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Involvement of nucleus accumbens AMPA receptor trafficking in augmentation of D- amphetamine reward in food-restricted rats

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Abstract

Rationale—Chronic food restriction (FR) increases behavioral responsiveness to drugs of abuse and associated environments. Pre- and postsynaptic neuroadaptations have been identified in the mesoaccumbens dopamine pathway of FR subjects but the mechanistic basis of increased drug reward magnitude remains unclear.

Objectives—Effects of FR on basal and D-amphetamine-induced trafficking of AMPA receptor subunits to the nucleus accumbens (NAc) postsynaptic density (PSD) were examined, and AMPA receptor involvement in augmentation of D-amphetamine reward was tested.

Materials and methods—FR and ad libitum fed (AL) rats were injected with D-amphetamine (2.5 mg/kg, i.p.) or vehicle. Brains were harvested and subcellular fractionation and Western analyses were used to assess AMPA receptor abundance in NAc homogenate and PSD fractions. A follow-up experiment used a curve-shift protocol of intracranial self-stimulation to assess the effect of 1-naphthylacetyl spermine (1-NASPM), a blocker of Ca^{2+}-permeable AMPA receptors, on rewarding effects of D-amphetamine microinjected in NAc shell.

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Results—FR increased GluA1 in the PSD, and D-amphetamine increased p-Ser845-GluA1, GluA1, GluA2, but not GluA3, with a greater effect in FR than AL rats. D-amphetamine lowered reward thresholds, with greater effects in FR than AL rats, and 1-NASPM selectively reversed the enhancing effect of FR.

Conclusions—Results suggest that FR leads to increased synaptic incorporation of GluA1 homomers to potentiate rewarding effects of appetitive stimuli and, as a maladaptive byproduct, D-amphetamine. The D-amphetamine-induced increase in synaptic p-Ser845-GluA1, GluA1, and GluA2 may contribute to the rewarding effect of D-amphetamine, but may also be a mechanism of synaptic strengthening and behavior modification.

Keywords

Food restriction; Nucleus accumbens; Reward; Self-stimulation; D-amphetamine; AMPA receptors; GluA1; Postsynaptic density; 1-NASPM

Animal models and human neuroimaging increasingly support a scheme in which drugs of abuse target and subvert the neurocircuitry that mediates appetitive motivation and reward (Kelley and Berridge 2002; Cardinal and Everitt 2004; DiChiara 2005; Volkow et al. 2008). This relationship is evident in the high comorbidity of disordered eating and drug abuse (Krahn et al. 1992; Wiederman and Prior 1996; Pisetsky et al. 2008; Root et al. 2010), the low prevalence of substance use among obese individuals (Warren et al. 2005; Simon et al. 2006), and the increased vulnerability to use, relapse, and drug-induced psychopathology among those who are dieting or have low body mass index (French et al. 1994; Austin and Gortmaker 2001; Cheskin et al. 2005; Rosse et al. 2005). A related and emerging public health concern is the possible switch to alcohol and drug abuse in gastric bypass patients (Ivezaj et al. 2012; Conason et al. 2013).

Animal studies indicate that food restriction (FR) increases sensitivity to drugs of abuse in self-administration (Carroll and Meisch 1984), conditioned place preference (Bell et al. 1997; Stuber et al. 2002; Liu et al. 2011; Zheng et al. 2012), motor activity (Deroche et al. 1995; Carr et al. 2003), and electrical brain stimulation reward paradigms (Cabeza de Vaca and Carr 1998; Cabeza de Vaca et al. 2004). In addition, FR interacts with episodic access to palatable food to generate enduring binge-like patterns of intake (Hagan and Moss 1997; Avena et al. 2008; Consoli et al. 2009). Most recently, it was observed that FR increases the incentive effects of an environment paired with cocaine during a prior ad libitum fed state (Zheng et al. 2012) and enhances cue-induced reinstatement of heroin seeking (D'Cunha et al. 2013). Consequently, food restriction may increase vulnerability to both initial use and relapse, and may do so by harnessing neuroadaptations that evolved to confer a selective advantage in an ecology of food scarcity (Carr 2011).

