Psychopharmacology
DOI 10.1007/s00213-009-1596-2

ORIGINAL INVESTIGATION

Systemic and intrabasalis administration of the orexin-1 receptor antagonist, SB-334867, disrupts attentional performance in rats

Karen E. Boschen · Jim R. Fadel · Joshua A. Burk

Received: 23 February 2009 / Accepted: 16 June 2009

Abstract
Rationale Orexin neurons project to a number of brain regions, including onto basal forebrain cholinergic neurons. Basal forebrain corticopetal cholinergic neurons are known to be necessary for normal attentional performance. Thus, the orexin system may contribute to attentional processing.
Objectives We tested whether blockade of orexin-1 receptors would disrupt attentional performance.
Methods Rats were trained in a two-lever sustained attention task that required discrimination of a visual signal (500, 100, 25 ms) from trials with no signal presentation. Rats received systemic or intrabasalis administration of the orexin-1 receptor antagonist, SB-334867, prior to task performance.
Results Systemic administration of the orexin-1 receptor antagonist, SB-334867 (5.0 mg/kg), decreased detection of the longest duration signal. Intrabasalis SB-334867 (0.60 μg) decreased overall accuracy on trials with longer signal durations.
Conclusions These findings suggest that orexins contribute to attentional processing, although neural circuits outside of basal forebrain corticopetal cholinergic neurons may mediate some of these effects.

Keywords Acetylcholine · Attention · Basal forebrain · Hypocretin · Hypothalamus · Vigilance

K. E. Boschen · J. A. Burk (✉)
Department of Psychology, College of William & Mary,
Williamsburg, VA 23187, USA
e-mail: jabur2@wm.edu

J. R. Fadel
Department of Pharmacology, Physiology and Neuroscience,
School of Medicine, University of South Carolina,
Columbia, SC 29208, USA

Introduction

Orexins (also known as hypocretins) are neuropeptides released from neurons located in the lateral hypothalamus and contiguous perifornical area (de Lecea et al. 1998; Sakurai et al. 1998). The orexinergic system projects to a number of neuronal targets, including to the basal forebrain (Peyron et al. 1998; Cutler et al. 1999), forming synapses onto cholinergic neurons in the basal forebrain (Fadel et al. 2005). Moreover, intrabasalis infusions of orexin A increase stimulated cortical acetylcholine release (Fadel et al. 2005). Intrabasalis orexin A stimulates acetylcholine release in the somatosensory cortex more potently than orexin B (Dong et al. 2006). Given that orexin B has preferential binding for the orexin-2 receptor, whereas orexin A has high affinity for the orexin-1 and orexin-2 receptors (Sakurai et al. 1998), it seems likely that the orexin-1 receptor contributes importantly to orexin A-induced increases in cortical acetylcholine release (Fadel and Frederick-Duus 2008). Consistent with this speculation, intrabasalis infusions of an orexin-1 receptor antagonist can block stimulated cortical acetylcholine release (Frederick-Duus et al. 2007). Despite clear evidence that orexins can stimulate cortical acetylcholine release via actions in the basal forebrain, the cognitive or behavioral impact of orexinergic projections to the basal forebrain has not been characterized.

The basal forebrain corticopetal cholinergic system is hypothesized to mediate aspects of attentional processing (Everitt and Robbins 1997; Sarter et al. 2005). In humans, systemic administration of drugs that decrease cholinergic function inhibit cognitive processing (Ebert and Kirch 1998) and those that increase cholinergic function can, under some conditions, enhance cognitive processing (Bentley et al. 2004). Experiments with rats have been

Published online: 03 July 2009

helpful in further demonstrating that cortically projecting basal forebrain cholinergic neurons represent an important neural component of the cholinergic system involved in attention. In attention-demanding tasks involving pressing one lever following a brief signal and a different lever following no signal, 192 IgG-saporin-induced lesions of basal forebrain corticopetal cholinergic neurons decrease signal detection accuracy (McGaughy et al. 1996, 2002). In similarly designed tasks, signal detection is also decreased by systemic administration of muscarinic receptor antagonists (McQuail and Burk 2006; Johnson and Burk 2006). Moreover, cortical acetylcholine release is elevated during attentional task performance compared with control tasks (Passetti et al. 2000; Dalley et al. 2001; Arnold et al. 2002). Choline-sensitive biosensors have provided evidence for enhanced cholinergic transmission following attention-demanding visual cues (Parikh et al. 2007). Collectively, these data implicate the basal forebrain corticopetal cholinergic system in attentional processing.

Several lines of evidence suggest a role for orexin peptides in cognition. Human narcolepsy is associated with dramatic—and fairly selective—loss of hypothalamic neurons (Nishino et al. 2000; Peyron et al. 2000; Thannickal et al. 2000) and studies suggest that narcoleptic patients show attentional deficits even during periods of normal wakefulness (Rieger et al. 2003). In rats, direct administration of orexin B into the prefrontal cortex improves performance in an attentional task (Lambe et al. 2005). Finally, recent data demonstrate that transnasal delivery of orexin A ameliorates sleep deprivation-induced cognitive deficits in monkeys (Deadwyler et al. 2007). Collectively, the literature suggests that both orexin A and orexin B may play roles in modulating cognitive function. However, given evidence suggesting that intrabasalis orexin A may be more effective than orexin B at increasing electroencephalographic, electromyographic, and behavioral indices of wakefulness (España et al. 2001; Thakkar et al. 2001; Dong et al. 2006) and the lack of a commercially available selective orexin-2 receptor antagonist, we have chosen to focus on orexin-1 receptor effects on attention that may be mediated via the basal forebrain. Several experiments have shown that intrahippocampal infusions of the orexin-1 receptor antagonist, SB-334867, can disrupt aspects of spatial working memory (Akbari et al. 2006, 2007, 2008). The contribution of orexin inputs to the basal forebrain in an attentional task that clearly depends on cholinergic transmission has not been assessed.

