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The hypocretin/orexin antagonist almorexant promotes sleep without impairment of performance in rats

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The hypocretin receptor (HcrtR) antagonist almorexant (ALM) has potent hypnotic actions but little is known about neurocognitive performance in the presence of ALM. HcrtR antagonists are hypothesized to induce sleep by disfacilitation of wake-promoting systems whereas GABA_A receptor modulators such as zolpidem (ZOL) induce sleep through general inhibition of neural activity. To test the hypothesis that less functional impairment results from HcrtR antagonist-induced sleep, we evaluated the performance of rats in the Morris Water Maze in the presence of ALM vs. ZOL. Performance in spatial reference memory (SRM) and spatial working memory (SWM) tasks were assessed during the dark period after equipotent sleep-promoting doses (100 mg/kg, po) following undisturbed and sleep deprivation (SD) conditions. ALM-treated rats were indistinguishable from vehicle (VEH)-treated rats for all SRM performance measures (distance traveled, latency to enter, time within, and number of entries into, the target quadrant) after both the undisturbed and 6 h SD conditions. In contrast, rats administered ZOL showed impairments in all parameters measured compared to VEH or ALM in the undisturbed conditions. Following SD, ZOL-treated rats also showed impairments in all measures. ALM-treated rats were similar to VEH-treated rats for all SWM measures (velocity, time to locate the platform and success rate at finding the platform within 60 s) after both the undisturbed and SD conditions. In contrast, ZOL-treated rats showed impairments in velocity and in the time to locate the platform. Importantly, ZOL rats only completed the task 23–50% of the time while ALM and VEH rats completed the task 79–100% of the time. Thus, following equipotent sleep-promoting doses, ZOL impaired rats in both memory tasks while ALM rats performed at levels comparable to VEH rats. These results are consistent with the hypothesis that less impairment results from HcrtR antagonism than from GABA_A-induced inhibition.

Keywords: hypocretins/orexins, cognitive impairment, memory impairment, hypnotics, water maze, spatial reference memory, spatial working memory, EEG

INTRODUCTION

Insomnia is a highly prevalent condition affecting 10–30% of the general population; (NIH, 2005; Roth, 2007; Mai and Buysse, 2008). Sleep loss and sleep disruption can lead to a degradation of neurocognitive performance as assessed by objective and subjective measures (Wesensten et al., 1999; Belenky et al., 2003; Lamond et al., 2007). Prescription sleep medications are often used to treat insomnia and obtain desired amounts of sleep. Presently, nonbenzodiazepine, positive allosteric modulators of the GABA_A receptor such as zolpidem (ZOL) are the most widely prescribed hypnotic medications. Although known to induce sleep, these compounds have been shown to significantly impair psychomotor and memory functions in rodents (Huang et al., 2010; Uslaner et al., 2013; Zanin et al., 2013), non-human primates (Makaron et al., 2013; Soto et al., 2013; Uslaner et al., 2013) and humans (Balkin et al., 1992; Wesensten et al., 1996, 2005; Mattila et al., 1998; Mintzer and Griffiths, 1999; Verster et al., 2002; Storm et al., 2007; Otmani et al., 2008; Gunja, 2013). Such impairment can be particularly troubling when there is an urgent

need for highly functional performance in the presence of drug such as with first responders, military personnel, and caregivers. Further, complex behaviors during the sleep period (e.g., eating, cooking, driving, conversations, sex) have been associated with these medications (Dolder and Nelson, 2008). Therefore, more effective hypnotics are needed that facilitate sleep that is easily reversible in the event of an unexpected awakening that demands unimpaired cognitive and psychomotor performance.

Recently, antagonism of the hypocretin (Hcrt; also called orexin) receptors has been identified as a target mechanism for the next generation of sleep medications (Brisbare-Roch et al., 2007; Dugovic et al., 2009; Whitman et al., 2009; Hoever et al., 2010, 2012a,b; Coleman et al., 2012; Herring et al., 2012; Winrow et al., 2012; Betschart et al., 2013). The Hcrt system is well known to play an important role in the maintenance of wakefulness (de Lecea, 2012; Inutsuka and Yamanaka, 2013; Mieda and Sakurai, 2013; Saper, 2013). Hcrt fibers project throughout the central nervous system (CNS), with particularly dense projections and receptor expression found in arousal centers including the locus

coeruleus, the tuberomammillary nucleus, dorsal raphe nuclei, laterodorsal tegmentum, pedunculopontine tegmentum, and the basal forebrain (Peyron et al., 1998; Marcus et al., 2001). The excitatory effects of the Hcrt peptides on these arousal centers is hypothesized to stabilize and maintain wakefulness. Therefore, blockade of the Hcrt system should disfacilitate these arousal centers, creating conditions that are permissive for sleep to occur.

