Effects of Systemic and Intrabasalis Administration of the Orexin-1 Receptor Antagonist, SB-334867, on Attentional Performance in Rats

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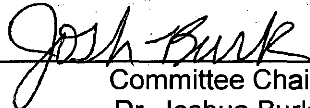

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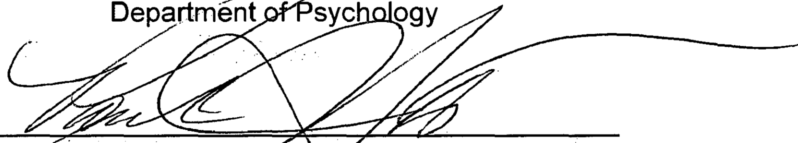

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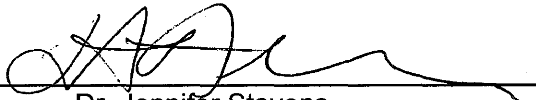

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ABSTRACT PAGE

The neuropeptides orexin A and B (also known as hypocretin 1 and 2) have been implicated in the regulation of feeding behavior, emotional arousal, and sleeping and waking behaviors. Previous research has indicated that orexin neurons projecting from the hypothalamus excite basal forebrain cholinergic neurons, resulting in increased awakefulness (Eggermann et al., 2001). The basal forebrain cholinergic system has been implicated in the regulation of attention (McGaughy et al., 1996). Collectively, these results suggest that orexins might play a role in attentional processing. The current studies assessed the effects of systemic and intrabasalis administration of an orexin-1 receptor anagonist, SB-334867 on attentional performance. Rats were trained on an attention task that required discrimination between visual signals (500 ms, 100 ms, and 25 ms) and non-signals. In Experiment 1, the rats were each given three injections: a vehicle solution and two levels of SB-334867, 1.0 mg/kg and 5.0 mg/kg, counterbalanced across subjects. The results revealed an interaction for signal duration and dose. Visual detection at the 500 ms signal duration was impaired at the highest dose; in addition, performance at the 25 ms signal duration improved at the highest dose. In Experiment 2, rats were given four intrabasalis infusions: a vehicle solution and three doses of SB-334867, 0.15 µG/side, 0.33 µG/side, and 0.6 µG/side. No significant effects were found for dose. The data suggest that orexin A does play a role in regulating attention, though the link might be more complicated than previously thought.

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As humans, we have a basic idea of what attention is and can report when it is being used. However, understanding the specific functions and processes of attention is more difficult. It is only relatively recently that scientific innovations have allowed scientists to map out some of the specific mechanisms of attention, as well as what brain regions make up the attentional system.

Classic attention research described attention as a process similar to decision-making (Broadbent & Gregory, 1963). Two models of attention were developed by researchers during this time: the early-selection model and the late-selection model (Broadbent & Gregory, 1963; Deutsch & Deutsch, 1963). Early-selection theory hypothesizes that selective attention places more emphasis on the selected stimulus, not by blocking out the unattended stimulus, but by reducing its intensity. This process takes place before the brain analyzes the content of the stimulus. Late-selection models hypothesize that the brain analyzes the content of both stimuli but filters out irrelevant information. Thus, unattended stimuli never reach a conscious level of cognition.

Attention has been compared to shining a spotlight on the object which is being scrutinized (Cavanaugh, 2004). Only the object within the spotlight has been selected for higher processing, and objects outside of the spotlight are not further processed. Multiple spotlights, or areas of selection, can be functioning at once. By having these specific areas of selection, the brain can filter out unimportant stimuli and send the more important information on for deeper processing. The attentional system displays limited capacity and acuity during selection tasks (Cavanaugh, 2004; Carr, 2004). One's ability to focus on multiple

objects is limited, and the detail with which one can describe the object decreases as the load on the attentional system increases. Increased numbers of distractors can inhibit one's ability to focus efficiently on the selected object, compromising deeper processing.

The neuroanatomy of attention was first explored using electrode recordings in the superior colliculus during visual attention shifts in the macaque monkey (Wurtz & Goldberg, 1972). The first imaging studies offered more insight into the neural systems that underlie attentional processing, including the posterior parietal lobe and the anterior cingulate, both of which were found to be important for target detection in attention tasks (Posner & Petersen, 1990).

Since then, numerous other neural systems have been implicated in the regulation of attentional processing. A growing body of literature has implicated the cholinergic system as a key neurotransmitter within the attentional system. Specifically, cholinergic projections from the basal forebrain, in particular the nucleus basalis of Meynert, to the neocortex are implicated in attention.

Basal Forebrain Corticopetal Cholinergic Neurons and Attention in the Rat

Various tasks have been designed to measure attentional performance in rats. Researchers often use a five-choice serial task, which is specifically designed to tax attentional demands (Robbins, 2002). In this task, rats are required to monitor five locations for a 0.5 s visual signal presentation. A nose poke into an aperture underneath the signal presentation location (as measured by infrared photobeams) is scored as a correct response and results in a food reinforcement.

An incorrect nose poke or no response results in a five second time out. This task tests the ability of the rat to maintain a level of spatial attention over 30 minutes.

Excitotoxic lesions to the basal forebrain result in reduced choice accuracy on this task, perhaps due to the removal of cholinergic input to the neocortex (Robbins, Everitt, Marston, Wilkinson, Jones, & Page, 1989). However, due to the nonspecificity of excitotoxic lesions, the learning impairments found in the five-choice serial reaction time task were not necessarily a result of the loss of cholinergic neurons. GABAergic and glutamatergic neurons are much more numerous within the basal forebrain than cholinergic neurons (Zaborsky, Gaykema, Swanson, & Cullinan, 1997). Since excitotoxic lesions completely destroy neurons within the target area and fibers of passage, it is reasonable to speculate that it is the loss of another neuronal system, or the cumulative loss of the neurons within the basal forebrain, that decrease task performance (Everitt & Robbins, 1997).

Immunotoxins have allowed more targeted lesions of specific neurotransmitter systems. Specifically, researchers developed the cholinotoxin 192 IgG-saporin, which selectively targets the p75 nerve growth factor receptors (Waite, Chen, Wardlow, Wiley, Lappi, & Thal, 1995). This nerve growth factor is expressed by cortically-projecting cholinergic neurons within the basal forebrain and some Purkinje cells within the cerebellum. Thus, this cholinotoxin selectively destroys neurons expressing p75 and leaves all other neurons intact. This allows for 192 IgG-saporin to be infused into cortical regions, such as the prefrontal cortex (PFC), and only destroy cholinergic neurons within the basal

forebrain, which receives projections from the PFC. These immunotoxic lesions reduce levels of choline acetyltransferase by up to 90% with increasing doses (Holley, Wiley, Lappi, & Sarter, 1994; Waite et al., 1995). On a behavioral level, extensive intrabasalis lesions caused by 192 IgG-saporin resulted in decreased choice accuracy and an increase in omissions on a five-choice serial reaction time task (McGaughy, Dalley, Morrison, Everitt & Robbins, 2002).

