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Nicotine and its withdrawal modify dorsal raphe 8-hydroxy-2-(di-*n*-propylamino) tetralin feeding

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Abstract

Nicotine (NIC) and its withdrawal modify dorsal raphe (DR) serotonin (5-HT) neurotransmission in ways that may contribute to the body weight loss vs. gain associated with cigarette smoking vs. cessation, respectively. Modifications in feeding to DR infusions of the 5-HT-1A receptor agonist, 8-hydroxy-2-(di-*n*-propylamino) tetralin (8-OH-DPAT), were used to characterize these potential relationships in the DR-5-HT system during NIC administration vs. withdrawal. Two groups of female rats (total $N=45$) were implanted for 14 days with subcutaneous Alzet minipumps containing NIC (6 mg/kg/day) or saline. Mid-light cycle (1300–1500 h) 8-OH-DPAT feeding tests occurred three times: (1) 2 days after pump implant, (2) 12 days after pump implant, and (3) 2 days after pump removal. Each feeding test consisted of a 1-h measure of pre-feeding, then a 1-h measure of feeding after DR injection of 8-OH-DPAT (0.6 nmol) or 0.4 μl saline. NIC administration produced acute hypophagia, weight loss, and attenuated 8-OH-DPAT-induced feeding. NIC withdrawal produced acute hyperphagia, weight gain, and a transient increase in 8-OH-DPAT feeding. These findings provide behavioral evidence that systemic NIC modifies the DR 5-HT system in ways that may contribute to NIC's ability to alter feeding and body weight.

Keywords: Dorsal raphe; Nicotine; Serotonin; 8-hydroxy-2-(di-*n*-propylamino) tetralin; Body weight; Feeding

1. Introduction

Tobacco smoking produces feeding and weight suppression in humans that often rebound following smoking cessation (Klesges , 1989; O'Hara et al., 1998). Similarly, nicotine (NIC) treatment in rats induces hypophagia and weight loss, while cessation of NIC treatment has been reported to elicit significant hyperphagia and rapid weight gain, particularly in female rats (Grunberg et al., 1984; Bowen et al., 1986; Levin et al., 1987).

Though NIC effects on body weight and feeding are well established, the specific mechanisms responsible remain elusive. Recent studies suggest that NIC's effects on energy balance correlate with its effects on the feeding-related peptides orexin and neuropeptide Y (NPY) (Frankish et al., 1995; Li et al., 2000; Kane et al., 2001; Bishop et al., 2002). NIC also alters brain levels of serotonin (5-HT), a biogenic amine that plays a significant role in satiety, body weight regulation, and macronutrient preference (Blundell, 1986; Samanin and Garattini, 1989; Leibowitz and Alexander, 1998). Systemic NIC administration consistently increases 5-HT levels in forebrain sites, including the frontal cortex (Ribeiro et al., 1993), striatum (Westfall et al., 1983), and hypothalamus (Hery et al., 1977; Miyata et al., 1999). NIC-induced increases in 5-HT may result from activating presynaptic nicotinic receptors located in the dorsal raphe (DR) (Aghajanian et al., 1978; Schwartz et al., 1984; Wonnacott, 1997; Li et al., 1998). In support of that possibility, NIC has been shown to acutely increase DR neuronal firing and DR-5-HT release, while administration of the NIC antagonist, mecamylamine, blocks these effects (Mihailescu et al., 1998; Li et al., 1998). Thus, decreases in feeding and body weight associated with NIC administration may result from increased 5-HT neurotransmission in feeding related brain sites (Grunberg, 1989; Schwid et al., 1992).

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There is also recent evidence that withdrawal of NIC influences DR-5-HT neurotransmission. Rasmussen and Czachura (1997) demonstrated electrophysiologically that NIC cessation increased DR sensitivity to the 5-HT-1A receptor agonist 8-hydroxy-2-(di-*n*-propylamino) tetralin (8-OH-DPAT). 5-HT-1A receptors within the DR are thought to be somatodendritic autoreceptors whose stimulation suppresses 5-HT neuronal activity (Sinton and Fallon, 1988), leading to reduced 5-HT release in terminal areas (Hjorth and Magnusson, 1988; Hjorth and Sharp, 1991). Because DR infusions of 8-OH-DPAT reliably increase feeding (Hutson et al., 1986; Currie and Coscina, 1993), hypersensitivity of 5-HT-1A receptors maybe a candidate mechanism for the overeating and weight gain that follow NIC cessation.

