

**Title Page:**

**Dopamine Agonist-Induced Yawning in Rats: A Dopamine D3  
Receptor Mediated Behavior.**

**Gregory T. Collins, Jeffrey M. Witkin, Amy H. Newman, Kjell A. Svensson, Peter  
Grundt, Jianjing Cao, and James H. Woods**

Department of Pharmacology  
1301 MSRB III  
1150 W. Medical Center Drive  
University of Michigan Medical School  
Ann Arbor, MI 48109-0632  
(GTC, JHW)

Psychiatric Drug Group  
Lilly Research Laboratories  
Indianapolis, IN 46285-0510  
(JMW, KAS)

Medicinal Chemistry Section  
National Institutes on Drug Abuse-Intramural Research Program  
National Institutes of Health  
5500 Nathan Shock Drive, Baltimore, MD 21224-0180  
(AHN, PG, JC)

**Running Title Page:**

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**Corresponding Author:** James H. Woods  
Department of Pharmacology  
1301 MSRB III  
University of Michigan Medical School  
Ann Arbor, MI 48109-0632  
734-764-9133 (phone)  
734-764-7118 (fax)  
jhwoods@umich.edu

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**Words in the Abstract:** 246

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**Words in the Discussion:** 1887

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**Abbreviations:**

**7-OH-DPAT:** ( $\pm$ )-7-Hydroxy-2-dipropylaminotetralin HBr; **Apomorphine:** (*R*)-(-)-5,6,6a,7-Tetrahydro-6-methyl-4*H*-dibenzo[*de,g*]quinoline-10,11-diol HCl; **Bromocriptine:** (+)-2-Bromo-12'-hydroxy-2'-(1-methylethyl)-5'-(2-methylpropyl) ergotaman-3',6'-18-trione methanesulfonate; **Haloperidol:** 4-[4-(4-Chlorophenyl)-4-hydroxy-1-piperidinyl]-1-(4-fluorophenyl)-1-butanone HCl; **L-741,626:** 3-[[4-(4-Chlorophenyl)-4-hydroxypiperidin-1-yl]methyl-1*H*-indole; **L-745,870:** 3-(4-[4-Chlorophenyl] piperazin-1-yl)-methyl-1*H*-

pyrrolo[2,3-b]pyridine trihydrochloride; **Nafadotride**: N-[(1-Butyl-2-pyrrolidinyl)methyl]-4-cyano-1-methoxy-2-naphthalenecarboxamide; **PD 128,907**: (S)-(+)-(4aR,10bR)-3,4,4a,10b-Tetrahydro-4-propyl-2*H*,5*H*-[1]benzopyrano-[4,3-b]-1,4-oxazin-9-ol HCl; **PD 128,908**: (R)-(-)-(4aS,10bS)-3,4,4a,10b-Tetrahydro-4-propyl-2*H*,5*H*-[1]benzopyrano-[4,3-b]-1,4-oxazin-9-ol HCl; **PG01037**: *N*-{4-[4-(2,3-Dichlorophenyl)-piperazin-1-yl]-*trans*-but-2-enyl}-4-pyridine-2-yl-benzamide HCl; **Pramipexole**: *N*'-propyl-4,5,6,7-tetrahydrobenzothiazole-2,6-diamine; **Quinelorane**: (5aR-*trans*)-5,5a,6,7,8,9,9a,10-Octahydro-6-propylpyrido[2,3-*g*]quinazolin-2-amine dihydrochloride; **Quinpirole**: *trans*-(-)-(4aR)-4,4a,5,6,7,8,8a,9-Octahydro-5-propyl-1*H*-pyrazolo[3,4-*g*]quinoline HCl; **SB-277011A**: *trans*-*N*-[4-[2-(6-Cyano-1,2,3, 4-tetrahydroisoquinolin-2-yl)ethyl]cyclohexyl]-4-quinolinecarboxamide; **SCH 23390**: (*R*)-(+)-7-Chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine HCl; **TFMPP**: *N*-[3-(Trifluoromethyl)phenyl]piperazine HCl; **U99194**: 2,3-Dihydro-5,6-dimethoxy-*N*, *N*-dipropyl-1*H*-inden-2-amine maleate

## Abstract:

A specific role for the dopamine D3 receptor in behavior has yet to be elucidated. We now report that dopamine D2/D3 agonists elicit dose-dependent yawning behavior in rats, resulting in an inverted U-shaped dose-response curve. A series of experiments was directed toward the hypothesis that the induction of yawning is a D3 receptor mediated effect, while the inhibition of the yawning observed at higher doses is due to competing D2 receptor activity. We compared several dopaminergic agonists with a range of *in vitro* D3 selectivity, including; PD-128,907, PD-128,908, quinelorane, pramipexole, 7-OH-DPAT, quinpirole, bromocriptine, and apomorphine with respect to their ability to induce yawning in rats. A series of D2/D3 antagonists differing in selectivity for D3 over D2 receptors were evaluated for their ability to alter the effects of the dopamine agonists. The antagonists L-741,626, haloperidol, nafadotride, U99194, SB-277011A, and PG01037 were used to determine effects on dose-response curves for D2/D3 agonist-induced yawning. In addition, the potential contribution of cholinergic and/or serotonergic mechanisms to the yawning response was investigated using a series of pharmacological tools including scopolamine, mianserin, and the D3-preferring antagonists; nafadotride, U99194, SB-277011A, and PG01037 to differentially modulate yawning induced by the PD-128,907, physostigmine, and TFMPP. The results of these experiments provide convergent evidence that dopamine D2/D3 agonist-induced yawning is a D3 agonist mediated behavior, with subsequent inhibition of yawning being driven by competing D2 agonist activity. Thus, dopamine agonist-induced yawning may represent an *in vivo* method for selectively identifying D3 and D2 receptor-mediated activities.

## Introduction:

Dopamine D3 receptors have received considerable interest since originally cloned (Sokoloff et al., 1990). The D3 receptor shares significant sequence homology with the dopamine D2 receptor, but displays a much more restricted, limbic pattern of distribution compared to that of the D2 receptor in the rat (Levesque et al., 1992) and human brain (Gurevich and Joyce, 1999). Based in large part on this restricted distribution and high sequence homology, it has been hypothesized that the D3 receptor may be of interest as a pharmacological target for antipsychotics and antiparkinsonian therapeutics (for review see Joyce, 2001). Additionally, the D3 receptor is thought to play a role in reinforcement pathways, as the D3 receptor is expressed in high levels within the mesolimbic dopaminergic system, and more specifically, the nucleus accumbens shell (Sokoloff et al., 1990; Stanwood et al., 2000).

However, progress in defining a role for the D3 receptor has been slowed by the inability to identify behavioral effects that can be linked exclusively to a D3 mechanism (Levant, 1997). This is, at least in part, due to the lack of pharmacologically selective compounds acting at either the D3 or D2 receptors, as well as the fact that potentially selective agonists have failed to elicit obvious, direct behavioral changes. While D2/D3 agonists and antagonists have been shown to produce changes in body temperature, locomotor activity, and other behavioral measures (Pugsley et al., 1995; Millan et al., 1995; Varty et al., 1998), a role for the D3 receptor in the regulation of these effects has typically not been confirmed by studies using D3 receptor deficient mice (Boulay et al., 1999a; Boulay et al., 1999b; Xu et al., 1999). Recently, increases in locomotor activity by MK-801 (Leriche et al., 2003) and blockade of the convulsant effects of dopamine uptake inhibitors (Witkin et al., 2004) have been proposed as *in vivo* models of D3 receptor activation. However, systematic replication of those findings or confirmation by other

means has not been reported. The studies reported herein provide evidence supporting the contention that yawning induced by D2/D3 agonists is mediated specifically through D3 receptor activation.

The ability of dopaminergic agonists to elicit biphasic yawning resulting in an inverted U-shaped dose-response curve in rats has been a long-studied phenomenon (e.g., Mogilnicka & Klimek, 1977; Holmgren & Urba-Holmgren, 1980; Yamada & Furkawa, 1980). An early hypothesis regarding the biphasic regulation of apomorphine-induced yawning behavior attributed the induction of yawning behavior to a D2 agonist activity, while the inhibition seen at higher doses was thought to be due to a competing D1 agonist activation (Yamada & Furkawa, 1980; Urba-Holmgren et al., 1982). The cloning of the dopamine D3 receptor and the development of agonists such as PD-128,907 (Pugsley et al., 1995) and 7-OH-DPAT (Levesque et al., 1992; Pugsley et al., 1995) as well as antagonists including U99194 (Cannon et al., 1982; Haadsma-Svensson and Svensson, 1998), SB-277011A (Stemp et al., 2000; Reavill et al., 2000) and PG01037 (Grundt et al., 2005) with greater degrees of *in vitro* selectivity for the D3 receptor have allowed greater insights into the regulation of dopaminergic agonist-induced yawning behavior to be made. Based on a series of studies examining the unconditioned behavioral effects of 7-OH-DPAT (Daly & Waddington, 1993; Kurashima et al., 1995; Ferrari & Guiliani, 1995), as well as binding studies (Levant et al., 1995), Levant (1997) hypothesized in an extensive review that, D2/D3 agonist-induced yawning may be a D3 agonist mediated effect, while the inhibition seen at higher doses was a result of concomitant D2 agonist activation.

This hypothesis was evaluated in the present studies using a host of pharmacological tools. The abilities of a series of compounds with varying *in vitro* selectivities for the D3

relative to D2 receptors to elicit yawning were examined. A series of antagonists, again defined by binding *in vitro* selectivity for the D3 and D2 receptors, were evaluated with respect to their modification of dose-response relationships for D2/D3 agonists, with the majority of the studies using PD-128,907 as a prototype D3 agonist.

Finally, in addition to dopaminergic mechanisms, yawning can be induced by cholinergic (Urba-Holmgren et al., 1977; Yamada and Furukawa, 1980) or serotonergic (Stancampiano et al., 1994) compounds. While the exact mechanisms and neural pathways involved in the regulation of yawning behavior have not been fully elucidated, there is a large set of data that suggests that dopaminergic, serotonergic, and cholinergic induction of yawning occur via distinct mechanisms. In addition both dopaminergic and serotonergic pathways are thought to eventually feed onto cholinergic neurons, thus allowing for differential regulation of dopaminergic and serotonergic yawning, with a cholinergic component common in all three pathways (for review see Argiolas and Melis, 1998). Therefore, some D3 antagonists that reduced PD-128,907-induced yawning were also assessed for their capacity to alter non-dopaminergic-induced yawning.

