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Editor's Summary

Sleep Without the After Effects

Currently available treatments for insomnia can produce a number of central nervous system–based cognitive side effects, including the potential to impair memory and attention. Recently, selective dual orexin receptor antagonists, such as suvorexant and almorexant, have been shown to promote sleep onset and maintenance in clinical trials for patients with insomnia. In new work, Uslaner and colleagues compared sleep-promoting doses to the cognitive-impairing doses for an orexin receptor antagonist, DORA-22, versus sleep drugs currently in use: zolpidem, diazepam, or eszopiclone. At doses that produced equivalent amounts of sleep in rat and rhesus monkey, zolpidem, diazepam, and eszopiclone significantly disrupted attention and memory, whereas DORA-22 promoted sleep at doses that did not exert measurable effects on cognition. Furthermore, when compared to the other insomnia treatments that modulate γ-aminobutyric acid (GABA) receptor function, the authors saw greater separation for orexin receptor antagonism between doses that promoted sleep and doses that reduced expression of a hippocampal gene involved in synaptic plasticity called Arc. These findings suggest that dual orexin receptor antagonists might provide an effective treatment for insomnia with a greater therapeutic margin for sleep versus cognitive disturbances compared to the $GABA_A$-positive allosteric modulators currently available.

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DRUG DISCOVERY

Orexin Receptor Antagonists Differ from Standard Sleep Drugs by Promoting Sleep at Doses That Do Not Disrupt Cognition

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Current treatments for insomnia, such as zolpidem (Ambien) and eszopiclone (Lunesta), are γ-aminobutyric acid type A ($GABA_A$)–positive allosteric modulators that carry a number of side effects including the potential to disrupt cognition. In an effort to develop better tolerated medicines, we have identified dual orexin 1 and 2 receptor antagonists (DORAs), which promote sleep in preclinical animal models and humans. We compare the effects of orally administered eszopiclone, zolpidem, and diazepam to the dual orexin receptor antagonist DORA-22 on sleep and the novel object recognition test in rat, and on sleep and two cognition tests (delayed match to sample and serial choice reaction time) in the rhesus monkey. Each compound's minimal dose that promoted sleep versus the minimal dose that exerted deficits in these cognitive tests was determined, and a therapeutic margin was established. We found that DORA-22 has a wider therapeutic margin for sleep versus cognitive impairment in rat and rhesus monkey compared to the other compounds tested. These data were further supported with the demonstration of a wider therapeutic margin for DORA-22 compared to the other compounds on sleep versus the expression of hippocampal activity–regulated cytoskeletal-associated protein (Arc), an immediate-early gene product involved in synaptic plasticity. These findings suggest that DORAs might provide an effective treatment for insomnia with a greater therapeutic margin for sleep versus cognitive disturbances compared to the $GABA_A$-positive allosteric modulators currently in use.

INTRODUCTION

Insomnia affects 10 to 15% of the adult population (*1*–*3*), and one-third of these patients use pharmacotherapy for their sleep disturbances (*4*, *5*). Most are prescribed nonbenzodiazepine hypnotics, such as zolpidem (Ambien) and eszopiclone (Lunesta), which are positive allosteric modulators of a subclass of γ-aminobutyric acid type A ($GABA_A$) receptors.

Although these compounds are effective for sleep induction, they are also associated with a number of side effects including cognitive disruption. Clinical studies show that zolpidem and eszopiclone impair attention and memory (*6*–*10*). These effects are most pronounced when drug concentrations are high, and therefore, short-acting compounds are sometimes preferred such that sleep is promoted but cognitive functioning is spared the following morning (*6*, *11*–*14*). Unfortunately, shorter-acting compounds are less effective for sleep maintenance because exposures are not sustained sufficiently to encourage sleep throughout the night (*15*). Clinicians are therefore forced to consider dose, half-life, or preparation such that insomnia is relieved without residual effects. Treatment is further complicated as patients may wake up shortly after taking the drug. In such circumstances, these compounds can produce significant cognitive disruption with potentially concerning consequences (*7*–*9*, *16*–*21*).

Reducing the activity of the orexinergic system has emerged as a therapeutic approach for insomnia that might provide enhanced specificity for sleep-related pathways with reduced potential for cognitive disruption. As a predominant arousal signal to nuclei controlling the sleep/wake cycle, orexins are important for the normal control of wakefulness and vigilance (*22*, *23*). Blocking orexin-mediated arousal using orexin 1 and 2 receptor antagonists represents a new mechanism to promote sleep onset and maintenance (*24*–*27*). In contrast to GABA, orexin A and B synthesis is localized. Orexins A and B are almost exclusively synthesized in the lateral hypothalamus (*28*, *29*) and project to brain regions primarily involved in sleep (*30*).

To compare the influence of dual orexin receptor antagonists (DORAs) and GABA modulators on cognitive endpoints, we characterized the effects of DORA-22 (*31*) compared to diazepam, zolpidem, and eszopiclone on the novel object recognition test and on sleep in rats and on the delayed match to sample test, serial choice reaction time, and sleep in rhesus monkeys. We also examined the effects of these compounds on the expression of hippocampal activity–regulated cytoskeletal-associated protein (Arc), an immediate-early gene involved in synaptic plasticity (*32*, *33*).