The present experiment determined if female rats first exposed to NIC and then withdrawal showed altered feeding in response to a threshold dose of DR-injected 8-OH-DPAT. To study this, a longitudinal experiment examined the effects of acute NIC, chronic NIC, and withdrawal of NIC on DR-8-OH-DPAT-induced feeding. We hypothesized that NIC administered in a dose sufficient to suppress body weight and food intake would blunt 8-OH-DPAT-induced feeding. Conversely, we postulated that NIC cessation would potentiate 8-OH-DPAT feeding in a manner consistent with rebound overeating and increases in weight gain.

2. Methods

2.1. Animals

Fifteen-week-old female Sprague–Dawley ($N=45$) rats, were purchased from a commercial supplier (Harlan, Indianapolis, IN) and housed individually in plastic cages (22 cm high, 45 cm deep, and 23 cm wide) within a temperature-controlled colony room illuminated on a 12-h-light/dark cycle (lights on 0600 h). Body weights and 24-h food intakes were measured daily throughout the experiment. Rats had ad libitum access to water and standard lab chow (Rodent Diet 5001, LabDiet, Brentwood, MO) throughout the experiment. Animals were maintained in accordance with the guidelines of the Institutional Animal Care and Use Committee of Wayne State University and the "Guide for the Care and Use of Laboratory Animals" (Institute of Laboratory Animal Resources, National Academy Press 1996; NIH publication number 85-23, revised 1996).

2.2. Cannula implant surgery

One week after arrival in the lab and adaptation to housing conditions, all rats were implanted unilaterally with chronic 22-gauge intracranial guide cannulae (C313G/SPC, Plastics One, Roanoke, VA) under sodium pentobarbital anesthesia (50 mg/kg, ip). With the incisor bar positioned 3.5 mm above the interaural line and the stereotaxic arm set at a 20° angle in the coronal plane towards the midline, cannulae were positioned 4 mm above the DR using coordinates 1.2 mm anterior to interaural zero, 1.0 mm lateral to the midline, 7.0 mm dorsal to interaural zero (Currie and Coscina, 1993). At the completion of surgery, guide cannulae were fitted with 28-gauge inner stylets to maintain patency.

2.3. Drugs and chemicals

(−)-1-Methyl-2-(3-pyridyl)pyrrolidine (NIC) hydrogen tartrate (Sigma, St. Louis, MO) dissolved in buffered 0.9% tartaric acid (pH ~ 7.0) or buffered tartaric acid alone was used to fill Alzet minipumps (model 2002, Durect, Cupertino, CA). 8-OH-DPAT–hydrogen bromide (Sigma) at doses of 0 and 0.6 nmol was used for DR injections. This dose was chosen because past work in our lab revealed it elicited a submaximal feeding response (Currie and Coscina, 1993). 8-OH-DPAT or its vehicle (0.9% NaCl) were administered in volumes of 0.4 μl.

2.4. Alzet pump surgery

Following 7–10 days of recovery from cannula implants, rats had sterile Alzet minipumps inserted subcutaneously between their shoulder blades under brief halothane anesthesia (Halocarbon, River Edge, NJ). Osmotic minipumps were filled to continuously dispense NIC (6 mg/kg/day, calculated free base) or its vehicle (0.9% tartaric acid) for 14 days. This dose of NIC was chosen because we have shown that it effectively suppresses body weight gain and food intake (Bishop et al., 2002). After 14 days, pumps were removed under brief halothane anesthesia and the evacuated subcutaneous space was flushed with 10 ml sterile saline (0.9% NaCl) to remove residual NIC.

2.5. Acute feeding tests

All feeding tests were conducted in wire mesh cages within the same room in which rats were normally housed. One week before the first feeding test, the female rats described above were acclimated to test procedures, including mock injections, at least three times. The test diet consisted of the rats' standard lab chow. A longitudinal design was used for each subject to determine food intake at three time points: (1) 2 days post-implant (Acute NIC), (2) 12 days post-implant (Chronic NIC), and (3) 2 days after NIC withdrawal (Acute WD). Rats were transferred to test cages at 1300 h and allowed free access to food for 1 h. This time was chosen because feeding induced by intra-DR 8-OH-DPAT at the dose chosen is particularly sensitive during the mid-light cycle when baseline feeding is low (Currie and Coscina, 1993). At 1400 h, 8-OH-DPAT or saline was infused into the DR, and then food intake was measured for 1 h (1400–1500 h). Over the three feeding tests, each rat received 8-OH-DPAT or saline on a given

testing day in a quasi-randomized fashion to minimize possible order effects.

