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Effects of Medial Prefrontal Cortical Administration of the Orexin-2 Receptor Antagonist, TCS-OX2-29, on Attentional Performance in Rats

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A thesis submitted in partial fulfillment of the requirement for the degree of Bachelor of Science in Neuroscience from The College of William and Mary

by

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A Thesis presented to the Graduate Faculty of The College of William and Mary in Candidacy for Departmental Honors for the Degree of Bachelor of Science

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Abstract

Orexins are excitatory neuropeptides that come in two isoforms, Orexin A and Orexin B, and serve as ligands for the G-protein coupled orexin 1 and orexin 2 receptors (Ox1R and Ox2R, respectively). Changes in orexinergic transmission are thought to contribute to attentional processing. While several studies have examined the role of Ox1Rs in attention, less research has assessed the contribution of Ox2Rs. Moreover, several lines of evidence suggest that the right medial prefrontal cortex is particularly critical for visual attentional performance. Taking all of this into consideration, the goal of the present experiment was to test the effects Ox2R blockade, via administration of TCS-OX2-29, in the left or right medial prefrontal cortex on visual attention. The results suggest that low dose administration of TCS-OX2-29 into the right, but not into the left, medial prefrontal cortex enhanced attentional performance. We speculate that relatively mild antagonism of Ox2Rs may have increased the sensitivity of these receptors to subsequent orexin transmission, thereby enhancing attentional performance. Ongoing projects in our laboratory are assessing whether these effects are observed when TCS-OX2-29 is infused into other brain regions known to be critical for attentional performance.

Introduction

The orexin/hypocretin family of neuropeptides, discovered in the late 1990s, impacts a variety of physiological phenomena (de Lecea et al., 1998, 2002; Sakurai et al., 1998). The cell bodies orexin/hypocretin neurons project are relatively confined to the lateral hypothalamus (LH) and contiguous perifornical area (CPA)(Peyron et al, 1998). These neurons project to the cortex, thalamus, brainstem, and the spinal cord (Peyron et al, 1998; Broberger et al., 1998; Elias et al., 1998; Nambu et al., 1999; van den Pol, 1999; Cluderay et al., 2002). The neuropeptides, Orexin A (OxA) and Orexin B (OxB), are produced by orexin neurons, which are translated from a 131-residue mRNA sequence known as preproorexin. Preproorexin mRNA is transcribed from the preproorexin gene, which consists of two exons and one intron distributed over 1432 base pairs (Heifetz et al., 2013). A 3.2-kb promoter region has been utilized with the preproorexin cDNA sequence to direct orexin expression in *Escherichia coli*. Orexin A has an identical sequence in humans and rodents, while Orexin B has two amino acid substitutions between species (Sakurai et al., 1999; Heifetz et al., 2013).

Orexins act on two G protein-coupled receptors: the orexin/hypocretin 1 receptor (Ox1R/HcrtR1) and the orexin/hypocretin 2 receptor (Ox2R/HcrtR2). These receptors bind orexin A/hypocretin 1 (OxA/HCRT-1) and orexin B/hypocretin 2 (OxB/HCRT-2) with relatively varied affinities depending on the receptor. The Ox1R is favorably selective for OxA, a 33-residue peptide with two intra-molecular disulfide bridges. Orexin B, a 28-residue peptide, has a 10 to 100-fold higher relative potency for the Ox2R binding compared to the Ox1R (Hirose et

al., 2003; Ammoun et al., 2003; Sakurai et al., 1998). Orexin B application has been shown to induce depolarization in orexin neurons of Ox1R knock-out mice at comparable levels to wild-type mice, but failed to depolarize orexin neurons in the Ox2R knock-out mice, providing further evidence that Ox2R is a primary receptor for orexin B neuropeptides (Yamanaka et al. 2010). The differences in binding may be due to a notable feature of the Ox1R structure not observed in Ox2R, an N-terminal extension before the first transmembrane domain that contains a linker and a two-turn α-helix (Yin et al., 2016). Specifically, Ox2R binding is believed to occur in a way that the residue L11 forms a nonpolar interaction with F346, L14 interacts with W214, and H350 forms a hydrophobic interaction with L15 of OxB. In this model, D211 is in the proximity of L14 and could potentially form a nonclassical hydrogen bond, and a potential salt bridge may form between R15 and D203. Unlike in the interactions with Ox1R, Y223 does not interact with N20 (Heifetz et al., 2013).

Neuropsychopharmacology of Orexins

Orexin/hypocretins are thought to regulate attention and arousal through interactions with a variety of ascending neuromodulatory systems (Fadel and Deutch, 2002). Specifically, the lateral hypothalamic region is crucial for the coordination of behavioral responses to interoceptive cues, and lateral hypothalamus projections to the midbrain may be critical for a number of behavioral states, ranging from appetite to arousal (Fadel and Deutch, 2002). It has been shown that more complex behaviors require the integration of interoceptive cues with activation of the forebrain via the reticular core (Fadel and Deutch, 2002).