The convergent evidence from the agonist and antagonist studies support the hypothesis that dopamine agonist-induced yawning is mediated specifically through activation of D3 receptors. Therefore, yawning in rats may provide a critical model for establishing the *in vivo* activities of putative D3 selective ligands, a first step toward defining their role in normal and pathological physiological states.

## **Methods:**

### **Subjects.**

Male Sprague-Dawley rats (250-400 g) were obtained from Harlan (Indianapolis, IN) and housed three to a cage for the duration of the study. Rats had free access to standard Purina rodent chow and water, and were maintained in a temperature and humidity controlled environment, on a 12-h dark/light cycle with lights on at 7:00 AM. 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.

### **Behavioral Observations.**

**Yawning.** Yawning behavior was defined as a prolonged (~1 sec.), wide opening of the mouth followed by a rapid closure. On the day of testing rats were transferred from their home cage to a test chamber (19 in. x 9 in. x 8 in. clear “shoebox” rodent cage with standard cob bedding), and allowed to habituate to the chamber for a period of 30 minutes. Antagonist or vehicle was administered as a 30 minute pretreatment prior to the injection of agonist or vehicle. Behavioral observations began 10 minutes after all injections, and the total number of yawns was recorded for a period of 20 minutes thereafter. Dose-response curves were first generated for all agonists with a vehicle pretreatment, with antagonists substituted for vehicle pretreatments in subsequent sets of experiments. Each rat was tested multiple times, with separate groups of rats used to establish dose-response curves for each agonist, or antagonist X agonist combination. At least 48 hours was allowed between experimental sessions to allow for a drug



washout period. Food and water were unavailable during individual test sessions, and all experiments were conducted between the hours of 12:00 PM and 6:00 PM.

***Dopamine D2/D3 agonist-induced yawning.*** A series of dopaminergic agonists with varying degrees of *in vitro* selectivity for the D3 and D2 receptors were used to assess the ability of D2/D3 agonists to induce yawning behavior in rats. The D2/D3 agonists used in this series of experiments included: 7-OH-DPAT (0.0032, 0.01, 0.032, and 0.1 mg/kg), apomorphine (0.001, 0.0032, 0.01, 0.032, 0.1, and 0.32 mg/kg), bromocriptine (0.32, 1.0, 3.2, and 10.0 mg/kg), PD-128,907 (0.0032, 0.01, 0.032, 0.1, and 0.32 mg/kg), PD-128,908 (0.01, 0.032, 0.1, 0.32, and 1.0 mg/kg), pramipexole (0.00032, 0.001, 0.0032, 0.01, 0.032, 0.1, 0.32, and 1.0 mg/kg), quinelorane (0.0001, 0.00032, 0.001, 0.0032, 0.01, and 0.032 mg/kg), and quinpirole (0.0032, 0.01, 0.032, 0.1, and 0.32 mg/kg). All agonists were investigated in separate groups of rats, with doses presented in a random order.

***Effects of dopaminergic antagonists on D2/D3 agonist-induced yawning behavior.*** The effects of dopaminergic antagonists on D2/D3 agonist-induced yawning were examined, with each antagonist X agonist combination determined in separate groups of rats. Agonist and antagonist dose combinations were presented in a random order, with dose-response curves for vehicle X agonist treatments determined before and after antagonist X agonist combinations to insure there were no changes in agonist-induced yawning behavior.

***D2 selective antagonists.*** The effects of L-741,626 (0.32 and 1.0 mg/kg) on yawning elicited by PD-128,907 (0.01, 0.032, 0.1, and 0.32 mg/kg), or quinelorane (0.00032,

0.001, 0.0032, 0.01, and 0.032 mg/kg) were determined. Only a dose of 1.0 mg/kg L-741,626 was used in the quinelorane induced yawning studies.

*Non-selective dopamine receptor antagonism.* Haloperidol was used to determine the effects of non-selective dopaminergic antagonist activity on PD-128,907 (0.032, 0.1, 0.32, and 1.0 mg/kg) and quinelorane (0.001, 0.0032, 0.01, 0.032, and 0.1 mg/kg) induced yawning. Doses of 0.01 and 0.032 mg/kg haloperidol were used in PD-128,907 experiments, while only the lowest active dose of 0.032 mg/kg was used in quinelorane studies.

*D3 selective antagonists.* The D3-preferring antagonists; nafadotride (0.01, 0.1, and 0.32 mg/kg), U99194 (1.0, 3.2, and 10.0 mg/kg), SB-277011A (3.2, 32.0, and 56.0 mg/kg), and PG01037 (10.0, 32.0, and 56.0 mg/kg) were used to examine their effects on PD-128,907 (0.01, 0.032, 0.1, and 0.32 mg/kg) induced yawning. In rats treated with 0.32 mg/kg nafadotride the range of doses used for PD-128,907-induced yawning was extended to 1.0 mg/kg.

*D1/D5 and D4 selective antagonists.* The D1/D5 antagonist, SCH 23390 (0.01 mg/kg), and the D4 antagonist, L-745,870 (3.2 mg/kg), were used to address the possible involvement of these receptors in yawning elicited by PD-128,907 (0.01, 0.032, 0.1, and 0.32 mg/kg).

***Effects of cholinergic and serotonergic agonists and antagonists on yawning.***

Yawning elicited by cholinergic and serotonergic mechanisms were established by administration of physostigmine (0.01, 0.32, 0.1, 0.32, and 1.0 mg/kg; i.p.), and TFMPP (0.32, 1.0, 3.2, and 10.0 mg/kg) respectively. Scopolamine (0.0001, 0.001, and 0.01

mg/kg) was used to examine the effects of muscarinic cholinergic antagonism on yawning elicited by physostigmine (0.1 mg/kg; i.p.), TFMPP (3.2 mg/kg), and PD-128,907 (0.1 mg/kg). Likewise, the ability of the 5-HT<sub>2</sub> receptor antagonist mianserin (0.0032, 0.032, and 0.32 mg/kg) to antagonize yawning induced by TFMPP (3.2 mg/kg), physostigmine (0.1 mg/kg; i.p.), and PD-128,907 (0.1 mg/kg) was determined.

**D3 selective antagonists on cholinergic and serotonergic yawning.** The ability of nafadotride (0.01, 0.1, and 1.0 mg/kg), U99194 (1.0, 3.2, and 10.0 mg/kg), SB-277011A (3.2, 32.0, and 56.0 mg/kg), and PG01037 (10.0, 32.0, and 56.0 mg/kg) to modulate yawning behavior induced by PD-128,907 (0.1 mg/kg), TFMPP (3.2 mg/kg) and physostigmine (0.1 mg/kg; i.p.) was determined in separate groups of rats.

## Drugs:

(±)-7-OH-DPAT [(±)-7-Hydroxy-2-dipropylaminotetralin HBr], (-)-apomorphine [(R)-(-)-5,6,6a,7-Tetrahydro-6-methyl-4*H*-dibenzo[*de,g*]quinoline-10,11-diol HCl], (+)-bromocriptine [(+)-2-Bromo-12'-hydroxy-2'-(1-methylethyl)-5'-(2-methylpropyl)ergotaman-3',6'-18-trione methanesulfonate], haloperidol [4-[4-(4-Chlorophenyl)-4-hydroxy-1-piperidinyl]-1-(4-fluorophenyl)-1-butanone HCl], mianserin [1,2,3,4,10,14b-Hexahydro-2-methyldibenzo[*c,f*]pyrazino[1,2-*a*]azepine HCl], PD-128,907 [(S)-(+)-(4*aR*,10*bR*)-3,4,4*a*,10*b*-Tetrahydro-4-propyl-2*H*,5*H*-[1]benzopyrano-[4,3-*b*]-1,4-oxazin-9-ol HCl], PD-128,908 [(R)-(-)-(4*aS*,10*bS*)-3,4,4*a*,10*b*-Tetrahydro-4-propyl-2*H*,5*H*-[1]benzopyrano-[4,3-*b*]-1,4-oxazin-9-ol HCl], physostigmine [(3*aS*)-*cis*-1,2,3,3*a*,8,8*a*-Hexahydro-1,3*a*,8-trimethylpyrrolo[2,3-*b*]indol-5-ol methylcarbamate hemisulfate], quinelorane [(5*aR-trans*)-5,5*a*,6,7,8,9,9*a*,10-Octahydro-6-propylpyrido[2,3-*g*]quinazolin-2-amine dihydrochloride], (-)-quinpirole [*trans*-(-)-(4*aR*)-4,4*a*,5,6,7,8,8*a*,9-Octahydro-5-propyl-1*H*-pyrazolo[3,4-*g*]quinoline HCl], SCH 23390 [(R)-(+)-7-Chloro-8-hydroxy-3-

methyl-1-phenyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine HCl], scopolamine [(*a,S*)-*a*-(Hydroxymethyl)benzeneacetic acid (1*a,2b,4b,5a,7b*)-9-methyl-3-oxa-9-azatricyclo[3.3.1.0<sup>2,4</sup>]non-7-yl ester hydrobromide], and TFMPP [N-[3-(Trifluoromethyl)phenyl]piperazine HCl] were obtained from Sigma Chemical Co (St. Louis, Mo). L-741,626 [3-[[4-(4-Chlorophenyl)-4-hydroxypiperidin-1-yl]methyl-1*H*-indole], L-745,870 [3-(4-[4-Chlorophenyl] piperazin-1-yl)-methyl-1*H*-pyrrolo[2,3-*b*]pyridine trihydrochloride], nafadotride [N-[(1-Butyl-2-pyrrolidinyl)methyl]-4-cyano-1-methoxy-2-naphthalenecarboxamide], and U99194 [2,3-Dihydro-5,6-dimethoxy-*N, N*-dipropyl-1*H*-inden-2-amine maleate] were obtained from Tocris (Ellisville, MO). Pramipexole [N'-propyl-4,5,6,7-tetrahydrobenzothiazole-2,6-diamine] was generously provided by Dr. Edward F. Domino, MD (University of Michigan Medical School, Ann Arbor, MI), SB-277011A [trans-N-[4-[2-(6-Cyano-1,2,3, 4-tetrahydroisoquinolin-2-yl)ethyl]cyclohexyl]-4-quinolinecarboxamide] by Dr. Deyi Zhang (Lily Research Labs, Indianapolis, IN), and PG01037 [N-{4-[4-(2,3-Dichlorophenyl)-piperazin-1-yl]-*trans*-but-2-enyl}-4-pyridine-2-yl-benzamide HCl] by Dr. Amy H Newman (Medicinal Chemistry Section-NIDA, Baltimore, MD). All drugs were dissolved in sterile water with the exception of haloperidol, which was dissolved in 5% ethanol, L-741,626, which was dissolved in 5% ethanol with 1M HCl, and SB-277011A, which was dissolved in 10%  $\beta$ -cyclodextrin. All drugs were administered sub-cutaneously (s.c.) in a volume of 1 ml/kg, with the exception of physostigmine, which was administered i.p. in a volume of 1 ml/kg. The 56.0 mg/kg doses of SB-277011A and PG01037 were administered in a volume of 3 ml/kg s.c. due to solubility limitations.