RESULTS

Effects of GABA modulators and DORA-22 on sleep in rats

The effects of the different drug treatments on the amount of time that rats spent asleep are shown in Fig. 1. DORA-22 [$F_{(3,49)} = 2.98$, $P < 0.05$], diazepam [$F_{(3,40)} = 9.29$, $P < 0.001$], or eszopiclone [$F_{(3,54)} = 63.98$, $P < 0.001$] exerted a significant effect on the amount of time spent asleep, whereas the effect of zolpidem did not reach significance

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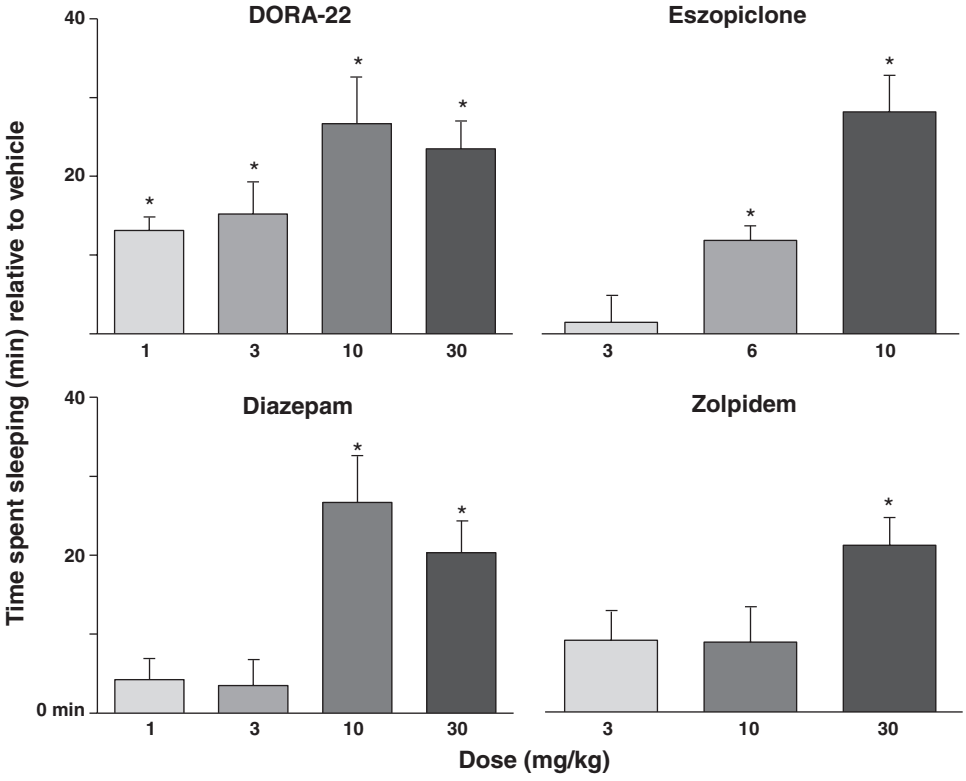

Fig. 1. Effect of oral administration of DORA-22, eszopiclone, diazepam, or zolpidem on time spent sleeping (minutes) in rat during the 2 hours after compound administration. Time spent sleeping after vehicle administration was subtracted from each group, such that 0-min sleeping is equal to the amount of sleep on vehicle. Data were analyzed using within-subjects analysis of variance (ANOVA) to determine main effects and one-sample *t* tests to compare to vehicle. n = 8 to 16 animals per group. *P < 0.05, significantly different than vehicle.

[$F_{(2,43)}$ = 3.20, P = 0.059]. Post hoc analysis revealed that all doses of DORA-22 (P < 0.01), diazepam (10 or 30 mg/kg) (P < 0.01), eszopiclone (6 or 10 mg/kg) (P < 0.01), or zolpidem (30 mg/kg) increased sleep relative to vehicle.

Effects of GABA modulators and DORA-22 on the novel object recognition test in rats

The impact of DORA-22, diazepam, eszopiclone, or zolpidem on a test of episodic-like memory, the novel object recognition test in the rat, was evaluated in four separate experiments, with scopolamine serving as a positive control in each experiment (Fig. 2). Diazepam [$F_{(6,87)}$ = 2.3, P < 0.05], eszopiclone [$F_{(5,74)}$ = 2.6, P < 0.05], or zolpidem [$F_{(3,40)}$ = 5.4, P < 0.05] exerted significant impairment on the novel object recognition test, whereas the effect of DORA-22 did not reach significance [$F_{(4,99)}$ = 1.66, P = 0.17]. Post hoc tests revealed that DORA-22 (30 mg/kg), diazepam (1, 3, or 10 mg/kg), eszopiclone (1, 3, or 10 mg/kg), or zolpidem (3 mg/kg) decreased recognition (P < 0.05). Scopolamine, the positive control, also reduced performance on the novel object recognition test in all of the studies (P < 0.05). None of the treatments for which recognition data were analyzed exerted an effect on exploration during E1, the session in which the rats were initially exposed to the arena and objects (Table 1). Zolpidem (10 mg/kg) was tested, but because it significantly reduced object exploration in E1 (Table 1), recognition data were not analyzed.

Drug effects on the expression of Arc protein in rat hippocampus

Arc protein is involved in synaptic plasticity and memory. To determine whether doses of compound that reduced recognition memory on the novel object recognition test were associated with changes in the expression of Arc protein in rat hippocampus, we examined Arc protein concentrations in the hippocampus of drug-treated rats after a low dose of drug that did not disrupt performance on the novel object recognition test and a high dose that significantly affected performance on this test (Fig. 3). For each experiment, a main effect of group was observed (P < 0.005). In all four experiments, introducing the animals to the novel object recognition arena and to objects significantly increased Arc expression in the hippocampus relative to the group that remained in their cage (home cage group) (P < 0.05). For each of the compounds tested, the doses that failed to significantly affect novel object recognition also failed to affect Arc expression in the hippocampus, whereas the dose that decreased novel object recognition also decreased Arc expression in the hippocampus (P < 0.05). Representative Western blots are shown in the Supplementary Materials (fig. S1).

