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Effects of Protein Kinase A Inhibitor and Activator on Rewarding Effects of SKF-82958 Microinjected into Nucleus Accumbens Shell of Ad Libitum Fed and Food-Restricted Rats

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Abstract

Rationale—Previous studies indicate that the rewarding effect of D-1 dopamine (DA) receptor stimulation in nucleus accumbens (NAc) shell is greater in food-restricted (FR) than in *ad libitum* fed (AL) rats. The D-1 receptor is positively coupled to adenylyl cyclase and activates protein kinase A (PKA).

Objectives—The purpose of this study was to determine whether PKA is involved in the rewarding effect of D-1 receptor stimulation and, if so, whether it is involved in the enhanced response of FR rats.

Materials and methods—Rats were stereotactically implanted with microinjection cannulae in NAc shell and a stimulating electrode in lateral hypothalamus. Rewarding effects of SKF-82958 (1.5 or 3.0 µg, bilaterally) in the presence and absence of PKA inhibitor, Rp-cAMPS (8.9 µg), and PKA activator, Sp-cAMPS (8.9 µg), were assessed using the curve-shift method of self-stimulation (ICSS). Basal NAc levels of DARPP-32 phosphorylated on Thr34 and Thr75 were measured.

Results—Rp-cAMPS increased the rewarding effect of SKF-82958 in AL but not FR rats, doubling the ICSS threshold-lowering effect of the 3.0 µg dose. Sp-cAMPS decreased the rewarding effect of SKF-82958 in FR but not AL rats. Levels of phospho-DARPP-32 (Thr75), which inhibits PKA, were higher in FR than AL rats.

Conclusions—Results indicate that inhibition of PKA enhances the unconditioned rewarding effect of D-1 receptor stimulation and that decreased PKA may be involved in the effect of FR on drug reward. Evidence for involvement of D-2 receptor-expressing neurons in the enhancing effect of PKA inhibition is discussed.

Keywords

food restriction; nucleus accumbens; reward; self-stimulation; D-1 receptor; protein kinase A; SKF-82958; Rp-cAMPS; Sp-cAMPS; DARPP-32

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More than 90% of neurons in the nucleus accumbens (NAc) dopamine (DA) terminal field are medium spiny neurons (MSN) (O'Donnell and Grace 1993), and less than 1% coexpress D-1- and D-2-like DA receptors (Shuen et al. 2008). Recent evidence suggests that excitation of D-1 DA receptor-expressing MSNs mediates reward-related behavior including cocaine-conditioned place preference and locomotor sensitization (Lobo et al. 2010). However, the effect of D-1 DA receptor stimulation on MSN excitability depends on whether cells are in a resting hyperpolarized “down state” or an activated depolarized “up state” (Gerfen and Surmeier 2010). When MSNs are in the down state, the effect of D-1 stimulation is inhibitory and it eliminates responsiveness to weak glutamatergic input. When MSNs are in the up state, the effect of D-1 stimulation is excitatory and it facilitates responsiveness to coordinated and strong glutamate signaling. The convergence of DA and glutamate in NAc (Groenewegen et al. 1999) mediates both goal-directed behavior and reward-related learning (Kelley 2004; Dalley et al. 2005; Hyman et al. 2006). Multiple intracellular signaling pathways mediate the latter, including D-1 receptor-linked adenylyl cyclase and its downstream target, protein kinase A (PKA) (Beninger and Gerdjikov 2004). PKA phosphorylates target proteins involved in synaptic plasticity and gene expression, including the AMPA receptor GluA1 subunit, the NMDA receptor NR1 subunit, DARPP-32 and CREB (Malenka and Bear 2004; Svenningsson et al. 2004; Wang et al. 2006; Lee and Messing 2008).

Chronic food restriction (FR) increases the reward magnitude of drugs whose primary rewarding effects are mediated by DA in NAc medial shell (Ikemoto 2007). Upregulation of D-1 DA receptor function is one of the neuroadaptations in NAc that may contribute to the increased behavioral effects of abused drugs in FR subjects (Carr 2007). Administration of a D-1 DA receptor agonist produces stronger locomotor-activating and reward-potentiating effects in FR relative to *ad libitum* fed (AL) rats (Carr et al. 2001; Carr et al. 2003; Carr et al. 2009). In addition, intracellular signaling and transcription responses downstream of D-1 receptor stimulation are increased in NAc of FR subjects (Carr et al. 2003; Haberny et al. 2004; Haberny and Carr 2005a; Carr et al. 2010). Illumination of mechanisms underlying the modulation of reward by FR may provide insight into the high comorbidity of disordered eating and substance abuse (Wiederman and Pryor 1996; Pisetsky et al. 2008; Root et al. 2010), and risks associated with use of tobacco and psychostimulants as diet aids (Klesges et al. 1997; Cochrane et al. 1998).

It was recently observed that similar to drugs of abuse, microinjection of the D-1 DA receptor agonist SKF-82958 into NAc shell produces a reward-potentiating effect in the intracranial self-stimulation (ICSS) paradigm (Carr et al. 2009). Dose-related rewarding effects were greater in FR than AL rats, suggesting an upregulation of D-1 DA receptor function or, perhaps, a greater abundance of D-1-expressing MSNs that are in the up state. While changes in adenylyl cyclase, PKA and the downstream molecular targets of the D-1 receptor-coupled signaling pathway play an important role in the NAc response to chronic drug treatment (Terwilliger et al. 1991; Unterwald et al. 1996), it is not clear whether PKA regulates the acute rewarding effect of DA in NAc. Should PKA activity modulate reward, it would be predicted that the corresponding effect is greater in FR than AL rats. Consequently, the reward-potentiating effect of SKF-82958 microinjected into NAc shell was evaluated in AL and FR rats in the presence and absence of the cell-permeable competitive inhibitor of cAMP-dependent PKA, Rp-cAMPS. Effects of the cell-permeable activator of cAMP-dependent PKA, Sp-cAMPS were also determined. Contrary to expectation, PKA inhibition enhanced the rewarding effect of SKF-82958 and this result was observed in AL but not FR rats. Complementing this effect, PKA activation diminished the rewarding effect of SKF-82958 in FR but not AL rats. These findings lead to a new set

of speculations regarding mechanistic changes in NAc of FR rats that may underlie the enhanced rewarding effects of abused drugs.

Materials and methods

Subjects and Surgical Procedures

Subjects were mature male Sprague-Dawley rats (Taconic Farms, Germantown, NY) initially weighing 375–425 g. Food (pelleted LabDiet #5001) and water were available *ad libitum* except when FR conditions applied. Animals were individually housed in clear plastic cages with bedding under a 12 h light:dark photoperiod with lights on at 0700 h. Several days after arrival in the central animal facility, all rats except those in Experiment 3 were anesthetized with ketamine (100 mg/kg, i.p.) and xylazine (10 mg/kg, i.p.) and were stereotactically implanted with a 0.25 mm diameter monopolar stimulating electrode (Plastics One, Roanoke, VA) in the lateral hypothalamic medial forebrain bundle (skull flat coordinates: 3.0 mm posterior to bregma, 1.6 mm lateral to the sagittal suture, and 8.5 mm ventral to skull surface). An anterior ipsilateral stainless steel skull screw served as ground. Rats were also implanted with two chronically indwelling guide cannulae (26 ga) which were placed bilaterally 2.0 mm dorsal to injection sites in the NAc medial shell (1.6 mm anterior to bregma; 2.1 mm lateral to the sagittal suture, tips angled 8° toward the midline, 5.8 mm ventral to skull surface). The electrode, ground, cannulae, and three additional mounting screws were then permanently secured to the skull by flowing dental acrylic around them. Postsurgical analgesia was achieved by administration of buprenorphine (2.0 mg/kg, s.c.) following recovery from anesthesia and the morning after. Experimental procedures were approved by the Institutional Animal Care and Use Committee at the New York University School of Medicine and were consistent with the *Principles of Laboratory Animal Care* (NIH Publication no. 85-23).