Other studies have suggested the lateral hypothalamus is a conduit for information from corticolimbic sites such as the prefrontal cortex, nucleus accumbens, amygdala and brainstem, thus, lateral hypothalamus orexin projections into the ventral tegmental area likely play a key role in attention (Fadel and Deutch, 2002; Borgland et al., 2006). The wide innervation of hypothalamic orexin neurons suggest that these orexin neurons are "a locus wherein humoral and interoceptive cues gain access to and influence over mesocorticolimbic dopamine neurons", whose terminal fields are most dense in orexin innervation at the prefrontal cortex (Fadel and Deutch, 2002). Along with this well-established innervation, orexin fibers and receptors are located within a variety of brainstem and basal forebrain regions, including the locus coeruleus, media septal area, medial preoptic area, and substantain innominat (Espana et al., 2005; Peyron et al., 1998; Sakurai et al., 1998; Date et al., 1999; Nambu et al., 1999; Taheri et al., 1999; Bourgin et al., 2000; Marcus et al., 2001; Hervieu et al., 2001; Cluderay et al., 2002). The large variety of innervations suggest implications of orexin neurons' role for a wide range of processes including appetite, arousal, sleep/wakefulness, circadian rhythm, and, especially, attention.

Attentional processing: importance of the medial prefrontal cortex

The prefrontal cortex (PFC) is essential for a variety of functions in the brain including attention, motor control, spatial orientation, short-term memory, temporal and source memory, metamemory, associative learning, creativity, perseveration and reasoning (Stuss and Benson, 1984; Fuster, 1988; Wise et al.,

1996; Roberts, et al. 1998; Engle and Kane, 2002). Findings from several studies highlight the importance of the prefrontal cortex in blocking the effects of distractors, and a strong positive correlation between working memory and the capacity for executive attention (Engle et al. 1999; Engle, 2001; Engle, 2002).

Deficits in attention may be produced by right prefrontal cortex abnormalities, which prevent inhibitory control over the anterior cingulate (Rothbart et al., 2011). The prefrontal cortex is also known to be the "first to malfunction in normal aging" and the cognitive abilities it supports decline at a greater degree than those supported by other cortical and noncortical structures (Fuster, 1988). When encountering novel information, reduced prefrontal cortex performance can lead to the inability to integrate temporal and contextual information into discrete memory traces (West, 1996). The ability to remain focused for an extended period of time in a resource-demanding task is vulnerable to the effects of increasing age, especially in the right-prefrontal region (Rothbart et al., 2011). The medial PFC (mPFC) tends to be more active during expectant spatial attention tasks, when internally generated expectations rather than ambient extra personal targets must regulate the distribution of attention. Studies of patients with mPFC damage suggest that the mPFC mediates the use of environmental cues to prepare for action and may interfere with the ability to benefit from spatial cues in target detection tasks as well as tasks that demand attentional shifting (Small et al., 2002). It is apparent that the prefrontal cortex is crucial for attention and a loss of neurons that project into the prefrontal cortex to provide support for its various cognitive functions is detrimental to attentional capabilities.

Orexin systems affect on attention

Orexins play a distinct role in sleep and wakefulness and are necessary for arousal and attention, because of their importance within the cholinergic system, which receives significant orexin innervation (Marcus et al., 2001). Orexin neuron's activity varies with the degree of arousal and is linked to heightened attentional states (Villano et al., 2017). Studies suggest that these orexin neurons form a positive feedback circuit through direct pathways, resulting in the preservation of the orexin neuron networks at high activity levels and for longer periods (Yamanaka et al. 2010). Application of OxB to orexin producing neurons significantly depolarized membrane potential, increased firing frequency, and induced depolarization even in the presence of tetrodotoxin, suggesting that orexin directly activates orexin neurons. This positive feedback circuit activation of orexin neurons through Ox2R suggests an important role in the maintenance of arousal and arousal systems. Most often, orexin neurons exposed to orexin show burst-type firing, in either clusters or repetitive bursts. Orexin neurons may also be indirectly activated through glutamatergic neurons within the lateral hypothalamus (Yamanaka et al. 2010).

Narcolepsy and orexin systems

Narcolepsy is a sleep-wake disorder characterized by excessive daytime sleepiness, cataplexy, REM sleep dysregulation, sleep paralysis and hypnagogic hallucinations (Kilduff 2001; Overeem et al., 2001; Rechtschaffen et al., 1963). Narcolepsy is believed to be caused by a general loss of orexins, as orexin knockout

mice have show behavior reminiscent of narcoleptic attacks. Narcoleptic phenotypes have been created in rats, dogs, and humans through dual oral OX1/Ox2 antagonist administration, and OxR2 mutations in dogs result in narcolepsy (de Lecea et al, 2002; Kilduff 2001). It has been demonstrated that human narcoleptics also have at least 85-95% reduction in the number of orexin neuron innervations to the cholinergic brainstem (Thannickal et al., 2000). Orexin-releasing neurons also innervate the locus coeruleus (Cluderay et al., 2002; Brisbare-Roch et al., 2007; Dugovic et al., 2009; de Lecea et al., 2002; Fadel and Fredrick-Duus., 2008). Lateral hypothalamic orexin-2 projections increase spike frequency to and are crucial for appropriate function of the locus coeruleus. Therefore, a loss of orexin 2 projections may result in dysregulation of the locus coeruleus. Densely innervated orexin projections distribute to regions of the brain including to the locus coeruleus, basal forebrain, and other arousal related structures. Without the presence of OxR2 systems, almost all behaviors related to sleep-wakefulness malfunction across multiple species. Loss of proper sleep-wakefulness regulation leads to cognitive decline and difficulties in attention tasks because compensatory processes and task-specific processing engage the same neuronal systems. Narcoleptics have deficits on most attentional tasks that assess attention and other tasks related to executive functions (Naumann et al. 2006). Despite the known role about Ox2Rs in subcortical regions, such as the locus coeruleus, much less is known about the role of these receptors in the mPFC.