Effects of GABA modulators and DORA-22 on sleep in monkeys

The effects of the different drug treatments on the amount of time rhesus monkeys spent asleep are shown in Fig. 4. DORA-22 [$F_{(4,35)}$ = 2.57, P < 0.05], diazepam [$F_{(2,27)}$ = 8.52, P < 0.005], or eszopiclone [$F_{(2,16)}$ = 11.89, P < 0.001] exerted a significant effect on sleep, whereas zolpidem failed to significantly increase sleep. As a result of its short half-life after intramuscular administration, zolpidem data were subjected to additional processing using 10-min bins, but again, no changes with respect to vehicle could be detected. Within-subjects *t* tests revealed that each dose of DORA-22 (P < 0.05), diazepam (5 or 10 mg/kg) (P < 0.05), and eszopiclone (3 or 10 mg/kg) (P < 0.05) significantly increased sleep relative to vehicle.

Effects of GABA modulators and DORA-22 on a test of working memory in monkeys

To characterize the effects of drug treatment on working memory in rhesus monkeys, we used the delayed match to sample task. Treatment with diazepam [$F_{(3,42)}$ = 9.58, P < 0.001], eszopiclone [$F_{(3,42)}$ = 10.57, P < 0.001], or zolpidem [$F_{(3,42)}$ = 7.55, P < 0.001] dose-dependently decreased the number of trials initiated in the delayed match to sample task in monkeys (Table 2). In contrast, DORA-22 did not disrupt task engagement at any of the doses examined [$F_{(3,42)}$ = 1.19, P > 0.05]. Monkeys making fewer than 30 choice responses were excluded from analysis of choice accuracy and latency (2 of 15 rhesus monkeys receiving diazepam and 3 of 15 receiving eszopiclone were excluded).

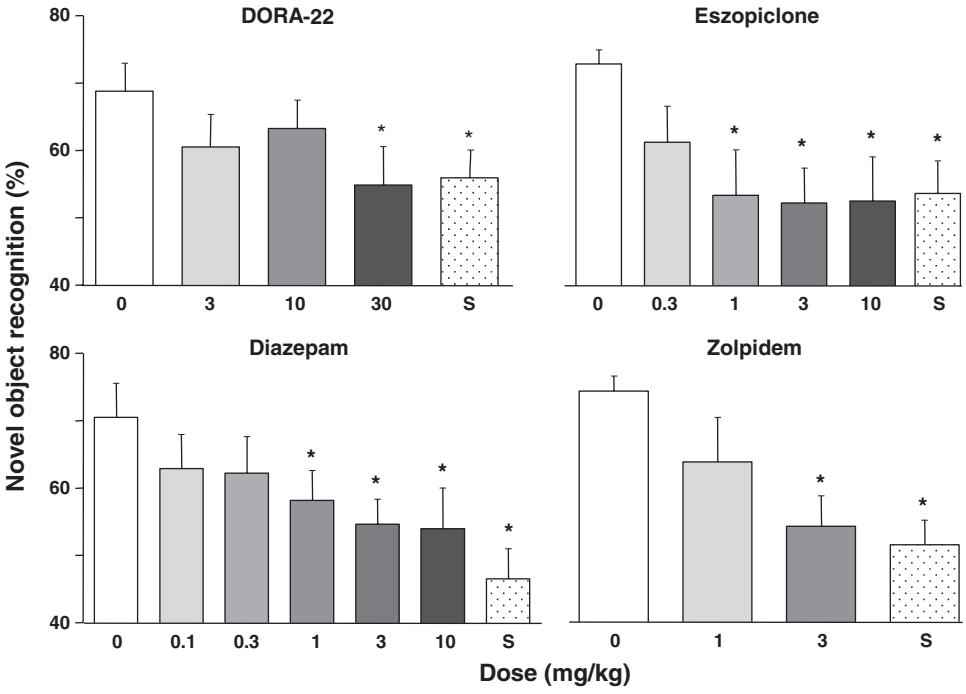

Fig. 2. Effect of oral administration of DORA-22, eszopiclone, diazepam, or zolpidem on rat performance in the novel object recognition test. Scopolamine administered intraperitoneally at 1 mg/kg was used as a positive control. Compounds were given 30 min before the first exposure to the objects and arena (E1), and animals were tested for novel object recognition 60 min after E1 (90 min after dosing). 50% = chance recognition. Recognition was analyzed using between-subjects ANOVA to determine main effects and Fisher's least significance difference post hoc tests to compare to vehicle. *n* = 10 to 22 animals per group. *$P < 0.05$, significantly lower than vehicle.

Table 1. The influence of compound and dose on exploration during E1. Values are given in seconds (SEM). nt, not tested.

Dose (mg/kg)	DORA-22	Eszopiclone	Diazepam	Zolpidem
0.0	22 (2)	12 (1)	15 (2)	17 (3)
0.1	nt	nt	13 (2)	nt
0.3	nt	15 (3)	20 (3)	nt
1.0	nt	13 (2)	14 (2)	11 (2)
3.0	17 (3)	12 (2)	14 (2)	10 (1)
10.0	18 (3)	8 (2)	11 (3)	4 (1)*
30.0	14 (3)	nt	nt	nt

*Significantly different than vehicle.