Feeding regimens

During the second week of training in the ICSS protocol, approximately half of the rats in Experiments 1, 2 and 4 were switched from unlimited access to food to a single 10-g meal each day, representing ~40% of the *ad libitum* intake. The daily meal was delivered at approximately 1700 h in the home cage. This regimen continued until body weights decreased by 20% (approximately two weeks). ICSS training continued throughout this period. For the remainder of each experiment, daily feeding was titrated to maintain stable body weights, typically requiring an upward adjustment to 12–16 grams. Drug testing did not begin until rats in the FR group had been stabilized for one week at 80% of pre-restriction body weight. Rats in Experiment 3 were also assigned to FR and AL diet conditions, however they did not undergo surgery or behavioral testing; these animals were used to obtain NAc biochemical measures.

Self-stimulation (ICSS) apparatus

Brain stimulation training and testing were conducted in eight standard test chambers (26 × 26 × 21 cm) placed within sound attenuating cubicles. Each chamber had a retractable lever mounted on one wall and a house light mounted on the opposite wall. Four constant current stimulators (PHM-152B/2; Med-Associates, Georgia, VT), with dual outputs, were used to deliver trains of 0.1 ms cathodal pulses, which were conducted to implanted electrodes by way of commutators and flexible cables. Electrical stimulation, contingencies, and data recording were controlled through Dell XPS R400 computers and interface (Med-Associates). All stimulation parameters were monitored on Tektronix (TAS 455) oscilloscopes.

Self-stimulation procedures

After one week of postsurgical recovery, rats were trained to lever press for 0.5 s trains of electrical stimulation at a frequency of 100 pulses per second (pps). The initial stimulation intensity of 120 μ A was systematically varied to locate the lowest intensity that maintained a high rate of lever pressing (e.g., 35–45/min). On subsequent days, rats were trained in sessions consisting of twenty four 60-s trials. Extension of the lever and a 2-s train of 'priming' stimulation initiated each trial. Each trial was terminated by retraction of the lever and followed by a 10-s intertrial interval. Each lever press produced a 1-s train of stimulation, except for those presses emitted during the stimulation train, which did not increase reinforcement density. The number of lever presses and reinforcements were recorded for each trial.

Initial training was followed by rate-frequency training (Miliaressis et al. 1986), which continued for approximately two weeks. Rate-frequency curves were generated by presenting 12 trials in which the frequency of brain stimulation decreased over successive trials (approximately 0.05 log units each trial) from an initial frequency of 100 pps to a terminal frequency of 28 pps. At least two series were presented in each training session. During the second week of training, subjects in Experiments 1, 2 and 4 were divided into two groups matched for body weight and M-50 (the brain stimulation frequency that supported 50% of the maximum reinforcement rate) and one of the groups was placed on the FR regimen. Training and mock microinjection test sessions (see below) continued during the ensuing ~3 week period during which the FR group achieved and then stabilized at the target body weight.

Intra-NAc microinjection procedure

For microinjections, solutions were loaded into two 30 cm lengths of PE-50 tubing attached at one end to 5- μ l Hamilton syringes filled with distilled water and at the other end to 31-gauge injector cannulae, which extended 2.0 mm beyond the implanted guides. The 0.5 μ l injection volumes were delivered over a period of 90 sec at a rate of 0.05 μ l/10 sec. In all experiments, subjects received two bilateral drug microinjections spaced 10-min apart. One minute following completion of the first microinjection, injector needles were removed from guides, stylets were replaced, and animals were returned to home cages for an additional 9-min prior to initiating the second microinjection. The second microinjection was procedurally identical to the first. Five minutes following completion of the second microinjection, rats were returned to test chambers for post-injection behavioral testing.

Behavioral testing

Each test session began with a pre-injection test consisting of three rate-frequency series (42 min). The first series in a session was considered a 'warm-up' and data were excluded. This was followed by intra-NAc microinjections which were followed, in 5 min, by a post-injection test consisting of two rate-frequency series (28 min). For each rate-frequency series, the number of reinforcements obtained as a function of brain stimulation frequency was recorded. For each rat, the two series from each test were averaged to yield a single rate-frequency function per test. Test sessions were conducted between 0900 and 1500 h.

Whole cell homogenates prepared from NAc for biochemical measurements

Rats were briefly exposed to CO₂, decapitated by guillotine, and NAc dissected from fresh brain on ice. Protease inhibitor cocktail and PMSF were added to 0.32 M sucrose solution containing 1mM NaHCO₃, 1mM MgCl₂ and 0.5 mM CaCl₂. Brain tissue was rinsed, homogenized and subsequently diluted to 10% weight/volume. After being well mixed, the whole cell homogenates were stored at – 80 °C until use.

Western blotting

Proteins were separated by electrophoresis on precast 4–12% sodium dodecyl sulfate polyacrylamide gels (Lonza, Rockland, ME Dual colored protein standards (Bio-Rad, Hercules, CA) were loaded to estimate the size of target proteins and to assure complete transfer of proteins from gel to membrane. Proteins were electrophoretically transferred to Polyvinylidene fluoride (PVDF) membranes and blocked for 60 minutes with 5% nonfat milk in phosphate buffered saline with 0.05% Tween-20 (PBST) with shaking at room temperature. The membranes were incubated with primary antibodies for target proteins by shaking overnight at 4 °C (in 3% nonfat milk prepared in PBST). Primary antibodies used were rabbit polyclonal anti-phospho-DARPP-32 (Thr75) (1:1,000; 2301S, Cell Signaling, Danvers, MA), rabbit polyclonal anti-phospho-DARPP-32 (Thr34) (1:1,000; 2304S, Cell Signaling, Danvers, MA), and mouse monoclonal anti- α -tubulin (1:5,000; T6199, Sigma-Aldrich, St. Louis, MO). Blots were washed 3×8 min in PBST, incubated with the appropriate secondary antibodies (1:15,000) for 1 h at room temperature (in 3% nonfat milk prepared in PBST), washed 2×8 min in PBST and 1×8 min in PBS, then treated with SuperSignal West Pico or Femto Substrate (Thermo Scientific Pierce, Rockford, IL, USA) and exposed membrane to visualize bands of interest. All Western blot analyses were run at least 3 times for each sample and results were averaged to yield one data point for each sample. Immunoblots were analyzed using NIH Image J software.

