an incremental count for that cage, registered by on-line input to a Cube System 10 microprocessor (Control Universal, Cambridge, UK), housed in an adjacent room. Water was freely available in each photocell cage.

Behavioural procedure. When mature (14 weeks old; mean body weight = 220 g), rats were gradually reduced to 80% of their free-feeding weight and maintained at this reduced weight for the duration of the experiment. All subjects (socials, $n = 7$; isolates, $n = 6$) were given daily 2-h tests for locomotor activity (between 10:00 and 14:00 hours). Allocation to individual photocell cages on each day was on a pseudo-random schedule.

For the first 9 days of testing, rats were maintained at their reduced body weight by restricted home cage feeding, at varying time intervals (2–6 h) after testing. This was to limit possible associations between activity testing and food presentation. The conditioning phase began on day 10. During this phase the food supplements to maintain body weight were presented in the photocell cages after the first 30 min of testing and no food was given in the home cage.

After day 30, when stable levels of conditioned activity had been established, rats were injected IP with 0.5, 2.0, 3.5, or 5.0 mg/kg *d*-amphetamine sulphate in 0.9% saline, with intervening days of vehicle injection. All injections were made immediately before testing and in a volume of 1.0 ml/kg body weight. On these test days, food presentation was delayed until 1 h after being placed in the photocell cages to prevent interruption of amphetamine-induced behaviours. Normal conditioning days were also interspersed between saline and amphetamine days to maintain the established conditioned anticipatory response to food.

In order to examine whether any increase in locomotor activity was due to food-related incentive-motivation, feeding was switched from the photocell cages back to the home cage (days 64–93), again at varying times after testing. Once the animals had extinguished, the conditioning procedure was reintroduced to investigate whether the anticipatory response to food could be reinstated and to determine the reproducibility of the original conditioning effect. Food was again presented in the photocell cages after the first 30 min of testing (days 94–98).

To investigate the relationship between the conditioned locomotor activity, amphetamine-induced locomotor activity, and deprivation state, all animals were given the following tests, both when the level of activity was high (during the conditioning phase) and when low (during extinction): food available for 2 h before testing; injection of 0.5 mg/kg *d*-amphetamine immediately before testing; food available 2 h before injection of 0.5 mg/kg *d*-amphetamine.

Data analysis. Locomotor activity scores for the initial 30-min period on each day were subjected to analysis of variance with repeated measures (ANOVA; Winer 1971). Activity during the conditioning and extinction phases was analysed by analysis of covariance, with the covariate being the appropriate baseline scores preceding the behavioural manipulation. The covariate for the analysis of conditioned activity (days 11–30) was the individual mean activity scores for the 10 days of spontaneous locomotor activity testing. The covariate for locomotor activity during extinction was the individual mean activity scores for the 4 days prior to extinction. For drug treatment days and pre-feeding days, activity scores for the 1st hour of testing were analysed.

Experiment 2

Apparatus. Three double-lever operant chambers (Campden Instruments) fitted with model 441 water dippers (0.06 ml) were used. The test chambers were controlled by on-line input to an BBC Master microcomputer programmed in SPIDER (Paul Fray Ltd, Cambridge, UK) housed in an adjacent room. The two levers were removable and could be replaced with metal plates which covered the aperture through which the levers normally protruded. Each of the levers was 3.8 cm wide and positioned 1.6 cm from the side walls and 5.5 cm from the grid floor. A food tray was situated midway between the two levers and contained a recessed dipper for the presentation of sucrose reward (10% W/V). Access to the dipper could be obtained by opening a hinged perspex panel, the movement of which was monitored by means of a microswitch. The food tray could be illuminated by a 2.4–W light inside the panel. Another 2.4–W light was fitted in the ceiling of the operant chamber and served as a house-light. Each test chamber was housed in a well ventilated sound-attenuating box.

Surgical procedures. Rats were anaesthetised with Equithesin (0.3 ml/100 g) and placed in a stereotaxic frame (David Kopf, Tujunga, California, USA). Bilateral stainless-steel guide cannulae (23-gauge) were implanted to gain access to the nucleus accumbens, AP +3.4 from Bregma, Lat ± 1.7, Vert −5.8 from dura with the incisor bar set at +5 mm (Pellegrino and Cushman 1967). The guide cannulae were secured in place with the use of skull screws and dental cement. Removable stylets were placed in the guide cannula. One week was allowed for recovery from the surgical procedures.

