Wake-Promoting Actions of Dopamine D1 and D2 Receptor Stimulation

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ABSTRACT

Multiple ascending neurotransmitter systems participate in the regulation of behavioral state. For example, noradrenergic, cholinergic, and serotonergic systems increase EEG and, in some cases, behavioral indices of arousal. The extent to which dopaminergic systems exert a similar activating influence on behavioral state remains unclear. The current studies examined the wake-promoting actions of centrally administered D1 and D2 receptor agonists. In these studies, intracerebroventricular infusions of a D1 (SKF-82958; 2.5 and 25 nmol) or D2 (quinpirole; 40 and 140 nmol)-agonist were made into sleeping animals. The effects of these infusions on electroencephalogram/electromyographic indices of sleep-wake state and behavior were examined. D1 agonist administration dose dependently increased time spent awake and suppressed rapid eye movement and slow-wave sleep in the 2 h immediately after infusion. D1 agonist administration also elicited modest increases in measures of locomotion and time spent grooming and eating. D2 agonist administration had similar wake-promoting actions, accompanied by modest effects on drinking and locomotion. Interestingly, D2 agonist administration also significantly increased time spent chewing on inedible material, an arousal/stress-related behavior. Overall, these results demonstrate that dopamine contributes to the alert waking state via actions of D1 and D2 receptors. Additionally or alternatively, these results further suggest a potential involvement of dopamine receptors in the induction of high-arousal states, including stress.

The induction of the alert waking state and the accompanying activation of the forebrain are dependent on a variety of ascending neurotransmitter systems, including noradrenergic, serotonergic, cholinergic, and histaminergic. These systems display state-dependent alterations in activity across sleep-wake state. For example, cholinergic and locus coeruleus-noradrenergic neurons display higher discharge rates during waking than in slow-wave sleep (Hobson et al., 1975; Foote et al., 1980; Vanderwolf, 1988; Steriade and McCarley, 1990). Experimentally induced reduction in rates of neurotransmission within any of these systems results in an increase in large-amplitude, slow-wave activity within forebrain electroencephalographic (EEG) activity and, in some cases, increases in behavioral indices of sedation (Vanderwolf and Robinson, 1981; Lin et al., 1988; Vanderwolf, 1988; Berridge and España, 2000). These observations suggest a causal relationship between neuronal activity rates within these ascending neuromodulatory systems and forebrain neuronal and behavioral activity states.

In contrast to that observed with the above-mentioned neurotransmitter systems, midbrain dopamine (DA) neurons do not seem to display robust alterations in firing rate between sleep and quiet waking (Trulson et al., 1981; Steinfels et al., 1983; Trulson and Preussler, 1984). Interestingly, despite this relative insensitivity of DA neuronal firing rate to sleep-wake state, rates of DA release seem to be sensitive to fluctuations in sleep-wake state (Trulson, 1985). For example, in vivo microdialysis studies indicate that during the light period, when rats typically sleep, extracellular DA levels in the striatum and prefrontal cortex are lower than during the dark period (Smith et al., 1992; Feenstra et al., 2000). These observations suggest potential wake-promoting actions of DA neurotransmission. Consistent with this hypothesis, systemic administration of D1 receptor agonists increases time spent awake and reduces slow-wave and REM sleep (Monti et al., 1990; Trampus et al., 1991, 1993). Conversely, systemic administration of D1 receptor antagonists increases slow-wave and REM sleep and reduces time spent awake (Monti et al., 1990; Trampus et al., 1991, 1993; Ongini et al., 1994). D2 receptor agonists exert more complex effects, likely reflecting both presynaptic and postsynaptic functions.

ABBREVIATIONS: EEG, electroencephalographic; DA, dopamine; REM, rapid eye movement; EMG, electromyographic; PE, polyethylene; SKF-82958, (±)-chloro-7,8-dihydroxy-3-allyl-1-phenyl-2,3,4,5-tetra-hydro-1H-benzazepine; SCH 23390, 1-phenyl-1H-3-benzazepine HCl; QUIN, quinpirole.
of this receptor family. Thus, sedation is observed after systemic administration of low dose (and possibly presynaptic-prefering) D2 agonists, whereas higher doses seem to promote waking (Monti et al., 1989; Python et al., 1996; Lagos et al., 1998; Olive et al., 1998).

To date, the effects of direct manipulation of central DA systems have not been examined systematically. Limited observations indicate sedative effects of D1 antagonist administration into the cerebral ventricles (i.c.v.; Bagetta et al., 1987). Similar effects were observed after suppression of dopaminergic neurotransmission induced by ventral tegmental area D2 autoreceptor stimulation (Bagetta et al., 1988). Therefore, available evidence suggests dopaminergic neurotransmission is necessary for the maintenance of alert waking. The degree to which increased rates of dopaminergic neurotransmission elicit waking remains unclear.