2.6. Histology

Upon completion of the experiment, rats were sacrificed with an overdose of sodium pentobarbital (120 mg/kg), their brains extracted and stored in formalin. After at least 3 days, 50-μm sections of brains were cut using a freezing microtome and viewed relative to the stereotaxic atlas of Paxinos and Watson (1986) using light microscopy and computer-enhanced imaging (Imascan Software, Foresight Imaging, Chelmsford, MA) to verify cannula placements.

2.7. Data analysis

Measures of daily body weights and food intakes were entered into mixed three-factor ANOVAs followed by mean comparisons using Tukey post hoc tests. The within-subjects factors analyzed were Implant status (Implant and

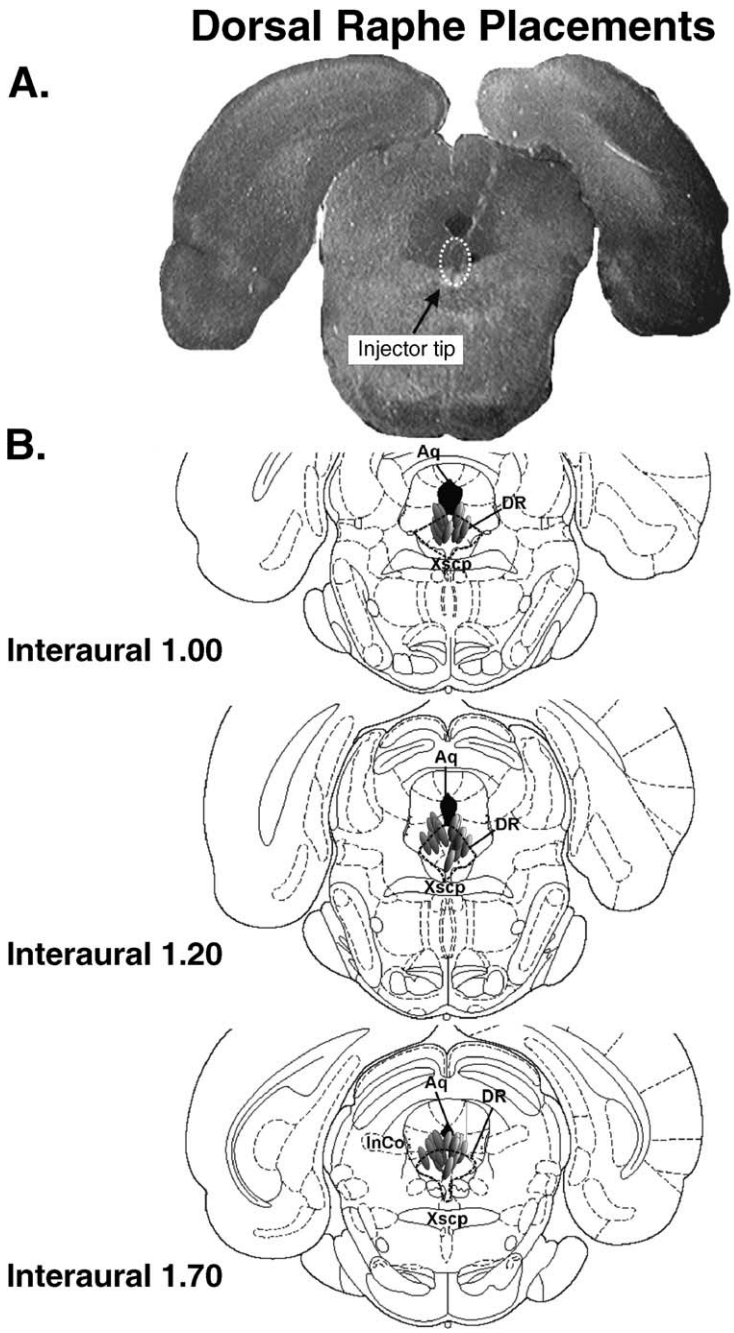

Fig. 1. (A) Histological verification of injector tip placement located within the dorsal raphe (DR). (B) Schematic representation of coronal sections of the rat brain depicting the distribution of DR microinfusion sites. Sections are taken from Paxinos and Watson (1986). Shaded ovals denote placements within the DR. Relevant anatomical structures are: Aq, aqueduct; InCo, intercollicular nucleus; Xscp, decussation of the superior cerebellar peduncle.