The current study tests the hypothesis that the dual Hcrt receptor antagonist almorexant (ALM) produces less functional impairment than ZOL. The rationale that underlies this hypothesis is that ZOL causes a general inhibition of neural activity whereas ALM specifically disfacilitates wake-promoting systems. We tested this hypothesis using tests of spatial reference memory (SRM) and spatial working memory (SWM) in the Morris Water Maze. Although the concentrations of ALM and ZOL administered prior to these tests were equipotent in hypnotic efficacy, the performance of rats treated with ALM were superior to that of rats treated with ZOL.

MATERIALS AND METHODS

ANIMALS

One hundred fifty three male Sprague Dawley rats (300 g at time of purchase; Charles River, Wilmington, MA) were distributed among the 12 groups as described in **Table 1**. All animals were individually housed in temperature-controlled recording chambers ($22 \pm 2^\circ\text{C}$, $50 \pm 25\%$ relative humidity) under a 12:12 light/dark cycle with food and water available *ad libitum*. All experimental procedures were approved by SRI International's Institutional Animal Care and Use Committee and were in accordance with National Institute of Health (NIH) guidelines.

SURGICAL PROCEDURES

Rats were instrumented with sterile telemetry transmitters (F40-EET, Data Sciences Inc., St Paul, MN) as previously described (Morairty et al., 2008, 2012; Revel et al., 2012, 2013). Briefly, under isoflurane anesthesia, transmitters were placed intraperitoneally and biopotential leads were routed subcutaneously to the head and neck. Holes were drilled into the skull at 1.5 mm anterior to bregma and 1.5 mm lateral to midline, and 6 mm posterior to bregma and 4 mm lateral to midline on the right hemisphere. Two biopotential leads used as EEG electrodes were inserted into the holes and affixed to the skull with dental acrylic. Two biopotential leads used as EMG electrodes were positioned bilaterally through the nuchal muscles.

IDENTIFICATION OF SLEEP/WAKE STATES

After at least 3 weeks post-surgical recovery, EEG, and EMG were recorded via telemetry using DQ ART 4.1 software (Data Sciences

Inc., St Paul, MN). Following completion of data collection, the EEG, and EMG recordings were scored in 10 s epochs as waking (W), rapid eye movement sleep (REM), or non-rapid eye movement sleep (NREM) by expert scorers blinded to the treatments using NeuroScore software (Data Sciences Inc., St Paul, MN). Sleep latency was defined as the first 60 s of continuous sleep following drug administration. Recordings were started at Zeitgeber time (ZT) 12 (lights off) and continued until animals performed the water maze tests.

SLEEP DEPRIVATION PROCEDURES

Animals were sleep deprived (SD) from ZT12-18 by progressive manual stimulation concurrent with EEG and EMG recording. The rats were continuously observed and, when they appeared to attempt to sleep, progressive interventions were employed to keep them awake: removal of cage tops, tapping on cages, placement of brushes inside the cage, or stroking of vibrissae or fur with an artist's brush.

DRUGS

Almorexant (ALM; ACT-078573), was synthesized at SRI International (Menlo Park, CA, USA) according to the patent literature. Zolpidem (ZOL) was a gift from Actelion Pharmaceuticals Ltd. For the SWM task, rats were dosed with ALM (100 mg/kg, p.o.), ZOL (100 mg/kg, p.o.) or vehicle (VEH; 1.25% hydroxypropyl methyl cellulose, 0.1% dioctyl sodium sulfosuccinate, and 0.25% methyl cellulose in water) at ZT18 and left undisturbed until time to perform memory tasks (see below). For the SRM task, most rats were also administered ALM, ZOL, and VEH p.o. at the concentrations above. However, one cohort of rats was administered drugs i.p. For these rats, ALM was administered at 100 mg/kg ($N = 6$), ZOL at 30 mg/kg ($N = 8$) and VEH ($N = 7$). ZOL is approximately 3X more potent i.p. than p.o. (Vanover et al., 1999) while ALM is equipotent through both routes of administration. Analysis of the sleep/wake data confirmed the equipotent effects of both drugs through both routes of administration at the concentrations tested.

