European Journal of Neuroscience, Vol. 22, pp. 697–705, 2005

Differential effects of calcineurin inhibition and protein kinase A activation on nucleus accumbens amphetamine-produced conditioned place preference in rats

Todor V. Gerdjikov[1] and Richard J. Beninger[1,2]
[1]Department of Psychology, Queen's University, Kingston, ON K7L 3N6, Canada
[2]Department of Psychiatry, Queen's University, Kingston, ON K7L 3N6, Canada

Keywords: dopamine, phosphatase, PKA, reward-related learning

Abstract

The nucleus accumbens (NAc) plays a critical role in amphetamine-produced conditioned place preference (CPP). In previous studies inhibition or activation of cyclic adenosine monophosphate-dependent protein kinase (PKA) blocked NAc amphetamine-produced CPP. PKA activation unrelated to ongoing DA transmission may disrupt reward-related learning. Calcineurin (CN) down-regulates downstream PKA targets. Unlike PKA activation, CN inhibition may preserve and enhance reward-related learning. The PKA signalling cascade is negatively regulated by calcineurin (CN). We tested the hypothesis that post-training CN inhibition in NAc will enhance NAc amphetamine-produced CPP and that PKA activation will block CPP. Eight but not four or two 30-min conditioning sessions were sufficient to establish significant CPP. Immediate post-training, NAc injection of the calcineurin inhibitor FK506 (5.0 but not 1.0 μg in 0.5 μL per side) led to a significant amphetamine CPP in rats receiving four but not two training sessions; the 5.0-μg dose had no effect on rats trained with eight sessions. Injections of the PKA activator Sp-cAMPS (2.5 or 10.0 μg in 0.5 μL per side) failed to affect CPP following two or four training sessions and blocked CPP produced by a standard 8-day conditioning schedule. Results suggest that CN acts as a negative regulator in the establishment of NAc amphetamine-produced CPP, a form of reward-related learning.

Introduction

Nucleus accumbens (NAc) dopamine (DA) is implicated in reward-related learning (Roberts & Koob, 1980; Everitt *et al*., 1991; Baldwin *et al*., 2000; Parkinson *et al*., 2000; Everitt & Wolf, 2002). Reward-related learning shares many of the same intracellular pathways known to mediate other forms of learning (for reviews see Beninger & Gerdjikov, 2004; Kelley, 2004) including the cyclic adenosine monophosphate (cAMP)-dependent protein kinase (PKA) pathway (Kandel, 2001). NAc PKA is necessary for the acquisition of conditioned approach, lever-pressing for food (Baldwin *et al*., 2002) and amphetamine-produced conditioned place preference (CPP) (Beninger *et al*., 2003). Reward-related learning probably involves DA-mediated PKA activation; however, direct PKA activation with Sp-adenosine 3′,5′-cyclic monophosphorothioate triethylammonium salt (Sp-cAMPS) does not enhance learning for most doses tested (but see Jentsch *et al*., 2002). Sp-cAMPS did not enhance CPP with subthreshold amphetamine doses and it impaired CPP with an amphetamine dose sufficient to establish CPP (Beninger *et al*., 2003). Furthermore, NAc Sp-cAMPS impaired acquisition of lever pressing for food (Baldwin *et al*., 2002). PKA is regulated by a number of neurotransmitter systems including norepinephrine, acetylcholine, opiates and serotonin. PKA is also regulated by hormones, Ca^{2+} and other kinases (Cooper, 2003). The DA–PKA signal may be lost when PKA is activated independently of DA, thus disrupting reward and impairing learning. In contrast to Sp-cAMPS, cholera toxin prolongs the ability of G_s-coupled receptors to activate PKA and may preserve the DA–PKA signal, resulting in enhanced learning. Cholera toxin enhanced reward-related learning in two different tasks (Kelley & Holahan, 1997; Jentsch *et al*., 2002). Thus inhibition and in some cases activation of PKA abolishes reward-related learning.

Learning requires a balance between kinase and phosphatase activity (Mansuy, 2003). Calcineurin (CN), a phosphatase enriched in the striatum (Winder & Sweatt, 2001), negatively regulates molecules implicated in memory, including glutamatergic *N*-methyl-D-aspartate (NMDA) receptors and PKA (Mansuy, 2003). NAc DA-mediated reward-related learning requires intact glutamatergic transmission (Wickens & Kötter, 1995; Beninger & Gerdjikov, 2005). Ca^{2+} entry through NMDA receptors may mediate such plasticity (Beninger & Gerdjikov, 2004). CN is activated by Ca^{2+} entry through NMDA channels and down-regulates NMDA channels (Mansuy, 2003). CN inhibition enhances learning in both invertebrates (Sharma *et al*., 2003) and rodents (Malleret *et al*., 2001). Thus, CN may play a role in reward-related learning.

CN modulates DA-dependent intracellular processes. Dopamine- and cAMP-regulated phosphoprotein of M(r) 32 kDa (DARPP-32) phosphorylation at Thr-34 by PKA provides a mechanism for amplification of D1 signalling. This effect is antagonized by D2 receptor-produced CN activation and dephosphorylation at this site (Nishi *et al*., 1997). These results complement findings implicating D1 but not D2 receptors in reward-related learning (Beninger & Miller,

Correspondence: Dr Richard J. Beninger, Department of Psychology, as [1]above.
E-mail: beninger@post.queensu.ca

Received 27 February 2005, revised 30 May 2005, accepted 1 June 2005

doi:10.1111/j.1460-9568.2005.04256.x

1998; Sutton & Beninger, 1999). Limited evidence has implicated the CN target DARPP-32 in reward-related learning. DARPP-32 knockout mice showed impaired cocaine-produced place preference (Zachariou *et al*., 2002). In another study, methamphetamine-sensitized rats showed decreased striatal CN immunoreactivity (Lin *et al*., 2002). This finding parallels results showing that sensitization enhances CPP and further supports the idea that CN may be negatively related to DA-produced reward-related learning (Narita *et al*., 2004). Further work is needed to investigate the contribution of CN to reward-related learning.

CN acts predominantly on downstream PKA targets. CN inhibition may therefore preserve and enhance PKA signalling, resulting in enhanced reward-related learning. Direct PKA activation, on the other hand, may disrupt learning (Baldwin *et al*., 2002; Beninger *et al*., 2003). We hypothesized that the CN inhibitor FK506 but not by the PKA activator Sp-cAMPS would enhance NAc amphetamine-produced CPP (Tzschentke, 1998). To test this hypothesis, rats were trained either in a full CPP protocol previously used to produce a reliable CPP effect (Gerdjikov *et al*., 2004) or partial training protocols consisting of fewer conditioning sessions. FK506 or Sp-cAMPS were injected immediately after amphetamine training sessions.

Materials and methods

Animals and surgery

Male Wistar rats (Charles River, St. Constant, Quebec, Canada) weighing between 200 and 250 g on arrival were housed in pairs on a 12-h reversed light–dark cycle (lights on at 19 : 00 h) at an average temperature of 21 °C, humidity 40–70%. Water and food (LabDiet 5001, PMI Nutrition Intl, Brentwood, MO, USA) were freely available. Rats were handled for ≈1 min on each of five consecutive days after arrival. The experimental protocol was approved by the Animal Care Committee at Queen's University. All animals were treated in full compliance with the Animals for Research Act and relevant guidelines set by the Canadian Council on Animal Care.