Removal) and Day (14 days repeated measures). The between-subjects factor was Group [Saline and NIC (6 mg/kg/day)]. A three-factor ANOVA analyzing the 1 h of feeding postinjection in the three NIC phases was run using the between-subjects factors Phase (Acute NIC, Chronic NIC, and Acute W), Group [Saline and NIC (6 mg/kg/day)], and 8-OH-DPAT dose (0 and 0.6 nmol). Planned post hoc comparisons on 1-h feeding effects were performed when appropriate. All data were analyzed using STATISTICA '98 (Statsoft, Tulsa, OK).

3. Results

3.1. Attrition and histology

Data from two rats who lost cannulae during testing were not included in the daily food intake and body weight analyses. Because of these exclusions, 43 rats were included in daily food intake, body weight analyses, and 8-OH-DPAT feeding test analyses. Fig. 1A shows a representative histological example of a correct placement, while Fig. 1B shows schematic representations of placements for rats included in the feeding tests.

3.2. Nicotine and body weight changes

There was a significant main effect of Group on body weight [$F(1,39)=5.81$, $P<.05$] (Fig. 2). Post hoc tests demonstrated that the NIC group had lower body weights than the Saline group ($P<.05$). There were also main effects of Implant status and Day [$F(1,39)=26.51$, $P<.001$ and $F(13,507)=25.42$, $P<.001$, respectively]. These indicated that rats weighed more after implant removal than during implant and that both groups gained weight over the course of the experiment. Significant two-way interactions were found for Group × Implant status [$F(1,39)=15.40$, $P<.001$], Group × Day [$F(13,507)=2.32$, $P<.01$], and Implant status × Day [$F(13,507)=9.05$, $P<.001$]. The Group × Implant status interaction reflected that NIC group body weights were significantly lower than the Saline group during pump implant, but not after implant removal. The Group × Day interaction indicated that the Saline group maintained a higher body weight over time than the NIC group. The Implant status × Day interaction revealed that body weight gain over time depended on whether animals had pump implants or not.

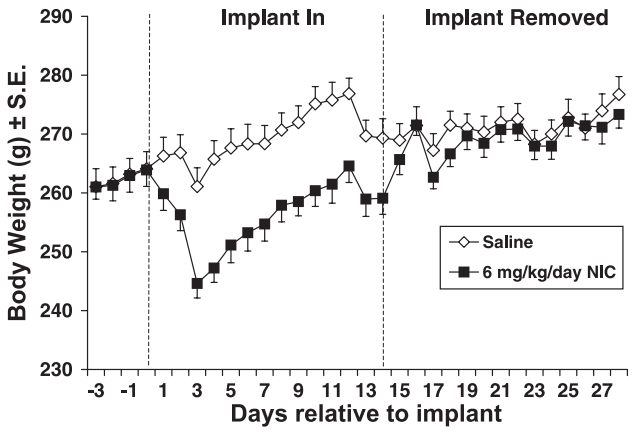

Fig. 2. Effects of nicotine (NIC) on body weight in grams (g) ± standard error (S.E.).

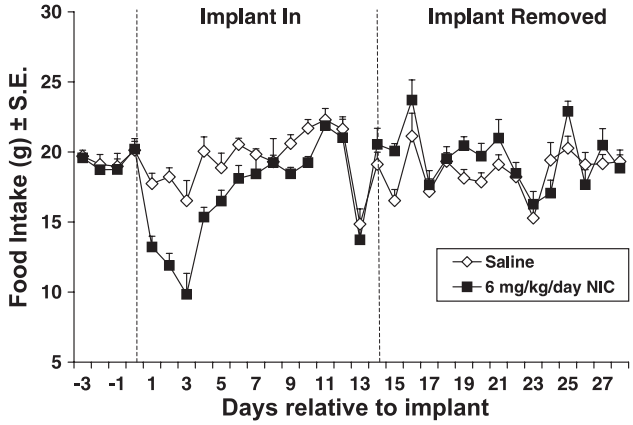

Fig. 3. Effects of nicotine (NIC) on daily food intake in grams (g) ± standard error (S.E.).

