

RENEWAL OF AN EXTINGUISHED INSTRUMENTAL RESPONSE: NEURAL CORRELATES AND THE ROLE OF D1 DOPAMINE RECEPTORS

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Abstract—Contexts play an important role in controlling the expression of extinguished behaviors. We used an ABA renewal design to study the neural correlates, and role of D1 dopamine receptors, in contextual control over extinguished instrumental responding. Rats were trained to respond for a sucrose reward in one context (A). Responding was then extinguished in the same (A) or different (B) context. Rats were tested for responding in the original training context (A). Return to the original training context after extinction (group ABA) was associated with a return of responding. Three distinct patterns of Fos induction were detected on test: 1) ABA renewal was associated with selective increases in c-Fos protein induction in basolateral amygdala, ventral accumbens shell, and lateral hypothalamus (but not in orexin- or melanin-concentrating hormone (MCH)–hypothalamic neurons); 2) being placed in the same context as extinction training (AAA or ABB) was associated with a selective decrease in c-Fos induction in rostral agranular insular cortex; 3) being placed in any context on test was associated with the up-regulation of c-Fos induction in anterior cingulate, dorsomedial accumbens shell, accumbens core, lateral septum, and substantia nigra. The return of responding in ABA renewal was prevented by pre-treatment with the D1 dopamine receptor antagonist SCH23390 (10 μ g/kg; s.c.). SCH23390 also suppressed basal and renewal-associated c-Fos protein induction throughout accumbens, and, selectively suppressed renewal-associated c-Fos induction in lateral hypothalamus. These results suggest that renewal of extinguished responding for a sucrose reward depends on a distributed neural circuit involving basolateral amygdala, ventral accumbens shell, and lateral hypothalamus. D1 dopamine receptors within this circuit are essential for renewal. The results also suggest that rostral agranular insular cortex may play an important role in suppressing reward-seeking after extinction training. © 2006 IBRO. Published by Elsevier Ltd. All rights reserved.

Key words: renewal, c-Fos, extinction, nucleus accumbens, lateral hypothalamus, rostral agranular insular cortex.

Contexts play an important role in regulating the performance of learned associations. This is exemplified by their

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Abbreviations: CS, conditioned stimulus; IR, immunoreactive/immunoreactivity; MCH, melanin-concentrating hormone; MOR, μ -opioid receptor; NHS, normal horse serum; PB, phosphate buffer; PBS, phosphate buffer saline; PBT-X, phosphate buffer with Triton X-100; RAIC, rostral agranular insular cortex; S.E.M., standard error of the mean; TH, tyrosine hydroxylase; US, unconditioned stimulus.

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role in regulating expression of extinguished responses. Extinction is context specific (Bouton, 2002; Bouton and Swartzendruber, 1991). For example, rats trained in Pavlovian conditioning preparations with conditioned stimulus (CS)–unconditioned stimulus (US) pairings in one context (A), then subjected to extinction training in a different context (B), show a loss of conditioned responding to the CS when tested in the extinction context (B), but a recovery or “renewal” of such responding when tested with the CS in the original training context (A) (ABA renewal; e.g. Bouton and Bolles, 1979; Bouton and King, 1983; Harris et al., 2000) or a novel context (C) (ABC renewal; e.g. Bouton and Bolles, 1979; Harris et al., 2000). The presence of renewal indicates that extinction training does not erase the original learning. It suggests instead that a context-dependent mask is placed on this learning. Moreover, renewal acts to reduce behavior change beyond the therapeutic context and therefore contributes to relapse. The behavioral and neural mechanisms for contextual control over extinguished responding have been studied extensively for Pavlovian conditioning, when an animal learns about the relation between a CS and a US (e.g. Bouton, 2004; Corcoran and Maren, 2001, 2004; Frohardt et al., 2000). By contrast, considerably less attention has been directed toward these mechanisms for instrumental conditioning, when an animal learns about the relations between its actions and their outcomes.

Extinguished instrumental responding is also subject to contextual control. There is evidence for renewal of extinguished instrumental responding for natural (e.g. Welker and McAuley, 1978; Nakajima et al., 2000, 2002) and drug rewards (e.g. Crombag and Shaham, 2002). Renewal has typically been observed when training and extinction occur in different contexts and test occurs in the training context (ABA renewal). The brain mechanisms for such renewal remain unclear. Studies of ABA renewal of instrumental responding based on drug reinforcers implicate D1 dopamine receptors (Crombag et al., 2002) as well as glutamatergic neurotransmission within the ventral tegmental area and nucleus accumbens (Bossert et al., 2004, 2005). Functional inactivation studies, using microinjections of the sodium channel blocker tetrodotoxin, further implicate a distributed neural network that includes the dorsomedial prefrontal cortex, basolateral amygdala, and dorsal hippocampus in ABA renewal of instrumental responding for a drug reward (Fuchs et al., 2005). Thus, knowledge about the brain mechanisms for renewal of extinguished instrumental responding is limited and based almost entirely on drug reinforcers.

These experiments studied the neural mechanisms for renewal of an extinguished instrumental response based on a natural reward (sucrose). The first aim was to visualize the induction of the c-Fos protein, a marker of neuronal activity, to identify the brain regions activated during ABA renewal of extinguished reward-seeking. To date there have been no published studies on the patterns of c-Fos induction associated with contextual control over extinguished instrumental responding. The second aim was to study the role of dopamine D1 receptors in contextual control over extinguished reward-seeking. There have been no investigations into the role of D1 receptors in renewal of instrumental responding, other than for drug reinforcers. It is unclear whether the previously described role for dopamine in renewal is specific to drug reinforcers, or if it is a more general role in contextual control over extinguished instrumental responding.

To address these aims, rats were first trained to respond for a sucrose reward in context A. In experiment 1 this responding was then extinguished in either the same context (group AAA) or in a different context, context B (group ABA). Rats were then tested for responding in the original training context, context A. In experiment 2 rats were trained in context A, extinguished in context B, and then were tested in either the original training context, context A (groups ABA), or in the extinction context, context B (groups ABB). Prior to test in experiment 2 rats were injected with the D1 dopamine receptor antagonist SCH23390 or with saline. In both experiments we studied the behavioral responses on test and the pattern of c-Fos protein induction associated with test. We used different designs in experiment 1 (AAA versus ABA renewal) and experiment 2 (ABB versus ABA renewal) to study the potential different consequences of testing in an extinction context with (AAA) and without (ABB) a history of prior response reinforcement. Finally, because any investigation of the patterns of c-Fos induction associated with instrumental renewal can confound the consequences of recovery of extinguished responding with those of re-exposure to the training context, we attempted to dissociate these two influences. In experiment 3 all rats were trained in context A, extinguished in context B, and tested in context A (ABA). For one group, group Response, the nose-poke manipulandum was present on this test and subjects were able to emit the trained response. For the second group, group No Response, the manipulandum was removed and subjects were unable to emit the trained response on test. The context was otherwise unaltered. Comparison between these groups permitted study of the potentially different role in c-Fos protein induction served by re-exposure to the training context (context A) versus that served by instrumental responding.

EXPERIMENTAL PROCEDURES

Experiment 1: neural correlates of renewal of an extinguished instrumental response

Subjects. Subjects were 12 experimentally naive male Long-Evans rats (240–260 g) obtained from a commercial sup-

plier (Monash Animal Services, Gippsland, Victoria, Australia). After arrival, rats were housed in groups of six to eight in plastic cages maintained on a 12-h reverse light/dark cycle (lights on at 19:00 h). Three days prior to the start of training rats were placed on a schedule of restricted access to water and food, with these available for 1 h each day between 17:00 and 18:00 h. The procedures were approved by the Animal Care and Ethics Committee at the University of New South Wales and conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80–23) revised 1996. The number of animals used in these experiments was the minimum required. All procedures were designed to reduce their impact on the animals.

Behavioral apparatus. Training, extinction, and test were conducted in two sets of four chambers which differed in their visual (brightly lit versus dark), tactile (Perspex versus grid floors), and olfactory (rose oil versus peppermint essence) properties. For all chambers (24 cm [length] × 30 cm [width] × 21 cm [height]), the front and rear walls as well as the hinged lid were constructed of clear Perspex and the end walls were made of stainless steel. In all chambers, two nose-poke holes, containing a white cue-light, were located on one side wall of the chamber 3 cm above a grid floor. A recessed magazine was located behind a 4 cm × 4 cm opening in the center of the same wall, between the two nose-pokes. Responding on one (active) nose-poke resulted in delivery of a sucrose-reward to the magazine whereas responding on the other (inactive) nose-poke had no programmable consequences.

In one set of chambers the floors consisted of stainless steel rods, 4 mm in diameter, spaced 15 mm apart (center to center). These chambers were located in sound- and light-attenuating cabinets equipped with fans providing constant ventilation and low level background noise. There was no illumination in these chambers other than that provided by the white cue light recessed in the nose pokes. A concentrated rose oil essence (1 ml) was placed in the bedding beneath these chambers. In the second set of four chambers the floors consisted of Perspex. These chambers were located in sound- and light-attenuating cabinets equipped with fans providing constant ventilation. The chambers were illuminated by a 100 mV white house light mounted on the side wall of the chamber opposite to the nose-pokes and magazine. A concentrated peppermint essence (1 ml) was placed in the bedding beneath these chambers. These two sets of chambers were fully counterbalanced within-subjects to serve as Contexts A and B.