Data analysis

As in previous studies (e.g., Carr et al. 2009) regression analysis of the descending portion of rate-frequency curves was used to calculate the M-50 and theta-0 reward thresholds which are defined as the log pulse frequency sustaining half the maximum reinforcement rate and x-axis intercept of the regression line, respectively. For threshold parameters, antilog transformations were applied and natural frequencies were used to calculate the percentage change occurring in post-injection tests relative to a pre-injection test. Drugs of abuse generally produce parallel leftward shifts in the rate-frequency curve with similar effects on the two threshold measures (Wise 1996).

Results of Experiment 1 (SKF-82958 at two doses, with and without Rp-cAMPS, in AL and FR rats) were analyzed by 3-way mixed ANOVA with diet and dose of SKF-82958 as the between subjects factors and presence/absence of Rp-cAMPS as the within subjects factor. In Experiment 2 (SKF-82958 with and without Sp-cAMPS in AL and FR rats), different doses of SKF-82958 were used to generate a similar baseline effect in the two diet groups. Consequently, results were analyzed by separate t-tests for correlated samples in AL and FR subjects. Results of Experiment 3 (levels of phospho-DARPP-32 (Thr75) and phospho-DARPP-32 (Thr34) in NAc of AL and FR rats) were separately analyzed by t-tests for independent samples. Results of Experiment 4 (SKF-82958 with and without quinpirole in AL and FR rats) were analyzed by 2-way mixed ANOVA with diet group as the between subjects factor and drug treatment as the within subjects factor. Analyses were performed using SYSTAT 8.0.

Histology

Upon completion of behavioral testing, rats were euthanized with CO₂ and decapitated. Brains were removed and fixed in 10% buffered formalin for at least 48 hr. Frozen coronal sections, 40 μ m thick, were cut on a Reichert-Jung Cryostat, thaw-mounted on gelatin-coated glass slides and stained with cresyl violet. Electrode placements and injection sites were determined by visual inspection of sections under an Olympus SZ40 microscope. In order for a rat's behavioral data to be included in the analysis, cannula placements were required to be judged as bilaterally accurate within the NAc medial shell (with some

unilaterally placed on the shell/core and shell/olfactory tubercle borders) as in recent studies (Carr et al. 2009; Carr et al. 2010).

Drugs

SKF-82958 (Sigma-Aldrich) was dissolved in a small volume of DMSO and diluted with sterile 0.9% saline (final DMSO concentration 5%) and microinjected bilaterally in a dose of 3.0 µg or 1.5 µg. Rp-cAMPS and SP-cAMPS (Enzo Life Sciences, Farmingdale, NY) were dissolved in sterile 0.9% saline and microinjected bilaterally in a dose of 8.9 µg (20 nmol). The doses of SKF-82958 were selected based on prior studies in which the drug was microinjected into NAc shell and produced greater rewarding effects in FR relative to AL rats (Carr et al. 2009; Carr et al. 2010). The dose of Rp-cAMPS was based on its efficacy in previous NAc microinjection studies (Baldwin et al. 2002; Lynch and Taylor 2005). Quinpirole (Sigma-Aldrich) was dissolved in sterile 0.9% saline and microinjected bilaterally in a dose of 0.5 µg or 1.0 µg.

Experiment 1 Intra-NAc shell microinjection of SKF-82958 with and without Rp-cAMPS

- a. 14 AL and 13 FR rats were tested in three sessions over an 8–10 day period to assess effects of a 1.5 µg dose of SKF-82958. Two additional groups of 11 AL and 13 FR rats were used to assess effects of a 3.0 µg dose. In the first and third test sessions, pre-injection testing was followed by bilateral microinjection of the vehicle for Rp-cAMPS (0.9% saline) which, 10-min later, was followed by bilateral microinjection of SKF-82958 (1.5 µg or 3.0 µg per side). In the second session, which was separated from the first and third by at least 3 days, pre-injection testing was followed by bilateral microinjection of Rp-cAMPS (8.9 µg, i.e., 20 nmol per side) which, 10-min later, was followed by bilateral microinjection of SKF-82958. In each SKF-82958 dose study, results of the two vehicle/SKF-82958 treatment sessions were averaged for purposes of data analysis. Because microinjections of vehicle in NAc shell do not alter reward threshold parameters (Carr et al. 2009; Carr et al. 2010) testing of the vehicle/vehicle combination was postponed and used to assess the effect of Rp-cAMPS/vehicle in Experiment 1 (b).
- b. At least 3 days after completion of “a” above, subjects tested with the 3.0 µg dose of SKF-82958 were tested in two additional sessions, three days apart. In one session animals received two intra-NAc microinjections of vehicle spaced 10-min apart, and in the other they received a microinjection of Rp-cAMPS followed, 10-min later, by vehicle. The order of the two treatments was counterbalanced and matched between groups.

Experiment 2 Intra-NAc shell microinjection of SKF-82958 with and without Sp-cAMPS

—9 AL and 9 FR rats were tested in three sessions over an 8 day period. In the first and third session pre-injection testing was followed by bilateral microinjection of the vehicle for Sp-cAMPS (0.9% saline) which, 10-min later, was followed by bilateral microinjection of SKF-82958. In the second session, which was separated from the first and third by at least 3 days, pre-injection testing was followed by bilateral microinjection of Sp-cAMPS (8.9 µg, i.e., 20 nmol per side) which, 10-min later, was followed by bilateral microinjection of SKF-82958. In order to obtain a similar magnitude of SKF-82958 effect in AL and FR rats as a baseline for evaluating the effect of Sp-cAMPS, AL rats were microinjected with the 3.0 µg and FR rats with the 1.5 µg bilateral dose of SKF-82958. Results of the two vehicle/SKF-82958 treatment sessions were averaged for purposes of data analysis.

Experiment 3 Effects of food restriction on NAc levels of DARPP-32 phosphorylated at Thr75 and Thr34

DARPP-32, and particularly the balance between DARPP-32 phosphorylated at Thr75 and Thr34, is an important regulator of NAc intracellular signaling (Walaas et al. 2011). Phospho-DARPP-32 (Thr34) is a phosphatase (PP-1) inhibitor, and phospho-DARPP-32 (Thr75) is a protein kinase A inhibitor. Consequently, an experiment was conducted to determine whether FR alters the balance between the two in NAc. 20 AL and 20 FR rats without history of surgery or drug exposure were sacrificed, brains were harvested, and NAc was dissected from fresh brain on ice. NAc from 4 subjects were pooled to obtain each sample for assay.

Experiment 4 Intra-NAc shell microinjection of SKF-82958 with and without quinpirole—In some behavioral studies, synergistic effects of concurrent D-1 and D-2 DA receptor stimulation have been observed (Wachtel et al. 1989; Ikemoto et al. 1997; LaHoste et al. 2000). Given that the D-2 DA receptor is coupled to G_i and inhibits adenylyl cyclase and PKA signaling, an experiment was conducted to determine whether the D-2/3 agonist, quinpirole, produces effects similar to those produced in Experiment 1 by Rp-cAMPS.

