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Food restriction alters pramipexole-induced yawning, hypothermia, and locomotor activity in rats: Evidence for sensitization of dopamine D2 receptor-mediated effects

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Abbreviations: L-741,626: 3-[4-(4-Chlorophenyl)-4-hydroxypiperidin-l-yl]methyl-1*H*-indole; PE: penile erection; PG01037: *N*-{4-[4-(2,3-Dichlorophenyl)-piperazin-1-yl]-trans-but-2-enyl}-4-pyridine-2-yl-benzamide hydrochloride; physostigmine: (3aS)-cis-1,2,3,3a,8,8a-hexahydro-1,3a,8-trimethylpyrrolo[2,3-b]indol-5-ol methylcarbamate hemisulfate; pramipexole: *N*'-propyl-4,5,6,7-tetrahydrobenzothiazole-2,6-diamine dihydrochloride; TFMPP: *N*-[3-(Trifluoromethyl)phenyl]piperazine hydrochloride

Abstract:

Food restriction enhances sensitivity to the reinforcing effects of a variety of drugs of abuse including opiates, nicotine, and psychostimulants. Food restriction has also been shown to alter a variety of behavioral and pharmacological responses to dopaminergic agonists including an increased sensitivity to the locomotor stimulatory effects of direct- and indirect-dopamine agonists, elevated extracellular dopamine levels in responses to psychostimulants, as well as suppression of agonist-induced yawning. Behavioral and molecular studies suggests that augmented dopaminergic responses observed in food-restricted animals result from a sensitization of the dopamine D2 receptor, however, little is known about how food restriction affects dopamine D3 receptor function. The current studies were aimed at better defining the effects of food restriction on D2 and D3 receptor function by assessing the capacity of pramipexole to induce yawning, penile erection (PE), hypothermia, and locomotor activity in free-fed and food-restricted rats. Food restriction resulted in a suppression of pramipexole-induced yawning, a sensitized hypothermic response, and an enhanced locomotor response to pramipexole, effects that are suggestive of an enhanced D2 receptor activity; no effect on pramipexole-induced PE was observed. Antagonist studies further supported a food restriction-induced enhancement of D2 receptor activity as the D2 antagonist, L-741,626, recovered pramipexole-induced yawning to free-fed levels, while yawning and PE were suppressed following pretreatment with the D3 antagonist, PG01037. The results of the current studies suggest that food restriction sensitized rats to the D2-mediated effects of pramipexole while having no effect on the D3-mediated effects of pramipexole.

Introduction:

Food restriction affects the function of a variety of neurotransmitter systems including dopaminergic (Carlson et al., 1988; Carr et al., 2003), serotonergic (Gur et al., 2003; Jahng et al., 2007), and cholinergic (Persinger et al., 2002) systems, and is known to alter the effects of drugs with diverse mechanisms of action. For instance, food restriction has been shown to enhance the reinforcing properties of opiates (Carroll et al., 1979), ethanol (Meisch and Thompson, 1973), nicotine (Donny et al., 1998), and psychostimulants (Carroll et al., 1981; Macenski and Meisch, 1999), elevate extracellular dopamine levels in the nucleus accumbens core in response to psychostimulants (Cadoni et al., 2003), and enhance the locomotor stimulatory effects of both direct- (Carr et al., 2001; 2003), and indirect-dopamine agonists (Deroche et al., 1993; Cadoni et al., 2003). A growing literature supports the notion that the sensitized behavioral responses to D2/D3 agonists, such as quinpirole, observed in food-restricted rats result from an enhancement of the functional coupling of Gi G-proteins to D2 receptors, and not an increase in D2 receptor expression (Pothos et al., 1995; Carr et al., 2003). Alternatively, changes in D3 receptor expression and/or function could also explain the behavioral sensitivity observed in food-restricted animals, however, little is known about how food restriction affects D3 receptors.

For example, previous studies suggest that the enhancement of quinpirole-induced locomotor activity observed in food-restricted rats results from an enhanced functional activity of the D2 receptor (Carr et al., 2003). However, this effect could also be explained by a tolerance, or down-regulation of the D3 receptor as the inhibition of locomotor activity by D2/D3 agonists has been hypothesized to be mediated by the D3 receptor (Svensson et al., 1994). Interpretation of changes in D2/D3 agonist-induced locomotor activity is further complicated by the fact that D2-like antagonists often alter locomotor activity on their own. In addition to their effects on

locomotor activity, D2/D3 agonists are known to possess a variety of other behavioral effects including the induction of yawning (Yamada et al., 1986), penile erection (PE) (Melis et al., 1987), and hypothermia (Faunt and Crocker., 1987). While post-synaptic D2/D3 receptors within the mesolimbic dopaminergic pathway are thought to mediate the locomotor effects of D2-like agonists (Levant, 1997), the induction of yawning and PE by D2-like agonists is thought to be mediated by postsynaptic D2-like receptors on oxytocinergic neurons in the paraventricular nucleus (Argiolas and Melis, 1998). Recently, D3-selective antagonists have been shown to produce selective rightward shifts of the ascending limbs, while D2-selective antagonists shifted only the descending limbs of the dose-response curves for D2-like agonist-induced yawning and PE (Collins et al., 2005; 2007; submitted) suggesting that the induction of yawning and PE by D2/D3 agonists is mediated by a selective activation of the D3 receptor while the inhibition of yawning and PE observed at higher doses is mediated by agonist activity at the D2 receptor. D2 receptors have also been reported to mediate the hypothermic effects of D2-like (Boulay et al., 1999; Chaperon et al., 2003; Collins et al., 2007). Interestingly, food restriction has been shown to suppress apomorphine-induced yawning (Nasello et al., 1995), an effect that is suggestive of a decrease in D3 receptor expression and/or function. However, based on the findings that yawning is differentially mediated by the D3 (induction) and D2 (inhibition) receptors, the suppression of D2/D3 agonist-induced yawning observed during food restriction could also result from an enhanced or sensitized D2 response.

The present studies were aimed at determining the effects of food restriction on D2 and D3 receptor function in rats. Thus, the capacity of pramipexole to induce yawning, PE, hypothermia, and locomotor activity was first assessed in free-fed rats, assessed in the same rats following 10 days of food restriction, and then reassessed following 7 days of free feeding. Additionally, antagonists selective for the D2 and D3 receptors were assessed for their capacity to alter the induction of yawning and PE in both free-fed and food-restricted rats to determine

whether changes in D2 and/or D3 receptor function and/or sensitivity could be observed. Finally, as yawning can be induced by a variety of mechanisms, the capacity of the cholinesterase inhibitor, physostigmine, and the 5-HT2 receptor agonist, TFMPP, to induce yawning was assessed in free-fed and food-restricted rats. Results from the study of the effects of food restriction on the behavioral effects of pramipexole alone, and in combination with antagonists suggest that food restriction effectively sensitized rats to the D2-mediated effects of pramipexole while not altering the function and/or sensitivity of the D3 receptor.