Intracerebral infusions were made bilaterally via 30-gauge injection cannulae which were lowered into the surgically implanted guide cannulae. The injection cannulae were attached via plastic (PE10) tubing to 5 µl syringes mounted on a Harvard Apparatus infusion pump. The infusions (2 × 1 µl) were delivered simultaneously over a 2-min period with an additional 2-min diffusion period allowed to elapse before withdrawing the injection cannulae. Doses of 3, 10, and 20 µg *d*-amphetamine sulphate (Sigma, Poole, UK) were dissolved in phosphate buffered saline (PBS), pH 7.4, which was used as the control solution. The rats were hand held (but not actively restrained) during the infusions and were immediately placed in the test chambers.

Data analysis. The number of responses on each lever, the number of CR presentations, the total number of panel-presses and the time spent at the panel were recorded for each test session. Lever presses were analysed by analysis of variance with the data subjected to a square-root transformation to achieve homogeneity of variance (Winer 1971). The untransformed data were also analysed and showed comparable effects.

Histology. At the completion of testing all rats were administered an overdose of barbiturate and perfused transcardially with 10% formalin. Following fixation, coronal sections (60 µm) were cut on a freezing microtome and every second section through the nucleus accumbens and associated structures was mounted on a glass slide and stained with cresyl violet for the determination of cannula placement.

Behavioural procedures. Before entering the experiment, the 17 subjects (social, $n=9$; isolates, $n=8$) were gradually reduced to 85% of their free-feeding weight and maintained at this weight throughout the experiment by restricted access to food in the home cage.

The experiment consisted of two distinct phases, training and testing. During the training phase the rats were subjected to a classical conditioning procedure in which an association between a previously neutral stimulus and sucrose reward was established. In the test phase, during which the sucrose reward was no longer presented, the ability of this stimulus (CR) to act as a reinforcer was examined on the acquisition and maintenance of a new response (Mackintosh 1974). The test was conducted following the administration of *d*-amphetamine or control (both no-injection and PBS-injection were tested) into the nucleus accumbens.

Initial training consisted of two sessions of panel training in which the panel was taped open and food-reward pellets placed inside the food tray. This was followed by two preliminary sessions of 30 trials in which a 5-s illumination of the tray light and house-light offset (conditioned stimuli; CS) occurred on a random-time 6-s schedule and was immediately followed by the 9-s elevation of the sucrose dipper (which made a characteristic sound and therefore formed part of the CS). Access to the dipper could now be obtained by making a panel-press response. This initial training was sufficient to establish panel-pressing and drinking from the dipper.

During the training phase proper, a reinforcement schedule (random-time 30-s schedule) was used in which there were 30 presentations of a 5-s tray-light stimulus and house light offset followed immediately by the 5-s elevation of the sucrose dipper. The subjects were trained not to panel-press at inappropriate times (i.e., outside the CS period) by delaying the next possible random presentation of the CS by 3 s if they responded during the variable interval period (VI; between 15 and 45 s). The presentation of the reward would also not occur if the rat nosepoked during these additional 3 s and was delayed until 3 s after the nosepoke had ended. After 11 sessions, the duration of the tray-light illumination and house light offset was reduced by 1 s per session from 5 s to 1 s. Two further sessions were given with this 1-s light illumination to establish a preoperative baseline performance.

The rats were then given a single test session in which the levers were now present in the test chamber for the first time. Responding on the CR lever, one of the two novel levers, resulted in the presentation of the compound stimulus that served as the CR (0.5-s tray-light illumination, 0.3-s dipper elevation). The probability of a CR presentation following a CR lever press was 0.5. The CR lever (left or right) was counterbalanced over subjects but remained constant for each rat across the test sessions. Responding on the NCR lever or panel pressing had no programmed consequences but were recorded. Sucrose was no longer delivered during the test phase.

After this initial test session, the rats underwent surgery for the bilateral implantation of guide cannulae aimed at the nucleus accumbens. At the time of surgery the rats were 18 weeks old (mean body weight = 206 g). Two of the socially reared rats died under postoperative anaesthesia. One week after surgery the animals were given three additional training sessions to confirm the preoperative level of appropriate panel-pushing during the CS and VI periods. The rats then re-entered the test phase. Each rat was assigned to receive a series of single counterbalanced *d*-amphetamine infusions into the nucleus accumbens (3, 10, 20 µg) and a control infusion of PBS. The order of infusions was determined by a Latin Square design, which allowed each rat to act as its own control. The four drug test sessions were separated by at least 48 h to ensure recovery from the effects of the drug.