Administration of DORA-22, at doses up to 30 times the minimum dose that significantly increased sleep, failed to significantly affect choice accuracy performance (main effect of compound; compound by retention interval interaction, $P > 0.05$; Fig. 5). In contrast, treatment with diazepam [$F_{(3,36)} = 10.09$, $P < 0.001$], eszopiclone [$F_{(3,33)} = 14.38$, $P < 0.001$], or zolpidem [$F_{(3,45)} = 37.53$, $P < 0.001$] dose-dependently decreased choice accuracy independent of retention interval (all compound by retention interval interaction, $P > 0.05$). Within-subjects *t* tests demonstrated that diazepam (1 or 5 mg/kg), eszopiclone (1, 3, or 10 mg/kg), and zolpidem (0.1 or 0.3 mg/kg) exerted a significant disruption on accuracy when the data were collapsed across retention interval.

A dose-dependent increase in both sample and choice response latencies was observed after administration of diazepam, eszopiclone, or zolpidem (all main effect of compound, $P < 0.001$; Table 2). Sample response latencies after DORA-22 administration were also increased [$F_{(3,42)} = 4.00$, $P < 0.05$], whereas choice response latencies were not significantly increased by DORA-22 [$F_{(3,42)} = 0.37$, $P > 0.05$; Table 2].

Effects of GABA modulators and DORA-22 on a test of attention in monkeys

To characterize the effects of drug treatment on attention in rhesus monkeys, we administered the serial choice reaction time task. The percentage of correct responses in the serial choice reaction time task as a function of dose and treatment are shown in Fig. 6. Because centering response omissions and premature responses were affected by diazepam, eszopiclone, or zolpidem, the analysis of percent correct responses and post-cue latency measures was restricted to monkeys who were exposed to at least 32 cues (4 of 15 rhesus monkeys receiving diazepam and 6 of 15 monkeys receiving eszopiclone were excluded). Administration of DORA-22, at up to 30 times the minimum dose that significantly increased sleep, failed to affect the percentage of correct responses in the serial choice reaction time task [$F_{(3,42)} = 0.06$, $P > 0.05$; compound by cue interaction, $F_{(9,126)} = 1.12$, $P > 0.05$]. In contrast, treatment with diazepam [compound by cue interaction, $F_{(9,90)} = 98.44$, $P < 0.05$] or zolpidem [compound by cue interaction, $F_{(9,108)} = 7.22$, $P < 0.001$] dose-dependently decreased the percentage of correct responses made at short, but not long, cue durations. Eszopiclone exerted a dose-dependent reduction in the percentage of correct responses [$F_{(3,24)} = 11.33$, $P < 0.001$], which was independent of cue duration [compound by cue interaction, $F_{(9,72)} = 0.90$, $P > 0.05$]. *T* tests revealed that diazepam (5 or 10 mg/kg), eszopiclone (10 mg/kg), and zolpidem (0.3 mg/kg) significantly reduced the percentage of correct responses (Table 3).

Cue reaction times were slowed by diazepam or eszopiclone ($P < 0.05$) but were unchanged by DORA-22 or zolpidem (Table 3). Similarly, diazepam, eszopiclone, or zolpidem slowed movement latencies ($P < 0.05$). Despite a trend toward slowing, DORA-22 failed to significantly affect movement latencies [$F_{(3,42)} = 2.51$, $P > 0.05$]. Table 3 further describes compound effects for additional ancillary measures such as centering response latency and pre-cue releases.

DISCUSSION

Here, we report that the $GABA_A$ receptor–positive allosteric modulators diazepam, zolpidem, and eszopiclone impair novel object recognition (a rodent memory test), reduce hippocampal Arc (a protein