Orexins and Alzheimer's Disease

Alzheimer's disease (AD), characterized by cognitive decline, also includes irregular sleep/wake patterns as major feature of the disease. Irregular sleep/wakefulness cycles tend to correlate with severity of dementia being suffered by the patient (Witting et al., 1990; Mirmiran et al., 1992). Along with disturbances in sleep, Alzheimer's patients also suffer from narcolepsy-like symptoms such as excessive napping in the daytime, daytime sleepiness, rapid eye movement dysregulation and circadian rhythm disturbances (Fronczek et al., 2012). Many of these symptoms may be due to failure of the locus coeruleus. The coexistence of narcolepsy, caused by a loss of orexins, and Alzheimer's disease progression is interrelated, shown by up to a 40% loss of orexin producing neurons and significantly lower levels of orexins in the cerebrospinal fluid of patients with AD (Scammell et al. 2012; Baldo et al., 2003; Horvath et al., 1999; Fronczek et al., 2012). Alzheimer's disease, sleep and circadian rhythm physiology display intricate relationships, where AD pathology may lead to sleep/circadian rhythm disturbances while sleep and circadian regulating systems may exert and influence on AD pathology. There is also significant evidence that orexins modulate AD pathophysiology and may influence sleep and circadian rhythm deterioration (Slats et al. 2013).

Current Study

Numerous neuropsychiatric disorders, such as Alzheimer's disease, schizophrenia and drug addiction are associated with disruptions of cholinergic

system function (Brousseau et al., 2007; Field and Cox, 2008; Sarter et al. 2005). In addition to the monoaminergic system, orexins also activate cholinergic neurons through both Ox1R and Ox2R (Fadel and Frederick-Duus, 2008). Taking the previous sections together, it can be hypothesized that orexin 2 projections are needed for proper function of the PFC and attentional tasks, as they are densely innervated at this region and related to arousal and appropriate cholinergic system function of the cortex. The study of this experiment is to determine if blocking of the orexin 2 receptors in the mPFC, which is crucial for visual attention, in either the right or the left hemisphere will result in a loss of attentional performance. The effects of administering the Ox2R antagonist, TCS-OX2-29, in the left or right mPFC on visual attention were tested in the present experiment. It was hypothesized that at low dose administration of TCS-OX2-29 into the right, but not into the left, medial prefrontal cortex attentional performance would be enhanced. Furthermore, at higher doses, the ORx2 antagonist would reduce attentional performance, suggesting a cognitive loss analogous to the deficits that narcolepsy patients experience, and provide strong implications that as AD progresses, resulting in a narcoleptic phenotype due to a lack of OxR2, attentional performance degradation also occurs due, in part, to a loss of orexin innervation to a variety of brain regions.

Methods

Subjects

Male FBNFI hybrid rats, 150-175 g at the beginning of the experiment were used (National Institute of Aging colony). The rats were individually housed in a

vivarium which was temperature and humidity controlled and operated on a 14:10 hour light/dark cycle (lights on 0600). All rats were water restricted throughout the experiment, receiving water during behavioral testing and for 10-15 minutes after each testing session.

Apparatus

Rats were trained five to seven days a week, and received at least twenty minutes of water access on days when no behavioral testing occurred. Rats were trained in one of 16 chambers, each located within a sound-attenuating box (Med Associates, Inc.). Each chamber contained a water port positioned with a dipper that could be raised to provide water access (0.01 ml tap water). Two retractable levers were located on either side of the water port. A panel light was located above each lever and above the water port. A house light was positioned on the opposite side of each chamber. Illumination levels of these chambers have been described (Burk, 2004). Behavioral testing programs and data collection was controlled by a personal computer using the Med-PC version IV software.

Presurgical Attention Task Training

Training occurred between 9:00 a.m. and 2:00 p.m. daily. Food was provided ad libitum throughout the experiment. Animals were treated in accordance with the guidelines of the Animal Care and Use Committee at the College of William and Mary. In the initial shaping procedure, rats were trained to press an extended lever using an FR-1 schedule of reinforcement, with the rule, to prevent a side bias, that if one lever was pressed five consecutive times, the other lever had to be pressed to

receive water access. Once animals received 120 rewards in a session for three sessions, they were moved to the next training stage, where there were two trial types, signal (1-s illumination of the central panel light) and nonsignal (no illumination of the central panel light) trials. On seconds following a signal or no signal, the rats were cued to respond by extension of the levers into the chamber. Rules for training were counterbalanced, such that half of the rats were reinforced (3-s access to 0.01ml tap water) for pressing the left lever following a signal, which was recorded as a hit. A miss was recorded for right lever presses after a signal. For nonsignal trials, a right lever press was considered a correct rejection and water access was provided, while a press of the left lever on these trials was recorded as a false alarm. The rules of the task were reversed for the other half of the rats such that a right lever press was considered a hit following a signal presentation whereas the left lever was considered a correct response on a nonsignal trial. The inter-trial interval (ITI) was 12-s and the houselight was illuminated throughout the session during this stage of training. An incorrect response during this training phase would be followed by a correction trial, which was the same trial type as that in which the error occurred. If the rat responded incorrectly for three consecutive trials, a forced trial occurred in which only the correct lever was extended into the chamber for 90-s or until the rat responded. If the errors occurred on signal trials, the central panel light was illuminated while the lever was extended. Each session lasted for 45 min and rats were trained with this task until reaching a criterion of >70% accuracy on signal and nonsignal trials for three consecutive sessions. After reaching criterion, rats were moved to the final stage of training prior to surgery. In the final task, the