Methods:

Subjects. Male Sprague-Dawley rats (250-300g) were obtained from Harlan (Indianapolis, IN) and individually housed for the duration of the experiments in a temperature and humidity controlled environment on a 12-h dark/light cycle with lights on at 7:00AM. With the exception of the food-restricted condition, and during observational periods, rats had free access to standard Purina rodent chow and water. All studies were performed in accordance with the Declaration of Helsinki and with the Guide for the Care and Use of Laboratory Animals, as adopted and promulgated by the National Institutes of Health, and all experimental procedures were approved by the University of Michigan Committee on the Use and Care of Animals.

Surgical implantation of temperature and locomotor probes. Rats were anesthetized with ketamine (90mg/kg, i.p.) and xylazine (10mg/kg; i.p.) and their abdominal area was shaved and cleaned with Betadine swabs and alcohol prior to implantation of the radio-telemetric probes (E-4000 E-Mitter, Mini-Mitter, Bend, OR, USA). A small rostral-caudal incision was made in the abdominal wall to allow for the insertion of the probe. The abdominal wall was closed with absorbable, 5.0 chromic gut sutures and the skin was closed with 5.0 Ethilon® suture. Rats were allowed at least one week to recover before experiments began.

Behavioral observations. On the day of testing, rats were weighed and transferred from their home cage to a test cage (48cm x 23cm x 20cm, clear rodent cage with cob bedding). Dose-response curves for agonist-induced yawning, PE, and hypothermia were generated using a multiple dosing procedure. Briefly, following a 30 min habituation period, rats were administered either vehicle or antagonist 30 min. prior to the first dose of agonist**,** with each successive dose of agonist separated by 35 min. Behavioral observations began immediately after each injection, and the total number of yawns and PE were recorded for 25 min thereafter. Yawning

was defined as a prolonged (~1s), wide opening of the mouth followed by a rapid closure, while PE was defined by an upright posture, repeated pelvic thrusts, and an emerging, engorged penis which was typically followed by genital grooming. All experiments were conducted between 12:00PM and 6:00PM.

Dietary conditions. Rats had free access to standard Purina rodent chow during free-fed conditions, and maintained at ~ 85% of their free-feeding weight with ~ 20 g of Purina rodent chow per day during food-restricted conditions; water was always freely available. Rats were fed at 6:00 each day during the food-restricted condition, and remained on the restricted diet for a period of 10 days prior to experimental sessions. Following the generation of dose-response curves in the food-restricted condition all rats were returned to the free-fed condition for a period of 7 days prior re-establishing the free-fed dose-response curves. Rats were subsequently returned to the food-restricted condition for a period of 10 days prior to antagonist studies.

Pramipexole-, physostigmine- and TFMPP-induced yawning and penile erection. The effect of food restriction on yawning and PE induced by the D3-preferring agonist, pramipexole, the cholinesterase inhibitor, physostigmine, and the non-selective 5HT-2 agonist, TFMPP, were assessed using a multiple dosing procedure as described above. Doses of pramipexole (vehicle, 0.01, 0.032, 0.1, 0.32, and 1.0 mg/kg; s.c.), physostigmine (vehicle, 0.032, 0.1, and 0.32 mg/kg; i.p.) and TFMPP (vehicle, 1.0, 3.2, and 10.0 mg/kg; s.c.) were administered at 35 min intervals with observations occurring for 25 min immediately after each injection. Doses for the multiple dosing procedure were based on doses that induced yawning in single dosing procedures (Collins et al., 2005). Dose-response curves for the free-fed, food-restricted, and free-fed conditions were generated in separate groups of rats (n=8) on the last day of each dietary condition (day 7 of free-fed and day 10 of food-restricted).

Effects of D3- and D2-selective antagonists on pramipexole-induced yawning and penile erection in free-fed and food-restricted rats. The ability of the D3 antagonist, PG01037, and the D2 antagonist, L-741,626, to alter the induction of yawning and PE induced by pramipexole was assessed in free-fed and food-restricted rats using the multiple dosing procedure described above with either PG01037 (32.0 mg/kg; s.c.), L-741,626 (1.0 mg/kg; s.c.), or vehicle was administered 30 min prior to the first dose of pramipexole. The food-restricted rats were the same group of rats that had previously been used to assess the effects of food restriction and re-feeding, while the free-fed rats were experimentally naïve. Following 10 days of food restriction, dose-response curves were generated for each rat with antagonists and vehicle administered in random order. Experimental sessions were separated by at least 72 hrs to allow for a drug washout period.

Effects of D2-selective antagonists on physostigmine- and TFMPP-induced yawning and penile erection during food restriction. The ability of the D2 antagonist, L-741,626 to alter the induction of yawning and PE by physostigmine or TFMPP during food restriction was assessed using the multiple dosing procedure described above with L-741,626 (1.0 mg/kg; s.c.) or vehicle administered, in random order, 30 min prior to the first dose of each agonist. Experimental sessions were separated by at least 72 hrs to allow for a drug washout period.

Pramipexole-induced hypothermia and locomotor activity. The effects of food restriction on pramipexole-induced hypothermia and locomotor activity were assessed using the same multiple dosing procedure as described for the yawning and PE studies. On the day of testing, rats were weighed and returned to their home cages which were placed onto a receiving pad (ER-4000 Receiver, Mini-mitter, Bend, OR) to allow for the real time detection and recording of core body temperature and ambulatory locomotor activity. Temperature and locomotor activity measurements were taken every min with at least 45 min of baseline data recorded prior to

vehicle injection. Doses of pramipexole (vehicle, 0.01, 0.032, 0.1, 0.32, and 1.0 mg/kg; s.c.) were administered every 35 min, and rats were removed from the receivers for a period of 5 min to allow for injections to be administered, but were otherwise uninterrupted. Dose-response curves for pramipexole-induced hypothermia and locomotor activity were generated in the free-fed, food-restricted, and free-fed conditions using the same experimental timeline as described above. All experiments were carried out between the hours of 9:00 AM and 3:00 PM.