7 AL and 8 FR rats were tested in four sessions over a 10–12 day period. In the first and fourth session pre-injection testing was followed by bilateral microinjection of SKF-82958 (1.5 μ g per side). In the second and third sessions, which were separated by at least 3 days, pre-injection testing was followed by bilateral microinjection of SKF-82958 (1.5 μ g per side) in combination with quinpirole (0.5 or 1.0 μ g per side). The order in which subjects received the two doses of quinpirole was counterbalanced and matched between diet groups. Results of the two SKF-82958-only treatment sessions were averaged for purposes of data analysis.

Results

Experiment 1 Intra-NAc shell microinjection of SKF-82958 with and without Rp-cAMPS

Effects of NAc microinjection of SKF-82958 preceded by Rp-cAMPS or its vehicle are displayed in Figure 1. Shifts in rate-frequency curves produced by the high (3.0 μ g) dose of SKF-82958 with and without Rp-cAMPS are displayed in Figure 2. Analysis of results by 3-way mixed factor ANOVA indicated no main effect of diet ($F_{1,47}=3.12$, $p=.08$), a main effect of SKF dose ($F_{1,47}=4.18$, $p<.05$), and a main effect of Rp-cAMPS ($F_{1,47}=8.97$, $p<.01$). A significant interaction between diet and Rp-cAMPS ($F_{1,47}=5.33$, $p<.05$) followed by 2-way mixed factor ANOVAs revealed that Rp-cAMPS enhanced the rewarding effect of SKF-82958 in the AL group ($F_{1,23}=20.4$, $p<.001$) but not in the FR group ($F_{1,24}=0.18$, $p>.05$). Mean maximum reinforcement rates remained in the range of 39.5 to 42 reinforcements per minute regardless of treatment. There were no main effects of diet ($F_{1,47}=1.1$, $p>.05$), SKF dose ($F_{1,47}=0.08$, $p>.05$), or Rp-cAMPS ($F_{1,47}=3.0$, $p=.085$), and no interaction effects on the maximum reinforcement rate measure (data not shown).

To assess whether Rp-cAMPS lowered reward thresholds in the absence of SKF-82958 two follow-up test sessions were conducted. There was no main effect of drug treatment ($F_{1,22}=3.8$, $p>.05$) and no interaction between diet and drug treatment ($F_{1,22}=0.6$; Figure 3).

Experiment 2 Intra-NAc shell microinjection of SKF-82958 with and without Sp-cAMPS

T tests for correlated samples indicated that Sp-cAMPS had no effect on the reward-potentiating effect of SKF-82958 in AL rats ($t(8)=0.5$) but decreased the effect in FR rats ($t(8)=5.99$, $p<.001$; Figure 4). There were no treatment effects on maximum reinforcement rate (data not shown).

Experiment 3 NAc levels of DARPP-32 phosphorylated at Thr75 and Thr34

FR rats displayed elevated levels of phospho-DARPP-32 (Thr75) in NAc relative to AL rats ($t(8)=3.2$, $p<.05$). Levels of phospho-DARPP-32 (Thr34) did not differ between diet groups ($t(8)=0.85$, $p>.05$). Results are displayed with representative blots in Figure 5.

Experiment 4 Intra-NAc shell microinjection of SKF-82958 with and without quinpirole

When the effect of SKF-82958 was analyzed separately, FR rats displayed a greater threshold-lowering effect than AL rats ($t(13)=2.81$, $p<.01$). However, in the full 2-way ANOVA (2 diets $\times$ 3 drug treatments) the difference between AL and FR was not significant ($F_{1,14}=2.93$, $p>.05$), there was no main effect of drug treatment ($F_{2,28}=0.47$), and no interaction ($F_{2,28}=0.65$; Figure 6). However, the x-axis intercept measure of reward threshold, which is usually redundant with the M-50 measure, yielded a different result. While there was no main effect of diet ($F_{1,14}=0.0$), there was a main effect of drug treatment whereby quinpirole, at the dose of 1.0 μg , increased the rewarding effect of SKF-82958 across diet groups ($F_{2,28}=5.17$, $p=.01$; $t(28)=2.58$, $p<.01$). There was no interaction between diet and drug treatment ($F_{2,28}=0.58$) although inspection of Figure 6 suggests that the effect of quinpirole was limited to the AL group.

Discussion

The observations that a PKA inhibitor selectively increased the rewarding effect of NAc D-1 DA receptor stimulation in AL rats, while a PKA activator selectively decreased the rewarding effect in FR rats suggest that PKA inhibition facilitates reward and that FR may decrease endogenous PKA activity. A potential mechanism of PKA inhibition is suggested by the finding that levels of phospho-DARPP-32 (Thr75) were elevated in NAc of FR relative to AL rats. PKA activity is often measured by inference from phosphorylation of specific substrates such as DARPP-32. PKA phosphorylates DARPP-32 on Thr34 which, in turn, amplifies phosphorylation of other PKA targets by its inhibition of protein phosphatase-1 (Lee and Messing 2008). On the other hand, DARPP-32 phosphorylated on Thr75 is a PKA inhibitor (Walaas et al. 2011) and the balance between DARPP-32 phosphorylated on these two threonine residues plays an important role in the integration of NAc neuronal signaling. The mechanism underlying elevated phospho-DARPP-32 (Thr75) is often initiated by increased expression of the transcription factor ΔFosB (Bibb et al. 2001), which is elevated in NAc of FR subjects (Stamp et al. 2008). Tenability of this scheme would require reconciliation with a number of previous findings. First, D-1 DA receptor-dependent PKA signaling in NAc has a well established *positive* role in the acquisition of Pavlovian and instrumental responses reinforced by food and psychostimulant drugs (Beninger and Gerdjikov 2004). Yet, compatible with the present results, opposite effects of PKA inhibition on reward-related learning and unconditioned behavioral responses to psychostimulant drug challenge have been reported. NAc microinjection of Rp-cAMPS inhibited acquisition of a conditioned response to d-amphetamine but potentiated the unconditioned locomotor response to the drug (Gerdjikov et al. 2007). NAc microinjection of Rp-cAMPS also shifted the cocaine self-administration dose-response curve to the left (Self et al. 1998), suggesting increased reward magnitude. Another finding that suggests dissociation of PKA regulation of learning and acute drug responsiveness was obtained in mice that are deficient in type 5 adenylyl cyclase, which is the predominant isoform in striatal MSNs. These animals displayed deficits in appetitive learning (Kheirbek et al. 2008) but an augmented locomotor response to D-1 agonist administration (Lee et al. 2002). Thus, the positive role of PKA in reward-related learning may not be inconsistent with a negative role in unconditioned behavioral responsiveness to DA agonists, particularly when considering that the aforementioned studies blocked PKA signaling in all NAc cell types.