A link between drug use and ingestive behavior is the common involvement of the mesoaccumbens dopamine (DA) pathway (Wise and Bozarth 1985; Pontieri et al. 1995; Hajnal et al. 2004; Palmiter 2007; Kenny 2011). A variety of neuroadaptations in the mesoaccumbens pathway are associated with the augmentation of drug reward in FR rats. Most of the evidence indicates that basal and evoked DA release are diminished, but postsynaptic intracellular signaling, gene expression and behavioral responses downstream

of D1 DA receptor stimulation are upregulated (Pothos et al. 1995; Carr et al. 2003; Haberny et al. 2004; Haberny and Carr 2005a, b; Pan et al. 2006; Stamp et al. 2008; Carr et al. 2010; Stouffer et al. 2012).

The DA innervation of nucleus accumbens (NAc) is convergent with several limbic forebrain glutamate inputs (Groenewegen et al. 1999), and the integration of DA- and glutamate-coded signals regulates medium spiny neuron (MSN) activity (Moyer et al. 2007; Surmeier et al. 2007), goal-directed behavior, reward-related learning, and addiction (Kelley 2004; Dalley et al. 2005; Hyman et al. 2006). Changes in glutamatergic AMPA receptor abundance in the synaptic membrane mediate dynamic tuning of synaptic transmission as well as enduring forms of synaptic plasticity (Choquet 2010; Kessels and Malinow 2009). AMPARs are co-expressed with DA receptors in NAc neurons (Bernard et al. 1997; Glass et al. 2008), and most are either GluA1/GluA2 or GluA2/GluA3 heteromers (Reimers et al. 2011). In cultured MSNs, D1 DA receptor agonist rapidly increases GluA1 surface expression in a PKA-dependent manner (Chao et al. 2002; Mangiavacchi and Wolf 2004). It is therefore of interest that FR was recently shown to increase phosphorylation of GluA1 on Ser845, the PKA site, in response to a D1 DA receptor agonist, sucrose consumption, and exposure to a cocaine-paired environment (Carr et al. 2010; Liu et al. 2011; Zheng et al. 2013). Phosphorylation on Ser845 increases GluA1 peak currents and channel open probability, stabilizes the receptor in the membrane, and facilitates synaptic insertion (Roche et al. 1996; Shi et al. 2001; Esteban et al. 2003; Oh et al. 2006; Ehlers et al. 2007; Man et al. 2007; He et al. 2009; He et al. 2011). Moreover, there is evidence that phosphorylation regulates behavioral responsiveness to D-amphetamine; hyperlocomotion was blocked by NAc overexpression of an alanine mutant GluA1 that prevents Ser845 phosphorylation (Li et al. 2011).

In addition to potentiating stimulus-induced Ser845 phosphorylation, FR may increase basal GluA1 abundance in the NAc postsynaptic density (PSD); in a study of sucrose consumption, the FR control group with access to tap water displayed higher levels of GluA1 than the AL control group (Peng et al. 2011). Moreover, involvement of this AMPAR subunit in reward modulation was supported by the finding that, within NAc shell, 1-NASPM, an antagonist of Ca^{2+}-permeable AMPARs, decreased the rewarding effect of a D1 DA receptor agonist in FR rats (Carr et al. 2010). The purpose of the present study was therefore to determine whether FR increases D-amphetamine-induced Ser845 phosphorylation, trafficking of GluA1-containing AMPARs to the NAc post-synaptic density, and 1-NASPM-reversible augmentation of D-amphetamine reward.

Materials and Methods

Subjects

Adult male Sprague–Dawley rats (Taconic Farms, Germantown, NY) received 40–50 % of the ad libitum intake of LabDiet 5001 (10–12 g) provided as a single meal daily, until body weight declined by 20 % (∼2 weeks). Experiments were initiated after FR rats had been stabilized for one week at their target body weight (i.e., ∼3 weeks after implementation of FR). Rats with ad libitum (AL) access to chow for the same period as paired FR rats served as controls. All rats had ad libitum access to water. In the biochemical experiment, 48

subjects were assigned to each diet condition. Twenty-four rats in each condition were injected with D-amphetamine (2.5 mg/kg, i.p.) and 24 with saline vehicle 30-min prior to sacrifice by brief exposure to CO_2 and decapitation by guillotine. In the behavioral experiment, 24 AL and FR subjects were tested. Experiments were conducted in accordance with the National Institutes of Health Guide for Care and Use of Laboratory animals and were approved by the New York University School of Medicine Institutional Animal Care and Use Committee.