Drugs. Pramipexole was generously provided by Drs. Shaomeng Wang and Jianyong Chen (University of Michigan, Ann Arbor, MI), and PG01037 (*N*-{4-[4-(2,3-Dichlorophenyl)-piperazin-1-yl]-trans-but-2-enyl}-4-pyridine-2-yl-benzamide hydrochloride) by Drs. Amy H. Newman and Peter Grundt (Medicinal Chemistry Section-NIDA, Baltimore, MD). L-741,626 (3-[4-(4-Chlorophenyl)-4-hydroxypiperidin-l-yl]methyl-1*H*-indole) was obtained from Tocris (Ellisville, MO), and physostigmine [(3aS)-cis-1,2,3,3a,8,8a-hexahydro-1,3a,8-trimethylpyrrolo[2,3-b]indol-5-ol methylcarbamate hemisulfate], and TFMPP [*N*-[3-(Trifluoromethyl)phenyl]piperazine hydrochloride] were obtained from Sigma Aldrich (St. Louis, MO). All drugs were dissolved in sterile water with the exception of L-741,626, which was dissolved in 5% ethanol with 1M HCl, and PG01037 which was dissolved in 10% β-cyclodextrin. All drugs were administered in a volume of 1 ml/kg, s.c., with the exception of physostigmine which was delivered i.p..

Data analysis. Dose-response curves for agonist-induced yawning, PE, hypothermia, and locomotor activity were generated with eight rats per drug. Yawning and PE are expressed as mean number of yawns or PE during the 25 min observation period ± standard error of the mean (S.E.M.). Change in core body temperature is expressed as the mean ± S.E.M. difference in core body temperature as measured 30 min after each injection as compared to the core body temperature measured 1 min prior to the vehicle injection. Locomotor activity is expressed as the mean ± S.E.M. of the total number of ambulatory locomotor activity counts

during the 30 min period between each injection. A one-way, repeated-measures ANOVA with post hoc Dunnett's tests were used to determine significant differences in agonist-induced yawning, hypothermia, and locomotor activity as compared to vehicle (GraphPad Prism; GraphPad Software Inc., San Diego, CA). A two-way, repeated-measures ANOVA with post hoc Bonferroni tests were used to determine significant differences in agonist-induced yawning, hypothermia, and locomotor activity between the three dietary conditions (free-fed 1, food-restricted, and free-fed 2), as well as between yawning in vehicle and antagonist pretreated rats. Friedman tests with post-hoc Dunn's tests were used to determine significant levels of agonist-induced PE as compared to vehicle, as well as the effects of dietary condition, and antagonist pretreatment on agonist-induced PE.

Results:

Food restriction on pramipexole-, physostigmine-, and TFMPP-induced yawning and penile erection. Dose-response curves for pramipexole-induced yawning and PE are shown in Figures 1a and b. Dose-dependent increases in pramipexole-induced yawning and PE were observed over low doses, with inhibition of both responses observed at higher doses resulting in an inverted U-shaped response curve for both yawning and PE in all dietary conditions. Significant increases in yawning during the free-fed 1 and free-fed 2 conditions were observed at doses of 0.032 ($p<0.01$ in Free-Fed1 and $p<0.05$ in Free-Fed2) and 0.1 mg/kg ($p<0.001$ for both), while significant increases in yawning were only observed with a dose of 0.032 mg/kg ($p<0.01$) pramipexole during the food-restricted condition. Pramipexole significantly increased the occurrence of PE at a dose of 0.1 mg/kg ($p<0.05$) in both free-fed conditions, however, this effect failed to reach significance in the food-restricted condition. As shown in Figure 1a, 10 days of food restriction resulted in a suppression of pramipexole-induced yawning, with significantly lower levels of yawning observed at doses of 0.032 and 0.1 mg/kg pramipexole ($p<0.001$ for both); yawning returned to baseline levels following 7 days of unrestricted access to food. Unlike with yawning, restricting daily food intake did not alter the capacity of pramipexole to induce PE (Figure 1b); however, slight increases in the number of PEs were observed in the vehicle condition, as well as at lower doses of pramipexole during food restriction. Importantly, the dose-response curves for pramipexole-induced yawning and PE obtained in the current studies using the multiple-dose procedure are similar to those obtained using single-dose procedures (Collins et al., 2005; 2007; submitted), however, slight differences in the magnitude of the yawning and PE response were observed.

Similar to previous reports (e.g., Collins et al., 2005), physostigmine and TFMPP induced dose-dependent increases in yawning behavior, with inhibition of yawning observed at higher doses

resulting in an inverted U-shaped dose-response curve during the initial free-fed condition (Figures 1c and 1e). While significant increases in yawning were observed at doses of 0.1 mg/kg physostigmine ($p<0.01$) and 3.2 mg/kg TFMPP ($p<0.01$) during the free-fed condition, physostigmine and TFMPP both failed to induce significant levels of yawning at any dose tested during the food-restricted condition. As with pramipexole, food restriction significantly suppressed physostigmine- and TFMPP-induced yawning, with significant inhibition of yawning observed at doses of 0.1 ($p<0.001$) and 0.32 mg/kg ($p<0.01$) physostigmine, and 1.0 ($p<0.05$) and 3.2 mg/kg ($p<0.001$) TFMPP. Re-feeding partially recovered physostigmine-induced yawning, however, TFMPP-induced yawning remained suppressed, even after 7 days of unrestricted access to food. No significant increases in PE were observed at any dose of physostigmine or TFMPP tested (Figure 1d and 1f).

Food restriction on pramipexole-induced hypothermia and locomotor activity. The effects of food restriction on pramipexole-induced changes in core body temperature and locomotor activity are shown in Figure 2. Food restriction had a significant effect on both the hypothermic and locomotor stimulatory effects of pramipexole. Significant decreases in core body temperature were observed in all dietary conditions, with doses of 0.32 and 1.0 mg/kg pramipexole ($p<0.01$ for both) resulting in significant decreases in core body temperature during both free-fed conditions, and doses of 0.1 ($p<0.05$), 0.32 ($p<0.01$), and 1.0 ($p<0.01$) mg/kg pramipexole resulting in significant decreases in core body temperature during the food-restricted condition. While the dose-response curve for pramipexole-induced hypothermia was shifted to the left, significant differences between the hypothermic responses in the food-restricted, and free-fed conditions were only observed at a dose of 1.0 mg/kg pramipexole; an effect that persisted even after rats were returned to the free-fed condition (Figure 2a). As with pramipexole-induced yawning and hypothermia, food restriction significantly altered the locomotor-stimulatory effects of pramipexole (Figure 2b). While there were no significant effects

of pramipexole on locomotor activity in either free-fed condition, a significant increase in locomotor activity was observed following a dose of 0.32 mg/kg pramipexole ($p<0.01$) during the food-restricted condition; locomotor activity returned to baseline levels following return to the free-fed condition. Importantly, the dose-response curves for pramipexole-induced hypothermia obtained in the current studies using the multiple-dose procedure are similar to those obtained using single-dose procedures (Collins et al., 2007).