The current studies characterized the wake-promoting actions of central D1 and D2 receptor stimulation. Specifically, these studies examined the EEG, electromyographic (EMG), and behavioral effects of i.c.v. administration of the D1 agonist SKF-82958 and the D2 agonist quinpirole. Importantly, accurate assessment of arousal-enhancing/wake-promoting actions of a given experimental intervention requires low-arousal baseline conditions (e.g., sleep) from which wake-promoting actions can be readily discerned and quantified. Therefore, in the current studies, remote-controlled infusions were used to permit drug administration in undisturbed, sleeping animals. Time spent in distinct sleep-wake states (determined from combined EEG and EMG recordings) and time engaged in specific behavioral activities were measured before and after i.c.v. infusions.

**Materials and Methods**

**Animals and Surgical Procedures.** Male Sprague-Dawley rats (250–280 g; Charles River Laboratories, Inc., Wilmington, MA) were housed in pairs for at least 7 days before surgery. Stereotaxic surgery was performed under halothane anesthesia delivered through a nose cone, with the incisor bar set at 11.5° below ear bar zero. Body temperature was maintained at approximately 36°C with a water-circulating heating blanket. A guide cannula (26-gauge; Plastics One, Roanoke, VA) was implanted into the lateral ventricle (A, –1.0; L, –1.2; V, –1.3), and EEG and EMG electrodes were implanted as described below. The guide cannula and EEG electrodes were secured to jeweler’s screws and cemented in place with acrylic cement (Plastics One). A stainless steel wire stylet was placed in the cannula to prevent occlusion. After surgery, rats were allowed 5 to 7 days to recover before experimentation. Animals were pair-housed in plastic cages before and after surgery on a 13:11-h light/dark cycle (lights on at 6:00 AM) and had free access to food and water. All surgical and experimental procedures were performed in accordance with U.S. Department of Agriculture and National Institutes of Health (1986) Guidelines for the Care and Use of Laboratory Animals.

**Drugs and Infusions.** On the day before testing, rats were transferred to Plexiglas testing chambers (32 × 32 × 40 cm) where they were housed individually. Each testing chamber was equipped with a 15-W light bulb operating on a 13:11-h light/dark cycle (lights on at 6:00 AM), a speaker emitting white noise (80 dB), and a ventilation fan. The outer chamber was equipped with two 10-cm holes, one on top to permit entry of infusion lines and recording cables, and one on the front to permit videotaping of behavior. The stylet was removed from the guide cannula, and a stainless steel coil spring was threaded onto the guide cannula. The other end of the spring was attached to a liquid swivel (Instech Laboratories Inc., Plymouth Meeting, PA) mounted on a counterbalance on the top of the outer chamber.

On the day of testing, between the hours of 8:00 and 10:00 AM, the stainless steel coil spring, and the distal end of the tubing was attached to the outlet of the liquid swivel. The inlet of the liquid swivel was connected to a 10-μL syringe via a length of PE20 tubing. The PE20 tubing and needle were housed within a stainless steel coil spring, and the distal end of the tubing was attached to the outlet of the liquid swivel. The liquid swivel was connected to a 10-μL syringe via a length of PE20 tubing filled with water. An air bubble was placed in the tubing above the liquid swivel to permit visualization of fluid displacement during advancement of the syringe plunger. The needle and tubing were then loaded with approximately 7 μL of vehicle or drug. The infusion needle was then inserted into the guide cannula and the stainless steel coil spring was secured to the guide cannula via plastic-threaded sleeves. The infusion needle extended 2.5 mm beyond the ventral tip of the cannula.

Drugs (SKF-82958-HCl, quinpirole-HBr; Sigma-Aldrich, St. Louis, MO) were dissolved in artificial extracellular fluid (147 mM NaCl, 1.3 mM CaCl₂, 0.9 mM MgCl₂, 2.5 mM KCl; pH 7.4). SKF-82958 (SKF) was dissolved at a concentration of 2.5 nmol/4-μL or 25 nmol/4-μL. Quinpirole (QUIN) was dissolved at a concentration of 40 or 140 nmol/5-μL. Doses were chosen on the basis of previously described behavioral actions of these drugs in these dose ranges as well as on a limited number of pilot studies indicating behavioral state-modulating actions of these doses. Infusions were made approximately 90 to 120 min after needle insertion and after at least 60-min during which the majority of time was spent asleep as determined by EEG and EMG measures. Infusions were made using a microprocessor-controlled pump located outside the wooden sound-attenuating chamber. Infusions were made at a rate of 1 μL/min. The flow of infusate was verified by observing the travel of the air bubble. Recording continued for 2 h after infusion. The majority of animals were tested once and only once. A subset of animals (n = 11) received two different treatments, with at least 2 days intervening between treatments. In these cases, treatments were randomized by dose and order of administration. Within each drug treatment group, data obtained from the 2nd day of testing did not differ from that obtained in animals tested once only.