Behavioral testing procedures. On the first two days, the animals received 20 min magazine training sessions in both chambers. During these sessions there were 10 non-contingent deliveries of 0.15 ml of a 10% sucrose solution, at time intervals variable around a mean of 1.2 min. On the next five days animals were placed in Context A for 1 h. During this time responding on the active nose-poke was rewarded, on an FR-1 schedule, by delivery of 0.6 ml of a 10% sucrose solution to the magazine. Responses on the inactive nose-poke were recorded but had no programmable consequences. We used an FR-1 schedule to facilitate comparison with published data on the brain mechanisms for ABA renewal of extinguished drug seeking (e.g. Bossert et al., 2004; Crombag et al., 2002; Fuchs et al., 2005). At the end of training rats were randomly allocated to group AAA ($n=4$), group ABA ($n=4$), or group AA– ($n=4$). Each day for the next 4 days rats were placed in context A (group AAA) or Context B (group ABA) for 1 h. During this training, responses on either nose-poke had no programmable consequences but were recorded. Twenty-four hours after the final extinction training session, all rats were placed in Context A for 1 h. During this time responses on either nose-poke had no programmable consequences but were recorded. The final group in this experiment, group AA– ($n=4$), was trained and extinguished in an identical manner to group AAA but never tested. Instead, on test they were

transported to the laboratory and simply given equivalent handling as groups AAA and ABA. This final group was intended to control for any effects of training history as well as handling and transport effects and so forth associated with testing.

Immunohistochemistry. At 2 h following placement in the chambers on test (group AAA and ABA) or 2 h after arrival in the laboratory (group AA–), rats were deeply anesthetized with sodium pentobarbital (100 mg/kg i.p.) and perfused transcardially with 50 ml of 0.9% saline, containing 1% sodium nitrite and heparin (5000 i.u./ml), followed by 500 ml of 4% paraformaldehyde in 0.1 M phosphate buffer (PB), pH 7.4. Brains were post-fixed for 1 h in the same fixative and placed in 20% sucrose solution overnight. Brains were blocked using a matrix aligned to the atlas of Paxinos and Watson (1997) and 40 μ m coronal sections were cut using a cryostat (Microm HM560, Microm International, Waldorf, Germany). Four serially adjacent sets of sections were obtained from each brain and stored in 0.1% sodium azide in 0.1 M phosphate buffer saline (PBS) pH 7.2. Two series of sections were selected from each rat and two-color peroxidase immunohistochemistry was used to reveal c-Fos in combination with tyrosine hydroxylase (TH) or the μ -opioid receptor (MOR).

Free floating sections were washed repeatedly in 0.1 M PB (pH 7.4), followed by two 30 min washes in 50% ethanol, the second of which contained 3% H_2O_2 , and were then incubated in 5% normal horse serum (NHS) in PB (pH 7.4) for 30 min. Sections were incubated in rabbit antiserum against c-Fos (1:5000; c-Fos (4), sc-52, Santa Cruz Biotechnologies, Santa Cruz, CA, USA), which were mixed with either a sheep antiserum against TH (1:3000; AB1542, Chemicon, Temecula, CA, USA) or a guinea-pig antiserum against the MOR (1:2000; AB1774, Chemicon). These primary antibodies were diluted in 0.1 M phosphate buffer (pH 7.4) containing 2% normal horse serum and 0.2% Triton X-100 (PBT-X), and incubations were for 48 h at 4 °C, with gentle agitation. After washing off unbound primary antibodies, sections were incubated overnight at room temperature in biotinylated donkey anti-rabbit IgG (1:1000; Jackson Immunoresearch Laboratories, West Grove, PA, USA) diluted in 2% NHS PBT-X. Sections were then incubated for two hours at room temperature in ABC reagent (Vector Elite kit: 6 μ l/ml avidin and 6 μ l/ml biotin; Vector Laboratories, Burlingame, CA, USA). Black immunoreactive (IR) nuclei labeled for c-Fos were revealed by a nickel-intensified diaminobenzidine reaction, with peroxide being generated by glucose oxidase. To do this sections were washed in PB, followed by 0.1 M acetate buffer (pH 6.0), and then incubated for 15 min in 0.1 M acetate buffer (pH 6.0) containing 2% nickel sulfate, 0.0025% 3,3'-diaminobenzidine, 0.004% ammonium chloride, and 0.02% D-glucose. The peroxidase reaction was started by adding 0.2 μ l/ml glucose oxidase and stopped using acetate buffer (pH 6.0). Brain sections were then washed in PB and processed again, in a similar manner using biotinylated donkey anti-sheep IgG or donkey anti-guinea-pig IgG (1:1000; Jackson Immunoresearch Laboratories) but without nickel-intensification to localize IR for the MOR and TH, revealed as a brown reaction product. Sections were mounted onto gelatin-treated slides, dehydrated, cleared in xylene, and coverslipped with DePeX.

Experiment 2: role of D1 dopamine receptors in renewal of an extinguished instrumental response

Subjects and apparatus. The subjects were 28 experimentally naive male Long-Evans rats obtained from the same source and maintained under the same conditions as described in experiment 1. All apparatus was identical to that described in experiment 1.

Behavioral testing procedures. On the first two days, rats received 20 min magazine training sessions in a manner identical to experiment 1. On the next five days animals were placed in Context A for 1 h and trained to nose-poke for sucrose in a manner

identical to experiment 1. Each day for the next 5 days rats were placed in context B for 1 h for extinction training. This training was identical to experiment 1. Twenty-four hours after the final extinction session rats were transported to the laboratory and injected s.c. with 0.9% w/v saline or 10 μ g/kg of the D1 receptor antagonist SCH23390 (Sigma-Aldrich, Sydney, NSW, Australia). This dose was chosen based on published work showing that it attenuated renewal of responding based on a drug reinforcer but was otherwise without effect (Crombag et al., 2002). Half the rats in each drug condition were placed in Context B for 1 h whereas the remainder was placed in Context A. Thus, there were four groups: group ABB-Saline ($n=7$), group ABB-SCH23390 ($n=6$), group ABA-Saline ($n=8$), and group ABA-SCH23390 ($n=7$).

Immunohistochemistry. At 2 h following placement in the chambers on test, four randomly selected rats from each group were perfused, brains removed, sectioned and processed for c-Fos and TH as described for experiment 1. A second and third series of sections through the lateral hypothalamus was selected from each rat and two-color immunofluorescence was used to reveal c-Fos and orexin in one series and c-Fos and melanin-concentrating hormone (MCH) in the other series. Free floating sections were washed repeatedly in 0.1 M PBS (pH 7.2), followed by 2 h incubation in PBS (pH 7.2) containing 10% NHS and 0.5% Triton X-100. Sections were then incubated in goat antiserum against c-Fos (1:2000; Santa Cruz Biotechnologies), mixed with either rabbit antiserum against orexin (1:20,000; Phoenix Pharmaceuticals, Belmont, CA, USA) or MCH (1:10,000; Phoenix Pharmaceuticals). These primary antibodies were diluted in 0.1 M PBS (pH 7.2) containing 0.1% sodium azide, 2% NHS and PBT-X, and incubations were for 48 h at room temperature, with gentle agitation. After washing off unbound primary antibodies, sections were then incubated for 4 h at room temperature in biotinylated donkey anti-sheep IgG (1:1000; Jackson Immunoresearch Laboratories), and donkey anti-rabbit FITC (1:200; Jackson Immunoresearch Laboratories), diluted in 2% NHS PBT-X. After washing off unbound secondary antibodies, sections were further incubated for 1 h in streptavidin CY3 (1:2000; Sigma). Sections were mounted onto chrome alum/gelatin-treated slides and coverslipped with buffered glycerol (pH 8.6).

Experiment 3: role of responding in c-Fos induction

The subjects were eight experimentally naive male Long-Evans rats obtained from the same source and maintained under the same conditions as described in experiment 1. All apparatus was identical to that described in experiment 1. The procedure for conditioning and extinction was identical to experiment 2. All rats were tested in context A. For rats in group Response, context A was identical on test and training. For rats in group No Response, the nose-poking manipulanda were removed from the chambers and replaced with steel panels. At 2 h following placement in the chambers on test, rats were deeply anesthetized with sodium pentobarbital. The procedures for immunohistochemistry were those described previously.

Data analysis

In all experiments, the number of responses was recorded during training, extinction, and test. These data were analyzed by means of analysis of variance adopting a multivariate approach to repeated measures where necessary (O'Brien and Kaiser, 1985). The type I error rate (α) was controlled at 0.05 for each contrast tested.

