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AMPA Receptor Subunit GluR1 Downstream of D-1 Dopamine Receptor Stimulation in Nucleus Accumbens Shell Mediates Increased Drug Reward Magnitude in Food-Restricted Rats

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Abstract

Previous findings suggest that neuroadaptations downstream of D-1 dopamine (DA) receptor stimulation in nucleus accumbens (NAc) are involved in the enhancement of drug reward by chronic food restriction (FR). Given the high co-expression of D-1 and GluR1 AMPA receptors in NAc, and the regulation of GluR1 channel conductance and trafficking by D-1-linked intracellular signaling cascades, the present study examined effects of the D-1 agonist, SKF-82958, on NAc GluR1 phosphorylation, intracranial electrical self-stimulation reward (ICSS), and reversibility of reward effects by a polyamine GluR1 antagonist, 1-NA-spermine, in *ad libitum* fed (AL) and FR rats. Systemically administered SKF-82958, or brief ingestion of a 10% sucrose solution, increased NAc GluR1 phosphorylation on Ser845, but not Ser831, with a greater effect in FR than AL rats. Microinjection of SKF-82958 in NAc shell produced a reward-potentiating effect that was greater in FR than AL rats, and was reversed by co-injection of 1-NA-spermine. GluR1 abundance in whole cell and synaptosomal fractions of NAc did not differ between feeding groups, and microinjection of AMPA, while affecting ICSS, did not exert greater effects in FR than AL rats. These results suggest a role of NAc GluR1 in the reward-potentiating effect of D-1 DA receptor stimulation and its enhancement by FR. Moreover, GluR1 involvement appears to occur downstream of D-1 DA receptor stimulation rather than reflecting a basal increase in GluR1 expression or function. Based on evidence that phosphorylation of GluR1 on Ser845 primes synaptic strengthening, the present results may reflect a mechanism via which FR normally facilitates reward-related learning to re-align instrumental behavior with environmental contingencies under the pressure of negative energy balance.

Keywords

SKF-82958; 1-NA-spermine; addiction; synaptic plasticity

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Regulation of drug rewarding effects by diet composition, energy balance, and body weight have become increasingly well substantiated, beginning with early demonstrations of enhancement by food restriction (Carroll et al., 1979; Carroll and Meisch, 1984) and more recent reports of inhibition by maintenance on high energy diets (Wellman et al., 2007; Davis et al., 2008). These effects are not surprising given the common involvement of ventral tegmental dopamine (DA) neurons and nucleus accumbens (NAc) microcircuitry in the incentive motivational and reward-related learning processes that underlie food- and drug-directed behavior (e.g., Kelley, 2004; Volkow and Wise, 2005; Hyman et al., 2006; Fields et al., 2007).

The enhancement of acute drug rewarding effects by chronic food restriction (FR) has been conceptualized as arising from neuroadaptations that otherwise facilitate foraging, procurement, and reward-related learning in the service of restoring energy balance and body weight (Carr, 2007). Indeed, a variety of changes in presynaptic DA dynamics (Pothos et al., 1995; Cadoni et al., 2003; Pan et al., 2006; Zhen et al., 2006) and postsynaptic intracellular signaling and transcriptional responses to DA receptor agonist administration (Carr et al., 2003; Haberny et al., 2004; Haberny and Carr, 2005a,b) are consistent with this hypothesis. One coherent set of reported changes consists of increased behavioral, intracellular signaling, and transcriptional responses to D-1 DA receptor stimulation in NAc.

Considering that the DA innervation of NAc is convergent with several major limbic forebrain glutamate inputs (Groenewegen et al., 1999; Kalivas et al., 2005), and the integration of DA- and glutamate-coded signals is involved in the regulation of medium spiny neuronal activity (e.g., Moyer et al., 2007; Surmeier et al., 2007), goal-directed behavior, reward-related learning, and addiction (Kelley, 2004; Malenka et al., 2004; Dalley et al., 2005; Hyman et al., 2006), the ability of D-1 receptor stimulation to regulate the phosphorylation state of specific NMDA and AMPA receptor subunits (Wang et al., 2006) is of general functional importance and may be of particular importance in understanding the mechanisms via which FR modulates behavioral responses to food and abused drugs.

Among the glutamate receptor types co-expressed with DA receptors in striatal neurons (Bernard et al., 1997; Glass et al., 2008), AMPA receptors mediate fast excitatory synaptic transmission (Hollman and Heinemann, 1994; Barry and Ziff, 2002). AMPA receptors are tetrameric and composed of combinations of four subunits, GluR1–4. GluR1 subunit-containing AMPA receptors undergo activity-dependent trafficking, and GluR1 homomers are Ca^{2+} permeable. Generally, insertion and removal of AMPA receptors from the neuronal membrane underlie changes in synaptic strength (Shi et al., 2001; Barry and Ziff, 2002; Derkach et al., 2007). Phosphorylation of GluR1 on Ser845 by D-1 receptor-regulated cAMP and NMDA receptor-regulated cGMP pathways enhances AMPA currents and facilitates rapid insertion into the postsynapse (Roche et al., 1996; Snyder et al., 2000; Banke et al., 2000; Man et al., 2007; Serulle et al., 2007). It is therefore of interest that *in vivo* administration of D-1 DA receptor agonists, cocaine, methamphetamine, and ingestion of sugar lead to a rapid increase in striatal GluR1 phosphorylation on Ser845 that is D-1 DA receptor-dependent (Rauggi et al., 2005; Snyder et al., 2000; Valjent et al., 2005).

