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Feeding conditions differentially affect the neurochemical and behavioral effects of dopaminergic drugs in male rats

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Abstract

The high comorbidity of eating disorders and substance abuse suggests that nutritional status can impact vulnerability to drug abuse. These studies used rats to examine the effects of food restriction on dopamine clearance in striatum and on the behavioral effects of amphetamine (locomotion, conditioned place preference), the dopamine receptor agonist quinpirole (yawning), and the dopamine receptor antagonist raclopride (catalepsy). Amphetamine increased locomotion and produced conditioned place preference in free-feeding and food-restricted rats. Food restriction reduced dopamine clearance, which was restored by repeated treatment with amphetamine or by free feeding. Food restriction also decreased sensitivity to quinpirole-induced yawning and raclopride-induced catalepsy; normal sensitivity to both drugs was restored by free feeding. The same amphetamine treatment that normalized dopamine clearance, failed to restore normal sensitivity to quinpirole or raclopride, suggesting that in food-restricted rats the activity of dopamine transporters and dopamine receptors is differentially affected by pathways that are stimulated by amphetamine. These studies show that modest changes in nutritional status markedly alter dopamine neurotransmission and the behavioral effects of direct-acting dopamine receptor drugs (agonist and antagonist). These results underscore the potential importance of nutritional status (e.g., glucose and insulin) in modulating dopamine neurotransmission and in so doing they begin to establish a neurochemical link between the high co-morbidity of eating disorders and drug abuse.

Keywords

dopamine; receptor; chronoamperometry; locomotion; conditioned place preference; quinpirole; raclopride; amphetamine

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1. Introduction

Nutritional status can affect dopamine systems, the significance of which might be found in the high co-morbidity of substance abuse and eating disorders where concentrations of glucose, insulin, and other molecules can fluctuate markedly (e.g., Wolfe and Maisto, 2000). The dopamine transporter regulates dopamine neurotransmission by high-affinity reuptake of dopamine and it is a site of action for some drugs, including amphetamine. Dopamine uptake is reduced in striatal synaptosomes from food-deprived rats, and treatment with insulin restores uptake (Patterson et al., 1998). Converging evidence suggests that insulin enhances dopamine transporter activity (Carvelli et al., 2002; Knusel et al., 1990), while hypoinsulinemia (e.g., streptozotocin) reduces dopamine transporter activity (Owens et al., 2005; Sevak et al., 2007b). Because food restriction also reduces insulin (Carr, 1996), we hypothesized that food restriction reduces *in vivo* dopamine transporter activity in the striatum.

Because the dopamine transporter is a target for amphetamine, understanding how amphetamine affects the transporter is critical for understanding the neurochemistry of stimulant abuse. Although others have examined modulation of dopamine transporter activity by amphetamine and cocaine (Zahniser and Doolen, 2001; Kahlig and Galli, 2003), little is known about modulation of dopamine transporter activity by amphetamine in animals with altered nutritional status. Owens et al. (2005) showed that reduced dopamine transporter activity in streptozotocin-treated rats was restored by amphetamine. Because food restriction causes hypoinsulinemia (Carr, 1996) and can reduce dopamine uptake (Patterson et al., 1998), we hypothesized that amphetamine restores the reduced dopamine transporter activity during food restriction. In addition to modulating dopamine transporter activity, food restriction can modify behavioral effects of drugs acting on this transporter; food restriction enhances positive reinforcing effects of amphetamine and cocaine, as indicated by increased sensitivity in conditioned place preference and self-administration studies (Carroll et al., 1981; Stuber et al., 2002; Bell et al., 1997). However, little is known about the relationship between dopamine transporter activity and behavioral effects of drugs acting on the transporter during altered nutritional status. Dopamine transporters are highly concentrated in striatum (Ciliax et al., 1995; Fuxe et al., 1985; Cass and Gerhardt, 1995) and converging lines of evidence implicate striatal dopamine systems in the effects of abused drugs (Belin and Everitt, 2008; Everitt and Robbins, 2005; McCann et al., 2008). Experiment 1 examined the effects of food restriction on amphetamine-induced locomotion and conditioned place preference; the same rats were used to evaluate whether dopamine transporter activity was altered by food restriction or by amphetamine.

Despite a growing literature on the relationship between nutritional status and behavioral effects of indirect-acting dopamine agonists (e.g., amphetamine), little is known about food restriction and behavioral effects of direct-acting dopamine receptor drugs. Experiment 2 used catalepsy by the dopamine receptor antagonist raclopride and yawning by the direct-acting dopamine receptor agonist quinpirole, as indices of dopamine receptor sensitivity in food-restricted rats. Streptozotocin and food restriction can reduce circulating insulin (Carr, 1996) and streptozotocin decreases sensitivity to catalepsy produced by haloperidol (Sevak et al., 2005); thus, we hypothesized that food restriction reduces sensitivity to the behavioral effects of dopamine receptor agonists and antagonists. Since dopamine transporter activity co-varies with dopamine (D₂) receptor function (Jones et al., 1999; Dickinson et al., 1999), and repeated treatment with amphetamine can normalize the reduced transporter activity that occurs after streptozotocin treatment (Owens et al., 2005; Sevak et al., 2007b), the last experiment (3) examined whether the same amphetamine treatment (i.e., dose, route, and frequency of administration) that restores transporter activity in food-restricted rats also restores sensitivity to the behavioral effects of direct-acting dopamine drugs.