The present experiments were designed to test whether orexins contribute to attentional processing. Rats were trained in a two-lever sustained attention (SAT)-demanding task that requires discrimination of brief and variable visual signals from trials with no signal presentation. After a signal, pressing one lever allowed access to a water dipper whereas, if no signal was presented, pressing another lever led to water dipper access. This task was chosen for several reasons. First, the task was developed and validated as a measure of attention (McGaughy and Sarter 1995) in rats based upon a taxonomy of vigilance (Parasuraman et al. 1987). Second, as mentioned above, selective lesions of basal forebrain corticopetal cholinergic neurons decrease signal detection in this task, without affecting accuracy on trials with no signal presentation (McGaughy et al. 1996). The lack of lesion effects on nonsignal trials is important as it suggests the lesions did not affect the animals' ability to respond based upon the task rules. Third, manipulations that decrease the activity of corticopetal cholinergic neurons, such as systemic or intrabasalis administration of benzodiazepine receptor agonists (Moore et al. 1995) or glutamate receptor antagonists (Fadel et al. 2001), decrease signal detection in this task (Holley et al. 1995; Turchi and Sarter 2001). Finally, some experiments have provided evidence that this task may be useful for translation to humans (Bushnell et al. 2003; Demeter et al. 2008). In the present experiment, after reaching stable performance levels in this attention task, rats were given either systemic or intrabasalis administration of the orexin-1 receptor antagonist, SB-334867, prior to task performance. We hypothesized that orexin-1 receptor blockade would disrupt signal detection accuracy.

Materials and methods

Subjects

A total of 24 male Long–Evans rats (13 for systemic administration, 11 for intrabasalis administration), weighing 151–175 g at the beginning of the experiment were used (Charles River Laboratories, Inc., Wilmington, MA, USA). The rats were housed individually in a temperature- and humidity-controlled environment with a 14:10-h light/dark cycle (lights on 0600–2000 hours). All behavioral testing took place between 0900 and 1200 hours, 5–6 days per week. Animals were water restricted throughout behavioral testing, receiving water during the task and for 30 min after the testing session. The rats were allowed at least 1 h of water access on days when no behavioral testing occurred. Food was available ad libitum for the duration of experiment. The experimental protocol was approved by the Institutional Animal Care and Use Committee at the College of William and Mary, and all animals were treated according to the Guidelines for the Care and Use of Laboratory Animals as set forth by the National Institutes of Health (National Research Council 1996).

Apparatus

The rats were trained in one of 12 chambers each housed within a sound-attenuating box. One side of the chamber contained two retractable levers, a water port with a water delivery dipper (0.01 ml) located between the two levers, and three panel lights. One panel light was positioned directly above each retractable lever, and the third panel light was located above the water port. Only the panel light above the water port was used for these experiments. A house light was located on the other side of the chamber. The house light remained illuminated during all behavioral testing sessions. Illuminance levels of the house and panel lights have been previously described (Burk 2004). Behavioral testing programs were controlled by a personal computer using the Med-PC version IV software.

Behavioral training prior to drug administration

During the first day of training, the retractable levers were extended into the chamber at all times. The water dipper was raised (3.0 s access to 0.01 ml tap water) as reinforcement for each lever press. To minimize a lever bias, five consecutive presses on a lever resulted in the discontinuation of water access until the other lever was pressed. Rats were required to meet a criterion of 120 reinforcers per session for three sessions in order to move to the next training stage. During the second stage, the rats were trained to discriminate between signals (1 s illumination of the panel light) and nonsignals (no illumination of the light). After a signal or no signal, the retractable levers were extended into the chamber. Half of the rats were given water access for pressing the left lever after a signal, which was recorded as a hit. If the rat pressed the right lever after a signal trial, the trial was recorded as a miss. Following no signal presentation, a press on the right lever was recorded as a correct rejection and water access was provided. A press to the left lever was recorded as a false alarm. The rules of the task were reversed for half of the rats. Thus, for these animals, the right lever was the correct response following signal presentation and the left lever was the correct response following no signal. Incorrect choices were followed by a correction trial that was identical to the previous trial. Three consecutive incorrect choices resulted in a forced choice trial in which only the correct lever was extended for 90 s. If the errors occurred on signal trials, the panel light remained illuminated for the duration of the lever extension during the forced choice trial. For all trials, if no lever press was made within 3 s after lever extension, the trial was scored as an omission. The intertrial interval (ITI) for this stage of training was 12 s. Criterion for completion of this stage of training was set at 70% hits and 70% correct rejections for three consecutive sessions.

During the final version of the task, three signal durations were used: 500, 100, and 25 ms. The presentation of these signal durations was randomly varied. In addition, the ITI was reduced to 9±3 s. The changes to the signal duration and to the ITI were designed to increase attentional demands (Parasuraman et al. 1987; Koelega et al. 1990). No correction trials or forced choice trials were used during the final task. There were a total of 162 trials each session (81 signal, 81 nonsignal). Trials were pseudorandomly presented such that for every 18 trials, half were signal trials (three trials with each signal duration) and half were nonsignal trials. Sessions lasted for approximately 40 min. The animals were trained in this task until a criterion of 70% hits on the 500-ms signal and 70% correct rejections for three consecutive sessions was met. Animals were considered prepared for drug administration after reaching criterion.

Procedures for SB-334867 preparation and systemic administration

The nonpeptide orexin-1 receptor antagonist SB-334867 (Tocris Bioscience, Ellisville, MO, USA) was suspended in a vehicle solution of 1.0 ml saline, 200 mg Hydroxy-beta-cyclodextran, and 125 μl 100% dimethyl sulfoxide. This drug has been well-documented as a selective orexin-1 receptor antagonist, with greater than 50-fold selectivity for orexin-1 receptors compared with orexin-2 receptors (Duxon et al. 2001) and no appreciable affinity for over 50 other G-protein coupled receptors and ion channels (Smart et al. 2001). The entire solution was placed on a vortex prior to injection. All drug preparation occurred immediately prior to the injection.