WATER MAZE

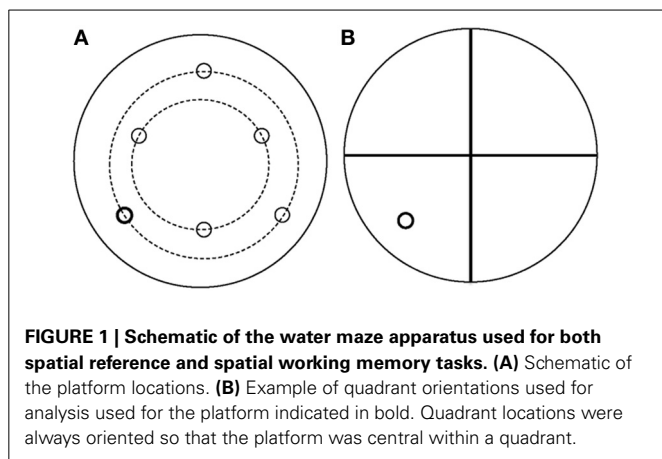
All water maze (WM) tasks occurred in a pool 68" in diameter and 25" in depth, containing water at $24 \pm 2^\circ\text{C}$ made opaque by the addition of non-toxic, water soluble black paint and milk powder. Since all tests took place during the dark period, distinctive spatial cues were made of small "rice" lights colored blue, yellow, and green. Patterns of lights in distinct shapes (circle, square, diamond, "T" shape) were clearly visible from within the pool. Preliminary studies determined the minimum number of lights that were needed for learning to occur. A 10 cm diameter platform was submerged approximately 1 cm below the surface of the water in one of 6 locations (**Figure 1**). The platform location determined the orientation of the 4 quadrants used for analysis. Both WM tasks were similar to previous reports (Wenk, 2004; Ward et al., 2009).

TEST OF SPATIAL REFERENCE MEMORY

The acquisition phase occurred in one session consisting of 12–15 consecutive trials with a 60 s inter-trial interval. For each trial,

Table 1 | The number of rats tested for each of the 12 experimental groups.

Test	No SD			6 h SD		
	VEH	ALM	ZOL	VEH	ALM	ZOL
Reference memory	14	13	17	16	16	8
Working memory	11	12	12	12	11	11



rats were placed in the WM facing the wall in one of three quadrants that did not contain the hidden platform. The location of the hidden platform remained constant across all trials. Rats were given 60 s to locate the platform. If the rats did not locate the platform within this period, they were guided to the platform location. When the rats reached the platform, they were allowed to remain on the platform for approximately 15 s before being placed in a dry holding cage for the next 60 s. This training sequence continued until the rats learned the task, typically 12–15 trials.

On the following day, rats were dosed with ALM, ZOL or VEH at ZT18 and a retention probe trial was performed 90 min later in which the rats were returned to the WM but the platform had been removed. A total of 40 rats were subjected to SD for 6 h prior to drug administration, and 42 were left undisturbed during this period (Table 1). Rats were started in the quadrant opposite the target quadrant and allowed to swim for 30 s. All trials were recorded by video camera and analyzed with Ethovision XT software (Noldus, Leesburg, VA). Test measures for the retention probe were time spent in target quadrant, latency to target quadrant, frequency of entrance into target quadrant, and total distance traveled. Swim speed was calculated to control for nonspecific effects.

TEST OF SPATIAL WORKING MEMORY

The SWM task consisted of 6 pairs of trials, one for each platform location (Figure 1A). In the first trial, a cued platform marked with a flag was placed in one of 6 positions in the WM. Rats were released facing the wall from one of the 3 quadrants not containing the platform and were allowed 120 s to locate the cued platform before the researcher guided the rats to the platform. This procedure provided all rats the opportunity to learn the platform location even if they did not find it on their own. After 15 s on the platform, the rats were removed from the WM and placed in a holding cage. The flag was then removed but the platform remained in the same location as in the first trial. Following a delay of 1, 5, or 10 min in the holding cage, the rats were placed back in the WM into one of the 2 quadrants that did not contain the platform and was not the starting quadrant during the first trial. Once the rats found the platform, they were removed after approximately 5 s and placed back in a holding cage for 10 min

before a new pair of trials with a novel platform location was given. The order of delays was counterbalanced so that each rat was tested twice at 1, 5, or 10 min delays between the cued and hidden platforms. All trials were recorded by video camera and analyzed with Ethovision XT software (Noldus, Leesburg, VA). Test measures were time to locate the platform and the swim velocity during all tests.

STATISTICAL ANALYSIS

Statistical analyses were performed using SigmaPlot 12.3 (Systat Software Inc., San Jose, CA). Sleep/wake data (W, NREM, and REM time) were analyzed in 30 min bins and compared between drug groups using Two-Way mixed-model ANOVA on factors “drug group” (between subjects) and “time” (within subjects). SRM performance parameters (latency, duration and frequency in target quadrant, total distance traveled) were analyzed using a One-Way ANOVA. SWM performance measures (velocity, time to platform, percent found) by delay time were analyzed using Two-Way mixed-model ANOVA on factors “drug group” (between subjects) and “time” (within subjects). Significance levels were set at $\alpha = 0.05$. When ANOVA indicated significance, Bonferroni *t*-tests were used for *post hoc* analyses.

