

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 nmol, 25 nmol) or D2 (quinpirole; 40 nmol, 140 nmol) agonist were made into sleeping animals. The effects of these infusions on EEG/EMG indices of sleep-wake state and behavior were examined. D1 agonist administration dose-dependently increased time spent awake and suppressed REM and slow-wave sleep in the two hours immediately following 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 DA receptors in the induction of high-arousal states, including stress.

The induction of the alert waking state and the accompanying activation of the forebrain is 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 (LC)-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 activity (EEG) 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 appear 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 appear 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 they are 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; Trampus *et al.*, 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; Ongini *et al.*, 1993; Trampus *et al.*, 1993). D2 receptor agonists exert more complex effects, likely reflecting

both presynaptic and postsynaptic functions of this receptor family. Thus, sedation is observed following systemic administration of low dose (and possibly presynaptic-preferring) D2 agonists, whereas higher doses appear 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 (ICV; Bagetta *et al.*, 1987). Similar effects were observed following 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 ICV 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 manipulation 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 prior to and following ICV infusions.

Methods

Animals and Surgical Procedures: Male Sprague-Dawley rats (250-280g; Charles River, Wilmington, MA) were housed in pairs for at least seven days prior to surgery. Stereotaxic surgery was performed under halothane anesthesia delivered through a nose cone, with the incisor bar set at 11.5 degrees below ear bar zero. Body temperature was maintained at approximately 36°C with a water-circulating heating blanket. A guide cannula (26 ga.; Plastics One, Roanoke, VA) was implanted over 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. Following surgery, rats were allowed 5-7 days to recover before experimentation. Animals were pair-housed in plastic cages before and after surgery on a 13-11-hr light-dark cycle (lights on 0600), 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 prior to testing, rats were transferred to Plexiglas testing chambers (32 x 32 x 40 cm) where they were housed individually. Each testing chamber was contained within a wooden sound-attenuating chamber equipped with a 15-W light bulb operating on a 13-11-hr light-dark cycle (lights on 0600), 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 08:00 and 10:00 AM, the stainless-steel coil spring, connected the previous day, was disconnected. A 33-gauge needle (Plastics One) was attached to 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 inlet of the liquid swivel was connected to a 10- μ l 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 μ l 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- μ l or 25-nmol/4- μ l. Quinpirole (**QUIN**) was dissolved at a concentration of 40 nmol/5- μ l or 140-nmol/5- μ l. 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 90-120 min following needle insertion and following 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- μ l/min. The flow of infusate was verified by observing the travel of the air bubble. Recording continued for two hours following infusion. The majority of animals were tested once and only once. A subset of animals (n = 11) received two different treatments, with at least two 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 second 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 ($A +3.0$; $L \pm 1.5$), and two EMG electrodes were implanted into the neck muscle, and a ground screw electrode was placed over the cerebellum. The free ends of each electrode were placed in a five-pin plastic connector that was cemented into place along with the guide cannula. EEG and EMG signals were amplified, filtered (0.5-100.0-Hz bandpass), and continuously recorded on a polygraph and on VHS tape using a 4-channel headstage FET amplifier connected to standard EEG amplifiers.

Cortical EEG and EMG were scored for the following behavioral state categories: (a) Slow-wave sleep (high-voltage EEG and low-voltage EMG activity), (b) 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), (c) Quiet Waking (low-voltage EEG and EMG amplitude approximately twice that of slow-wave sleep), (d) 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 fifteen seconds. Time spent in each behavioral state was aggregated over 30-min periods, both pre- and post-infusion (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 video camera (Panasonic WV-BL2000). Behavioral and EEG data were recorded onto VHS tape with a modified VCR (Vetter, Model 620). 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 recorder by a trained observer who was blind to experimental conditions (Noldus Information, Wageningen, The Netherlands). The following behaviors were scored for all rats: (a) *Sleep*: body resting on floor, head resting on floor; (b) *Head-Up*: head raised off of

floor, body resting on floor; (c) *Inactive-Up*: any point in which the body was raised off of the floor, and the rat was inactive; (d) *Active-Up*: any point in which the body was raised off of the floor and the rat was active, though not engaged in any of the scored behaviors (grooming, rears, eating, or drinking) other than horizontal locomotion; (e) *grooming*; (f) *rears* (both free and wall); (g) quadrant entries: a measure of horizontal locomotion defined by hind legs crossing into a new quadrant; (h) *eating*; (i) *chewing*: chewing of inedible objects, including bedding material and feces; (j) *drinking*; and (k) *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 prior to infusion and for the three 30-min epochs immediately following infusion.