The rats received three intraperitoneal (ip) injections: vehicle solution, 1.0 mg/kg SB-334867, and 5.0 mg/kg SB-334867 in a counterbalanced order. This dose range has been shown to block stimulated cortical acetylcholine release (Frederick-Duus et al. 2007). All injections were administered immediately prior to the beginning of each testing session. After the injection, each rat was placed in its appropriate testing chamber and the program was activated. The task began after a 10-min delay. The house light was illuminated throughout the delay period. At least 2 days of behavioral training took place between drug administrations in order to reestablish baseline task performance, which was defined as 70% hits on the 500-ms signal and 70% correct rejections.

Surgical procedures

For rats receiving intrabasalis drug administration, after reaching baseline criterion in the attention task, animals

received bilateral intrabasalis guide cannulae implantations. Prior to surgery, rats received 2.7 mg/ml acetaminophen diluted in water overnight. Animals were anesthetized using ip injections of 90.0 mg/kg ketamine and 9.0 mg/kg xylazine. After the surgical area was shaved with an electric razor, rats were placed in a stereotaxic device (Kopf Instruments, Tujunga, CA, USA) with the incisor bar set 3.3 mm below the interaural line. An incision was made along the midline from anterior to posterior (AP), exposing the skull. Holes were drilled over the target coordinates for guide cannulae implantation (AP and medial–lateral (ML) from bregma, dorsal–ventral (DV) from the interaural line; AP −1.3 mm, ML ±2.7 mm, DV +3.5). Eight-millimeter guide cannulae (22 gauge) were used, with each internal cannula extending 1 mm beyond the guide cannula. Three stainless steel screws were also inserted into the skull. Dental cement was used to secure guide cannulae placements. Dummy cannulae were inserted to prevent clogging. Rats were given free access to food and water for 1 week after surgery, after which the rats were returned to water restriction and began to retrain on the task. Infusion procedures began once the rats reestablished criterion performance.

Procedures for SB-334867 infusions

The drug preparation procedures were identical to those described for systemic drug administration. Animals received two to three sham infusion sessions prior to drug infusions, in which a short (5.0 mm) internal cannula (28 gauge) was inserted but no drug was delivered. These sham infusion sessions were designed to acclimate the rats to the infusion procedures prior to drug administration sessions. The rats received four drug doses: vehicle solution, 0.15, 0.30, and 0.60 μg SB-334867, infused bilaterally into the basal forebrain in a randomized order. These values are within the range of intracranial doses of SB-334867 shown to block the development of morphine conditioned place preference (Harris et al. 2007) but are well below those that reduce spontaneous motor activity (Kiwaki et al. 2004). Infusions were made through internal cannula attached to a 1.0-μl Hamilton syringe via polyethylene tubing. A total volume of 0.5 μl was infused into each cannula at a rate of 1.0 μl/min. After the infusions were completed, the internal cannula remained in place for 1 min to allow drug diffusion. Animals were then placed into the behavioral testing chambers and the task began 1 min after the rats were placed in the chambers. At least 2 days of behavioral training took place between each infusion to reestablish baseline task performance.

Histological procedures

Rats were deeply anesthetized with 90.0 mg/kg ketamine and 9.0 mg/kg xylazine (ip). Rats were then transcardially perfused with 10% sucrose and then 10% formalin at a pressure of 300 mmHg using a Perfusion One instrument (myneurolab.com, St. Louis, MO, USA). The brains were then removed, put into formalin for not more than 48 h and then into a 30% sucrose solution in phosphate-buffered saline for at least 3 days. The tissue was sectioned (50 μm) using a freezing microtome (Leica, Wetzlar, Germany). Sections near the cannula sites were stained using cresyl violet. Sections were viewed using an Olympus BX-51 Research microscope to assess cannulae placement.

Behavioral measures and statistical analyses

The number of hits (*H*), misses (*M*), correct rejections (CR), false alarms (FA), and omissions were recorded for each testing session. Each session was divided into three blocks (trials 1–54, trials 55–108, and trials 109–162) to assess the effect of the drug within each session. The relative number of hits per block at each signal duration, as well as for the overall session, was calculated as $[H/(H+M)]$, and the relative number of correct rejections per block and for the overall session was calculated as [CR/(CR+FA)]. Relative hits can range from 0 (the correct rejection/miss lever was pressed every time a signal was presented) to 1 (the hit/false alarm lever was pressed following every signal). Relative correct rejections have a similar range, with the opposite lever being pressed following no signal presentation for values of 0 or 1. An angular transformation ($X'=2\times \arcsin \sqrt{x}$) of the relative hits and correct rejections was conducted to normalize all accuracy data (Zar 1974). A measure of overall SAT accuracy was also calculated using the formula: $SAT=(H-FA)/(2\times(H+FA)-(H+FA)^2)$ (McGaughy et al. 1996; Nuechterlein et al. 2009). The SAT measure takes into account accuracy on signal and nonsignal trials. The SAT scores can vary from −1 to 1, with a value of 1 indicating that responses were correct to all signal and nonsignal trials. A value of 0 indicates an inability to discriminate between signals and nonsignals. Omissions were analyzed separately from measures of response accuracy.

The SAT measure and the relative number of hits were analyzed using repeated-measures analysis of variance (ANOVA) with the factors of signal duration, block, and dose. The relative number of correct rejections and omissions were analyzed using repeated-measures ANOVA with the factors of block and dose. Data analyses were conducted with SPSS 15.0 for Windows (SPSS, Chicago, IL, USA). A level of $\alpha=0.05$ was used to determine statistical significance.