Approximately one week after arrival at the colony, rats were anaesthetized in an induction chamber using an inhalable anaesthetic (5% isoflurane; Bimeda, Cambridge, ON, Canada) mixed with oxygen in a vaporizer system (Benson, Merkham, ON, Canada) and administered at 1.0 L/min. Anaesthetized animals were fitted to a stereotaxic apparatus and isoflurane was administered at a concentration of 2% or as needed to maintain anaesthesia. The head was adjusted so that lambda and bregma were on the same horizontal plane. For analgesia, buprenorphine hydrochloride in solution (0.15 mg/kg; Reckitt & Colman, Richmond, VA, USA) was injected subcutaneously preoperatively. Ketoprofen (1.5 mg/kg; Merial, Baie d'Urfé, QC, Canada) was injected immediately after surgery and on three subsequent days postoperatively. The experimenter shaved the rat's head and applied betadine solution with a cotton tip applicator before incising the skin. Holes were drilled into the skull and 23-gauge (0.64 mm diameter) stainless-steel guide cannulae were chronically implanted bilaterally into the NAc, with coordinates 1.6 mm anterior to bregma, 1.4 mm lateral to the midline and 6.7 mm ventral from the skull surface (Paxinos & Watson, 1998). The guide cannulae were held in place by four stainless-steel screws and dental acrylic. Stainless steel wire stylets (0.31 mm diameter) flush with the end of the guide cannulae were put in place to prevent occlusion. Rats were allowed ≈1 week to recover before the start of behavioural testing.

Drug infusion

FK506 (Biomol; Plymouth Meeting, PA, USA) was dissolved in dimethyl sulfoxide (DMSO) before the beginning of the experiment and stored at −20 °C; Sp-cAMPS (Sigma-Aldrich, Oakville, Ontario, Canada) was dissolved in saline before the beginning of the experiment and stored at −20 °C; amphetamine sulphate (USP; Rockvill, MD, USA) was dissolved in saline daily before each set of injections. Central injections into the NAc were made with a microinfusion pump (KD Scientific, Holliston, MA, USA). Injectors were glued to polyethylene tubing (0.75 mm o.d.) filled with distilled water. The tubing was connected to two 10-μL microsyringes (Microliter #701; Hamilton, Reno, NV, USA) mounted on the microinfusion pump. Drugs were backloaded into the injectors by aspiration with the two syringes. Rats were hand-held as the experimenter removed the stylets from the guide cannulae and inserted the two injectors (0.31 mm o.d.). The injectors projected 1.2 mm beyond the guide cannulae.

Amphetamine (20.0 μg in 0.5 μL on each side) was injected bilaterally in saline over 30 s. We have previously reported reliable CPP effects using this dose (Beninger *et al*., 2003). After the drug was delivered, the injectors were left in place for another 30 s to allow for diffusion, after which they were slowly retracted from the guide cannulae. Place conditioning began immediately after amphetamine injection. FK506 (1.0 or 5.0 μg in 0.5 μL per side; i.e. 1.2 or 6.2 nmol in 0.5 μL per side) or Sp-cAMPS (2.5 or 10.0 μg in 0.5 μL per side; i.e. 5.6 or 22.4 nmol in 0.5 μL per side) were injected immediately after training using the same procedure. The maximum FK506 dose was identical to an amygdala dose previously shown to impair the extinction of fear-potentiated startle (Lin *et al*., 2003). Sp-cAMPS doses were similar to NAc doses previously reported to produce an impairment in the acquisition of lever pressing for food (Baldwin *et al*., 2002). DMSO was used for vehicle injections for groups receiving FK506 after conditioning. Rats trained with amphetamine only or amphetamine plus post-training Sp-cAMPS received saline as the vehicle. Groups were tested drug-free.

Animals tested with drug combinations may show altered CPP for reasons other than changes in intracellular signalling. Thus, if FK506 or Sp-cAMPS produce rewarding or aversive physiological states on their own, they may cause animals to either approach or avoid the amphetamine-plus-inhibitor-paired side. To test this possibility we injected two groups of rats with vehicle on both vehicle-paired sessions and what would normally have been amphetamine-paired sessions and with post-training FK506 (5.0 μg in 0.5 μl per side) or Sp-cAMPS (10.0 μg in 0.5 μl per side).

Apparatus

The four testing chambers each consisted of two rectangular compartments (38 × 27 × 34 cm) connected with a tunnel (8 × 8 × 8 cm). Two different spatial features were varied across the four testing chambers. The compartment walls were either urethane-sealed wood or alternating 1-cm-wide black and white vertical stripes and were covered with clear Plexiglas. The floor was either wire bars 1.0 cm apart running perpendicular to the tunnel, or a wire grid with openings of 1.0 cm^2. This resulted in four possible compartment types, distributed as left or right compartment across four different testing chambers. Each compartment had a Plexiglas top with a number of circular ventilation holes. The tunnel was fitted with guillotine-type doors, which could be closed to prevent movement from one compartment to the other. Two infrared emitters

and detectors (height 5.0 cm) in each compartment and two in the tunnel (height 3.0 cm) were used to monitor movement between and within compartments and to record time spent in each compartment and the tunnel. The number of beam breaks during conditioning sessions was used as a measure of locomotion. Each of the four testing chambers was housed in a dimly lit (7.5 W) sound-attenuated and ventilated wooden box. Indirect light reached the rat though the Plexiglas tops of the compartments. Data from the sensors were collected on a 6809 microcontroller using custom-made software and transferred to a Macintosh computer for analyses. For further details of the apparatus see Brockwell *et al*. (1996).

Behavioural procedure

Training and testing occurred during the day (07.00–19.00 h). Rats were tested in groups of four using a different testing chamber for each rat. The protocol for experiments using the full conditioning paradigm consisted of three habituation sessions, eight conditioning sessions and one test session. To test the effects of the CN inhibitor FK506 and the PKA activator Sp-cAMPS, partial protocols, consisting of two or four instead of eight conditioning sessions, were used for some groups. Thus, each rat completed one session per day for a total of 12 days if tested in the full protocol or 8 or 6 days if tested in the partial protocols.

Habituation

At the start of each 15-min session the rat was placed in one compartment of the box; left compartment for half the rats and right compartment for the other half. Tunnel doors were open allowing the animals to move freely between the two compartments. Activity sensors recorded the amount of time spent on each side and in the tunnel.

Conditioning

In the full conditioning protocol, consisting of eight 30-min sessions carried out on eight consecutive days, pretraining amphetamine (20.0 μg in 0.5 μL per side) and post-training FK506 (5.0 μg in 0.5 μL per side) or Sp-cAMPS (10.0 μg in 0.5 μL per side) were injected into the NAc on days 1, 3, 5 and 7, and vehicle was injected on days 2, 4, 6 and 8. In the 4-day partial training protocol, pretraining amphetamine and post-training FK506 (1.0 or 5.0 μg in 0.5 μL per side) or Sp-cAMPS (2.5 or 10.0 μg in 0.5 μL per side) were injected on days 1 and 3, and vehicle was injected on days 2 and 4. In the 2-day partial training protocol, amphetamine and FK506 (5.0 μg in 0.5 μL per side) or Sp-cAMPS (10.0 μg in 0.5 μL per side) were injected on day 1 and vehicle was injected on day 2. Rats received two vehicle injections on vehicle days, one before conditioning and one after conditioning.