Statistical Analyses: Behavioral and EEG/EMG effects of drug infusion were analyzed using two-way mixed-design analysis of variance (ANOVA), 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 pre-infusion and the four post-infusion epochs of the experiment. For behavioral analyses, time had four levels corresponding to one pre-infusion and three post-infusion epochs. When statistical significance was indicated, ($p < 0.05$), simple main effects analyses were conducted to determine between-group effects during each testing interval. *Post hoc* analyses were conducted using Tukey's HSD test to determine differences between individual treatment groups.

Histology and Data Selection Criteria: Following testing, approximately 2 μ l of artificial extracellular fluid containing 0.5% Pontamine Sky Blue dye was infused ICV. Rats were then deeply anesthetized and perfused transcardially with 4% formaldehyde. Brains were removed and placed in fixative. After a minimum of 24 hours, brains were frozen and 50- μ m 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 was used to assess needle placement within the lateral ventricle. Data were included in the above 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

A. EEG/EMG effects of D1-agonist infusion

Experiments I and II examined the wake-promoting actions of ICV infusions of the D1 agonist, SKF-82958 (**SKF**), and the D2 agonist, quinpirole (**QUIN**), respectively. Due to differing solubility limits for the two drugs, the infusion volume was 4 μ l for SKF and 5 μ l for QUIN infusions. Statistical analyses indicated no significant difference in time spent awake between 4 μ l (n = 4) and 5 μ l (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 prior to infusions (Figs. 1 and 2). Vehicle treatment had no significant effect on time spent awake (Fig. 2; $F_{(5,35)}=0.656$, $p=0.659$). 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 x 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 nmol 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 first hour following 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 sec (range = 85-1262 sec) for the 2.5 nmol group and 8 ± 27 sec (range = 0-67 sec) 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 x 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 x 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 x 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 x time, $F_{(10,105)}=2.6$, $p=0.007$), being nearly eliminated in the 25-nmol group in POST1 and POST2.

B. 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 x 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 x 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 x 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 x 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 x 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 x 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 x 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 x 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 x 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 x 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 (España *et al.*, 2002; Berridge and Foote, 1996).

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 one-half of 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

A. EEG/EMG effects of D2-agonist infusion

To assess the wake-promoting actions of D2 receptor stimulation, ICV infusions of vehicle ($n = 8$) or 40 nmol QUIN ($n = 8$) or 140 nmol QUIN ($n = 7$) were made in sleeping rats (Fig 4). As described above, animals in all groups spent the majority of time asleep prior to 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 x 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 sec (range = 0 – 293 sec) for high-dose animals and 209.4 ± 74.0 sec (range = 0 – 600 sec) for low-dose animals.

QUIN-induced changes in total waking were derived primarily from significant 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 x 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, $F_{(5,105)}=4.4$, $p=0.001$; treatment, $F_{(2,21)}=0.2$, $p=0.819$; treatment x 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 x 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 in REM sleep (treatment, $F_{(2,21)}=0.3$, $p=0.753$, time, $F_{(5,105)}=5.3$, $p<0.001$, treatment x time, $F_{(10,105)}=2.2$, $p=0.025$).

B. 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 x time, $F_{(6,63)}=10.0$, $p<0.001$). These changes in total waking resulted from increases in both Active-Up (Figure 5; treatment, $F_{(2,21)}=31.9$, $p<0.001$; time, $F_{(3,63)}=21.5$, $p<0.001$; treatment x 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 x time, $F_{(6,63)}=5.3$, $p<0.001$).

Drinking was modestly, though significantly, increased by the high dose of QUIN during POST1 (treatment, $F_{(2,21)}=5.6$, $p=0.012$; time, $F_{(3,63)}=4.1$, $p=.011$; treatment x 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 x 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 x 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 x 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 x time, $F_{(6,63)}=0.9$, $p=0.479$). This level of motor activity is comparable to that seen during normal waking (España *et al.*, 2002) and waking induced by a noradrenergic β -agonist (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 x 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 following QUIN infusion. The only exception to this was that in the high-dose group five out of eight animals exhibited an unusual posture, in which the animals appeared 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 ten to twenty times in the first hour following 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 addressing the arousal-enhancing actions of dopaminergic neurotransmission typically employed peripheral administration of DA receptor-selective drugs utilizing procedures that result in alert, awake animals. The current studies employed 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, ICV 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 following ICV administration of the D2 receptor agonist, quinpirole.