Subcellular fractionation

NAc was dissected from fresh brain on ice and three per treatment condition were pooled for fractionation. The whole cell homogenate (WC) and PSD fraction were obtained as reported previously (Peng et al. 2011). Briefly, protease inhibitor cocktail and PMSF were added to 0.32 M sucrose containing 1 mM $NaHCO_3$, 1 mM $MgCl_2$, and 0.5 mM $CaCl_2$ (Solution A). Brain tissue was rinsed, homogenized and diluted to 10 % weight/volume in Solution A. After being well mixed, 50 μl of the WC were stored at −80 °C until use. Following preparation of a synaptosomal fraction, the PSD fraction was obtained using an equal volume solution containing 1 % Triton X-100, 0.32 M sucrose, and 12 mM Tris, pH 8.1, which was added to the resuspended sample. The mixture was rocked at 4 °C for 15 min, followed by centrifugation at 13,800g for 30 min. 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. This method of PSD purification yields a fraction enriched in proteins that are preferentially localized or novel to the PSD (Jordan et al. 2004). In the present study, PSD fractions were confirmed to contain abundant PSD95 relative to the WC.

Western blotting

Gel electrophoresis and Western blotting were as described (e.g., Peng et al. 2011) using rabbit polyclonal anti-phospho-Ser845-GluA1 (1:1,500; AB5849, Millipore, Temecula, CA), mouse monoclonal anti-GluA1 (1:1,500; MAB2263, Millipore), rabbit polyclonal anti-GluA2 (1:1,000; PA1-4659, Thermo Scientific, Rockford, IL), rabbit polyclonal anti-PSD95 (1:1,000; AB9708, Millipore), mouse monoclonal anti-GluA3 (1:500; MAB5416, Millipore), and mouse monoclonal anti-α-tubulin (1:5,000; T6199, Sigma-Aldrich, St. Louis, MO). Immunoblots were analyzed using NIH Image J software. Following densitometry, intensities of bands corresponding to GluA1, GluA2, GluA3, and p-Ser845-GluA1 for each sample were divided by intensities of the corresponding α-tubulin bands. Results were expressed in comparison to the normalized control, defined as the AL group that received the control treatment. Results were analyzed by two-way ANOVA, with significant interaction effects followed by comparison of cell means of interest using the error term from the ANOVA in the denominator of a *t*-statistic.

Surgical procedures

Several days after arrival in the central animal facility, rats were anesthetized with ketamine (100 mg/kg, i.p.) and xylazine (10 mg/kg, i.p.) and were stereotaxically 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 gauge) 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). Cannula patency was maintained with occlusion stylets. 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 banamine (2.0 mg/kg, s.c.) following recovery from anesthesia and the morning after.

Lateral hypothalamic self-stimulation

Lateral hypothalamic self-stimulation (LHSS) was used to obtain a learning-free measure of drug reward magnitude. Subjects lever pressed to obtain 1-s trains of 0.1 ms duration cathodal pulses via the indwelling electrode. Brain stimulation frequency was varied systematically over 1-min trials, generating a rate-frequency curve analogous to the pharmacological dose-response curve. Two threshold parameters were determined: M-50, the pulse frequency that elicits half the maximum reinforcement rate, and *x*-axis intercept, the lowest frequency at which stimulation is rewarding. Drugs of abuse produce a decrease in reward threshold, expressed as a parallel left shift of the rate-frequency curve. Training and stabilization of baseline performance began ∼1 week after surgery and FR was initiated ∼1 week into training. Testing began once the diet had been in place for a minimum of 3 weeks. Test sessions included a pre-injection LHSS rate-frequency test of 28 min duration, followed by bilateral microinjection of D-amphetamine (5.0 μg, bilaterally in 0.5 μl) in the presence or absence of 1-NASPM (25.0 μg). Microinjection was followed, 5 min later, by a post-injection LHSS rate-frequency test. Tests were conducted a minimum of 72 h apart. Half of subjects in each diet group were first tested with co-microinjection of 1-NASPM and half without. The % changes in post-injection relative to pre-injection tests for each parameter were calculated as before (e.g., Cabeza de Vaca and Carr 1998). A second pair of test sessions followed to confirm the prior finding that neither saline nor 1-NASPM microinjected in the absence of D-amphetamine-altered LHSS thresholds in AL or FR rats (Carr et al. 2010). Results were analyzed by mixed two-way ANOVA (diet×drug treatment). Based on prior results obtained with intra-NAc D-amphetamine (Carr et al. 2009) and SKF-82958 in the presence and absence of 1-NASPM (Carr et al. 2010), significant interaction effects were followed by planned comparison of cell means using the error term from the ANOVA in the denominator of a one-tailed *t*-statistic.