3.3. Nicotine and food intake changes

Fig. 3 depicts the effects of NIC administration on daily food intake. Significant main effects for Implant status [$F(1,39)=4.74$, $P<.05$] and Day [$F(13,507)=11.13$, $P<.005$] were found. Post hoc tests indicated that animals during pump implants consumed less chow per day than when implants were removed and that daily food intake differed over time. A significant two-way interaction of Group × Implant status [$F(1,39)=33.36$, $P<.001$] showed that NIC-treated rats consumed less food daily during implant compared to the Saline group. A significant Implant status × Day effect was also shown [$F(13,507)=14.27$, $P<.001$]. This revealed that daily food intake patterns differed during implant compared to days following its removal. A significant Group × Day effect [$F(13,507)=2.13$, $P<.05$] indicated that the feeding patterns of the groups differed across time. Finally, a significant three-way interaction between Group × Implant status × Day was demonstrated [$F(13,507)=3.96$, $P<.001$]. As seen in Fig. 3, this interaction showed that the NIC group consumed less during pump implants, while conversely, pump removal resulted in a mild hyperphagia in comparison to the Saline group.

3.4. Effects of NIC on DR-8-OH-DPAT-induced feeding

Fig. 4 shows the effects of NIC and its withdrawal on 8-OH-DPAT feeding. Analyses revealed a significant main effect of 8-OH-DPAT dose [$F(1,117)=13.06$, $P<.001$],

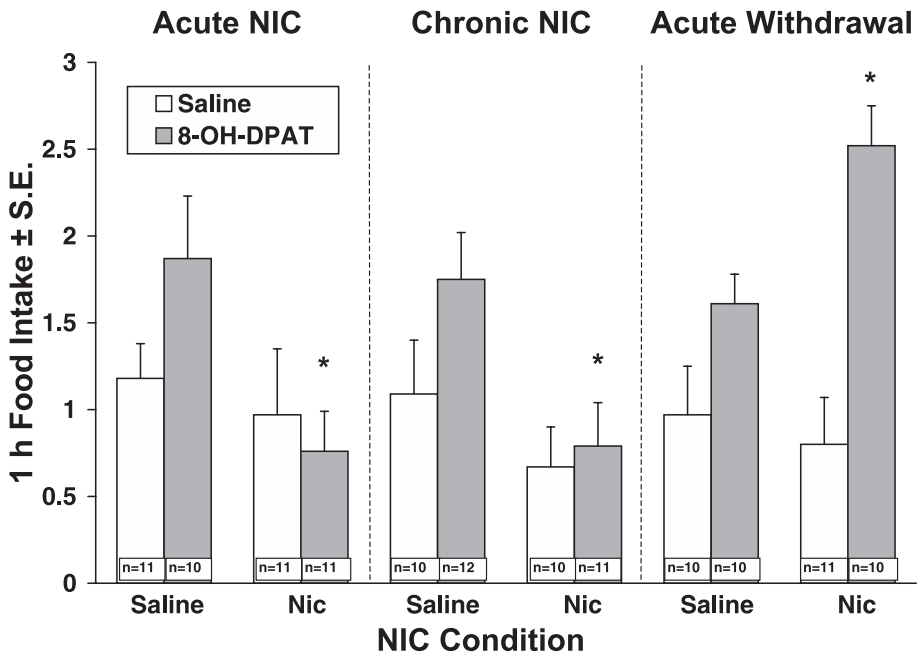

Fig. 4. Effects of nicotine (NIC) group (Saline and NIC [6 mg/kg/day]) on dorsal raphe 8-OH-DPAT-induced food intake in grams (g) ± standard error (S.E.). Symbol denotes comparisons of 1 h food intake results. * $P < .05$ vs. Saline–8-OH-DPAT.

with post hocs demonstrating that rats consumed more to 8-OH-DPAT than saline ($P < .05$). A significant Phase × Group interaction was also found [$F(2,117) = 4.60$, $P < .05$] indicating that NIC-treated rats consumed less overall than controls in the Acute and Chronic NIC phases (both $P < .05$). Further, a Phase × 8-OH-DPAT dose interaction [$F(2,117) = 4.33$, $P < .05$] showed that feeding to 8-OH-DPAT was increased during the Acute WD period compared to all other phases (all $P < .01$). Finally, a significant three-way interaction between Phase × Group × 8-OH-DPAT dose was found [$F(2,117) = 3.13$, $P < .05$]. Post hoc testing revealed a number of significant effects. First, feeding to 8-OH-DPAT was significantly lower in NIC-treated rats compared to controls during both Acute and Chronic NIC phases (both $P < .05$). Second, feeding to 8-OH-DPAT in NIC-treated rats during these phases was no different from saline. Finally, in Acute WD, NIC-treated rats consumed more that Saline-treated rats following 8-OH-DPAT infusions ($P < .05$).