Counts of neurons IR for c-Fos were conducted through the rostro-caudal extent of each brain region of interest by an observer unaware of group allocations. All sections counted were 160 μ m apart. Chemoarchitecture was revealed by TH or MOR labeling and aided identification of anatomical boundaries. The brain regions analyzed were: prelimbic and infralimbic cortex over

three sections beginning at +3.20 mm; rostral agranular insular cortex (RAIC) over four sections beginning at +2.70; nucleus accumbens (rostral pole) over three sections beginning at +2.70 mm; nucleus accumbens core and shell subnuclei over six sections beginning at +1.70 mm; anterior cingulate over four sections beginning at +1.20 mm; lateral septum over six sections beginning at +1.20; ventral pallidum over four sections beginning at +0.20 mm; paraventricular thalamus over eight sections beginning at –1.30 mm; hippocampus over eight sections beginning at –1.80 mm; lateral hypothalamus and the basolateral and central amygdala over six sections beginning at –1.80 mm; habenula over six sections beginning at –3.30; substantia nigra, ventral tegmental area, paranigral, and interfascicular nucleus over six sections beginning at –5.20 mm. All coordinates given are distance from bregma according to the rat brain atlas of Paxinos and Watson (1997).

To determine the distribution of c-Fos-positive neurons relative to orexin and MCH containing neurons in the lateral hypothalamus eight-bit monochrome images were taken of c-Fos and orexin or c-Fos and MCH throughout the rostro-caudal extent of the lateral hypothalamus (Olympus BX51 camera running ImagePro software). Images were exported into ImageJ software (NIH), where colorized image stacks of corresponding c-Fos and orexin or c-Fos and MCH images were combined for analysis (see Fig. 7 for example).

RESULTS

Experiment 1: neural correlates of renewal of an extinguished instrumental response

Behavior. All animals acquired responding for sucrose that increased across days ($F(1, 6)=25.9$, $P<0.05$). There was significantly more responding on the active than the inactive nose-poke ($F(1, 6)=91.4$, $P<0.05$). At the end of training rats were randomly allocated to group AAA or ABA (there were no differences between these groups on levels of responding during training). The left panel of Fig. 1 shows the mean and standard error of the mean (S.E.M.) number of responses during extinction training. There were no differences between group AAA and group ABA in overall responding during extinction ($F(1, 6)<1$, $P>0.05$). There was more responding on the active than inactive nose-poke during extinction ($F(1, 6)=90.1$; $P<0.05$) and this difference approached, but did not sig-

nificantly interact with the difference between groups ($F(1, 6)=5.8$, $P=0.052$). Overall levels of responding decreased across extinction ($F(1, 6)=21.0$, $P<0.05$) and there were no differences between group AAA and group ABA in this decrease ($F(1, 6)<1$, $P>0.05$). Not surprisingly, this decrease was significantly greater for responding on the active than the inactive nose poke ($F(1, 6)=38.7$, $P<0.05$) but this did not interact with group ($F(1, 6)<1$, $P>0.05$).

The right panel of Fig. 1 shows the mean and S.E.M. number of responses when all rats were tested in context A. Overall, there was significantly greater responding in group ABA than group AAA ($F(1, 6)=6.9$, $P<0.05$). There was also overall more responding on the active than the inactive nose-poke ($F(1, 6)=14.6$, $P<0.05$). Importantly, there was an interaction ($F(1, 6)=7.1$, $P<0.05$) so that the differences between group AAA and group ABA were significantly greater for the active than the inactive nose-poke. This indicates the presence of renewal of extinguished responding which was selective for the response previously associated with delivery of the sucrose reward.

c-Fos-IR. c-Fos-IR was examined in 29 brain regions. Quantification was performed in two serially adjacent series of tissue sections taken from the same animals, in which c-Fos-IR was combined with either TH- or MOR-IR.

The accumbens was separated into three main subdivisions: shell, core, and rostral pole (Zahm and Brog, 1992). The accumbens shell was further subdivided into its dorsomedial and ventral regions for analysis (Fig. 2). Of the four accumbal subregions analyzed, the only region where there was a significant increase in the number of c-Fos-IR neurons associated with ABA renewal was the ventral region of the accumbens shell (group ABA vs. groups AAA/AA–, $F(2, 9)=5.3$; $P<0.05$). In the dorsomedial region of the accumbens shell and in the accumbens core increases in c-Fos-IR were detected in rats that were placed in the test environment compared with control rats (dorsomedial shell: groups AAA/ABA vs. AA–, $F(2, 9)=9.5$, $P<0.05$; core: AAA/ABA vs. AA–, $F(2, 9)=6.4$, $P<0.05$), however no further increase was observed in the group ABA compared with group AAA in either structure ($P_s<0.05$) (Fig. 2). Compartmental analysis of the distribution c-Fos-IR neurons relative to the patch (MOR-positive) and matrix (MOR-negative) regions of the accumbens revealed that the majority of c-Fos-IR neurons were located in MOR-negative matrix regions in both the AAA and ABA groups. There was no change in the patch/matrix distribution of c-Fos-IR in any of the accumbens subnuclei as a function of group (Table 1).

Activity of accumbens neurons depends on glutamatergic innervation from amygdala, hippocampus, and cortex, as well as innervations from midbrain dopaminergic regions. Interaction between dopamine and glutamate release in the accumbens is critical to the induction of transcription factors including Fos-related proteins (Cole et al., 1992; Liu et al., 1994). We therefore examined c-Fos-IR neurons in the cortical, thalamic, and amygdaloid structures that provide excitatory glutamate input to the accu-

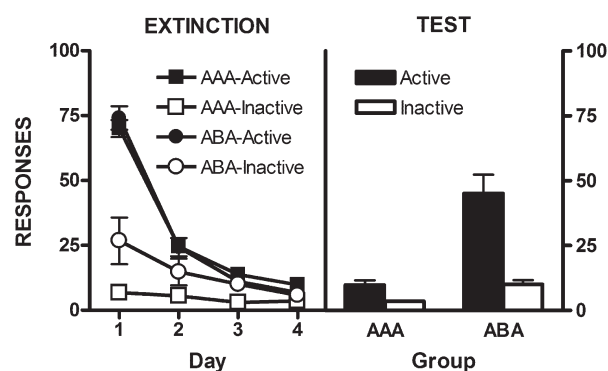


Fig. 1. Mean ($\pm$ S.E.M.) responses on active and inactive nose-poke during extinction of instrumental responding (left panel) and on test (right panel) in experiment 1 in rats trained, extinguished and tested in context A (AAA) or trained and tested in context A and extinguished in context B (ABA).