2. Materials and Methods

2.1 Subjects

Eighty-four male rats (Harlan Sprague-Dawley Inc., Indianapolis, Indiana, USA), weighing 250–350 g, were individually housed in a room ($24 \pm 1^\circ\text{C}$, $50 \pm 10\%$ relative humidity) under a 12 h light/dark cycle with water available continuously (experiments were conducted during the light period). Food was continuously available in the home cage, with the exception of 51 rats that received 10 g/day of food during part of the study (see below). An additional 9 animals were handled identically, received amphetamine under free-feeding conditions, and were studied at the same time; their data from a prior publication are included for comparison (Sevak et al., 2007b). Animals were maintained and experiments were conducted in accordance with the Institutional Animal Care and Use Committee, The University of Texas Health Science Center at San Antonio, and with the 1996 Guide for Care and Use of Laboratory Animals (Institute of Laboratory Animal Resources on Life Sciences, the National Research Council and the National Academy of Sciences).

2.2 Procedures

2.2.1 Amphetamine-induced locomotion and conditioned place preference—

Locomotion and conditioned place preference were studied according to methods described previously (Sevak et al., 2007b) using Lexan® polycarbonate (General Electric Structured Products, Mt. Vernon, Indiana, USA) chambers ($26 \times 61 \times 23$ cm high) located in sound-attenuating cubicles (MED Associates Inc., St. Albans, Vermont, USA). The floors of the chambers were removable and varied in texture across conditions. For half of the rats the drug was paired with the hole floor-texture (6 mm diameter, 9 mm center-to-center) and vehicle with the grid floor-texture (6×6 mm wire mesh supported by 5 mm diameter metal rods spaced 16 mm); pairings were opposite for the remaining rats. On test days, half (26×30.5 cm) of the floor had the hole texture and half the grid texture. Horizontal activity and position in the chamber were measured with four pairs of infrared photobeams (Multi-Varimex, Columbus Instruments, Columbus, Ohio, USA) positioned 4 cm above the floor of the chamber. The photo beams were separated by 15 cm with two photo beams located 8 cm from the ends of the chamber. Data were recorded and experimental events controlled by a computer, Multi-Varimex software, and a computer interface.

Conditioning sessions were preceded by a single 30-min habituation session (during which the floor was covered with a sheet of paper to prevent tactile contact with the conditioning stimuli). For the next 8 daily 30-min sessions free-feeding ($n=21$) and food-restricted ($n=19$; 10g/day; testing began one week after the start of restriction) rats received either amphetamine (1.78 mg/kg, i.p.) or saline (1 ml/kg, i.p.) and were placed in a chamber equipped with a floor with only one texture (hole or grid, alternating across days and specifically paired with either drug or vehicle). A single dose of 1.78 mg/kg amphetamine was chosen for the following reasons: this dose reliably produced conditioned place preference in normal and in streptozotocin-treated rats (Sevak et al., 2008); and four every-other-day injections of this dose normalized dopamine clearance and partially restored sensitivity to amphetamine-induced locomotion in streptozotocin-treated rats (Owens et al., 2005). Some rats (12 free-feeding and 12 food-restricted) received only saline in all eight conditioning trials to determine whether there was a preference for floor texture or side of the chamber. The conditioned place preference test was performed on the day after the last (eighth) conditioning trial, when all rats received saline (i.p.) immediately before being placed in a chamber with a floor having both textures; the times spent on the floor textures paired either with the amphetamine or with saline were recorded for 30 min.

2.2.2 *In vivo* chronoamperometric recordings of dopamine clearance—Clearance of exogenously applied dopamine was measured by high-speed chronoamperometry using the FAST-12 system (Quanteon, Nicholasville, Kentucky, USA) as described elsewhere (Owens et al., 2005). Oxidation potentials consisted of 100 msec pulses of +0.55 V with each pulse separated by 100 msec during which resting potential was held at 0.0 V. Carbon fiber electrodes were coated with Nafion® (5%) to prevent interference from anionic substances in the extracellular fluid. Electrodes were tested for sensitivity to the dopamine metabolite 3, 4-dihydroxyphenylacetic acid (DOPAC, 100 μ M). Using DOPAC as the challenge compound, all electrodes were calibrated *in vitro* with increasing concentrations of dopamine in 1 μ M increments. Only electrodes displaying a selectivity ratio greater than 1000:1 (i.e., dopamine:DOPAC) were used. Rats were anesthetized by chloralose (85 mg/kg, i.p.) and urethane (850 mg/kg, i.p.) and prepared for chronoamperometry (Owens et al., 2005). The electrode-micropipette recording assembly was lowered into the striatum (AP, +1.2; ML, +2.2; DV, -3.5 to -5.5) (Paxinos and Watson, 1986). Barrels were filled with dopamine (200 μ M) dissolved in 0.1 M phosphate buffered saline with 100 μ M ascorbic acid added as an antioxidant (pH 7.3–7.4). Different volumes were pressure ejected at 5-min intervals to deliver 5, 10, 20, 40 or 50 pmol dopamine, in randomized order among rats. Other than an anesthetic, no drug was present at the time of chronoamperometric measurements and rats had not received any other drug for at least one day. Chronoamperometry was performed the day after the place preference test in free-feeding rats and in food-restricted rats (i.e., 18 days after the beginning of food restriction). Six food-restricted rats that received only saline during behavioral testing were given free-access to food beginning on day 18 with chronoamperometry performed on day 23.