AMPA currents are also increased by CaMKII- and PKC-mediated phosphorylation of GluR1 on Ser831 (Derkach et al., 1999), although D-1 agonists, psychostimulants, and sugar do not normally lead to phosphorylation of GluR1 on this serine residue. However, the increased phosphorylation of the NMDA receptor NR1 subunit in NAc of FR rats following D-1 DA receptor stimulation, and the consequent NMDA receptor-dependent activation of CaMKII (Haberny and Carr, 2005a), raise the possibility of GluR1 phosphorylation on Ser831, selectively in FR subjects.

The purpose of the present study was to determine whether *in vivo* administration of a D-1 DA receptor agonist leads to greater phosphorylation of GluR1 on Ser845 and/or Ser831 in NAc of FR relative to ad libitum fed (AL) subjects, and whether NAc microinjection of a polyamine GluR1 homomer antagonist attenuates the reward-potentiating effect of a D-1 DA receptor agonist and diminishes the difference otherwise observed between AL and FR subjects. Thus, Experiment 1 examines whether systemically administered SKF-82958, at a dose verified to produce a greater behavioral effect in FR relative to AL rats, increases phosphorylation of GluR1 on Ser845 and Ser831, with a difference between feeding groups. Experiment 2 examines the effect of sucrose ingestion on these measures. In Experiment 3, acute reward-potentiating effects of SKF-82958 microinjected into NAc are compared between AL and FR rats in an intracranial electrical self-stimulation (ICSS) paradigm, and the involvement of GluR1 is assessed by co-infusion of a polyamine GluR1 antagonist, 1-NA spermine. To ascertain the specificity of 1-NA spermine effects to D-1 agonist-induced behavioral responses, acute reward-potentiating effects of the D-2/3 agonist, quinpirole, with and without co-infused 1-NA spermine, are also determined. To assess whether an apparent D-1 DA receptor stimulation-dependent involvement of GluR1 in the enhanced reward-potentiating effect may instead be due to a D-1 receptor-independent increase in GluR1 function, Experiment 4 examines GluR1 abundance in the synaptosomal fraction of NAc tissue samples and the reward-potentiating effect of AMPA microinjection in NAc.

EXPERIMENTAL PROCEDURES

Subjects and surgical procedures

All subjects were male Sprague–Dawley rats (Taconic Farms, Germantown, NY, USA) weighing 350–400 g at the time of arrival in the central animal facility where they were housed in individual plastic cages, with free access to Purina rat chow (St. Louis, MO, USA) and water unless otherwise noted. The animal room was maintained on a 12-h light/dark cycle, with lights on at 0700 h. Approximately half the subjects in each experiment were placed on a chronic food restriction regimen whereby daily food allotment was limited to 10 g of chow, delivered at 1700 h, until a 20% decrease in body weight was attained. The remaining subjects continued to have ad libitum access to chow. In the biochemical experiments, in which no surgical preparation of subjects was required, food restriction was implemented 3–5 days after arrival of rats in the animal facility. In the behavioral experiments, in which rats were implanted with chronically indwelling stimulating electrodes and microinjection cannulas, food restriction was implemented 10–15 days following surgery. In all experiments, the target body weight of food-restricted rats, which took 15–20 days to achieve, was maintained by titrating the daily food allotment.

In preparation for the behavioral experiments, each rat was anesthetized with ketamine (100 mg/kg, i.p.) and xylazine (10 mg/kg, i.p.) and 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 cannulas (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, cannulas, and three additional mounting screws were then permanently secured to the skull by flowing dental acrylic around them.

All experimental procedures were approved by the New York University School of Medicine Institutional Animal Care and Use Committee and were performed in accordance with the “Principles of Laboratory Animal Care” (NIH publication number 85–23). All efforts were

made to minimize animal suffering, to reduce the number of animals used and to utilize alternatives to *in vivo* techniques.

Tissue preparation and Western blotting

Whole cell homogenates—Brains were rapidly frozen in powdered dry ice, 500- μ m sections were cut using an IEC Minotome cryostat, and NAc and CPu were micropunched, under an Olympus dissecting microscope, from a series of six consecutive frozen sections. The tissue was then sonicated in 2X Laemmli sample buffer (10 sec, on ice), heated for 5 min at 95°C, centrifuged (14,000g, 5 min) and the supernatant stored at –80°C until use.

Synaptosomal fraction—NAc was dissected from fresh brain on ice. NAc of two rats per treatment condition were pooled for fractionation. Protease inhibitor and PMSF were added to 0.32 M sucrose solution containing 1mM NaHCO₃, 1mM MgCl₂ and 0.5 mM CaCl₂ (Solution A). Brain tissue was rinsed, homogenized and subsequently diluted to 10% weight/volume in Solution A. The homogenate was centrifuged at 1,400g for 10 minutes, after which intact cells and nuclei formed a pellet at the bottom of the tube. The supernatant was saved and the pellet resuspended in Solution A. The homogenate was again centrifuged at 1,400g for 10 minutes. The supernatant was collected and combined with the previously collected supernatant. They were centrifuged together at 710g for 10 minutes and then at 13,800g for 20 minutes. The pellet was collected and homogenized in 0.32M sucrose solution containing 1mM of NaHCO₃ (Solution B). This homogenate was placed on a sucrose gradient and centrifuged for 2 hours at 82,500g. The synaptosomal layer was collected from the interface of the 1M and 1.2M sucrose layers. The sample was then resuspended in Solution B and centrifuged at 82,500g for 45 minutes. After centrifugation, the upper liquid was discarded and the pellet resuspended in a solution of 25 mM Tris pH 7.4 with 2% SDS and stored at –80 C until use.