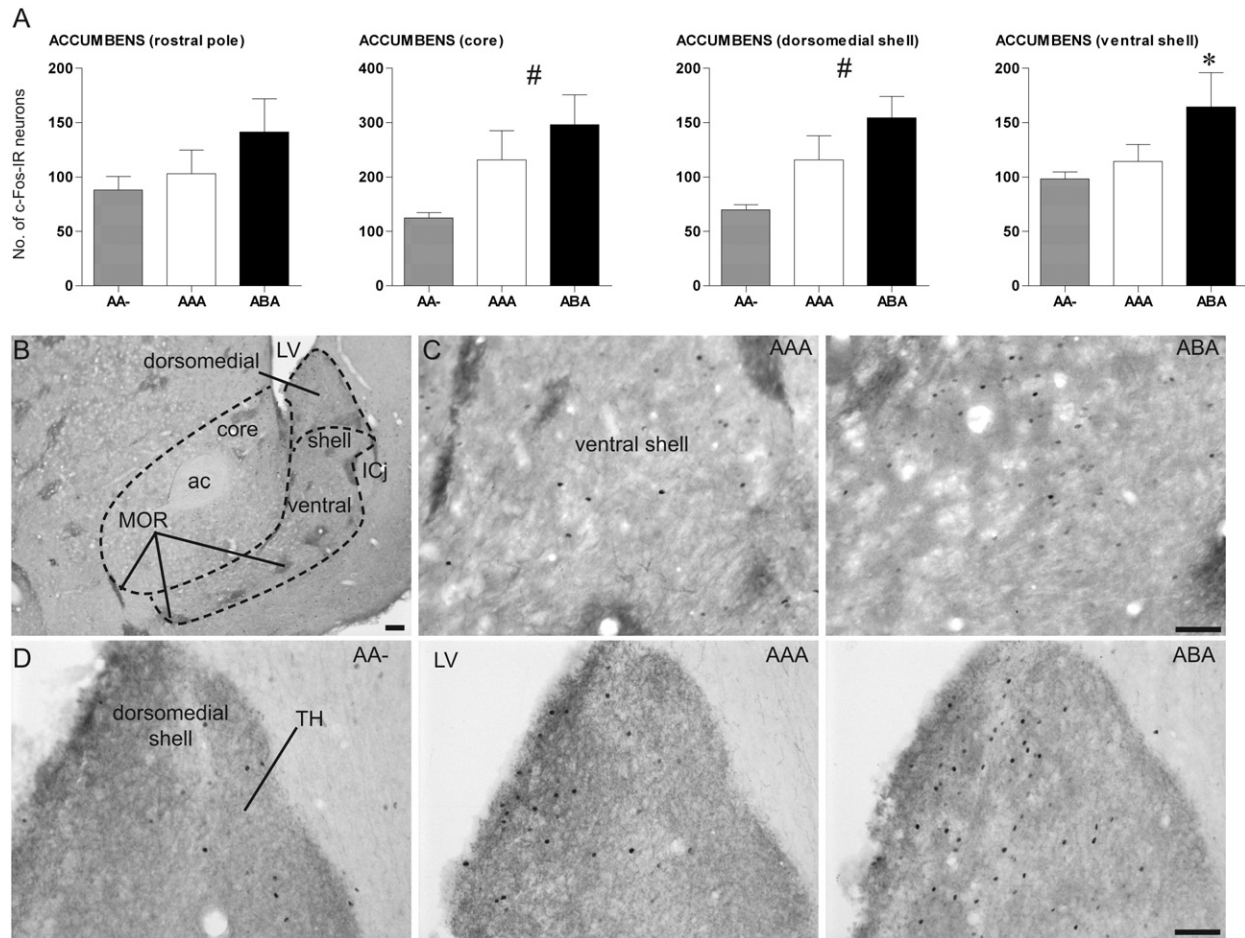


Fig. 2. (A) c-Fos-IR in the nucleus accumbens in experiment 1. Bars show mean ($\pm$ S.E.M.) number of c-Fos-IR neurons counted for each of the treatment groups in the major subnuclei of the nucleus accumbens. #, (AAA/ABA vs. AA–, $P < 0.05$), *, (ABA vs. AAA/AA–, $P < 0.05$). (B) Photomicrograph of a nucleus accumbens tissue section showing IR for the MOR and the major subdivisions of the nucleus accumbens analyzed. (C) Photomicrographs showing MOR-IR and c-Fos-IR neurons in representative AAA and ABA animals in the ventral accumbens shell. (D) Photomicrographs showing TH-IR and c-Fos-IR neurons in representative AA–, AAA and ABA animals in the dorsomedial accumbens shell. *Abbreviations:* ac, anterior commissure; ICj, islands of Calleja; LV, lateral ventricle. Scale bars=100 μ m B, C and D=50 μ m.

bens and the midbrain regions that provide dopaminergic innervation to the accumbens.

Renewal of sucrose seeking failed to increase the number of c-Fos-IR neurons in any of the cortical structures examined (Fig. 3). However, exposure to the test environment increased the number of c-Fos-IR neurons in groups AAA and ABA relative to AA– in anterior cingulate cortex ($F(1, 9)=6.2$, $P < 0.05$) but no further difference was observed between groups AAA and ABA in this region

($P > 0.05$). Interestingly, there were fewer c-Fos-IR neurons in RAIC in group AAA than groups ABA and AA– ($F(2, 9)=17.1$, $P < 0.05$) which did not differ from each other ($P > 0.05$) (Fig. 3).

Renewal was associated with increases in number of c-Fos-IR neurons in basolateral amygdala (group ABA vs. groups AAA/AA–, $F(2, 9)=12.3$, $P < 0.05$) (Fig. 4). No other changes were observed in lateral amygdala, amygdalostratial transition zone, or in any subregion of central

Table 1. Mean ($\pm$ S.E.M.) numbers of c-Fos-IR neurons relative to the patch (MOR+) and matrix (MOR–) compartments of the nucleus accumbens in experiment 1

Structure	AAA		ABA	
	MOR+	MOR–	MOR+	MOR–
Rostral pole	37.01 $\pm$ 1.10	102.50 $\pm$ 23.56	48.25 $\pm$ 14.88	106.50 $\pm$ 27.17
Core	48.25 $\pm$ 13.76	198.25 $\pm$ 34.26	71.25 $\pm$ 10.09	197.25 $\pm$ 31.38
Dorsomedial shell	36.50 $\pm$ 2.06	66.25 $\pm$ 19.88	55.25 $\pm$ 11.25	68.50 $\pm$ 8.93
Ventral shell	25.50 $\pm$ 7.26	76.25 $\pm$ 12.13	31.25 $\pm$ 2.78	124.50 $\pm$ 26.00

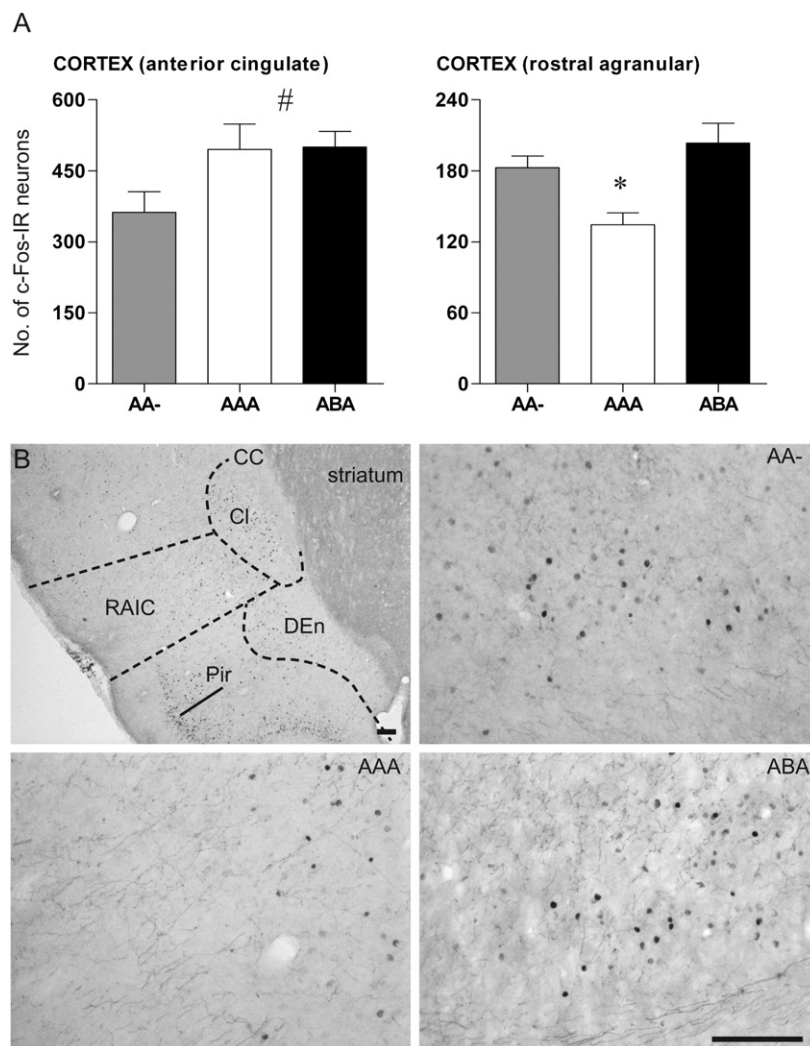


Fig. 3. (A) c-Fos-IR in the anterior cingulate and rostral agranular insular cortices in experiment 1. Bars show mean ($\pm$ S.E.M.) number of c-Fos-IR neurons counted for each of the treatment groups. #, (AAA/ABA vs. AA-, $P < 0.05$), *, (AAA vs. ABA/AA-, $P < 0.05$). (B) Photomicrographs showing regions analyzed and c-Fos-IR neurons in representative AA-, AAA and ABA animals in the RAIC. Abbreviations: CC, corpus callosum; Cl, claustrum; DEn, dorsal endopiriform nucleus; Pir, piriform cortex. Scale bars=100 μ m.

amygdala (Table 2). There was no change in c-Fos in any hippocampal region. Furthermore, being placed in the testing environment failed to increase the number of c-Fos-IR neurons in either the AAA or ABA groups relative to group AA- (Table 2). Within midbrain dopamine regions, exposure to the test environment increased the number of c-Fos-IR neurons in groups AAA and ABA relative to group AA- in substantia nigra ($F(1, 9) = 8.9$, $P < 0.05$) (Table 2) but no further difference was observed between groups AAA and ABA in this region ($P > 0.05$). No changes were seen in ventral tegmental area, interfascicular nucleus or paranigral nuclei (Table 2).

Lateral hypothalamus is a major target of accumbens shell controlling behavioral and autonomic responses to motivational signals. Like ventral accumbens shell, renewal was associated with significantly increased c-Fos-IR in lateral hypothalamus (group ABA vs. groups AAA/AA-, $F(2, 9) = 5.6$, $P < 0.05$) (Fig. 4) but no further difference

was observed between groups AAA and AA- in this region ($P > 0.05$). Being placed in the testing environment significantly increased the number of c-Fos-IR neurons in lateral septum (groups AAA/ABA vs. AA-, $F(2, 9) = 19.7$, $P < 0.05$) but no further difference was observed between groups AAA and ABA in this region ($P > 0.05$) (Table 2). No changes were seen in ventral pallidum, habenular, or paraventricular hypothalamus (Table 2).