2.2.3 Quinpirole-induced yawning and raclopride-induced catalepsy—Yawning was defined as opening of the mouth such that the lower incisors were completely visible, followed by a closure in not more than 3 s. An earlier study (Sevak et al., 2007a) determined complete time- and dose-response functions for quinpirole-induced yawning and found that maximal effects were obtained 21–30 min after drug administration. This observation period (10 min beginning 20 min after acute drug administration) was used in the current study.

Catalepsy was studied using a bar test, whereby the forelimbs were placed on a horizontal, cylindrical metal bar (diameter, 1.0 cm; height, 10 cm) and the time until both forelimbs touched the table surface was recorded, up to a maximum of 120 s (Sevak et al., 2005). Raclopride-induced catalepsy was studied for 5 consecutive 25-min cycles. Saline (1 ml/kg) was administered i.p. at the beginning of the first cycle; increasing doses of raclopride were administered at the beginning of subsequent cycles such that the cumulative dose increased by 0.25 log units per injection (0.056, 0.178, 0.56, 1.78 mg/kg); catalepsy was assessed 24 min after each injection. For yawning and catalepsy, drugs were studied 3 days before food restriction (day -3), during food restriction (days 1, 3, 8, 13, 18), and after free access to food (days 23, 28).

Experiment 3 examined whether the same amphetamine treatment (dose, route, and frequency of administration) that restored dopamine clearance in food-restricted rats also restored sensitivity to quinpirole-induced yawning and raclopride-induced catalepsy in food-restricted rats. The behavioral effects of quinpirole and raclopride were studied in food-restricted rats that were treated with amphetamine and in the same rats after they were given free access to food (the effects of quinpirole and raclopride were not studied before food restriction in order to avoid any lasting effect that quinpirole or raclopride might have had on treatment with amphetamine). Thus, for comparison a separate group of 12 free-feeding rats was used to study quinpirole and raclopride. Other rats were given 10 g/day of food for 24 days with amphetamine-induced locomotion and conditioned place preference studied on days 8–17. The day after the place preference test (day 18) as well as 2 and 4 days thereafter (days 20 and 22),

quinpirole-induced yawning was assessed; raclopride-induced catalepsy was assessed on day 24, prior to rats being given free access to food.

2.2.4 Body weight and blood glucose concentration—On days when behavioral experiments were conducted rats were weighed immediately before experimental sessions and, on some occasions, blood glucose was measured immediately after sessions. Blood was collected from the tail vein and glucose concentration was measured with a commercially-available glucose testing kit (Prestige LX®; Home Diagnostics Inc., Ft. Lauderdale, Florida, USA). For food restricted rats, food was provided in the home cage immediately after glucose measurements.

2.3 Drugs

d-Amphetamine hydrochloride was provided by The Research Technology Branch, National Institute of Drug Abuse (Rockville, Maryland, USA). Raclopride tartrate, quinpirole dihydrochloride, dopamine hydrochloride, 3,4-dihydroxyphenylacetic acid (DOPAC), ascorbic acid, Nafion® (5%), chloralose and urethane were purchased from Sigma-Aldrich (St. Louis, Missouri, USA). Chloralose, urethane, dopamine, DOPAC and ascorbic acid were dissolved in 0.1 M phosphate buffered saline. Amphetamine, quinpirole and raclopride were dissolved in sterile 0.9% saline. Amphetamine, quinpirole and the solution of chloralose with urethane were administered i.p. in a volume of 1 ml/kg. Raclopride was administered s.c. in a volume of 1 ml/kg.

2.4 Data analyses

The effects of amphetamine on locomotor activity were analyzed by a three-factor ANOVA (feeding condition, treatment, and conditioning trial). Data for locomotor activity of rats that received only saline across 8 conditioning trials were analyzed by a two-factor ANOVA (feeding condition and conditioning trial). A two-factor ANOVA also was used to analyze difference scores in the conditioned place preference study.