Western blotting—Proteins were separated by electrophoresis on precast 10% polyacrylamide gels (Lonza, Rockland, ME). Dual-colored protein standard molecular weight markers (Bio-Rad, Hercules, CA) were loaded to assure complete electrophoretic transfer and estimate the size of bands of interest. Proteins were electrophoretically transferred to nitrocellulose membranes, and blots cut at the 70kDa mark and incubated in blocking buffer (3% bovine serum albumin in Tris-buffered saline with 0.05% Tween-20 (TBST) when detecting phosphoproteins and 5% nonfat dry milk/TBST for all other proteins) for 1 hour with shaking at room temperature. The upper halves of the blots were incubated with phospho-specific primary antibodies by shaking overnight at 4°C (in 3% bovine serum albumin/TBST). The lower halves of the blots were left in blocking buffer overnight at 4°C and incubated with anti-tubulin antibody the next day for 1 hour with shaking at room temperature (in 5% milk/TBST). Blots were washed 3×5 min in TBST, incubated with the appropriate secondary antibody for 1 hour at room temperature, washed 3×5 min in TBST, then treated with West Pico enhanced chemiluminescence (ECL) reagent (Pierce) and exposed to film. The upper halves of the blots were then stripped of antibodies by incubation for 15 minutes at room temperature in Restore Western Blot Stripping Buffer (Pierce), re-blocked, washed, and probed for 1–3 hours at room temperature with the corresponding antibody that recognized total antigen protein. Blots were washed, incubated with the appropriate secondary antibody, washed again, and treated with ECL reagent and exposed to film to visualize bands of interest. Antibodies used included rabbit anti-GluR1 (1:5000; Upstate), anti-phospho-Ser831-GluR1 (1:1000; Upstate), and anti-phospho-Ser845 (1:1000; Upstate).

Data analysis—For each blot, relative protein levels were calculated from the ratio of optical density of the GluR1s to tubulin (1:40,000; Sigma Aldrich) to correct for small differences in protein loading. Immunoblots were analyzed using NIH Image J software. Differences between feeding groups were analyzed by 2-way ANOVA followed by protected t-tests where

appropriate. To assess the substantive importance of some effects, Cohen's d was used to estimate effect size.

Intracranial self-stimulation and intracerebral microinjection

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 post-surgical 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, for each rat, the lowest intensity that maintained vigorous lever pressing. On subsequent days, rats were trained in a discrete-trials procedure. Each training session consisted 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. Discrete trials training was followed by rate–frequency training, which continued for approximately 2 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 such series were presented in each training session. During the second week of training, subjects 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 described above. Training and mock microinjection test sessions (see below) continued, at least twice per week, for all rats, during the ensuing ~3-week period during which the FR group achieved and then stabilized at the target body weight.

Intracerebral microinjection procedure—Solutions were loaded into two 30 cm lengths of PE-50 tubing attached at one end to 25- μ l Hamilton syringes filled with distilled water and at the other end to 31-gauge injector cannulas, which extended 2.0 mm beyond the implanted guides. The syringes were mounted on the twin holders of a Harvard 2272 microliter syringe pump which delivered the 0.5 μ l injection volumes over a period of 100 s. One minute following completion of injections, injector cannulas were removed from guides, stylets were replaced, and animals were returned to test chambers where the post-injection test began 5-min after completion of the microinjections.

Experimental 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.

Data analysis—For each test, the rate–frequency function was used to derive three parameters. The maximum reinforcement rate, described by a line that parallels the x-axis, was defined as the mean of all consecutive values within 10% of the highest rate for the curve. All remaining values comprised the descending portion of the curve, with the lowest point being at the highest frequency to produce fewer than 2.5 reinforcements per minute. Regression analysis of the descending portion of the curve 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. The M-50 is a conventional threshold measure in this protocol, though the theta-0 indicates the lowest frequency at which stimulation becomes rewarding. Changes in the reinforcing efficacy of stimulation produced by drugs of abuse are typically reflected as parallel leftward shifts in the rate–frequency curve with similar effects on the M-50 and theta-0 measures (Wise 1996). For each ICSS parameter, results were analyzed by 2-way ANOVA with repeated measures on one factor, followed by protected t-tests where appropriate. In some cases, estimates of effect size were calculated using Cohen's *d*.

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 µm 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.

Experiment 1

AL and FR rats (*n*=5 per group) were briefly removed from home cages and injected with either SKF-82958 (1.0 mg/kg, i.p.) or vehicle (5% DMSO in 0.9% saline). Fifteen minutes later, subjects were briefly exposed to CO₂ and decapitated by guillotine. The dose of SKF-82958 administered was chosen based on confirmed differential behavioral effects in a separate set of AL and FR rats; e.g., this dose produced a markedly greater locomotor-activating effect in FR relative to AL rats (post-injection minus pre-injection beam interruptions/30 min: FR: 9,317 ± 789 *vs* AL: 4,740 ± 677, *t*(5) = 3.32, *p* < .025). The 15-min time-point was chosen for sacrifice because it is within the time frame of behavioral measurement in the experiments of the present study (see below) and GluR1 phosphorylation in response to a systemically administered psychostimulant has been reported to dissipate by 30-min post-injection (Valjent et al., 2005).

Experiment 2

AL and FR rats (*n*=6–7 per group) were placed in novel cages on 4 occasions, spaced 2–3 days apart, and after 30 min acclimation, drinking tubes containing either 10% sucrose solution (one FR group, one AL group) or tap water (one FR group, one AL group) were inserted through wire cage tops. The period of fluid access was decreased from one occasion to the next, beginning with 30-min on the first occasion, to establish the access periods that would produce an equal average intake of 12 ml sucrose solution in AL and FR subjects. Consequently, on the terminal day of the experiment, AL subjects had access to fluid (sucrose or water) for 8-min prior to sacrifice and FR subjects had access for 5-min prior to sacrifice.

Experiment 3

To achieve adequate sample sizes, Experiment 3 was conducted using two sets of AL and FR subjects which underwent identical ICSS training and testing but were separated in time by ~ 2 months. Data from subjects whose cannulas became occluded in the course of the experiment,

or whose histologically localized cannula placements did not satisfy anatomical criteria (see above) were excluded from analysis, yielding a total of 13 AL and 9 FR subjects.