Experiment 2: role of D1 dopamine receptors in renewal of an extinguished instrumental response

Behavior. All animals acquired responding for sucrose that increased across days ($F(1, 27) = 30.2$; $P < 0.05$) and there was significantly more responding on the active than the inactive nose-poke ($F(1, 27) = 168.1$, $P < 0.05$). At the end of training, rats were randomly allocated to groups

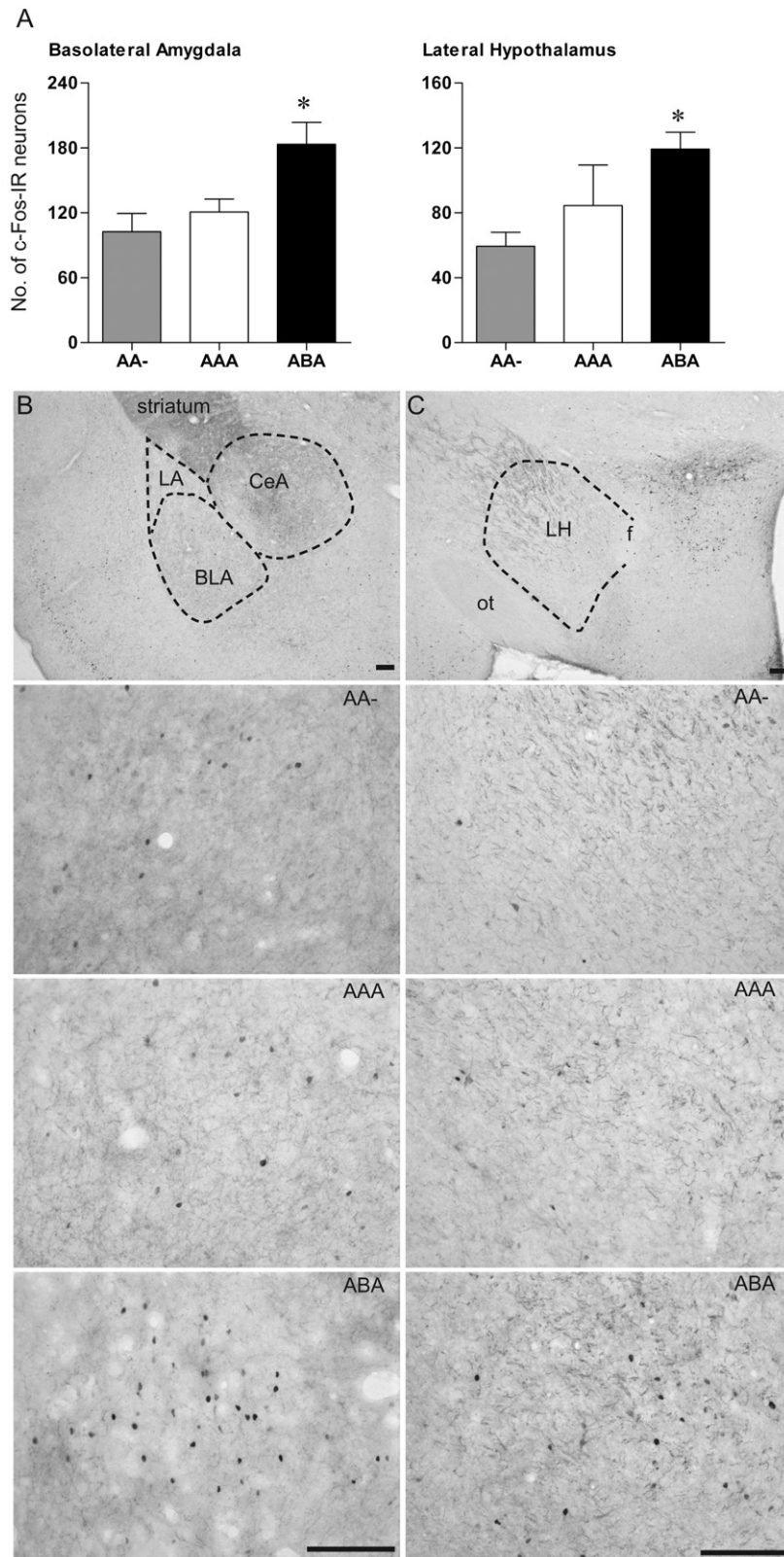


Fig. 4. (A) c-Fos-IR in the BLA and LH in experiment 1. Bars show the mean ($\pm$ S.E.M.) number of c-Fos-IR neurons counted for each of the treatment groups. *, (ABA vs. AAA/AA-, $P < 0.05$). (B) Photomicrographs showing the regions of the amygdala analyzed and c-Fos-IR neurons in representative AA-, AAA and ABA animals in the BLA. (C) Photomicrographs showing regions analyzed and c-Fos-IR neurons in representative AA-, AAA and ABA animals in the LH. Abbreviations: BLA, basolateral amygdala; CeA, central amygdala; f, fornix; LA, lateral amygdala; LH, lateral hypothalamus; ot, optic tract. Scale bars = 100 μ m.

Table 2. Mean ($\pm$ S.E.M.) number of c-Fos-IR neurons in experiment 1

Structure	AA–	AAA	ABA
Cortex			
Prelimbic	230.50 $\pm$ 26.81	325.00 $\pm$ 48.19	435.00 $\pm$ 100.02
Infralimbic	141.00 $\pm$ 16.77	167.00 $\pm$ 21.75	198.75 $\pm$ 18.45
Orbital	376.00 $\pm$ 32.55	383.50 $\pm$ 13.99	486.25 $\pm$ 80.38
Amygdala			
Lateral	23.50 $\pm$ 2.53	30.50 $\pm$ 6.74	38.00 $\pm$ 4.42
AStr	22.75 $\pm$ 4.23	20.25 $\pm$ 1.93	25.00 $\pm$ 1.68
CeC	31.75 $\pm$ 3.97	26.25 $\pm$ 3.20	33.75 $\pm$ 6.94
CcL	37.25 $\pm$ 6.49	41.00 $\pm$ 3.72	52.75 $\pm$ 10.31
CeM	51.75 $\pm$ 12.09	79.25 $\pm$ 31.04	86.75 $\pm$ 17.25
Hippocampus			
DG	146.50 $\pm$ 41.52	158.25 $\pm$ 15.69	175.00 $\pm$ 17.64
CA1	4.50 $\pm$ 0.87	8.50 $\pm$ 0.50	5.50 $\pm$ 2.96
CA2	5.25 $\pm$ 2.59	5.50 $\pm$ 0.65	7.00 $\pm$ 1.78
CA3	10.75 $\pm$ 3.82	9.25 $\pm$ 3.59	11.25 $\pm$ 4.96
Midbrain			
SN	3.00 $\pm$ 0.71	14.25 $\pm$ 3.71	11.50 $\pm$ 2.75
VTA	22.75 $\pm$ 6.52	13.00 $\pm$ 1.87	18.50 $\pm$ 6.20
PN	4.75 $\pm$ 1.65	10.50 $\pm$ 6.17	14.00 $\pm$ 4.73
IF	11.75 $\pm$ 4.53	23.00 $\pm$ 9.61	21.25 $\pm$ 3.71
Habenula	56.50 $\pm$ 9.13	71.00 $\pm$ 21.71	63.75 $\pm$ 17.35
PvTh	541.50 $\pm$ 74.63	472.75 $\pm$ 74.52	537.75 $\pm$ 40.60
Lateral septum	111.50 $\pm$ 21.41	338.00 $\pm$ 43.10	382.75 $\pm$ 62.85
Ventral pallidum	10.50 $\pm$ 1.71	29.00 $\pm$ 6.16	28.75 $\pm$ 9.96

Abbreviations: AStr, amygdalostratial transition zone; CeC, capsular central amygdala; CeL, lateral central amygdala; CeM, medial central amygdala; DG, dentate gyrus; IF, interfascicular nucleus; PN, parafascicular nucleus; PvTh, paraventricular thalamus; SN, substantia nigra; VTA, ventral tegmental area.

ABB or ABA (there were no differences between these groups on levels of responding during training). The left panel of Fig. 5 shows the mean and S.E.M. number of responses during extinction training. There were no differences between group ABB and group ABA in overall responding during extinction ($F(1, 26) < 1$, $P > 0.05$). There was more responding on the active than inactive nose-poke during extinction ($F(1, 26) = 42.0$, $P < 0.05$) but this did not interact significantly with the difference between groups ($F(1, 26) < 1$, $P > 0.05$). Overall levels of responding decreased across extinction ($F(1, 26) = 69.7$, $P < 0.05$) and there were no differences between groups in this decrease ($F(1, 26) < 1$, $P > 0.05$). Not surprisingly, the decrease in responding was significantly greater for the active than the inactive nose-poke ($F(1, 26) = 43.9$, $P < 0.05$) but this did not interact with group ($F(1, 26) < 1$, $P > 0.05$).