Two days after the last test day, all rats were placed in individual cages and their water bottles replaced with bottles containing 10% sucrose solution. The bottles were weighed before being placed in the cages and were weighed after 30 min and 1 h had elapsed.

All subjects were given three tests for locomotor activity in photocell cages (described in experiment 1). After a 2-h habituation period, rats were given intra-accumbens infusions of 3 or 10 µg *d*-amphetamine or PBS and immediately replaced in the photocell cages. Drug-induced locomotor activity was monitored for 2 h and at least 48 h separated each infusion.

Results

The analysis of the effects of social isolation on body weight gain and spontaneous activity was conducted on the 30 animals from the two experiments. Isolated rats

gained weight faster than controls, $F(2,56)=3.93$, $P<0.05$, and were spontaneously hyperactive during the 2-h test conducted 1 week after housing individually, $t(28)=4.00$, $P<0.001$.

Experiment 1

Spontaneous and conditioned locomotor activity. Isolation-reared rats were spontaneously hyperactive when compared to socially-reared controls, as indicated by a significant main effect of Rearing condition, $F(1,11)=23.60$, $P<0.001$. However, the isolates did show habituation to the test environment as indicated by an attenuation of this increased locomotor response. By day 10 the two groups did not differ (Fig. 1A).

Covariate analysis of locomotor activity during the conditioning phase revealed a significant Rearing × Days interaction, $F(19,196)=2.73$, $P<0.001$, confirming a greater increase in activity scores across conditioning days in the isolation-reared rats (Fig. 1A). This response was almost 70% greater than in the social control animals. The mean individual activity scores for both spontaneous and conditioned activity were also analysed in six blocks of 5 days, two blocks of unconditioned activity and four blocks of conditioned activity. This indicated a significant main effect of Block, $F(5,55)=17.08$, $P<0.001$ and a Rearing × Block interaction, $F(5,55)=8.42$, $P<0.001$; appropriate post hoc comparisons confirmed a significant effect of the conditioning procedure in both rearing condition groups, as the activity counts in all four conditioned blocks were greater than in the two unconditioned blocks.

There was no difference in the activity counts for the two rearing groups in the 30-min period following the presentation of food, Rearing condition, $F(1,11)=0.03$, ns; Rearing × Days, $F(19,199)=0.70$, ns, with mean scores for the social and isolation groups of 367 and 371, respectively.

Extinction and reconditioning. During the extinction phase (64–83), when food was no longer paired with testing (Fig. 1B), both groups of rats exhibited a decline in locomotor activity. Analysis of covariance indicated a significant effect of the extinction procedure with a main effect of Days, $F(18,197)=6.10$, $P<0.001$. This reduction in locomotor activity was significantly greater in the isolation-reared rats, $F(18,197)=2.75$, $P<0.001$ and eventually the activity scores of the two groups did not differ. When the data from the rearing condition groups were analysed separately this revealed that the extinction procedure reduced locomotor activity in both the socials, $F(6,18)=2.77$, $P<0.001$ and the isolates, $F(5,18)=3.52$, $P<0.001$.

During the reconditioning phase (days 93–98), the increased locomotor activity could be rapidly reinstated, which again revealed a markedly greater response in the isolation-reared rats, $F(5,55)=3.51$, $P<0.01$ (Fig. 1B). The scores for the socially-reared rats increased from a mean of 530 counts/30 min prior to reconditioning to a mean score of 655 counts/30 min on the last day of testing, $F(6,5)=5.57$, $P<0.001$. The scores for the isolation-reared rats increased from a mean of 514 to a mean of 1208 counts/30 min over the same time period, $F(5,5)=6.35$, $P<0.001$.