D2- and D3-selective antagonism of pramipexole-induced yawning and penile erection in free-fed and food-restricted rats. The effects of the D2-selective antagonist, L-741,626, and the D3-selective antagonist, PG01037, on pramipexole-induced yawning and PE induced are shown in Figure 3. Similar to previous reports using single dosing procedures (Collins et al., 2005; 2007; submitted), in free-fed rats, pretreatment with the D3-selective antagonist resulted in a selective rightward shift of the ascending limbs of the yawning and PE dose response curves with significant reductions in the levels of yawning and PE observed following a dose of 0.1 mg/kg pramipexole ($p<0.05$ for both). Pretreatment with the D2 antagonist, L-741,626, resulted in a reversal of the inhibition of yawning and PE by higher doses, while having no effect on yawning or PE induced by lower doses of pramipexole (Figure 3a and 3b). Similar to the effects of the antagonists in free-fed rats, pretreatment of food-restricted rats with the PG01037 resulted in a significant inhibition of pramipexole-induced yawning and PE with significant reductions in the levels of yawning and PE observed following doses of 0.032 ($p<0.05$) and 0.1 mg/kg pramipexole ($p<0.01$), respectively (Figure 3c and 3d). However, unlike in the free-fed condition in which the effects of L-741,626 were only observed at a dose of 0.32 mg/kg pramipexole, pretreatment of food-restricted rats with L-741,626 effectively restored the capacity of pramipexole to induce yawning, with the resulting dose-response curve (Figure 3c) very similar to that observed in free-fed rats (Figure 1). Pretreatment with L-741,626 also

significantly altered pramipexole-induced PE with a significant increase in the number of PEs observed following a dose of 0.32 mg/kg pramipexole (Figure 3d).

D2-selective antagonism of physostigmine- and TFMPP-induced yawning and penile erection during food restriction. Similar to the effects of food restriction on pramipexole-induced yawning, food restriction also suppressed physostigmine- and TFMPP-induced yawning. However, unlike with pramipexole-induced yawning and PE, the inhibition of physostigmine- and TFMPP-induced yawning resulting from food restriction was not reversed by pretreatment with L-741,626 (Table 1), although a non-significant increase the number of yawns observed following a dose of 0.1 mg/kg physostigmine from 1.4 ± 0.6 yawns to 4.8 ± 2.3 yawns was observed.

Discussion:

Food restriction has been shown to enhance and/or sensitize the D2-mediated behavioral and molecular effects of dopaminergic agonists (Deroche et al., 1993; Candoni et al., 2003; Carr et al., 2001; 2003), however, the effects of food restriction on the function and/or sensitivity of D3 receptors is not well understood. The current studies were aimed at characterizing the effects of food restriction on the induction of putative D3- (yawning and PE), and D2-mediated (hypothermia and locomotor activity) effects by the D3-preferring agonist, pramipexole (~90-fold selective for D3 over D2 receptors in vitro; Millan et al., 2002). Food restriction differentially affected the D3-mediated effects of pramipexole, suppressing pramipexole-induced yawning while not altering pramipexole-induced PE. Food restriction had similar effects on the D2-mediated effects of pramipexole, enhancing and/or sensitizing rats to the hypothermic and locomotor stimulatory effects of pramipexole. While food restriction altered both D2- and D3-mediated behavioral effects of pramipexole, convergent evidence from the effects of pramipexole alone, and in combination with D2- and D3-selective antagonists suggests that food restriction sensitized rats to the D2-mediated effects of pramipexole while not altering the function and/or sensitivity of D3 receptors.

Similar to previous reports in free-fed rats (Collins et al., 2005; 2007; submitted), pramipexole induced yawning and PE over low doses with inhibition of both behaviors occurring at higher doses that also corresponded to the induction of hypothermia, suggestive of a selective activation of D3 receptors at low doses, and a concomitant D2 receptor activation at higher doses. Food restriction affected pramipexole-induced yawning, locomotor activity, and hypothermia, but did not alter pramipexole-induced PE. While the enhanced and/or sensitized locomotor stimulatory and hypothermic effects of pramipexole suggest that food restriction enhanced the function and/or sensitivity of D2 receptors in the mesolimbic pathway (Ouagazzal

and Creese, 2000) and anterior hypothalamus/preoptic area (Lin et al., 1982), respectively, the effects of food restriction on pramipexole-induced yawning and PE are less clear. Previous studies (Melis et al., 1987; Collins et al., submitted) suggest that D2-like agonist-induced yawning and PE are similarly mediated by D3 (induction) and D2 (inhibition) receptors within the paraventricular nucleus of the hypothalamus, yet food restriction differentially affected pramipexole-induced yawing and PE, suppressing yawning while not affecting the induction of PE. While it is possible that these effects represent a decreased function and/or sensitivity of only some D3 receptors, the effects of food restriction on pramipexole's D2-mediated effects, as well as a comparison of the effects of D3- and D2-selective antagonists on pramipexole-induced yawning and PE suggest that the food restriction-induced suppression of yawning resulted from changes in the function and/or sensitivity of D2, but not D3 receptors.

Unlike the hypothermic effects of D2-like agonists which have been shown to be mediated by D2, but not D3 receptors (Boulay et al., 1999; Chaperon et al., 2003; Collins et al., 2007), the induction of yawning by D2-like agonists has been shown to be mediated by the D3 receptor, with the subsequent inhibition of yawning resulting from a concomitant D2 receptor activation (Collins et al., 2005; 2007; submitted). Therefore, although decreases in D3 receptor function could explain the suppressed yawning response in food-restricted rats, increases in D2 receptor function and/or sensitivity would also be expected to suppress pramipexole-induced yawning. Support for the notion that food restriction-induced changes in D2, but not D3 receptor function and/or sensitivity was provided by the effects of the D3-selective, PG01037 (~133-fold selective for D3 over D2 receptors in vitro; Grundt et al., 2005; 2007), and D2-selective, L-741,626 (~13-fold selective for D2 over D3 receptors in vitro; Millan et al., 2000) antagonists on pramipexole-induced yawning.