**EEG and EMG Recording and Analyses.** A bipolar EEG electrode was implanted into the frontal cortex (A, +3.0; L, ±1.5), and two EMG electrodes were implanted into the neck muscle, and a ground screw electrode was placed over the cerebellum. The free electrode wire was placed in a five-pin plastic connector that was cemented into place along with the guide cannula. EEG and EMG signals were amplified, filtered (0.5–100-Hz bandpass) and recorded continuously on a polygraph and on VHS tape using a four-channel headstage FET amplifier connected to standard EEG amplifiers.

Cortical EEG and EMG were scored for the following behavioral state categories: 1) slow-wave sleep (high-voltage EEG and low-voltage EMG activity); 2) REM sleep (low-voltage EEG activity combined with EMG amplitude of approximately 50% that observed in slow-wave sleep, with occasional short-duration, larger-amplitude deflections due to muscle twitches); 3) quiet waking (low-voltage EEG and EMG amplitude approximately twice that of slow-wave sleep); and 4) active waking (low-voltage EEG and EMG amplitude approximately twice that of quiet waking). To be scored as a distinct epoch, each activity pattern had to persist for at least 15 s. Time spent in each behavioral state was aggregated over 30-min periods, both pre- and postinfusion (PRE1, 0–30 min; PRE2, 30–60 min; POST1, 60–90 min; POST2, 90–120 min; POST3, 120–150 min; POST4, 150–180 min). Scoring was conducted by observers blind to experimental condition.

**Behavioral Analyses.** Behavior was videotaped with a black-and-white videocamera (Panasonic WV-BL2000). Behavioral and EEG data were recorded onto VHS tape with a modified VCR (model 620; Vetter Instruments, Rebersburg, PA). The output of the camera...
was sent to a time-and-date imprinter, a black-and-white monitor, and to a VCR. Behavior was scored from videotape with a computer-based event recording by a trained observer who was blind to experimental conditions (Noldus Information, Wageningen, The Netherlands). The following behaviors were scored for all rats: 1) sleep: body resting on floor, head resting on floor; 2) head-up: head raised off of floor, body resting on floor; 3) inactive-up: any point in which the body was raised off of the floor, and the rat was inactive; 4) active-up: any point in which the body was raised off of the floor and the rat was active, although not engaged in any of the scored behaviors (grooming, rears, eating, or drinking) other than horizontal locomotion; 5) grooming; 6) rears (both free and wall); 7) quadrant entries: a measure of horizontal locomotion defined by hind legs crossing into a new quadrant; 8) eating; 9) chewing: chewing of indelible objects, including bedding material and feces; 10) drinking; and 11) total time spent awake: defined as the total observation period minus time spent asleep. The frequency and duration of all behaviors except quadrant entries (frequency only) were scored for the 30-min epoch immediately before infusion and for the three 30-min epochs immediately after infusion.

Statistical Analyses. Behavioral and EEG/EMG effects of drug infusion were analyzed using two-way mixed design analysis of variance, with drug treatment as the between-subjects variable (three levels corresponding to vehicle, low dose, and high dose) and time as the within-subjects variable. For EEG/EMG analyses, the time variable had six levels corresponding to the two preinfusion and the four postinfusion epochs of the experiment. For behavioral analyses, time had four levels corresponding to one preinfusion and three postinfusion epochs. When statistical significance was indicated (p < 0.05), simple effect analyses were conducted to determine between-group effects during each testing interval. Post hoc analyses were conducted using Tukey’s honestly significant difference test to determine differences between individual treatment groups.

Histology and Data Selection Criteria. After testing, approximately 2 µl of artificial extracellular fluid containing 0.5% Pontamine Sky Blue dye was infused i.c.v. Rats were then deeply anesthetized and perfused transcardially with 4% formaldehyde. Brains were frozen and 50-µm sections were cut and collected through the region containing the needle track. Location of the cannula and needle as well as the presence and spread of dye through the ventricular system were used to assess needle placement within the lateral ventricle. Data were included in the above-mentioned analyses only when EEG recordings were electrically adequate and infusion needle placement was accurate.