Chronoamperometry data were analyzed using three signal parameters: 1) the maximal amplitude (in μM) of the signal; 2) clearance rate (T_c in nM/s), defined as the slope of the decay curve from 20–60% of maximal signal amplitude (i.e., the approximately linear portion of the decay); and 3) T_{80} , the time (s) for the signal to decline by 80% of the maximal amplitude. The effects of amphetamine on dopamine clearance (T_c) were analyzed using a three-factor ANOVA (group, treatment, and dopamine concentration). The effects of free-feeding on dopamine clearance (T_c) in previously food-restricted rats were analyzed using a two-factor ANOVA (group and dopamine concentration). The effects of amphetamine treatment on T_{80} and signal amplitude for 50 pmol dopamine were analyzed separately using a two-factor ANOVA (group and treatment) and the effects of free-feeding on T_{80} and signal amplitude of 50 pmol dopamine were analyzed using a one-factor ANOVA for groups. Depending on the experiment, body weight and glucose concentration data were analyzed by a one-factor (feeding condition) or three-factor (feeding condition, drug treatment, and day) ANOVA. Quinpirole-induced yawning and raclopride-induced catalepsy were analyzed using a two-factor ANOVA (dose and day). The data for raclopride-induced catalepsy were analyzed using a two-factor ANOVA (dose and days). Data for locomotor activity were analyzed using a two-factor ANOVA (treatment and trial). The effects of amphetamine on quinpirole-induced yawning were analyzed using a two-factor ANOVA (dose and group [free-feeding, food-restricted rats that received amphetamine]). The effects of free-access to food on quinpirole-induced yawning in food-restricted rats were analyzed using a two-factor ANOVA (dose and group). The effects of amphetamine treatment and feeding condition on raclopride-induced catalepsy were each analyzed using a two-factor ANOVA (dose and feeding condition). For

all tests, Tukey-Kramer post hoc tests were conducted when appropriate, and a two-tailed $P < 0.05$ was considered significant.

3. Results

3.1 Experiment 1

3.1.1 Food restriction did not affect amphetamine-stimulated locomotion but attenuated amphetamine-induced conditioned place preference—Locomotion was not different between free-feeding and food-restricted rats (no main effect of feeding conditions: $P > 0.20$) across four saline conditioning trials (no interaction between feeding condition and trial: $P > 0.20$; Fig. 1, left panel). Amphetamine (1.78 mg/kg) increased locomotor activity in free-feeding and food-restricted rats (significant main effect: $F_{(1,14)} = 156.4$, $P < 0.001$) across all four conditioning trials (no interaction; Fig. 1, left panel, compare open and closed triangles). In rats receiving only saline across all eight conditioning trials, locomotion did not vary across feeding conditions; however, locomotion varied across trials with significantly less locomotion on trial 6 as compared to trial 2 ($F_{(7,147)} = 2.1$, $P < 0.05$). Thus, food restriction did not affect baseline or amphetamine-stimulated locomotion.

Amphetamine produced conditioned place preference in free-feeding rats and in food-restricted rats (Fig. 1, right panel). There was a significant main effect of treatment ($F_{(1,36)} = 6.65$, $P < 0.05$) but not of feeding condition ($F_{(1,36)} = 0.24$), and there was no interaction ($F_{(1,36)} = 1.90$). Rats that received only saline across eight conditioning trials, did not show a significant preference for either floor-texture (data not shown).

3.1.2 Reduced dopamine clearance in food-restricted rats was normalized by repeated treatment with amphetamine and by free feeding—Decay of the dopamine signal was markedly slower in food-restricted rats that received only saline (Fig. 2, left panel, closed circles) as compared to other groups, showing notable effects of food restriction to decrease dopamine clearance rate in the striatum. The rate of dopamine clearance (T_c) was significantly decreased in saline-treated food-restricted rats (Fig. 2, right panel) across the range of dopamine concentrations tested ($F_{(4,64)} = 9.4$, $P < 0.001$). Changes in the T_c values for dopamine clearance were also reflected in the T_{80} time-course for dopamine clearance. T_{80} was significantly longer in saline-treated food-restricted rats as compared to saline-treated free-feeding rats ($F_{(1,16)} = 14.6$, $P < 0.01$), consistent with reduced dopamine clearance during food restriction. Although dopamine clearance was significantly reduced by food restriction, there was no significant difference in the signal amplitude produced by 50 pmol of dopamine between saline-treated food-restricted and saline-treated free-feeding rats ($P > 0.20$). Table 1 summarizes the values obtained for T_{80} values and signal amplitude at the highest concentration of dopamine ejected (50 pmol).