In addition, 192 IgG-saporin has been shown to cause behavioral deficits in other tasks designed to tax attentional demands (Bushnell, Oshiro, & Padnos, 1997; McGaughy & Sarter, 1998; Arnold, Burk, Hodgsom, Sarter, & Bruno, 2002). Intrabasalis infusions of 192 IgG-saporin decreased signal detection on a visual discrimination task designed to have high attentional demands (McGaughy, Kaiser, & Sarter, 1996). This task is comprised of signal and non-signal trials. The signal trials are comprised of three signal durations, 500 ms, 100 ms, and 25 ms. Rats are trained to press one lever in response to a signal presentation, and trained to press another lever in response to a non-signal or “blank” trial. Compared to the five-choice serial task, the visual discrimination task minimized the spatial and locomotor aspects of the task, while still being a valid measure of attentional performance (McGaughy & Sarter, 1995). In addition, the task is very sensitive to cholinergic manipulations. Rats that received 192 IgG-saporin lesions displayed significantly impaired visual detection of all signal durations. However, the percentage of correct lever responses to non-signal trials was not affected, indicating that the rats were still able to perform within the rules of the task.

Neuroanatomy of the Orexinergic System

In the late 1990's, two groups of researchers independently discovered a population of neuropeptides later known as orexin A and B (hypocretin 1 and 2), (Sakurai, Amemiya, Ishii, Matsuzaki, Chemelli, Tanaka, Williams, Richardson, Kozlowski, Wilson, Arch, Buckingham, Haynes, Carr, Annan, McNulty, Liu, Terrett, Elshourbagy, Bergsma, & Yanagisawa, 1998; de Lecea, Kilduff, Peyron, Gao, Foye, Danielson, Fukuhara, Battenberg, Gautvik, Bartlett, Frankel, van den Pol, Bloom, Gautvik, Sutcliffe, 1998). Orexin A and B are produced in the lateral hypothalamus and medially contiguous perifornical area (Sakurai, 2007; Mignot, Taheri, & Nishino, 2002). The orexin system projects to numerous brain regions including the locus coeruleus (LC), the preoptic area, paraventricular nucleus of the thalamus, basal forebrain, and spinal cord (Sakurai, 2007; Chemelli, Willie, Sinton, Elmquist, Scammell, Lee, Richardson, Williams, Xiong, Kisanuki, Fitch, Nakazato, Hammer, Saper, & Yanagisawa, 1999). Orexin A and B bind differentially to the two subtypes of orexin receptors, Orexin-1 and Orexin-2 (Sakurai et al., 1998). Orexin A binds to Orexin-1 receptors with high affinity, while Orexin B binds with 100- to 1000-fold lower affinity. The orexin-2 receptor has a high affinity for both peptides.

The hypothalamus is important for regulating metabolic functions, as well as hunger, thirst, and circadian rhythms (Sakurai, 2007). Orexins are neuroexcitatory in nature, in that they increase axonal activity, specifically within the hypothalamus (de Lecea et al, 1998). They have been implicated in the

regulation of energy homeostasis, hormone secretion, and feeding behaviors. In addition, orexins seem to play an important role in the maintenance of waking and sleeping patterns, as well as arousal states.

Orexin neurons have been implicated in autonomic homeostasis and hormone secretion. Orexins project to brain areas thought to be important for the regulation of heart rate and blood pressure, including the ventrolateral medulla and the locus coeruleus (Dampney, 1994). Orexins also might play a role in the regulation of body temperature, as they project to the raphe magnus and subcoeruleus (Werner & Bienek, 1990). Lesions of the rostral raphe nuclei have been shown to cause a loss of thermoreactiveness within preoptic neurons. In addition, the location of orexinergic projections also supplies evidence that these neuropeptides are important in hormone secretion. Orexin neurons project to the arcuate nucleus, an area that houses numerous populations of hormone-secreting neurons, including gonadatropin-releasing hormone (Iqbal, Pompolo, Sakurai, & Clarke, 2001). Orexin A has been shown to decrease concentrations of growth hormone and prolactin, while Orexin B seems to have a direct effect on the function of the pituitary, adrenal, and pineal glands (Mikkelsen et al., 2001; Randeva, Karteris, Grammatopoulos, & Hillhouse, 2001; Samson & Taylor, 2001).

When first discovered, one of the primary functions of orexins was thought to be related to feeding behavior (Sakurai et al., 1998). Orexin neurons express leptin receptors, a peptide hormone encoded on the *obese* gene that suppresses appetite via action within the hypothalamus (Hakansson, de Lecea,

Sutcliffe, Yanagisawa, & Meister, 1999; Horvath, Diano, & Van den Pol, 1999). Leptin knockout mice (*ob/ob*) also have decreased levels of the orexin precursor preprohypocretin mRNA (Yamamoto et al., 1999). In addition, periods of fasting increase orexin peptides levels within the hypothalamus (Sakurai et al., 1998; Mondal, Nakazato, Date, Murakami, Yanagisawa, & Matsukura, 1999).

Orexin's effects on feeding have also been seen through behavioral studies. Short-term food consumption in rats has been found to be increased following intracerebroventricular administration of both orexin A and B (Sakurai et al., 1998). Similarly, orexin B increases food consumption in sheep (Sartin et al., 2001). Feeding behavior can also be increased following the intracranial administration of orexin A to various brain regions involved in feeding, including the lateral hypothalamus, perifornical area, and the dorsomedial hypothalamic nucleus. However, many researchers believe that orexin's role in feeding is not as important as was first believed. Increases in feeding behavior could be a byproduct of orexin's role in the sleep-wake cycle (Yamanaka, Sakurai, Katsumoto, Yanagisawa, & Goto, 1999; Fujiki, Yoshida, Ripley, Honda, Mignot, & Nishino, 2001). Orexins seem to promote wakefulness, which could in turn cause greater food intake.

Pathology of orexin neurons has been implicated in the sleep disorder narcolepsy with cataplexy. Narcolepsy is a rare neurological disorder characterized by excessive daytime sleepiness, sleep paralysis, hypnogogic hallucinations, and in some cases, cataplexy (NINDS, 2008). Orexin's role in the sleep-wake cycle was first discovered through genetic mapping projects

concerning the defective canine narcolepsy gene (Lin et al., 1999). The absence of orexins within the cerebrospinal fluid is a characteristic of narcolepsy (Nishino, Ripley, Overeem, Lammers, & Mignot, 2000; Ripley et al., 2001). Ripley and colleagues found that 37 out of 42 narcoleptics studied did not have detectable levels of orexins within their cerebrospinal fluid.

Orexin neurons within the lateral and perifornical hypothalamus receive projections from the superchiasmatic nucleus (SCN), which generates the circadian rhythm (Abrahamson, Leak, & Moore, 2001). Orexin levels within the brain fluctuate throughout the circadian rhythm, becoming more active during wakefulness. Sleep deprivation has been shown to increase orexin levels even further, suggesting that one of the primary functions of orexin is to promote wakefulness (Yoshida et al., 2001).

Activation of monoamine neurons in the LC, dorsal raphe (DR), and tuberomamillary nucleus (TMN) are critical for maintaining wakefulness, and are referred to as REM-off cells. These neurons are most active during wakefulness, less active during non-REM sleep, and are completely inactive during REM sleep (Saper, Chou, & Scammell, 2001; Mileykovskiy, Kiyashchenko, & Siegel, 2005). Administration of orexin A and B increases the activity of histaminergic neurons within the TMN, and orexin A has an excitatory effect on noradrenergic neurons within the LC and seratonergic neurons in the DR (Hagan et al., 1999). The excitation of these monoamines prolongs their activity, which decreases REM sleep and increases arousal.

Behaviorally, intracerebroventricular injections of orexin A have been found to increase arousal as measured by increased locomotion in an X-maze and increased startle responses (Hagan et al., 1999). In addition, support for orexin's role in cognitive processing has been shown in sleep-deprived rhesus monkeys (Deadwyler, Porrino, Siegel & Hampson, 2007). Systemic and intranasalis administration of orexin A significantly improved performance on a delayed match-to-sample task following 30 to 36 hours of sleep deprivation. No improvements were seen after drug administration following normal sleep.