Results

Systemic SB-334867 administration

Systemic SB-334867 produced differential effects across signal duration and dose, without affecting detection of nonsignals (Fig. 1; Table 1). To test this observation, a signal duration (500, 100, 25 ms)×dose (0.0, 1.0, 5.0 mg/kg) ANOVA was conducted for the signal trials. This analysis yielded main effects of signal duration ($F(2, 24)=172.5$, $p<0.05$) and a significant signal duration×dose interaction for the hits ($F(4, 48)=2.87$, $p<0.05$), but the main effect of dose was not significant. The main effect of signal duration reflects decreases in accuracy when the signal duration was shorter (Fig. 1). Follow-up one-way ANOVAs of the hits with dose as a factor at each signal duration revealed a significant effect of dose at the 500-ms signal ($F(2, 24)=3.43$, $p<0.05$), but not at the 100- or 25-ms signals ($p>0.10$). Compared to vehicle administration, the hits following 5.0 mg/kg SB-334867 were lower following the 500-ms signal ($t(12)=2.26$, $p<0.05$; Cohen's $d=0.63$; Fig. 1). A similar signal duration×dose ANOVA was conducted for the SAT measure. This analysis yielded a main effect of signal duration ($F(2, 24)=169.7$, $p<0.05$) and the signal duration×dose interaction approached, but did not reach statistical significance ($F(4, 48)=2.51$, $p=0.062$). The reason for the discrepancy in the analyses of SAT and hits is that the SAT is a measure of accuracy on signal and nonsignal trials and accuracy on nonsignal trials (correct rejections) was unaffected by drug administration (Table 1). No significant effects of SB-334867 were found for correct rejections (Table 1). Finally, although there was an increase in omissions following higher SB-334867 doses, this effect was not statistically significant ($F(2, 24)=1.89$, $p=0.184$; Table 1).

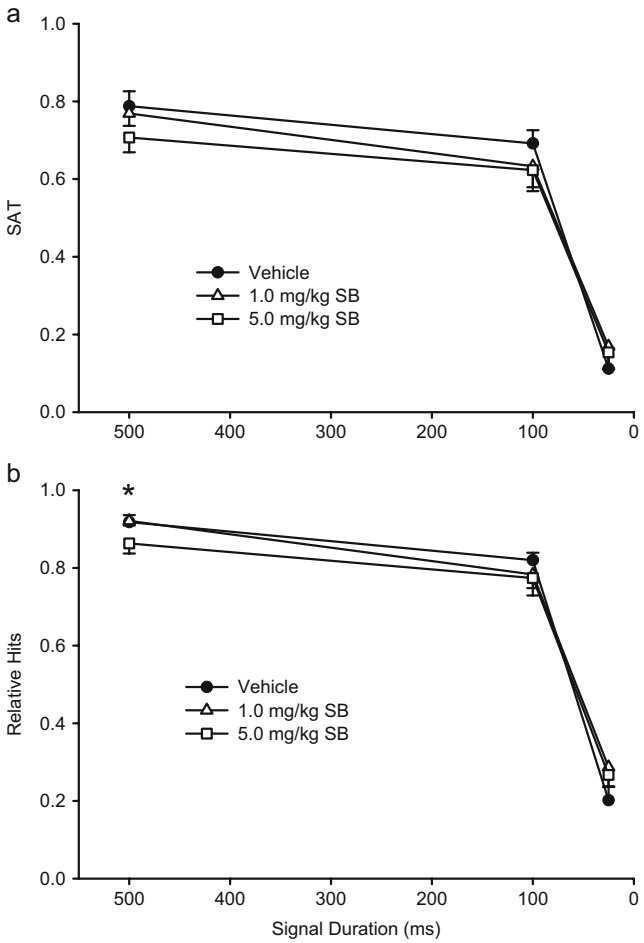

Fig. 1 The SAT measure (**a**; see text for calculations of the SAT measure) and relative hits (**b**) during attention task performance following systemic administration of the orexin-1 receptor antagonist, SB-334867 (0.0, 1.0, 5.0 mg/kg; $n=13$). There were no significant effects of SB-334867 on the SAT measure, although there was a trend for SB to decrease overall accuracy. SB-334867 did significantly decrease relative hits on 500-ms signal trials. The *asterisk* indicates significant difference in detecting the 500-ms signal between vehicle and 5.0 mg/kg. Error bars represent standard errors of the mean

Table 1 Summary of nonsignificant effects of systemic (ip; $n=13$) or intrabasalis SB-334867 ($n=8$) administration on relative correct rejections and omissions per session (mean±standard errors of the mean)

SB Dose	Correct rejections	Omissions per session
Vehicle, ip	0.86±0.03	9.4±4.3
1.0 mg/kg, ip	0.83±0.03	8.8±4.6
5.0 mg/kg, ip	0.84±0.02	15.1±4.8
Vehicle, intrabasalis	0.87±0.03	10.0±3.7
15 μg, intrabasalis	0.86±0.03	11.5±4.7
30 μg, intrabasalis	0.85±0.02	26.5±15.5
60 μg, intrabasalis	0.82±0.04	32.2±17.3

Intrabasalis SB-334867 administration

The data from one rat were not included in behavioral analyses due to inappropriate cannulae placements, and the data from two rats were not analyzed because they did not maintain stable task performance. Thus, there were a total of eight rats included in the behavioral analyses. Cannulae were located dorsal to the horizontal limb of the diagonal band, medial to the striatum, and ventral to the external globus pallidus (Fig. 2). Cannula tracks were characterized by loss of tissue and gliosis.

Signal duration (500, 100, 25 ms)×dose (vehicle, 0.15, 0.30, 0.60 μg) ANOVAs were conducted for the SAT measure and for the relative hits. These analyses did not yield main effects or significant interactions involving dose for the SAT measure or for hits. To test whether there were any effects of SB-334867 at the longer signal durations, as

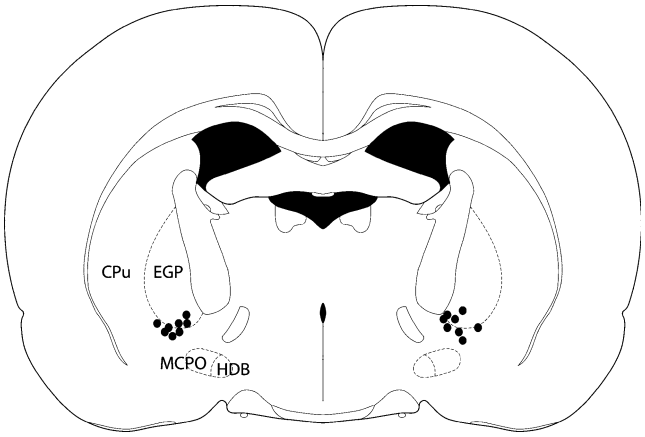

Fig. 2 Cannula placements within the basal forebrain at −1.3 mm to bregma (modified from Paxinos and Watson 2005). *Circles* indicate the location of the infusion cannulae, which extended 1.0 mm beyond the guide cannula. Substantial variability in anterior–posterior locations was not observed. All cannulae were located dorsal to the magnocellular preoptic nucleus. *CPu* caudate/putamen, *EGP* external globus pallidus, *MCPO* magnocellular preoptic nucleus, *HDB* nucleus of the horizontal limb of the diagonal band