Amphetamine administration. The effect of *d*-amphetamine treatment is shown in Fig. 2. The saline scores

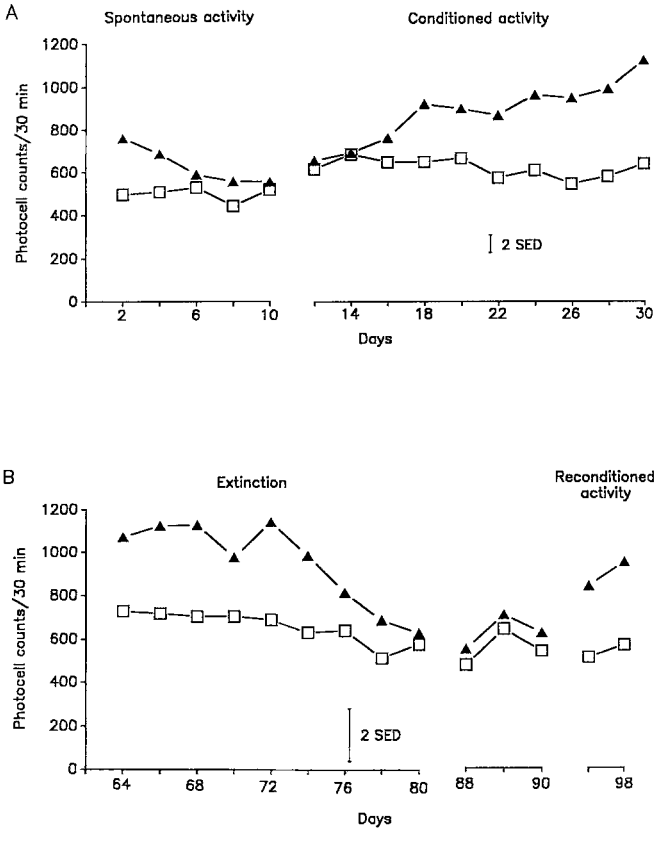

Fig. 1A Spontaneous and conditioned locomotor activity. Photocell counts for the first 30 min of each test session for both socially-reared and isolation reared groups. In **A** and **B**, for clarity of presentation, each point represents the mean score of 2 days and the *bars* represent 2 standard errors of the difference between means (SED) for the interaction between group and Days. **B** Extinction of the conditioned locomotor response and its reinstatement. □ Social; ▲ isolate

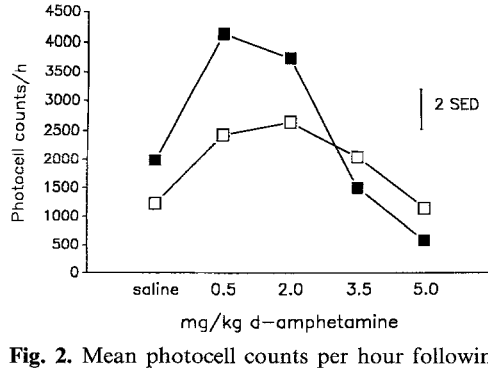

Fig. 2. Mean photocell counts per hour following IP injection of *d*-amphetamine during the conditioning phase. The *bar* represents 2 standard errors of the difference between means. □—□ Social; ■—■ isolate

represent the mean of the 5 saline days between the individual amphetamine injection days. Amphetamine injection altered locomotor activity in a dose-dependent fashion in both groups of animals, $F(4,38)=20.61$, $P<0.001$. ANOVA also indicated a significant Rearing × Dose interaction, $F(4,38)=5.90$, $P<0.001$, with an apparent shift in the dose-response curve to the left in isolation-reared rats. The isolates were more active at the lower doses and less active at the higher doses.

Figure 3 shows the results of manipulating motivational factors on the interaction of isolation-rearing and amphetamine treatment for locomotor activity. The effect of 0.5 mg/kg *d*-amphetamine and pre-feeding, both separately and combined, on locomotor activity conditioned to food presentation (high baseline) is shown in Fig. 3A. For comparison, data from the dose-response study are included (i.e., the photocell counts for saline and amphetamine while food deprived). Fig. 3A shows that the greater sensitivity of isolates to amphetamine is abolished by satiation, in parallel with the attenuation of conditioned locomotor activity [Rearing condition × Feeding, $F(1,10)=14.86$, $P=0.003$]. Appropriate post hoc comparisons revealed that the isolated rats did not differ in activity scores when sated prior to testing, either with saline or amphetamine treatment. When hungry, the isolates were more active than the socially-reared controls, in both drug conditions. Irrespective of rearing conditions, locomotor activity was increased by *d*-amphetamine treatment, $F(1,10)=47.17$, $P<0.001$, and was decreased by pre-feeding, $F(1,10)=41.72$, $P<0.001$. In confirmation of previous studies (Campbell and Fibiger 1971), food deprivation markedly potentiated the locomotor stimulating effects of *d*-amphetamine, $F(1,10)=14.70$, $P=0.003$.