Four every-other-day injections of amphetamine did not affect chronoamperometric parameters in free-feeding rats (Fig. 2, open squares and open circles; Table 1). However, in food-restricted rats amphetamine significantly increased T_c values ($F_{(4,44)} = 9.3$, $P < 0.001$), and decreased T_{80} ($F_{(1,30)} = 11.8$, $P < 0.01$), such that these values in amphetamine-treated food-restricted rats were not different from corresponding values obtained in free-feeding rats. These results show that modest treatment with amphetamine normalizes dopamine clearance in food-restricted rats. Analysis of signal amplitude revealed a significant interaction of feeding condition with treatment ($F_{(1,30)} = 4.9$, $P < 0.05$); however, post-hoc tests failed to show a significant difference across feeding conditions in rats treated with amphetamine or saline. Thus, despite marked changes in other neurochemical parameters, amphetamine did not affect the signal amplitude of 50 pmol dopamine across feeding conditions ($P > 0.20$). After 18 days of restricted access to food followed by 5 days of free-access to food, T_c values increased significantly across the full range of dopamine concentrations so that these values were not

different from Tc values obtained in saline-treated free-feeding rats ($F_{(8,84)} = 6.1$, $P < 0.001$). T_{80} ($F_{(2,21)} = 9.1$, $P < 0.01$) was also normalized after free-access to food, such that there was no difference between values obtained in previously food-restricted rats and rats that were never food restricted (Table 1). The signal amplitude obtained with 50 pmol of dopamine did not change across feeding conditions ($P > 0.20$, Table 1). These data show that restricted access to food reduces dopamine clearance and that subsequent administration of amphetamine or free access to food normalizes dopamine clearance.

3.2 Experiment 2

3.2.1 Food restriction reduced sensitivity to quinpirole-induced yawning and raclopride-induced catalepsy—Quinpirole produced yawning in free-feeding rats (Fig. 3, left panel, symbols above “-3”). Yawning produced by a dose of 0.056 mg/kg quinpirole decreased over 18 days of food restriction ($F_{(7,35)} = 4.8$, $P < 0.001$), such that on days 13 and 18, yawning was significantly reduced as compared to results obtained in the same rats prior to food restriction. After 5 days of free feeding (day 23), yawning produced by a dose of 0.056 mg/kg quinpirole was no longer different from that observed before food restriction; after 10 days of free feeding (day 28) quinpirole-induced yawning (0.056 mg/kg) was significantly increased as compared to day 18 and not different from control (day -3). A similar pattern was obtained with a smaller dose of quinpirole, although these effects did not reach statistical significance ($P > 0.20$).

Raclopride produced catalepsy in free-feeding rats (Fig. 3, right panel, symbols above “-3”). Raclopride-induced catalepsy decreased over 18 days of food restriction ($F_{(28,196)} = 2.2$, $P < 0.001$), such that on day 18 catalepsy produced by 1.78 mg/kg of raclopride was significantly decreased as compared to results obtained prior to food restriction (Fig. 3, right panel, squares). Raclopride-induced catalepsy increased after only 5 days of free feeding, and after 10 days of free feeding, catalepsy produced by 1.78 mg/kg raclopride was significantly different from catalepsy observed on day 18 (i.e., last day of food restriction) and not different from control (i.e., free feeding on day -3). These data show that modest food restriction markedly reduces behavioral effects of a direct-acting dopamine receptor agonist (quinpirole) and antagonist (raclopride); that these effects were due to limited access to food was confirmed by a complete restoration of behavioral effects for quinpirole and raclopride after a brief period of free access to food (Fig. 3).

3.3 Experiment 3

3.3.1 Repeated treatment with amphetamine did not restore sensitivity to quinpirole-induced yawning or raclopride-induced catalepsy in food-restricted rats—Consistent with the results obtained in Experiment 1, in food-restricted rats amphetamine increased locomotion similarly across four conditioning trials ($P > 0.20$) and did not produce place preference (6.3 [-1.7, 14.3]; mean [95% confidence limits]; data not shown). Four every-other-day injections of amphetamine did not restore sensitivity to quinpirole-induced yawning ($P < 0.05$; Fig. 4, left panel, open circles and closed diamonds) or to raclopride-induced catalepsy ($P < 0.001$; Fig. 4, right panel, open circles and closed diamonds). Thus, the same amphetamine treatment that normalized dopamine clearance in food-restricted rats (Fig. 2) did not restore sensitivity to these behavioral effects of quinpirole and raclopride.

When the same (food-restricted) rats that received amphetamine were given unrestricted access to food (beginning on day 24), sensitivity was restored to quinpirole-induced yawning and to raclopride-induced catalepsy (Fig. 4, left and right panels, respectively). The quinpirole and raclopride dose-response curves determined in rats that had been food restricted, treated with amphetamine, then given free access to food (open diamonds, both panels, Fig. 4) were not significantly different from dose-response curves determined in a separate group of free-

feeding rats (open circles, both panels, Fig. 4). Results from this study indicate that free-access to food, and not amphetamine treatment, normalizes sensitivity to quinpirole-induced yawning and to raclopride-induced catalepsy, thereby demonstrating the marked effect that feeding condition has on the behavioral actions of direct-acting dopamine receptor drugs.

3.3.2 Food restriction decreased body weight and did not affect blood glucose concentration—Body weight decreased significantly when rats were restricted to 10 g/day of food and recovered when rats again had free access to food, regardless of drug treatment ($F_{(10,300)}=2.93$, $P<0.01$; Table 2). Blood glucose concentration did not change significantly across the same feeding conditions in the same rats ($F_{(2,60)}=0.54$, $P>0.20$; Table 2).