signal durations (500, 100, 25 ms) and ITI (9 ± 3 s) were shorter and varied in order to increase explicit attentional demands (Parasuraman et al., 1987; Koelega et al. 1990). Manipulating the signal duration and causing signal variability requires a greater cumulative demand of subject's attentional capacity and vigilance when performing the task (Parasuraman et al., 1987). Each training session was comprised of 162 total trials (81 signal, 81 non-signal). For the signal trials, each of the three signal durations was presented for 27 trials within a session. Trials were presented in blocks of 18 (9 non-signal, 9 signal, with 3 of each signal duration) and trial types were selected randomly without replacement. Rats were considered trained for surgery when a criterion of >70% accuracy on trials when the 500-ms signal was presented and on nonsignal trials for three consecutive sessions.

Surgical Procedures

On the night prior to surgery, rats were provided with 2.7 mg/ml acetaminophen in their drinking water. Rats were anesthetized via intraperitoneal (IP) injections of 90.0 mg/kg ketamine combined with 9.0 mg/kg xylazine. Once the rats were sufficiently anesthetized, the surgical area was shaved and rats were positioned in a stereotaxic device with the incisor bar set at 3.3 mm below the interaural line. All surgical procedures were conducted under aseptic conditions.

A group of five rats received unilateral implantation of guide cannulae into the right medial prefrontal cortex. For these subjects, 8-mm guide cannulae (22 gauge) were implanted at +3.0mm anterior-posterior (AP) and +0.7mm medial-lateral (ML) from bregma and -2.8mm from dura.

In addition to the first five, five different rats received unilateral implantation of 8-mm guide cannulae into the left medial prefrontal cortex at +3.0mm anterior-posterior (AP) and -0.7 mm medial-lateral (ML) from bregma and -2.8mm from dura.

Three stainless steel screws and dental cement were used to secure the cannulae; bone wax was applied above the skull holes to prevent the dental cement from entering these holes. Dummy cannulae were inserted to prevent blockage within the guide cannulae. Following surgery, animals were given a one-week recovery period in which food and water were available ad libitum. Rats were then returned to water restriction and began to retrain on the attention task. Rats were retrained in the same attention task as before surgery.

Postsurgical behavioral testing procedures prior to TCS-Ox2-29 administration

After re-establishing criterion performance rats were then exposed to a form of the attention task where the houselight was flashed (1-s on/1-s off) during the middle block of trials within a testing session (trials 55-108).

Drug Administration Procedures

Infusions were made through the insertion of an internal cannula (28 guage), which was attached to a Hamilton syringe by polyethylene tubing. A total volume of 1 µl solution was infused through the cannula at a rate of 0.5 µl/min. The internal cannula was left in place for one minute following the completion of each infusion to allow for drug diffusion. Animals were then immediately loaded into the chambers to begin behavioral testing. At least one day of training was allowed between each

testing session to re-establish baseline performance. TCS-0x2-29 (Tocris, Inc.) was dissolved in dimethyl sulfoxide (DMSO) and alliquotted into small vials that were stored at -20º until being used for an infusion. Each vial was used only once on an infusion day and thus, TCS-0x2-29 was not repeatedly thawed and frozen. Rats with both right and left cannula received 0nM (DMSO only), 1nM, 10nM, and 20nm of TCS-0x2-29 via the internal cannulae that extended 1.0 mm beyond the guide cannulae, with each dose being administered one time prior to task performance in an order that was randomized for each rat (Haghparast, et al. 2013). On drug infusion sessions, rats were tested in a version of the attention task with the flashing houselight distracter presented during the second block of trials (trials 55-108) within the session.

Histological procedures and analysis

After being deeply anesthetized via an IP injection of 100.0 mg/kg ketamine and 10.0 mg/kg xylazine, rats were transcardially perfused with 10% sucrose followed by 4% paraformaldehyde at a pressure of 300mmHg using a Perfusion One apparatus. The brains were then removed and placed in the same fixative for 48 hours before being put into a 30% sucrose solution in phosphate buffered saline for at least three days. The tissue was then sectioned in 50 µM slices using a freezing microtome. Sections near the medial prefrontal cortex will be stained with cresyl violet in order to confirm cannula location.

Behavioral Measures and Statistical Analyses

The number of hits (H), misses (M), correct rejections (CR), false alarms (FA) and omissions were recorded for each animal during each testing session. To assess accuracy separately on signal and nonsignal trials, the percentage of hits and correct rejections were calculated. During TCS-OX2-29 (or vehicle) infusion sessions, the baseline version of the task, with the houselight consistently illuminated, occurred for the first 54 trials and then, for the next 54 trials, the houselight was flashed as a distracter. To determine the effect of the distracter for each rat, for each measure the difference between block 1 (standard task with no distracter) and block 2 (distracter presentation) was calculated as a distracter score. Positive values indicate greater accuracy during block 1 and that the distracter decreased performance. Omissions were analyzed separately from measures of accuracy. Data were analyzed with mixed factor ANOVAs, which included factors dose, lesion and signal duration (where appropriate). Data were analyzed with SPSS 19.0 for Windows (SPSS, Chicago, IL, USA). A level of $\alpha=0.05$ was used as the criterion for statistical significance.