RESULTS

Drug concentrations were chosen to be equipotent at sleep promotion based on our previous experience (Morairty et al., 2012). Although ZOL produced a more rapid onset to sleep under both SD and undisturbed conditions (No SD: ZOL = 6.6 min, VEH = 32.2 min, ALM = 25.4 min; SD: ZOL = 5.9 min, VEH = 20.0 min, ALM = 15.5 min), ALM- and ZOL-treated rats slept equivalent amounts during the last hour before the WM test (Figure 2; No SD: ZOL = 69.4%, ALM = 62.3%, VEH = 37.6%; SD: ZOL = 69.6%, ALM = 71.5%, VEH = 52.0%).

TEST OF SPATIAL REFERENCE MEMORY

For all performance measures analyzed, rats treated with ZOL showed significant impairments while ALM- and VEH-treated rats were indistinguishable (Figure 3). Following ZOL, the latency to the target zone increased (No SD: ZOL = 14.1 s, VEH = 5.7 s, ALM = 5.8 s; SD: ZOL = 18.4 s, VEH = 4.2 s, ALM = 3.6 s) and the duration in the target zone (No SD: ZOL = 5.5 s, VEH = 8.4 s, ALM = 7.9 s; SD: ZOL = 4.8 s, VEH = 7.7 s, ALM = 7.8 s), frequency entering the target zone (No SD: ZOL = 1.2, VEH = 2.7, ALM = 2.5; SD: ZOL = 0.9, VEH = 2.8, ALM = 2.9) and the distance traveled (No SD: ZOL = 472 cm, VEH = 666 cm, ALM = 725 cm; SD: ZOL = 343 cm, VEH = 709 cm, ALM = 775 cm) all decreased compared to VEH and ALM-treated rats. ALM-treated rats did not differ from VEH-treated rats on any of these four measures. Performance in the SRM task was not significantly affected by 6 h SD for any measure within any group.

Swim patterns in the WM were different for ZOL-treated rats compared to VEH- and ALM-treated rats (Figure 4). Both VEH and ALM rats repeatedly swam across the WM and typically swam through the area where the hidden platform was present on the previous day (Figure 4A). In contrast, ZOL-treated rats primarily swam around the perimeter of the WM, a pattern typical of a rat during its first exposure to the WM.

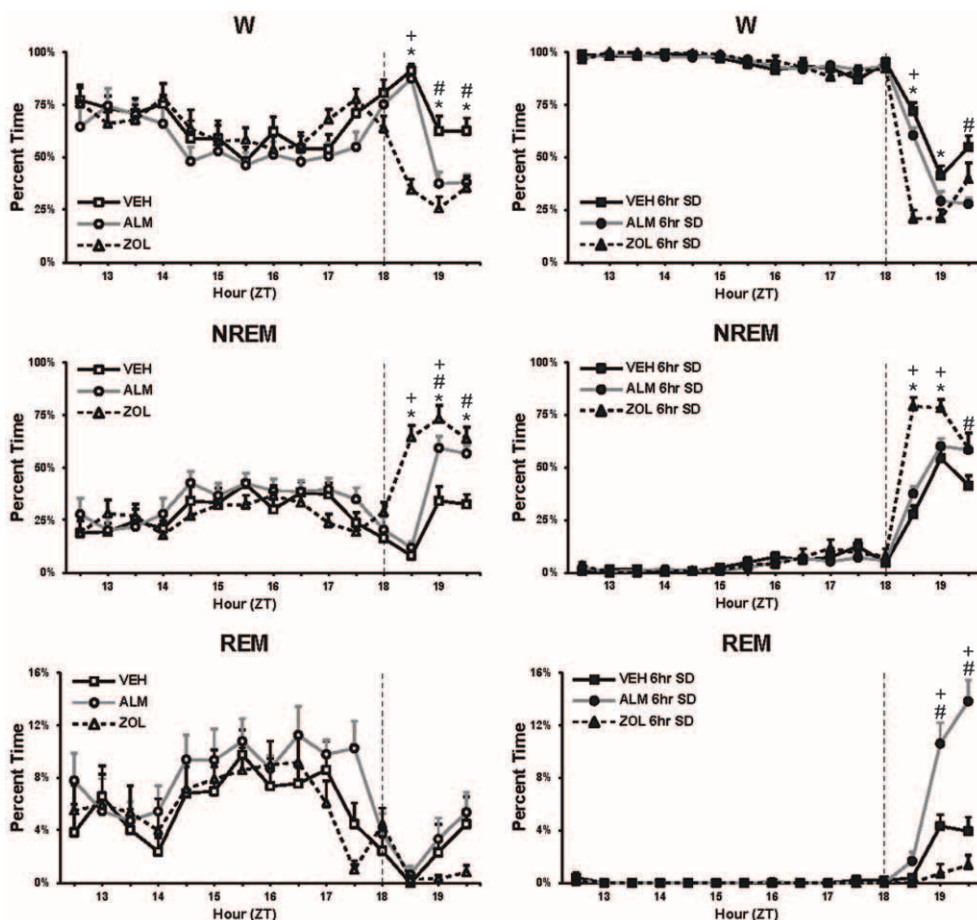


FIGURE 2 | Percent time spent in W, NREM, and REM during baseline (left panels) and during 6h SD (right panels). The vertical line in each panel at ZT18 depicts the time of drug administration. At the end of the recording time displayed in these panels, rats were

tested in the water maze. Note that, for the 60 min prior to testing (ZT19.5), the ALM and ZOL groups slept similar amounts. *, ZOL different from VEH; +, ZOL different ALM; #, ALM different from VEH; $p < 0.05$.