Similar to previous reports (Collins et al., 2005; 2007; submitted), pretreatment with the D3-selective antagonist, PG01037, inhibited pramipexole-induced yawning and PE in both the free-fed and food-restricted conditions, regardless of whether the responses were affected by food restriction. These data not only support a role for the D3 receptor in the induction of PE by pramipexole, but also suggest that food restriction does not alter, at least some of, the D3-mediated behavioral effects of pramipexole. Likewise, the D2-selective antagonist, L-741,626, had similar effects in both free-fed and food-restricted rats, reversing the inhibition of yawning and PE observed at higher doses while not altering their induction at lower doses of pramipexole. However, while L-741,626 increased the low levels of yawning observed at higher doses in both free-fed and food-restricted rats, this effect was observed at a lower dose of pramipexole in the food-restricted (0.1 mg/kg) compared to free-fed condition (0.32 mg/kg), suggestive of a leftward shift in the D2-mediated effects of pramipexole when food was restricted. Moreover, comparison of the effects of L-741,626 on pramipexole-induced yawning in food-restricted and free-fed rats suggests that the D2-selective antagonist was not only effective at reversing the D2-mediated inhibition of yawning in both conditions, but also that it was capable of unmasking pramipexole's D3-mediated effects, effectively restoring the food restricted yawning dose-response curve to that of free fed levels. When taken together with the enhanced hypothermic and locomotor stimulatory effects of pramipexole, these data strongly suggest that food restriction enhanced the function and/or sensitivity of D2 receptors in mesolimbic (locomotor activity) and hypothalamic (hypothermia and yawning) brain regions, while not altering the function and/or sensitivity of D3 receptors.

Interestingly, dopaminergic, cholinergic, and serotonergic systems within the corticostriatal and hypothalamic regions have been implicated in a variety of aspects of feeding behavior including, motor control, motivation to obtain food, food intake, and satiation (e.g., Leibowitz and Alexander, 1998; Kelley et al., 2005). Thus, food restriction-induced increases in the function

and/or sensitivity of mesolimbic and/or hypothalamic D2 receptors may be beneficial for several reasons. First, increased D2 receptor activity within the nucleus accumbens may serve to increase the motivational aspects of food or the orientation towards food-related stimuli (e.g., Robinson and Berridge, 1993; Kelley et al., 2005), while changes in D2 receptor activity affecting the integration of accumbal and hypothalamic dopamine systems may also alter motor control, food intake, and feeding duration (Kelley et al., 2005; Meguid et al., 2000). Moreover, dopaminergic neurons within the hypothalamus are known to interact with other neurotransmitters and neurohormones (i.e., serotonin and orexin) and thus changes in the function and/or sensitivity of hypothalamic D2 receptors may indirectly influence a variety of behaviors including arousal, food preference (i.e., carbohydrate vs. protein / palatable vs. non-palatable), and satiety (Leibowitz et al., 1990; Meguid et al., 2000; Isaac and Berridge, 2003; Alberto et al., 2006; Palmiter et al., 2007).

Unlike the effects of food restriction on pramipexole-induced behaviors which generally returned to baseline levels following 7 days of re-feeding, decrements in physostigmine- and TFMPP-induced yawning were still evident following 7 days of unrestricted access to food suggesting a prolonged effect of food restriction on cholinergic and serotonergic function. Interestingly, as both cholinergic and serotonergic systems have been strongly implicated in satiety mechanisms (e.g., Leibowitz et al., 1990; Meguid et al., 2000; Kelley et al., 2005), it is possible that a persistent decrease in cholinergic and serotonergic function may allow for increased levels of food intake once food is available. Thus, while these studies were not primarily aimed at the effects of food restriction on cholinergic and serotonergic function they do suggest that food restriction induced a prolonged decrease in cholinergic and serotonergic receptor function and/or sensitivity.

To summarize, evidence was provided in support of the notion that food restriction sensitized rats to the D2-mediated effects of pramipexole while not altering their sensitivity to the D3-mediated effects of pramipexole. Food restriction suppressed pramipexole-induced yawning while resulting in a sensitization and/or enhancement of the hypothermic and locomotor stimulatory effects of pramipexole; all of which suggest an increased function and/or sensitivity of the D2 receptor. This notion is further supported by the finding that the effects of food restriction on pramipexole-induced yawning were reversed by the D2 antagonist, L-741,626, and when combined with the finding that food restriction did not alter pramipexole-induced PE, these data strongly suggest that food restriction altered the D2-, but not D3-mediated effects of pramipexole. Importantly, while food restriction suppressed dopaminergic-, cholinergic-, and serotonergic-mediated behaviors, differences in the duration of these effects were observed and may be reflective of differential roles for dopamine, acetylcholine, and serotonin in feeding behaviors. For instance, while food restriction-induced changes in dopaminergic function may serve to increase the motivation to obtain food when food is unavailable, sensitization of D2 receptors would serve little purpose once food is readily available. Conversely, prolonged decreases in cholinergic and serotonergic sensitivity may allow for a sustained increase in meal frequency and size following extended periods of food deprivation. Moreover, while food restriction altered a variety of D2-mediated behaviors, food restriction failed to alter the pro-erectile effects of pramipexole suggesting that food restriction-induced changes in D2 receptors may serve a more general purpose to increase arousal and/or enhance dopamine-mediated reward (or prediction of reward), while allowing for other behaviors (reproduction) to be maintained. In conclusion, these studies suggest that food restriction enhanced the function and/or sensitivity of D2 receptors while having no effect on the function and/or sensitivity of D3 receptors.

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References:

Alberto CO, Trask RB, Quinlan ME, Hirasawa M (2006) Bidirectional dopaminergic modulation of excitatory synaptic transmission in orexin neurons. J Neurosci. 26:10043-10050

Argiolas A, Melis MR (1998) The neuropharmacology of yawning. Eur J Pharmacol. 343:1-16

Boulay D, Depoortere R, Perrault G, Borrelli E, Sanger DJ (1999) Dopamine D2 receptor knock-out mice are insensitive to the hypolocomotor and hypothermic effects of dopamine D2/D3 receptor agonists. Neuropharmacology. 38:1389-1396

Cadoni C, Solinas M, Valentini V, Di Chiara G (2003) Selective psychostimulant sensitization by food restriction: differential changes in accumbens shell and core dopamine. *Eur J Neurosci.* **18**:2326-2334

Carlson JN, Glick SD, Hinds PA, Baird JL (1988) Food deprivation alters dopamine utilization in the rat prefrontal cortex and asymmetrically alters amphetamine-induced rotational behavior. *Brain Res.* **454**:373-377

Carr KD, Kim GY, Cabeza de Vaca S (2001) Rewarding and locomotor-activating effects of direct dopamine receptor agonists are augmented by chronic food restriction in rats. *Psychopharmacology (Berl).* **154**:420-428