Results

Overall ANOVA. ANOVAs including hemisphere, dose, block and (for hits) signal duration, were conducted. There were no significant effects for correct rejections. There was a dose x block x hemisphere interaction significant at $F(6,48) = 3.502$, $p=0.006$ for hits. This three-way interaction was further assessed by conducting separate dose X hemisphere ANOVAs at each block.

Standard task (Block 1). TCS-Ox2-29 did not affect performance on the standard task without the distracter task (Block 1). A dose (0, 1, 10, and 20nM TCS-Ox2-29) x hemisphere ANOVA for hits yielded no significant effects of drug treatment during block 1.

Distracter task (Block 2). TCS-Ox2-29 did not affect performance during the second block of trials when a visual distracter was presented. A dose (0, 1, 10, and 20nM TCS-Ox2-29) x hemisphere duration ANOVA for hits yielded no significant effects of drug treatment during block 2.

Recovery from distracter task (Block 3). In block 3, a dose x hemisphere ANOVA showed there was a significant dose x hemisphere interaction for hits $F(3,24) = 3.898$ ($p = 0.021$). A t-test confirmed that the 1 nM dose administration improved the performance of rats with their right hemispheres cannulated when compared to the left hemisphere $t(8) = 3.290$, $p = .011$.

Discussion

The present experiments tested whether TCS-Ox2-29 infusion could inhibit attentional performance via direct infusions into the medial prefrontal cortex, particularly in the right hemisphere. The present results extend previous findings by showing that the right medial prefrontal cortex plays a crucial role in attention-demanding aspects of task performance. Right hemisphere cannulated subjects, who had some damage to the right medial prefrontal cortex due to cannula

implantation, tended to perform worse than subjects with left hemisphere cannulae (Fig. 1, Fig. 4). Surprisingly, improvement of attentional performance was seen in hits during the recovery block after low dose administration of TCS-Ox2-29 into the right medial prefrontal cortex (Fig. 2). Selectivity of hit improvement may show that the drug is helping in recovery from the distracter, which suggests that it is not motivation or motor effects that are leading to improvement (Fig. 5). The effects of Ox2R blockade on attentional performance tended to follow an inverted "U" shaped curve (Fig. 4). Specifically, low doses of TCS-Ox2-29 enhanced performance when attentional demands were augmented by a visual distracter, while high doses diminished performance. The benefits of low dose TCS-Ox2-29 were observed during the final block of trials, when subjects are recovering from the effects of the distracter.

The mPFC, specifically the right hemisphere, appears to be one brain structure that mediates attentional performance, thus, the right mPFC was chosen as a target of this attentional performance study (Small et al 2002). The mPFC contains a significant number of cholinergic projections, which are believed to be mediated by orexin producing neurons. These projections to the mPFC are thought to be important for the proper functionality of the mPFC especially with regard to attention (Small et al., 2002).

Aside from impacts on a particular brain region, orexins are needed for general arousal and attention and play distinct roles in sleep and wakefulness because of their importance within the cholinergic system (Marcus et al., 2001; Villano et al., 2017). Within this system, and others, it is suggested that orexins

directly activate orexin neurons, creating positive feedback circuit activation and suggesting an important role of orexins in the maintenance of arousal (Yamanaka et al. 2010). This arousal system is thought to be maintained through orexin neurons that have specific types of firing or neurons that may be indirectly activated through glutamatergic neurons within the lateral hypothalamus (Yamanaka et al. 2010). The lateral hypothalamic region, which is densely filled with orexin producing neurons, provides innervations to corticolimbic sites (Peyron et al, 1998). Included within these sites is the prefrontal cortex, an area necessary for attention (Cluderay et al., 2002). This suggests that lateral hypothalamus orexin projections area likely to play a key role in attention and events that require attentional processing power.

Narcolepsy, Alzheimer's disease, and drug addiction are diseases related to the malfunction of the orexinergic systems (de Lecea et al, 2002; Kilduff 2001; Witting et al., 1990; Mirmiran et al., 1992). It seems that many of these diseases occur, in part, due to improper function of OxR2 systems. The OxR2 systems assist in modulating behaviors related to sleep-wakefulness in many species. Irregular sleep/wakefulness cycles due to a loss of orexin receptors within the locus coeruleus tend to cause Alzheimer's patients to suffer from narcolepsy-like symptoms (Fronczek et al., 2012). These symptoms of sleep irregularities also correlate with the severity of dementia being suffered by AD patients and suggest sleep and circadian regulating systems controlled by orexins may exert and influence AD pathology. Because orexin 2 projections are critical for appropriate functionality of the PFC and attentional tasks, the blocking or disruption of the

orexin 2 receptors in the mPFC, especially within the right mPFC, is associated with reduced attentional performance (Rothbart et al., 2011).