4. Discussion

These studies show that modest food restriction markedly reduces DA clearance and sensitivity to drugs acting at dopamine receptors. Amphetamine normalizes dopamine clearance in food-restricted rats without normalizing sensitivity to the behavioral effects of direct-acting dopamine receptor drugs, suggesting that dopamine transporter activity and dopamine receptor sensitivity are regulated differentially by pathways stimulated by amphetamine. Given the important role of dopamine in motivated behavior and of insulin-related pathways in the effects of drugs of abuse (e.g., Russo et al., 2007), these data suggest that nutritional status could be a critical determinant of vulnerability to drug abuse and that the same neurochemical substrates might contribute to the high comorbidity of eating disorders and substance abuse.

Food restriction decreased dopamine uptake, consistent with previous studies showing reduced dopamine transporter activity during food restriction (Zhen et al., 2006) or after experimentally-induced hypoinsulinemia (Patterson et al., 1998; Owens et al., 2005). This reduced transporter activity is not due to a loss of transporter, because binding of a high-affinity dopamine transporter ligand ($[^{125}\text{I}]$ RTI-121) does not change in the striatum or the nucleus accumbens of food-deprived rats (Patterson et al., 1998). RTI-121 can bind to cell surface or intracellular dopamine transporters and the activity of the transporter can be regulated by trafficking between the plasma membrane and intracellular regions (Zahniser and Doolen, 2001). Thus, food restriction might decrease dopamine transporter number at the cell surface, thereby reducing transport capacity.

Although blood glucose was unchanged in this study, insulin levels are known to vary across feeding conditions. For example, Carr (1996) reported that 14 days of 10 g/day of food (same as used in this study) significantly reduced plasma insulin. That decreased insulin might contribute to reduced dopamine transporter activity is supported by Patterson et al. (1998) who showed that dopamine uptake was reduced in striatal synaptosomes from fasted rats, and that addition of a physiological concentration of insulin normalized dopamine uptake. More recently, *in vivo* dopamine transporter activity was reduced in the striatum of rats made hypoinsulinemic by streptozotocin (Owens et al., 2005). Together with the observation that insulin can enhance dopamine transporter activity (Carvelli et al., 2002; Knusel et al., 1990), these data suggest that reduced insulin could underlie reduced dopamine clearance in food-restricted rats. However, food restriction can increase plasma corticosterone and decrease plasma leptin (Carr, 1996; Havel et al., 1998); although corticosterone directly affects striatal dopamine transporter activity, long-term elevations in corticosterone are correlated with increased dopamine uptake (Copeland et al., 2005). Like insulin, leptin can induce translocation of Glut4 to the plasma membrane and activate glucose uptake in a PI 3-kinase dependent manner (Benomar et al., 2006). PI 3-kinase pathways, which can be activated by insulin, increase dopamine uptake and recruit dopamine transporters to the plasma membrane (Carvelli et al., 2002). However, it is not clear whether leptin has a similar effect on dopamine transporters through a PI-3 kinase mechanism. Decreased food intake can result in decreased

water intake (Oatley and Tonge, 1969), potentially resulting in dehydration. Moreover, decreased circulating insulin can promote dehydration in brain which can be reversed by insulin replacement (Haraldseth et al., 1997). Thus, it is possible that dehydration altered diffusion of pressure-ejected dopamine.

Free access to food or administration of amphetamine (that restored dopamine clearance in streptozotocin-treated rats [Owens et al., 2005; Sevak et al., 2007b]) fully restored dopamine clearance in food-restricted rats. Although insulin is decreased by streptozotocin as well as by food restriction (Carr, 1996; Patterson et al., 1998), restoration of normal dopamine transporter activity by amphetamine suggests that non-insulin dependent mechanisms can dramatically alter (in this case restore) dopamine transporter activity. Amphetamine and insulin can modulate dopamine D₂ receptor density and activity in the brain (Amano et al., 2003; Seeman et al., 2002). Activation of dopamine D₂ receptors can enhance dopamine transporter activity (Cass and Gerhardt, 1994) and the dopamine D₂ receptor antagonist raclopride can prevent amphetamine-induced restoration of dopamine clearance in streptozotocin-treated rats (Sevak et al., 2007b); thus, dopamine D₂ receptors appear to indirectly mediate, at least in part, amphetamine-induced restoration of dopamine transporter activity in food-restricted rats.

A primary site of action for amphetamine is the dopamine transporter (e.g., Giros et al., 1996) yet despite reduced transporter activity, food restriction did not affect either spontaneous locomotion or amphetamine-stimulated locomotion (see also Stuber et al., 2002). The locomotor-stimulating effect of the first amphetamine injection in food-restricted rats suggests that mechanisms other than or in addition to the dopamine transporter contribute to this behavioral effect of amphetamine. Other components of dopaminergic systems could be modified by food restriction or other monoaminergic systems might play a role. Amphetamine binds to norepinephrine transporters (Rothman et al., 2001), resulting in increased neurotransmitter release (Han and Gu, 2006; Rothman and Baumann, 2003) that is correlated with locomotion and reward-related effects (Drouin et al., 2002; Hilber et al., 2005; Kuczenski and Segal, 2001). Dopamine transporter mRNA is decreased by streptozotocin whereas norepinephrine transporter mRNA is increased by streptozotocin (Figlewicz et al., 1996). Thus, norepinephrine transporters could provide a compensatory mechanism for regulating dopamine and other neurotransmitters under conditions where dopamine transporter activity is reduced.