Immediately after pretraining amphetamine or vehicle injection, depending on the conditioning day, the rat was placed in one of the two compartments with the tunnel doors closed, preventing movement into the other compartment or the tunnel. Half the rats were confined to the left side on drug days and to the right side on vehicle days. The other half were confined to the right side on drug days and to the left side on vehicle days. In this way, any given compartment was paired with drug for some animals and with vehicle for others. Number of beam breaks was recorded for each rat to assess locomotion. FK506 or Sp-cAMPS were injected immediately after completion of the conditioning session.

Testing

Testing occurred on the day immediately following conditioning. The session lasted 15 min and was identical to habituation sessions. The start side was the same for each rat on habituation and test days. Time spent on each side and in the tunnel was recorded.

Data analysis

The interpretation of CPP results is not straightforward if animals have a natural avoidance of the to-be-drug-paired side. In such a case, an apparent increase in time spent on that side after conditioning may be the result of decreased avoidance of the drug-paired side or simply habituation (Tzschentke, 1998). To check for side bias, paired-samples *t*-tests compared time spent on the drug-paired side to time spent on the vehicle-paired side before conditioning.

A change in the time spent in the drug-paired side from habituation to test cannot be interpreted unambiguously as a change in place preference if time spent in the tunnel also changes. Therefore planned paired-samples *t*-tests were used to compare tunnel time before and after conditioning.

The amount of time spent on the drug-paired side before conditioning was averaged across the three habituation days. The average was compared to time spent on the same side on the test day using planned dependent-samples *t*-tests. CPP occurred if rats showed a significant increase in time spent on the drug-paired side after conditioning. Between-group comparisons were performed using one-way ANCOVA. For each conditioning schedule, time spent on the drug-paired side was compared among all groups tested using time spent on the same side during habituation as a covariate. If there was a significant main effect, ANCOVA was performed on pairs of groups to locate the source of the effect.

Total number of beam breaks for each rat on each of the two, four or eight conditioning days was used as an index of motor activity. Activity data was analysed using one-way (dose) or three-way (dose × session × day) mixed ANOVA.

Histology

After completion of the experiment, rats were placed in an airtight chamber and killed with CO_2. Brains were removed and preserved in a 10% formalin solution for at least 72 h. Coronal sections 60 μm thick from throughout the cannulated region were obtained by slicing the brains on a cryostat at −20 °C. The sections were mounted on gelatin-coated glass slides and stained with Cresyl Violet. Judgements about NAc cannulae placements were made by an observer blind to the results for individual animals. Brains with cannula tips in the shell and/or core regions of the NAc were included in subsequent analyses.

Results

Histology

A total of 174 rats were tested. Four rats from the FK506 experiment and two rats from the Sp-cAMPS experiment failed to complete the study due to illness or technical problems. There was no relationship between the type or dose of drug and illness observed in these animals.

Cannula placements were assessed for the remaining rats. A total of 26 rats were excluded, 16 from the FK506 experiment and 10 from the Sp-cAMPS experiment, leaving 148 rats for subsequent analyses. Figure 1 shows the location of cannulae tips for all rats included in the

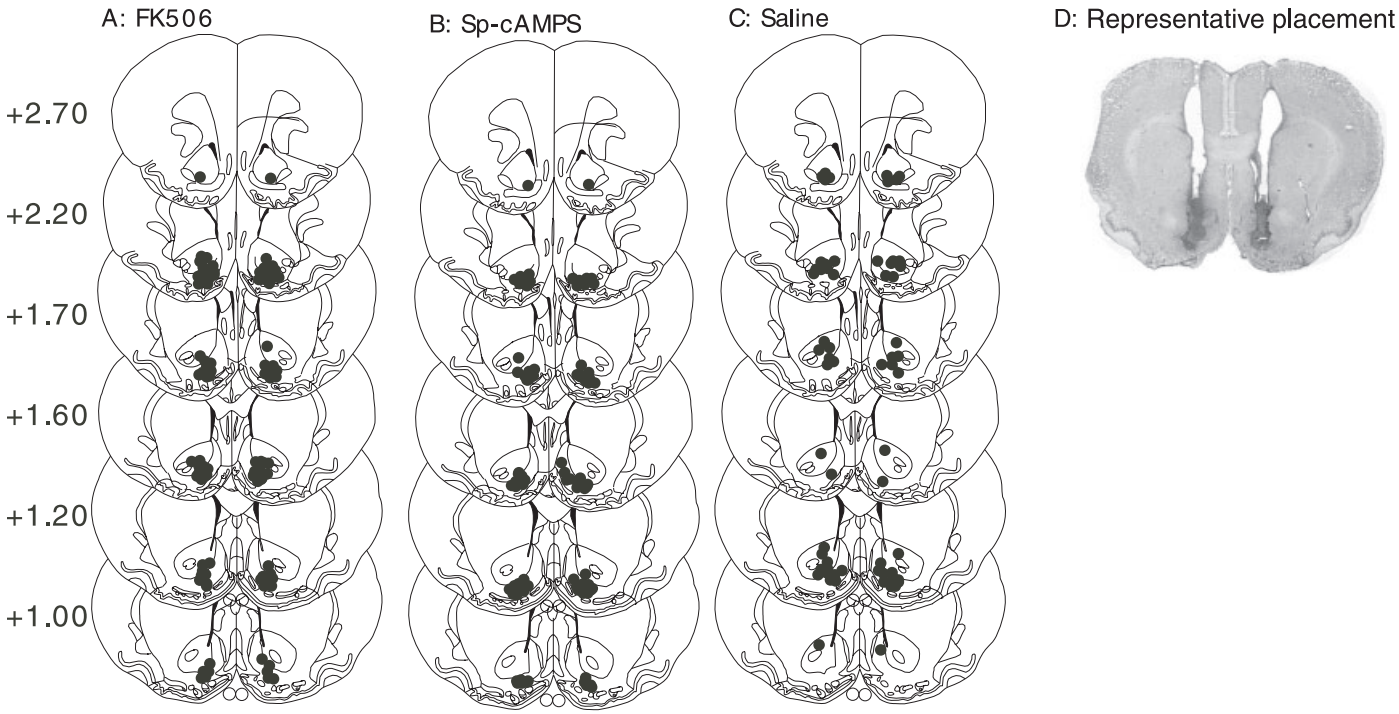

FIG. 1. Drawings of coronal sections through the nucleus accumbens, indicating sites of infusion. Injector sites may appear fewer than the reported number of rats because of overlap of placements. Numbers to the left indicate distance (mm) from bregma. (A) Injector sites for infusion of amphetamine (20 μg in 0.5 μL per side) before and FK506 (1.0 μg or 5.0 μg in 0.5 μL DMSO) after conditioning or 5.0 μg FK506 alone. (B) Injector sites for amphetamine before and Sp-cAMPS (2.5 μg or 10.0 μg in 0.5 μL saline) after conditioning or 10.0 μg Sp-cAMPS alone. (C) Injector sites for amphetamine before and saline after conditioning. (D) Photomicrograph depicting a representative NAc injector placement.

analyses. Animals were classified as hits if the tips of both cannulae were located in the core or shell region of NAc.