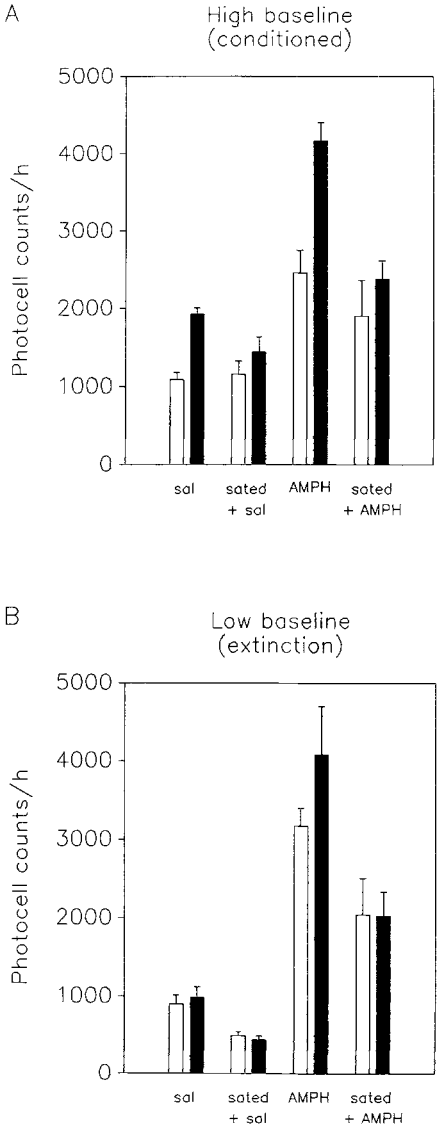

Fig. 3A,B. Mean photocell counts per hour following either saline or 0.5 mg/kg *d*-amphetamine IP, both when deprived or when sated. The bars indicate the standard error of the mean. **A** Activity measured during the conditioned phase when the test environment was predictive of reinforcement. For comparison the data for saline and *d*-amphetamine scores while deprived are reproduced from Fig. 2. **B** Locomotor activity scores measured during the extinction phase. □ Social; ■ isolate

The complementary manipulation of testing the rats in extinction (i.e., without food presentation) abolished the effects of isolation-rearing on locomotor activity. Thus, during these low baseline conditions (Extinction; Fig. 3B) the two rearing condition groups did not differ in locomotor activity scores for any treatment (saline or amphetamine; sated or hungry) as indicated by non-significant interactions of Rearing condition × Feeding, $F(1,10)=1.37$, ns and Rearing condition × Drug treatment, $F(1,10)=1.59$, ns.

Experiment 2

Acquisition of stimulus-reward association. The acquisition of the association between the light/noise compound stimulus and the presentation of the sucrose reward can be inferred from the behavioural control exhibited by the animals during the training phase. The effects of social isolation on the learning of this association, as indicated by the percentage of the total time spent at the panel that was during the 10-s CS period, are shown in Fig. 4. As can be seen, both groups of rats

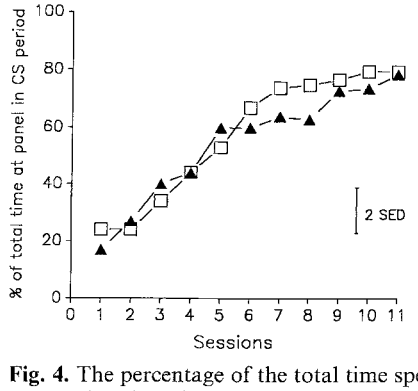

Fig. 4. The percentage of the total time spent at the panel that was appropriate i.e., during the 10-s CS period. The *bar* represents 2 standard errors of the difference between means for the interaction between Group and Sessions. □ Social; ▲ isolate

rapidly acquired this task so that the proportion of their panel-pressing time that is appropriate increased across training days, $F(10,147)=62.23$, $P<0.001$, reaching about 80% by day 11. There was no difference between the groups on this measure, $F(10,147)=1.56$, ns. The amount of time inappropriately spent at the panel (i.e., during the VI period) which is a measure of the ability to withhold responses until the CS is presented, reduced from about 200 s per 30 trials to less than 50 s by day 11, $F(10,147)=2.99$, $P=0.002$, but again, did not differ between groups, $F(10,147)=1.43$, ns.

The percentage of the possible time during the CS and VI periods that is spent at the panel was about 80% and 15%, respectively, and did not differ between the groups. These measures were not affected by the surgical implantation of the cannula. The amount of time at the panel in the VI period increased slightly on the first session after recovery from surgery, but thereafter retained preoperative values.