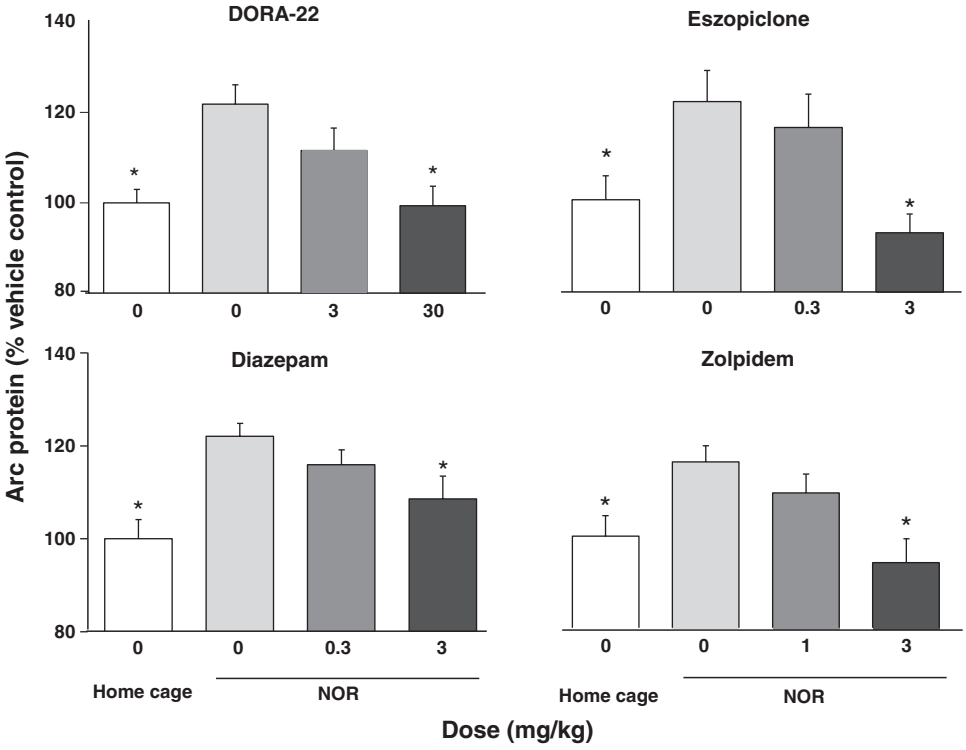

Fig. 3. Effect of oral administration of DORA-22, eszopiclone, diazepam, or zolpidem on Arc protein expression in rat hippocampus 1.5 hours after drug treatment and after being placed into the novel object recognition (NOR) arena. The "home cage" group of rats was administered vehicle and placed back into their home cage for 1.5 hours. Arc expression in rat hippocampus was analyzed using between-subjects ANOVA to determine main effects and Fisher's least significance difference post hoc tests to compare to vehicle. $n = 16$ animals per group. *$P < 0.05$, significantly different from NOR group treated with vehicle.

involved in synaptic plasticity), disrupt rhesus monkey delayed match to sample performance (a working memory task), and impair rhesus monkey serial choice reaction time accuracy (a measure of attention). All of these effects occurred at doses below or similar to those that increased sleep in these same species. Specifically, the minimum dose of each compound that impaired the novel object recognition test and reduced Arc expression in the rat hippocampus was $^1/_2$ to $^1/_{10}$ the minimum dose that increased sleep in rat. In rhesus monkeys, the minimum doses of diazepam or eszopiclone that impaired delayed match to sample accuracy were $^1/_5$ and the same as those that increased sleep, respectively. Serial choice reaction time accuracy was impaired either at the same dose or at a threefold higher dose than that which exerted effects on sleep after diazepam or eszopiclone, respectively. Finally, zolpidem failed to affect sleep in rhesus monkeys at doses well above those that impaired delayed match to sample or serial choice reaction time accuracy.

In stark contrast to diazepam, zolpidem, or eszopiclone, the dual orexin receptor antagonist DORA-22 increased sleep in rat and rhesus monkey at doses much lower than those exerting an effect on the novel object recognition test, Arc expression, or accuracy measures in the serial choice reaction time or delayed match to sample tests. Specifically, DORA-22 increased sleep in rat at a dose 30-fold lower than doses affecting the novel object recognition test or Arc expression in the hippocampus. None of the doses of DORA-22 tested, even a dose 30-fold greater than that which increased sleep, impaired accuracy on serial choice reaction time or delayed match to sample tasks in rhesus monkeys.

Zolpidem and eszopiclone, which are considered to be better tolerated than the classic benzodiazepines such as diazepam, elicited similar effects as diazepam with regard to cognitive disruption in this study. Given the perceived clinical differences between these compounds, one might be inclined to question whether the preclinical data reported here have relevance to the clinical situation. The better tolerability of the more subtype-selective sedative hypnotics has been demonstrated with respect to their muscle relaxant, anxiolytic, and anticonvulsant effects and their ability to elicit tolerance and withdrawal effects (*34*), but meaningful differences have generally not been observed with respect to cognition. For example, Wesensten *et al.* (*35*) report that zolpidem and the benzodiazepine triazolam impair memory to a similar extent. Similar findings have been reported by others for both eszopiclone (or zopiclone) and zolpidem using additional tests of memory and attention (*6*, *36*–*42*). Indeed, Wesensten *et al.* (*43*) reviewed the clinical results with these compounds and concluded that there is not "… an advantage to BZ(benzodiazepine)-receptor-subtype-selective drugs such as zolpidem over nonselective drugs such as triazolam. Rather, the results suggest that the hypnotic efficacy of these medications are functionally coupled to their performance-impairing effects."

The cognitive-impairing effects of the sedative hypnotics appear to be dependent, at least partially, on their activity at the $GABA_A$ α1 receptor subunit (*44*–*46*). Importantly then, activity on receptors containing the $GABA_A$ α1 subunit might be necessary for the sleep-promoting effects of these compounds [(*44*, *47*–*49*), but see (*50*)]. If this is indeed the case, it might be difficult to develop a treatment for insomnia acting on $GABA_A$ α1 subunit–containing receptors that has a reasonable therapeutic margin with regard to cognitive impairment. For this reason, short half-life compounds have been pursued to promote sleep induction but limit cognitive disturbance as a residual effect during wakefulness the following morning. Of course, the problem with this strategy is that shorter half-life compounds have limited efficacy for sleep maintenance because compound exposure is not sustained to encourage sleep throughout the night (*15*).