Results

Experiment I: Effects of D1 Receptor Activation on Sleep and Waking

EEG/EMG Effects of D1 Agonist Infusion. Experiments I and II examined the wake-promoting actions of i.c.v. infusions of the D1 agonist SKF and the D2 agonist QUIN, respectively. Due to differing solubility limits for the two drugs, the infusion volume was 4 µl for SKF and 5 µl for QUIN infusions. Statistical analyses indicated no significant difference in time spent awake between 4 µl (n = 4) and 5 µl (n = 4) vehicle infusions. Therefore, vehicle-treated animals from experiments I and II were combined for statistical analyses.

In experiment I, animals received infusions of vehicle (n = 8) or SKF (2.5 nmol, n = 8 or 25 nmol, n = 8). As reported previously, animals spent the majority of time asleep before infusions (Figs. 1 and 2). Vehicle treatment had no significant effect on time spent awake [Fig. 2; F(2,35) = 0.656, p = 0.659]. In contrast, the D1 agonist SKF elicited a dose-dependent increase in time spent awake [treatment, F(2,21) = 23.9, p < 0.001; time, F(5,105) = 25.4, p < 0.001; treatment × time, F(10,105) = 9.3, p < 0.001]. Post hoc analyses indicated that total time spent awake was significantly increased during the POST1 and POST2 epochs of the 2.5- and 25-nmol groups and during the POST3 epoch of the 25-nmol group (Fig. 2). Animals in the 25-nmol group were awake for nearly the entire 1 st h after infusion, and spent significantly more time awake than the 2.5-nmol group in POST1-POST3. By the end of the recording period, all animals had returned to baseline or near-baseline levels of waking. Latency to waking, measured from the end of the 4-min infusion, was 628 ± 375 s (range = 85–1262 s) for the 2.5-nmol group and 8 ± 27 s (range = 0–67 s) for the 25-nmol group.

The SKF-induced increase in total time spent awake resulted in part from a significant increase in active waking [Fig. 2; treatment, F(2,21) = 12.4, p < 0.001; time, F(5,105) = 24.1, p < 0.001; treatment × time, F(10,105) = 8.1, p < 0.001]. Post hoc analyses indicated that the high dose significantly elevated active waking in the POST1 and POST2 epochs, whereas the low dose significantly increased active waking in POST2. In contrast, SKF administration had only a marginal effect on time spent in quiet waking [Fig. 2; treatment, F(2,21) = 2.5, p = 0.111; time, F(5,105) = 2.0, p = 0.088; treatment × time, F(10,105) = 2.2, p = 0.025]. SKF-induced waking was associated with a significant decrease in slow-wave sleep [Fig. 2; treatment, F(2,21) = 23.7, p < 0.001; time, F(5,105) = 27.0, p < 0.001; treatment × time, F(10,105) = 8.7, p < 0.001], with both doses significantly reducing time spent in slow-wave sleep during POST1 and POST2. High-dose animals also spent significantly less time in slow-wave sleep than low-dose animals in POST1-POST3. REM sleep was significantly decreased by D1 agonist infusion [Fig. 2; treatment, F(2,21) = 1.9, p = 0.168; time, F(5,105) = 1.8, p = 0.126; treatment × time, F(10,105) = 2.6, p = 0.007], being nearly eliminated in the 25-nmol group in POST1 and POST2.
Behavioral Effects of D1 Agonist Infusion. The effects of D1 agonist infusion on a variety of behavioral activities were also assessed (Fig. 3). Consistent with results obtained with EEG/EMG measures, behavioral indices of total waking were significantly increased by SKF infusion [treatment, F(2,21) = 34.7, p < 0.001; time, F(3,63) = 21.4, p < 0.001; treatment × time, F(6,63) = 10.6, p < 0.001]. This resulted primarily from an increase in time spent in active-up, a measure of active waking [treatment, F(2,21) = 52.8, p < 0.001; time, F(3,63) = 49.3, p < 0.001; treatment × time, F(6,63) = 23.5, p < 0.001]. Behavioral measures of quiet waking were only moderately affected by SKF treatment [head-up (data not shown): treatment, F(2,21) = 1.9, p = 0.174; time, F(3,63) = 3.8, p = 0.015; treatment × time, F(6,63) = 3.4, p = 0.006; inactive-up: treatment, F(2,21) = 3.6, p = 0.046; time, F(3,63) = 1.8, p = 0.162; treatment × time, F(6,63) = 2.8, p = 0.017]. These mild effects on head-up and inactive-up are consistent with those observed with EEG/EMG-based measures of quiet waking.