4. Discussion

The findings of the present investigation confirm previous reports that NIC administration decreases food intake and body weight gain of female rats, while its withdrawal produces transient increases in food intake and weight gain (Grunberg et al., 1987; Bishop et al., 2002). The present report also provides evidence for one mechanism that might account for some of NIC's effects on food intake by demonstrating that the responsiveness of the DR to 8-OH-DPAT-induced feeding varies depending upon NIC status.

NIC-treated rats exhibited significant weight loss compared to the control group during pump implant. Cessation of NIC was followed within 2 days by body weight regain back to control levels. Similar NIC-associated body weight changes have been demonstrated by other researchers using similar doses of NIC (Grunberg et al., 1986; Levin et al., 1987; Miyata et al., 1999; Bishop et al., 2002).

NIC administration to rats of both sexes has been reported produce hypophagia. (Grunberg et al., 1986; Levin et al., 1987, 1993; Miyata et al., 1999; Li et al., 2000). The presence of a three-way interaction between group, implant status, and day suggested that NIC's effects on daily food intake were transient. In the NIC group, daily food intake was suppressed for approximately 1 week, followed by a return to control levels. Throughout the remainder of the implant period, rebound overeating did not occur in the NIC-treated rats. Therefore, during the second week, although NIC no longer exerted as strong a suppressant effect on food consumption as it initially had, these animals did not consume amounts that would indicate NIC withdrawal. Instead, the NIC group demonstrated suppressed body weight throughout the entire NIC administration phase. That the NIC group's body weight also remained suppressed beyond the recovery of food intake suggests that a portion of NIC's effects may have been due to suppression of energy expenditure (Bishop et al., 2002).

The first week of withdrawal from NIC appeared to produce a mild, transient hyperphagia in the daily food intake of the NIC group. However, these effects did not have a significant impact on long-term weight gain. These NIC-mediated feeding changes support previous findings from other researchers using similar doses and administration

routes (Grunberg et al., 1986, 1987; Levin et al., 1987; Miyata et al., 1999; Bishop et al., 2002).

The novelty of the present report is its attempt to explain NIC-induced changes in food intake by probing its interaction with the DR-5-HT system. NIC administration has been repeatedly shown to increase 5-HT levels in the brain (Hery et al., 1977; Westfall et al., 1983; Ribeiro et al., 1993; Miyata et al., 1999). This seems to occur largely by stimulating presynaptic NIC receptors located within the DR (Deutch et al., 1987; Mihailescu et al., 1998; Li et al., 1998). Because increased 5-HT neurotransmission often results in decreased feeding and body weight (Blundell, 1986; Leibowitz and Alexander, 1998), NIC-induced increases in 5-HT might contribute to changes in energy balance. Despite this possibility, few researchers have directly studied interactions between NIC, DR-5-HT, and indices of energy balance.

One consistent finding in the feeding literature is that reliable enhancement of feeding occurs following administration of the 5-HT-1A receptor agonist 8-OH-DPAT. Both peripheral and central injections of 8-OH-DPAT stimulate 5-HT-1A somatodendritic autoreceptors located on raphe cell bodies found in the midbrain and brainstem (Hjorth and Magnusson, 1988; Hjorth and Sharp, 1991). Stimulation of these receptors acts to inhibit 5-HT neuron firing and release of 5-HT at terminal areas (Sprouse and Aghajanian, 1987). Decreased 5-HT neurotransmission at feeding-relevant brain sites is thought to account for the increased feeding seen after administering 8-OH-DPAT (Hutson et al., 1986). Because NIC exerts its influence on 5-HT systems in part through the raphe, and 5-HT-1A receptors are involved in modulating DR neural activity, 8-OH-DPAT-induced feeding changes were used to functionally investigate potential NIC alterations in feeding via the 5-HT system.