Food restriction can enhance the effects of some drugs: decreased threshold for electrical brain stimulation after amphetamine is enhanced by food restriction (Carr, 1996); limited access to food can increase self-administration of amphetamine and other drugs (Carroll et al., 1981); and cocaine-induced conditioned place preference is enhanced with food restriction. Because the dose-response curve for amphetamine-induced conditioned place preference is inverted-U shaped (Stuber et al., 2002), an apparent decrease in the effectiveness of amphetamine in food-restricted rats could reflect an increase (i.e., shift leftward) or a decrease (shift rightward) in sensitivity. A single dose of amphetamine was used in this study specifically to test whether the treatment regimen that is known to restore dopamine transporter activity in streptozotocin-treated rats also restores transporter activity and sensitivity to direct-acting dopamine receptor agonists and antagonist in food-restricted rats.

One striking result from these studies is that food restriction significantly decreased quinpirole-induced yawning and raclopride-induced catalepsy, consistent with a recent study with other dopamine receptor agonists (Collins et al., 2008). Similar to results obtained with food restriction, streptozotocin-treated rats were less sensitive to catalepsy produced by the dopamine receptor antagonist haloperidol (Sevak et al., 2005). Decreased sensitivity to agonist-induced yawning and antagonist-induced catalepsy in streptozotocin-treated rats were reversed by insulin (Sevak et al., 2007a), providing support for the view that reduced circulating insulin

might contribute to a decreased sensitivity to the behavioral effects of direct-acting dopamine receptor drugs.

Dopamine D₂ receptor density in the caudate putamen was not increased by food restriction (Pothos et al., 1995), although the concentration-response curve for quinpirole-stimulated [³⁵S]GTPγS binding was slightly, but significantly, increased (Carr et al., 2003), and the locomotor-stimulating effects of quinpirole were enhanced (Carr et al., 2001). These data suggest that increased sensitivity to dopamine receptor drugs could result from increased dopamine receptor signaling. Results from the present study fail to confirm earlier reports showing increased sensitivity of food-restricted rats to the locomotor-stimulating effects of indirect-acting dopamine agonists (amphetamine); however, the current study clearly shows that the behavioral effects of direct-acting dopamine agonists and antagonists are markedly attenuated by food restriction. The bi-phasic nature of the dose-response curve for quinpirole-induced locomotion (Frantz and Van Hartesveldt, 1999) and quinpirole-induced yawning might contribute to apparent inconsistencies in the literature regarding the effect of food restriction on the behavioral actions or direct-acting dopamine receptor drugs. The responses to direct-acting dopamine receptor agonists and antagonists were both attenuated by food restriction and restored by free feeding, clearly demonstrating an effect of nutritional status on dopaminergic systems.

There is a complex interplay between dopamine transporter activity and dopamine receptor function. Deletion of dopamine transporters decreased dopamine D₂ receptor gene expression (Fauchey et al., 2000) and function (Jones et al., 1999) and either blockade or deletion of dopamine D₂ receptors reduced dopamine transporter activity (Cass and Gerhardt, 1994; Dickinson et al., 1999). Amphetamine restored dopamine transporter activity in food-restricted rats without restoring sensitivity to drugs acting directly at dopamine receptors, suggesting that dopamine transporter activity and dopamine D₂ receptor sensitivity do not always co-vary.

In summary, modest changes in food intake can profoundly affect dopamine transporter activity and sensitivity to the behavioral effects of direct-acting dopaminergic drugs. Several mechanisms could underlie the effects of food restriction on dopamine transporter activity and receptors, including reduced plasma insulin and leptin, increased plasma corticosterone, as well as compensatory changes in dopamine or other neurotransmitter systems. Elucidating the mechanisms that contribute to altered transporter activity and behavioral responsiveness as a consequence of altered feeding conditions may help identify biological risk factors contributing to the high co-morbidity of substance abuse and eating disorders.

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Fig. 1.

Locomotor activity (left panel; mean $\pm$ S.E.M. counts per 30-min session) during each of four every-other-day amphetamine (1.78 mg/kg, triangles) conditioning trials in free-feeding (open symbols, $n=9$; from Sevak et al., 2007b) and in food-restricted (closed symbols, $n=7$) rats that received saline (circles) on intervening days. Conditioned place preference produced by amphetamine (right panel) in free-feeding (open) and food-restricted (closed) rats. The same rats subsequently were studied with chronoamperometry. Data in the right panel represent the mean difference (min) between the time spent on the amphetamine-paired floor and the time spent on the saline-paired floor. Each value is the mean $\pm$ S.E.M. * $P<0.05$ compared with locomotion of food-restricted rats in saline-conditioning sessions. # $P<0.05$ compared with locomotion of free-feeding rats in saline-conditioning sessions.