Both eating and grooming were moderately increased by D1 agonist treatment [eating: treatment, F(2,21) = 2.5, p = 0.110; time, F(3,63) = 2.5, p = 0.065; treatment × time, F(6,63) = 3.2, p < 0.01; grooming, treatment, F(2,21) = 7.8, p = 0.003; time, F(3,63) = 21.0, p < 0.001; treatment × time, F(6,63) = 4.3, p = 0.001]. High-dose-treated animals spent significantly more time eating than vehicle controls in POST2. High-dose-treated animals spent significantly more time grooming in POST1 than did vehicle-treated animals, whereas low-dose-treated animals spent significantly more time grooming in POST2. SKF treatment had little effect on time spent drinking [treatment, F(2,21) = 1.3, p = 0.302; time, F(3,63) = 2.6, p = 0.063; treatment × time, F(6,63) = 1.2, p = 0.339] and chewing, an arousal/stress-related behavior [treatment, F(2,21) = 2.1, p = 0.151; time, F(3,63) = 1.7, p = 0.171; treatment × time, F(6,63) = 2.1, p = 0.068].

D1 agonist administration had only a mild, yet significant, locomotor-activating effect, as measured by quadrant entries [treatment, F(2,21) = 5.2, p = 0.014; time, F(3,63) = 9.9, p < 0.001; treatment × time, F(6,63) = 5.1, p < 0.001] and rears [treatment, F(2,21) = 10.3, p = 0.001; time, F(3,63) = 9.9, p < 0.001; treatment × time, F(6,63) = 5.1, p < 0.001]. This level of motor activity is comparable to that observed during spontaneous waking or noradrenergic β-agonist-induced waking (Berridge and Foote, 1996; España et al., 2002).

In general, no evidence of stereotypy or other atypical behavioral activity was observed in SKF-treated animals. The only exception to this was that approximately half the animals in each drug group exhibited wet dog shakes at a low frequency of approximately 20 per hour, independent of dose, usually in conjunction with episodes of grooming.

II. Effects of D2 Receptor Activation on Sleep and Waking

EEG/EMG Effects of D2 Agonist Infusion. To assess the wake-promoting actions of D2 receptor stimulation, i.c.v.
infusions of vehicle (n = 8) or 40 nmol of QUIN (n = 8) or 140 nmol of QUIN (n = 7) were made in sleeping rats (Fig. 4). As described above, animals in all groups spent the majority of time asleep before infusion, and vehicle infusion had no significant effect on time spent awake. In contrast, QUIN infusion dose-dependently increased total time spent awake [treatment, F(2,21) = 5.0, p < 0.017; time, F(5,105) = 13.1, p < 0.001; treatment × time, F(10,105) = 5.8, p < 0.001]. Low-dose animals spent significantly more time awake than vehicle-treated animals in POST1. High-dose animals spent significantly more time awake than vehicle controls in POST1 and POST2 and spent significantly more time awake than low-dose animals in POST2. Latency to waking was 109.5 ± 38.7 s (range = 0–293 s) for high-dose animals and 209.4 ± 74.0 s (range = 0–600 s) for low-dose animals.

QUIN-induced changes in total waking were derived primarily from increases in active waking [Fig. 4; treatment, F(2,21) = 13.6, p < 0.001; time, F(5,105) = 10.7, p < 0.001; treatment × time, F(10,105) = 5.5, p < 0.001]. QUIN infusion increased EEG-EMG indices of quiet waking, although this effect was fairly moderate [time, F(5,105) = 4.4, p < 0.01; treatment, F(2,21) = 0.2, p = 0.819; treatment × time, F(10,105) = 2.3, p = 0.017]. The QUIN-induced increase in waking was accompanied by a significant decrease in slow-wave sleep [Fig. 4; treatment, F(2,21) = 5.0, p = 0.017; time, F(5,105) = 11.9, p < 0.001; treatment × time, F(10,105) = 5.0, p < 0.001]. Low-dose animals spent significantly less time in slow-wave sleep in POST1 than vehicle-treated animals, whereas high-dose animals spent significantly less time in slow-wave sleep than vehicle-controls in POST1 and POST2. Furthermore, high-dose animals spent significantly less time in slow-wave sleep in POST2 than low-dose animals. Finally, QUIN infusion elicited a significant decrease
behavioral indices of total waking and in slow-wave sleep, quiet waking, active waking, and REM sleep. Symbols represent mean (±S.E.M.) time spent in a given behavioral state per 30-min epoch. PRE1 and PRE2 represent preinfusion epochs. POST1-POST4 represent postinfusion epochs, beginning immediately after infusion. QUIN infusion elicited a dose-dependent increase in total time spent awake that resulted from increases in active waking. Associated with this increase in waking were significant decreases in slow-wave and REM sleep.