The right panel of Fig. 5 shows the mean and S.E.M. number of responses when all rats were tested. Overall, there was significantly greater responding in group ABA than group ABB ($F(1, 26) = 6.4$, $P < 0.05$). There was also an overall difference between SCH23390 and vehicle ($F(1, 26) = 9.7$, $P < 0.05$). Finally, there was an overall interaction between group ABA versus group ABB and injection (SCH23390 vs. Saline) ($F(1, 26) = 7.4$, $P < 0.05$) so that the effects of the D1 antagonist were significantly greater in group ABA than ABB. Overall, there was more responding on the active than the inactive nose-poke ($F(1, 26) = 38.3$, $P < 0.05$). Importantly, there was an interaction between

group (ABA vs. ABB) and responding on active versus inactive nose-poke ($F(1, 26) = 9.4$, $P < 0.05$) so that the differences between group ABB and group ABA were significantly greater for the active than the inactive nose-poke. This confirms the renewal of extinguished responding in group ABA. There was also an interaction between injection (SCH-23390 versus saline) and responding on active versus inactive nose-poke ($F(1, 26) = 5.1$, $P < 0.05$), so that the effects of the D1 antagonist were significantly greater for responses on the active nose-poke. Finally, there was a 2 (group ABA versus ABB) $\times$ 2 (SCH-23390 versus saline) $\times$ 2 (active versus inactive nose-poke) interaction ($F(1, 26) = 9.5$, $P < 0.05$). This indicates that the D1 antagonist acted to prevent renewal of extinguished responding which was selective for the response previously associated with delivery of the sucrose reward. It did not act non-selectively to suppress overall levels of responding. Further evidence for this selective effect of the D1 antagonist was the absence of any significant difference in responding between group ABB-SCH23390 and group ABB-Saline ($F(1, 11) < 1$, $P > 0.05$).

c-Fos-IR. c-Fos-IR was examined in nucleus accumbens, lateral hypothalamus, and RAIC. There were two questions of interest. The first was whether differences between groups ABA and ABB in c-Fos expression could be modulated by the D1 antagonist. Renewal was again associated with increased c-Fos expression in ventral accumbens shell: group ABA-Saline was significantly different to group ABB-Saline ($F(3, 14) = 18.6$, $P < 0.05$) (Fig. 6). c-Fos expression in both the ABA and ABB conditions was modulated by D1 dopamine receptors because SCH23390 significantly reduced Fos in group ABB-SCH23390 ($F(3, 14) = 5.2$, $P < 0.05$) and group ABA-SCH23390 ($F(3, 14) = 40.2$, $P < 0.05$) compared with their respective saline controls. Renewal was again associated with increased c-Fos expression in lateral hypothalamus: group ABA-Saline was significantly different to group ABB-Saline ($F(3, 14) = 5.9$, $P < 0.05$) (Fig. 6). However, only Fos expression in ABA condition was dependent upon D1 dopamine receptors because there was a significant difference be-

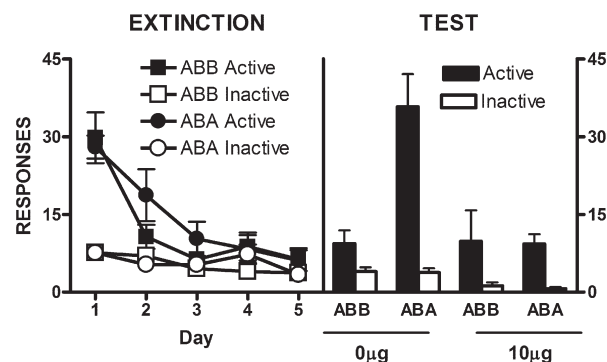


Fig. 5. Mean ($\pm$ S.E.M.) responses on active and inactive nose-poke during extinction of instrumental responding (left panel) and on test (right panel) in experiment 2 for rats trained in context A and extinguished as well as tested in context B (ABB) or trained and tested in context A and extinguished in context B (ABA). Rats were injected s.c. with 0 or 10 μ g/kg SCH23390 prior to test.

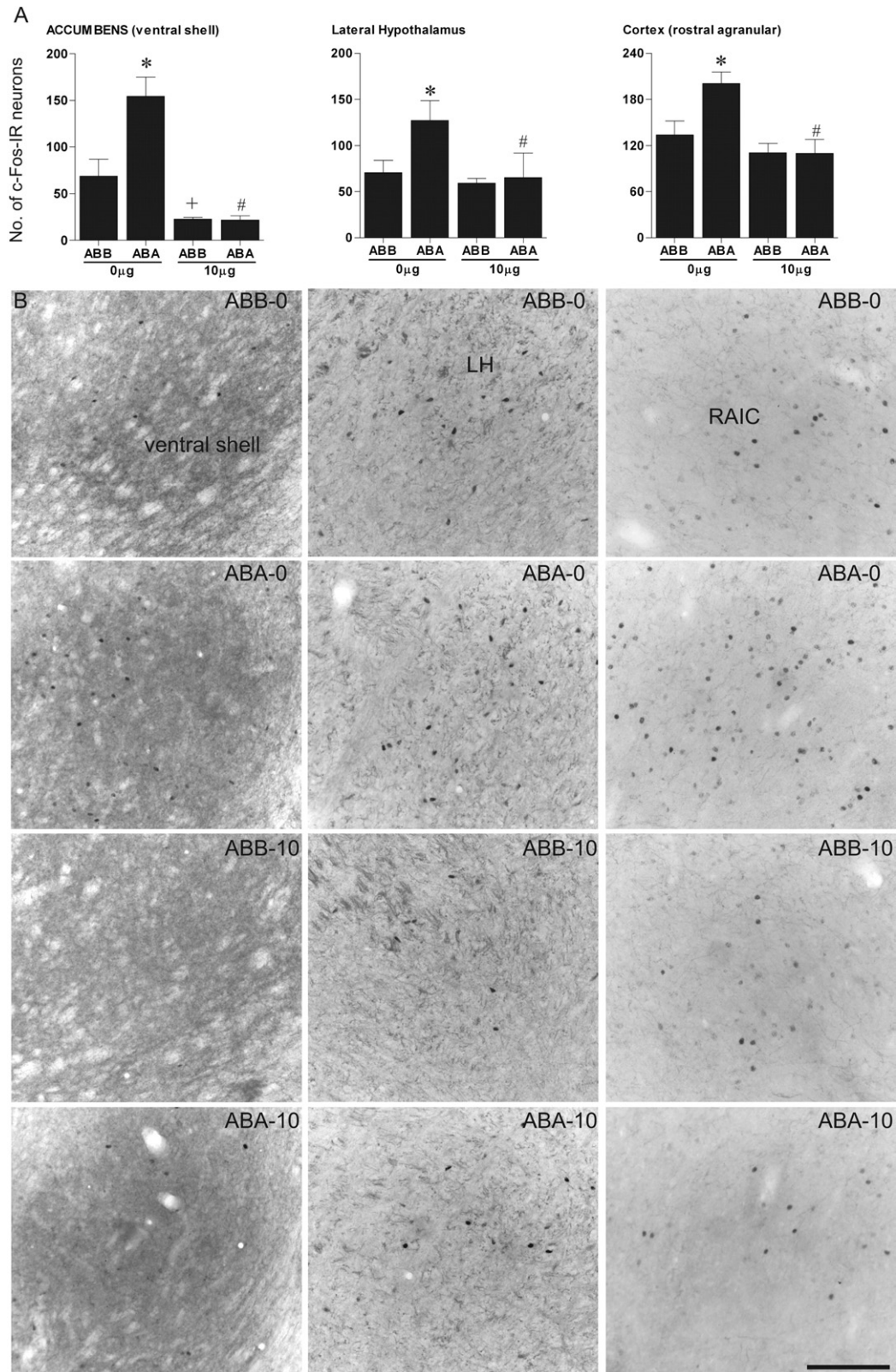


Fig. 6. (A) Effect of ABA renewal on c-Fos-IR in the accumbens ventral shell, LH, and RAIC following vehicle or SCH23390 (10 μ g/kg s.c.). Bars show the mean ($\pm$ S.E.M.) number of c-Fos-IR neurons counted for each of the treatment groups. *, (ABA-0 vs. ABB-0, $P < 0.05$) #, (ABA-10 vs. ABA-0, $P < 0.05$), +, (ABB-10 vs. ABB-0, $P < 0.05$). B, Photomicrographs showing c-Fos-IR neurons in representative ABB-0, ABA-0, ABB-10 and ABA-10 animals in the accumbens ventral shell, LH, and RAIC. Abbreviations: LH, lateral hypothalamus. Scale bar = 100 μ m.

tween group ABA-SCH23390 and group ABA-Saline ($F(3, 14)=6.1$, $P<0.05$), but not between group ABB-SCH23390 and group ABB-Saline ($F(3, 14)<1$, $P>0.05$). Being placed in the extinction context (group ABB) was again associated with a reduction in c-Fos expression in RAIC because group ABB-Saline were significantly different to group ABA-Saline ($F(3, 14)=8.7$, $P<0.05$) (Fig. 6). Interestingly, the D1 receptor antagonist significantly reduced c-Fos levels in the renewal but not control condition: there was a significant difference between group ABA-SCH23390 and group ABA-Saline ($F(3, 14)=14.5$, $P<0.05$), but not between group ABB-SCH23390 and group ABB-Saline ($F(3, 14)=1.1$, $P>0.05$).