Over a 3 week period, subjects underwent 6 test sessions conducted 3–4 days apart. Treatments included bilateral microinjection of SKF-82958 (3.0 µg; Sigma-Aldrich, St. Louis, MO) in solution with 1-naphthylacetyl spermine (Sigma-Aldrich) at doses of 2.5 and 25.0 µg on separate occasions in counterbalanced order. These two tests were flanked by tests of SKF-82958 (3.0 µg) infused alone, with the series of drug test sessions preceded and followed by mock injection and vehicle-only (5% DMSO in sterile 0.9% saline) test sessions.

Three to five days following completion of testing in the first set of animals, a follow-up test was conducted to assess the effect of 1-naphthylacetyl spermine (25.0 µg) microinjected alone. Five days following completion of testing in the second set of animals, a follow-up test was conducted on two occasions to compare the effect of quinpirole (3.0 µg; Sigma-Aldrich) with and without 1-naphthylacetyl spermine (25.0 µg) in the microinjected solution.

The single dose of SKF-82958 administered in this study was chosen on the basis of a prior intra-NAc dose-response experiment (Carr et al., 2009a), and the doses of 1-naphthylacetyl spermine were chosen on the basis of efficacy in decreasing cue-induced cocaine-seeking when microinjected into NAc (Conrad et al., 2008).

Experiment 4

To examine the effect of FR on GluR1 abundance in NAc synaptosomes, 10 AL and 10 FR subjects were habituated to transport to the laboratory from animal facility on five occasions. On the terminal day, subjects were sacrificed to obtain bilateral NAc tissue samples.

To assess the behavioral response to AMPA microinjection in NAc shell, 8 AL and 7 FR subjects were trained and tested in a manner similar to Experiment 3. Based on pilot testing in a separate group of subjects, it was determined that bilateral microinjection of (S)-AMPA (Sigma-Aldrich) at doses of 0.02 and 0.1 µg were below threshold for producing overt signs of convulsant activity while a 0.2 µg dose was suprathreshold in some animals. Consequently, the two lower doses were administered in Experiment 4. These doses are within the range or below those microinjected into NAc core to reinstate cocaine-seeking following extinction training (Cornish et al., 1999; Cornish and Kalivas, 2000; Suto et al., 2004).

RESULTS

Experiments 1 and 2: GluR1 phosphorylation under basal and stimulated conditions in AL and FR rats

Following vehicle administration, NAc tissue samples obtained from AL and FR rats did not differ in abundance of total GluR1 protein, phospho-Ser845 (Figure 1), or phospho-Ser831 GluR1 (Figure 2). Systemic administration of the D-1 DA receptor agonist, SKF-82958 (1.0 mg/kg, i.p.), increased GluR1 phosphorylation on Ser845 in NAc ($F_{1,16} = 24.4$, $p < .001$). Feeding groups differed ($F_{1,16} = 7.1$, $p < .02$), and an interaction between drug treatment and feeding condition ($F_{1,16} = 4.8$, $p < .05$), followed by Fisher's protected t-test (LSD), indicated that SKF-82958 treatment led to greater phosphorylation in FR than AL subjects ($p < .01$; Figure 1-A). A similar effect was observed in caudate-putamen (CPu); SKF-82958 administration led to increased phosphorylation on Ser845 ($F_{1,16} = 5.0$, $p < .05$) and feeding groups differed ($F_{1,16} = 45.1$, $p < .001$). Although the interaction between drug treatment and feeding condition for CPu was not significant ($F_{1,16} = 2.34$, $p = .14$) the main effect of FR was due in large part to the differential effect of SKF-82958 in the two feeding groups in as much as the effect size was $d = 1.31$, while for vehicle treatment it was $d = 0.56$ (Figure 1-B). No differences were seen in phosphorylation of GluR1 on Ser831 (Figure 2).

Based on results of Experiment 1 it was predicted that sucrose, which releases DA in NAc (Hajnal et al., 2004), would similarly increase phospho-Ser845 GluR1 with a greater effect in FR than AL rats. To test this prediction, the pooled error term was obtained from the two-way ANOVA and used in the denominator of one-tailed t-tests to conduct planned comparisons. Accordingly, phosphorylation on Ser845 following sucrose intake in FR rats was greater than following water intake ($t(21)=2.51$, one-tailed, $p<.01$) and greater than following sucrose intake in AL rats ($t(21)=2.72$, one-tailed $p<.01$; Figure 3-A). Sucrose intake also increased phosphorylation of GluR1 on Ser845 in CPu ($F_{1,21} = 6.91$, $p=.016$) but without difference between feeding groups (Figure 3-B). No differences were seen in phosphorylation of GluR1 on Ser831 (Figure 4).

Experiment 3: Reward-potentiating effects of DA receptor agonists microinjected into NAc alone and in combination with a polyamine GluR1 antagonist in AL and FR rats

Previously, it was observed that microinjection of SKF-82958 in sites dorsal to NAc shell had little effect on ICSS, and microinjection in NAc core, which lowered the ICSS threshold, produced only a marginally greater effect in FR than AL rats (Carr et al., 2009a). Consequently, in order for a rat's behavioral data to be included in the present analysis, cannula placements were required to be judged as bilaterally accurate within the NAc medial shell (with several placements on the shell/core and shell/olfactory tubercle borders included).