TEST OF SPATIAL WORKING MEMORY

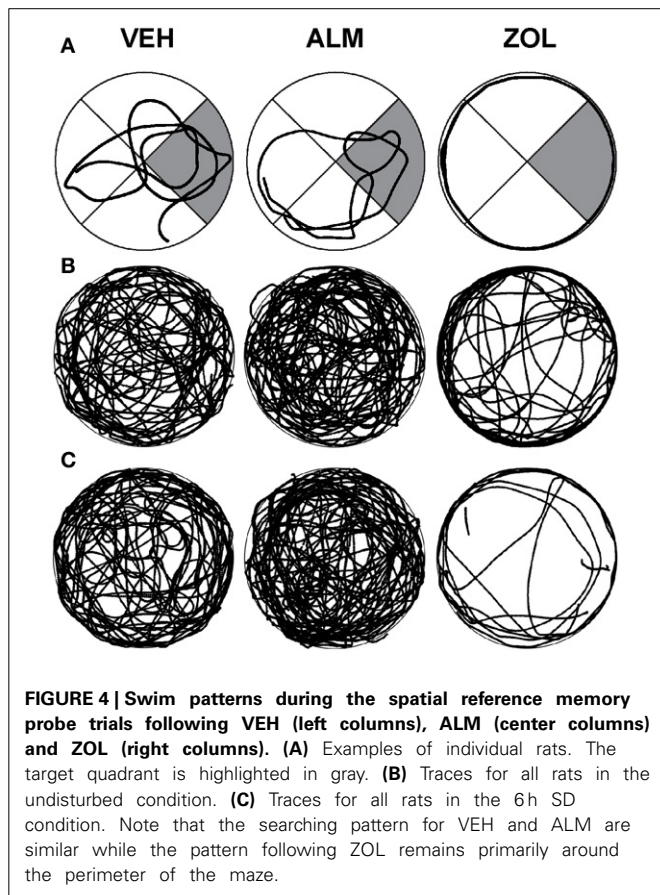
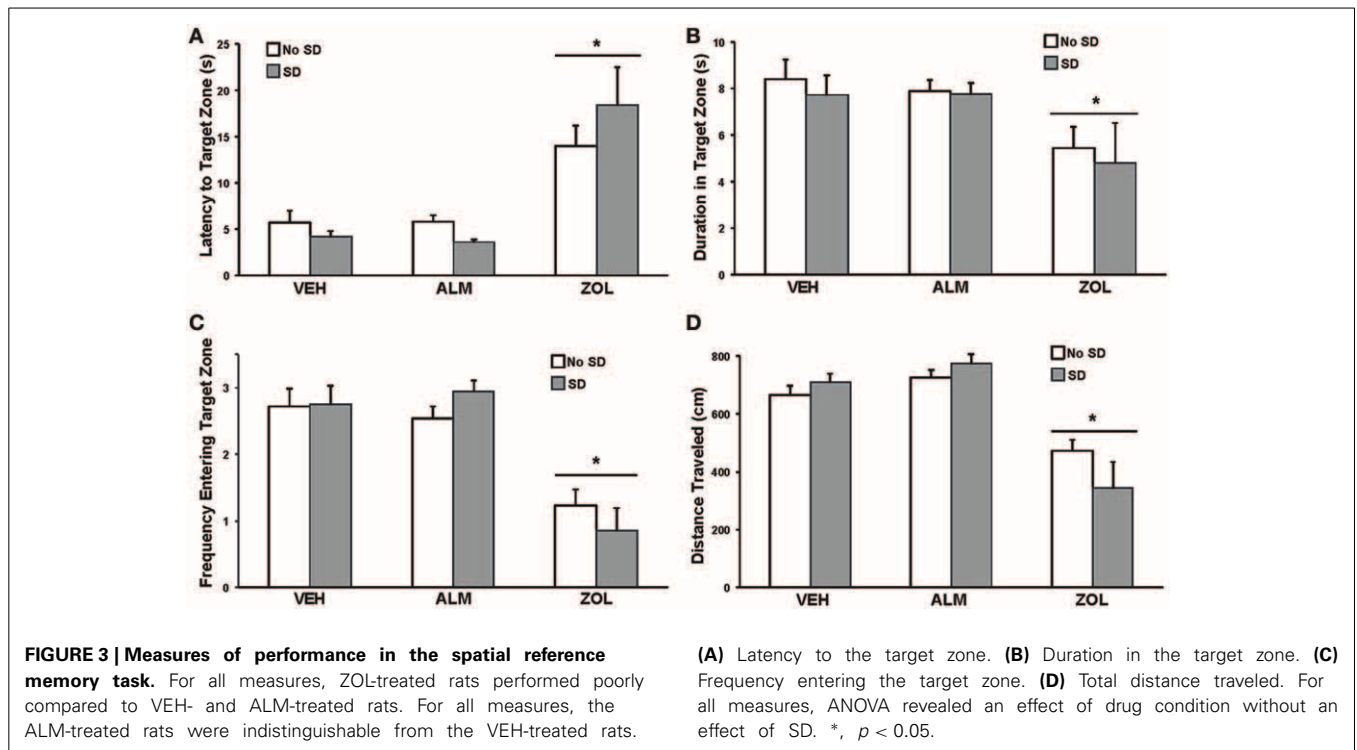
ZOL-treated rats performed poorly in the SWM task compared to either VEH- or ALM-treated rats (Figures 5, 6). ZOL-treated rats took longer to find the platform (No SD: ZOL = 43.4–47.3 s, VEH = 20.6–30.0 s, ALM = 22.5–30.7 s; SD: ZOL = 48.0–55.5 s, VEH = 26.9–31.0 s, ALM = 25.6–28.2 s) and swam more slowly (No SD: ZOL = 14.0–14.2 cm/s, VEH = 18.0–19.6 cm/s, ALM = 18.9–20.4 cm/s; SD: ZOL = 9.9–10.9 cm/s, VEH = 15.7–16.8 cm/s, ALM = 17.5–18.1 cm/s) than the VEH or ALM rats (Figure 5). These measures were not affected by increasing the delay from 1 to 5 min or 10 min for any of the 6 groups of rats.