Basal Forebrain Cholinergic Neurons and Orexins

Neuroanatomical research has found numerous axonal hypothalamic projections onto cholinergic neurons in the basal forebrain (Cullinan & Zaborsky, 1991). Fadel, Pasumarthi, and Reznikov (2005) found orexin-immunoreactive fibers positioned closely to cholinergic neurons, suggesting that orexin projections from the hypothalamus map directly onto cholinergic neurons within the basal forebrain. Infusions of orexin A into the basal forebrain result in increased wakefulness in rats (España, Baldo, Kelley, & Berridge, 2001). Electrodes monitored brain wave patterns during sleeping and waking cycles, showing that total time awake increased, and total time spent in slow wave sleep and rapid eye movement sleep decreased. More specifically, Eggermann and colleagues (2001) found that orexins excite cholinergic neurons in the basal forebrain, resulting in increased cortical activation and awakefulness.

Fadel and colleagues (2005) used microdialysis to assess the effect of orexin A on cholinergic release in the PFC. Levels of ACh in the PFC

significantly increased after intra-basalis administration of orexin A. Infusions of orexin A directly into the PFC did not increase PFC ACh. These data suggest that intrabasalis infusions of orexin A can affect levels of ACh in brain areas important for various cognitive functions, including attention. Fadel and Frederick-Duus (2008) theorize that orexins modulate basal forebrain ACh in response to visual stimuli that are relevant to one's physiological state, including hunger or thirst level. This would be especially relevant to the current study, in which appetitive motivation is being assessed using a visual discrimination task.

The Current Experiments

The current experiments use a previously validated attention task. The task has been tested with various manipulations (McGaughy & Sarter, 1995), including background noise, high event rates, and event asynchrony. During this task, rats are required to visually discriminate between three signal durations and a nonsignal trial, and then respond by pressing the correct lever. The introduction of "background noise" (in the form of a flashing house light) resulted in impaired signal detection between signal and non-signal trials, indicating that flashing light distracted the rats from achieving optimal performance. In addition, the flashing houselight resulted in increased rate of poorer signal detection of the shortest signal duration during the last block of trials. This vigilance decrement could be a form of attentional fatigue. Rats often respond to the shortest signal duration as a non-signal trial, by pressing the incorrect lever. This signal length could require more attentional resources to detect, thus being affected by a vigilance decrement before the other signal durations, especially in the presence of a distraction. High

event rates, or a higher number of trials per minute (defined as having 9 ± 3 s in between trials), also augmented this vigilance decrement. In addition, event asynchrony was used to test the rat's ability to time trial onset. To achieve this, the inter-trial interval (ITI) was varied across trials (12 ± 6 s). Highly varied ITI had no effect on attentional performance, as it did not affect signal detection or the number of trials omitted (not responded to). Thus, it is unlikely that the rats were timing trial onset. McGaughy and Sarter also used animal age as a variable to test task validity. Normal aging results in attentional deficits (Verhaeghen & Cerella, 2002). Twenty month old rats were less able to discriminate between the shortest signal duration and the nonsignal trials as compared to young rats, as displayed by an increased number of incorrect lever responses following the shortest signal duration. However, no deficits were found during the nonsignal trials, indicating that the rats knew the rules of the task. The previous data support that the current task is a valid measure of attention in the rat.

To examine differences in cortical ACh release during and after task performance, Arnold and colleagues (2002) compared two control procedures that did not explicitly tax attention to the current task. First, a basic operant control task using a fixed ratio schedule of 9, but in which only 60% of these intervals were rewarded; this task was designed to explore the effects of simple operant responding. The second task was designed to assess the effects of lever extension as a prompt for responding. This task mirrored the first control procedure, except that only one lever was extended into the chamber. ACh levels within the cortex were measured using microdialysis during and after task performance. The two

control procedures resulted in dramatically smaller increases in cortical ACh efflux (about 50%) compared to increases following the current task (about 140%). These data support that the current task requires high attention demands which in turn elevate cortical ACh levels.

Specifically important for the current experiments, visual signal detection in this task has been shown to be sensitive to various basal forebrain cholinergic manipulations, including cortical cholinergic deafferentation produced by intrabasalis infusions of 192 IgG-saporin (McGaughy et al., 1996). Moreover, signal detection in this task is also sensitive to other manipulations of basal forebrain neurons that depress cortical ACh release, including intrabasalis infusions of an NMDA receptor antagonist (Turchi & Sarter, 2001) or a benzodiazepine receptor agonist (Holley, Turchi, Apple, & Sarter, 1995; McGaughy & Sarter, 1995).

The current experiments assessed the effects of systemic and intrabasalis administration of the orexin-1 receptor antagonist SB-334867. While orexin A has been implicated in increased wakefulness and cognitive functioning, an orexin-1 receptor antagonist was used in this study due to the ceiling effect observed in the attentional task used. Rats often perform with over 90% accuracy on the longer signal durations, making attentional enhancements difficult to observe. Experiment 1 investigates the effects of systemic injections of SB-334867. Administration of the orexin-1 receptor antagonist was expected to decrease ACh release within the cortex, resulting in impaired visual signal detection. Experiment 2 investigates the effects of localized infusions of SB-

334867 into the basal forebrain. Infusions of the orexin-1 receptor antagonist were expected to result in impaired signal detection, similar to the impaired signal detection expected in Experiment 1.

Experiment 1

Methods

Subjects. Subjects were 16 male Long-Evans rats, approximately two months old at the beginning of the experiment (Charles River Laboratories, Inc., Wilmington, MA). All animals were experimentally naïve. The rats were housed individually in a temperature- and humidity-controlled environment with a 14/10 hour light/dark cycle. All behavioral testing took place between 0900 and 1200, five days per week. Animals were water restricted for the duration of the behavioral testing, only receiving water during the task and for 30 minutes after the testing session. The rats were allowed free access to water 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 handled according to 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 12 chambers each housed within a sound-attenuating box. One side of the chamber contained two retractable levers, a water port with an automated water delivery dipper (0.01 ml) located between the two levers, and three panel lights. One panel light was positioned directly

over each retractable lever, and one panel light was centrally located above the water port. Only the centrally located panel light was used for this experiment. A house light was located on the other side of the chamber. The house light remained illuminated during all behavioral training and testing. Luminance levels of the house and panel lights have been previously described (Burk, 2004). Behavioral training and testing programs were administered by a personal computer using the Med-PC version IV software.