Microinjection of SKF-82958 lowered the M-50 ($F_{1,20}=48.2$, $p<.001$) and theta-0 ($F_{1,20}=54.5$, $p<.001$) measures of reward threshold, and in both cases FR subjects differed from AL subjects (M-50: $F_{1,20}=11.3$, $p<.01$; theta-0: $F_{1,20}=5.9$, $p<.025$). Planned comparisons based on prior results (Carr et al., 2009a), using the pooled error term from the ANOVA in the denominator of a t-statistic, indicated that SKF-82958 had a greater threshold-lowering effect on the M-50 measure ($t(20)=2.87$, $p<.005$, one-tailed) and theta-0 measure ($t(20)=3.13$, $p<.005$, one-tailed) in FR relative to AL rats, while vehicle treatment did not ($t(20)=1.3$, $t(20)=0.9$, respectively; Figure 5-A,C; Figure 6-A,B). No differences were seen in maximum reinforcement rates (Figure 6-C).

Co-infusion of 1-NA spermine decreased the threshold-lowering effect of SKF-82958 on both the M-50 ($F_{2,40}=10.8$, $p<.001$) and theta-0 ($F_{2,40}=5.16$, $p<.01$) measures. For the M-50 measure, interaction between drug treatment and feeding condition ($F_{2,40}=3.28$, $p<.05$), followed by Fisher's protected t-tests, indicated that both doses of 1-NA spermine decreased the response to SKF-82958 in FR rats ($p<.01$; Figure 5-B,D; Figure 6-A) while neither significantly affected the response to SKF-82958 in AL rats ($p>.05$). For the theta-0 measure, Fisher's protected t-tests indicated that the 25.0 μg dose of 1-NA-spermine decreased the effect of SKF-82958 across feeding conditions ($p<.01$), though the effect size was substantially greater in FR ($d=1.94$) than in AL rats ($d=0.32$; Figure 6-B).

Microinjection of quinpirole had no effect on the M-50 measure of reward threshold ($F_{1,12}=2.0$, $p>.05$; Figure 7-A) but did lower the theta-0 measure ($F_{1,12}=10.0$, $p<.01$), with a nearly significant difference between feeding groups ($F_{1,12}=4.53$, $p=0.05$; Figure 7-B). Quinpirole tended to lower the maximum reinforcement rate in both feeding groups, but the effect was not significant ($F_{1,12}=3.8$, $p>.05$; Figure 7-C). Co-infusion of 1-NA spermine had no effect on the theta-0-lowering effect of quinpirole ($F_{1,12}=0.25$). However, 1-NA spermine increased the tendency of quinpirole to lower maximum reinforcement rate, yielding a significant net decreasing effect across feeding conditions ($F_{1,12}=9.95$, $p<.01$).

In a subset of AL and FR subjects microinjected with 25.0 μg 1-NA spermine after completion of the SKF-82958 sequence, no changes were observed in any ICSS parameter (Table 1). This differs from a prior experiment in which a 10-fold lower dose of 1-NA spermine had no effect

on M-50 or maximum reinforcement rates but did produce a modest decrease in theta-0 reward threshold in FR but not AL rats (Carr et al., 2009a).

Experiment 4: GluR1 in NAc synaptosomal fraction and reward-potentiating effect of NAc microinjection of AMPA in AL and FR rats

Under basal conditions, the whole cell and synaptosomal fraction obtained from NAc tissue samples of AL and FR rats did not differ in GluR1 protein abundance (Figure 8). Microinjection of AMPA significantly lowered the M-50 measure of reward threshold ($F_{2,28} = 8.5$, $p < .01$; Figure 9-A), with a nearly significant greater effect in AL than FR subjects ($F_{1,14} = 4.4$, $p = .054$). Fisher's protected t-tests indicated that only the 0.1 μ g dose of AMPA decreased threshold ($p < .01$). AMPA also lowered the theta-0 measure of reward threshold ($F_{2,28} = 18.5$, $p < .001$; Figure 9-B) with no difference between feeding groups ($F_{1,14} = 1.9$, $p > .05$). Fisher's protected t-tests indicated that both the 0.02 and 0.1 μ g doses lowered threshold ($p < .01$). AMPA also decreased the maximum reinforcement rate ($F_{2,28} = 4.3$, $p < .025$; Figure 9-C), with no difference between feeding groups ($F_{2,28} = 2.65$, $p > .10$), and this effect occurred in response to the 0.1 μ g dose ($p < .025$).

DISCUSSION

The enhanced rewarding effects of abused drugs in FR animals have been demonstrated in self-administration, conditioned place preference, and ICSS paradigms (Carroll and Meisch, 1984; Bell et al., 1997; Cabeza de Vaca and Carr, 1998; Carr et al., 2000; Cabeza de Vaca et al., 2004). The mechanistic importance of NAc neuroadaptations downstream of D-1 DA receptor stimulation in these behavioral effects is suggested by findings that FR enhances SKF-82958-induced activation of several intracellular signaling cascades and transcriptional responses, and microinjection of SKF-82958 in NAc medial shell duplicates the differential reward-potentiating and locomotor-activating effects of systemically administered psychostimulants (and other abused drugs) in AL and FR rats (Carr et al., 2009a). However, the cellular mechanism(s) via which D-1 receptor stimulation in NAc leads to acute rewarding effects that are greater in FR than AL rats is currently unknown. For example, although upregulated ERK 1/2 MAP kinase signaling has been implicated in the enhanced activation of CREB and c-fos in NAc of FR rats following D-1 DA receptor stimulation, its involvement in the enhanced reward-potentiating and locomotor-activating effects of d-amphetamine and SKF-82958 have not been supported (Carr et al., 2009b).

In light of the high coexpression of D-1 DA receptors and GluR1 in NAc shell (Glass et al., 2008), plus the rapid effects of D-1 receptor stimulation on GluR1 phosphorylation (Snyder et al., 2000), and the role of GluR1 phosphorylation in regulating neuronal excitability (Derkach et al., 2007), the initial purpose of the present study was to test the hypothesis that D-1 DA receptor stimulation leads to increased phosphorylation of NAc GluR1 on two serine residues implicated in regulation of channel conductance and surface expression. These include Ser845, which is targeted by D-1 DA receptor/cAMP-dependent protein kinase A signaling, and Ser831, targeted by NMDA receptor-dependent CaMKII signaling, which has been shown to follow D-1 DA receptor stimulation in FR rats (Haberny and Carr, 2005b).