Carr KD, Tsimberg Y, Berman Y, Yamamoto N (2003) Evidence of increased dopamine receptor signaling in food-restricted rats. *Neuroscience.* **119**:1157-1167

Carroll ME, France CP, Meisch RA (1979) Food deprivation increases oral and intravenous drug intake in rats. *Science.* **205**:319-321

Carroll ME, France CP, Meisch RA (1981) Intravenous self-administration of etonitazene, cocaine and phencyclidine in rats during food deprivation and satiation. *J Pharmacol Exp Ther.* **217**:241-247

Chaperon F, Tricklebank MD, Unger L, Neijt HC (2003) Evidence for regulation of body temperature in rats by dopamine D2 receptor and possible influence of D1 but not D3 and D4 receptors. *Neuropharmacology.* **44**:1047-1053

Collins GT, Witkin JM, Newman AH, Svensson KA, Grundt P, Cao J, Woods JH (2005) Dopamine agonist-induced yawning in rats: a dopamine D3 receptor-mediated behavior. *J Pharmacol Exp Ther.* **314**:310-319

Collins GT, Newman AH, Grundt P, Rice KC, Husbands SM, Chauvignac C, Chen J, Wang S, Woods JH (2007) Yawning and hypothermia in rats: effects of dopamine D3 and D2 agonists and antagonists. *Psychopharmacology (Berl).* **193**:159-170

Deroche V, Piazza PV, Casolini P, Le Moal M, Simon H (1993) Sensitization to the psychomotor effects of amphetamine and morphine induced by food restriction depends on corticosterone secretion. *Brain Res.* **611**:352-356

Donny EC, Caggiula AR, Mielke MM, Jacobs KS, Rose C, Sved AF (1998) Acquisition of

nicotine self-administration in rats: the effects of dose, feeding schedule, and drug contingency. Psychopharmacology (Berl). **136**:83-90

Faunt JE, Crocker AD (1987) The effects of selective dopamine receptor agonists and antagonists on body temperature in rats. *Eur J Pharmacol.* **133**:243-247

Grundt P, Carlson EE, Cao J, Bennett CJ, McElveen E, Taylor M, Luedtke RR, Newman AH (2005) Novel heterocyclic trans olefin analogues of N-{4-[4-(2,3-dichlorophenyl)piperazin-1-yl]butyl}arylcarboxamides as selective probes with high affinity for the dopamine D3 receptor. J Med Chem. 48:839-848

Grundt P, Prevatt KM, Cao J, Taylor M, Floresca CZ, Choi JK, Jenkins BG, Luedtke RR, Newman AH (2007) Heterocyclic analogues of N-(4-(4-(2,3-dichlorophenyl)piperazin-1-yl)butyl)arylcarboxamides with functionalized linking chains as novel dopamine D3 receptor ligands: potential substance abuse therapeutic agents. J Med Chem. 50:4135-4146

Gur E, Newman ME, Avraham Y, Dremencov E, Berry EM (2003) The differential effects of food restriction on 5-HT1A and 5-HT1B receptor mediated control of serotonergic transmission in the hippocampus and hypothalamus of rats. *Nutr Neurosci.* **6**:169-175

Isaac SO, Berridge CW (2003) Wake-promoting actions of dopamine D1 and D2 receptor stimulation. J Pharmacol Exp Ther. 307:386-394

Jahng JW, Kim JG, Kim HJ, Kim BT, Kang DW, Lee JH (2007) Chronic food restriction in young rats results in depression- and anxiety-like behaviors with decreased expression of serotonin reuptake transporter. *Brain Res.* **1150**:100-107

Kelley AE, Baldo BA, Pratt WE, Will MJ (2005) Corticostriatal-hypothalamic circuitry and food motivation: integration of energy, action and reward. *Physiol Behav.* **86**:773-795

Leibowitz SF, Weiss GF, Suh JS (1990) Medial hypothalamic nuclei mediate serotonin's inhibitory effect on feeding behavior. *Pharmacol Biochem Behav.* **37**:735-742

Leibowitz SF, Alexander JT (1998) Hypothalamic serotonin in control of eating behavior, meal size, and body weight. *Biol Psychiatry.* **44**:851-864

Levant B (1997) The D3 dopamine receptor: neurobiology and potential clinical relevance. *Pharmacol Rev.* **49**:231-252

Lin MT, Chandra A, Tsay BL, Chern YF (1982) Hypothalamic and striatal dopamine receptor activation inhibits heat production in the rat. *Am J Physiol.* **242**:R471-481

Macenski MJ, Meisch RA (1999) Cocaine self-administration under conditions of restricted and unrestricted food access. *Exp Clin Psychopharmacol.* **7**:324-337

Meguid MM, Fetissov SO, Varma M, Sato T, Zhang L, Laviano A, Rossi-Fanelli F (2000) Hypothalamic dopamine and serotonin in the regulation of food intake. Nutrition. 16:843-857

Meisch RA, Thompson T (1973) Ethanol as a reinforcer: effects of fixed-ratio size and food deprivation. *Psychopharmacologia.* **28**:171-183

Melis MR, Argiolas A, Gessa GL (1987) Apomorphine-induced penile erection and yawning: site of action in brain. *Brain Res.* **415**:98-104

Millan MJ, Gobert A, Newman-Tancredi A, Lejeune F, Cussac D, Rivet JM, Audinot V, Dubuffet T, Lavielle G (2000) S33084, a novel, potent, selective, and competitive antagonist at dopamine D(3)-receptors: I. Receptorial, electrophysiological and neurochemical profile compared with GR218,231 and L741,626. J Pharmacol Exp Ther. 293:1048-1062

Millan MJ, Maiofiss L, Cussac D, Audinot V, Boutin JA, Newman-Tancredi A (2002) Differential actions of antiparkinson agents at multiple classes of monoaminergic receptor. I. A multivariate analysis of the binding profiles of 14 drugs at 21 native and cloned human receptor subtypes. J Pharmacol Exp Ther. 303:791-804

Nasello AG, Tieppo CA, Felicio LF (1995) Apomorphine-induced yawning in the rat: influence of fasting and time of day. *Physiol Behav.* **57**:967-971

Ouagazzal AM, Creese I (2000) Intra-accumbens infusion of D(3) receptor agonists reduces spontaneous and dopamine-induced locomotion. Pharmacol Biochem Behav. 67:637-645

Palmiter RD (2007) Is dopamine a physiologically relevant mediator of feeding behavior? Trends Neurosci. 30:375-381

Persinger MA, Stewart LS, Richards PM, Harrigan T, O'Connor RP, Bureau YR (2002) Seizure onset times for rats receiving systemic lithium and pilocarpine: sources of variability. *Pharmacol Biochem Behav.* **71**:7-17