Behavioral training procedures. The house light was illuminated during all training procedures and the test task. During the first stage of training, the retractable levers were extended into the chamber at all times. The water dipper was raised after each lever press. To discourage side 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 lever presses per session for three sessions in order to move on to the next training stage. During the second training stage, the rats were trained to discriminate between signals (1 s illumination of the panel light) and non-signals (no illumination of the light). After a signal or non-signal was presented, the retractable levers were extended into the chamber. Half of the rats were trained to press the left lever after a signal presentation, which would be scored as correct, or a hit, and water access would be provided. If the rat pressed the right lever after a signal trial, this was considered to be a miss. Following a non-signal presentation, a press on the right lever was considered to be a correct rejection and water access was provided. A press to the left lever was considered to be a false alarm. The rules of the task

were reversed for half of the rats, meaning that the right lever was the correct response following signal presentation and the left lever was the correct response following a non-signal. Incorrect choices were followed by a correction trial that was identical to the previous trial; three incorrect choices in a row resulted in a forced choice trial in which only the correct lever was extended for 90 s. During the forced choice trial, the panel light remained illuminated for the duration of the lever extension. For all trials, if no lever press was made within three seconds after lever extension, the trial was scored as an omission. The inter-trial interval (ITI) for this stage of training was 12±3 s, to reduce anticipation of the beginning of the next trial. 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 ms, 100 ms, and 25 ms. The presentation of these signal durations was randomly varied. There were a total of 162 trials each session (81 signal, 81 non-signal). In addition, the ITI was reduced to 9±3 s. No correction trials or forced choice trials were used during the final task. The animals were trained on this task until the criterion of 70% hits on the 500 ms signal and 70% correct rejections for three consecutive sessions was met. Once criterion was reached, drug administration for the present experiment began.

Procedures for SB-334867 administration. The orexin-1 receptor antagonist SB-334867 (Tocris Bioscience, Ellisville, MO) was suspended in a vehicle solution of 1.0 mL saline, 200 mg Hydroxy-beta-cyclodextran, and 125 μL 100% DMSO. After the addition of SB-334867, the solution was vortexed for

30 minutes prior to injection. Fresh vehicle solution was prepared the day of injection, with excess solution being discarded after use.

The rats received three i.p. injections: vehicle solution, 1.0 mg/kg SB-334867, and 5.0 mg/kg SB-334867 in a counterbalanced order. All injections were administered immediately prior to the beginning of each testing session. The testing program had a 10-min delay included at the beginning of each testing session to allow time for the drug to cross the blood-brain barrier. Satiety studies have shown that significant effects of SB-334867 begin 20 minutes after i.p. administration and peak after one hour (Rodgers, Halford, Nunes de Souza, Canto de Souza, Piper, Arch, Upton, Porter, Johns, & Blundell, 2001). At least two days of behavioral training took place between drug administrations in order to reestablish baseline task performance, which was defined as three consecutive days of 70% hits on the 500 ms signal and 70% correct rejections.

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; sessions were also divided by signal duration (500 ms, 100 ms, and 25 ms). The relative number of hits per block at each signal duration, as well as 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). Omissions were analyzed separately from measures of response accuracy. The mean response latencies for hits, misses, correction rejections, and

false alarms were calculated and analyzed using as multi-factor ANOVA with the trial outcomes as factors.

The relative number of hits was analyzed using a repeated-measures analysis of variances (ANOVA) with the factors of signal duration, block, and dose. The relative number of correct rejections was also analyzed using a repeated-measures ANOVA with the factors of block and dose.

Results

The data reported here are from the 13 rats that maintained stable performance levels in between drug administration sessions. This study assessed the effects of systemic administration of orexin antagonist SB-334867 on performance on an attention task. A repeated measures ANOVA on the relative number of hits for the three signal durations found a significant main effect for signal duration ($F(2, 24) = 212.9, p < .001$). Rats exhibited signal duration-dependent accuracy, with the hit rate higher following longer signal durations (Figure 1). For hits, a significant interaction was found for signal duration and dose ($F(4,48) = 3.06, p < 0.05$). Compared to vehicle administration, the hits following 5.0 mg/kg SB-334867 were lower on 500 ms signal trials ($t(12) = 2.36$, $p < 0.05$). The hit rate to the 25-ms signal was elevated following 1.0 mg/kg SB-334867 compared to vehicle administration ($t(12) = 2.58, p < 0.05$; Figure 2). No other significant effects were found for the relative hits. A repeated-measures ANOVA for the relative number of correct rejections found no significant main effects for block or dose (Figure 3). No significant effects were found for lever press latency or omissions.

Discussion

Experiment 1 investigated the effect of the orexin-1 receptor antagonist SB-334867 on performance on an attention task. Systemic administration of the orexin-1 antagonist resulted in decreased signal detection at the highest dose (5.0 mg/kg SB-334867) for the longest signal duration (500 ms). In addition, signal detection of the shortest signal duration (25 ms) was improved at 1.0 mg/kg SB-334867 dose. A trend showing increased signal detection of the 25 ms signal flowing the highest dose was also found. No deficits were found for the non-signal trials as the number of correct rejections did not change across dose. The lack of drug-induced or, in previous experiments, lesion-induced effects on detection of non-signals has been used as an indicator that the subjects displayed no lever bias and were continuing to respond based on the task rules (McGaughy et al., 1996). No severe motor or motivational deficits due to the effects of the drug were detected, as evidenced by the lack of change in response latencies or trial omissions.

The above factors are important to consider when interpreting the changes in signal detection found. The decrease in signal detection at the 500 ms signal duration indicates that the highest dose of SB-334867 may decrease cortical ACh release. McGaughy & Sarter (1998) found a similar pattern after moderate ablations of cortical cholinergic inputs in 192 IgG-saporin-lesioned rats; only the hit rate to the longest signal duration (also 500 ms) was impaired. Administration of SB-334867 might have resulted in a moderate impairment of the cholinergic

system, resulting in the pattern of detection deficits at the longest signal duration reported here.

One unexpected result in the present experiment was that 1.0 mg/kg SB-334867 elevated detection of the 25 ms signal. This increase in signal detection is not necessarily indicative of attention enhancements. Signal detection remained stable at the 100 ms signal duration for all doses; if attentional enhancement took place, increased signal detection could be expected at the 100 ms signal duration as well. In addition, signal detection was decreased following the 500 ms signal. Thus, the reason for this improved signal detection remains speculative. The signal-driven and cognitive modulation hypotheses of signal detection offer potential explanations for the current results, specifically the improved detection at the 25 ms signal duration (Sarter, Hasselmo, Bruno, & Givens, 2005). According to this model, cortical acetylcholine release, functioning in response to signal properties and cognitive context, is involved in determining whether a particular event is responded to as a signal or non-signal. In the current study, rats appear to typically respond to the 25 ms signal as a non-signal during baseline task performance, resulting in a low number of hits at this signal duration. The average percentage of correct responses at the 25 ms signal following the vehicle dose was 21%, indicating on 79% of the trials, rats were responding to the 25 ms signal as if it was a non-signal. Administration of SB-334867 may have decreased cortical ACh release, altering this top-down process, changing the rat's criterion for identifying events as signals. A "riskier" criterion may have led to the increase in hits following the 25 ms signal.

The PFC has been implicated in the regulation of top-down attentional processing via the cholinergic and glutamatergic systems (Nelson, Sarter, & Bruno, 2005). The corticopetal cholinergic system of the basal forebrain aids in sensory optimization that filters out interfering information such as distractors and background noise, and receives glutamatergic and cholinergic input originating from the PFC (Sarter, Givens, & Bruno, 2001). The orexin receptor antagonist SB-334867 could affect PFC outputs to the basal forebrain, resulting in the shift bias seen in the current data. The neurochemical basis for this shift in bias remains unsettled.

Another explanation of the elevated detection of the 25 ms signal at the 1.0 mg/kg SB-334867 dose can be found in dopamine-related research. Amphetamine-induced dopamine release has been shown to increase the number of hits at the shortest signal duration (also 25 ms) by an average of 20% on the attention task used in the present study (Deller & Sarter, 1998), which is similar the results found in this study. No changes at the longer signal durations (500 ms and 50 ms) were found. Deller and Sarter (1998) suggested that behavioral sensitization caused by repeated exposure to amphetamines led to the increase in signal detection at the shortest duration. Orexin A within the ventral tegmental area has been implicated in having a critical role in behavioral sensitization after cocaine exposure (Borgland, Taha, Sarti, Fields, & Bonci, 2006). While this evidence suggests that a link between orexins and the dopamine system could account, at least in part, for the results found in the current study, amphetamine administration also led to an increase in false alarms, which was not observed in

the current study. Thus, the cause of the increased hit rate at the 25 ms signal duration remains speculative.