Time spent on each side during pre-exposure

The interpretation of CPP results is not straightforward if animals have a natural avoidance of the to-be-drug-paired side. In such a case, an apparent increase in time spent on that side after conditioning may be the result of decreased avoidance of the drug-paired side or simply habituation (Tzschentke, 1998). To check for bias, we averaged time spent on the side that would be paired with drug across the three habituation days and compared it to average time spent on the side that would be paired with vehicle for each group. Paired samples *t*-tests revealed no significant differences for all but one group. One group of rats receiving 5 μg of FK506 on two out of four conditioning days showed a preference for the to-be-drug-paired side: $t_8 = 2.70$, $P < 0.05$. Another group showed a similar magnitude of difference between means but failed to reach significance because variability was higher (see Table 1). Thus, with the exception of one group, rats did not avoid the to-be-drug-paired side during habituation and the CPP paradigm was unbiased.

TABLE. 1. Time spent on the to-be-drug-paired and to-be-vehicle-paired sides before conditioning with amphetamine

Experiment	Dose per side† (μg)	*n*	Time (s)	
			Drug-paired	Vehicle-paired
Saline				
2-day	–	10	427.3 ± 27.9	407.8 ± 27.2
4-day	–	16	414.6 ± 20.8	418.2 ± 15.1
8-day	–	11	408.8 ± 17.3	429.7 ± 18.4
FK506				
2-day	5.0	10	423.1 ± 20.4	415.8 ± 21.6
4-day	1.0	9	459.1 ± 33.4	387.4 ± 34.4
	5.0	12	413.8 ± 18.8	403.1 ± 18.4
	5.0 (replication)	9	445.9 ± 11.5	388.2 ± 10.7*
	5.0 (alone)	11	436.0 ± 15.8	410.5 ± 15.2
8-day	5.0	8	424.8 ± 14.8	418.6 ± 12.8
Sp-cAMPS				
2-day	10.0	8	438.8 ± 11.4	418.5 ± 12.1
4-day	2.5	11	423.6 ± 19.6	428.2 ± 19.4
	10.0	11	418.0 ± 22.6	424.2 ± 20.4
8-day	10.0	10	416.7 ± 17.0	436.6 ± 17.8
	10.0 (alone)	12	426.7 ± 27.4	428.0 ± 28.4

Time values are mean ± SEM. †In 0.5 μL each side. *$P < 0.05$ vs. drug-paired side (paired-samples *t*-tests).

Tunnel time

A change in the time spent in the drug-paired side from habituation to test cannot be interpreted unambiguously as a change in place preference if time spent in the tunnel also changes. Thus, additional analyses were performed to compare tunnel time before and after conditioning (see Table 2). Four groups showed a change in time spent in the tunnel. Both groups receiving 5 μg of FK506 in the 4-day schedule and rats receiving 10 μg of Sp-cAMPS in the 4-day schedule spent significantly less time in the tunnel after conditioning: $t_{11} = 4.74$, $P < 0.001$, $t_8 = 3.68$, $P < 0.01$ and $t_{10} = 4.98$, $P < 0.001$, respectively. In addition, rats receiving 10.0 μg of Sp-cAMPS alone spent significantly more time in the tunnel after conditioning: $t_{11} = 3.15$, $P < 0.01$. We analysed place preference data

TABLE 2. Time spent in tunnel before and after conditioning with amphetamine

Experiment	Dose per side† (μg)	*n*	Time (s) Before	Time (s) After
Saline				
2-day	–	10	64.8 ± 5.7	69.7 ± 9.4
4-day	–	16	69.1 ± 4.5	70.0 ± 7.4
8-day	–	11	61.4 ± 5.8	65.4 ± 10.7
FK506				
2-day	5.0	10	61.1 ± 5.6	48.7 ± 4.8
4-day	1.0	9	53.4 ± 7.5	43.4 ± 6.4
	5.0	12	83.2 ± 6.9	46.3 ± 7.5**
	5.0 (replication)	9	66.0 ± 6.4	45.4 ± 4.9**
	5.0 (alone)	11	53.3 ± 3.7	55.6 ± 4.4
8-day	5.0	8	56.6 ± 4.6	62.0 ± 12.6
Sp-cAMPS				
2-day	10.0	8	42.7 ± 3.2	51.1 ± 7.4
4-day	2.5	11	48.2 ± 6.9	45.2 ± 3.7
	10.0	11	57.9 ± 3.6	43.6 ± 3.2**
8-day	10.0	10	46.7 ± 4.6	44.0 ± 5.3
	10.0 (alone)	12	45.6 ± 5.4	63.3 ± 8.5**

Time values are mean ± SEM. †In 0.5 μL each side. **$P < 0.01$ vs. tunnel time before conditioning (paired-samples *t*-tests).

for these two groups using both raw scores and percentages (reported below). Percentages were based on total time spent in the compartments disregarding tunnel time to evaluate the possibility that the change in tunnel time biased the results. Both methods of analysing the data yielded the same results.

Place conditioning

Amphetamine alone

Rats receiving only amphetamine on drug days and saline on vehicle days did not show significant place preference on the test day if trained in the 2- or 4-day conditioning schedule. Rats trained in the 8-day schedule showed significant place preference for the drug-paired side: $t_{10} = 2.26$, $P < 0.05$ (open bars in Fig. 2).

FK506

Rats receiving 5.0 μg of FK506 in the 2-day schedule and rats receiving 1.0 μg of FK506 in the 4-day schedule did not show significant CPP. In contrast, rats receiving 5.0 μg of FK506 in the 4- or 8-day schedule showed significant CPP: $t_{11} = 3.11$, $P < 0.001$ and $t_7 = 3.39$, $P < 0.05$, respectively. We replicated the 4 day−5.0 μg FK506 condition and rats again showed a significant CPP effect: $t_8 = 3.53$, $P < 0.01$ (Fig. 2). Analysing the place preference data as a percentage of total time minus tunnel time for rats receiving 5.0 μg of FK506 in the 4-day schedule and for the replication group yielded similar results: $t_{11} = 2.50$, $P < 0.05$ and $t_8 = 3.11$, $P < 0.01$, respectively (data not shown). Rats receiving 5.0 μg of FK506 alone in the 4-day schedule did not show a significant place preference or avoidance.

Sp-cAMPS

Rats receiving 2.5 or 10.0 μg of the PKA activator Sp-cAMPS did not show significant place preference regardless of the number of training days (Fig. 2). Analysing the place preference data as a percentage of total time minus tunnel time for rats receiving 10.0 μg of Sp-cAMPS in the 4-day schedule also did not reveal significant increases in place preference (data not shown). Rats receiving 10.0 μg of Sp-cAMPS alone in the 8-day schedule did not show a significant place preference or avoidance.