Intracerebral microinjection procedures

Solutions were loaded into two 30-cm lengths of PE-50 tubing attached at one end to 5-μl Hamilton syringes filled with distilled water and at the other end to 33-gauge injector cannulae, which extended 2.0 mm beyond the implanted guides. The 0.5 μl injection volumes were delivered manually over a period of 90 s at a rate of 0.05 μl/10 s. One minute following completion of the microinjection, injector needles were removed from guides, stylets were replaced, and animals were returned to test chambers for an additional 4 min prior to behavioral testing.

Drugs and dose selection

D-amphetamine (Sigma-Aldrich) was dissolved in sterile 0.9 % saline and injected intraperitoneally in the biochemical experiment, and microinjected into NAc medial shell in the behavioral experiment. 1-Naphthylacetyl spermine (1-NASPM; Sigma-Aldrich) was dissolved in sterile 0.9 % saline and microinjected into NAc medial shell alone or in combination with D-amphetamine in the behavioral experiment.

In the biochemical experiment, the D-amphetamine dose of 2.5 mg/kg, and the 30-min interval to brain harvesting were chosen based on two considerations. First, it had been reported that at 30-min, this dose of D-amphetamine produced a nearly significant increase in NAc surface expression of GluA1 in AL rats (Nelson et al. 2009); it was predicted that any D-amphetamine-induced AMPA receptor trafficking, measured here in the PSD, would be augmented and therefore significant in FR rats. Second, in order to associate AMPAR findings with behavioral effects, it was necessary to probe at a time that coincided with post-injection testing of D-amphetamine reward magnitude. In the current behavioral experiment, the 5.0 μg dose of D-amphetamine was administered based on a previous observation that, when microinjected into NAc shell, it lowered LHSS thresholds with a significantly greater effect in FR than AL rats (Carr et al. 2009). Finally, the 25.0 μg dose of 1-NASPM, used to challenge the effect of D-amphetamine, was chosen based on effectiveness of a somewhat higher dose in blocking cue-induced reinstatement of cocaine seeking in subjects with increased NAc surface expression of GluA1 (Conrad et al. 2008), and effectiveness of this specific dose in reversing the enhancing effect of FR on the reward magnitude of SKF-82958 (Carr et al. 2010).

Histology

Rats were briefly exposed to CO_2 and decapitated by guillotine. Brains were removed and fixed in 10 % buffered formalin for 48 h. Frozen 40 μm coronal sections were cut on a Reichert-Jung 2800 cryostat, thaw-mounted on gelatin-coated slides and stained with cresyl violet. Microinjection sites were determined by visual inspection under an Olympus SZ40 microscope. Only subjects whose cannulae were accurately placed within the NAc medial shell, including the shell/core and shell/olfactory tubercle borders were included in data analysis.

Results

Neither FR, D-amphetamine nor their combination altered levels of GluA1, GluA2, or GluA3 in the NAc whole cell homogenate (Fig. 1), suggesting that treatments did not alter synthesis or degradation of AMPAR proteins. However, in the NAc postsynaptic density fraction (Fig. 2), FR increased levels of p-Ser845-GluA1 ($F_{diet;1,28}$=6.13, p<.025) and GluA1 ($F_{diet;1,28}$=5.2, p<.05). The planned comparison of GluA1 abundance in vehicle-treated groups confirmed greater abundance in FR relative to AL rats (t(14)=1.95, p<.05). D-amphetamine increased levels of p-Ser845-GluA1 ($F_{drug;1,28}$=15.5, p<.001) and GluA1($F_{drug;1,28}$=8.78, p<.01) across diet conditions. A significant interaction between diet and drug treatment ($F_{diet;1,28}$=6.84, p<.025; $F_{drug;1,28}$=17.7, p<.001; $F_{diet\times drug;1,28}$=3.97, p=.05), followed by comparison of cell means, revealed an increased GluA2 abundance in D-

amphetamine-treated groups relative to corresponding vehicle-treated groups ($t_{AL}(28)=3.8$, $p<.001$; $t_{FR}(28)=5.2$, $p<.001$) and a greater effect of D-amphetamine in FR relative to AL rats ($t_{FR\ vs\ AL}(28)=4.5$, $p<.001$). There were no effects of FR or D-amphetamine on levels of GluA3 or PSD95.