Injections of a threshold dose (0.6 nmol) of 8-OH-DPAT can produce significant feeding enhancement during the mid-light cycle. Similar effects have been demonstrated before at doses ranging from 0.4 to 0.8 nmol (Currie and Coscina, 1993). Of particular interest here was whether NIC administration and its withdrawal would modify this 8-OH-DPAT-induced feeding response. A significant three-way interaction between phase, group, and 8-OH-DPAT dose demonstrated the dynamic modification of this effect in NIC-treated rats. In both Acute and Chronic NIC phases, the normal 8-OH-DPAT-induced feeding response was blunted. This appeared to be due to specific effects observed in NIC-treated rats. Acute NIC administration (2 days) seemed to significantly decrease 8-OH-DPAT-induced feeding in NIC-treated rats compared to Saline-treated rats. Similarly, chronic NIC administration also appeared to suppress 8-OH-DPAT feeding to levels significantly less than those seen in the other groups.

The blunted 8-OH-DPAT-induced feeding responses in NIC-treated rats suggest that continued NIC administration alters 5-HT-1A receptor function in the DR. Local NIC administration into the DR has been shown to stimulate DR neuron firing and 5-HT release (Mihailescu et al., 1998). NIC-induced 5-HT release, in turn, stimulates local DR-5-HT-1A autoreceptors, resulting in DR neural inhibition, an effect that can be blocked by the selective 5-HT-1A antagonist WAY100635 (Li et al., 1998). If 5-HT-1A receptors are continuously stimulated by local NIC-induced 5-HT release, they might downregulate or desensitize. In support of this, Kennett et al. (1987) demonstrated that 5-HT-1A receptors rapidly desensitize to repeated 8-OH-DPAT stimulation. Likewise, 8-OH-DPAT's initial hypothermic effect has been shown to be attenuated by chronic (14 days sc) 8-OH-DPAT administration (De Souza et al., 1986). Therefore, the blunted 8-OH-DPAT feeding effects shown in this study provide additional behavioral evidence that NIC desensitizes 5-HT-1A receptors in the DR. This may, in turn, result in less overall inhibition of DR-projecting neurons, more 5-HT release in feeding-relevant sites, and consequently, less food intake which contributes to weight loss.

Opposite changes in DR-5-HT-1A receptor sensitivity may also explain the acute increases in feeding and body weight we observed after NIC withdrawal. Benwell and Balfour (1979) found that NIC withdrawal decreased 5-HT concentrations in the hippocampus. This supports the notion that NIC withdrawal decreases midbrain raphe cell firing and 5-HT release. This could lead to a decrease in the tonic level of 5-HT-1A stimulation and produce hypersensitivity to 8-OH-DPAT and, possibly, endogenous 5-HT stimulation. Rasmussen and Czachura (1997) reported data compatible with this interpretation. In that study, rats received 6 mg/kg/day NIC via minipumps for 14 days, after which, pumps were removed, and 8-OH-DPAT-induced changes in DR cell firing and 5-HT release were measured. Three and four days following NIC withdrawal, DR neurons were hypersensitive to 8-OH-DPAT inhibition. It was therefore hypothesized in the present study that 8-OH-DPAT-induced feeding might be enhanced during acute NIC withdrawal. In support of that possibility, 8-OH-DPAT feeding was potentiated in the NIC group 2 days after NIC withdrawal compared to controls. Interestingly, Rasmussen and Czachura (1997) found no exaggerated electrophysiological effects of 8-OH-DPAT 2 days following withdrawal when our tests occurred. This suggests that the feeding enhancement effects of NIC withdrawal may precede and/or be more sensitive to 8-OH-DPAT stimulation in the paradigm used here.

In conclusion, NIC administration appears to modify DR-8-OH-DPAT-induced feeding in a manner consistent with changes in feeding and body weight that accompany NIC administration and its withdrawal. These findings provide another potential mechanism for NIC's apparent effects on energy balance. Because the present study was restricted to female rats, male rats, which have been reported to respond differently to NIC administration and withdrawal (Grunberg et al., 1987; Levin et al., 1987), should be tested as well. These novel findings also suggest that certain symptoms associated with NIC usage and its discontinuation may be linked to altered brain 5-HT function, indicating a possible

mechanism and pharmacological target for the treatment of smoking cessation (Levin et al., 1993; Schatzberg, 2000).

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