The second question of interest concerned the phenotype of lateral hypothalamic neurons expressing c-Fos during ABA renewal. We were interested in whether these neurons co-expressed either orexin or MCH. Both orexin and MCH are expressed at high levels in hypothalamus and regulate feeding and energy balance (Kalra et al., 1999). Two further series of sections through lateral hypothalamus were taken from ABA-Saline animals for dual-immunofluorescence. c-Fos positive nuclear IR was not co-localized with either orexin or MCH (Fig. 7). As can be seen in Fig. 7, c-Fos-positive neurons were predominantly located rostrally, diminishing in number caudally. By contrast, orexin and MCH neurons were found predominantly at mid to caudal regions. c-Fos was segregated from orexin and MCH except for a small number of double-labeled c-Fos/orexin neurons in the perifornical region. This region of double labeling was distinct to the rostral regions where renewal-associated c-Fos was expressed.

Experiment 3: role of responding in c-Fos induction

This experiment studied whether the increased c-Fos expression observed in the accumbens shell, basolateral amygdala, and lateral hypothalamus for group ABA was due to re-exposure to the training context, context A, or whether it was due to the increased nose-poking observed in these animals. To distinguish between these possibilities, rats were trained to nose-poke for a sucrose reward in context A, this behavior was then extinguished in context B. On test day all rats were placed back in context A. During this test in context A, group Response had access to the nose poke manipulandum and so was able to emit the trained response. Group No Response did not have access to the nose-poke manipulandum and so was unable to emit the trained response. The context was otherwise unaltered for group No Response.

Training and extinction proceeded normally in both groups. There was more responding on the active than inactive nose-poke during extinction ($F(1, 17)=36.1$, $P<0.05$). There was an overall decrease in responding during extinction ($F(1, 7)=6.1$, $P<0.05$).

This decrease in responding was significantly greater for the active than the inactive nose-poke ($F(1, 7)=9.5$, $P<0.05$). Group Response showed significantly more responding on test than during last day of extinction training ($t(3)=5.8$; $P<0.05$) (Table 3), indicating renewal of extinguished responding. We restricted analysis of c-Fos IR to

the three structures selectively activated in group ABA: accumbens, basolateral amygdala, and lateral hypothalamus. There were no differences between the two groups in the number of c-Fos neurons in accumbens ventral shell ($F(1, 7)=4.9$; $P>0.05$) or other accumbens subnuclei ($P>0.05$), basolateral amygdala ($F(1, 7)=1.4$; $P>0.05$), or lateral hypothalamus ($F(1, 7)=1.4$; $P>0.05$). This shows that the increase in c-Fos expression is due to exposure to the training context and not the nose-poking that this context elicits.

DISCUSSION

These experiments studied the neural correlates of contextual control over an extinguished instrumental response. Rats were trained to respond for a sucrose reward in context A. Responding was then extinguished. In experiment 1 this extinction training occurred in either the same or different context to training, and all rats were tested in the original training context (i.e. ABA versus AAA). In experiment 2, this extinction training occurred in a different context, and rats were either tested in the extinction context or the original training context (i.e. ABA versus ABB). In both experiments, return to the original training context after extinction in a different context (group ABA) elicited renewal of extinguished responding that was selective for the response previously associated with delivery of the sucrose reward. These results confirm previous reports of the context-specificity of extinguished instrumental responding for natural (Welker and McAuley, 1978; Nakajima et al., 2000, 2002) and drug rewards (Bossert et al., 2004; Crombag et al., 2002; Crombag and Shaham, 2002; Fuchs et al., 2005). They show that extinction training does not erase the original learning. Instead, extinction training appears to impose a mask on the original learning that is removed with a change in physical context between extinction and test (Bouton, 2002; Rescorla, 2001).

Renewal of extinguished responding was accompanied by discrete patterns of induction of the c-Fos protein (Table 4). Renewal was associated with selective increases in the number of c-Fos-IR neurons in basolateral amygdala, ventral accumbens shell, and lateral hypothalamus. The same pattern of changes was seen regardless of whether ABA renewal was assessed relative to an AAA (experiment 1) or an ABB (experiment 2) control condition. Up-regulation of c-Fos in these structures during ABA renewal was caused by re-exposure to the contextual cues rather than the specific nose-poke responses emitted by subjects on test because preventing such responses, by removing the manipulandum, did not significantly alter expression of c-Fos. The basolateral amygdala plays an important role in encoding specific affective properties of rewards and in allowing stimuli and actions to be associated with these properties (Corbit and Balleine, 2005; Balleine et al., 2003; Hatfield et al., 1996). Of particular relevance to the present experiments, this role includes the augmentation of instrumental responding for a reward by Pavlovian conditioned stimuli previously paired with that reward (Corbit and Balleine, 2005), an effect that also

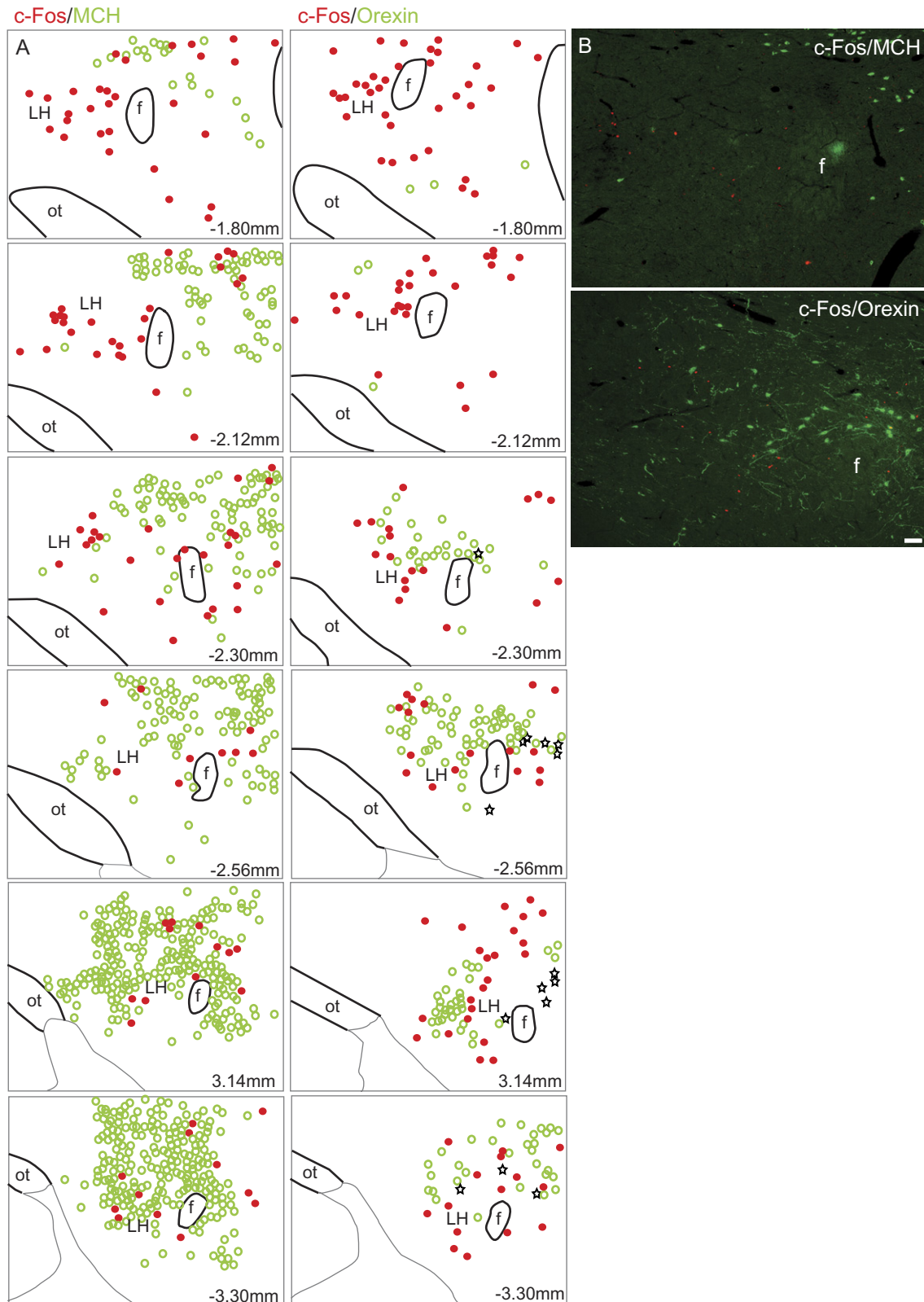


Fig. 7. (A) Illustration of the distribution of c-Fos-IR neurons relative to neurons IR for MCH and orexin in the LH following ABA renewal. Red dots show the distribution of single-labeled c-Fos-IR neurons, green circles represent single-labeled cytoplasmic staining of MCH or orexin, black stars represent dual-labeling of c-Fos and orexin. (B) Examples of MCH, orexin, and c-Fos dual-fluorescence immunohistochemistry in the LH following ABA renewal of sucrose seeking. *Abbreviations:* f, fornix; LH, lateral hypothalamus; ot, optic tract. Distance from bregma Paxinos and Watson (1997). Scale bar=100 μm.