Behavioral Effects of D2 Agonist Infusion. Similar to that observed with EEG/EMG indices, QUIN infusion elicited dose-dependent increases in behavioral indices of total waking [treatment, \(F_{(2,21)} = 26.8, p < 0.001\); time, \(F_{(3,63)} = 30.3, p < 0.001\); treatment \& time, \(F_{(6,63)} = 10.0, p < 0.001\)]. These changes in total waking resulted from increases in both active-up [Fig. 5; treatment, \(F_{(2,21)} = 31.9, p < 0.001\); time, \(F_{(3,63)} = 21.5, p < 0.001\); treatment \& time, \(F_{(6,63)} = 8.9, p < 0.001\)] and inactive-up [treatment, \(F_{(2,21)} = 8.2, p = 0.002\); time, \(F_{(3,63)} = 15.2, p < 0.001\); treatment \& time, \(F_{(6,63)} = 5.3, p < 0.001\)].

Drinking was modestly, although significantly, increased by the high dose of QUIN during POST1 [treatment, \(F_{(2,21)} = 5.6, p = 0.012\); time, \(F_{(3,63)} = 4.1, p = 0.011\); treatment \& time, \(F_{(6,63)} = 1.5, p = 0.187\)]. QUIN-administration did not significantly alter time spent eating [treatment, \(F_{(2,21)} = 1.4, p = 0.264\); time, \(F_{(3,63)} = 1.1, p = 0.351\); treatment \& time, \(F_{(6,63)} = 1.9, p = 0.090\)] or grooming [treatment, \(F_{(2,21)} = 0.7, p = 0.516\); time, \(F_{(3,63)} = 2.3, p = 0.086\); treatment \& time, \(F_{(6,63)} = 1.1, p = 0.387\)].

QUIN administration had only a mild locomotor stimulatory effect apparent in a small increase in the frequency of quadrant entries [treatment, \(F_{(2,21)} = 16.8, p < 0.001\); time, \(F_{(3,63)} = 13.4, p < 0.001\); treatment \& time, \(F_{(6,63)} = 6.4, p < 0.001\)] and time spent rearing [treatment, \(F_{(2,21)} = 4.3, p = 0.028\); time, \(F_{(3,63)} = 2.7, p = 0.055\); treatment \& time, \(F_{(6,63)} = 0.9, p = 0.479\)]. This level of motor activity is comparable to that seen during spontaneous waking (España et al., 2002) or noradrenergic β-agonist-induced waking (Berridge and Foote, 1996).

In contrast to that observed with D1 agonist infusions, QUIN elicited a significant increase in the chewing of inedible objects [treatment, \(F_{(2,21)} = 12.6, p < 0.001\); time, \(F_{(3,63)} = 8.9, p < 0.001\); treatment \& time, \(F_{(6,63)} = 4.0, p = 0.002\)]. Thus, low-dose-treated animals spent significantly more time chewing than vehicle animals in POST1. High-dose-treated animals spent more time chewing than vehicle-treated animals in POST1 and POST2, although only the increase in POST2 was statistically significant. These levels of chewing are similar to those observed in stress (Berridge et al., 1999a).

Stereotypy or other atypical behavior was generally not observed after QUIN infusion. The only exception to this was that in the high-dose group five of eight animals exhibited an unusual posture, in which the animals seemed to rise up onto their toes, with an arched back. Often associated with this was a pivoting motion, involving a partial turn of the body leading with the hind legs. In those animals in which this was observed, this occurred approximately 10 to 20 times in the 1st h after infusion.
These behaviors were not present in vehicle- or low-dose-treated animals.

**Discussion**

The current studies characterized the wake-promoting actions of D1 and D2 receptor stimulation. Previous studies examining the arousal-enhancing actions of dopaminergic neurotransmission typically used peripheral administration of DA receptor-selective drugs using procedures that result in alert, awake animals. The current studies used recording and infusion procedures that ensure a low-level arousal baseline state (i.e., sleep) from which wake-promoting actions of centrally administered D1 and D2 agonists could be readily discerned and quantified. Under these conditions, i.c.v. infusion of the D1 agonist SKF-82958 exerted robust and dose-dependent, wake-promoting actions while simultaneously suppressing slow-wave and REM sleep. Largely similar dose-dependent, wake-promoting and sleep-suppressing actions were observed after i.c.v. administration of the D2 receptor agonist quinpirole.