The goal for the SWM task was to locate the platform. VEH- and ALM-treated rats found the platform the majority of the time in both SD and undisturbed conditions (83.3–100% for VEH and 79.2–87.5% for ALM; Figure 6). Conversely, ZOL-treated rats failed to find the platform most of the time (22.7–50.0% success rate). Interestingly, ZOL-treated rats also often failed to find the cued platform during the training phase of each pair of trials (Figure 7). The ZOL-treated rats in the baseline group found

the cued platform 54.4% of the time while the SD ZOL-treated group were successful 53.8% of the time as compared to 98.6% for ALM-treated rats in the baseline group and 100% following SD and 100% of the time for all VEH-treated rats. A trend toward improved performance was observed with progressive trials in the ZOL-treated rats.

DISCUSSION

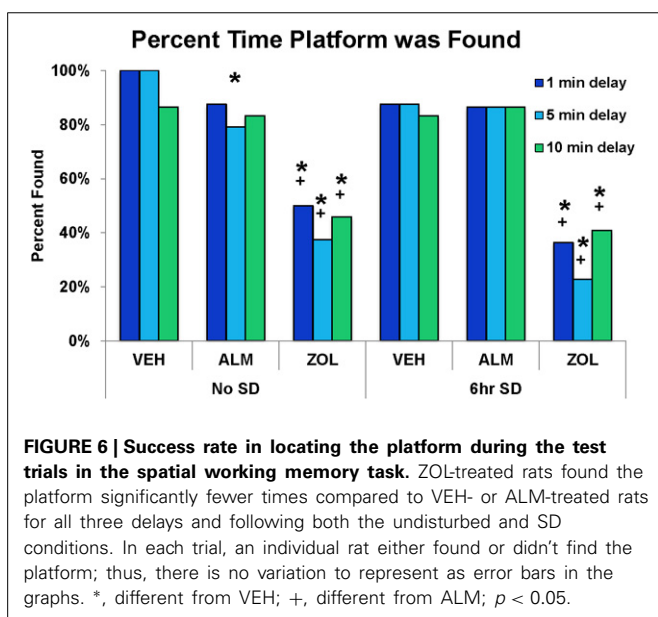
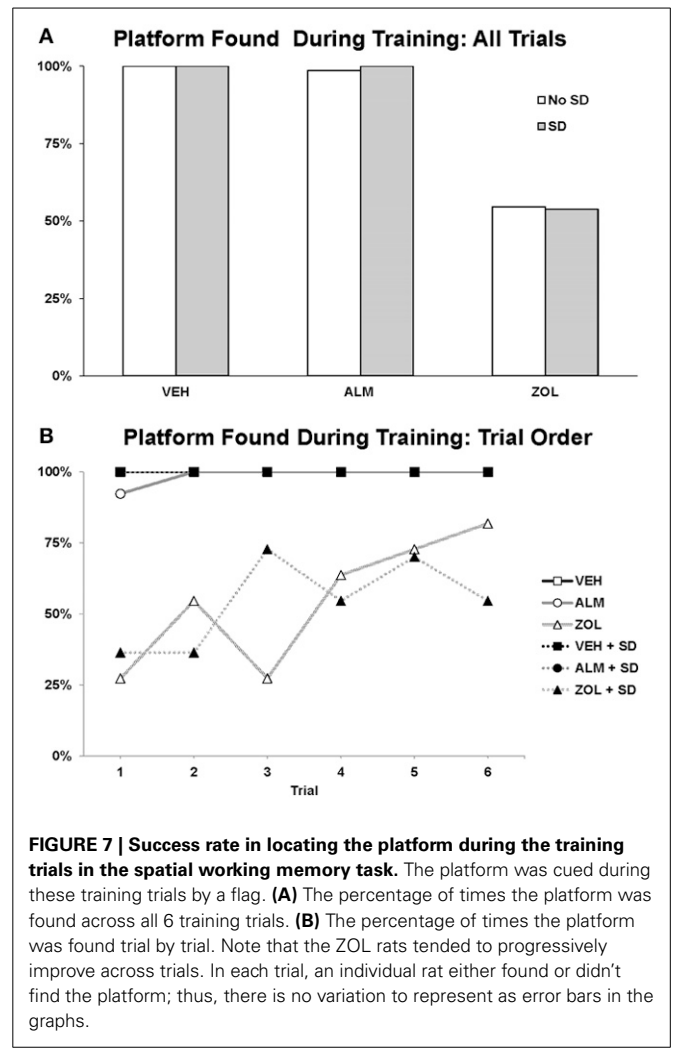