NAc medial shell microinjection of D-amphetamine produced a greater rewarding effect in FR than AL rats on both the M-50 ($F_{diet\times drug;1,16}=4.26$, $p=.05$; $t_{FR\ vs\ AL}(16)=3.1$, $p<.01$) and *x*-axis intercept ($F_{diet\times drug;1,16}=7.41$, $p<.025$; $t_{FR\ vs\ AL}(16)=2.67$, $p<.01$) measures of reward threshold (Fig. 3). Co-microinjection of 1-NASPM decreased these rewarding effects in FR but not AL rats (M-50, $t_{Amph\ vs\ Amph/NASPM}(16) = 1.89$, $p < .05$; *x*-intercept, $t_{Amph\ vs\ Amph/NASPM}(16)=3.56$, $p<.005$). Threshold measures were unaffected by saline vehicle or 1-NASPM microinjection in the absence of D-amphetamine (Fig. 4). Schematic diagrams indicating microinjection sites are provided in Fig. 5.

Discussion

Three main findings were obtained in this study. First, FR subjects receiving acute injection of saline vehicle displayed elevated levels of GluA1, but not GluA2 or GluA3, in the NAc PSD relative to AL subjects receiving the same treatment. This result is consistent with the previous finding that FR subjects with brief access to tap water, as a control for sucrose solution, displayed elevated levels of GluA1, but not GluA2, in the NAc PSD (Peng et al. 2011). Most NAc AMPARs are either GluA1/GluA2 or GluA2/GluA3 heteromers (Reimers et al. 2011). GluA2-lacking AMPARs, which are Ca^{2+}-permeable, make up only ∼7 % of the total (Reimers et al. 2011). Yet, it appears that FR is associated with increased synaptic incorporation of homomeric GluA1. This effect is reminiscent of the synaptic incorporation of GluA1 in primary visual cortex following visual sensory deprivation (Goel et al. 2006), and the cross-modal compensatory delivery of GluA1 into barrel cortex synapses to sharpen the functional whisker-barrel map (Jitsuki et al. 2011). AMPARs are the main excitatory postsynaptic glutamate receptors, and their trafficking is an established mechanism for regulating neuronal excitability (Lee 2012) and synaptic homeostasis following sustained inactivity (Man 2011; Lee 2012; Shepherd 2012). Consequently, the mechanism underlying increased synaptic GluA1 in Nac of FR subjects may be tied, at least in part, to diminished DA transmission during FR, and the deprivation of input via D1 receptors which exist in a low affinity state and require high DA concentrations for activation. When MSNs receive strong glutamatergic input, D1 stimulation facilitates the transition from a hyperpolarized downstate to the upstate where membrane potential is near spike threshold (Surmeier et al. 2007). Decreased D1 signaling during FR may therefore decrease excitatory activity and contribute to a compensatory synaptic accumulation of GluA1.

The second finding of this study is that acute administration of D-amphetamine rapidly delivered AMPARs into the NAc PSD. The dose and interval to brain harvesting were based on the study of Nelson et al. (2009) who, using a protein cross-linking method, observed a ∼10 % increase in surface expression that approached statistical significance. A more robust increase was seen 2 h after D-amphetamine administration, but that latency to measurement would have fallen outside the time frame of behavioral testing in the present and previous comparisons of AL and FR subjects. In both diet groups, D-amphetamine increased levels of

GluA1 and GluA2, but not GluA3, with an overall greater effect in FR than AL rats. In light of the high prevalence of GluA1/GluA2 heteromers in NAc, and their well demonstrated activity-dependent trafficking into synapses in hippocampal models (Barry and Ziff 2002), it is likely that D-amphetamine delivered GluA1/GluA2 heteromers into the PSD.