Future research into the link between dopamine and orexins would be beneficial in explaining the results found in the present study. While orexins seem to regulate dopamine release within several brain regions, a clear cut pattern of effect has not been established. Infusions of an orexin antagonist into the ventral tegmental area during the attention task would assess the specific effects of the drug on the dopaminergic neurons in this region. Recent research has suggested that dopamine negatively modulates acetylcholine release in the basal forebrain via the PFC-nucleus accumbens pathway (Brooks, Sarter, & Bruno, 2007). However, the interaction between dopamine and ACh, as well as the neural circuits involved, needs to be explored in more depth.

Experiment 2

One of the limitations of Experiment 1 was that i.p. injections were used, causing the drug spread throughout the brain and the periphery, making it difficult to know which neurotransmitter systems were affected and how this, in turn, affected task performance. To address this, as well as to explore acetylcholine's role in Experiment 1's results, Experiment 2 was designed to assess the effects of localized infusions of SB-334867 into the basal forebrain.

Methods

Subjects. Subjects were 11 male Long-Evans rats, approximately two months old at the beginning of the experiment (Charles River Laboratories, Inc., Wilmington, MA). All animals were experimentally naïve. Animal housing and

care procedures were identical to those described in Experiment 1. The experimental protocol was approved by the Institutional Animal Care and Use Committee at the College of William and Mary, and all animals were handled according to Guidelines for the Care and Use of Laboratory Animals as set forth by the National Institutes of Health (National Research Council, 1996).

Apparatus and behavioral training procedures. The experimental apparatus and training procedures used were identical to those described in Experiment 1. The animals were trained on this task until the criterion of 70% hits on the 500 ms signal and 70% correct rejections for three consecutive sessions was met.

Surgical procedures. 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 intraperitoneal (i.p.) injections of 90.0 mg/kg ketamine and 9.0 mg/kg xylazine. Animals were shaved using an electric razor, and placed in a stereotaxic device (Kopf Instruments, Tujunga, CA) with the incisor bar set 3.3mm below the interaural line (IA). An incision was made along the midline from anterior to posterior, exposing the skull. Holes were made over the target coordinates for guide cannulae implantation (AP: -1.3, ML: +/- 2.7 from bregma; DV: +3.5 from IA). Eight mm guide cannulae were used, with internal cannulae extending 1 mm beyond the guide cannulae. Three jeweler's screws were also inserted into the skull. Dental cement was used to secure guide cannulae placement. Dummy cannulae were inserted to prevent clogging. Rats

were given free access to food and water for one week after surgery, after which the rats were returned to water restriction and began to retrain on the task. Once the rats reached reestablished performance criterion, infusion procedures began.

Procedures for SB-334867 infusions. The vehicle solution and preparation procedures were identical to those described in Experiment 1. The rats received four drug doses: vehicle solution, 0.15 μg/μl, 0.3 μg/μl, and 0.6 μg/μl SB-334867, in a counterbalanced order. Infusions were given through an internal cannulae attached to a 1.0 μl Hamilton syringe via polyethylene tubing at a rate of 0.5 μl/minute (0.5 μl per hemisphere). Due to technical difficulties, a subset of animals (N=7) received all infusion types at a rate of 1.0 μl/minute. All infusions were administered immediately prior to each testing session. At least two 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. Rats were then transcardially perfused with 10% sucrose and 10% formalin at a pressure of 300mmHg using a Perfusion One instrument (myneurolab.com, St. Louis, MO). The brains were then removed and put into formalin for no more than 48 hours. The tissue was then put into a 30% sucrose solution in phosphate-buffered saline for at least three days. The tissue was then frozen and sectioned (40 μm) using a freezing microtome. Every section was saved and mounted. The tissue was then stained using cresyl violet. Slides were analyzed using an Olympus BX-51 Research microscope to assess cannulae placement.

Behavioral measures and statistical analyses. The behavioral measures used in Experiment 2 were the same as those used in Experiment 1. As in Experiment 1, the relative number of hits, correct rejections, and omissions were analyzed using repeated-measures analysis of variances (ANOVA).

Results

Histological analyses. Analyses confirmed that cannulae were correctly placed in ten animals. Only these animals were included in the analyses. The tips of the guide cannulae were located approximately 1 mm above the target brain region. Internal cannulae extended 1 mm beyond the ends of the guide cannulae. Among the animals included in the statistical analyses, only minor variations in placement were observed.

Statistical analyses. This study assessed the effects of intrabasalis infusions of the orexin antagonist SB-334867 on attentional performance. One animal was discarded from behavioral data analysis due to incorrect cannulae placement. The data reported here are from the ten rats with cannulae located within the basal forebrain. A repeated-measures ANOVA on the relative number of hits for the three signal durations found a main effect for signal duration ($F(2,18) = 113.73$, $p < 0.001$). Rats exhibited a signal duration-dependent accuracy, with the hit rate higher following longer signal durations. For hits, no significant main effects were found for dose or block ($p = 0.84$ and $p = 0.56$) (see Figure 4).

In addition, no significant interactions were found. However, inspection of the means revealed some trends concerning the effects of drug administration

on the hit rate, mainly during the third block of trials within a session. The relative number of hits following the 500 ms signal duration for the third block of trials following the 0.6 μl SB dose was slightly lower for the third block following vehicle administration (see Figure 5). However, exploratory *t*-tests comparing signal detection during the third block following vehicle and the 0.6 μG/side infusion did not find any significant effects.

A repeated measures ANOVA on the relative number of correct rejections found a significant main effect for block ($F(2,18) = 7.26, p < 0.01$) (see Figure 6). Rats exhibited a higher correct rejection rate during the second block of trials as compared to the first and third blocks. No other significant main effects or interactions were found for correct rejections. Overall, no differences in performance on non-signal trials were found between dose levels (see Figure 7). In addition, no significant effects were found for omissions.

Discussion

Experiment 2 investigated the specific action of orexins within the basal forebrain on attentional performance in rats. Intrabasalis infusions of the orexin-1 receptor antagonist SB-334867 did not result in any significant dose-related results. Significant main effects for signal duration were found, in that rats performed better at the longer signal durations compared to the shorter signal durations. However, these effects were expected on this task and remained constant across dose. Signal detection at the 500 ms signal duration tended to be lower during the third block of trials following the 0.6 mg/μL dose, however this effect was not significant. It is possible that the highest dose of SB-334867 could

have caused a vigilance decrement after prolonged attentional performance. Deficits in attention performance have been found in humans as a function of time (Paus, Zatorre, Hofle, Caramanos, Gotman, Petrides, & Evan, 1997). Participants showed decreased correct responses and increased reaction time during a 60 minute auditory attention task. In addition, there was a decrease in cerebral blood flow to the substantia innominata, a region of the basal forebrain. More specific to the current attention task, high event rates and lower intensity signals have been shown to perpetuate a vigilance decrement on signal trials (McGaughy & Sarter, 1995; Bushnell, 1999).