Pothos EN, Creese I, Hoebel BG (1995) Restricted eating with weight loss selectively decreases extracellular dopamine in the nucleus accumbens and alters dopamine response to amphetamine, morphine, and food intake. *J Neurosci.* **15**:6640-6650

Robinson TE, Berridge KC (1993) The neural basis of drug craving: an incentive-sensitization theory of addiction. Brain Res Brain Res Rev. 18:247-291

Svensson K, Carlsson A, Waters N (1994) Locomotor inhibition by the D3 ligand R-(+)-7-OH-DPAT is independent of changes in dopamine release. *J Neural Transm Gen Sect.* **95**:71-74

Yamada K, Tanaka M, Shibata K, Furukawa T (1986) Involvement of septal and striatal dopamine D-2 receptors in yawning behavior in rats. *Psychopharmacology (Berl).* **90**:9-13

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Legends for Figures:

Figure 1. Dose-response curves for yawning (left panels) and PE (right panels) induced by pramipexole, physostigmine and TFMPP in Free-Fed and Food-Restricted rats. Characterization of A and B) pramipexole- (0.01-1.0 mg/kg); C and D) physostigmine- (0.032-0.32 mg/kg); and E and F) TFMPP- (1.0-3.2 mg/kg) induced yawning and PE was conducted using a multiple-dose procedure in separate groups of rats with data presented as mean (±SEM), n=8, number of PEs and yawns observed during a 25 min observation period. *, $p<0.05$; **, $p<0.01$. Significant differences in agonist-induced yawning and PE compared to vehicle condition as determined by one-way, repeated-measures ANOVA with post-hoc Dunnett's tests, and Friedman tests with post-hoc Dunn's tests, respectively. +, $p<0.05$; ++, $p<0.01$; +++, $p<0.001$. Significant effect of food restriction on agonist-induced yawning or PE as compared to the Free-Fed 1 condition determined by two-way, repeated-measures ANOVA with post-hoc Bonferroni tests, and Friedman tests with post-hoc Dunns tests, respectively.

Figure 2. Dose-response curves for pramipexole-induced A) hypothermia and B) locomotor activity in Free-Fed and Food-Restricted rats. Characterization of the hypothermic and locomotor effects of pramipexole was conducted concurrently with data presented as mean (±SEM), n=8, change in core body temperature as measured 30 minutes after each injection as compared to the core body temperature 1 minute prior to the first injection, total number of ambulatory locomotor activity counts recorded during the 30 minutes after each injection. *, $p<0.05$; **, $p<0.01$. Significant differences in agonist-induced hypothermia or locomotor activity compared to vehicle treated animals were determined using a one-way, repeated-measures ANOVA with post-hoc Dunnett's tests. +, $p<0.05$; ++, $p<0.01$; +++, $p<0.001$. Significant differences in agonist-induced hypothermia or locomotor activity during the Food-Restricted and

Free-Fed 2 conditions compared to the Free-Fed 1 condition were determined using a two-way, repeated-measures ANOVA with post-hoc Bonferroni tests.

Figure 3. Effects of D3- and D2-selective antagonists on pramipexole- (0.01-1.0 mg/kg) induced yawning and PE in Free-Fed (A and B), and Food-Restricted (C and D) rats. Effects of the D3-selective antagonist, PG01037 (32.0 mg/kg), and the D2-selective antagonist, L-741,626 (1.0 mg/kg), on pramipexole-induced A and C) yawning, and B and D) penile erection. Data are presented as mean (±SEM), n=8, number of PEs and yawns observed during a 25 min observation period. *, $p<0.05$; **, $p<0.01$; ***, $p<0.001$. Significant effects of antagonist pretreatment on pramipexole-induced yawning or PE as determined by two-way ANOVA with post-hoc Bonferroni tests, and Friedman tests with post-hoc Dunn's tests, respectively.

Table 1. Effects of the D2-selective antagonist, L-741,626, on yawning induced by physostigmine or TFMPP in food-restricted rats[a].

	Yawning		PE	
Compound	**Vehicle**	**1.0 L-741,626**	**Vehicle**	**1.0 L-741,626**
physostigmine				
Vehicle	0.8 (±0.4)	1.0 (±0.7)	0.3 (±0.2)	0.1 (±0.1)
0.032 mg/kg	1.9 (±0.9)	2.8 (±1.2)	0.3 (±0.3)	0.3 (±0.2)
0.1 mg/kg	1.4 (±0.6)	4.8 (±2.3)	0.1 (±0.1)	0.1 (±0.1)
0.32 mg/kg	0.9 (±0.7)	1.1 (±0.7)	0.1 (±0.1)	0.0 (±0.0)
TFMPP				
Vehicle	0.1 (±0.1)	0.8 (±0.5)	0.1 (±0.1)	0.0 (±0.0)
1.0 mg/kg	0.1 (±0.1)	0.6 (±0.6)	0.9 (±0.5)	0.5 (±0.3)
3.2 mg/kg	0.1 (±0.1)	0.3 (±0.3)	0.1 (±0.1)	0.0 (±0.0)
10.0 mg/kg	0.1 (±0.1)	0.0 (±0.0)	0.0 (±0.0)	0.0 (±0.0)

[a]Data represent mean number of yawns observed over successive 25 min observation ± S.E.M.