The third finding of this study was the selective decrease of D-amphetamine reward by 1-NASPM microinjection in the NAc medial shell of FR rats. D-amphetamine decreased the minimum frequency at which brain stimulation became rewarding (*x*-axis intercept) and the frequency supporting 50 % of the maximal reinforcement rate (M-50). Most importantly, both threshold-lowering effects were augmented by FR, and the augmenting effect was blocked by 1-NASPM, a synthetic analogue of Joro Spider toxin that selectively blocks Ca^{2+}-permeable AMPARs (Tsubokawa et al. 1995; Koike et al. 1997). The biochemical results of this study, suggesting that this type of AMPAR may be driven into the PSD by FR rather than by D-amphetamine, suggests that a basal increase in GluA1, rather than enhanced D-amphetamine-induced trafficking of AMPARs to the PSD, is the mechanistic underpinning of increased drug reward magnitude in FR subjects. Several findings support this scheme. Unlike D-amphetamine, acute administration of cocaine and D1 DA receptor agonists do not increase surface expression of GluA1 in NAc of AL rats (albeit, FR rats were not tested; Ferrario et al. 2011). Yet, amphetamine, cocaine, D1 agonists, and cocaine-paired environmental stimuli are all subject to the enhancing effect of FR, and parsimony would point to a common underlying mechanism. In fact, the enhanced rewarding effect of the D1 agonist, SKF-82958, in FR rats was blocked by microinjection of 1-NASPM in NAc shell (Carr et al. 2010). Because the phenotype(s) of NAc MSNs in which FR increases PSD GluA1 abundance cannot be discerned here, the pharmacologic evidence implicating Ca^{2+}-permeable AMPARs in the enhanced rewarding effect of a D1 agonist is important. Unlike the D2 receptor, D1 requires high extracellular DA concentrations for activation and, when activated, can facilitate MSN transition from the hyperpolarized downstate to near threshold for firing. Increased GluA1 abundance would be expected to increase responsiveness of NAc neurons to excitatory inputs (Conrad et al. 2008) that potentially remove extracellular Mg^{2+} blockade of NMDAR-associated channels (Ozawa et al. 1998), and facilitate transition to the upstate (Wolf et al. 2005). Consequently, the present findings point to mechanisms via which FR may promote the occurrence of upstates and enhance responsiveness to DA. Recent optogenetic stimulation studies in D1- and D2-Cre BAC transgenic mice indicate that excitation of D1-expressing MSNs facilitates, while excitation of D2-expressing MSNs inhibits, cocaine's rewarding and stimulant effects (Lobo et al. 2010). Thus, FR-induced upregulation of D1 DA receptor function and increased synaptic abundance of GluA1 may combine to increase ventral striatal and behavioral responsiveness to appetitive reward stimuli and their pharmacological proxies.

FR and D-amphetamine both increased levels of p-Ser845-GluA1 in the NAc PSD. Phosphorylation of GluA1 on Ser845 is necessary for maintaining Ca^{2+}-permeable AMPARs on the surface (He et al. 2009) and may therefore contribute to the 1-NASPM-reversible augmentation of D-amphetamine reward in FR rats. However, phosphorylation on Ser845 also facilitates trafficking of GluA1-containing AMPARs to the perisynaptic membrane as the first step toward synaptic incorporation (Boehm et al. 2006). It is therefore

possible that enhanced behavioral responses to reward stimuli in FR rats are mediated not only by an increase in basal synaptic GluA1 but also an upregulation of stimulus-induced phosphorylation and synaptic delivery. AMPA receptors in NAc have previously been implicated in sensitization, craving, and relapse to cocaine seeking (Cornish and Kalivas 2000; Boudreau and Wolf 2005; Conrad et al. 2008; Famous et al. 2008). In light of the documented involvement of AMPAR trafficking in synaptic strengthening that underlies acquisition of aversive behavior (Whitlock et al. 2006; Nedelescu et al. 2010), upregulation of stimulus-induced AMPAR trafficking by FR may play a role in the enhanced acquisition and ingraining of appetitive behavior.

Results of the present study suggest that FR upregulates basal and stimulus-induced trafficking of GluA1-containing AMPARs to the NAc PSD. In the wild, these mechanisms would be expected to enhance behavioral responsiveness to appetitive reward stimuli and facilitate synaptic plasticity that mediates the learning of survival behavior. However, in modern societies, severe dieting is prevalent in a context of abundant appetitive rewards. As such, the present findings offer a new mechanistic focus for research aimed at clarifying the basis of dieting as a risk factor for development and reinstatement of maladaptive reward-directed behavior, including drug addiction.