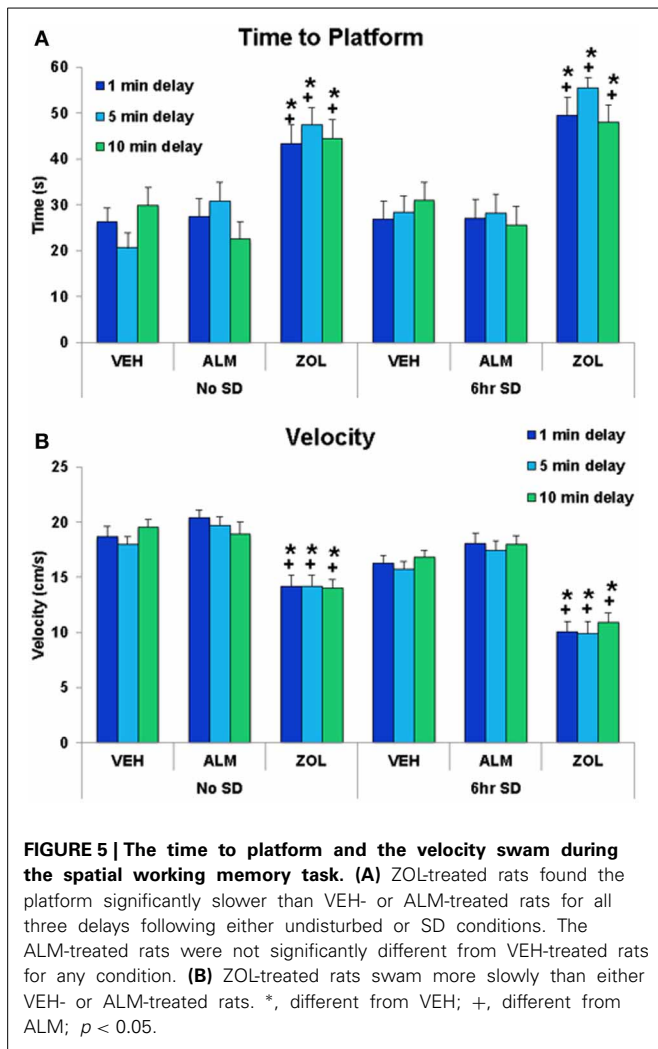
Though differing in the latency to induce sleep at the doses tested, ALM, and ZOL were equally effective at promoting sleep during the 90 min period prior to performance testing and both compounds significantly increased sleep compared to VEH. ALM-treated rats were indistinguishable from VEH-treated rats in their performance of both the SRM and SWM tasks. In contrast, ZOL caused significant impairments in both tasks. Specifically, in the SRM task, ZOL increased the latency to, the duration in, and the frequency of entering the target zone. In the SWM task, ZOL increased the time to find the platform, decreased the swim velocity and decreased the success rate in finding the platform. These results support the hypothesis that dual Hcrt receptor antagonism



effectively promotes sleep without the functional impairments observed following GABA_A receptor modulation.