### Data Analysis:

All yawning studies were conducted with 8 rats per group, and results are expressed as mean number of yawns during the 20 minute observation period  $\pm$  standard error of the

mean (SEM). A one-way, repeated-measures ANOVA with post-hoc Dunnett's tests was used to determine if agonist-induced yawning was significantly greater compared to vehicle (GraphPad Prism; GraphPad Software Inc., San Diego, CA). Significant differences in the maximal amount of yawning elicited were determined by one-way repeated-measures ANOVA with post-hoc Tukey's HSD tests. Significant effects of antagonist pretreatment on agonist-induced yawning was determined using an unbalanced, two-way ANOVA with post-hoc Bonferroni tests to determine significant differences among antagonist and vehicle treated groups (SPSS, SPSS Inc., Chicago, IL). One-way repeated-measures ANOVAs with post-hoc Dunnett's tests were also used to determine if D3-preferring, cholinergic, or serotonergic antagonists significantly inhibited yawning elicited by the maximal effective dose of D2/D3, cholinergic, or serotonergic agonists (GraphPad Prism).

## Results:

**Dopamine D2/D3 agonists on yawning behavior.** D2/D3 agonists generally elicited dose-dependent increases in yawning behavior, with a subsequent inhibition of yawning seen at higher doses resulting in a characteristic inverted U-shaped dose-response curve as shown in figure 1. PD-128,907 [F(5,35)=19.86;  $p<0.0001$ ], quinelorane [F(6,42)=29.68;  $p<0.0001$ ], pramipexole [F(8,56)=14.50;  $p<0.0001$ ], 7-OH-DPAT [F(4,28)=39.68;  $p<0.0001$ ], quinpirole [F(5,35)=42.47;  $p<0.0001$ ], and apomorphine [F(6,42)=3.81;  $p<0.01$ ] all elicited significant, dose-dependent increases in yawning behavior compared to vehicle, while yawning induced by bromocriptine [F(4,28)=1.14;  $p>0.05$ ] failed to reach significance. PD-128,908, the inactive enantiomer of PD-128,907 (DeWald et al., 1990) did not elicit yawning at any dose tested [F(4,28)=0.30;  $p>0.05$ ]. Significantly greater amounts of yawning compared to vehicle were observed for PD-128,907 (0.032 and 0.1 mg/kg;  $p<0.01$ ), quinelorane (0.001 and 0.0032 mg/kg;  $p<0.01$ ), pramipexole [(0.01, 0.032 and 0.1 mg/kg;  $p<0.01$ ); (0.32 mg/kg;  $p<0.05$ )], 7-OH-DPAT (0.01, and 0.032 mg/kg;  $p<0.01$ ), quinpirole (0.01, and 0.032 mg/kg;  $p<0.01$ ), and apomorphine (0.032 mg/kg;  $p<0.05$ ).

There were no significant differences [F(4,28)=1.70;  $p>0.05$ ] in the amount of yawning elicited by the maximal effective doses of PD-128,907 (0.1 mg/kg;  $20.0\pm1.7$ ), quinelorane (0.0032 mg/kg;  $29.3\pm3.1$ ), pramipexole (0.1 mg/kg;  $24.5\pm4.4$ ), 7-OH-DPAT (0.032 mg/kg;  $23.4\pm3.0$ ), and quinpirole (0.032 mg/kg;  $27.5\pm2.9$ ); however, the maximal effective dose of apomorphine (0.032 mg/kg;  $10.4\pm3.1$ ) [F(5,42)=4.67;  $p<0.01$ ] produced significantly lower levels of yawning compared to all other D2/D3 agonists that elicited significant amounts of yawning.

**D2 selective antagonism of D2/D3 agonist-induced yawning.** The effects of L-741,626, a D2-preferring antagonist approximately 50-fold selective for D2 compared to D3 receptors *in vitro* (Kulagowski et al., 1996), at behaviorally active doses (Chaperon et al., 2003), on PD-128,907- and quinelorane-induced yawning and are shown in Figures 2A and 2B respectively. An analysis of variance determined that there was an overall significant effect of L-741,626 on PD-128,907-induced yawning, and that the effect was dependent on both the dose of L-741,626 and PD-128,907 administered [main antagonist-dose effect,  $F(2,103)=8.29$ ,  $p<0.001$ ; main agonist-dose effect,  $F(4,103)=20.34$ ,  $p<0.001$ ; antagonist-dose x agonist-dose interaction,  $F(6,103)=7.52$ ,  $p<0.001$ ]. Likewise, L-741,626 significantly modified quinelorane-induced yawning, an effect that was dependent on both the dose of L-741,626, as well as the dose of quinelorane [main antagonist-dose effect,  $F(1,79)=11.91$ ,  $p<0.001$ ; main agonist-dose effect,  $F(4,79)=18.64$ ,  $p<0.001$ ; antagonist-dose x agonist-dose interaction,  $F(4,79)=11.81$ ,  $p<0.001$ ]. L-741,626 significantly increased the amount of yawning elicited by high doses of both PD-128,907 (0.32 mg/kg;  $p<0.001$ ) and quinelorane (0.01 mg/kg;  $p<0.001$ ), while having no effect on yawning induced by lower doses of either PD-128,907 or quinelorane.

***Non-selective dopaminergic antagonism of D2/D3 agonist-induced yawning.***

Haloperidol, a non-selective dopaminergic antagonist with high affinities for all DA receptor subtypes (Sokoloff et al., 1992; Kulagowski et al., 1996), was used at behaviorally active doses (e.g., Leriche et al., 2003) to examine the effects of dopaminergic antagonism on yawning induced by PD-128907 and quinelorane (figures 2C and 2D respectively). Pretreatment with haloperidol modified PD-128,907-induced yawning in a manner that was dependent on the dose of agonist administered [main antagonist-dose effect,  $F(2,79)=1.86$ ,  $p>0.05$ ; main agonist-dose effect,  $F(3,79)=12.52$ ,

$p < 0.001$ ; antagonist-dose  $\times$  agonist-dose interaction,  $F(4,79)=21.30$ ,  $p < 0.001$ ]. The effects of haloperidol on quinelorane-induced yawning were similar to those on PD-128,907-induced yawning, and were dependent on both the dose of haloperidol and the dose of quinelorane [main antagonist-dose effect,  $F(1,71)=10.78$ ,  $p < 0.01$ ; main agonist-dose effect,  $F(4,71)=13.50$ ,  $p < 0.001$ ; antagonist-dose  $\times$  agonist-dose interaction,  $F(3,71)=22.55$ ,  $p < 0.001$ ]. Unlike L-741,626, haloperidol produced differential effects on D2/D3 agonist-induced yawning. Pretreatment with 0.032 mg/kg haloperidol resulted in significant decreases in yawning elicited by low doses of PD-128,907 (0.032 mg/kg;  $p < 0.05$ ) and quinelorane (0.001 mg/kg;  $p < 0.01$ ), while producing significant increases in the amount of yawning elicited by high doses of PD-128,907 (0.32 mg/kg;  $p < 0.001$ ) and quinelorane (0.01 and 0.032 mg/kg;  $p < 0.001$  and  $p = 0.001$  respectively).

**D3 preferring antagonists on D2/D3 agonist-induced yawning.** Nafadotride, U99194, SB-277011A, and PG01037 have been shown to preferentially bind the D3 receptor over the D2 receptor *in vitro*, with D3 selectivities of approximately 3-, 30-, 100-, and 133-fold respectively (Sautel et al., 1995; Flietstra and Levant, 1998; Audinot et al., 1998; Stemp et al., 2000; Grundt et al., 2005), and were used at behaviorally active doses (Waters et al., 1993; Vorel et al., 2002; Di Ciano et al., 2003; Leriche et al., 2003; Millan et al., 2004) to examine their effects on yawning behavior in rats.

The effects of nafadotride (0.01, 0.1, and 0.32 mg/kg) on PD-128,907-induced yawning are shown in figure 3A. An analysis of variance revealed that nafadotride altered PD-128,907 induced yawning in a manner that was dependent on the dose of agonist administered [main antagonist-dose effect,  $F(3,135)=0.34$ ,  $p > 0.05$ ; main agonist-dose effect,  $F(4,135)=20.48$ ,  $p < 0.001$ ; antagonist-dose  $\times$  agonist-dose interaction,  $F(9,135)=3.92$ ,  $p < 0.001$ ]. While slight reductions in yawning elicited by low doses of PD-



128,907 were observed with doses of 0.1 and 0.32 mg/kg nafadotride, these effects were not significant at either dose. However, pretreatment with 0.32 mg/kg nafadotride did produce significant increases in yawning elicited by 0.32 mg/kg of PD-128,907 ( $p < 0.001$ ).

The effects of U99194 (1.0 mg/kg, 3.2 mg/kg, and 10.0 mg/kg) on PD-128,907-induced yawning are shown in figure 3B. U99194 modified PD-128,907-induced yawning in a manner that was dependent on both the dose of U99194 and dose of PD-128,907 [main antagonist-dose effect,  $F(3,119)=40.08$ ,  $p < 0.001$ ; main agonist-dose effect,  $F(3,119)=42.26$ ,  $p < 0.001$ ; antagonist-dose x agonist-dose interaction,  $F(8,119)=4.69$ ,  $p < 0.001$ ]. At a dose of 3.2 mg/kg, U99194 decreased the amount of yawning elicited by low doses of PD-128,907 (0.032 and 0.1 mg/kg;  $p < 0.05$  for both) while there was no effect on yawning elicited by 0.32 mg/kg PD-128,907. At the highest dose of U99194 tested (10.0 mg/kg), PD-128,907-induced yawning was completely inhibited at all doses tested [(0.032 mg/kg;  $p < 0.001$ ); (0.1 mg/kg;  $p < 0.001$ ) and (0.32 mg/kg;  $p > 0.05$ )].