In Experiment 1 it was observed that systemic administration of SKF-82958, at a behaviorally significant dose, increased phosphorylation of GluR1 on Ser845, but not Ser831, with a greater effect in NAc of FR relative to AL rats. This result is consistent with the hypothesized involvement of GluR1 in downstream behavioral effects of D-1 DA receptor stimulation. It seems particularly significant that in Experiment 2 brief intake of sucrose solution also increased phosphorylation of GluR1 on Ser845, but not Ser831, in FR but not AL rats. This correspondence between drug and sucrose effects is compatible with the schema in which

enhanced drug responsiveness is viewed as a consequence of neuroadaptations that normally facilitate ingestive behavior and the restoration of energy balance.

It had been expected that in FR subjects SKF-82958 might also increase phosphorylation on Ser831. This expectation was based on the prior finding that SKF-82958 increased an NMDA receptor-dependent phosphorylation of CaMKII in the NAc of FR but not AL rats. However, there is no indication that a behaviorally significant dose of SKF-82958 administered in the present study increased phosphorylation of GluR1 on Ser831; nor did sucrose – a natural releaser of DA which would be expected to preferentially stimulate D-1 DA receptors in so far as tonic DA release stimulates D-2 receptors but stimulus-induced (i.e., phasic) DA release appears necessary to stimulate the relatively low affinity D-1 receptor population (Goto and Grace, 2005). Moreover, in FR subjects, sucrose, even after it has become a familiar reward, preferentially increases extracellular DA concentrations in NAc shell relative to core (Bassareo and Di Chiara, 1999). This differs from the AL subject, in which the DA response transitions from shell to core as the palatable tastant becomes familiar. Consequently, microinjection of SKF-82958 and ingestion of sucrose may, to some extent, be interchangeable stimuli at the level of NAc shell in FR subjects. While it is possible that increased phosphorylation of GluR1 in NAc following sucrose intake by FR rats may reflect increased DA release, the similar effects of sucrose and a direct D-1 DA receptor agonist, along with the prior evidence of upregulated D-1 DA receptor-dependent cell signaling, suggest that important neuroadaptations are to be found in the D-1 DA receptor-expressing postsynaptic cells. Thus, the signaling pathways that lead to phosphorylation on Ser845, namely cAMP-dependent protein kinases downstream of D-1 DA receptor stimulation (Roche et al., 1996), and cGMP-dependent protein kinases downstream of NMDA receptor and nitric oxide stimulation (Serulle et al., 2007), merit investigation in the continuing effort to elucidate functionally important striatal neuroadaptations that develop as a result of chronic FR.

In Experiment 3 the previously reported enhancement of the reward-potentiating effect of SKF-82958 in NAc shell of FR relative to AL rats (Carr et al., 2009a) was replicated. Expression of this enhanced effect using the lateral hypothalamic (LH) ICSS paradigm is of interest because LH ICSS thresholds can be lowered by concurrent orosensory stimulation with sweet solution (Conover and Shizgal, 1994) or passive administration of abused drugs (Wise, 1996). This convergent modulation is likely to arise from a shared ability of sweet taste and abused drugs to increase extracellular DA concentrations in NAc, and underscores the relationship between adaptive changes in appetitive motivational/reward circuitry and drug reward magnitude. Anatomical localization to NAc medial shell is also consistent with a role in drug reward modulation, given that multiple approaches to the modeling of drug abuse in rodent subjects have identified NAc medial shell as fundamentally involved in the primary reinforcing effects (Pontieri et al., 1995; Carlezon and Wise, 1996; Ikemoto, 2007).

The novel finding of Experiment 3 is that co-injection of a polyamine GluR1 homomer antagonist decreased the reward-potentiating effect of SKF-82958. To our knowledge, this is the first evidence that receptors composed of the GluR1 subtype are involved in mediating the acute rewarding effect of DA receptor stimulation. While results suggest that GluR1 involvement may apply to both the AL and FR condition, 1-NA spermine preferentially decreased the effect of SKF-82958 in FR rats. This not only suggests that GluR1 is downstream of D-1 en route to behavioral expression, but also that a critical neuroadaptation in FR subjects is to be found within one or more of the molecular signaling pathways that intervene between D-1 and GluR1, as suggested above.

Microinjection of the D-2/D-3 agonist, quinpirole, in Experiment 3 recapitulated the effect of systemically administered quinpirole (Carr et al., 2001), lowering the theta-0 and maximum reinforcement rate, and having no effect on the M-50 measure of reward threshold. There are

a number of possible explanations of a drug effect that is limited to the theta-0 (x-axis intercept) measure. For example, the drug may selectively lower the brain stimulation detection threshold. Alternatively, it may selectively increase responding to the conditioned stimulus -lever extension- that initiates each trial; this would be sufficient to yield a few nonreinforced lever presses on trials in which the response-contingent brain stimulation is, itself, subthreshold. However, the important point for the present study is that co-infusion of 1-NA-spermine had no effect on the theta-0-lowering effect of quinpirole in FR rats, and rather than reversing the tendency of quinpirole to lower maximum reinforcement rates, enhanced this effect in both feeding groups. These observations, along with the absence of effect when the 25 µg dose of 1-NA-spermine was microinjected alone, support the conclusion that when 1-NA-spermine is co-infused with SKF-82958 it exerts a specific pharmacological effect and reverses the reward-potentiating effect initiated by D-1 DA receptor stimulation. It is of interest that D-2 receptor stimulation leads to decreased phosphorylation of GluR1 at Ser845 (Hakansson et al., 2006). Thus, to the extent that any behavioral effect of quinpirole is mediated by inhibition of GluR1 phosphorylation, 1-NA-spermine might be expected to exacerbate the effect; this may explain the decrease in maximum reinforcement rate when the two drugs were combined.