A significant main effect was found for block during non-signal trials. Rats performed better during the second block of trials as compared to the first and third blocks. This pattern could be due in part to a "warm up" effect following the infusion procedure. This effect can be observed as the rats performing more poorly during the beginning of a testing session as compared to later trials. In addition, vigilance could deteriorate after prolonged task performance, resulting in the decreased rate of correct rejections during the third block of trials, similar to the trend found during the third block following the 500 ms signal duration after the highest dose.

General Discussion

The first experiment was designed to assess the overall effects of the orexin-1 receptor antagonist SB-334867 on attentional performance via the use of systemic injections. However, it is unknown how much of the drug was absorbed in the periphery. The orexin-1 receptor antagonist affected many brain areas, not

just regions specialized for attention. To address this problem, the second experiment was designed to investigate the effects of localized infusions of SB-334867 into the basal forebrain. These infusions did not produce the effects observed in Experiment 1. One suggestion would be to use higher doses of SB-334867. However, the doses used are within the range of those previously shown to increase wakefulness (Thakkar, Ramesh, Strecker, & McCarley, 2001). In addition, Fadel and colleagues (2005) showed increased cortical ACh efflux after 0.1 μM infusions. Due to the trend found during the third block following the 500 ms signal duration after the 0.6 μg infusion, increasing the time between the initial infusion and onset of behavioral training might uncover significant attentional deficits. However, it is not know if the trend observed is due simply to time after the initial drug infusion, if orexin's actions within the basal forebrain interact with time-on-task, or simply due to the increasing number of trials. It is also possible that the changes in signal detection seen in Experiment 1 are due to SB-334867's action in a brain area other than the basal forebrain or on a different neurotransmitter system, such as glutamate or GABA.

The PFC is important for attention processing, and receives cholinergic projections from the basal forebrain (Fadel et al., 2005). Intrabasalis infusions of orexin A have been shown to increase ACh efflux within the PFC. While no significant dose-related results were found in Experiment 2, a microdialysis study might be useful in monitoring the effect of the orexin-1 antagonist on cholinergic levels in the PFC during the task. Previous microdialysis research (Fadel et al, 2005) found that infusions of orexin A increased ACh efflux within the cortex,

suggesting that a similar pattern would be found during task performance on an ACh-dependent task. An orexin-1 receptor antagonist such as SB-334867 would block this efflux of ACh, perhaps keeping ACh at lower levels (perhaps similar to those seen during non-attenionally demanding tasks by Burk & Sarter, 2001).

The statistical power of the present experiments is also limited due to the small number of subjects used in both cases. In Experiment 1, a larger number of animals may have revealed a significant increase in signal detection following a 25 ms signal at the highest dose of SB-334867. In Experiment 2, there was quite a bit of variability among subjects, specifically with the number of omissions at the higher doses of SB-334867. The standard error for overall omissions following the vehicle dose was 3.69 (calculated as $SD/\sqrt{(10)}$, where SD is the standard deviation of the sample). The standard error for overall omissions following the 0.6 μG/side infusion was 13.77. There was also a high level of variability for hits across blocks in Experiment 2 as compared to the hits in Experiment 1. This variability could be a result of SB-334867 administration (though not found to be significant as a function of dose); it could also be obscuring any effects the drug might have had. A larger number of animals could result in more clear cut patterns within the data.

In summary, systemic injections of orexin-1 receptor antagonist SB-334867 did decrease performance on an attention task in the hypothesized manner. Detection of the 500 ms signal was lower following the highest dose (5.0 mg/kg) of SB-334867 compared to vehicle administration. In addition, signal detection of the shortest signal duration (25 ms) was significantly improved at the

1.0 mg/kg dose. No significant dose-dependent results were found following intrabasalis infusions of SB-334867. Thus, it is likely that orexin's interaction with another neural system, such as the dopaminergic pathway in the PFC, contributed to the current results. The present data offer a starting point into understanding the relationship between orexins and the cholinergic system during an attention task.

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Figure Caption

Figure 1. Correct detection of the 500 ms signal was decreased following the highest dose (5.0 mg/kg SB-334867) as compared to vehicle administration.

Figure 2. Correct detection of the 25 ms signal was increased following the 1.0 mg/kg dose of SB-334867 as compared to vehicle administration.

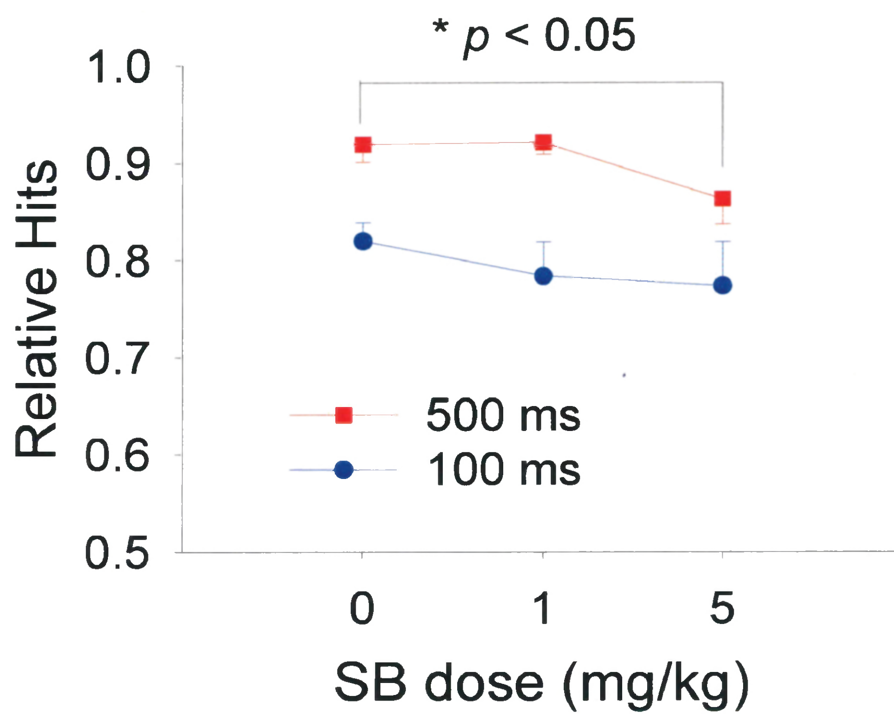
Figure 3. The percentage of correct rejections was not significantly affected by administration of SB.334867.