The effects of SB-277011A (3.2, 32.0 and 56.0 mg/kg) on PD-128,907-induced yawning are shown in figure 3C, and they were dependent on both the dose of SB-277011A as well as the dose of PD-128,907 administered [main antagonist-dose effect,  $F(3,119)=29.18$ ,  $p < 0.001$ ; main agonist-dose effect,  $F(3,119)=37.29$ ,  $p < 0.001$ ; antagonist-dose x agonist-dose interaction,  $F(8,119)=4.40$ ,  $p < 0.001$ ]. SB-277011, at a dose of 32.0 mg/kg, significantly inhibited PD-128,907-induced yawning at doses corresponding to the ascending limb of the dose-response curve [(0.01 mg/kg;  $p < 0.05$ ); (0.032 mg/kg;  $p = 0.001$ ); and (0.1 mg/kg;  $p < 0.001$ )]. Likewise, 56.0 mg/kg SB-277011 further reduced PD-128,907 elicited yawning at both 0.032 ( $p < 0.001$ ) and 0.1 mg/kg

( $p < 0.001$ ). There were no effects of any dose of SB-277011A on yawning induced by a high dose of 0.32 mg/kg PD-128,907.

PG01037, a D3-preferring antagonist with similar *in vitro* selectivity for the D3 receptor compared to SB-277011A, was administered at doses of 10.0, 32.0, and 56.0 mg/kg, and the effects on PD-128,907 elicited yawning are shown in figure 3D. Pretreatment with PG01037 altered PD-128,907-induced yawning in a manner that was dependent on both the dose of PG01037 and dose of PD-128,907 administered [main antagonist-dose effect,  $F(3,119)=17.68$ ,  $p < 0.001$ ; main agonist-dose effect,  $F(3,119)=33.10$ ,  $p < 0.001$ ; antagonist-dose x agonist-dose interaction,  $F(8,119)=2.69$ ,  $p < 0.05$ ]. Similar to SB-277011A, PG01037, at a dose of 32.0 mg/kg, significantly reduced yawning elicited by low doses of PD-128,907 [(0.032mg/kg;  $p < 0.01$ ) and (0.1 mg/kg;  $p < 0.001$ )]. Further decreases in yawning induced by low doses of PD-128,907 [(0.032mg/kg;  $p < 0.001$ ) and (0.1 mg/kg;  $p < 0.001$ )] were observed with a dose of 56.0 mg/kg PG01037. There were no effects of any dose of PG01037 on yawning induced by a high dose of 0.32 mg/kg PD-128,907.

**Other dopamine receptor antagonists.** The D1-like receptor selective antagonist SCH 23390 (Barnett et al., 1986) and the D4 selective antagonist L-745,870 (Kulagowski et al., 1996) were used at behaviorally active doses (Patel et al., 1997; Chaperon et al., 2003) to assess the ability of D1/D5 and D4 antagonism respectively, to modulate the dose response curve for D2/D3 agonist-induced yawning. SCH 23390, at a dose of 0.01 mg/kg did not produce any significant change in the amount of yawning elicited by any dose of PD-128,907 tested (0.01 – 0.32 mg/kg; data not shown). Likewise, at a dose of 3.2 mg/kg, the D4 selective antagonist L-745,870 failed to alter PD-128,907-induced yawning at any dose tested (0.01 – 0.32 mg/kg; data not shown).

**Cholinergic and serotonergic induced yawning.** Both physostigmine [ $F(4,28)=7.11$ ;  $p<0.001$ ] and TFMPP [ $F(4,28)=7.15$ ;  $p<0.001$ ] also elicited, inverted U-shaped, dose-dependent yawning behavior in rats, as shown in figure 4A; however, both the cholinergic and serotonergic agonists were significantly less effective compared to PD-128,907 [ $F(2,14)=9.50$ ;  $p<0.01$ ]. Maximal amounts of yawning induced by physostigmine and TFMPP occurred at doses of 0.1 mg/kg i.p. and 3.2 mg/kg, respectively, and were the only doses to elicit significantly greater amounts of yawning compared to vehicle treated rats ( $p<0.01$  for both).

The effects of the non-selective, muscarinic antagonist, scopolamine (0.0001, 0.001, and 0.01 mg/kg), on yawning elicited by physostigmine (0.1 mg/kg; i.p.), PD-128,907 (0.1 mg/kg), and TFMPP (3.2 mg/kg) are shown in figure 4B. Scopolamine produced significant, dose-dependent antagonism of physostigmine-induced yawning [ $F(3,21)=16.89$ ;  $p<0.0001$ ], with a dose of 0.01 mg/kg scopolamine significantly inhibiting physostigmine-induced yawning compared to vehicle treated rats ( $p<0.01$ ). In addition, scopolamine dose-dependently, and significantly inhibited yawning elicited by both PD-128,907 [ $F(3,21)=17.25$ ;  $p<0.0001$ ], and TFMPP [ $F(3,21)=22.40$ ;  $p<0.0001$ ]. Significantly lower levels of PD-128,907-induced yawning were observed with doses of 0.001, and 0.01 mg/kg scopolamine ( $p<0.01$  for both). Scopolamine significantly reduced TFMPP elicited yawning at all doses tested ( $p<0.01$  for all).

The effects of the 5-HT<sub>2</sub> receptor subtype antagonist, mianserin (0.0032, 0.032, and 0.32 mg/kg), on yawning elicited by TFMPP (3.2 mg/kg), PD-128,907 (0.1 mg/kg), and physostigmine (0.1 mg/kg; i.p.) are shown in figure 4C. Mianserin produced a dose-dependent and significant inhibition of TFMPP-induced yawning [ $F(3,21)=9.85$ ;  $p<0.001$ ],

with doses of 0.032 and 0.32 mg/kg mianserin significantly inhibiting TFMPP-induced yawning compared to vehicle treated rats ( $p < 0.01$  for both). Mianserin did not significantly effect yawning elicited by either PD-128,907 [ $F(3,21)=0.84$ ;  $p > 0.05$ ] or physostigmine [ $F(3,21)=0.26$ ;  $p > 0.05$ ], at any dose tested.

***D3-preferring antagonists on dopaminergic, cholinergic and serotonergic agonist induced yawning.*** Figure 5 shows the effects of the D3-preferring antagonists; nafadotride, U99194, SB-277011A, and PG01037 on yawning elicited by PD-128,907 (0.1 mg/kg), physostigmine (0.1mg/kg; i.p.), and TFMPP (3.2 mg/kg). Nafadotride (figure 5A), dose-dependently and significantly inhibited yawning elicited by PD-128,907 [ $F(3,21)=5.36$ ;  $p < 0.01$ ] with a dose of 1.0 mg/kg significantly reducing yawning compared to vehicle treated rats ( $p < 0.01$ ). There were no significant effects of nafadotride on either physostigmine- [ $F(3,21)=0.32$ ;  $p > 0.05$ ] or TFMPP- [ $F(3,21)=0.60$ ;  $p > 0.05$ ] induced yawning. As shown in figure 5B, U99194 dose dependently and significantly reduced the amount of yawning elicited by PD-128,907 [ $F(3,21)=29.78$ ;  $p < 0.0001$ ], with doses of 3.2 and 10.0 mg/kg U99194 significantly inhibiting yawning compared to vehicle treated rats ( $p < 0.01$  for both). Unlike nafadotride, U99194 also significantly inhibited the amount of yawning elicited by physostigmine [ $F(3,21)=11.91$ ;  $p < 0.0001$ ], and TFMPP [ $F(3,21)=7.07$ ;  $p < 0.01$ ], with a dose of 10.0 mg/kg U99194 resulting in a significant reductions in the amount of yawning elicited by both physostigmine ( $p < 0.01$ ) and TFMPP ( $p < 0.01$ ). The effects of SB-277011A on PD-128,907-, physostigmine-, and TFMPP-induced yawning are shown in figure 5C. SB-277011A dose-dependently and significantly reduced the amount of yawning elicited by PD-128,907 [ $F(3,21)=12.09$ ;  $p < 0.0001$ ], with doses of 32.0 and 56.0 mg/kg ( $p < 0.01$  for both) significantly inhibiting yawning compared to vehicle treated rats. No significant effects of SB-277011A were seen on yawning elicited by either physostigmine

[F(3,21)=0.68;  $p>0.05$ ] or TFMPP [F(3,21)=2.20;  $p>0.05$ ]. Similarly, PG01037 significantly and dose-dependently inhibited yawning elicited by PD-128,907 [F(3,21)=29.43;  $p<0.0001$ ], with doses of 32.0 and 56.0 mg/kg ( $p<0.05$  for both) PG01037 significantly reducing yawning compared to vehicle treated rats (figure 5D). PG01037 did not significantly effect yawning elicited by either 0.1 mg/kg physostigmine [F(3,21)=0.16;  $p>0.05$ ], or 3.2 mg/kg TFMPP [F(3,21)=0.07;  $p>0.05$ ] at any dose tested.

## **Discussion:**

Evidence has been provided in the present paper to support the hypothesis that D2/D3 agonist-induced yawning behavior in rats is mediated by agonist activation of the dopamine D3 receptor, while the inhibition of yawning is a result of a competing agonist activation of the dopamine D2 receptor. In agreement with the majority of previous studies, all of the D2/D3 agonists tested with exception of bromocriptine and PD-128,908 (Figure 1c), the inactive enantiomer of PD-128,907, elicited significant, dose-dependent increases in yawning behavior with inhibition seen at higher doses, resulting in the characteristic inverted U-shaped dose response curve for yawning in rats. Evidence is also provided for the selective antagonism of the induction of yawning behavior by D3-preferring antagonists, and the inhibition of yawning by D2-preferring antagonists. In addition, the current studies demonstrate that inhibition of D3 agonist-induced yawning by D3-preferring antagonists is a result of their selective antagonist activity at the D3 receptor, and not through antagonist effects at D2, serotonergic, or muscarinic cholinergic receptors.

### **Yawning is a D3 mediated behavior.**

Several lines of evidence have been provided in support of the hypothesis that yawning is a D3 agonist mediated behavior. In general, all D3-preferring D2/D3 agonists induced significant amounts of yawning at low doses. While there were no significant differences in the effectiveness of the agonists with respect to induction of yawning behavior with the exception of apomorphine, there were differences in the potency of the D2/D3 agonists to induce yawning. The rank-order potency of the D2/D3 agonists to elicit yawning behavior was as follows; quinelorane, apomorphine, quinpirole, 7-OH-DPAT, pramipexole, and PD-128,907, while bromocriptine and PD-128,908 failed to elicit significant levels of yawning. The stereoselectivity of the yawning response with regard

to PD-128,907 [and PD-128,908] is an important finding, as dopamine receptors are selective with respect to more rigid agonists (DeWald et al., 1990). Taken together with the findings of Stahle and Ungerstedt (1984), who showed that (+)-3-PPP, but not (-)-3-PPP, will elicit yawning, our current findings provide further evidence that D2/D3 agonists are inducing yawning via dopaminergic agonist mechanisms. Differences in yawning induced by bromocriptine may be a result of pharmacokinetic differences, as bromocriptine has been shown to induce significant levels of yawning in studies using a 60 minute observation period (Protais et al., 1983; Zarrindast and Jamshidzadeh, 1992).