To supplement the results of the *t*-tests three separate one-way ANCOVA were performed for the 2-, 4- and 8-day schedules, respectively, using time in the drug-paired side during test as the dependent variable and time in the same side during habituation as a covariate. The use of ANCOVA followed recommendations by Stevens (2002). For the 2-day schedule, the ANCOVA did not yield significant differences between the saline, 5.0 μg FK506 and 10.0 μg Sp-cAMPS groups. For the 4-day schedule the ANCOVA yielded significant differences among the six groups (amphetamine alone, 1.0 μg FK506, 5.0 μg FK506 totaled over the two replications, 5 μg FK506 alone,

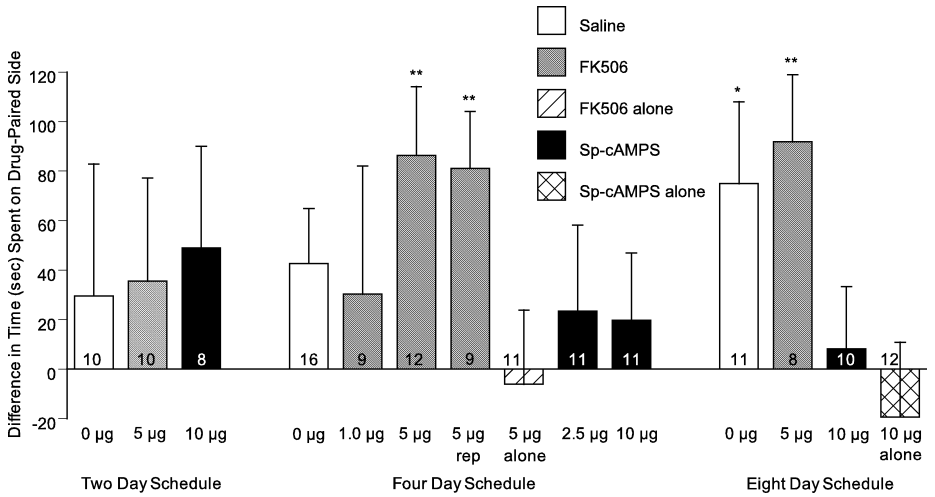

FIG. 2. Mean (+ SEM) difference scores for various doses of the calcineurin inhibitor FK506 or the PKA activator Sp-cAMPS infused into NAc after amphetamine conditioning sessions. Two groups received FK506 or Sp-cAMPS alone, i.e. no amphetamine was administered before conditioning sessions. To obtain difference scores, time spent on the drug-paired side was averaged across the three preconditioning days. The average preconditioning time was subtracted from time spent on the drug-paired side recorded on test. Numbers in each bar indicate number of animals tested. *$P < 0.05$, **$P < 0.01$ for increase in time spent on the drug-paired side on test day compared to preconditioning. Rep, replication.

2.5 μg Sp-cAMPS and 10.0 μg Sp-cAMPS): $F_{5,72} = 5.15$, $P < 0.05$. To locate the source of the significant main effect, ANCOVA was performed on pairs of groups showing that the 5.0 μg FK506 group differed significantly from the other groups (all $P < 0.05$) except the group receiving the lower (1.0 μg) dose of FK506. For the 8-day schedule the ANCOVA comparing rats receiving amphetamine only, 5.0 μg of FK506, 10.0 μg of Sp-cAMPS or Sp-cAMPS alone yielded significant differences among the groups: $F_{3,36} = 3.99$, $P < 0.01$. Follow-up ANCOVA showed that amphetamine differed significantly from Sp-cAMPS alone ($P < 0.05$) but not from the 10 μg Sp-cAMPS dose, and that FK506 differed significantly from both the 10.0 μg Sp-cAMPS and the Sp-cAMPS alone group ($P < 0.01$). In summary, these results are consistent with the coclusions from the *t*-tests and show that FK506 but not Sp-cAMPS enhanced CPP in the 4-day schedule and that Sp-cAMPS but not FK506 impaired CPP in the 8-day schedule.

Post hoc CPP analyses of NAc subregions

The NAc is divided into core and shell regions. These regions have been shown to play differential roles in reward-related learning and drug addiction. Our data did not permit a direct investigation of the relative contribution of the core and shell. To assess the possible role of these subregions we identified subsets of rats with placements exclusively in the core or shell and performed *post hoc* analyses of the place preference data. For drug groups in which sufficient numbers ($n = 5$) of rats with exclusively core or shell placements could be found, we re-analysed the place preference data and found results consistent with the effects reported above (analyses not shown; Table 3). Results suggests that either the core or shell may mediate the observed effects.

Locomotor activity

Two-day schedule

All groups showed increased activity on the amphetamine day compared to the saline day (Fig. 3). A 3 (group: saline vs. 5.0 μg FK506 or 10.0 μg Sp-cAMPS) × 2 (conditioning session: amphetamine vs. saline) ANOVA revealed a significant main effect for session: $F_{1,25} = 83.04$, $P < 0.001$. The interaction was also significant: $F_{2,25} = 4.21$, $P < 0.05$. On the amphetamine day, rats receiving 5.0 μg of FK506 showed higher activity than those receiving the amphetamine alone or 10 μg Sp-cAMPS (Tukey *post hoc* tests). We attribute this effect to sampling error because all groups received identical treatment at the time of conditioning; FK506 or Sp-cAMPS administration occurred only after the amphetamine conditioning session.

TABLE 3. Increase in time spent on the drug-paired side from habituation to test for rats classified as having both cannula placements exclusively in the core or shell subregion

Placement of cannulae and Experiment	Dose per side† (μg)	*n*	Increase (s)
Core			
Saline (4-day)	–	5	9.93 ± 30.54
FK506 (4-day)	5.0	8	120.54 ± 31.84**
Shell			
FK506 (4-day)	5.0 (replication)	6	86.39 (33.28)*
FK506 (8-day)	5.0	7	77.64 ± 24.88*
Sp-cAMPS (2-day)	10.0	5	76.80 ± 63.03
Sp-cAMPS (4-day)	2.5	9	27.89 ± 42.59
Sp-cAMPS (4-day)	10.0	9	17.48 ± 33.47
Sp-cAMPS (8-day)	10.0	8	1.71 ± 29.04

Values of increase are mean ± SEM. †In 0.5 μL each side. rep, replication. Only groups with $n \geq 5$ are shown. *$P < 0.05$, **$P < 0.01$ for increase in place preference from habituation to test (paired-samples *t*-tests).

Four-day schedule

All groups that received amphetamine showed increased activity on amphetamine days compared to saline days (Fig. 3). Activity was higher on the first amphetamine day than on the second. FK506 and Sp-cAMPS doses were compared to saline in a 6 (group: sal. vs. 5.0 μg FK506 alone vs. 1.0 or 5.0 μg FK506 vs. 2.5 μg or 10.0 μg Sp-cAMPS) × 2 (session: amph vs. sal) × 2 (conditioning day) mixed ANOVA. All groups showed increased activity on the amphetamine session compared to the saline session: $F_{1,62} = 199.68$, $P < 0.001$. Across both session types, activity was lower on the second day: $F_{1,62} = 25.01$, $P < 0.001$. The group × session interaction was also significant: $F_{5,62} = 2.43$, $P < 0.05$. This effect reflected the significantly higher activity of the first FK506 5.0 μg group than most other groups during the amphetamine sessions (the exceptions were the 1.0 μg FK506 and the 2.5 μg Sp-cAMPS group; Tukey *post hoc* tests). The higher activity of the first FK506 group was attributable largely to the unusually high activity of one rat. It is noteworthy that the CPP effect of this group remained significant without this rat. For the control group receving 5.0 μg of FK506 and no amphetamine before conditioning sessions, activity did not differ between sessions or days (session × day within-subjects ANOVA).