A second potential encumbrance to proposing a negative involvement of PKA in the unconditioned rewarding effect of SKF-82958 is that excitation of D-1 DA receptor-expressing MSNs in NAc is involved in the rewarding effect of cocaine and optogenetic stimulation (Lobo et al. 2010), and the known mechanisms through which D-1 receptor stimulation facilitates the MSN up state and spike firing appear to involve PKA (Surmeier et al. 1995; Snyder et al. 2000; Sun et al. 2005; Hallett et al. 2006). However, it was recently demonstrated that D-1 DA receptor stimulation and cAMP elicit membrane depolarization in NAc neurons, and these effects were not blocked by Rp-cAMPS; they were attributed to direct interaction of cAMP with inwardly rectifying K⁺ channels (Podda et al. 2010). Thus, inhibition of PKA need not prevent D-1 DA receptor agonist-induced activation of MSNs. In addition, previous findings indicate an upregulation of NMDA receptor-dependent intracellular signaling downstream of D-1 DA receptor stimulation in NAc of FR subjects (Haberny and Carr 2005b). Evidence that D-1 and NMDA receptors can form heteromeric complexes (Fiorentini et al. 2003), and the D-1 receptor can modulate NMDA receptor function via direct protein-protein interactions (Lee et al. 2002), and regulate NMDA receptor trafficking in a DARPP-32-independent manner (Dunah et al. 2004), suggest mechanisms through which D-1 receptor stimulation could increase NMDA receptor function independently of PKA.

A third, and perhaps key, consideration when interpreting the present results is that Rp-cAMPS microinjected into NAc not only blocks SKF-82958-induced activation of PKA in D-1-expressing MSNs but also mimics the effect of stimulating D-2 DA receptors which are coupled to G_i and inhibit adenylyl cyclase/PKA signaling (Stoof and Keibian 1982). D-2 DA receptor stimulation decreases inward depolarizing currents via L-type Ca²⁺ channels, increases outward hyperpolarizing K⁺ currents, and decreases both AMPA and NMDA receptor currents (for review see: Gerfen and Surmeier 2011). Several of these effects may be due to inhibition of PKA and the dephosphorylation of NMDA receptor NR1, AMPA receptor GluA1 (Cepeda et al. 1993; Hakansson et al. 2006), and L-type Ca²⁺ channels (Fernandez et al. 2006). Quinpirole and Rp-cAMPS produce similar inhibitory effects on evoked NA⁺ spikes in NAc neurons and the latter occludes the effect of the former (Perez et al. 2006), indicating that D-2 receptor stimulation inhibits NAc cell firing by inhibiting PKA. Moreover, quinpirole increases levels of phospho-DARPP-32 (Thr75) selectively in D-2 DA receptor-expressing MSNs (Bateup et al. 2008). These findings offer an explanation of the present results based on evidence that inhibition of D-2 DA receptor-expressing MSNs promotes the rewarding effect of cocaine (Lobo et al. 2010), and concurrent stimulation of D-1 and D-2 DA receptors yields synergistic effects (Wachtel et al. 1989; Ikemoto et al. 1997; LaHoste et al. 2000). It may be that when assaying an unconditioned response to SKF-82958, the inhibition of PKA in D-2 DA receptor expressing MSNs is functionally dominant and yields synergy. On the other hand, when assaying a response to chronic drug treatment and/or learning reinforced by drug treatment, the inhibition of PKA in D-1 DA receptor expressing MSNs dominates by preventing synaptic plasticity. Under this scheme the lack of response of FR rats to Rp-cAMPS in the present study implies that D-2 DA receptor signaling and consequent inhibition of cAMP and PKA are already maximal and synergize with the effect of administered SKF-82958. This idea seems plausible based on the higher affinity of D-2 than D-1 receptors for DA (Missale et al. 1998), evidence of D-2 DA receptor supersensitivity in FR subjects (Carr et al. 2003; Collins et al. 2008; Baladi and France 2009), and recent evidence that SKF-82958, in addition to being a full and potent agonist at D-1 receptors, also behaves as a weak partial agonist at D-2 receptors (Mannoury La Cour et al. 2007). Together, these factors could enable SKF-82958 and/or physiological DA to exert D-2-mediated effects in FR rats that can be duplicated in AL rats only by administering a PKA inhibitor or D-2 agonist. It was for this latter reason that the effect of quinpirole on the response to SKF-82958 was assessed.

Coadministration of SKF-82958 and quinpirole did not provide strong evidence of synergy or an enhanced effect in AL rats. However, quinpirole did enhance the effect of SKF-82958 on the x-axis intercept measure of reward threshold across diet groups, and inspection of Figure 6 suggests that this was due to an effect in AL rats, as would be predicted. The utility of this test may have been compromised by the inability to disentangle the effect of quinpirole on postsynaptic D-2 receptors from the effect on presynaptic D-2 receptors and DA release. One additional mechanistic possibility bears mention. Adenosine A2A receptors exist in a heteromeric complex with D-2 receptors (Hillion et al. 2002; Fuxe et al. 2003), are coupled to Gs/olf and Gi/o, respectively, and mediate opponent effects on adenylyl cyclase, PKA, and DARPP-32 (Svenningsson et al. 1998; Diaz-Cabiale et al. 2001). Further, the A2A receptor antagonist, caffeine, increases phospho-DARPP-32 (Thr75) specifically in D-2 receptor-expressing cells with no effect on (Thr34) (Bateup et al. 2008; Hsu et al. 2009). This is of interest given the previous finding that FR rats are hypersensitive to A2A-agonist induced behavioral and NAc cellular effects (Cabeza de Vaca et al. 2007). A2A hypersensitivity may be compensation for adenosine/A2A transmission deficiency in FR subjects, which would be expected to upregulate phospho-DARPP-32 (Thr75), inhibit PKA, and synergize with D-1 DA receptor agonist administration to yield an enhanced net behavioral effect relative to AL subjects.

The present study demonstrates that PKA inhibition in NAc shell enhances the rewarding effect of the D-1 DA receptor agonist SKF-82958 in AL but not FR rats. The decreased rewarding effect of SKF-82958 in FR but not AL rats following PKA activation supports the importance of altered PKA signaling in the enhanced rewarding effect of D-1 DA receptor stimulation. Phospho-DARPP-32 (Thr75) may be the endogenous PKA inhibitor that occludes the effect of Rp-cAMPS in FR rats and contributes to the enhanced behavioral responsiveness of FR subjects to SKF-82958. Whether the effect of inhibiting PKA pharmacologically with Rp-cAMPS or physiologically with FR is dependent upon inhibiting PKA activity in D-1-expressing or D-2-expressing MSNs cannot be discerned from the present study. However, these results focus attention on altered PKA function in NAc shell as another component of the neuroadaptive response to FR that contributes to increased drug reward magnitude. Other experimental approaches may help clarify which NAc neurons display PKA-related changes in FR subjects, how those changes relate to other known FR-induced neuroadaptations, and how resultant alterations of neuronal function impact the integrative microcircuitry of NAc shell and the balance of activity between functionally opponent D-1 and D-2 DA receptor-expressing MSNs.