In general, D1 and D2 agonist-induced waking seemed similar to normal, spontaneous waking. For example, intense locomotor activation or motor stereotypes typically associated with moderate-to-high doses of amphetamine-like stimulants and high doses of D2 receptor agonists were not observed. Moreover, the pattern of behavioral activities associated with D1 and D2 agonist-induced waking was typical of that observed in spontaneous waking during both the light and dark portions of the circadian cycle (España et al., 2002). Although there were not marked differences overall in the behavioral actions of the two drugs, there were a few

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**Fig. 5.** Effects of D2 receptor stimulation on behavior. Shown are the effects of vehicle and either 40 or 140 nmol of QUIN infused i.c.v. on time spent in active up, inactive up, rearing, grooming, eating, chewing, and drinking. Also shown is the frequency of quadrant crossing. Symbols represent mean (S.E.M.) time spent in a given behavioral activity per 30-min epoch. PRE represents the 30-min epoch immediately preceding infusion. POST1-POST3 represent postinfusion epochs, beginning immediately after infusion. QUIN infusion significantly increased time spent in active up, inactive up, drinking, and chewing (an arousal/stress-related behavior). QUIN infusion also significantly increased the frequency of quadrant crossing (a measure of locomotion). *, p < 0.05 compared with vehicle-treated controls. †, p < 0.05 compared with 140 nmol of QUIN.
exceptions to this. For example, D1 agonist infusion elicited substantially greater levels of grooming than did the D2 agonist, although the magnitude of grooming observed after D1 agonist infusion was nonetheless moderate. Perhaps the most intriguing difference between the behaviors of animals treated with the D1 or D2 agonist was the greater level of chewing of inedible objects observed after D2 agonist treatment compared with D1 agonist treatment. Levels of chewing observed after D2 agonist administration were similar to levels of chewing observed during novelty stress (Berridge et al., 1999a; see below for further discussion).

In general, neither drug elicited atypical behavioral activity. For the D1 agonist, the only exception to this was the occurrence of wet dog shakes in approximately half the animals of each dose group. Comparable numbers of wet dog shakes were observed with both doses, occurring approximately 20 to 30 times in the 1st h after infusion. This is consistent with a previous study demonstrating that D1 antagonist treatment blocked wet dog shakes induced by exogenous DA infusion into the substantia nigra pars reticulata (Fan et al., 2000). However, because the incidence of wet dog shakes was not dose-dependent, occurred relatively infrequently, and only in approximately half the animals receiving drug, it is unlikely that the observed dose-dependent wake-promoting actions of this D1 agonist result from non-specific effects related to this behavior. Approximately half the high-dose D2 agonist-treated animals displayed a postural abnormality accompanied by a pivoting motion (see Results). This seems consistent with previous reports of pivoting after administration of a mixture of D1 and D2 agonists into the nucleus accumbens shell (Kitamura et al., 2001). Given that these behaviors were only observed within half the animals of the high-dose group, the dose-dependent increase in waking observed after D2 agonist administration is unlikely to be related to these latter behavioral effects of the drug.

Circuitry Underlying the Wake-Promoting Actions of D1 and D2 Receptors. The above-described studies indicate that stimulation of either D1 or D2 receptors globally within the brain elicits alert waking. Presently, the terminal fields involved in the dopaminergic modulation of behavioral state remain unknown. Candidate sites include cortical, thalamic, and basal forebrain regions. Previous studies indicate that noradrenergic systems promote waking via actions within a circumscribed region of the medial basal forebrain (Alam and Mallick, 1990; Berridge and Foote, 1996; Berridge et al., 2003). This region extends from the anterior aspect of the medial septal area to the posterior aspect of the medial preoptic area and receives a moderate dopaminergic innervation (Bjorklund et al., 1975; Fallon et al., 1978; Charuchinda et al., 1987). Interestingly, amphetamine, which increases synaptic/extracellular DA (as well as norepinephrine) levels, acts within this same basal forebrain region to exert arousal-enhancing actions (Berridge et al., 1999b). Combined, these observations suggest the hypothesis that DA-dependent modulation of behavioral state involves, at least in part, actions of DA within this region of the medial basal forebrain. DA exerts locomotor stimulatory and stereotypy-inducing effects that involve actions within the nucleus accumbens (core subregion) and the striatum, respectively. Interestingly, amphetamine infusions into the core subregion of the nucleus accumbens or the striatum did not alter behavioral state (Berridge et al., 1999b). These latter observations suggest the wake-promoting action of D1 and D2 receptor stimulation does not involve either of these regions.