Eight-day schedule

Again all groups that received amphetamine showed higher activity on amphetamine days than on saline days (Fig. 3). Amphetamine-produced activity was higher for rats not receiving FK506 or Sp-cAMPS. A 3 (group: saline vs. FK506 or Sp-cAMPS) × 2 (conditioning session: amph vs. saline) × 4 (conditioning day) mixed ANOVA revealed a three-way interaction: $F_{6,78} = 5.21$, $P < 0.001$. Following recommendations by Keppel & Wickens (2004) we broke down the interaction to further analyse activity in this schedule; main effects and lower interactions were not considered except we noted that, as expected, there was a main effect of conditioning session with amphetamine treatment enhancing activity: $F_{1,26} = 166.63$, $P < 0.001$. The three-way interaction was further analysed by carrying out a two-way group × day ANOVA for each session.

For the drug session, the group × day mixed ANOVA revealed a main effect for group ($F_{2,26} = 8.96$, $P < 0.001$) and day ($F_{3,78} = 8.22$, $P < 0.001$), and an interaction ($F_{6,78} = 5.78$, $P < 0.001$). Activity did not differ among groups on day 1. On days 2 and 3, activity for both the FK506 and Sp-cAMPS groups was lower than activity for the saline group. On day 4, activity for the Sp-cAMPS group was lower than activity for both the FK506 and saline groups. On the saline session, there was a main effect for group, $F_{2,26} = 7.27$, $P < 0.01$, with the Sp-cAMPS group showing lower activity than the saline group. Overall these results show a decrease in amphetamine-produced locomotion with repeated post-training administration of the CN inhibitor FK506 or the PKA activator Sp-cAMPS.

A session × day within-subjects ANOVA for the control group receving no amphetamine before conditioning sessions and 10.0 μg of Sp-cAMPS after conditioning sessions revealed only a main effect for day: $F_{3,33} = 4.54$, $P < 0.01$. This effect occurred when sessions were

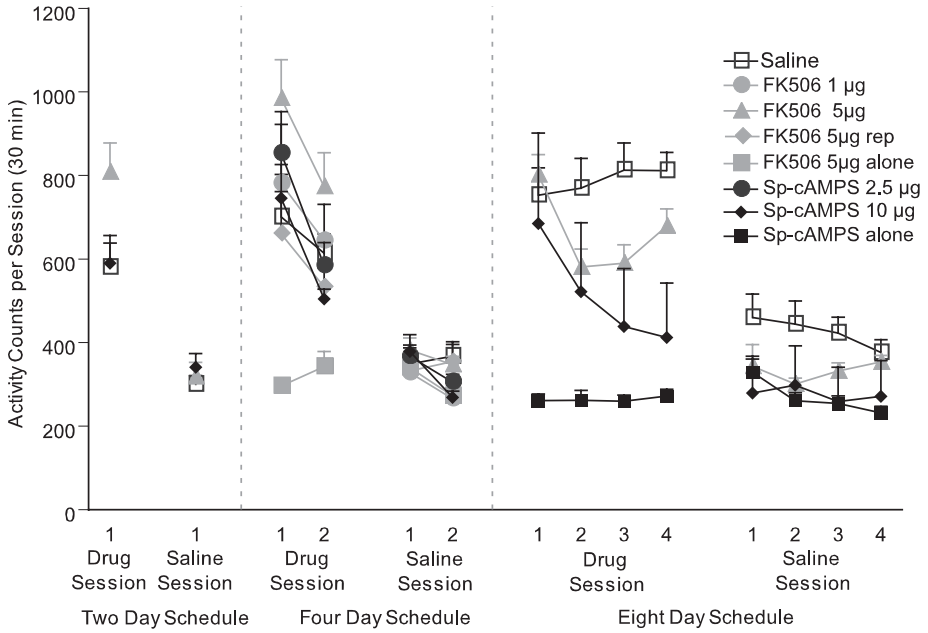

FIG. 3. Mean (+ SEM) activity counts (per 30 min) during conditioning on the drug- or the vehicle-paired side for rats receiving two (left panel), four (middle panel) or eight (right panel) conditioning sessions. Groups were treated with Sp-cAMPS (2.5 or 10.0 µg in 0.5 µL per side) or FK506 (1.0 or 5.0 µg in 0.5 µL per side) immediately after amphetamine (20 µg in 0.5 µL per side) conditioning sessions and with vehicle after saline sessions. Two groups received FK506 or Sp-cAMPS alone, i.e. no amphetamine was administered before conditioning sessions. Rep, replication.

combined, the first day being higher than the third and fourth days (Tukey *post hoc* tests).

Discussion

The present studies demonstrated the dependence of NAc amphetamine-produced CPP on the number of conditioning sessions. CPP was not seen in the 2- or 4-day conditioning schedule. Training in the 8-day schedule, on the other hand, produced significant CPP. We have previously reported robust effects using the 8-day protocol and an identical experimental apparatus (Beninger *et al*., 2003; Gerdjikov *et al*., 2004). Post-training injections of a PKA activator produced no significant CPP in the 2- or 4-day schedules and blocked the effects of amphetamine in the standard 8-day schedule. In contrast, post-training injections of the CN inhibitor resulted in a dose-dependent enhancement of the CPP effect in the 4-day schedule and failed to significantly affect the CPP effect observed in the 8-day schedule.

The CPP paradigm was unbiased with the exception of one group; overall rats did not show a systematic preference for one side of the apparatus over the other before conditioning. In addition, the time rats spent in the tunnel before and after conditioning did not differ for most groups, suggesting that changes in time spent on the drug-paired side were in fact due to changes in preference. For the four groups that showed a decrease in time spent in the tunnel after conditioning, we analysed time spent on the drug-paired side as a percentage of the total time minus tunnel time. The results were the same as those obtained using raw scores; therefore, tunnel times did not significantly affect the current findings. These results, combined with the multiple replications of the amphetamine CPP with and without a CN inhibitor, emphasize the reliability of the CPP methodology.

Post-training CN inhibition with FK506 enhanced CPP whereas PKA activation with Sp-cAMPS impaired it. If FK506 produced place preference on its own and the Sp-cAMPS activator produced place avoidance on its own then simple additivity of these putative effects and the positive effect of amphetamine might explain the observed pattern of results. To test this, we injected two groups of rats with saline on both vehicle days and what would normally have been amphetamine conditioning days and gave post-training injections of FK506 or Sp-cAMPS in doses that were found to affect amphetamine CPP. On test day these rats did not show a preference or avoidance for the chamber normally paired with amphetamine. Thus place conditioning properties of Sp-cAMPS or FK506 do not appear to account for the observed results.

In the current experiment, repeated post-training Sp-cAMPS administration resulted in reduced amphetamine-produced locomotion. We cannot rule out the possibility that the loss of CPP was related to this diminution in amphetamine-produced locomotion. However, a direct causal relationship between attenuation of locomotion and loss of place preference is unlikely. Groups receiving the CN inhibitor FK506 also showed reduced locomotion during amphetamine conditioning sessions but showed a CPP. Thus, there was a dissociation of the effects of these drugs on CPP vs. locomotor stimulation produced by NAc amphetamine. A similar dissociation of the effects on reward-related learning vs. locomotor stimulation has previously been reported using inhibitors of PKA or PKC (Aujla & Beninger, 2003; Beninger *et al*., 2003), suggesting that amphetamine's ability to produce conditioning is dissociable from its effects on unconditioned locomotion. On the other hand, work with mice has shown that a strain susceptible to amphetamine-produced CPP shows higher amphetamine-produced locomotion (Orsini *et al*., 2004). Perhaps these differences can be explained by differences in species and the use of systemic vs. central drug administrations. Further work is needed to elucidate the relationship between amphetamine-produced locomotion and CPP.