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References

- Baladi MG, France CP. High fat diet and food restriction differentially modify the behavioral effects of quinpirole and raclopride in rats. *Eur J Pharmacol.* 2009; 610:55–60. [PubMed: 19327348]
- Baldwin AE, Sadeghian K, Holahan MR, Kelley AE. Appetitive instrumental learning is impaired by inhibition of cAMP-dependent protein kinase within the nucleus accumbens. *Neurobiol Learn Mem.* 2002; 77:44–62. [PubMed: 11749085]
- Bateup HS, Svenningsson P, Kuroiwa M, Gong S, Nishi A, Heintz N, Greengard P. Cell type-specific regulation of DARPP-32 phosphorylation by psychostimulant and antipsychotic drugs. *Nat Neurosci.* 2008; 11:932–939. [PubMed: 18622401]
- Beninger RJ, Gerdjikov T. The role of signaling molecules in reward-related incentive learning. *Neurotox Res.* 2004; 6:91–104. [PubMed: 15184110]

- Bibb JA, Chen J, Taylor JR, Svenningsson P, Nishi A, Snyder GL, Yan Z, Sagawa ZK, Ouimet CC, Nairn AC, Nestler EJ, Greengard P. Effects of chronic exposure to cocaine are regulated by the neuronal protein Cdk5. *Nature*. 2001; 410:376–380. [PubMed: 11268215]
- Cabeza de Vaca S, Kannan P, Pan Y, Jiang N, Sun Y, Carr KD. The adenosine A2a receptor agonist, CGS-21680, blocks excessive rearing, acquisition of wheel running, and increases nucleus accumbens CREB phosphorylation in chronically food-restricted rats. *Brain Res*. 2007; 1142:100–109. [PubMed: 17292868]
- Carr KD. Food restriction: enhancing effects on drug reward and striatal cell signaling. *Physiol Behav*. 2007; 91:459–472. [PubMed: 17081571]
- Carr KD, Cabeza de Vaca S, Sun Y, Chau LS. Reward-potentiating effects of D-1 dopamine receptor agonist and AMPAR GluR1 antagonist in nucleus accumbens shell and their modulation by food restriction. *Psychopharmacol*. 2009; 202:731–743.
- Carr KD, Chau LS, Cabeza de Vaca S, Gustafson K, Stouffer M, Tukey D, Restituto S, Ziff E. AMPA receptor subunit GluR1 downstream of D-1 dopamine receptor stimulation in nucleus accumbens shell mediates increased drug reward magnitude in food-restricted rats. *Neurosci*. 2010; 165:1074–1086.
- Carr KD, Kim G-Y, Cabeza de Vaca S. Rewarding and locomotor-activating effects of direct dopamine receptor agonists are augmented by chronic food restriction in rats. *Psychopharmacol*. 2001; 154:420–428.
- Carr KD, Tsimberg Y, Berman Y, Yamamoto N. Evidence of increased dopamine receptor signaling in food-restricted rats. *Neurosci*. 2003; 119:1157–1167.
- Cepeda C, Buchwald NA, Levine MS. Neuromodulatory actions of dopamine in the neostriatum are dependent upon the excitatory amino acid receptor subtypes activated. *Proc Natl Acad Sci USA*. 1993; 90:9576–9580. [PubMed: 7692449]
- Cochrane C, Malcolm R, Brewerton T. The role of weight control as a motivation for cocaine abuse. *Addict Behav*. 1998; 23:201–207. [PubMed: 9573424]
- Collins GT, Calinski DM, Newman AH, Grundt P, Woods JH. Food restriction alters N⁷-propyl-4,5,6,7-tetrahydrobenzothiazole-2,6-diamine dihydrochloride (pramipexole)-induced yawning, hypothermia, and locomotor activity in rats: evidence for sensitization of dopamine D2 receptor-mediated effects. *J Pharmacol Exp Ther*. 2008; 325:691–697. [PubMed: 18305018]
- Dalley JW, Laane K, Theobald DEH, Armstrong HC, Corlett PR, Chudasama Y, Robbins TW. Time-limited modulation of appetitive Pavlovian memory by D1 and NMDA receptors in the nucleus accumbens. *Proc Natl Acad Sci*. 2005; 102:6189–6194. [PubMed: 15833811]
- Diaz-Cabiale Z, Hurd Y, Guidolin D, Finnman U-B, Zoli M, Agnati LF, Vanderhaeghen J-J, Fuxe K, Ferre S. Adenosine A_{2A} agonist CGS 21680 decreases the affinity of dopamine D2 receptors for dopamine in human striatum. *Neuroreport*. 2001; 12:1831–1834. [PubMed: 11435907]
- Dunah AW, Sirianni AC, Fienberg AA, Bastia E, Schwarzschild MA, Standaert DG. Dopamine D1-dependent trafficking of striatal N-methyl-D-aspartate glutamate receptors requires Fyn protein tyrosine kinase but not DARPP-32. *Mol Pharmacol*. 2004; 65:121–129. [PubMed: 14722243]
- Fernandez E, Schiappa R, Girault J-A, Novere Le N. DARPP-32 is a robust integrator of dopamine and glutamate signals. *PloS Comp Biol*. 2006; 2:1619–1633.
- Fiorentini C, Gardoni F, Spano P, Di Luca M, Missale C. Regulation of dopamine D1 receptor trafficking and desensitization by oligomerization with glutamate N-methyl-D-aspartate receptors. *J Biol Chem*. 2003; 278:20196–20202. [PubMed: 12646556]
- Fuxe K, Agnati LF, Jacobsen K, Hillion J, Canals M, Torvinen M, Tinner-Staines B, Staines W, Rosin D, Terasmaa A, Popoli P, Leo G, Vergoni V, Lluís C, Ciruela F, Franco R, Ferre S. Receptor heteromerization in adenosine A2A receptor signaling: relevance for striatal function and Parkinson's disease. *Neurol*. 2003; 61:S19–S23.
- Gerdjikov TV, Giles AC, Swain SN, Beninger RJ. Nucleus accumbens PKA inhibition blocks acquisition but enhances expression of amphetamine-produced conditioned activity in rats. *Psychopharmacol*. 2007; 190:65–72.
- Gerfen CR, Surmeier DJ. Modulation of striatal projection systems by dopamine. *Ann Rev Neurosci*. 2011; 34:441–466. [PubMed: 21469956]