In contrast to the GABA modulators, DORA-22 promoted sleep at doses much lower than those that exerted cognitive impairment in the tests used here. We speculate that the differences between these mechanisms might be due to neuroanatomical differences between the orexinergic and GABAergic systems. $GABA_A$ α1 subunit–containing receptors account for ~40 to 60% of $GABA_A$ receptors (*49*, *51*, *52*) and are heavily expressed in the amygdala, hippocampus (particularly interneurons), and throughout the cerebral cortex, including the prefrontal and entorhinal cortex (*53*–*55*), brain regions involved in both attention and memory including the cognition tests used in the studies described here. Furthermore, the cortex, hippocampus, and amygdala

receive dense GABAergic input, such that positive allosteric modulation would elicit hyperpolarization in these brain regions by increasing GABA receptor activation. In contrast, neurons synthesizing orexin A and B, the endogenous ligands for orexin 1 and 2 receptors, are relatively discrete, having cell bodies almost exclusively located in the lateral hypothalamus (*28*, *29*). Although orexin-containing neurons project to a number of brain regions, the densest projections are onto the hypothalamus, locus coeruleus, dorsal raphe nucleus, and pedunculopontine nucleus, which are involved primarily in regulating the sleep and awake states. Orexinergic projections to other brain regions are relatively diffuse (*30*). Correspondingly, orexin 1 and 2 receptors are heavily expressed in the brain regions involved in sleep and wake and to a lesser extent in brain regions involved in cognition (*29*, *56*). Therefore, differences between the receptor expression pattern and density of efferents are such that antagonizing orexin signaling appears to have more selective effects on sleep, in contrast to potentiating GABA transmission, which exerts global effects beyond sleep, including mood, coordination, and cognition.

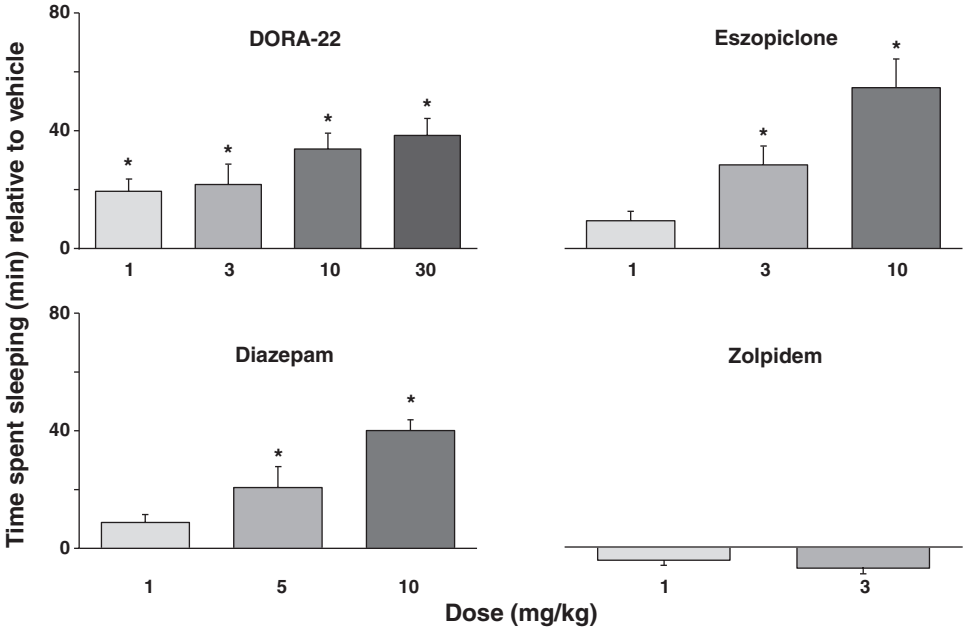

Fig. 4. Total time spent asleep in rhesus monkeys during the first 2 hours after drug administration. Sleep was measured using ECoG, EMG, EOG, and locomotor activity signals, as described in Materials and Methods. DORA-22, eszopiclone, diazepam, and zolpidem were administered orally, and zolpidem was administered intramuscularly to rhesus monkeys. Data were analyzed using within-subjects ANOVA to determine main effects and one-sample *t* tests to compare to vehicle. Values represent means ± SEM, expressed as a change from appropriate vehicle. $n = 6$ to 12 animals per group. *$P < 0.05$, significantly different from vehicle.

Our data regarding the relatively small effect of dual orexin receptor antagonism on cognition are consistent with one other report examining the effects of a DORA on learning and memory. Specifically, the DORA almorexant had no effect on the acquisition of the Morris water maze task or passive avoidance learning in rat (*57*) at doses that, in another report, were

Table 2. Delayed match to sample task measures. All values are means (SEM).

	Dose (mg/kg)	Sample responses	Sample response latency (ms)	Choice response latency (ms)	Percent correct
DORA-22	0	96 (0)	1227 (87)	1829 (124)	63 (1)
	3	95 (1)	1548 (140)*	2009 (144)	62 (2)
	10	92 (3)	1679 (170)*	1934 (123)	63 (2)
	30	95 (1)	1390 (106)	1852 (106)	66 (2)
Eszopiclone	0	95 (1)	1570 (151)	1967 (152)	62 (2)
	1	81 (7)	2170 (242)	2099 (145)	59 (2)*
	3	70 (8)*	3069 (360)*	2608 (293)*	50 (2)*
	10	61 (8)*	3773 (410)*	2976 (222)*	47 (2)*
Diazepam	0	95 (1)	1547 (146)	1940 (116)	59 (2)
	1	85 (5)	2340 (251)*	2399 (170)	53 (2)*
	5	73 (4)*	4433 (555)*	3540 (362)*	48 (2)*
	10	62 (8)*	4591 (590)*	3273 (258)*	46 (3)*
Zolpidem	0	94 (1)	1535 (150)	1848 (105)	63 (1)
	0.03	93 (2)	1572 (120)	1864 (120)	63 (2)
	0.10	94 (1)	1704 (137)	1860 (115)	59 (1)*
	0.30	81 (4)*	3165 (355)*	2538 (164)*	50 (1)*

*$P < 0.05$, statistically different from vehicle, ANOVA followed by paired two-tailed *t* test.