Alternative mechanistic explanations of the present results do exist. For example, D-1 DA receptor agonists may increase hippocampal glutamatergic input to NAc (Goto and Grace, 2008). If FR increases either the glutamate releasing effect or GluR1 response to released glutamate, an explanation based on postsynaptic D-1/GluR1 interaction need not be invoked. To begin assessment of postsynaptic GluR1 status, Experiment 4 measured GluR1 abundance in NAc synaptosomes and the reward-potentiating effects of AMPA microinjection in NAc shell. GluR1 abundance did not differ between feeding groups, although it must be allowed that a difference localized to the postsynaptic density (PSD) could be masked by an absence of difference in internal or presynaptic receptors (though striatal presynaptic AMPA heteroreceptors appear to be few in number (Tarazi et al., 1998)). It is also possible that a difference that is exclusive to NAc shell is masked by an absence of difference in NAc core. Focused analyses in PSD and NAc shell should allow a more definitive determination. While microinjection of AMPA did produce a lowering of both measures of ICSS reward threshold, neither effect was greater in FR than AL rats. This finding, along with the findings that feeding groups did not differ in whole cell GluR1 or baseline phospho-Ser845- or phospho-Ser831-GluR1, suggests that differences between feeding groups do not exist at the level of basal AMPA receptor surface expression or function.

Substantial research has accrued to implicate NAc AMPA receptors in the reinstatement of cocaine-seeking, following extinction, in response to environmental cues and a priming dose of cocaine (Cornish and Kalivas, 2000; Di Ciano and Everitt, 2001; Anderson et al., 2008; Conrad et al., 2008). Cocaine withdrawal is associated with increased GluR1 surface expression in NAc (Boudreau and Wolf, 2005; Boudreau et al., 2007; Conrad et al., 2008), increased stimulus-induced glutamate release in NAc (for discussion see: Kalivas et al., 2009), and internalization of GluR1 in response to cocaine challenge (Boudreau et al., 2007). The mechanisms of GluR1 involvement in reinstatement are still under intense investigation, though it appears that vulnerability to reinstatement is set up by increased GluR1 surface expression during cocaine abstinence in conjunction with increased release of glutamate in response to triggering stimuli. Importantly, the glutamatergic mechanism mediating cue-induced relapse has been localized to the NAc core (Kalivas et al., 2009; Conrad et al., 2008; Di Ciano and Everitt, 2001). In contrast, the present results suggest a role of GluR1 in regulating the reward magnitude of acute drug effects in otherwise drug naïve subjects, and this function may be localized to NAc shell, and amplified by FR. Recently, however, it has been shown that cocaine-induced reinstatement of drug-seeking is mediated not only by AMPA receptor mechanisms in NAc core (Famous et al., 2008; Bachtell et al., 2008) but also by a D-1

DA receptor-dependent mechanism in NAc shell that involves phosphorylation of GluR1 on Ser831 (Anderson et al., 2008).

In addition to increasing channel open probability, phosphorylation of GluR1 on Ser845 delivers GluR1-containing AMPA receptors to extrasynaptic sites in the plasma membrane (Oh et al., 2006; Serulle et al., 2007; Sun et al., 2008). This may “prime” synaptic strengthening by increasing the pool of GluR1 for subsequent NMDA-dependent synaptic insertion (Derkach et al., 2007; Sun et al., 2008). Thus, concurrent activation of DA and glutamate inputs to NAc may mediate synaptic strengthening that underlies formation of new context- and response-reward associations. A variety of evidence suggests that the capacity for synaptic plasticity is decreased in the NAc of “addicted” animals and may contribute to the persistence of drug-directed behavior even after long periods of abstinence (Kalivas, 2009). Present results suggest that FR may have the opposite effect, increasing the capacity for synaptic plasticity, consequent to stimulus-induced DA release, and thereby facilitate reward-related learning to re-align instrumental behavior with environmental contingencies under the pressure of negative energy balance and threat of starvation. This possibility leads to interesting predictions regarding the interaction between food restriction and drug abuse. Specifically, if food restriction –or key physiological concomitants- were to precede or coincide with initial drug exposure, vulnerability to compulsive drug use might be increased. On the other hand, if food restriction without malnutrition were to be introduced during drug abstinence, a restoration of NAc capacity for synaptic plasticity might assist in the therapeutic displacement of drug-directed behavior by more productive alternatives.

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ABBREVIATIONS

AMPA	α -amino-3-hydroxyl-5-methyl-4-isoxazole-propionate
DA	dopamine
NAc	nucleus accumbens
CPu	caudate-putamen
FR	food-restriction
AL	ad libitum fed
1-NA-spermine	1-naphthylacetyl spermine

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Figure 1.

Effects of food restriction (FR) on GluR1 phosphorylation on Ser845 in nucleus accumbens (NAc) and caudate-putamen (CPu). Subjects were injected with SKF-82958 (1.0 mg/kg, i.p.) or vehicle ($n=5/\text{group}$). Whole cell homogenates were immunoblotted with anti-phospho-Ser845 GluR1 or anti-GluR1 antibodies. Following densitometry, intensities of bands corresponding to phospho-Ser845 GluR1 for each subject were divided by the intensities of the corresponding total GluR1 bands to control for small differences in protein loading. Results (mean $\pm$ s.e.m.) are expressed in comparison to the normalized control, which was defined as the ad libitum fed (AL) group injected with vehicle. Results are displayed for NAc (panel "A")

and CPu (panel “B”) with representative immunoblots. * Effect of SKF-82958 greater in FR than AL group, $p < .01$. Additional treatment effects are listed.