Antagonists with a high degree of selectivity for the D3 compared to the D2 receptor selectively antagonized the induction of yawning behavior. Three of the four D3-preferring antagonists (U99194, SB-277011A, and PG01037) tested in the current studies possess the ability to dose-dependently and selectively antagonize the induction of yawning by PD-128,907, while having no effect on the inhibition of yawning observed at higher doses. As shown in figures 3C and 3D respectively, SB-277011A and PG01037, D3-preferring antagonists with similarly high degrees of *in vitro* D3 selectivity (100- and 133-fold respectively) produced almost identical effects on PD-128,907-induced yawning; significant, dose-dependent, downward/rightward shifts of the ascending limb of the yawning dose-response curve were observed, while the descending limb of the dose-response curve for PD-128,907-induced yawning was not changed. Similar effects were seen with the moderately selective (30-fold) D3-preferring antagonist U99194, however, unlike SB-277011A and PG01037, at relatively high-dose of 10.0 mg/kg, U99194 completely inhibited PD-128,907-induced yawning; however, it should be noted that at this dose U99194 effectively antagonized not only dopaminergic, but cholinergic and serotonergic yawning as well. Nafadotride, the least selective (3-fold) of the D3-preferring antagonists, was the only D3 antagonist to produce a non-selective

antagonism of yawning behavior; shifting both the ascending and descending limbs of the dose-response curve for PD-128,907-induced yawning at the highest dose tested. This effect was similar to that observed with haloperidol, a non-selective dopamine antagonist, and suggests that at a dose of 0.32 mg/kg, nafadotride is no longer selective for the D3 receptor, but rather is active at both the D3 and D2 receptors. Taken together, these data provide strong support for the hypothesis that the induction of yawning by D2/D3 agonists is mediated by an agonist activation of the D3 receptor.

### **Inhibition of yawning is a D2 mediated effect.**

We have also provided evidence in support of the hypothesis that inhibition of D2/D3 agonist-induced yawning occurring at higher doses is mediated by an agonist activity at the D2 receptor. As shown in figure 2A and 2B, the D2-preferring antagonist L-741,626, at the first behaviorally active dose (1.0 mg/kg), selectively antagonized the inhibitory effects of high doses of PD-128,907 and quinelorane, resulting in a rightward shift in the descending limbs while having virtually no effect on the ascending limbs of the dose-response curves for both PD-128,907- and quinelorane-induced yawning. In addition L-741,626 produced a rightward shift in the maximal effective dose of PD-128,907 and quinelorane, resulting in an increased effectiveness for both agonists. These data not only suggest that L-741,626, at a dose of 1.0 mg/kg, is an effective D2 antagonist *in vivo*, but that it is also devoid of D3 antagonist activity.

Further support for the differential regulation of yawning behavior by the D3 and D2 receptors was provided by the effects of the non-selective DA antagonist haloperidol. As D3- and D2-preferring antagonists selectively antagonize the ascending and descending limbs of the dose-response curve for D2/D3 agonist-induced yawning respectively, it would be expected that antagonists with mixed D2/D3 actions, such as haloperidol,



would shift both the ascending and descending limbs of yawning dose-response curves at their initial active doses. Indeed, at the first behaviorally active dose (0.032 mg/kg), haloperidol produced rightward shifts in both the ascending and descending limbs of the dose-response curves for both PD-128,907- and quinelorane-induced yawning (Figures 2C and 2D). This not only suggests that the effects of D3- and D2-preferring antagonists are a result of selective antagonist activity, but that non-selective D2/D3 antagonists produce effects distinct from those of other dopaminergic antagonists on D3 agonist-induced yawning.

However, it should be noted that in addition to possessing high affinities for the D3 and D2 receptors, haloperidol also has significant affinities for the D1, D4, and D5 receptors. It is, however, unlikely that activity at these receptors is influencing PD-128,907-induced yawning behavior as the D1/D5-selective antagonist, SCH 23390, and the D4-selective antagonist, L-745,870, at behaviorally active doses (Patel et al., 1997; Chaperon et al., 2003) did not alter yawning elicited by either low (0.032-0.1 mg/kg) or high (0.32 mg/kg) doses of PD-128,907. This provides further evidence that D2/D3 agonist-induced yawning behavior is under the direct control of the D3 (induction) and D2 (inhibition) receptors, but not the D1, D4, or D5 receptors. However, the possibility remains that other dopaminergic receptors may modulate D3 agonist-induced yawning elicited by other D2/D3 agonists, as several of the agonists tested, such as apomorphine, quinelorane, and quinpirole, possess significant affinities for the D1, D4, and D5 receptors (apomorphine), or D4 receptor (quinelorane and quinpirole) in addition to the D3 and D2 receptors.

**Dopaminergic, serotonergic, and cholinergic regulation of yawning.**

The findings of the current study confirm, and extend those of earlier studies (e.g. Yamada and Furukawa, 1980; Ushijima et al., 1984; Zarrindast and Poursoltan, 1989; Stancampiano et al., 1994), and demonstrate that while scopolamine will dose-dependently antagonize yawning induced by cholinergic, serotonergic, and dopaminergic agonists (figure 4B), serotonergic and dopaminergic antagonists are able to selectively antagonize yawning elicited by their respective agonists. More specifically, nafadotride, SB-277011A, and PG01037, D3-preferring antagonists with a wide range (3-133 fold) of selectivities for the D3 receptor over the D2 receptor *in vitro*, were able to selectively antagonize PD-128,907-induced yawning, while having no effect on yawning elicited by either physostigmine or TFMPP (Figure 5). This suggests that SB-277011A and PG01037 are not only selective for the D3 over the D2 receptor, but that they are also selective for the D3 receptor over certain serotonergic and cholinergic receptors at doses up to 56.0 mg/kg. Similarly, while nafadotride demonstrated little or no preference for the D3 compared to the D2 receptor *in vivo*, no serotonergic or cholinergic antagonist activity was detected at doses up to 1.0 mg/kg. However, in contrast to the effects of the other D3-preferring antagonists, U99194, at a dose of 10.0 mg/kg, significantly antagonized yawning elicited by PD-128,907, TFMPP and physostigmine, suggesting that at higher doses, it is no longer selective for dopaminergic receptors. While U99194 is unique in this regard within this group of D3-preferring antagonists, clozapine, an antagonist with significant affinities for dopaminergic, serotonergic and cholinergic receptors has also been shown to antagonize both dopaminergic and cholinergic yawning (Dubuc et al., 1982), suggesting that antagonism of physostigmine-induced yawning may be a reliable measure of anti-cholinergic activity. Further evidence of an *in vivo* anti-muscarinic activity of U99194 has been demonstrated by Goudie and colleagues (2001) who showed in discrimination studies that U99194 generalized to a scopolamine cue, suggesting that U99194 may possess anti-cholinergic activity at higher

doses. Although it has been suggested that U99194 functions as a D3 selective antagonist *in vivo* at doses ranging from 13.0 to 40.0 mg/kg based on its inability to increase plasma prolactin, to induce catalepsy, and to inhibit the induction of hypothermia by PD-128,907 (Audinot et al., 1998), the results of the current study suggest that while U99194 may be selective for the D3 compared to the D2, a significant anti-cholinergic effect is apparent at 10.0 mg/kg. Thus the current studies support the hypothesis that dopaminergic, serotonergic and cholinergic agonists induce yawning via distinct mechanisms, and furthermore that yawning induced by D2/D3 agonists is a result of agonist activation of D3 receptors, and not serotonergic or cholinergic receptors.

To summarize the results of the studies reported herein, evidence has been provided in support of the hypothesis that the induction of yawning by D2/D3 agonists is mediated through an agonist activity at the D3 receptor, while the subsequent inhibition of yawning seen at higher doses is a result of an increasing D2 agonist activity. Based on these findings several conclusions can be drawn: First, the ascending limb of the dose-response curves corresponds to doses that are selectively activating D3 receptors over D2 receptors, while the descending limb corresponds to those activating both the D3 and D2 receptors. Additionally, determinations of *in vivo* D3 potency and effectiveness may be possible, based on the onset and maximal amount of yawning elicited. Furthermore, inhibition of yawning may provide useful information regarding *in vivo* D2 potency, and lastly, the shape of the dose-response curves may allow for determinations of *in vivo* D3 selectivity of D3-preferring D2/D3 agonists to be made. The results of the current set of studies have demonstrated that D3 selective antagonism will only shift the ascending limb of the yawning dose-response curve, that D2 selective antagonism will only shift the descending limb of the yawning dose-response curve, while non-selective D2/D3

antagonism will shift both the ascending and descending limbs of the dose-response curve for D2/D3 agonist-induced yawning behavior in rats. In conclusion, as the current studies have provided evidence that the induction of yawning behavior by D2/D3 agonists is mediated by the D3 receptor, yawning may be an important pharmacological effect that can be used in the characterization, classification, and discovery of *in vivo* D3 agonist and antagonist actions. Thus, it may be possible to relate other behavioral effects of D2/D3 agonists and antagonists to their ability to modulate yawning. Whether the potency and selectivity measures of these compounds can be utilized across behavioral measures will need to be explored in the future.

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## References:

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

Audinot V., Newman-Tacnredi A., Gobert A., Rivet J.M., Brocco M., Lejeune F., Gluck L., Desposte I., Bervoets K., Dekeyne A., and Millan M.J. (1998) A comparative in vitro and in vivo pharmacological characterization of the novel dopamine D3 receptor antagonists (1)-S 14297, nafadotride, GR 103,691 and U 99194. *J Pharmacol Exp Ther* **287**:187-197.

Barnett A, Ahn HS, Billard W, Gold EH, Kohli JD, Glock D, Goldberg LI. (1986) Relative activities of SCH 23390 and its analogs in three tests for D1/DA1 dopamine receptor antagonism. *Eur J Pharmacol.* **128**(3):249-253.

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**(9):1389-1396.

Boulay D, Depoortere R, Rostene W, Perrault G, Sanger DJ. (1999) Dopamine D3 receptor agonists produce similar decreases in body temperature and locomotor activity in D3 knock-out and wild-type mice. *Neuropharmacology.* **38**(4):555-565.

Cannon JG, Perez JA, Bhatnagar RK, Long JP, Sharabi FM. (1982) Conformationally restricted congeners of dopamine derived from 2-aminoindan. *J Med Chem.* **25**(12):1442-1446.

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**(8):1047-1053.

Daly SA, Waddington JL. (1993) Behavioural effects of the putative D-3 dopamine receptor agonist 7-OH-DPAT in relation to other "D-2-like" agonists. *Neuropharmacology*. **32**(5):509-510.