The NAc can be subdivided into core and shell regions (Zahm, 2000) and a number of studies have shown that these subregions may be differentially involved in reward-related learning (Parkinson *et al*., 1999; Di Chiara, 2002). Recently the medial shell region was

implicated in amphetamine reward (Sellings & Clarke, 2003). Our experimental design did not include a direct investigation of the relative contribution of these regions. To assess the possible role of the core and shell we identified subsets of rats with placements exclusively in the core or shell and performed *post hoc* analyses of the place preference data. Subsets of rats with placements exclusively in the core or shell showed CPP in a manner that confirmed the general pattern of results. It should be pointed out, however, that the amount of vehicle injected and the possibility that some of the drug diffused up the cannula tip, as well as the *post hoc* nature of these analyses, preclude us from making conclusions about the relative importance of the two NAc subregions. A more thorough investigation of the role of NAc subregions in reward produced by local injections of amphetamine and drugs that affect signalling pathways is awaited.

The current findings confirm previous work in which Sp-cAMPS injections impaired reward-related learning (Baldwin *et al*., 2002; Beninger *et al*., 2003). Interestingly, amygdala injections of doses that impaired amphetamine CPP and the acquisition of lever-pressing for food enhanced Pavolvian appetitive conditioning (Jentsch *et al*., 2002). Higher doses of amygdala Sp-cAMPS blocked conditioning. These differences in dose efficacy may be explained by task- or region-specific factors. Overall our data are in excellent agreement with previous work in NAc and amygdala. PKA activation is triggered by D1 receptor activation but also by a host of other cell-surface receptors and intracellular signals. The Sp-cAMPS block of reward-related learning may have been produced by the uncoupling of PKA activation from the DA reward signal.

CN inhibition enhanced amphetamine-produced CPP. This finding is in agreement with a growing body of literature implicating this phosphatase in memory and synaptic plasticity. CN down-regulation has resulted in improved performance in a discrimination task, enhanced PKA-dependent hippocampal LTP (Malleret *et al*., 2001), enhanced contextual fear conditioning (Ikegami & Inokuchi, 2000) and impaired extinction of fear conditioning (Lin *et al*., 2003). Extinction of fear conditioning also resulted in increased amygdalar CN activity and decreased phosphatidylinositol 3 (I-3) kinase activity. The activation of other kinase families was found to be necessary for extinction training in other work (Hugues *et al*., 2004). These findings underscore the complexity of kinase–phosphatase interactions mediating learning and memory. In *Aplysia* CN inhibition resulted in enhanced learning of shock-induced sensitization of gill withdrawal and this effect was blocked by MAPK inhibition (Sharma *et al*., 2003). These results are consistent with the current findings and with previous work with rats, in which NAc MAPK inhibition impaired amphetamine-produced CPP (Gerdjikov *et al*., 2004) and amphetamine-produced MAPK activation depended on DARPP-32, a protein negatively regulated by CN (Valjent *et al*., 2005). Results suggest a role for CN as a negative regulator of learning in rats (see Bennett *et al*., 2002, for some conflicting evidence in chicks). The current findings show that CN may also negatively regulate NAc DA-mediated reward-related learning.

Conclusion

We investigated the role of CN and PKA in reward-related learning using the CPP paradigm. NAc amphetamine-produced CPP was impaired by a PKA activator and enhanced by a CN inhibitor. These results are consistent with previous research showing a role for CN and PKA in memory and plasticity and with the role of these molecules in mediating cellular processes initiated by DA. Previous studies had not looked at the role of CN in reward-related learning. Importantly, the current results demonstrate that CN negatively regulates consolidation of NAc amphetamine-produced CPP, a form of reward-related learning.

Acknowledgements

This research was funded by a grant from the Natural Sciences and Engineering Research Council of Canada to R.J.B.

Abbreviations

cAMP, cyclic adenosine monophosphate; CN, calcineurin; CPP, conditioned place preference; DA, dopamine; DARPP-32, phosphoprotein of M(r) 32 kDa; DMSO, dimethyl sulfoxide; NAc, nucleus accumbens; NMDA, *N*-methyl-D-aspartate; PKA, protein kinase A; Sp-cAMPS, Sp-adenosine 3′,5′-cyclic monophosphorothioate triethylammonium salt.

References

Aujla, H. & Beninger, R.J. (2003) Intra-accumbens protein kinase C inhibitor NPC 15437 blocks amphetamine-produced conditioned place preference in rats. *Behav. Brain Res.*, **147**, 41–48.

Baldwin, A.E., Holahan, M.R., Sadeghian, K. & Kelley, A.E. (2000) N-methyl-D-aspartate receptor-dependent plasticity within a distributed corticostriatal network mediates appetitive instrumental learning. *Behav. Neurosci.*, **114**, 84–88.

Baldwin, A.E., Sadeghian, K., Holahan, M.R. & Kelley, A.E. (2002) Appetitive instrumental learing is impaired by inhibition of cAMP-dependent protein kinase within the nucleus accumbens. *Neurobiol. Learn. Mem.*, **77**, 44–62.

Beninger, R.J. & Gerdjikov, T. (2004) The role of signaling molecules in reward-related incentive learning. *Neurotox. Res.*, **6**, 91–104.

Beninger, R.J. & Gerdjikov, T.V. (2005) Dopamine–glutamate interactions in reward-related incentive learning. In Schmidt, W.J. & Reith, M.E. (ed.), *Dopamine and Glutamate in Psychiatric Disorders*. Humana Press Inc., Totawa NK, pp. 315–350.

Beninger, R.J. & Miller, R. (1998) Dopamine D1-like receptors and reward-related incentive learning. *Neurosci. Biobehav. Rev.*, **22**, 335–345.

Beninger, R.J., Nakonechny, P.L. & Savina, I. (2003) cAMP-dependent protein kinase and reward-related learning: intra-accumbens Rp-cAMPS blocks amphetamine-produced place conditioning in rats. *Psychopharmacology (Berl.*, **170**, 23–32.

Bennett, P.C., Schmidt, L., Lawen, A., Moutsoulas, P. & Ng, K.T. (2002) Cyclosporin A, FK506 and rapamycin produce multiple, temporally distinct, effects on memory following single-trial, passive avoidance training in the chick. *Brain Res.*, **927**, 180–194.

Brockwell, N.T., Ferguson, D.S. & Beninger, R.J. (1996) A computerized system for the simulatneous monitoring of place conditioning and locomotor activity in rats. *J. Neurosci. Meth.* **64**, 227–232.

Cooper, D.M. (2003) Regulation and organization of adenylyl cyclases and cAMP. *Biochem. J.*, **375**, 517–529.

Di Chiara, G. (2002) Nucleus accumbens shell and core dopamine: differential role in behavior and addiction. *Behav. Brain Res.*, **137**, 75–114.

Everitt, B.J., Morris, K.A., O'Brien, A. & Robbins, T.W. (1991) The bsolateral amygdala–ventral striatal system and conditioned place preference: further evidence of limbic–striatal interactions underlying reward-related processes. *Neuroscience*, **42**, 1–18.