Fig. 2.

Chronoamperometric parameters in free-feeding and in food-restricted rats that received only saline (open circles, $n=12$, data for 9 of 12 rats from Sevak et al., 2007b); closed circles, $n=6$) or amphetamine and saline on alternate days (open squares, $n=9$, from Sevak et al., 2007b; closed squares, $n=7$) during behavioral testing. The left panel shows the oxidation current (converted to a micromolar concentration using a calibration factor determined *in vitro*) produced by pressure ejection of 50 pmol of dopamine into the striatum. The right panel shows the corresponding summary data where the rates of dopamine clearance (T_c) are plotted as functions of increasing concentrations of dopamine. Data represent the mean $\pm$ S.E.M.; * $P<0.05$ compared with T_c values in all other groups at the corresponding concentration of dopamine.

Fig. 3.

Quinpirole-induced yawning (left panel; $n=6$ for each dose) and raclopride-induced catalepsy (right panel; $n=8$, cumulative dosing with all rats receiving all doses in each test) before (day -3) and during (days 1, 3, 8, 13, 18) food restriction as well as after (days 23 and 28) free access to food was resumed on day 18. Data represent the mean $\pm$ S.E.M. * $P<0.05$ compared with yawning produced by 0.056 mg/kg quinpirole prior to food restriction (day -3). ** $P<0.05$ compared with yawning produced by 0.056 mg/kg quinpirole on the last day of food restriction (day 18). # $P<0.05$ compared with catalepsy produced by 1.78 mg/kg raclopride prior to food restriction (day -3). ## $P<0.05$ compared with catalepsy produced by 1.78 mg/kg raclopride on the last day of food restriction (day 18).

Fig. 4.

Quinpirole-induced yawning (left panel) and raclopride-induced catalepsy (right panel) in free-feeding rats (open circles) and in the separate group of rats that had restricted access to food and received amphetamine (closed diamonds) and after these rats were given 5 days of free access to food (open diamonds). Each symbol represents the mean $\pm$ S.E.M. of 4 rats (left panel; each rat received one of three doses of quinpirole [acute-dosing procedure]) or 12 rats (all rats received all doses of raclopride [cumulative-dosing procedure], right panel). In the right panel, the points above 'S' indicate the effects obtained with saline in the first cycle. * $P < 0.05$ compared with yawning produced by 0.056 mg/kg quinpirole in all other groups. # $P < 0.05$ compared with catalepsy produced by the corresponding dose of raclopride in all other groups.

Table 1

Chronoamperometric parameters for clearance of 50 pmol dopamine in striatum of rats treated with saline or amphetamine (1.78 mg/kg) across feeding conditions.

Group	N	Signal amplitude (μM)	T ₈₀ (s)
Free feeding, saline	12	12.1 ± 1.1 ^a	52.8 ± 4.8
Free feeding, amphetamine	9	10.5 ± 0.6	57.9 ± 4.9
Food restricted, saline	6	10.3 ± 1.8	162.5 ± 40.7 ^b
Food restricted, amphetamine	7	14.0 ± 1.1	60.1 ± 6.9
Free feeding (prior restricted)	6	14.3 ± 1.2	66.7 ± 7.6

^aData represent the mean ± S.E.M. for the number of animals indicated. All rats had undergone behavioral testing (locomotion and conditioned place preference) prior to chronoamperometry studies.

^bP<0.05 compared to T₈₀ in all other groups.

Table 2

Body weight and blood glucose concentration across feeding conditions.

	Free feeding	Food-restricted	Free-feeding
<u>Experiment 1</u>			
Weight	292.8 ± 2.9 ^a (1) ^b	243.8 ± 2.1 ^c (17)	297.7 ± 3.3 (23)
Glucose	86.5 ± 2.0 ^d (1) ^b	86.0 ± 13.5 (17)	84.5 ± 6.3 (23)
<u>Experiment 2</u>			
Weight	295.3 ± 3.3 (-3)	239.9 ± 4.1 ^c (18)	291.0 ± 3.7 (23)
Glucose	94.3 ± 6.1 (-3)	86.7 ± 7.1 (18)	93.9 ± 6.8 (23)
<u>Experiment 3</u>			
Weight	291.2 ± 2.2 (1)	226.5 ± 2.2 ^c (24)	287.8 ± 1.9 (30)
Glucose	107.2 ± 15.1 (1)	114.9 ± 14.3 (17)	95.9 ± 13.4 (30)

^aWeight in g (mean ± S.E.M.) for rats in each study that sequentially were studied under all three conditions (n=6 for Experiment 1, n=20 for Experiment 2, and n=12 for Experiment 3).

^bValues in parentheses indicate day of the study the measurement was made relative to the beginning of food restriction (see Methods for details).

^cP<0.05 compared to free-feeding conditions.

^dBlood glucose concentration in mg/dl (mean + S.E.M.).