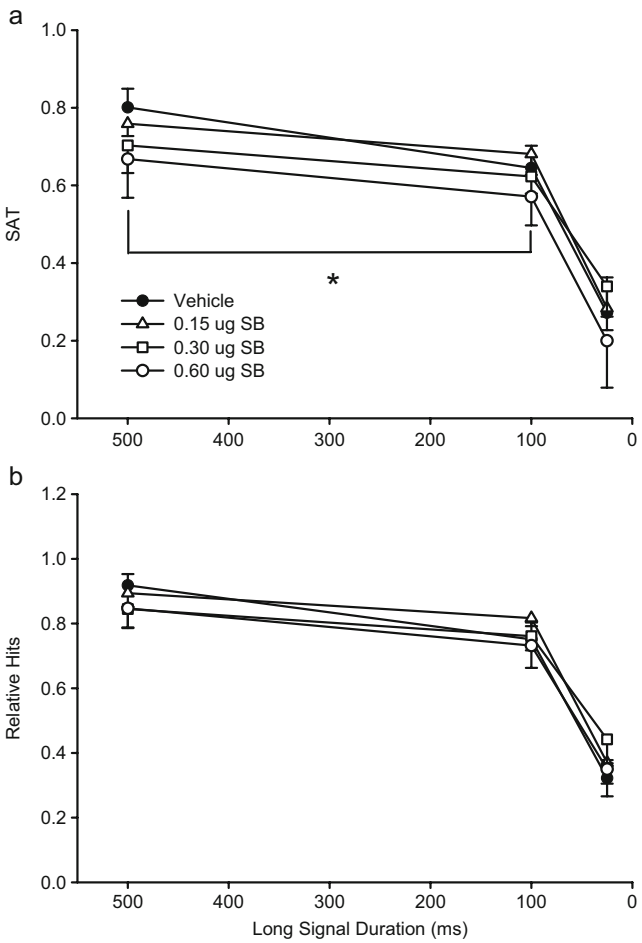

Fig. 3 The SAT measure (**a**; see text for calculations of the SAT measure) and relative hits (**b**) during attention task performance following intrabasalis administration of the orexin-1 receptor antagonist, SB-334867 (0.0, 0.15, 0.30, 0.60 μg; $n=8$). The *asterisk* indicates that, compared to vehicle, intrabasalis SB-334867 (0.60 μg) significantly decreased overall accuracy in the task at the longer signal durations, as indicated by the SAT measure ($p<0.05$). There were no effects of SB-334867 on the relative hits. Error bars represent standard errors of the mean

observed following systemic SB-334867, we conducted planned comparisons that compared vehicle administration with each drug dose including only the longer signal durations in the ANOVAs for the SAT measure and hits. This analysis yielded a main effect of dose ($F(1, 7)=6.012$, $p<0.05$; partial $\eta^2=0.54$) for the SAT measure when vehicle was compared with 0.60 μg SB-334867. Thus, 0.60 μg SB-334867 decreased overall accuracy, as assessed by the SAT measure, but this effect was restricted to analyses in which the shortest signal duration was omitted. Similar analyses did not yield any significant effects of drug on the hit rate nor were there any effects of drug at the 25-ms signal duration for either the SAT measure or hits (Fig. 3; Table 1). There were no significant effects of drug dose on correct rejections (Table 1). Finally, although there was a trend for elevated omissions following higher SB-334867 doses (Table 1), omissions were not significantly affected by drug administration ($F(3, 21)=0.848$, $p=0.45$).

Discussion

The present experiments tested whether orexin-1 receptor blockade affects performance in an attention-demanding task that is dependent upon the integrity of basal forebrain corticopetal neurons. Systemic orexin-1 receptor blockade decreased detection following presentation of the longest signal duration. Infusions of SB-334867 into the basal forebrain decreased overall task performance, as assessed with the SAT score, without selective effects on detection of signals or nonsignals. The effect sizes suggest that systemic SB-334867 produced a "medium" effect on task performance whereas intrabasalis SB-334867 produced a "large" effect (Cohen, 1988). The lack of effects on omissions in either experiment suggests that drug administration did not substantively affect motivation for the reinforcement or the motoric capabilities necessary for task performance. Although 0.6 μg SB-334867 did increase omissions for some animals, on average, the animals completed approximately 80% of the trials following this drug dose. The effects of orexin-1 receptor blockade in either experiment cannot be explained as a side bias because, for example, if the rat was pressing exclusively the correct rejection/miss lever, then a decrease in hits would be accompanied by an increase in correct rejections. The fact that systemic SB-334867 decreased signal detection following the 500-ms signal duration may seem counterintuitive. The hit rate following

the 25-ms signal was relatively low (20–44% across both experiments), indicating that rats most commonly responded as if no signal was presented on these trials. This relatively low hit rate may have contributed to difficulty observing further drug-induced declines in accuracy. However, the lack of the effects of systemic SB-334867 following the 100-ms signal cannot be attributed to a floor effect. Sarter et al. (2005) distinguish between signal-driven processing and cognitive modulation of signal detection, with cognitive modulation including the rules for appropriate responding based on signal-driven processing. SB-334867 may have disrupted the ability to generate the appropriate signal-guided response (as opposed to signal-driven processing per se), which would be expected to produce the largest deficit following the 500-ms signal, when a signal-guided response most often has to be generated.