- Groenewegen HJ, Wright CI, Beijer AV, Voorn P. Convergence and segregation of ventral striatal inputs and outputs. *Ann NY Acad Sci.* 1999; 877:49–63. [PubMed: 10415642]
- Haberny S, Berman Y, Meller E, Carr KD. Chronic food restriction increases D-1 dopamine receptor agonist-induced ERK1/2 MAP kinase and CREB phosphorylation in caudate-putamen and nucleus accumbens. *Neurosci.* 2004; 125:289–298.
- Haberny SL, Carr KD. Comparison of basal and D-1 dopamine receptor agonist-stimulated neuropeptide gene expression in caudate-putamen and nucleus accumbens of ad libitum fed and food-restricted rats. *Molec Brain Res.* 2005a; 141:121–127. [PubMed: 16257473]
- Haberny SL, Carr KD. Food restriction increases NMDA receptor-mediated CaMK II and NMDA receptor/ERK 1/2-mediated CREB phosphorylation in nucleus accumbens upon D-1 dopamine receptor stimulation in rats. *Neurosci.* 2005b; 132:1035–1043.
- Hakansson K, Galdi S, Hendrick J, Snyder G, Greengard P, Fisone G. Regulation of phosphorylation of the GluR1 AMPA receptor by dopamine D2 receptors. *J Neurochem.* 2006; 96:482–488. [PubMed: 16336634]
- Hallett PJ, Spoelgen R, Hyman BT, Standaert DG, Dunah AW. Dopamine D1 activation potentiates striatal NMDA receptors by tyrosine phosphorylation-dependent subunit trafficking. *J Neurosci.* 2006; 26:4690–4700. [PubMed: 16641250]
- Hillion J, Canals M, Torvinen M, Cadado V, Scott R, Terasmaa A, Hansson A, Watson S, Olah ME, Mallol J, Canela EI, Zoli M, Agnati LF, Ibanez CF, Lluís C, Franco R, Ferre S, Fuxe K. Coaggregation, cointernalization and codesensitization of adenosine A_{2A} receptors and dopamine D₂ receptors. *J Biol Chem.* 2002; 277:18091–18097. [PubMed: 11872740]
- Hyman SE, Malenka RC, Nestler EJ. Neural mechanisms of addiction: the role of reward-related learning and memory. *Ann Rev Neurosci.* 2006; 29:565–598. [PubMed: 16776597]
- Hsu CW, Chen CY, Wang C-S, Chiu TH. Caffeine and a selective adenosine A_{2A} receptor antagonist induce reward and sensitization behavior associated with increased phospho-Thr75-DARPP-32 in mice. *Psychopharmacol.* 2009; 204:313–325.
- Ikemoto S. Dopamine reward circuitry: two projection systems from the ventral midbrain to the nucleus accumbens-olfactory tubercle complex. *Brain Res Rev.* 2007; 56:27–78. [PubMed: 17574681]
- Ikemoto S, Glazier BS, Murphy JM, McBride WJ. Role of dopamine D1 and D2 receptors in the nucleus accumbens in mediating reward. *J Neurosci.* 1997; 17:8580–8587. [PubMed: 9334429]
- Kelley AE. Ventral striatal control of appetitive motivation: role in ingestive behavior and reward-related learning. *Neurosci Biobehav Rev.* 2004; 27:765–776. [PubMed: 15019426]
- Kheirbek MA, Beeler JA, Ishikawa Y, Zhuang X. A cAMP pathway underlying reward prediction in associative learning. *J Neurosci.* 2008; 28:11401–11408. [PubMed: 18971482]
- Klesges RC, Elliott VE, Robinson LA. Chronic dieting and the belief that smoking controls body weight in a biracial, population-based adolescent sample. *Tob Control.* 1997; 6:89–94. [PubMed: 9291216]
- LaHoste GJ, Henry BL, Marshall JF. Dopamine D1 receptors synergize with D2, but not D3 or D4, receptors in the striatum without the involvement of action potentials. *J Neurosci.* 2000; 20:6666–6671. [PubMed: 10964971]
- Lee AM, Messing RO. Protein kinases and addiction. *Ann NY Acad Sci.* 2008; 1141:22–57. [PubMed: 18991950]
- Lee FJ, Xue S, Pei L, Vukusic B, Chery N, Wang Y, Wang YT, Niznik HB, Yu XM, Liu F. Dual regulation of NMDA receptor functions by direct protein-protein interactions with the dopamine D1 receptor. *Cell.* 2002; 111:219–230. [PubMed: 12408866]
- Lobo MK, Covington HE 3rd, Chaudhury D, Friedman AK, Sun H, Dames-Werno D, Dietz DM, Zaman S, Koo JW, Kennedy PJ, Mouzon E, Mogri M, Neve RL, Deisseroth K, Han MH, Nestler EJ. Cell type-specific loss of BDNF signaling mimics optogenetic control of cocaine reward. *Science.* 2010; 330:385–390. [PubMed: 20947769]
- Lynch WH, Taylor JR. Persistent changes in motivation to self-administer cocaine following modulation of cyclic AMP-dependent protein kinase A (PKA) activity in the nucleus accumbens. *Eur J Neurosci.* 2005; 22:1214–1220. [PubMed: 16176364]

- Malenka RC, Bear MF. LTP and LTD: An embarrassment of riches. *Neuron*. 2004; 44:5–21. [PubMed: 15450156]
- Mannoury La Cour C, Vidal S, Pasteau V, Cussac D, Millan MJ. Dopamine D1 receptor coupling to Gs/olf and Gq in rat striatum and cortex: A scintillation proximity assay (SPA)/antibody-capture characterization of benzazepine agonists. *Neuropharmacol*. 2007; 52:1003–1014.
- Miliaressis E, Rompre PP, Laviolette P, Philippe L, Coulombe D. The curve-shift paradigm in self-stimulation. *Physiol Behav*. 1986; 37:85–91. [PubMed: 3016774]
- Missale C, Nash SR, Robinson SW, Jaber M, Caron MG. Dopamine receptors: from structure to function. *Physiol Rev*. 1998; 78:189–225. [PubMed: 9457173]
- O'Donnell P, Grace AA. Physiological and morphological properties of accumbens core and shell neurons recorded in vitro. *Synapse*. 1993; 13:135–160. [PubMed: 8446922]
- Perez MF, White FJ, Hu XT. Dopamine D(2) receptor modulation of K(+) channel activity regulates excitability of nucleus accumbens neurons at different membrane potentials. *J Neurophysiol*. 2006; 96:2217–2228. [PubMed: 16885524]
- Pisetsky EM, Chao YM, Dierker LC, May AM, Striegel-Moore RH. Disordered eating and substance use in high-school students: results from the Youth Risk Behavior Surveillance System. *Int J Eat Disord*. 2008; 41:464–470. [PubMed: 18348283]
- Podda MV, Riccardi E, D'Ascenzo M, Azzena GB, Grassi C. Dopamine D1-like receptor activation depolarizes medium spiny neurons of the mouse nucleus accumbens by inhibiting inwardly rectifying K+ currents through a cAMP-dependent protein kinase A-independent mechanism. *Neurosci*. 2010; 167:678–690.
- Root TL, Pinheiro AP, Thornton L, Strober M, Fernandez-Aranda F, Brandt H, Crawford S, Fichter MM, Halmi KA, Johnson C, Kaplan AS, Klump KL, La Via M, Mitchell J, Woodside DB, Rotondo A, Berrettini WH, Kaye WH, Bulik CM. Substance use disorders in women with anorexia nervosa. *Int J Eat Disord*. 2010; 43:14–21. [PubMed: 19260043]
- Self DW, Genova LM, Hope BT, Barnhart WJ, Spencer JJ, Nestler EJ. Involvement of cAMP-dependent protein kinase in the nucleus accumbens in cocaine self-administration and relapse of cocaine-seeking behavior. *J Neurosci*. 1998; 18:1848–1859. [PubMed: 9465009]
- Shuen JA, Chen M, Gloss B, Calakos N. Drd1a-tdTomato BAC transgenic mice for simultaneous visualization of medium spiny neurons in the direct and indirect pathways of the basal ganglia. *J Neurosci*. 2008; 28:2681–2685. [PubMed: 18337395]
- Snyder GL, Allen PB, Fienberg AA, Valle CG, Huganir RL, Nairn AC, Greengard P. Regulation of phosphorylation of the GluR1 AMPA receptor in the neostriatum by dopamine and psychostimulants in vivo. *J Neurosci*. 2000; 20:4480–4488. [PubMed: 10844017]
- Stamp JA, Mashoodh R, van Kampen JM, Robertson HA. Food restriction enhances peak corticosterone levels, cocaine-induced locomotor activity, and DeltaFosB expression in the nucleus accumbens of the rat. *Brain Res*. 2008; 1204:94–101. [PubMed: 18342839]
- Stoof JC, Kebabian JW. Independent in vitro regulation by the D-2 dopamine receptor of dopamine-stimulated efflux of cyclic AMP and K⁺-stimulated release of acetylcholine from rat neostriatum. *Brain Res*. 1982; 250:263–270. [PubMed: 6293642]
- Sun X, Zhao Y, Wolf ME. Dopamine receptor stimulation modulates AMPA receptor synaptic insertion in prefrontal cortex neurons. *J Neurosci*. 2005; 25:7342–7351. [PubMed: 16093384]
- Surmeier DJ, Bargas J, Hemmings HC Jr, Nairn AC, Greengard P. Modulation of calcium currents by a D1 dopaminergic protein kinase/phosphatase cascade in rat neostriatal neurons. *Neuron*. 1995; 14:385–397. [PubMed: 7531987]
- Svenningsson P, Lindskog M, Rognoni F, Fredholm BB, Greengard P, Fisone G. Activation of adenosine A_{2A} and Dopamine D₁ receptors stimulates cyclic AMP-dependent phosphorylation of DARPP-32 in distinct populations of striatal projection neurons. *Neurosci*. 1998; 84:223–228.
- Svenningsson P, Nishi A, Fisone G, Girault J-A, Nairn AC, Greengard P. DARPP-32: An integrator of neurotransmission. *Annu Rev Pharmacol Toxicol*. 2004; 44:269–296. [PubMed: 14744247]
- Terwilliger RZ, Beitner-Johnson D, Sevarino KA, Crain SM, Nestler EJ. A general role for adaptations in G-proteins and the cyclic AMP system in mediating the chronic actions of morphine and cocaine on neuronal function. *Brain Res*. 1991; 548:100–110. [PubMed: 1651140]