The present study found that low dose administration of TCS-OX2-29 into the right, but not into the left, medial prefrontal cortex somewhat enhanced attentional performance. Based on prior studies it is speculated that relatively mild antagonism of Ox2Rs may have increased the sensitivity of these receptors to subsequent orexin transmission, thereby improving attentional performance. Other forms of sensitivity are commonly seen in nicotine receptors, particularly during the use of drugs that bind allosterically (Maelicke, et al., 2001). Recent studies using cholinesterase inhibitors, considered allosterically potentiating ligands, sensitize nicotinic receptors by increasing the probability of channel opening induced by acetylcholine and nicotinic agonists, and by slowing down receptor desensitization through directly interacting with nicotinic acetylcholine receptors (Maelicke, et al., 2001). In the present study, the beneficial effects of low dose TCS-Ox2-29 were primarily observed on signal trials during block 3, which potentially support the conclusion that the effects of orexin release by orexin neuron innervation in the medial prefrontal cortex improves attentional performance when exposed to a similar sensitization mechanism. That mechanism may be the result of orexin antagonists sitting on the orthosteric site of the receptor, and, when the antagonists release, generating a greater binding affinity for natural orexin agonists. Further investigation of the neural basis of the beneficial effects of low dose TCS-Ox2-29 is needed.

These results add to the growing literature about the potential beneficial cognitive effects of targeting orexinergic neurotransmission (Piantadosi et al., 2015). One caveat in interpreting these data is that the data set would benefit from a larger sample size; the current size is two groups, left and right hemisphere cannulated subjects, of 5. Also, the histological processing is not complete and, thus, cannula locations have not been verified. Collectively, these findings support the conclusion that orexin 2 receptor systems are a potential target for future research into treatments for disorders characterized by attentional deficits.

Figures

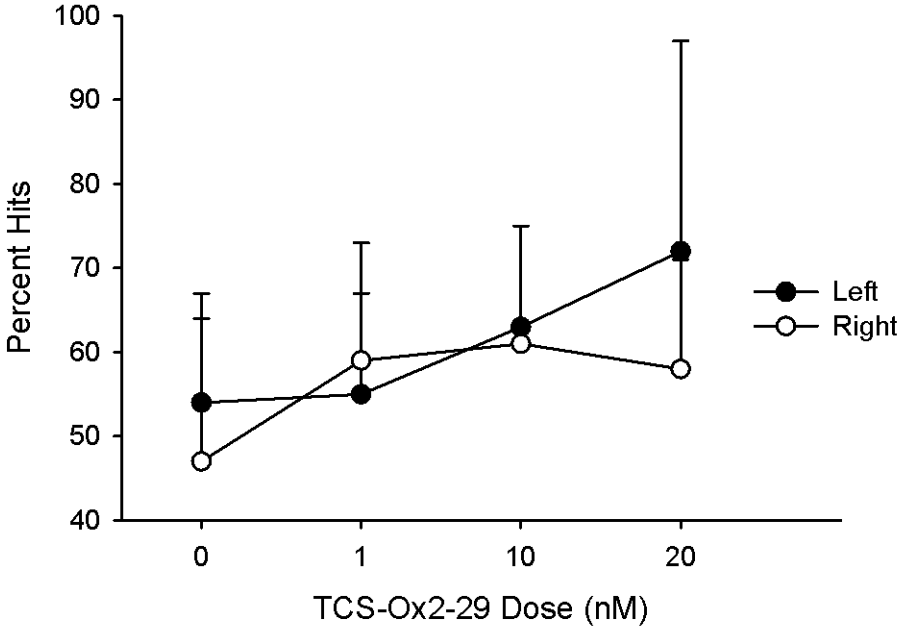

Fig. 1 The figure depicts the differences between left (n=5) and right (n=5) hemisphere cannula implantation groups on percentage hits related to varied dose administration of TCS-Ox2-29.

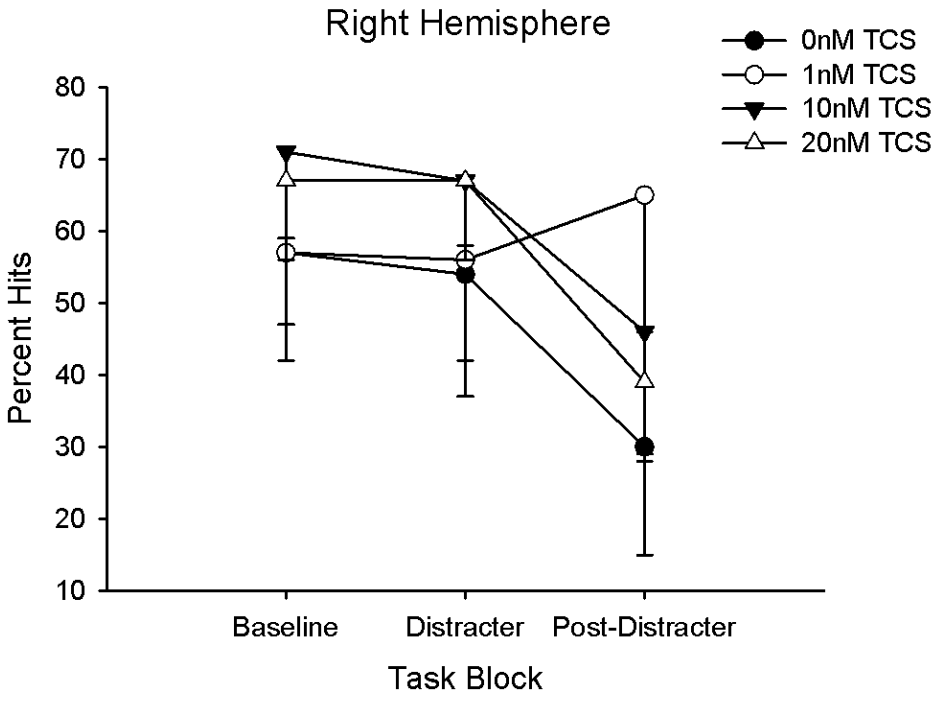

Fig. 2 The figure depicts the differences of dose administration and percentage hits for subjects with right hemisphere cannula implantation during the three blocks of the distracter task (Block 1 – Baseline, Block 2 – Distracter, Block 3 – Post-Distracter/Recovery).