In general, D1 and D2 agonist-induced waking appeared similar to normal, spontaneous waking. For example, intense locomotor activation or motor stereotypies 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 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 following D1 agonist infusion was nonetheless moderate (España *et al.*, 2002). 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 following D2 agonist treatment compared to D1 agonist treatment. Levels of chewing observed following 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 one-half of the animals of each dose group. Comparable numbers of wet dog shakes were observed with both doses, occurring approximately 20-30 times in the first hour following infusion. This is consistent with a previous study demonstrating that D1 antagonist treatment blocked the induction of wet dog shakes by exogenous DA infusion into the substantia nigra pars reticulata (Fan *et al.*, 2000). However, because the incidence of wet dog shakes did not appear dose-dependent, occurred relatively infrequently and only in approximately one-half of 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 one-half of the high-dose D2 agonist-treated animals displayed a postural abnormality accompanied by a pivoting motion (see Results above). This appears consistent with previous reports of pivoting following 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 one-half of the animals of the high-dose group, the dose-dependent increase in waking observed following 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 NE levels)

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 which 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 efferents.

Postsynaptic vs. presynaptic actions of D2 receptor activation. Given that 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 ICV administration of the D2 autoreceptor-preferring agonist, (-)-3PPP, elicits increased time spent asleep (Bagetta *et al.*, 1987). Similar effects were observed following infusion of the D2 agonist (+)-3PPP into the ventral tegmental area, a region containing DA-synthesizing neurons. This effect was blocked by pre-treatment, 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 normal spontaneous waking. As such, these data provide further evidence that cerebral DA systems are involved in the initiation and maintenance of alert waking. Alternatively, or additionally, D1 and/or D2 receptors may participate in the induction of high-arousal states, associated with aversive and/or appetitive conditions.

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Footnotes

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Figure Legends

Figure 1. Effects of D1-receptor stimulation in the sleeping rat on cortical EEG and EMG activity. Shown are 5-min traces of cortical EEG and EMG recorded immediately before and after an infusion of 25 nmol SKF-82958. Before the infusion, the rat spent the majority of time in slow-wave sleep (resting with large-amplitude, slow-wave EEG activity and low-amplitude EMG activity). Shortly following the infusion, the animal awoke, as reflected by a decrease in large-amplitude, slow-wave EEG activity and the increase in EMG amplitude.

Figure 2. Effects of D1-receptor stimulation on EEG/EMG-defined sleep-wake states. Shown are the effects of ICV vehicle and 2.5 or 25 nmol SKF-82958 (SKF) on total time spent awake (total waking) and in slow-wave sleep, quiet waking, active waking, and REM sleep. Symbols represent mean (\pm SEM) time spent in a given behavioral state per 30-min epoch. PRE1 and PRE2 represent pre-infusion epochs. POST1-POST4 represent post-infusion epochs, beginning immediately following infusion. SKF infusions elicited a dose-dependent increase in total time spent awake that resulted largely from increases in active waking. Associated with this increase in waking were significant decreases in slow-wave and REM sleep. * $p < 0.05$ compared with vehicle-treated controls. † $p < 0.05$ compared with 2.5 nmol SKF.

Figure 3. Effects of D1-receptor stimulation on behavior. Shown are the effects of ICV vehicle and 2.5 or 25 nmol SKF-82958 (SKF) on time spent in active up, inactive up, rearing, grooming, eating, chewing, and drinking. Also shown is the frequency of quadrant entries. Symbols represent mean (\pm SEM) time (or frequency) spent in a given behavioral state per 30-min epoch. PRE represents the 30-min. epoch immediately preceding infusion. POST1-POST3 represent post-infusion epochs, beginning immediately following infusion. SKF infusion significantly increased time spent in active up, grooming, eating, and rearing. SKF infusion also significantly increased the frequency of quadrant entries. * $p < 0.05$ compared with vehicle-treated controls. † $p < 0.05$ compared with 2.5 nmol SKF.

Figure 4. Effects of D2-receptor stimulation on electroencephalographic/electromyographic-defined sleep-wake states. Shown are the effects of ICV vehicle and 40 or 140 nmol quinpirole (QUIN) on total time spent awake (total waking) and in slow-wave sleep, quiet waking, active waking, and REM sleep. Symbols represent mean (\pm SEM) time spent in a given behavioral state per 30-min epoch. PRE1 and PRE2 represent pre-infusion epochs. POST1-POST4 represent post-infusion epochs, beginning immediately following infusion. QUIN infusions 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. * $p < 0.05$ compared with vehicle-treated controls. † $p < 0.05$ compared with 40 nmol QUIN.

Figure 5. Effects of D2-receptor stimulation on behavior. Shown are the effects of vehicle and either 40 or 140 nmol quinpirole (QUIN) infused ICV on time spent in states of active up, inactive up, rearing, grooming, eating, chewing, drinking. Also shown is the frequency of quadrant crossing. Symbols represent mean (SEM) time spent in a given behavioral state per 30-min epoch. PRE represents the 30-minute epoch immediately preceding infusion. POST1-POST3 represent post-infusion epochs, beginning immediately following 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 QUIN.