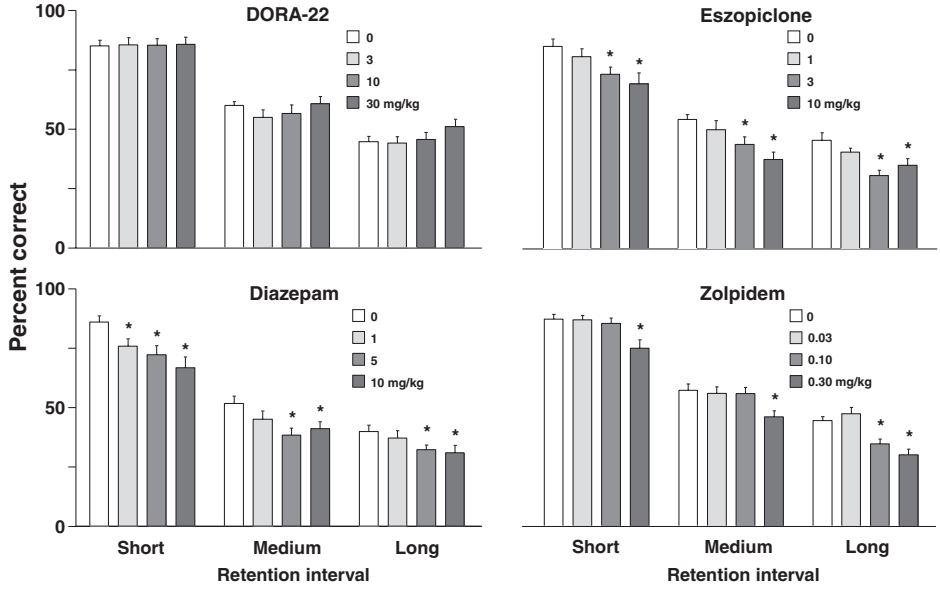

Fig. 5. Choice accuracy performance in the delayed match to sample task in monkeys after drug treatment. DORA-22, eszopiclone, and diazepam were administered orally, and zolpidem was administered intramuscularly to monkeys. Diazepam and eszopiclone were administered 90 min before testing, DORA-22 was administered 40 min before testing, and zolpidem was administered 30 min before testing. Data were analyzed using within-subjects ANOVA to determine main effects and paired *t* tests to compare to vehicle. Retention intervals ranged from 0.25 to 0.5 s, 2.5 to 14 s, and 9 to 39 s for the short, medium, and long retention interval, respectively, and were titrated for each animal to give performance of about 80 to 100%, 55 to 65%, and 35 to 45%, respectively. Values are means ± SEM; chance recognition corresponds to 25%. $n = 12$ to 16 animals per group. *$P < 0.05$, significantly different from vehicle.

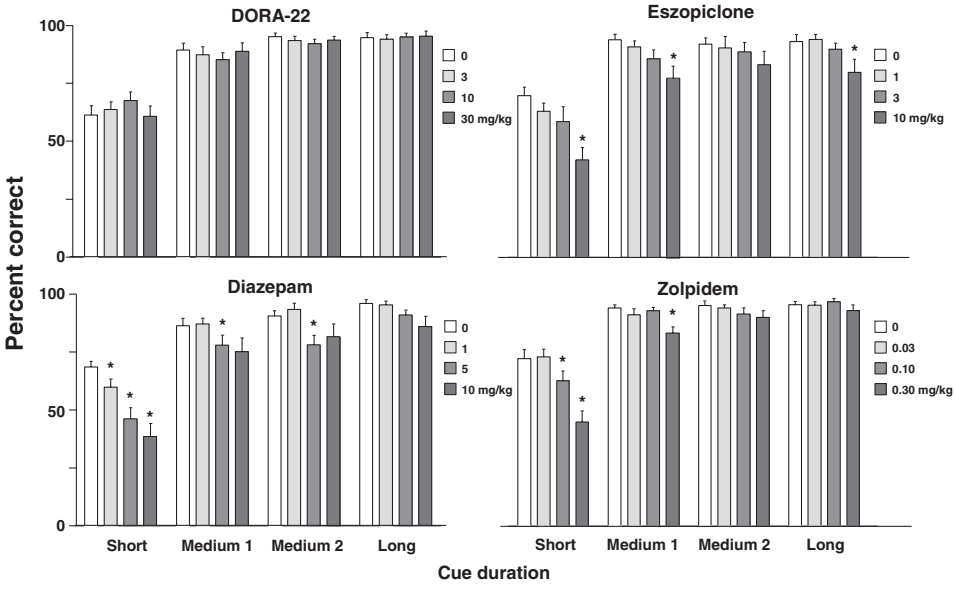

Fig. 6. Percentage of correct responses in the serial choice reaction time task in monkeys after drug treatment. DORA-22, eszopiclone, and diazepam were administered orally, and zolpidem was administered intramuscularly to monkeys. Diazepam and eszopiclone were administered 150 min before testing, DORA-22 was administered 100 min before testing, and zolpidem was administered 30 min before testing. Cue duration and the target size were titrated for each subject on the basis of performance during previous baseline sessions to yield a performance of 60 to 80% correct responses for trials. The briefest cue duration varied from 0.04 to 0.1 s, and the cues varied in size from 0.2 to 0.7. Data were analyzed using within-subjects ANOVA to determine main effects and paired *t* tests to compare to vehicle. Values are means ± SEM; chance responding corresponds to 10%. $n = 9$ to 16 animals per group. *$P < 0.05$, significantly different from vehicle.

shown to be greater than the minimum effective dose that increased sleep (*58*).