Table 3. Mean ($\pm$ S.E.M.) number of responses on active nose-poke during last day of extinction and on test and mean ($\pm$ S.E.M.) c-Fos-positive neurons in experiment 3

	Group Response	Group no response
Responses		
Last day extinction	10.3 $\pm$ 1.4	16.0 $\pm$ 4.6
Test	43.8 $\pm$ 5.8	n/a
Accumbens		
Rostral pole	104.00 $\pm$ 12.12	135.50 $\pm$ 20.84
Dorsomedial shell	59.67 $\pm$ 14.17	90.00 $\pm$ 11.29
Ventromedial shell	84.33 $\pm$ 20.85	128.50 $\pm$ 10.81
Core	196.33 $\pm$ 56.13	231.75 $\pm$ 26.94
Basolateral amygdala	105.00 $\pm$ 19.22	137.00 $\pm$ 18.59
Lateral hypothalamus	75.00 $\pm$ 17.58	99.00 $\pm$ 12.01

depends on the nucleus accumbens shell (Corbit et al., 2001). We found a renewal-associated increase in c-Fos induction in ventral accumbens shell, a region receiving extensive projections from basolateral amygdala (McDonald, 1991). We also found a renewal-associated increase in c-Fos induction in lateral hypothalamus, a region receiving extensive projections from basolateral amygdala as well as from accumbens shell (Zahm and Heimer, 1993; Usuda et al., 1998) and which has been implicated in reward and regulation of food intake (Kelley, 2004; Maldonado-Irizarry et al., 1995; Petrovich et al., 2005; Stratford and Kelley, 1999). Interestingly, renewal-associated c-Fos was not observed in hypothalamic orexin or MCH neurons. Orexin and MCH are important components of hypothalamic networks for feeding and energy balance (e.g. Kalra et al., 1999), and orexin has recently been implicated in neural and behavioral responsiveness to drug rewards (Borgland et al., 2006; Narita et al., 2006). Renewal-associated c-Fos was induced at levels of the hypothalamus rostral to the majority of orexin- and MCH expressing cells, and there were only a few c-Fos/orexin double-labeled cells caudally in the perifornical region. The pattern of ABA renewal-associated c-Fos induction detected here suggests that return to the context previously associated with delivery of a sucrose reward elicited renewal of extinguished reward-seeking via projections from the basolateral amygdala to lateral hypothalamus directly, or indirectly via the accumbens ventral shell.

The actions of dopamine at the D1 dopamine receptor within this circuit are critical for renewal of extinguished reward seeking. Renewal of extinguished responding was prevented by systemic injection of the D1 receptor antagonist SCH23390. These injections also altered the patterns

of c-Fos induction associated with test. For accumbens, injection of SCH23390 suppressed c-Fos in both groups ABB and ABA. This result is consistent with the role of D1 receptor in c-Fos expression in the nucleus accumbens (Cole et al., 1992; Liu et al., 1994). For LH, the D1 antagonist acted selectively to reduce c-Fos in group ABA and did not alter levels of c-Fos expression in group ABB. This finding indicates an important role for the D1 receptor in LH renewal-associated c-Fos expression. SCH23390 also prevents the ABA renewal of extinguished cocaine-seeking (Crombag et al., 2002). Therefore, dopamine actions at the D1 receptor may be a common substrate for the recovery of extinguished reward-seeking with a change in context after extinction.

It is worth emphasizing that prevention of ABA renewal by D1 receptor antagonism did not appear attributable to any potential motoric effects of SCH23390 because the antagonist had no significant effect on responding in the control group, group ABB. It might be suggested that any motoric effects of SCH23390 would be more likely to interfere with the high levels of responding in group ABA rather than the lower levels of responding in group ABB. However, prior studies have shown SCH23390, including at the same dose used here, does not interfere with performance of instrumental responses. For example, Crombag et al. (2002) trained rats to lever press for sucrose. During a 60 min test of responding, for which responses were rewarded by sucrose, Crombag et al. (2002) showed that 10 μ g of SCH23390 did not affect responding. In the present experiments rats were tested for the same duration with the same dose of SCH23390. The only difference between the present experiment and that reported by Crombag et al. (2002) was that the sucrose reward was absent here. Taken together, these observations argue strongly against the possibility that the prevention of renewal by SCH23390 was due to non-specific effects on behavior.

A number of structures, including anterior cingulate, dorsomedial accumbens shell, accumbens core, lateral septum, and substantia nigra pars compacta, showed increased c-Fos expression in groups ABA and AAA compared with a control group trained and extinguished and handled on test but never tested (group AA–) (Table 4). Given that group AAA and group ABA differed significantly in behavior, the fact that up-regulation of c-Fos protein in these structures was similar is striking. Whereas it is clear that c-Fos in these structures is unrelated to renewal of an extinguished response, it is unclear whether this c-Fos induction simply represents the effect of being exposed to

Table 4. Summary of changes in c-Fos expression observed during contextual control over extinguished reward-seeking

ABA renewal ABA>AAA=AA– and ABA>ABB	Test in extinction context AAA<ABA=AA– and ABB<ABA	Test versus no test ABA=AAA>AA–
Accumbens, ventral shell	Rostral agranular insular cortex	Anterior cingulate
Basolateral amygdala		Accumbens, dorsomedial shell
Lateral hypothalamus		Accumbens, core
		Lateral septum
		Substantia nigra, pars compacta

a context, or whether it additionally reflects the fact that for groups ABA and AAA the context was previously associated with responding for, and delivery of, a sucrose reward. Further research is needed to investigate these possibilities.

Our results revealed a suppression of c-Fos expression in RAIC (Table 4). This suppression was due specifically to being tested in the same context where instrumental responding had been extinguished. Experiment 1 showed that RAIC c-Fos induction was significantly lower in group AAA compared with groups ABA and AA–, which did not differ from each other. This pattern of differences shows that there was a reduction in c-Fos induction among group AAA. Experiment 2 showed that RAIC c-Fos expression was also reduced in group ABB compared with group ABA. This shows that the critical variable influencing RAIC c-Fos was placement in the same context where extinction training occurred (e.g. context B in experiment 2) rather than placement in a context with a mixed reinforcement history (e.g. context A in experiment 1). It is difficult to interpret reductions in c-Fos induction; however, the finding of reduced RAIC c-Fos induction following exposure to the extinction context is interesting for at least three reasons. First, the RAIC was the only structure where such differences were observed. Second, the RAIC is reciprocally connected with each of the three structures showing c-Fos induction during ABA renewal—basolateral amygdala, nucleus accumbens and lateral hypothalamus (Jasmin et al., 2004). Finally, extinction training does not erase or overwrite the original training memory (Bouton, 2002; Bouton and Swartzendruber, 1991). Rather, extinction training imposes a mask on the original learning in a context-dependent manner. One view of this mask is that it is memorial so that retrieval of the original training memory is actively inhibited when rats are tested in the extinction context (Bouton, 2002; Bouton and Swartzendruber, 1991). One possibility, therefore, is that RAIC is involved in regulating retrieval of the original training memory or reinforcer memory after extinction. We are currently studying this possibility.

Dopamine may be central to the role of RAIC in contextual control over extinguished instrumental responding because the D1 antagonist not only prevented ABA renewal but also suppressed RAIC c-Fos induction in ABA animals to levels seen in ABB animals. That is, the D1 antagonist rendered the behavioral and RAIC responses of ABA animals identical to animals tested in the extinction context. The RAIC is a target of midbrain dopamine projections (O'Hara et al., 2003) and it expresses D1 dopamine receptors (Richfield et al., 1989; Gaspar et al., 1995). Our data fit neatly with the previously described role of this dopamine input in tonic regulation of the RAIC (Burkey et al., 1996). Specifically, exposure to the extinction context (i.e. AAA or ABB) could cause an active suppression of tonic dopamine input to the RAIC, in turn causing a reduction in basal c-Fos expression and instrumental responding. Injections of the D1 antagonist in group ABA mimic such exposure to the extinction context to reduce both renewal of responding and RAIC c-Fos.

It is worth noting that the data reported here were obtained from designs that compared ABA groups to either AAA (trained, extinguished, and tested in the same environment) (experiment 1) or to ABB (trained in context A but extinguished and tested in context B) (experiment 2) groups. In both cases, the pattern of behavioral and neural differences between groups was very similar. We did not employ an AAB group whereby rats were trained and then extinguished in context A prior to being tested in context B. From a neurobiological perspective, an AAB group might be worth considering in future research because it may potentially control for any influence on c-Fos induction of changing contexts between extinction and test in group ABA.

CONCLUSION

In conclusion, our experiments show for the first time that ABA renewal of extinguished responding for a sucrose reward depends critically on the D1 dopamine receptor and is associated with an up-regulation of c-Fos protein in basolateral amygdala, ventral shell of the nucleus accumbens, and lateral hypothalamus. Our results also show that being placed in the context where extinction training occurred was associated with a selective reduction of c-Fos induction in RAIC. Combined with previous data, it appears that dopamine actions at the D1 receptor are a common substrate for the recovery of extinguished responding for natural and drug rewards with a change in context. Our results suggest that recovery of such responding for natural rewards depends on a distributed neural circuit including basolateral amygdala, ventral shell of the nucleus accumbens, and lateral hypothalamus, that is regulated by RAIC.

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