Functional Significance Underlying the Wake-Promoting Actions of D1 and D2 Receptors. The current results suggest a potential role of cerebral DA systems in the initiation and maintenance of the waking state. However, the degree to which the wake-promoting effects of D1 and D2 agonists reflect direct or indirect actions on arousal-enhancing circuits is unknown. For example, D1 and D2 receptors may be located within circuits directly involved in the regulation of behavioral state. Alternatively, these receptors may be located within circuits that support any number of specific behavioral processes, each requiring the alert waking state. In this case, these receptors may indirectly impact behavioral state via pathways that ensure coordination of the appropriate behavioral state with DA-sensitive state-dependent behavior.

A specific role of DA in the regulation of the waking state is consistent with the results of previous studies demonstrating increases in indices of DA release during the dark phase of the sleep-wake cycle, when rats spend more time awake (Smith et al., 1992; Feenstra et al., 2000). Alternatively, increased DA release is also associated with high-arousal states associated with aversive (e.g., stress; Thierry et al., 1976; Roth et al., 1988) and appetitive conditions (Cousins et al., 1999; Di Chiara et al., 1999). Thus, D1 and D2 agonist-induced waking may reflect the induction of a higher arousal state than that typical of spontaneous (normal) waking. Interestingly, chewing of inedible material attenuates stressor-induced increases in prefrontal cortical DA levels, as well as other physiological indices of stress (Hennessy and Foy, 1987; Berridge et al., 1999a). Thus, the D2 agonist-induced increase in chewing may reflect the induction of a high arousal (and possibly stress-like) state and the subsequent activation of this coping response. Alternatively, the increase in chewing could reflect direct activation of circuits associated with coping/displacement behaviors in the absence of elevated arousal/stress levels. Also consistent with a role of DA in high-arousal processes is the observation that DA neuronal discharge activity is increased during active waking and not quiet waking, relative to sleep (Trulson et al., 1981; Steinfels et al., 1983; Trulson and Preussler, 1984). Consistent with these observations, in the current studies, D1 and D2 agonist administration tended to increase active waking to a greater extent than quiet waking. However, it is important to note that in these studies, D1 and D2 agonist-induced waking was associated with levels of locomotor activity typically observed with spontaneous waking (España et al., 2002), levels that were well below those associated with moderate-to-high doses of psychostimulants (Segal, 1975). These observations suggest dissociation between the wake-promoting and locomotor-stimulating effects of DA efficients.

Postsynaptic versus Presynaptic Actions of D2 Receptor Activation. Given D2 type receptors exist presynaptically as well as postsynaptically, it could be argued that the wake-promoting effects of the D2 agonist, quinpirole, observed in this study were due to actions at presynaptic receptors. Arguing against a presynaptic site of action in the wake-promoting actions of quinpirole is the finding that i.c.v. administration of the D2 autoreceptor-prefering agonist (−).
3-propylpiperidine HCl, elicits increased time spent asleep (Bagetta et al., 1987). Similar effects were observed after infusion of the D2 agonist (+)-3-propylpiperidine HCl into the ventral tegmental area, a region containing DA-synthesizing neurons. This effect was blocked by pretreatment, within the same site, of the D2 antagonist haloperidol, but not the D1 antagonist SCH 23390 (Bagetta et al., 1988). These results indicate that activation of presynaptic D2 receptors reduces waking, suggesting that the wake-promoting actions of quinpirole observed in the current studies likely result from activation of postsynaptic D2 receptors.

Summary. The current results indicate that, under conditions of low arousal (e.g., sleep), activation of D1 or D2 receptors promotes a waking state that largely resembles conditions of low arousal (e.g., sleep), activation of D1 or D2 actions of quinpirole observed in the current studies likely not the D1 antagonist SCH 23390 (Bagetta et al., 1988). This effect was blocked by pretreatment, within the same site, of the D2 antagonist haloperidol, but not the D1 antagonist SCH 23390 (Bagetta et al., 1988). These results indicate that activation of presynaptic D2 receptors reduces waking, suggesting that the wake-promoting actions of quinpirole observed in the current studies likely result from activation of postsynaptic D2 receptors.

References
Bagetta G, De Sarro G, Priolo E, and Nistico G (1987) Similar effects were observed after infusion of the D2 agonist (+)-3-propylpiperidine HCl into the ventral tegmental area, a region containing DA-synthesizing neurons. This effect was blocked by pretreatment, within the same site, of the D2 antagonist haloperidol, but not the D1 antagonist SCH 23390 (Bagetta et al., 1988). These results indicate that activation of presynaptic D2 receptors reduces waking, suggesting that the wake-promoting actions of quinpirole observed in the current studies likely result from activation of postsynaptic D2 receptors.

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