Systemic SB-334867 decreased signal detection accuracy whereas intrabasalis SB-334867 did not. The decrease in signal detection following systemic SB-334867 could be mediated via the basal forebrain corticopetal cholinergic system, as previous studies have shown that pharmacological blockade of muscarinic receptors (Johnson and Burk 2006; McQuail and Burk 2006) or selective loss of corticopetal cholinergic neurons decrease signal detection in the present task (McGaughy et al. 1996). The systemic SB-334867-induced decrease in hits at the 500-ms signal is similar to effects observed in experiments that reported relatively more minor loss of cortical cholinergic inputs (40–70% loss of cortical acetylcholinesterase-positive fibers; McGaughy and Sarter 1998; McGaughy et al. 1999). Of course, it is possible that systemic orexin 1 receptor blockade affected circuitry outside of basal forebrain corticopetal cholinergic neurons. For example, the orexin system has extensive interactions with norepinephrine (Baldo et al. 2003), a neurotransmitter system implicated in aspects of attention (Foote et al. 1991; Aston-Jones et al. 1999).

Intrabasalis SB-334867 decreased the SAT measure, whereas systemic SB-334867 did not significantly decrease the SAT measure. The SAT measure includes accuracy on signal and nonsignal trials. Without a selective decrease in hits or correct rejections, the SAT measure typically reflects a nonsignificant decrease in accuracy on both signal and nonsignal trials (Fig. 3; Table 1). Lesions that predominantly destroy noncholinergic basal forebrain neurons, using ibotenic acid, decrease correct rejections in this task (Burk and Sarter 2001). Although speculative, it is possible that the effects of intrabasalis SB-334867 administration on attentional performance reflect the actions of orexin-1 receptor blockade on basal forebrain cholinergic and noncholinergic neurons (Sarter and Bruno 2002). Some orexin-positive projections to the basal forebrain appear to form synapses onto neurons that were not immunopositive for choline acetyltransferase (Fadel et al. 2005). Many of these neurons appear to be magnocellular and parvalbumin-immunoreactive (unpublished data) suggesting that they may contribute to the GABAergic component of the basalocortical projection system (Gritti et al. 1993, 1997; Zaborszky et al. 1997). In summary, the present experiments provide evidence that orexin-1 receptor blockade can disrupt accuracy in the present attention-demanding task, but clearly, additional work is needed to identify the mechanisms underlying the observed accuracy impairments. Moreover, the present results may underestimate the contribution of orexinergic projections to the basal forebrain in attention because only orexin-1 receptors were blocked.

Implication of orexin effects on attention for pathological conditions

In addition to a clear role in narcolepsy, orexins have been strongly implicated in a number of other pathologies with an attentional component, including drug addiction (Harris et al. 2005) and schizophrenia (Deutch and Bubser 2007; Lambe et al. 2007). Furthermore, several lines of evidence indicate declines in markers of orexin function in aging (Terao et al. 2002; Zhang et al. 2002; Porkka-Heiskanen et al. 2004; Downs et al. 2007), suggesting that orexin dysfunction may contribute to age-related decline in cholinergic-dependent attentional function.

In summary, the data support the conclusion that orexins can modulate attentional performance. Some of the effects of orexin-1 receptor blockade on attention may be mediated by basal forebrain corticopetal neurons although it seems likely that there are other neural circuits that also contribute to the effects of SB-334867 observed in the present experiments. Alterations of orexinergic functioning may represent a common neural substrate of the attentional dysfunction that accompanies such diverse conditions as drug relapse and age-related cognitive decline.

Acknowledgments This work was supported by AG030646 (JRF and JAB) and awards from the Jeffress Memorial Trust (JAB). The authors wish to thank Andrea Robinson, Usmaan Bashir, Sarah Baum, Will Bleser, Fuad Bohsali, Aileen Kim, David Mangini, Puja Parekh, Jon Weeks, and Bethany Wilson for technical assistance.

References

Akbari E, Naghdi N, Motamedi F (2006) Functional inactivation of orexin 1 receptors in CA1 region impairs acquisition, consolidation and retrieval in Morris water maze task. Behav Brain Res 173:47–52

Akbari E, Naghdi N, Motamedi F (2007) The selective orexin 1 receptor antagonist SB-334867-A impairs acquisition and consolidation but not retrieval of spatial memory in Morris water maze. Peptides 28:650–656

Akbari E, Motamedi F, Naghdi N, Noorbakhshnia M (2008) The effect of antagonization of orexin 1 receptors in CA1 and dentate