- Unterwald EM, Fillmore J, Kreek MJ. Chronic repeated cocaine administration increases dopamine D1 receptor-mediated signal transduction. *Eur J Pharmacol.* 1996; 318:31–35. [PubMed: 9007509]
- Wachtel SR, Hu XT, Galloway MP, White FJ. D1 dopamine receptor stimulation enables the postsynaptic, but not autoreceptor, effects of D2 dopamine agonists in nigrostriatal and mesoaccumbens dopamine systems. *Synapse.* 1989; 4:327–346. [PubMed: 2532422]
- Walaas SI, Hemmings HC Jr, Greengard P, Nairn AC. Beyond the dopamine receptor: regulation and roles of serine/threonine protein phosphatases. *Front Neuroanat.* 2011; 5:1–17. [PubMed: 21373368]
- Wang JQ, Liu X, Zhang G, Parelkar NK, Arora A, Haines M, Fibuch EE, Mao L. Phosphorylation of glutamate receptors: A potential mechanism for the regulation of receptor function and psychostimulant action. *J Neurosci Res.* 2006; 84:1621–1629. [PubMed: 16983660]
- Wiederman MW, Pryor T. Substance abuse and impulsive behaviors among adolescents with eating disorders. *Addict Behav.* 1996; 21:269–272. [PubMed: 8730530]
- Wise RA. Addictive drugs and brain stimulation reward. *Ann Rev Neurosci.* 1996; 19:319–340. [PubMed: 8833446]

Figure 1.

Mean ($\pm$ SEM) percentage changes in the reward threshold following microinjection of SKF-82958 (upper panel: 1.5 μ g per side; lower panel: 3.0 μ g per side) preceded by vehicle (clear bars) or Rp-cAMPS (8.9 μ g per side; hatched bars) in NAc medial shell of *ad libitum* fed (left) and food-restricted (right) rats. *Effect of Rp-cAMPS/SKF-82958 was greater than vehicle/SKF-82958 in *ad libitum* fed rats, $p < .001$. 1.5 μ g dose of SKF-82958: $n_{AL}=14$, $n_{FR}=13$; 3.0 μ g dose: $n_{AL}=11$, $n_{FR}=13$.

Figure 2.

ICSS rate-frequency curves of *ad libitum* fed ($n=11$; top) and food-restricted ($n=13$; bottom) rats (filled circles), and post-injection curves obtained following NAc shell microinjection of SKF-82958 ($3.0 \mu\text{g}$) preceded by vehicle (filled triangles) or Rp-cAMPS ($8.9 \mu\text{g}$; filled squares.) The reward-potentiating effect of SKF-82958, indicated by the leftward shift of rate-frequency curves, was enhanced by Rp-cAMPS in *ad libitum* fed but not food-restricted rats. Points indicated by symbols correspond to mean theta-0 (x-axis intercept), M-50 (frequency supporting 50% of maximum reinforcement rate) and maximum/asymptotic reinforcement rate. Post-injection curves obtained following microinjection of vehicle preceded by vehicle do not differ from pre-injection curves and are omitted from this figure.

Figure 3.

Mean ($\pm$ SEM) percentage changes in reward threshold following microinjection of vehicle preceded by vehicle (clear bars) or Rp-cAMPS (8.9 μ g; hatched bars), bilaterally, in NAc medial shell of *ad libitum* fed (left) and food-restricted (right) rats. $n_{AL}=10$, $n_{FR}=13$.

Figure 4.

Mean ($\pm$ SEM) percentage changes in reward threshold following microinjection of SKF-82958 preceded by vehicle (clear bars) or Sp-cAMPS (8.9 μ g; hatched bars), bilaterally, in NAc medial shell of *ad libitum* fed (left) and food-restricted (right) rats. To achieve similar baseline effects of SKF-82958, *ad libitum* fed rats were microinjected with 3.0 μ g and food-restricted rats were microinjected with 1.5 μ g bilaterally. *Effect of SKF-82958 was decreased by Sp-cAMPS in food-restricted rats, $p < .005$. $n_{AL}=9$, $n_{FR}=9$.

Figure 5.

Effect of chronic food restriction on levels of phosphorylated DARPP-32 in NAc. Lysates were immunoblotted with anti-phospho-DARPP-32 (Thr75; left pair of bars), anti-phospho-DARPP-32 (Thr34; right pair of bars), and anti- α -tubulin antibodies. Following densitometry, intensities of bands corresponding to phospho-DARPP-32 for each subject were divided by the intensities of the corresponding α -tubulin bands to correct for small differences in protein loading. Results (mean $\pm$ SEM) are expressed in comparison to the normalized control, which was defined as the *ad libitum* fed group. Representative blots are included beneath corresponding bars. *Food-restricted (hatched bar) greater than *ad libitum* fed (clear bar), $p < .05$.

Figure 6.

Mean ($\pm$ SEM) percentage changes in M-50 (upper panel) and x-axis intercept (lower panel) measures of reward threshold following microinjection of SKF-82958 alone (SKF; 1.5 μ g), in combination with quinpirole 0.5 μ g (SKF/Q-0.5), and in combination with quinpirole 1.0 μ g (SKF/Q-1.0) bilaterally in NAc medial shell of *ad libitum* fed (clear bars) and food-restricted (hatched bars) rats. *Effect of SKF/Q-1.0 was greater than SKF across diet conditions, $p < .01$. $n_{AL}=7$, $n_{FR}=8$.