Histology. The results of the histological analysis (Fig. 5) showed that the majority of guide cannulae were correctly placed and gave access to the nucleus accumbens (the most ventral position of the injection cannulae is shown). All placements were in a anterior-posterior range of less than 1 mm. Two of the rats in the isolated group were rejected on the basis of their histology. One of these animals had one placement too ventral and outside of the accumbens, the other cannula placement in this rat appeared to be within the lateral ventricle. The other animal excluded from this group was found to have both cannulae in the olfactory tubercles. Examination of the locomotor activity scores of these two animals supported this analysis, as they had the two lowest scores in this group following intra-accumbens *d*-amphetamine and this response is well known to depend on the nucleus accumbens (Pijnenburg et al. 1976).

Responding with conditioned reinforcement. The effects of social isolation on responding with conditioned reinforcement, following a range of doses of intra-accumbens *d*-amphetamine are shown in Fig. 6. *d*-amphetamine infused into the nucleus accumbens produced a selective dose-dependent increase in responding on the CR lever, Lever × Dose, $F(4,44)=13.38$, $P<0.001$. This enhancement by *d*-amphetamine was significantly greater in isolation-reared rats, $F(4,44)=3.62$, $P=0.012$, with a mean of 96 lever presses following saline, increasing to a mean of 284 after the dose of 20 µg, compared to an increase from 47 to 105 in the socially-reared rats. Although isolates responded more on the CR lever than the socially-reared rats at all doses, $F(1,11)=5.02$, $P=0.047$, analysis of the simple main effect of Rearing at each dose showed this to be significant only at the 20 µg dose. When the rearing condition groups were analysed separately this revealed a significant, selective effect of intra-accumbens amphetamine on responding with CR in both the socials, $F(4,24)=4.16$, $P=0.01$, and the isolates, $F(4,28)=5.72$, $P=0.002$.

Both groups of rats responded more on the CR lever than on the NCR lever, $F(1,11)=51.06$, $P<0.001$, although appropriate post hoc comparisons revealed that in the socially-reared rats this difference only reached significance for the three doses of *d*-amphetamine, whereas in the isolation-reared rats CR responding was also significantly greater than NCR responding following saline infusions.

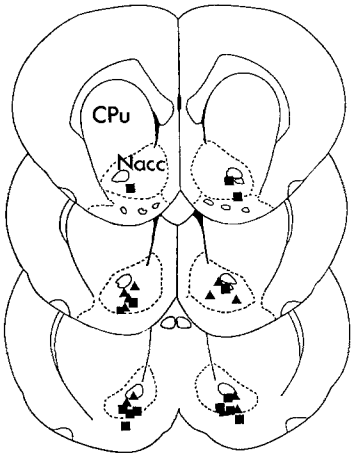

Fig. 5. Placement of the most ventral position of the injection cannulae. Brain sections are modified from Paxinos and Watson (1982). ■ socials; ▲ isolates. *Nacc* = nucleus accumbens, *CPu* = caudate putamen

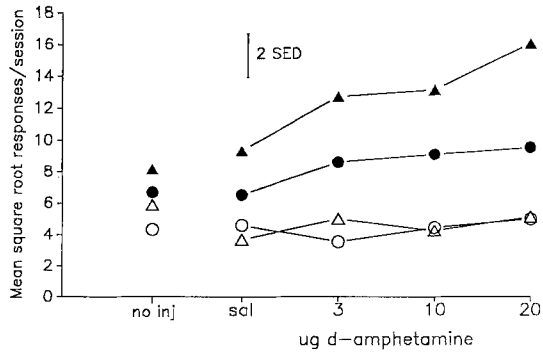

Fig. 6. Effects of social isolation and microinfusions of *d*-amphetamine on responding on a lever providing conditioned reinforcement *(CR)* and a lever providing no conditioned reinforcement *(NCR)*. The *bar* represents the standard error of the difference between means for the three-way interaction between Group, Dose and Lever. ▲ isol-CR; △ isol-NCR; ● soc-CR; ○soc-NCR

Sucrose consumption. There were no significant differences between the two groups in the amount of sucrose consumed in the 1-h test, although there was a non-significant trend for the isolates to consume less than the socially-reared controls, main effect of Rearing, $F(1,11)=3.09$, ns; Rearing × Time, $F(1,11)=1.24$, ns. The mean volumes consumed after 1 h were 16.3 ml and 13.8 ml for the socially-reared and isolated rats, respectively.