gyrus regions on memory processing in a passive avoidance task. Behav Brain Res 187:172–177
Arnold HM, Burk JA, Hodgson EM, Sarter M, Bruno JP (2002) Differential cortical acetylcholine release in rats performing a sustained attention task versus behavioral control tasks that do not explicitly tax attention. Neuroscience 114:451–460
Aston-Jones G, Rajkowski J, Cohen J (1999) Role of locus coeruleus in attention and behavioral flexibility. Biol Psychiatry 46:1309–1320
Baldo BA, Daniel RA, Berridge CW, Kelley AE (2003) Overlapping distributions of orexin/hypocretin- and dopamine beta-hydroxylase immunoreactive fibers in rat brain regions mediating arousal, motivation, and stress. J Comp Neurol 464:220–237
Bentley P, Husain M, Dolan RJ (2004) Effects of cholinergic enhancement on visual stimulation, spatial attention, and spatial working memory. Neuron 41:969–982
Burk JA (2004) Introduction of a retention interval in a sustained attention task in rats: effects of presenting a visual distracter and increasing the inter-trial interval. Behav Processes 67:521–531
Burk JA, Sarter M (2001) Dissociation between the attentional functions mediated via basal forebrain cholinergic and GABAergic neurons. Neuroscience 105:899–909
Bushnell PJ, Benignus VA, Case MW (2003) Signal detection behavior in humans and rats: a comparison with matched tasks. Behav Processes 64:121–129
Cohen J (1988) Statistical power: analysis for the behavioral sciences. Erlbaum, Hillsdale
Cutler DJ, Morris R, Sheridhar V, Wattam TA, Holmes S, Patel S, Arch JR, Wilson S, Buckingham RE, Evans ML, Leslie RA, Williams G (1999) Differential distribution of orexin-A and orexin-B immunoreactivity in the rat brain and spinal cord. Peptides 20:1455–1470
Dalley JW, McGaughy J, O'Connell MT, Cardinal RN, Levita L, Robbins TW (2001) Distinct changes in cortical acetylcholine and noradrenaline efflux during contingent and noncontingent performance of a visual attentional task. J Neurosci 21:4908–4914
de Lecea L, Kilduff TS, Peyron C, Gao X, Foye PE, Danielson PE, Fukuhara C, Battenberg EL, Gautvik VT, Bartlett FS, Frankel WN, van del Pol AN, Bloom FE, Gautvik KM, Sutcliffe JG (1998) The hypocretins: hypothalamus-specific peptides with neuroexcitatory activity. Proc Natl Acad Sci U S A 95:322–3277
Deadwyler SA, Porrino L, Siegel JM, Hampson RE (2007) Systemic and nasal delivery of orexin-A (hypocretin-1) reduces the effects of sleep deprivation on cognitive performance in nonhuman primates. J Neurosci 27:14239–14247
Demeter E, Sarter M, Lustig C (2008) Rats and humans paying attention: cross-species task development for translational research. Neuropsychology 22:787–799
Deutch AY, Bubser M (2007) The orexins/hypocretins and schizophrenia. Schizophr Bull 33:1277–1283
Dong HL, Fukuda S, Murata E, Zhu Z, Higuchi T (2006) Orexins increase cortical acetylcholine release and electroencephalographic activation through orexin-1 receptor in the rat basal forebrain during isoflurane anesthesia. Anesthesiology 104:1023–1032
Downs JL, Dunn MR, Borok E, Shanabrough M, Horvath TL, Kohama SG, Urbanski HF (2007) Orexin neuronal changes in the locus coeruleus of the aging rhesus macaque. Neurobiol Aging 28:1286–1295
Duxon MS, Stretton J, Starr K, Jones DN, Holland V, Riley G, Jerman J, Brough S, Smart D, Johns A, Chan W, Porter RA, Upton N (2001) Evidence that orexin-A-evoked grooming in the rat is mediated by orexin-1 (OX1) receptors, with downstream 5-HT2C receptor involvement. Psychopharmacology (Berl) 153:203–209
Ebert U, Kirch W (1998) Scopolamine model of dementia: electroencephalogram findings and cognitive performance. Eur J Clin Invest 28:944–949
España RA, Baldo BA, Kelley AE, Berridge CW (2001) Wake-promoting and sleep-suppressing actions of hypocretin (orexin): basal forebrain sites of action. Neuroscience 106:699–715
Everitt BJ, Robbins TW (1997) Central cholinergic systems and cognition. Annu Rev Psychol 48:649–684
Fadel J, Frederick-Duus D (2008) Orexin/hypocretin modulation of the basal forebrain cholinergic system: insights from in vivo microdialysis studies. Pharmacol Biochem Behav 90:156–162
Fadel J, Sarter M, Bruno JP (2001) Basal forebrain glutamatergic modulation of cortical acetylcholine release. Synapse 39:201–212
Fadel J, Pasumarthi R, Reznikov LR (2005) Stimulation of cortical acetylcholine release by orexin A. Neuroscience 130:541–547
Foote SL, Berridge CW, Adams LM, Pineda JA (1991) Electrophysiological evidence for the involvement of the locus coeruleus in alerting, orienting, and attending. Prog Brain Res 88:521–532
Frederick-Duus D, Guyton MT, Fadel J (2007) Food-elicited increases in cortical acetylcholine release require orexin transmission. Neuroscience 149:499–507
Gritti I, Mainville L, Jones BE (1993) Codistribution of GABA- with acetylcholine-synthesizing neurons in the basal forebrain of the rat. J Comp Neurol 329:438–457
Gritti I, Mainville L, Mancia M, Jones BE (1997) GABAergic and other non-cholinergic basal forebrain neurons, together with cholinergic neurons, project to the mesocortex and isocortex in the rat. J Comp Neurol 383:163–177
Harris GC, Wimmer M, Aston-Jones G (2005) A role for lateral hypothalamic orexin neurons in reward seeking. Nature 437:556–559
Harris GC, Wimmer M, Randall-Thompson JF, Aston-Jones G (2007) Lateral hypothalamic orexin neurons are critically involved in learning to associate an environment with morphine reward. Behav Brain Res 183:43–51
Holley LA, Turchi J, Apple C, Sarter M (1995) Dissociation between the attentional effects of infusions of a benzodiazepine receptor agonist and an inverse agonist into the basal forebrain. Psychopharmacology (Berl) 120:99–108
Johnson RT, Burk JA (2006) Effects of gonadectomy and androgen supplementation on attention in male rats. Neurobiol Learn Mem 85:219–227
Kiwaki K, Kotz CM, Wang C, Lanningham-Foster L, Levine JA (2004) Orexin A (hypocretin 1) injected into hypothalamic paraventricular nucleus and spontaneous physical activity in rats. Am J Physiol Endocrinol Metab 286:E551–E559
Koelega HS, Brinkman JA, Zwep B, Verbaten MN (1990) Dynamic vs static stimuli and their effect on visual vigilance performance. Percept Mot Skills 70:823–831
Lambe EK, Liu RJ, Aghajanian GK (2007) Schizophrenia, hypocretin (orexin), and the thalamocortical activating system. Schizophr Bull 33:1284–1290
Lambe EK, Olausson P, Horst NK, Taylor JR, Aghajanian GK (2005) Hypocretin and nicotine excite the same thalamocortical synapses in prefrontal cortex: correlation with improved attention in rat. J Neurosci 25:5225–5229
McGaughy J, Sarter M (1995) Behavioral vigilance in rats: task validation and effects of age, amphetamine, and benzodiazepine receptor ligands. Psychopharmacology (Berl) 117:340–357
McGaughy J, Sarter M (1998) Sustained attention performance in rats with intracortical infusions of 192 IgG-saporin-induced cortical cholinergic deafferentation: effects of physostigmine and FG 7142. Behav Neurosci 112:1519–1525
McGaughy J, Kaiser T, Sarter M (1996) Behavioral vigilance following infusions of 192 IgG-saporin into the basal forebrain: selectivity of the behavioral impairment and relation to cortical AChE-positive fiber density. Behav Neurosci 110:247–265
McGaughy J, Decker MW, Sarter M (1999) Enhancement of sustained attention performance by the nicotinic acetylcholine receptor