Figure 2.

Effects of food restriction on GluR1 phosphorylation on Ser831 in nucleus accumbens. Subjects were injected with SKF-82958 (1.0 mg/kg, i.p.) or vehicle. Whole cell homogenates were immunoblotted with anti-phospho-Ser831 GluR1 or anti-GluR1 antibodies. Following densitometry, intensities of bands corresponding to phospho-Ser831 GluR1 for each subject were divided by the intensities of corresponding total GluR1 bands. Results (mean $\pm$ s.e.m.) are expressed in comparison to the normalized control, which was defined as the ad libitum fed group injected with vehicle. No differences were observed as a function of feeding condition or drug treatment.

Figure 3.

Effects of food restriction (FR) on GluR1 phosphorylation on Ser845 in nucleus accumbens (NAc) and caudate-putamen (CPu). Subjects ingested ~12 ml of 10% sucrose (Suc) or had access to tap water (H₂O) for an equal period (n=6–7/group). Whole cell homogenates were immunoblotted with anti-phospho-Ser845 GluR1 or anti-GluR1 antibodies. Following densitometry, intensities of bands corresponding to phospho-Ser845 GluR1 for each subject were divided by the intensities of corresponding total GluR1 bands. Results (mean ± s.e.m.) are expressed in comparison to the normalized control, defined as the ad libitum fed group with access to H₂O. Results are displayed for NAc (panel “A”) and CPu (panel “B”) with a

representative immunoblot for NAc. Sucrose increased phosphorylation of GluR1 in NAc of FR subjects (* $p < .01$ relative to FR/H₂O and AL/Suc) and in CPu of both feeding groups.

Figure 4.

Effect of food restriction on GluR1 phosphorylation on Ser831 in nucleus accumbens. Subjects ingested 10% sucrose or had access to H₂O for equal duration. Whole cell homogenates were immunoblotted with anti-phospho-Ser831 GluR1 or anti-GluR1 antibodies. Following densitometry, intensities of bands corresponding to phospho-Ser831 GluR1 for each subject were divided by the intensities of the corresponding total GluR1 bands. Results (mean $\pm$ s.e.m.) are expressed in comparison to the normalized control, defined as the ad libitum fed group with access to H₂O. No differences were observed as a function of feeding condition or sucrose intake.

Figure 5.

Average pre- and post-injection ICSS rate-frequency curves for ad libitum fed ($n=13$) and food-restricted ($n=9$) rats that received bilateral microinjections of SKF-82958 alone ($3 \mu\text{g}$ in $0.5 \mu\text{l}$) (A= ad libitum fed; C= food-restricted) or in combination with 1-NA-spermine ($25 \mu\text{g}$; B= ad libitum fed; D= food-restricted) in NAc medial shell. Points indicated by symbols correspond to mean theta-0 (x-axis intercept), M-50 (brain stimulation frequency supporting 50% of the maximum reinforcement rate), and maximum/asymptotic reinforcement rate.

Figure 6.

Mean ($\pm$ s.e.m.) percentage change in M-50 (A) and theta-0 (B) measures of ICSS reward threshold, and maximum reinforcement rate (C), following bilateral nucleus accumbens microinjection of vehicle, SKF-82958 (3 μ g), and SKF-82958 in combination with 2.5 μ g and 25 μ g 1-NA-spermine in ad libitum fed (AL) and food-restricted (FR) rats. * Threshold-lowering effects of SKF-82958 were greater in FR than AL rats, $p<.005$. Additional treatment effects are listed.

Figure 7.

Mean ($\pm$ s.e.m.) percentage change in M-50 (A) and theta-0 (B) measures of ICSS reward threshold, and maximum reinforcement rate (C), following bilateral microinjection of vehicle, quinpirole (5 μ g), and quinpirole in combination with 25 μ g 1-NA-spermine in ad libitum fed and food-restricted rats ($n=7$ /group). Treatment effects are listed.

Figure 8.

Ratio of total GluR1 to tubulin in whole cell homogenates (A) of nucleus accumbens obtained from ad libitum fed and food-restricted subjects. Results are based on pooling of data from control subjects of Experiments 1 and 2 (i.e., subjects that did not receive SKF-82958 or sucrose prior to sacrifice; n=11 per group). Feeding groups displayed no difference on this measure, either within a single experiment or when pooled as above.

Ratio of total GluR1 to tubulin in the synaptosomal fraction (B) obtained from nucleus accumbens (NAc) tissue samples of ad libitum fed and food-restricted subjects that received no acute drug or ingestion treatment prior to sacrifice. N=10 per group with pooling of two NAc to obtain each data point. Feeding groups displayed no difference on this measure.

Figure 9.

Mean ($\pm$ s.e.m.) percentage change in M-50 (A) and theta-0 (B) measures of ICSS reward threshold, and maximum reinforcement rate (C), following bilateral microinjection of vehicle, 0.02 μ g and 0.1 μ g AMPA in nucleus accumbens of ad libitum fed and food-restricted rats ($n=8$ AL, 7 FR). Treatment effects are listed.

Table 1

Percentage changes in ICSS parameters produced by Nucleus Accumbens shell microinjection of 1-NA-spermine in *ad libitum* fed and food-restricted rats

	Theta-0			Max		
	M-50					
		<i>Ad libitum</i>	Restricted	<i>Ad libitum</i>	Restricted	Restricted
Vehicle		0.0 ± 2.4	-2.8 ± 2.1	3.2 ± 4.3	-5.1 ± 4.3	-0.9 ± 0.9
1-NA-Sp (25 µg)		-1.1 ± 2.3	1.6 ± 1.3	- 6.6 ± 5.3	-4.4 ± 3.9	6.9 ± 3.0

* Data represent mean ± SEM