The effect of intra-accumbens d-amphetamine on locomotor activity. The two rearing condition groups did not differ in their scores for the 2-h habituation periods, $F(22,209)=0.39$, ns, (Fig. 7). Doses of 3 and 10 µg *d*-

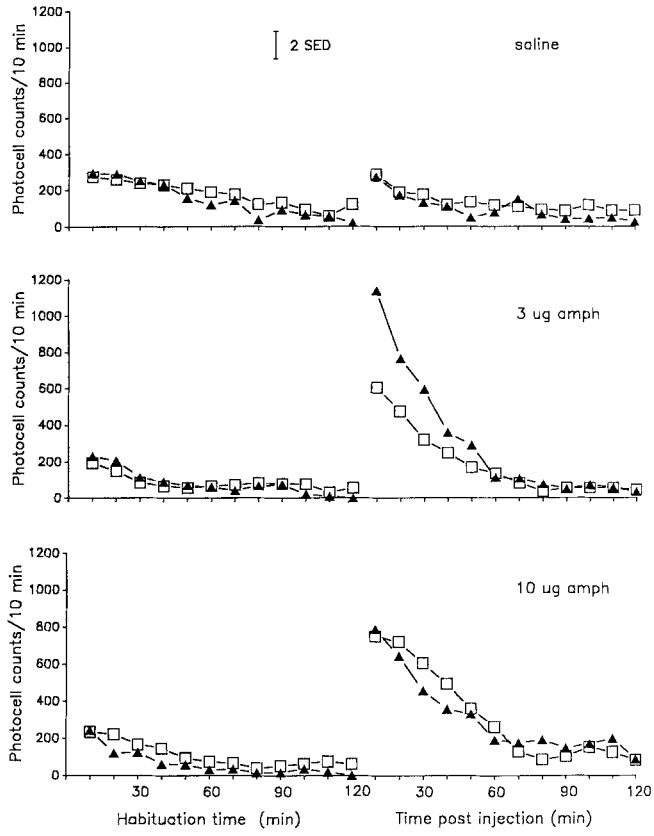

Fig. 7. The effects of intra-accumbens infusions of *d*-amphetamine or vehicle (phosphate buffered saline) on locomotor activity. The left-hand side of each panel indicates the photocell counts per 10 min for the 2-h habituation period before microinjection. The right-hand side shows the activity immediately following infusions. The bar indicates the standard error of the difference between the means for the three-way interaction between Group, Dose and Time. □ social; ▲ isolate

amphetamine produced a marked increase in activity compared to saline infusions, in both groups of rats, $F(2,19) = 17.21$, $P < 0.001$. ANOVA also indicated a Dose × Time interaction, $F(11,121) = 83.11$, $P < 0.001$, indicating that the locomotor stimulating effects of intra-accumbens *d*-amphetamine lasted only about 1 h. Isolated rats showed a significantly greater response to a dose of 3 μg *d*-amphetamine, but did not differ at a dose of 10 μg or following saline. This was confirmed by a significant three-way interaction of Rearing condition × Dose × Time, $F(22,209) = 3.45$, $P < 0.001$. Thus, in isolated rats there was an apparent increased sensitivity to a low dose of intra-accumbens *d*-amphetamine.

Discussion

These experiments demonstrate, in two complementary paradigms, changes in the processing of stimuli associated with reward following social isolation. In both experiments isolated rats showed an enhanced response to reward-related stimuli, which was further exaggerated by either systemic or intra-accumbens *d*-amphetamine, indicating a possible mesolimbic dopamine substrate. The isolation-induced sensitivities to these signals of reward and to *d*-amphetamine were both critically dependent on motivational variables. Thus, in Experiment 1 the difference in anticipatory locomotor activity and response to *d*-amphetamine between isolates and socially-reared rats was abolished by pre-feeding or extinction. In Experiment 2, the differential response to intra-accumbens *d*-amphetamine was restricted to the lever providing conditioned reinforcement (CR); thus it did not arise from a non-specific stimulation of behaviour. Although intra-accumbens *d*-amphetamine also stimulated locomotor activity to a greater extent in the isolated animals, it is pertinent to note that the animals were tested food-deprived and in a relatively novel situation, factors conducive to the development of incentive motivation (Campbell and Sheffield 1953).