DeWald HA, Heffner TG, Jaen JC, Lustgarten DM, McPhail AT, Meltzer LT, Pugsley TA, Wise LD. (1990) Synthesis and dopamine agonist properties of (+)-trans-3,4,4a,10b-tetrahydro-4-propyl-2H,5H-[1]benzopyrano [4,3-b]-1,4-oxazin-9-ol and its enantiomers. *J Med Chem*. **33**(1):445-450.

Di Ciano P, Underwood RJ, Hagan JJ, Everitt BJ. (2003) Attenuation of cue-controlled cocaine-seeking by a selective D3 dopamine receptor antagonist SB-277011-A. *Neuropsychopharmacology*. **28**(2):329-338.

Dubuc I, Protais P, Colboc O, Costentin J. (1982) Antagonism of the apomorphine-induced yawning by "atypical" neuroleptics. *Neuropharmacology*. **21**(11):1203-1206.

Ferrari F, Giuliani D. (1995) Behavioural effects of the dopamine D3 receptor agonist 7-OH-DPAT in rats. *Pharmacol Res*. **32**(1-2):63-68.

Flietstra RJ, Levant B. (1998) Comparison of D2 and D3 dopamine receptor affinity of dopaminergic compounds in rat brain. *Life Sci*. **62**(20):1825-1831.

Goudie AJ, Baker LE, Smith JA, Prus AJ, Svensson KA, Cortes-Burgos LA, Wong EH, Haadsma-Svensson S. (2001) Common discriminative stimulus properties in rats of muscarinic antagonists, clozapine and the D3 preferring antagonist PNU-99194a: an analysis of possible mechanisms. *Behav Pharmacol.* **12**(5):303-315.

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**(3):839-848.

Gurevich EV, Joyce JN. (1999) Distribution of dopamine D3 receptor expressing neurons in the human forebrain: comparison with D2 receptor expressing neurons. *Neuropsychopharmacology.* **20**(1):60-80.

Haadsma-Svensson SR, Cleek KA, Dinh DM, Duncan JN, Haber CL, Huff RM, Lajiness ME, Nichols NF, Smith MW, Svensson KA, Zaya MJ, Carlsson A, Lin CH. (2001) Dopamine D(3) receptor antagonists. 1. Synthesis and structure-activity relationships of 5,6-dimethoxy-N-alkyl- and N-alkylaryl-substituted 2-aminoindans. *J Med Chem.* **44**(26):4716-4732.

Holmgren B, Urba-Holmgren R. (1980) Interaction of cholinergic and dopaminergic influences on yawning behavior. *Acta Neurobiol Exp (Wars).* **40**(3):633-642.

Joyce JN. (2001) Dopamine D3 receptor as a therapeutic target for antipsychotic and antiparkinsonian drugs. *Pharmacol Ther.* **90**(2-3):231-259.



Kulagowski JJ, Broughton HB, Curtis NR, Mawer IM, Ridgill MP, Baker R, Emms F, Freedman SB, Marwood R, Patel S, Patel S, Ragan CI, Leeson PD. (1996) 3-((4-(4-Chlorophenyl)piperazin-1-yl)-methyl)-1H-pyrrolo-2,3-b-pyridine: an antagonist with high affinity and selectivity for the human dopamine D4 receptor. *J Med Chem.* **39**(10):1941-1942.

Kurashima M, Yamada K, Nagashima M, Shirakawa K, Furukawa T. (1995) Effects of putative dopamine D3 receptor agonists, 7-OH-DPAT, and quinpirole, on yawning, stereotypy, and body temperature in rats. *Pharmacol Biochem Behav.* **52**(3):503-508.

Lerich L, Schwartz JC, Sokoloff P. (2003) The dopamine D3 receptor mediates locomotor hyperactivity induced by NMDA receptor blockade. *Neuropharmacology.* **45**(2):174-181.

Levant B, Grigoriadis DE, De Souza EB. (1995) Relative affinities of dopaminergic drugs at dopamine D2 and D3 receptors. *Eur J Pharmacol.* **278**(3):243-247.

Levant B. (1997) The D3 dopamine receptor: neurobiology and potential clinical relevance. *Pharmacol Rev.* **49**(3):231-252.

Levesque D, Diaz J, Pilon C, Martres MP, Giros B, Souil E, Schott D, Morgat JL, Schwartz JC, Sokoloff P. (1992) Identification, characterization, and localization of the dopamine D3 receptor in rat brain using 7-[3H]hydroxy-N,N-di-n-propyl-2-aminotetralin. *Proc Natl Acad Sci U S A.* **89**(17):8155-8159.

Millan MJ, Audinot V, Melon C, Newman-Tancredi A. (1995) Evidence that dopamine D3 receptors participate in clozapine-induced hypothermia. *Eur J Pharmacol.* 280(2):225-229.

Millan MJ, Seguin L, Gobert A, Cussac D, Brocco M. (2004) The role of dopamine D3 compared with D2 receptors in the control of locomotor activity: a combined behavioural and neurochemical analysis with novel, selective antagonists in rats. *Psychopharmacology (Berl)*. 174(3):341-357.

Mogilnicka E, Klimek V. (1977) Drugs affecting dopamine neurons and yawning behavior. *Pharmacol Biochem Behav.* 7(4):303-305.

Patel S, Freedman S, Chapman KL, Emms F, Fletcher AE, Knowles M, Marwood R, Mcallister G, Myers J, Curtis N, Kulagowski JJ, Leeson PD, Ridgill M, Graham M, Matheson S, Rathbone D, Watt AP, Bristow LJ, Rupniak NM, Baskin E, Lynch JJ, Ragan CI. (1997) Biological profile of L-745,870, a selective antagonist with high affinity for the dopamine D4 receptor. *J Pharmacol Exp Ther.* 283(2):636-647.

Protais P, Dubuc I, Costentin J. (1983) Pharmacological characteristics of dopamine receptors involved in the dual effect of dopamine agonists on yawning behaviour in rats. *Eur J Pharmacol.* 94(3-4):271-280.

Pugsley TA, Davis MD, Akunne HC, MacKenzie RG, Shih YH, Damsma G, Wikstrom H, Whetzel SZ, Georgic LM, Cooke LW, et al. (1995) Neurochemical and functional characterization of the preferentially selective dopamine D3 agonist PD 128907. *J Pharmacol Exp Ther.* 275(3):1355-1366.

Reavill C, Taylor SG, Wood MD, Ashmeade T, Austin NE, Avenell KY, Boyfield I, Branch CL, Cilia J, Coldwell MC, Hadley MS, Hunter AJ, Jeffrey P, Jewitt F, Johnson CN, Jones DN, Medhurst AD, Middlemiss DN, Nash DJ, Riley GJ, Routledge C, Stemp G, Thewlis KM, Trail B, Vong AK, Hagan JJ. (2000) Pharmacological actions of a novel, high-affinity, and selective human dopamine D(3) receptor antagonist, SB-277011-A. *J Pharmacol Exp Ther.* **294**(3):1154-1165.

Sautel F, Griffon N, Sokoloff P, Schwartz JC, Launay C, Simon P, Costentin J, Schoenfelder A, Garrido F, Mann A, et al. (1995) Nafadotride, a potent preferential dopamine D3 receptor antagonist, activates locomotion in rodents. *J Pharmacol Exp Ther.* **275**(3):1239-1246.

Sokoloff P, Giros B, Martres MP, Bouthenet ML, Schwartz JC. (1990) Molecular cloning and characterization of a novel dopamine receptor (D3) as a target for neuroleptics. *Nature.* **347**(6289):146-151.

Sokoloff P, Andrieux M, Besancon R, Pilon C, Martres MP, Giros B, Schwartz JC. (1992) Pharmacology of human dopamine D3 receptor expressed in a mammalian cell line: comparison with D2 receptor. *Eur J Pharmacol.* **225**(4):331-337.

Stahle L, Ungerstedt U. (1984) Assessment of dopamine autoreceptor agonist properties of apomorphine, (+)-3-PPP and (-)-3-PPP by recording of yawning behaviour in rats. *Eur J Pharmacol.* **98**(2):307-310.

Stancampiano R, Melis MR, Argiolas A. (1994) Penile erection and yawning induced by 5-HT<sub>1C</sub> receptor agonists in male rats: relationship with dopaminergic and oxytocinergic transmission. *Eur J Pharmacol.* **261**(1-2):149-155.

Stanwood GD, Artymyshyn RP, Kung MP, Kung HF, Lucki I, McGonigle P. (2000) Quantitative autoradiographic mapping of rat brain dopamine D<sub>3</sub> binding with [(125)I]7-OH-PIPAT: evidence for the presence of D<sub>3</sub> receptors on dopaminergic and nondopaminergic cell bodies and terminals. *J Pharmacol Exp Ther.* **295**(3):1223-1231.

Stemp G, Ashmeade T, Branch CL, Hadley MS, Hunter AJ, Johnson CN, Nash DJ, Thewlis KM, Vong AK, Austin NE, Jeffrey P, Avenell KY, Boyfield I, Hagan JJ, Middlemiss DN, Reavill C, Riley GJ, Routledge C, Wood M. (2000) Design and synthesis of trans-N-[4-[2-(6-cyano-1,2,3, 4-tetrahydroisoquinolin-2-yl)ethyl]cyclohexyl]-4-quinolinecarboxamide (SB-277011): A potent and selective dopamine D(3) receptor antagonist with high oral bioavailability and CNS penetration in the rat. *J Med Chem.* **43**(9):1878-1885.

Urba-Holmgren R, Gonzalez RM, Holmgren B. (1977) Is yawning a cholinergic response? *Nature.* **267**(5608):261-262.

Urba-Holmgren R, Holmgren B, Anias J. (1982) Pre- and post-synaptic dopaminergic receptors involved in apomorphine-induced yawning. *Acta Neurobiol Exp (Wars).* **42**(2):115-125.

Ushijima I, Yamada K, Inoue T, Tokunaga T, Furukawa T, Noda Y. (1984) Muscarinic and nicotinic effects on yawning and tongue protruding in the rat. *Pharmacol Biochem Behav.* **21**(2):297-300.

Varty GB, Higgins GA. (1998) Dopamine agonist-induced hypothermia and disruption of prepulse inhibition: evidence for a role of D3 receptors? *Behav Pharmacol.* **9**(5-6):445-455.

Vorel SR, Ashby CR Jr, Paul M, Liu X, Hayes R, Hagan JJ, Middlemiss DN, Stemp G, Gardner EL. (2002) Dopamine D3 receptor antagonism inhibits cocaine-seeking and cocaine-enhanced brain reward in rats. *J Neurosci.* **22**(21):9595-9603.