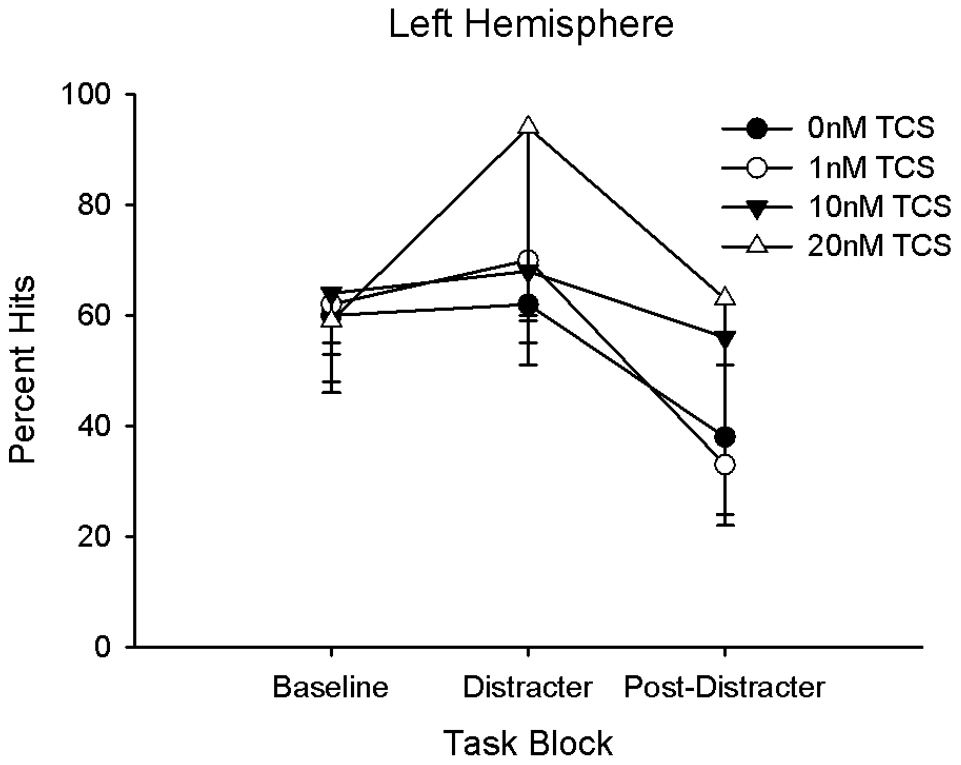

Fig. 3 The figure depicts the differences of dose administration and percentage hits for subjects with left hemisphere cannula implantation during the three blocks of the distracter task (Block 1 – Baseline, Block 2 – Distracter, Block 3 – Post-Distracter/Recovery).

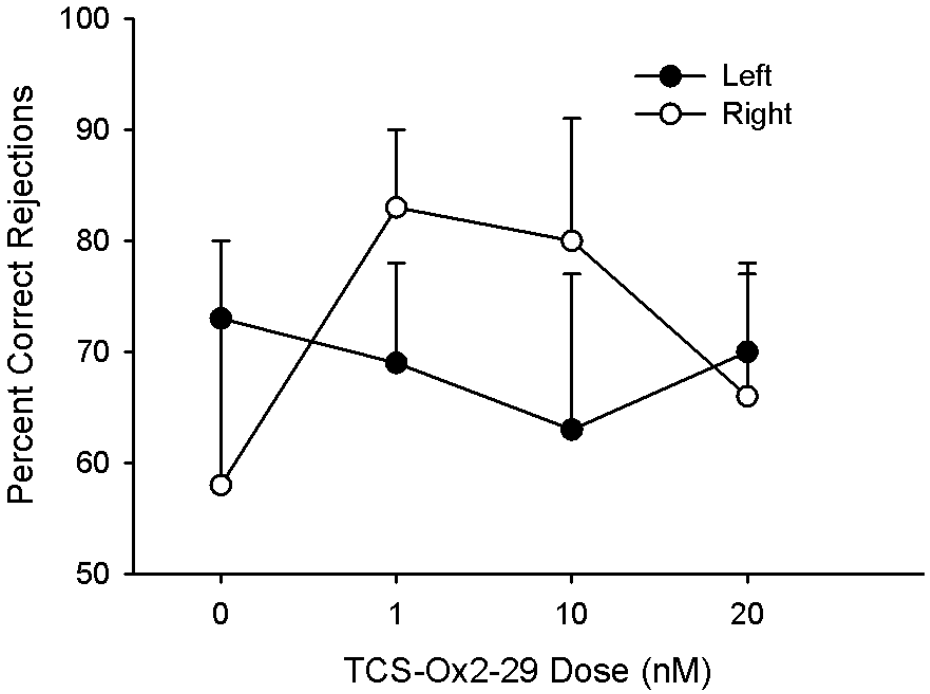

Fig. 4 The figure depicts the differences between left (n=5) and right (n=5) hemisphere cannula implantation groups on correct rejections related to varied dose administration of TCS-Ox2-29.