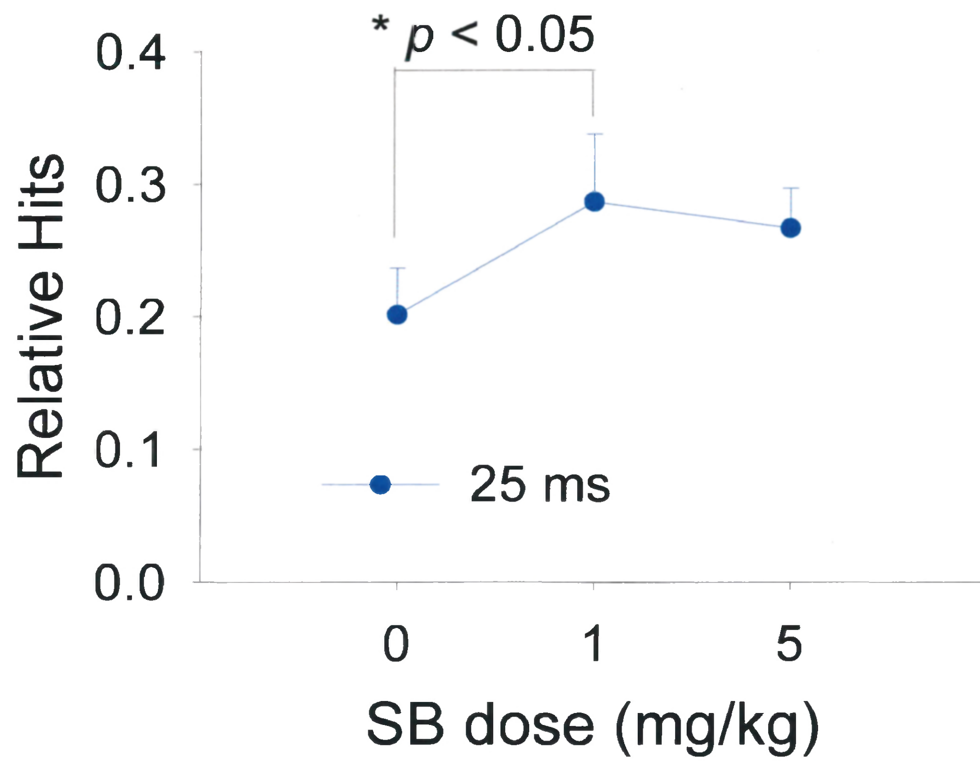
Figure 4. Overall, correction detection of signals was not affected by dose.

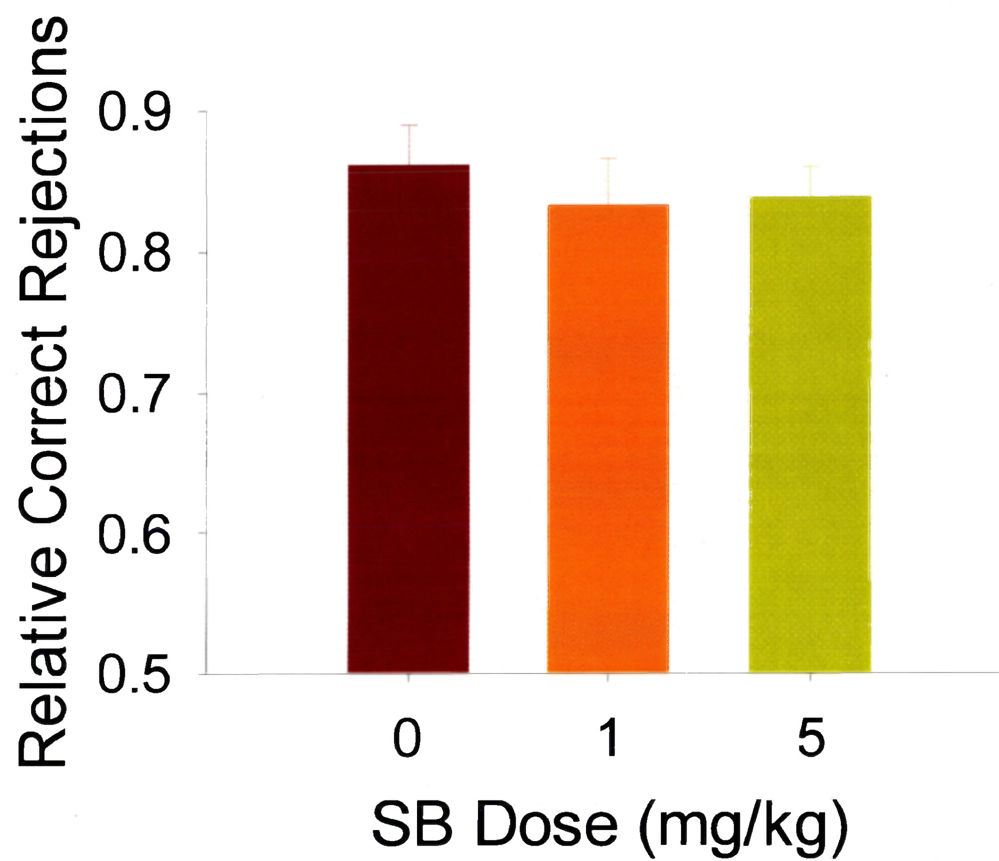
Figure 5. A decrease in signal detection at the 500 ms signal duration was present following the 0.6 μg/μl infusion of SB-334867. This effect was not significant ($p > 0.05$).

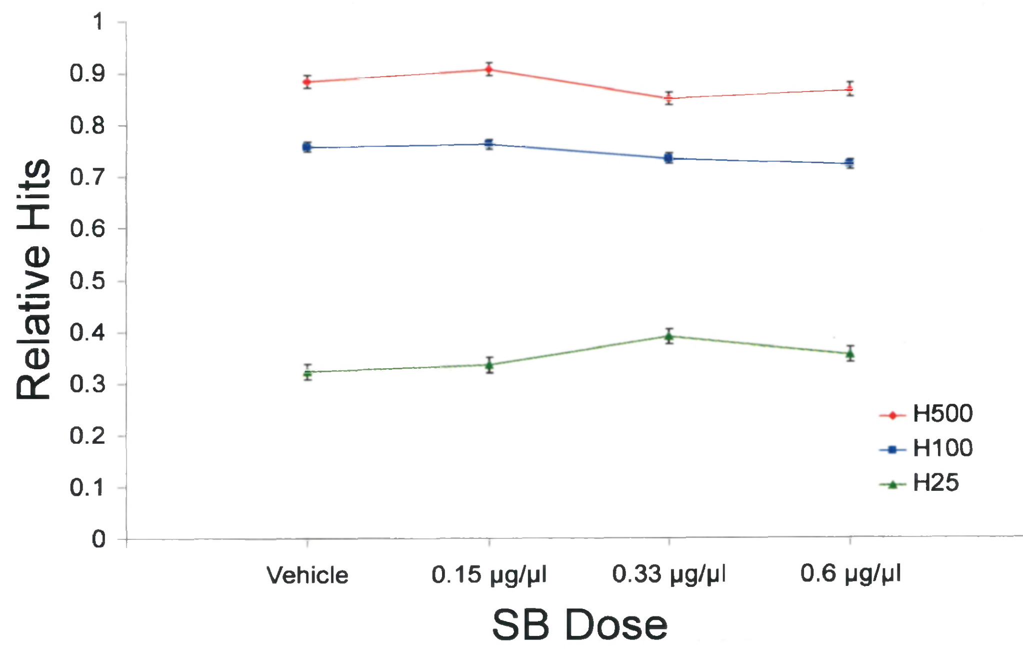
Figure 6. The percentage of correct rejections was increased during the second block of trials as compared to the first and second block of trials. This effect was not affected by SB-334867 dose. * Indicates $p < 0.01$.

Figure 7. The overall percentage of correct rejections was not significantly affected by intrabasalis infusions of SB-334867.