These results cannot be attributed to differences in primary motivation, since in experiment 2 isolates did not drink more sucrose than controls. In fact there was a tendency for them to drink less, thus emphasizing an involvement of disturbances in appetitive rather than consummatory behaviour. The suggested involvement of mesolimbic dopamine in these incentive-motivational processes (Fibiger and Phillips 1986) supports the view that the effects of isolation interact directly or indirectly with dopamine-dependent mechanisms of the nucleus accumbens. Evidence in favour of this hypothesis includes findings that the facilitation of the effects of CR by systemic (Robbins and Everitt 1982) or intra-accumbens *d*-amphetamine (Taylor and Robbins 1986), and locomotor activity conditioned to presentation of either food (Jones, Marsden and Robbins, unpublished results) or drug (Gold et al. 1988), are dependent upon the functional integrity of the mesolimbic dopamine projection.

Rearing in isolation neither altered the acquisition of the association between the stimulus and reward nor impaired the ability to withhold responding, thereby indicating that the results in these experiments are not due to a general disruption of normal behaviour (for example, behavioural disinhibition or stimulus control) but depend on situation specific variables.

The isolation-reared rats appeared to show an altered response to *d*-amphetamine in at least three separate tests in this study. There was a shift in the dose-response curve both to systemic and to intra-accumbens *d*-amphetamine. In the latter case the effect was shown both for acquisition of responding for conditioned reinforcement and for locomotor activity. This altered sensitivity has been observed previously for a number of psychomotor stimulants, including amphetamine and cocaine, but primarily for stereotyped behaviours (Sahakian et al. 1975), which were not assessed directly in this study. However, the reductions in locomotor activity at the highest systemic doses are consistent with the possibility of increased stereotyped behaviours in the isolates. Whereas these amphetamine-induced stereotyped behaviours have been localised in the caudate putamen (Kelly et al. 1975), the present results indicate that another site of altered sensitivity to amphetamine in isolates is the nucleus accumbens. The additional contribution of this study has been to show that the altered sensitivity to certain of the nucleus accumbens dependent effects of

amphetamine in isolates is determined by motivational factors. This is also consistent with observations of apparent increases in the reinforcing efficacy of cocaine and amphetamine in a self-administration procedure (Schenk et al. 1987, 1988).

The precise mechanisms underlying the apparent isolation-induced dysregulation of mesolimbic dopamine still remain to be elucidated and potentially could include changes in post-synaptic mechanisms, such as D_1 or D_2 receptor number, or presynaptic processes such as dopamine synthesis, re-uptake or release. The increased locomotor response to intra-accumbens administration of 3 μg *d*-amphetamine, but not to 10 μg, in isolates indicates that these effects are probably not due to increases in the size of the releasable dopamine pool. A further possibility is disruption in the balance between dopamine and other neurotransmitters, such as serotonin, within the nucleus accumbens, which is known to influence both spontaneous (Jones et al. 1981; Carter and Pycock 1979) and amphetamine-induced locomotor activity (Lyness and Moore 1981).

It should also be pointed out that differences may have arisen due to the effects of food deprivation on dopaminergic sensitivity and thus the present results may not apply to animals that have food continuously available.

Both conditioned activity and the effects of conditioned reinforcers depend on processing by structures such as the amygdala (Cador et al. 1989) and hippocampus (Devenport et al. 1981), probably in conjunction with dopamine-dependent mechanisms of the ventral striatum, to which they project (Kelley and Domesick 1982; Kelley et al. 1982). Thus, the effects of social isolation could also be mediated by changes in these modulatory influences on the ventral striatum from the limbic system (Mogenson et al. 1980; Yim and Mogenson 1982; Yang and Mogenson 1984). Indeed, alterations in catecholamine function in both the amygdala and hippocampus have been reported following isolation-rearing in rats (Thoa et al. 1977).

These results could have implications for understanding the basis for a number of psychopathological conditions, including social factors influencing drug abuse and psychotic states such as schizophrenia, which similarly may depend on a conjunction of neuropathological changes in the limbic system (e.g., Farley et al. 1978; Reynolds 1983) and dopaminergic changes in the nucleus accumbens (Crow et al. 1979; Mackay et al. 1982). The current findings may also have relevance to reports that children with attention-deficit hyperactivity disorder show increased sensitivity to reward (Douglas and Peters 1979; Douglas 1983), environmental variables being a significant component in the aetiology of this syndrome (Werner and Smith 1977).

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