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Figure 1

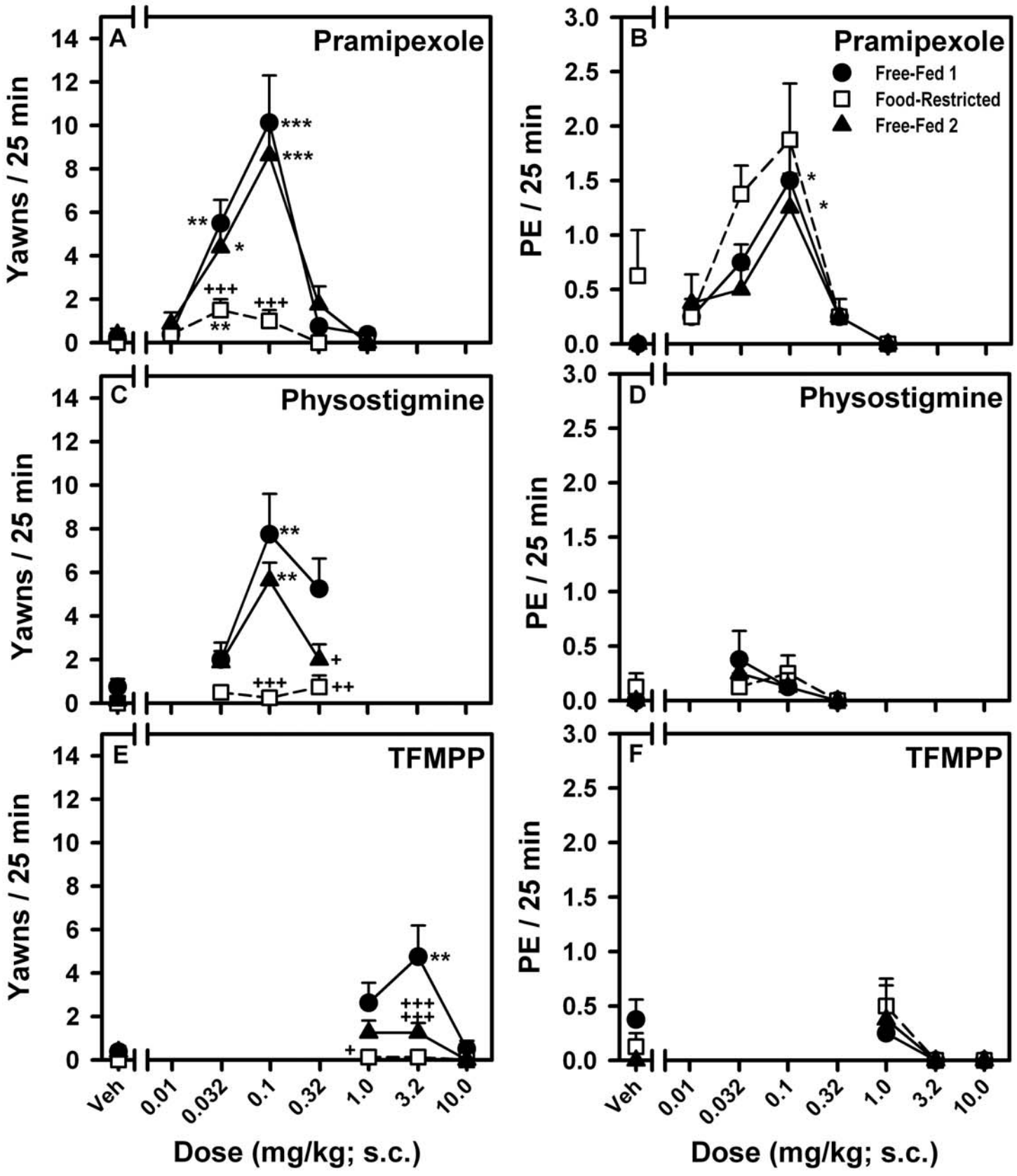

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Figure 2

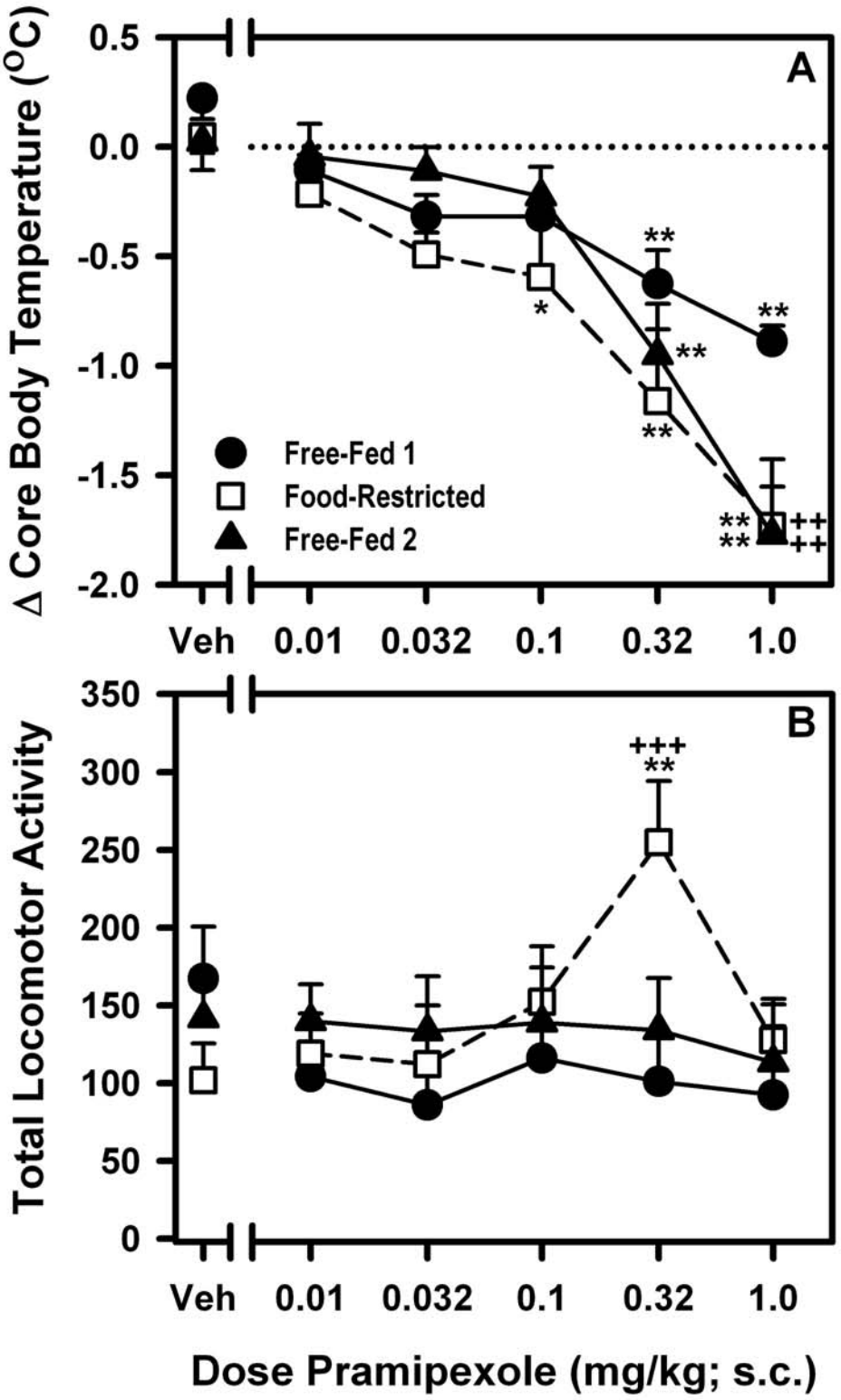

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Figure 3