An alternative explanation of the results obtained is that ZOL-treated rats were not motivated to perform the tasks rather than having memory/cognitive deficits. ZOL-treated rats had decreased distance traveled during the SRM task and decreased velocity during the SWM task, which could indicate a lack of motivation to escape the WM. Further, the lower success rate in finding the cued platform during the training trials for the SWM task could be interpreted as an absence of motivation to escape. However, ZOL rats did not simply float in the WM; they swam continuously, primarily circling the perimeter of the WM. As mentioned above, this swim pattern is typical of an untrained rat during its first exposure to the WM. Although not measured in this study, it is possible that the decreased distance traveled during the SRM task and decreased velocity during the SWM task are due to motor deficits produced by ZOL. This hypothesis is supported by previous studies that found prominent motor effects following ZOL administration (Depoortere et al., 1986; Steiner et al., 2011; Milic et al., 2012).

The SD protocol in these studies was included to assess whether moderate increases in sleep drive would exacerbate any cognitive deficits found following ALM or ZOL administration and also produce deficits in VEH-treated rats. While the primary active period of nocturnal rodents such as the rat is during the dark phase, rats still sleep approximately 30% of the time during this period and increasing wake duration during the dark period should create a mild sleep deficit (see Figure 2). Therefore, a portion of our experimental protocol involved SD during the 6 h of the dark period just prior to drug administration at ZT18.



Although we did not find significant effects of SD vs. non-SD within any of the 3 dosing conditions, these results are likely due to the fact that we allowed the rats to sleep after drug administration until water maze testing began. This undisturbed period lasted only 60–90 min but provided an opportunity for the experimental subjects to recover from this mild sleep deprivation. If the SD were continued until testing, increased memory deficits might have been observed. Further studies are needed to determine whether this is indeed the case.

ZOL is a widely prescribed hypnotic medication that can be well-tolerated when taken as directed (Greenblatt and Roth, 2012). However, numerous adverse effects associated with ZOL usage have been reported including driving impairment (Verster et al., 2006; Gunja, 2013), effects on balance and postural tone (Zammit et al., 2008), interference with memory consolidation (Balkin et al., 1992; Wesensten et al., 1996, 2005; Mintzer and Griffiths, 1999; Morgan et al., 2010) and increased incidence of complex behaviors during sleep (Hoever et al., 2010). Some studies investigated the effects of daytime administration of ZOL and tested psychomotor function upon arousal from naps (Wesensten et al., 2005; Storm et al., 2007), a protocol which our experiments closely mimic. In these studies, ZOL or melatonin was

administered at either 10:00 or 13:00. Following a 1.5–2 h nap opportunity, subjects were awakened and required to perform a series of psychomotor and cognitive tests. Significant performance decrements were observed following ZOL in cognitive, vigilance and memory tasks while little to no decrements were found following melatonin. The results of ZOL administration on rat cognitive performance in the current study correlate well with these deficits found in humans.

In contrast, the high level of performance following ALM in both of our memory tasks suggests a high degree of safety at concentrations with hypnotic efficacy. Indeed, a recent study found no performance decrements in a variant of the WM SRM task at three-fold the concentration of ALM that we used (Dietrich and Jenck, 2010). Furthermore, another recent study found no effect of ALM at 300 mg/kg on motor function (Steiner et al., 2011). In humans, however, psychometric test battery assessment of the effect of ALM administered in the daytime found reductions in vigilance, alertness, and visuomotor and motor coordination at dose concentrations of 400–1000 mg (Hoever et al., 2010, 2012a). Notably, 400 mg ALM is within the therapeutic dose range required to improve sleep in patients with primary insomnia (Hoever et al., 2012b). Therefore, performance deficits following ALM occur within the range of hypnotic efficacy in humans. In one report, pharmacokinetic/pharmacodynamic modeling suggests that doses of 500 mg ALM and 10 mg ZOL are equivalent with respect to subjectively assessed alertness (Hoever et al., 2010). Since we find hypnotic efficacy to be achieved at roughly similar dose concentrations, there may be species differences in pharmacokinetic/pharmacodynamics of ALM and/or ZOL. While not uncommon, this makes direct translational interpretations of the present data more difficult. Regardless, in both rodents and humans, ALM appears to have a significantly better safety profile than ZOL with regards to cognitive/memory domains.

CONCLUSION

ALM and ZOL are effective hypnotics in multiple mammalian species (Brisbare-Roch et al., 2007; Hoever et al., 2010, 2012a,b; Morairty et al., 2012). They act through entirely different mechanisms of action, and their effects on cognition, psychomotor vigilance and memory are in stark contrast to one another. We found that at equipotent hypnotic concentrations, ZOL impaired SRM and SWM but ALM did not. These results support the hypothesis that antagonism of the Hcrt system can provide hypnotic efficacy without the impairments found by inducing sleep through GABA_A modulation.

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