Waters N, Svensson K, Haadsma-Svensson SR, Smith MW, Carlsson A. (1993) The dopamine D3-receptor: a postsynaptic receptor inhibitory on rat locomotor activity. *J Neural Transm Gen Sect.* **94**(1):11-19.

Witkin JM, Dijkstra D, Levant B, Akunne HC, Zapata A, Peters S, Shannon HE, Gasior M. (2004) Protection against cocaine toxicity in mice by the dopamine D3/D2 agonist R-(+)-trans-3,4a,10b-tetrahydro-4-propyl-2H,5H-[1]benzopyrano[4,3-b]-1,4-oxazin-9-ol [(+)-PD 128,907]. *J Pharmacol Exp Ther.* **308**(3):957-964.

Xu M, Koeltzow TE, Cooper DC, Tonegawa S, White FJ. (1999) Dopamine D3 receptor mutant and wild-type mice exhibit identical responses to putative D3 receptor-selective agonists and antagonists. *Synapse.* **31**(3):210-215.

Yamada K, Furukawa T. (1980) Direct evidence for involvement of dopaminergic inhibition and cholinergic activation in yawning. *Psychopharmacology (Berl)*. **67**(1):39-43.

Zarrindast MR, Poursoltan M. (1989) Interactions of drugs acting on central dopamine receptors and cholinceptors on yawning responses in the rat induced by apomorphine, bromocriptine or physostigmine. *Br J Pharmacol*. **96**(4):843-848.

Zarrindast MR, Jamshidzadeh A. (1992) Inhibitory effect of morphine on yawning induced by cholinceptor and dopamine D2 receptor activation in rats. *Br J Pharmacol*. **105**(3):675-678.

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Reprint requests:

James H. Woods

Department of Pharmacology

1301 MSRB III

1150 West Medical Center Drive

University of Michigan Medical School

Ann Arbor, MI 48109-0632

<sup>1</sup> Department of Pharmacology, 1301 MSRB III, 1150 W. Medical Center Drive,  
University of Michigan Medical School, Ann Arbor, MI 48109-0632

<sup>2</sup> Psychiatric Drug Group, Lilly Research Laboratories, Indianapolis, IN 46285-0510

<sup>3</sup> Medicinal Chemistry Section, National Institutes on Drug Abuse-Intramural Research  
Program, National Institutes of Health, 5500 Nathan Shock Drive, Baltimore, MD 21224-  
0180

## Legends for Figures:

**Figure 1.** Dose-dependent induction of yawning by dopamine D3-preferring agonists A) PD-128,907 (0.0032 – 0.32 mg/kg), quinlorane (0.0001 – 0.032 mg/kg), and pramipexole (0.00032 – 1.0 mg/kg); B) PD-128,907 (0.0032 – 0.32 mg/kg), 7-OH-DPAT (0.0032 – 0.1 mg/kg), and quinpirole (0.0032 – 0.32 mg/kg); C) PD-128,907 (0.0032 – 0.32 mg/kg), bromocriptine (0.32 – 10.0 mg/kg), apomorphine (0.001 – 0.32 mg/kg), and PD-128,908 (0.01 – 1.0 mg/kg). Data are presented as mean ( $\pm$ SEM), n=8, number of yawns during a 20 minute observation period.

**Figure 2.** Effects of the D2-selective antagonist L-741,626 (0.32 and 1.0 mg/kg) on A) PD-128,907 (0.0032 – 1.0 mg/kg) induced yawning, and B) quinlorane (0.0001 – 0.032 mg/kg) induced yawning. Effects of the non-selective dopamine receptor antagonist haloperidol (0.01 and 0.032 mg/kg) on C) PD-128,907 (0.0032 – 1.0 mg/kg) induced yawning, and D) quinlorane (0.0001 – 0.1 mg/kg) induced yawning. Data are presented as mean ( $\pm$ SEM), n=8, number of yawns during a 20 minute observation period. \*  $p < 0.05$ ; \*\*  $p < 0.01$ ; \*\*\*  $p < 0.001$ ; Significant difference from vehicle-treated animals was determined by unbalanced, two-way ANOVA with post-hoc Bonferroni tests.



**Figure 3.** Effects of D3-preferring antagonists on PD-128,907 (0.0032 – 0.32 mg/kg) induced yawning in rats. A) Nafadotride at doses of 0, 0.001, 0.1, and 0.32 mg/kg; B) U99194 at doses of 0, 1.0, 3.2, and 10.0 mg/kg; C) SB-277011A at doses of 0, 3.2, 32.0, and 56.0 mg/kg; and D) PG01037 at doses of 0, 10.0, 32.0, and 56.0 mg/kg. Data are presented as mean ( $\pm$ SEM), n=8, number of yawns during a 20 minute observation period. \*  $p<0.05$ ; \*\*  $p<0.01$ ; \*\*\*  $p<0.001$ ; Significant difference from vehicle-treated animals was determined by unbalanced, two-way ANOVA with post-hoc Bonferroni tests.

**Figure 4.** A) Dose-response curves for PD-128,907 (0.0032 – 0.32 mg/kg), physostigmine (0.01 – 1.0 mg/kg; i.p.), and TFMPP (0.32 – 10.0 mg/kg) induced yawning in rats. B) Effects of scopolamine (0, 0.0001, 0.001, and 0.01 mg/kg) on yawning induced by PD-128,907 (0.1 mg/kg), physostigmine (0.1 mg/kg; i.p.) and TFMPP (3.2 mg/kg). C) Effects of mianserin (0, 0.0032, 0.032, and 0.32 mg/kg) on yawning induced by PD-128,907 (0.1 mg/kg), physostigmine (0.1 mg/kg; i.p.) and TFMPP (3.2 mg/kg). Data are presented as mean ( $\pm$ SEM), n=8, number of yawns during a 20 minute observation period. \*  $p<0.05$ ; \*\*  $p<0.01$ ; \*\*\*  $p<0.001$ ; Significant difference from vehicle-treated rats was determined by one-way repeated-measures ANOVAs with post-hoc Dunnett's tests.

**Figure 5.** Effects of D3-preferring antagonists on yawning induced by PD-128,907 (0.1 mg/kg), physostigmine (0.1 mg/kg; i.p.) and TFMPP (3.2 mg/kg). A) Nafadotride at doses of 0, 0.01, 0.1, and 1.0 mg/kg; B) U99194 at doses of 0, 1.0, 3.2, and 10.0 mg/kg; C) SB-277011A at doses of 0, 3.2, 32.0, and 56.0 mg/kg; and D) PG01037 at doses of 0, 10.0, 3.2, and 56.0 mg/kg. Data are presented as mean ( $\pm$ SEM), n=8, number of yawns during a 20 minute observation period. \*  $p<0.05$ ; \*\*  $p<0.01$ ; \*\*\*  $p<0.001$ ; Significant difference from vehicle-treated rats was determined by one-way repeated-measures ANOVAs with post-hoc Dunnett's tests.

Figure 1.