Everitt, B.J. & Wolf, M.E. (2002) Psychomotor stimulant addiction: a neural systems perspective. *J. Neurosci.*, **22**, 3312–3320.

Gerdjikov, T.V., Ross, G.M. & Beninger, R.J. (2004) Place preference induced by nucleus accumbens amphetamine is impaired by antagonists of ERK or p38 MAP kinases in rats. *Behav. Neurosci.*, **118**, 740–750.

Hugues, S., Deschaux, O. & Garcia, R. (2004) Postextinction infusion of a mitogen-activated protein kinase inhibitor into the medial prefrontal cortex impairs memory of the extinction of conditioned fear. *Learn. Mem.* **11**, 540–543.

Ikegami, S. & Inokuchi, K. (2000) Antisense DNA against calcineurin facilitates memory in contextual fear conditioning by lowering the threshold for hippocampal long-term potentiation induction. *Neuroscience*, **98**, 637–646.

Jentsch, J.D., Olausson, P., Nestler, E.J. & Taylor, J.R. (2002) Stimulation of protein kinase A activity in the rat amygdala enhances reward-related learning. *Biol. Psychiatry*, **52**, 111–118.

Kandel, E. (2001) The molecular biology of memory storage: a diologue between genes and synapses. *Science*, **294**, 1030–1038.

Kelley, A.E. (2004) Memory and addiction: shared neural circuitry and molecular mechanisms. *Neuron*, **44**, 161–179.

Kelley, A.E. & Holahan, M.R. (1997) Enhanced reward-related responding following cholera toxin infusion into the nucleus accumbens. *Synapse*, **26**, 46–54.

Keppel, G. & Wickens, T.D. (2004) *Design and Analysis: a Researcher's Handbook*. Prentice Hall, Englewood Cliffs, NJ.

Lin, X.H., Hashimoto, T., Kitamura, N., Murakami, N., Shirakawa, O. & Maeda, K. (2002) Decreased calcineurin and increased phosphothreonine-DARPP-32 in the striatum of rats behaviorally sensitized to methamphetamine. *Synapse*, **44**, 181–187.

Lin, C.H., Yeh, S.H., Leu, T.H., Chang, W.C., Wang, S.T. & Gean, P.W. (2003) Identification of calcineurin as a key signal in the extinction of fear memory. *J. Neurosci.*, **23**, 1574–1579.

Malleret, G., Haditsch, U., Genoux, D., Jones, M.W., Bliss, T.V., Vanhoose, A.M., Weitlauf, C., Kandel, E.R., Winder, D.G. & Mansuy, I.M. (2001) Inducible and reversible enhancement of learning, memory, and long-term potentiation by genetic inhibition of calcineurin. *Cell*, **104**, 675–686.

Mansuy, I.M. (2003) Calcineurin in memory and bidirectional plasticity. *Biochem. Biophys. Res. Commun.*, **311**, 1195–1208.

Narita, M., Akai, H., Nagumo, Y., Sunagawa, N., Hasebe, K., Nagase, H., Kita, T., Hara, C. & Suzuki, T. (2004) Implications of protein kinase C in the nucleus accumbens in the development of sensitization to methamphetamine in rats. *Neuroscience*, **127**, 941–948.

Nishi, A., Snyder, G.L. & Greengard, P. (1997) Bidirectional regulation of DARPP-32 phosphorylation by dopamine. *J. Neurosci*., **17**, 8147–8155.

Orsini, C., Buchini, F., Piazza, P.V., Puglisi-Allegra, S. & Cabib, S. (2004) Susceptibility to amphetamine-induced place preference is predicted by locomotor response to novelty and amphetamine in the mouse. *Psychopharmacology (Berl.)*, **172**, 264–270.

Parkinson, J.A., Olmstead, M.C., Burns, L.H., Robbins, T.W. & Everitt, B.J. (1999) Dissociation in effects of lesions of the nucleus accumbens core and shell on appetitive pavlovian approach behavior and the potentiation of conditioned reinforcement and locomotor activity by D-amphetamine. *J. Neurosci.*, **19**, 2401–2411.

Parkinson, J.A., Willoughby, P.J., Robbins, T.W. & Everitt, B.J. (2000) Disconnection of the anterior cingulate cortex and nucleus accumbens core impairs pavlovian approach behavior: further evidence for limbic cortical–ventral striatopallidal systems. *Behav. Neurosci.*, **114**, 42–63.

Paxinos, G. & Watson, C. (1998) *The Rat Brain in Stereotaxic Coordinates*. Academic Press, San Diego, CA.

Roberts, D.C.S. & Koob, G.F. (1980) Extinction and recovery of cocaine self-administration following 6-hydroxydopamine lesions of the nucleus accumbens. *Pharmacol. Biochem. Behav.*, **12**, 781–787.

Sellings, L.H. & Clarke, P.B. (2003) Segregation of amphetamine reward and locomotor stimulation between nucleus accumbens medial shell and core. *J. Neurosci.*, **23**, 6295–6303.

Sharma, S.K., Bagnall, M.W., Sutton, M.A. & Carew, T.J. (2003) Inhibition of calcineurin facilitates the induction of memory for sensitization in Aplysia: requirement of mitogen-activated protein kinase. *Proc. Natl Acad. Sci. USA*, **100**, 4861–4866.

Stevens, P.J. (2002) *Applied Multivariate Statistics for the Social Sciences*. Lawrence Erlbaum, Mahwah, New Jersey.

Sutton, M.A. & Beninger, R.J. (1999) Psychopharmacology of conditioned reward; evidence for a rewarding signal at D1-like dopamine receptors. *Psychopharmacology*, **144**, 95–110.

Tzschentke, T.M. (1998) Measuring reward with the conditioned place preference paradigm: a comprehensive review of drug effects, recent progress and new issues. *Prog. Neurobiol.*, **56**, 613–672.

Valjent, E., Pascoli, V., Svenningsson, P., Paul, S., Enslen, H., Corvol, J.C., Stipanovich, A., Caboche, J., Lombroso, P.J., Nairn, A.C., Greengard, P., Herve, D. & Girault, J.A. (2005) Regulation of a protein phosphatase cascade allows convergent dopamine and glutamate signals to activate ERK in the striatum. *Proc. Natl Acad. Sci. USA*, **102**, 491–496.

Wickens, J.R. & Kötter, R. (1995) Cellular models of reinforcement. In Houk, J.C., Davis, J.L. & Beiser, D.G. (Eds), *Models of Information Processing in the Basal Ganglia.* MIT, Boston, pp. 187–214.

Winder, D.G. & Sweatt, J.D. (2001) Roles of serine/threonine phosphatases in hippocampal synaptic plasticity. *Nature Neuroscience*, **2**, 461–474.

Zachariou, V., Benoit-Marand, M., Allen, P.B., Ingrassia, P., Fienberg, A.A., Gonon, F., Greengard, P. & Picciotto, M.R. (2002) Reduction of cocaine place preference in mice lacking the protein phosphatase 1 inhibitors DARPP 32 or Inhibitor 1. *Biol. Psychiatry*, **51**, 612–620.

Zahm, D. (2000) An integrative neuroanatomical perspective on some subcortical substrates of adaptive responding with emphasis on the nucleus accumbens. *Neurosci. Biobehav. Rev.*, **24**, 85–105.