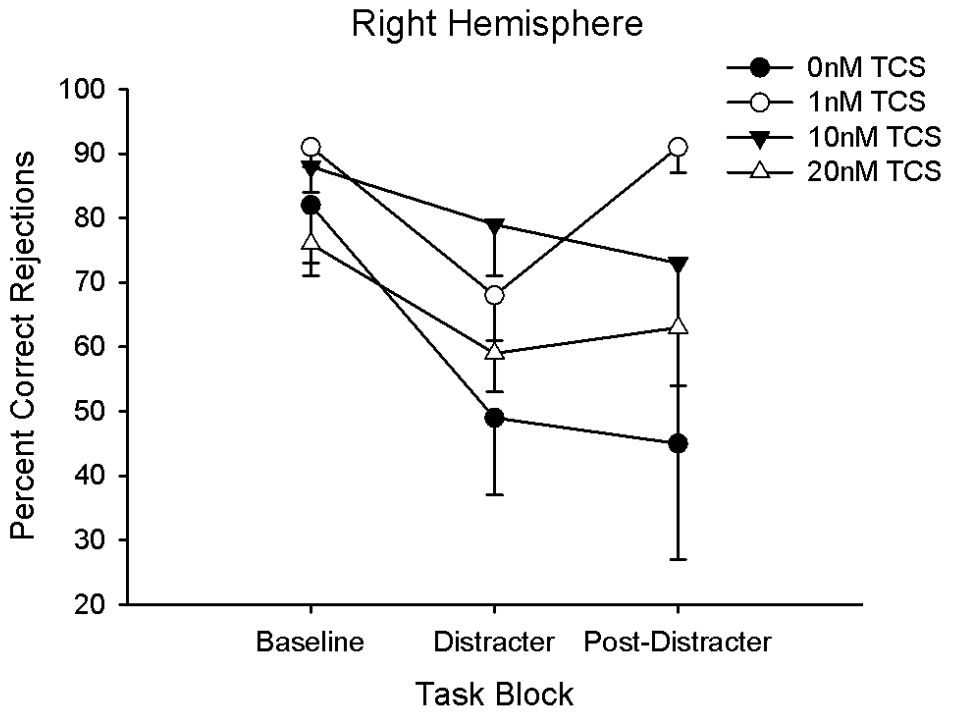

Fig. 5 The figure depicts the differences of dose administration and correct rejections for subjects with right hemisphere cannula implantation during the three blocks of the distracter task (Block 1 – Baseline, Block 2 – Distracter, Block 3 – Post-Distracter/Recovery).

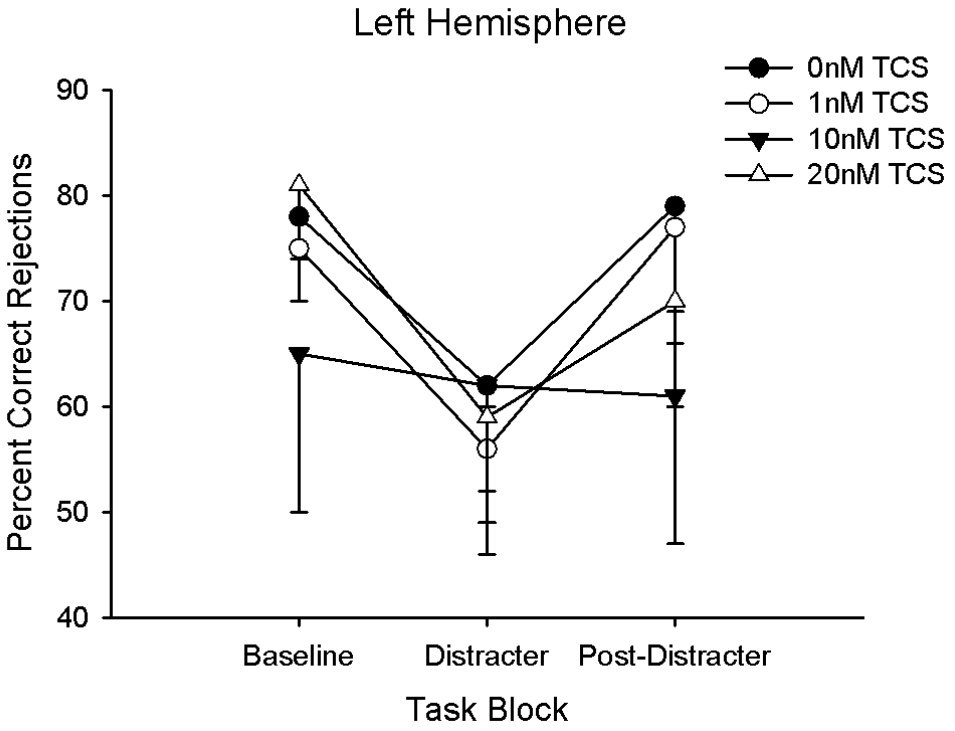

Fig. 6 The figure depicts the differences of dose administration and correct rejections for subjects with left hemisphere cannula implantation during the three blocks of the distracter task (Block 1 – Baseline, Block 2 – Distracter, Block 3 – Post-Distracter/Recovery).

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