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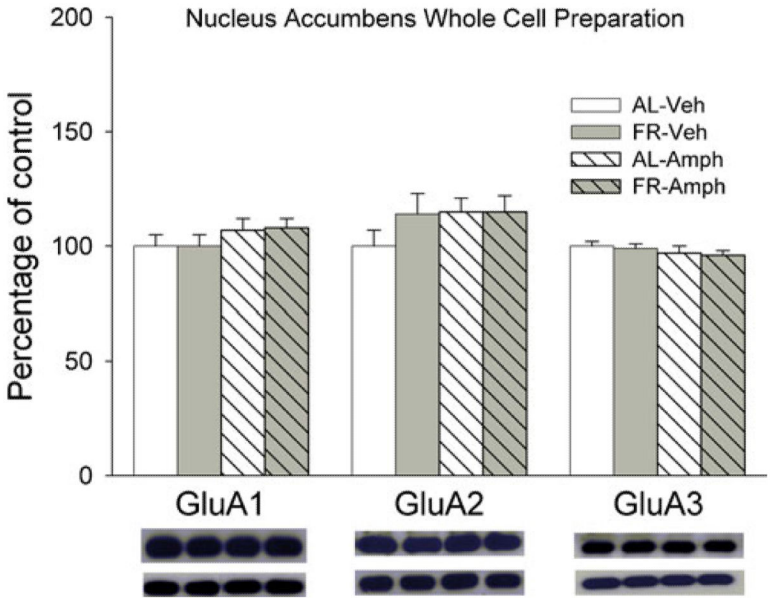

Fig. 1.

Effects of food restriction and D-amphetamine (2.5 mg/kg, i.p.) on GluA1, GluA2, and GluA3 abundance in nucleus accumbens whole cell homogenate. Results (mean±s.e.m.) are expressed in comparison to the normalized control, defined as the ad libitum fed group injected with vehicle. Representative immunoblots for target proteins, with corresponding α-tubulin beneath, are included in the same sequence as the *data bars* immediately above. *n*=24 per group, with 3 NAc pooled per tube for fractionation

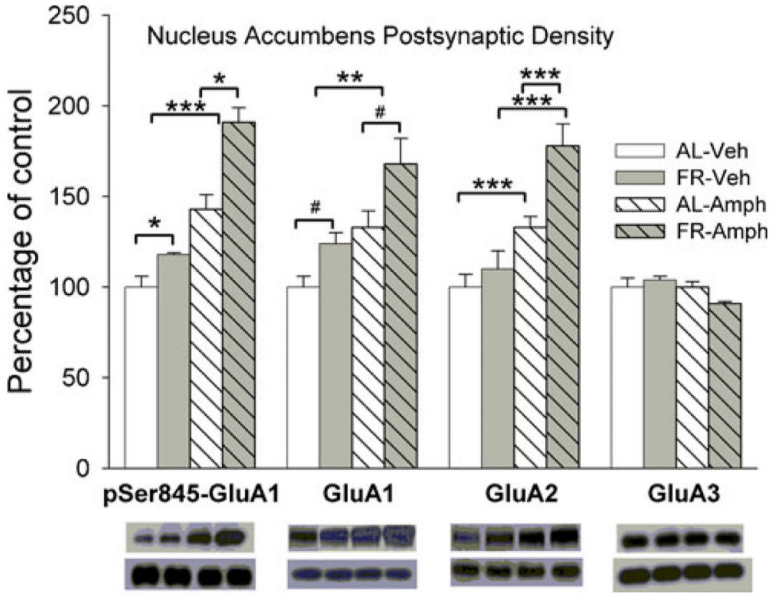

Fig. 2.

Effects of food restriction and D-amphetamine (2.5 mg/kg, i.p.) on p-Ser845-GluA1, GluA1, GluA2, and GluA3 abundance in nucleus accumbens postsynaptic density. Results (mean ±s.e.m.) are expressed in comparison to the normalized control, defined as the ad libitum fed group injected with vehicle. Representative immunoblots for target proteins, with corresponding *α*-tubulin beneath, are included in the same sequence as the *data bars* immediately above. p-Ser845-GluA1, GluA1, GluA2, and GluA3 were identified as bands at ∼100, 110, 100, and 110 kDA, respectively. *n*=24 per group, with 3 NAc pooled per tube for fractionation. $^{\#}p<.05$, $*p<.025$, $**p<.01$, $***p<.001$