agonist ABT-418 in intact but not basal forebrain-lesioned rats. Psychopharmacology 144:175–182
McGaughy J, Dalley JW, Morrison CH, Everitt BJ, Robbins TW (2002) Selective behavioral and neurochemical effects of cholinergic lesions produced by intrabasalis infusions of 192 IgG-saporin on attentional performance in a five-choice serial reaction time task. J Neurosci 22:1905–1913
McQuail JA, Burk JA (2006) Evaluation of muscarinic and nicotinic receptor antagonists on attention and working memory. Pharmacol Biochem Behav 85:796–803
Moore H, Sarter M, Bruno JP (1995) Bidirectional modulation of cortical acetylcholine efflux by infusion of benzodiazepine receptor ligands into the basal forebrain. Neurosci Lett 189:31–34
National Research Council (1996) Guide for the care and use of laboratory animals. National Academy Press, Washington, D. C.
Nishino S, Ripley B, Overeem S, Lammers GJ, Mignot E (2000) Hypocretin (orexin) deficiency in human narcolepsy. Lancet 355:39–40
Nuechterlein KH, Luck SJ, Lustig C, Sarter M (2009) CNTRICS final task selection: control of attention. Schizophr Bull 25:182–196
Parasuraman R, Warm JS, Dember WN (1987) Vigilance: taxonomy and utility. In: Mark LS, Warm JS, Huston RL (eds) Ergonomics and human factors. Springer, New York, pp 11–32
Parikh V, Kozak R, Martinez V, Sarter M (2007) Prefrontal acetylcholine release controls cue detection on multiple timescales. Neuron 56:141–154
Passetti F, Dalley JW, O'Connell MT, Everitt BJ, Robbins TW (2000) Increased acetylcholine release in the rat medial prefrontal cortex during performance of a visual attentional task. Eur J Neurosci 12:3051–3058
Paxinos G, Watson C (2005) The rat brain in stereotaxic coordinates, 5th edn. Elsevier, Amsterdam
Peyron C, Tighe DK, van den Pol AN, de Lecea L, Heller HC, Sutcliffe JG, Kilduff TS (1998) Neurons containing hypocretin (orexin) project to multiple neuronal systems. J Neurosci 18:9996–10015
Peyron C, Faraco J, Rogers W, Ripley B, Overeem S, Charnay Y, Nevsimalova S, Aldrich M, Reynolds D, Albin R, Li R, Hungs M, Pedrazzoli M, Padigaru M, Kucherlapati M, Fan J, Maki R, Lammers GJ, Bouras C, Kucherlapati R, Nishino S, Mignot E (2000) A mutation in a case of early onset narcolepsy and a generalized absence of hypocretin peptides in human narcoleptic brains. Nat Med 6:991–997
Porkka-Heiskanen T, Alanko L, Kalinchuk A, Heiskanen S, Stenberg D (2004) The effect of age on prepro-orexin expression and contents of orexin A and B in the rat brain. Neurobiol Aging 25:231–238
Rieger M, Mayer G, Gauggel S (2003) Attention deficits in patients with narcolepsy. Sleep 26:36–43
Sakurai T, Amemiya A, Ishii M, Matsuzaki I, Chemelli RM, Tanaka H, Williams SC, Richarson JA, Kozlowski GP, Wilson S, Arch JR, Buckingham RE, Haynes AC, Carr SA, Annan RS, McNulty DE, Liu WS, Terrett JA, Elshourbagy NA, Bergsma DJ, Yanagisawa M (1998) Orexins and orexin receptors: a family of hypothalamic neuropeptides and G protein-coupled receptors that regulate feeding behavior. Cell 92:573–585
Sarter M, Bruno JP (2002) The neglected constituent of the basal forebrain corticopetal projection system: GABAergic projections. Eur J Neurosci 15:1867–1873
Sarter M, Hasselmo ME, Bruno JP, Givens B (2005) Unraveling the attentional functions of cortical cholinergic inputs: interactions between signal-driven and cognitive modulation of signal detection. Brain Res Brain Res Rev 48:98–111
Smart D, Sabido-David C, Brough SJ, Jewitt F, Johns A, Porter RA, Jerman JC (2001) SB-33867-A: the first selective orexin-1 receptor antagonist. Br J Pharmacol 132:1179–1182
Terao A, Apte-Deshpande A, Morairty S, Freund YR, Kilduff TS (2002) Age-related decline in hypocretin (orexin) receptor 2 messenger RNA levels in the mouse brain. Neurosci Lett 332:190–194
Thakkar MM, Ramesh V, Strecker RE, McCarley RW (2001) Microdialysis perfusion of orexin-A in the basal forebrain increases wakefulness in freely behaving rats. Arch Ital Biol 139:313–328
Thannickal TC, Moore RY, Nienhuis R, Ramanathan L, Gulyani S, Aldrich M, Cornford M, Siegel JM (2000) Reduced number of hypocretin neurons in human narcolepsy. Neuron 27:469–474
Turchi J, Sarter M (2001) Bidirectional modulation of basal forebrain N-methyl-D-aspartate receptor function differentially affects visual attention but not visual discrimination performance. Neuroscience 104:407–417
Zaborszky L, Gaykema RP, Swanson DJ, Cullinan WE (1997) Cortical input to the basal forebrain. Neuroscience 79:1051–1079
Zar JH (1974) Biostatistical analysis. Prentice Hall, Englewood Cliffs
Zhang JH, Sampogna S, Morales FR, Chase MH (2002) Age-related changes in hypocretin (orexin) immunoreactivity in the cat brainstem. Brain Res 930:206–211