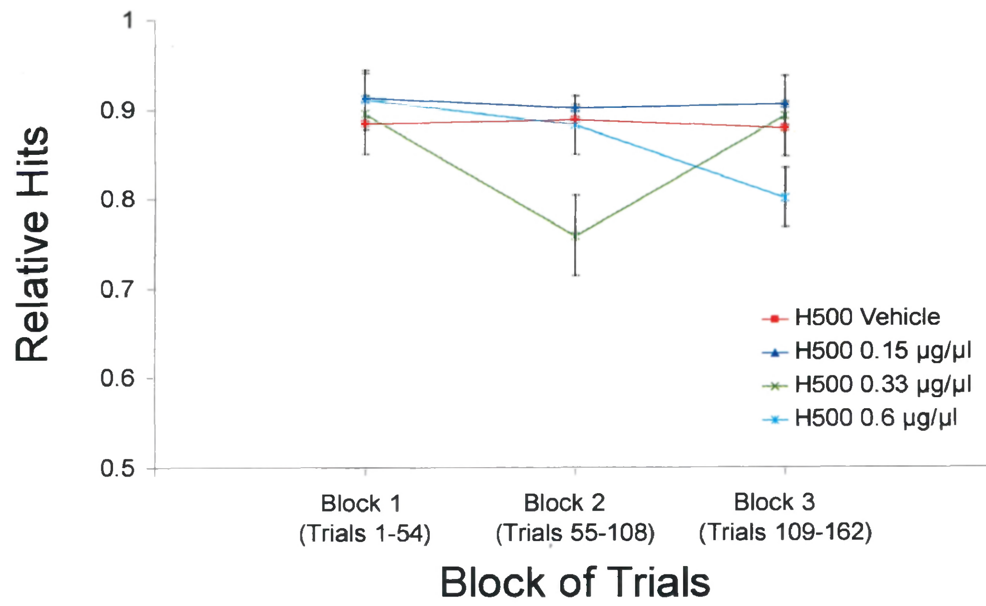

* p < 0.05
1.0
0.9
0.8
0.7
0.6
0.5
Relative Hits
500 ms
100 ms
0
1
5
SB dose (mg/kg)

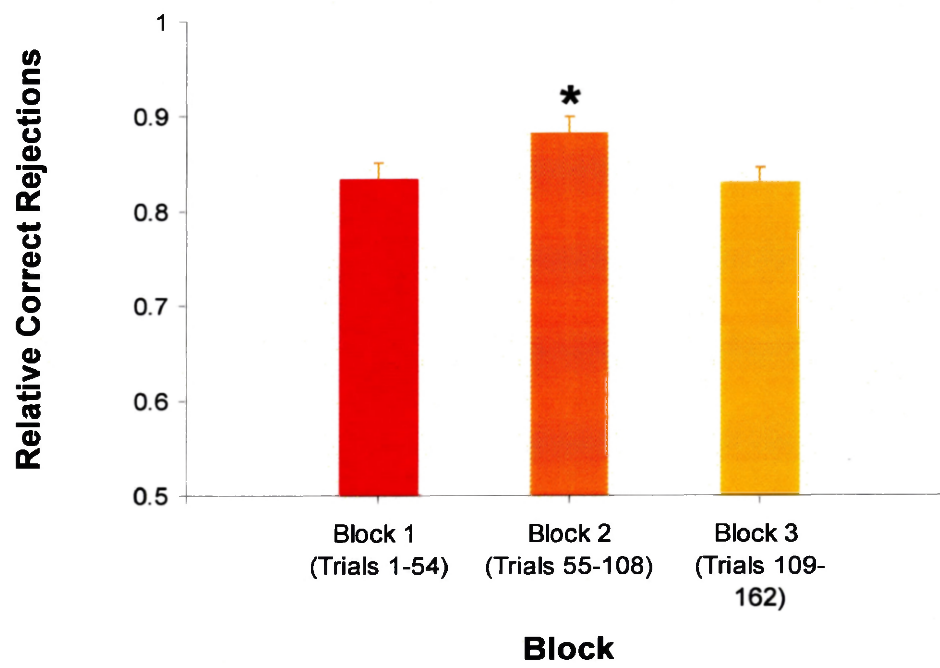

* p < 0.05
Relative Hits
0.4
0.3
0.2
0.1
0.0
25 ms
0
1
5
SB dose (mg/kg)


Relative Correct Rejections
0.9
0.8
0.7
0.6
0.5
0
1
5
SB Dose (mg/kg)

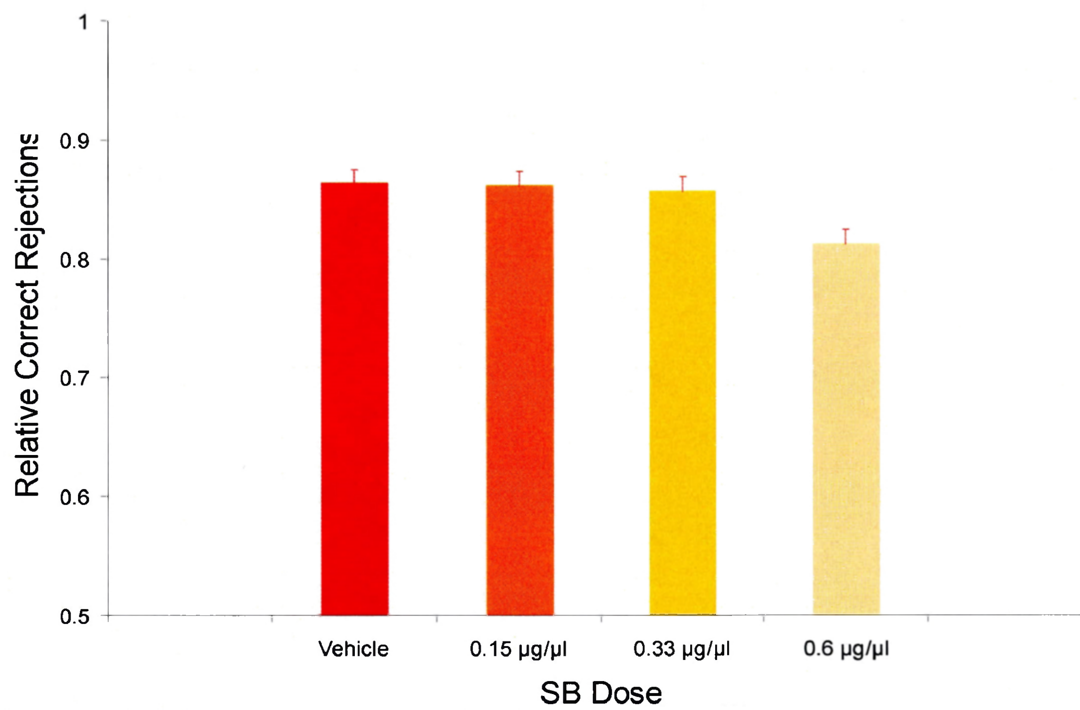


1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Relative Hits
H500
H100
H25
Vehicle
0.15 μg/μl
0.33 μg/μl
0.6 μg/μl
SB Dose


1
0.9
0.8
0.7
0.6
0.5
Relative Hits
H500 Vehicle
H500 0.15 µg/µl
H500 0.33 µg/µl
H500 0.6 µg/µl
Block 1
(Trials 1-54)
Block 2
(Trials 55-108)
Block 3
(Trials 109-162)
Block of Trials



Relative Correct Rejections
1
0.9
0.8
0.7
0.6
0.5
*
Block 1
(Trials 1-54)
Block 2
(Trials 55-108)
Block 3
(Trials 109-
162)
Block



1
0.9
0.8
0.7
0.6
0.5
Relative Correct Rejections
Vehicle
0.15 µg/µl
0.33 µg/µl
0.6 µg/µl
SB Dose

Karen Elizabeth Boschen

Karen Boschen was born in New Orleans, LA on June 26, 1984. She graduated from Illinois Wesleyan University in April, 2006 with a degree in Psychology. In August, 2006, she entered the College of William and Mary to pursue a Master of Arts degree in Experimental Psychology. She defended her thesis in August, 2008. She is currently working as a Research Specialist in the Pharmacology and Toxicology Department at Virginia Commonwealth University.