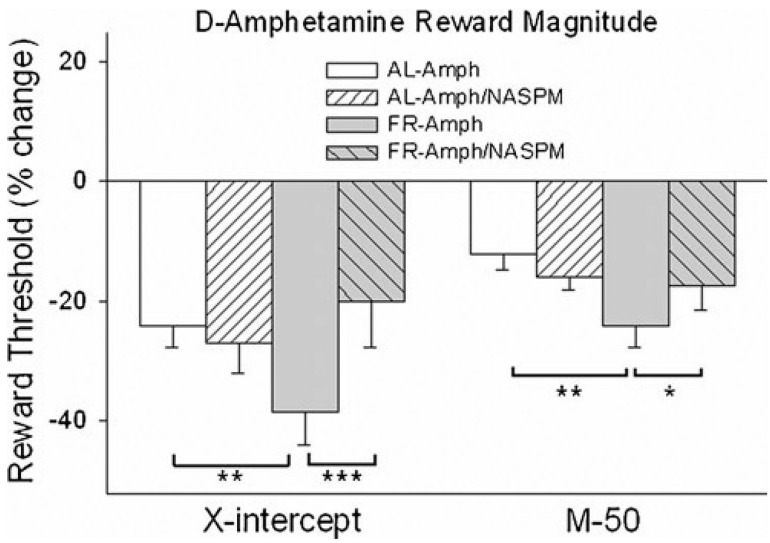

Fig. 3.

Effects of D-amphetamine (5.0 μg/ 0.5 μl), microinjected bilaterally in nucleus accumbens medial shell in the presence and absence of 1-NASPM (25.0 μg), on two measures of threshold (*left x*-axis intercept; *right* M-50) in the curve-shift protocol of LHSS. *n*=9 per group. **p*<.05, ***p*<.01, ****p*<.005

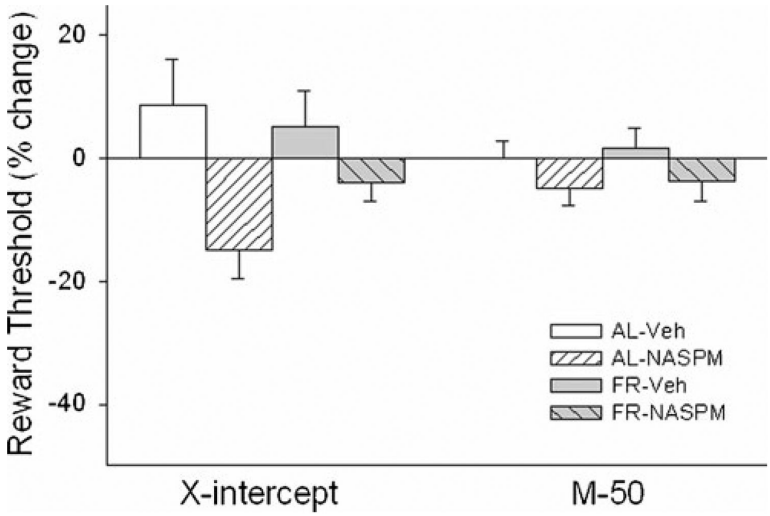

Fig. 4.

Effects of saline vehicle and 1-NASPM (25.0 μg) microinjected bilaterally in nucleus accumbens medial shell on two measures of threshold (*left x*-axis intercept; *right* M-50) in the curve-shift protocol of LHSS. *n*=9 per group

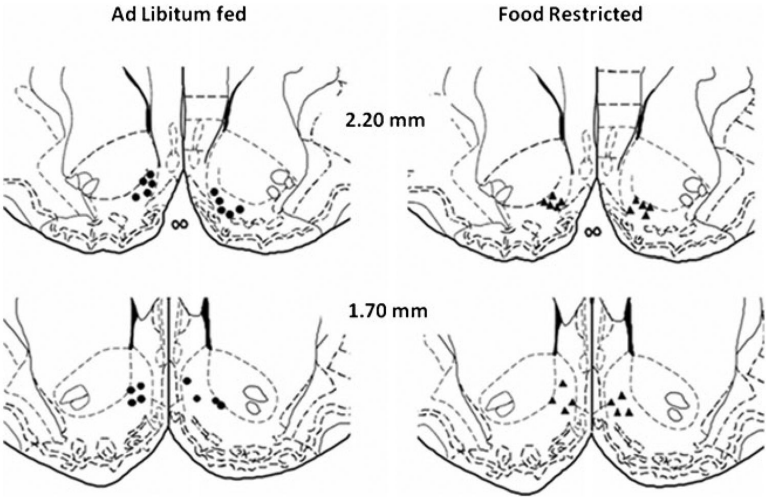

Fig. 5.

Schematic diagrams adapted from Paxinos and Watson (1998), indicating microinjection sites in NAc shell. *Closed circles in the left panel* and *closed triangles in the right panel* indicate sites in AL and FR rats, respectively