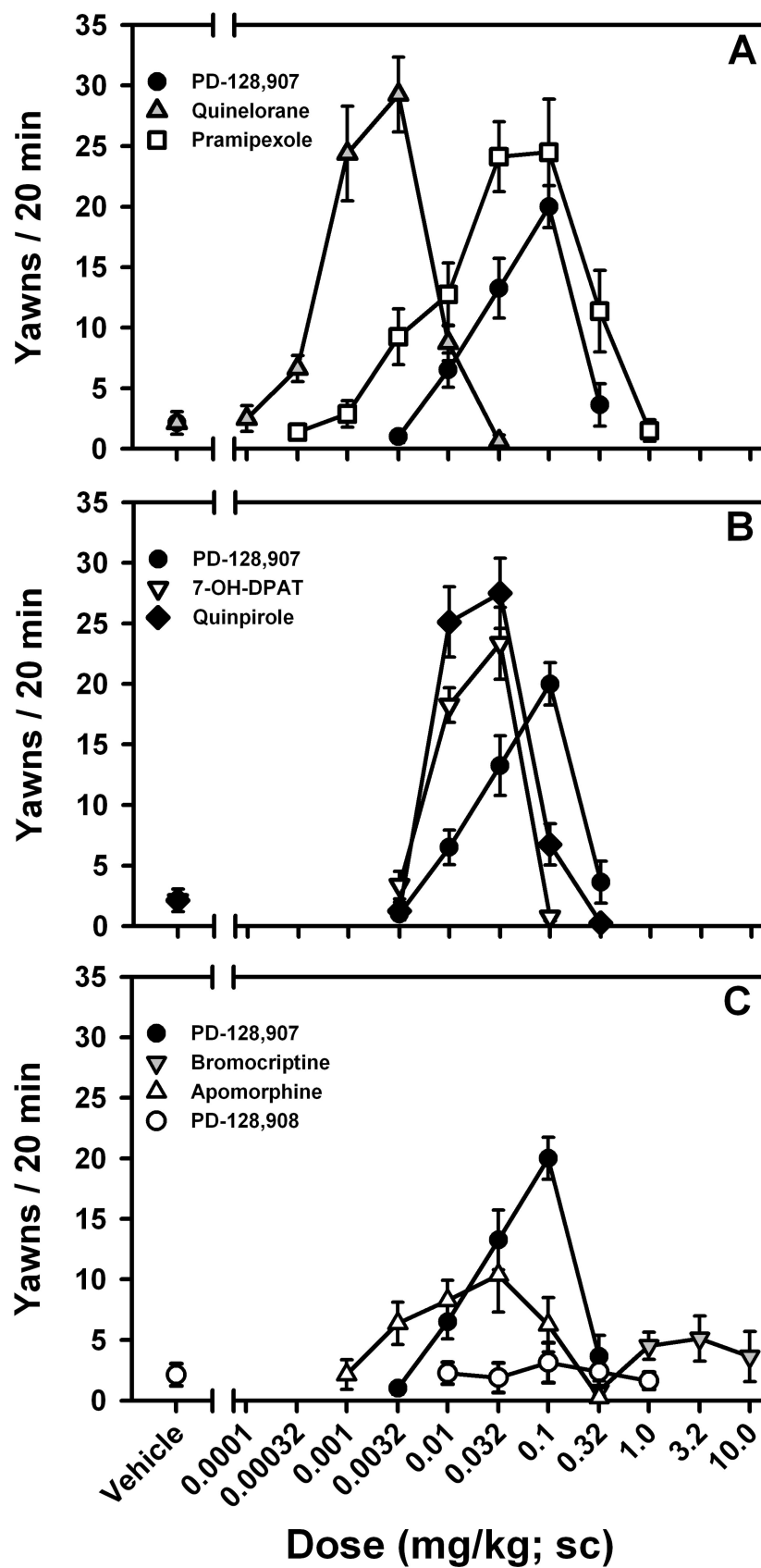
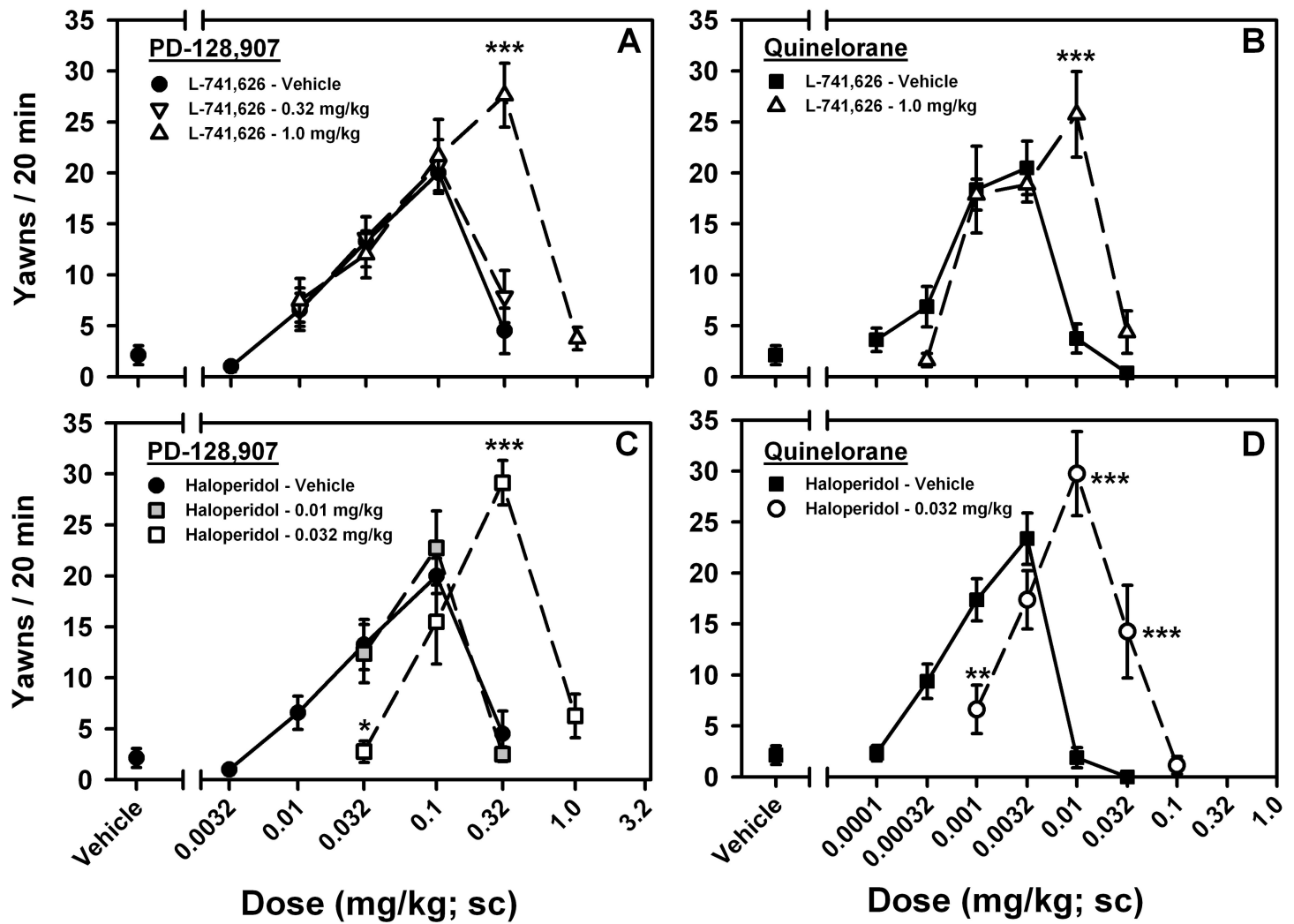


Figure 2.



**Figure 3.**

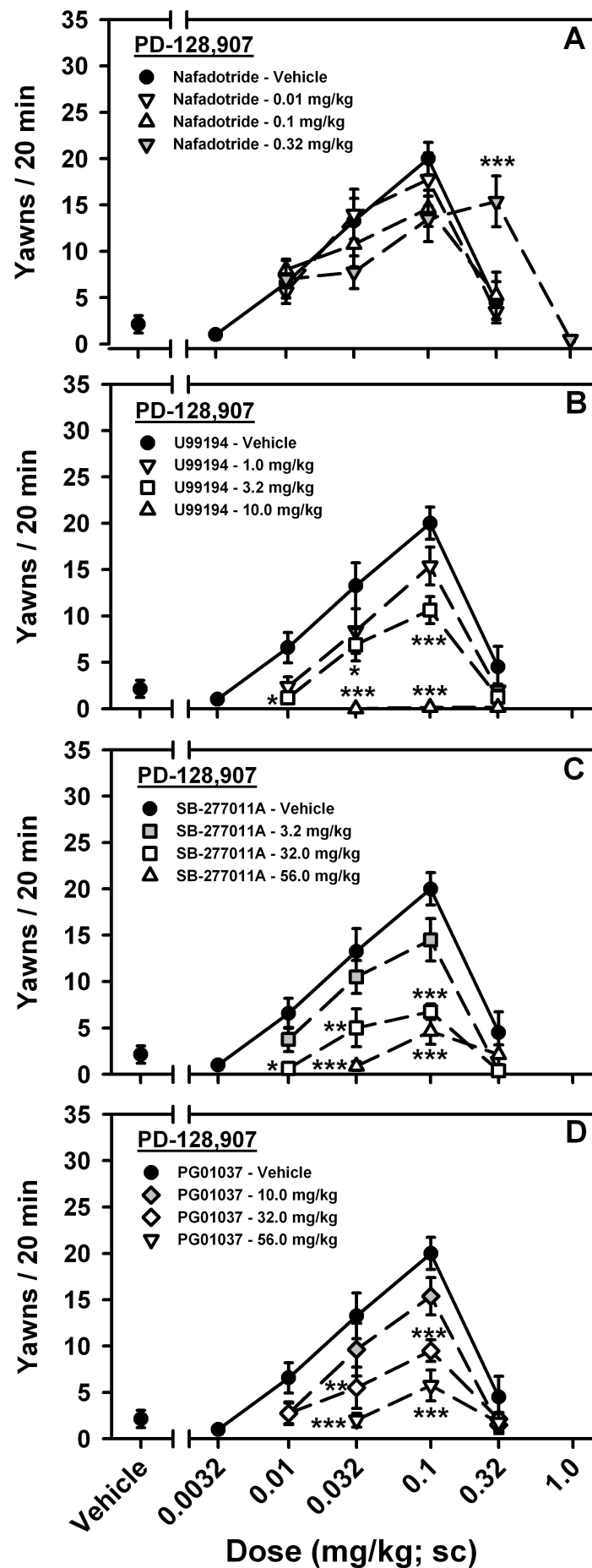


Figure 4.

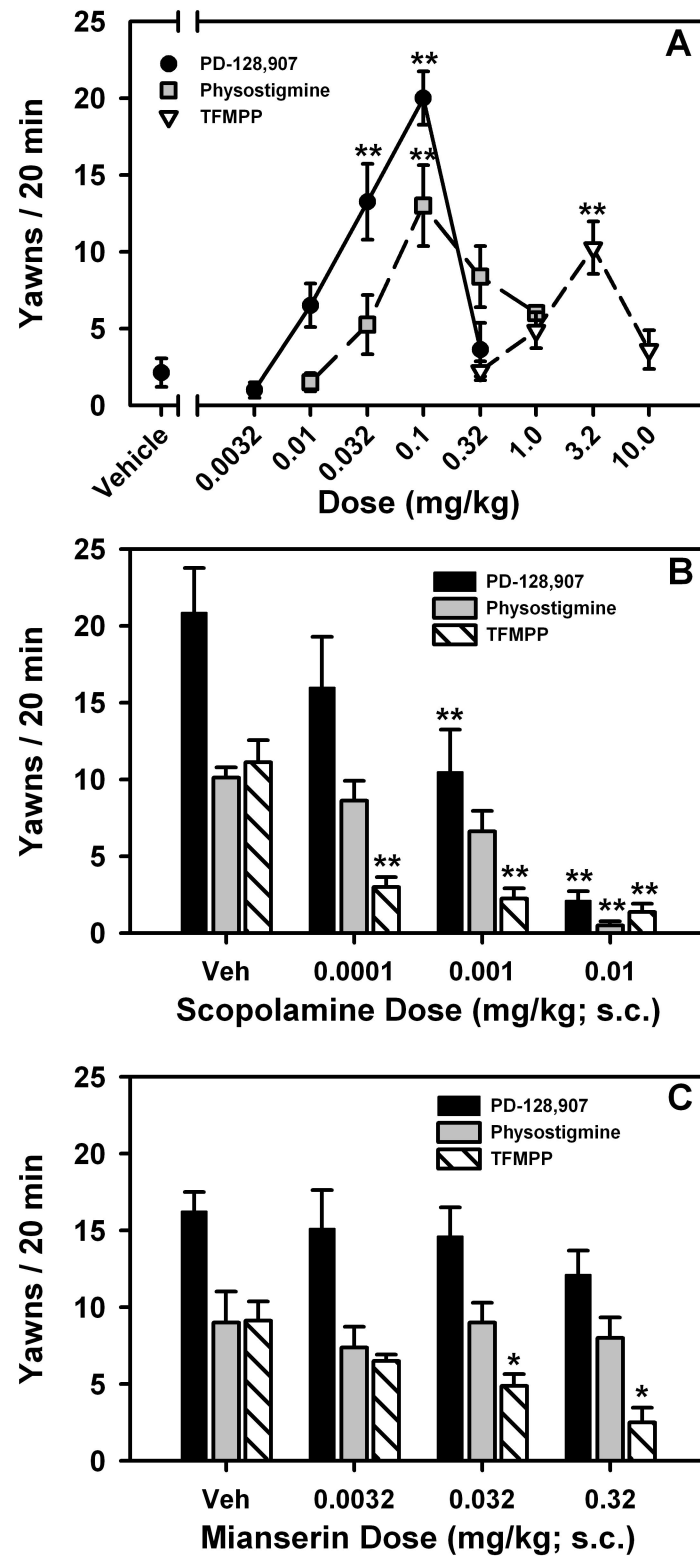


Figure 5.