In contrast to the effect of dual orexin 1 and 2 receptor antagonism, the effects of selective antagonism of orexin 1 receptors (no relevant data with a selective orexin 2 receptor antagonist have been published) and the effects of orexin receptor stimulation are less consistent and difficult to integrate with the current findings. On the one hand, the relatively selective orexin 1 receptor antagonist SB-334867-A has been found to impair acquisition of performance on the Morris water maze (*59*, *60*) and passive avoidance learning in rats (*61*) after intrahippocampal infusions and exert small but significant impairment on an attention task in rats after both intrabasalis and systemic administration (*62*). However, it is unknown whether the doses administered are meaningful in terms of the effects of this compound on sleep, making it difficult to compare them with the current findings. Furthermore, orexin A, an agonist that has a similar affinity for orexin 1 and 2 receptors (*29*), has been found to stimulate cortical acetylcholine release (*63*), activate septal hippocampal cholinergic neurons after direct administration onto the cholinergic neurons in the basal forebrain (*64*), improve passive avoidance learning when given intracerebroventricularly (*65*), and reduce the effects of sleep deprivation on delayed match to sample performance in rhesus monkey (*66*); however, it also has been found to impair Morris water maze performance when infused intracerebroventricularly and impair long-term potentiation when applied directly to the rat hippocampus (*67*). It is presently unclear whether these apparent discrepancies are due to differences in the behavioral and neurobiological endpoints being measured or the species being examined. Indeed, in the current study, we observed differences between the effects of DORA-22 as a function of cognition tests (novel object recognition versus delayed match to sample and serial choice reaction time) and the species (rat versus rhesus monkey) being examined.

DORA-22, diazepam, and eszopiclone/zolpidem were chosen to represent DORAs, benzodiazepines, and nonbenzodiazepine GABA modulators, respectively. The results reported here represent the comparative mechanistic differences between these

Table 3. Serial choice reaction time task measures. All values are means (SEM).

	Dose (mg/kg)	Percent centering omissions	Centering response latency (ms)	Percent pre-cue releases	Cue reaction time (ms)	Movement latency (ms)	Percent correct
DORA-22	0	0 (0)	1263 (94)	7 (2)	509 (30)	372 (16)	85 (2)
	3	0 (0)	1320 (89)	7 (2)	530 (35)	392 (19)	85 (2)
	10	0 (0)	1290 (114)	6 (2)	545 (32)	392 (15)	85 (2)
	30	0 (0)	1389 (95)	6 (1)	502 (26)	384 (16)	85 (2)
Eszopiclone	0	0 (0)	1390 (97)	7 (1)	492 (25)	392 (20)	88 (2)
	1.	2 (2)	1935 (239)*	10 (2)	490 (23)	405 (25)	85 (2)
	3	13 (7)	2970 (828)	15 (5)	562 (39)*	446 (22)*	81 (3)
	10	20 (9)*	5114 (923)*	23 (4)*	565 (44)	537 (38)*	69 (4)*
Diazepam	0	0 (0)	1555 (162)	8 (1)	507 (17)	384 (16)	86 (1)
	1	1 (1)	2707 (586)	13 (4)	541 (31)	449 (27)*	84 (1)
	5	6 (4)	4518 (745)*	24 (5)*	620 (27)*	671 (93)*	74 (2)*
	10	12 (6)	6001 (789)*	33 (8)*	631 (28)*	643 (112)*	70 (4)*
Zolpidem	0	0 (0)	1576 (171)	5 (1)	497 (25)	403 (19)	90 (2)
	0.03	0 (0)	1512 (160)	4 (1)	498 (21)	389 (18)	89 (1)
	0.10	0 (0)	2211 (362)	4 (1.)	514 (23)	404 (21)	86 (2)
	0.30	8 (5)	5410 (1005)*	12 (3)*	518 (23)	432 (17)*	79 (2)*

*$P < 0.05$, statistically different from vehicle, ANOVA followed by paired two-tailed t test.

various mechanisms of action at or near each compound's respective C_{max}, and would therefore translate most accurately to situations in which patients are awake or awakened after taking these medications and are expected to execute tasks that demand attention and memory. The current study design is limited in that it does not address the issue of next-day "hangover" or carryover effects that are mediated by compound pharmacokinetics, as well as by its mechanism of action. Clinically, the potential for residual effects is a significant concern with currently prescribed sedative hypnotics. Additional comparative translational studies will be needed to further clarify the potential differential cognitive effects after conclusion of the sleep period to better understand this clinical aspect. Of additional note, diazepam was used in the current studies as the representative benzodiazepine GABA receptor modulator. Although diazepam is a well-studied preclinical and clinical benzodiazepine, it is less often used as a sleep medication in outpatient settings compared to other benzodiazepines, particularly in the United States. Future studies should further inform differentiation among mechanisms of action through the inclusion of additional representative benzodiazepines that have more common clinical use as sedative hypnotics in outpatient settings.

In conclusion, our findings demonstrate that the orexin 1 and 2 receptor antagonist DORA-22 has a much greater therapeutic margin than diazepam, zolpidem, or eszopiclone with regard to its therapeutic margin on sleep versus the novel object recognition test and hippocampal Arc expression in rat and the serial choice reaction time and delayed match to sample tests in monkeys. These findings could have important clinical implications. $GABA_A$ modulators disrupt cognition in humans at doses similar to those that promote sleep, forcing clinicians to carefully monitor dose, pharmacokinetics, and individual differences in sleep magnitude and maintenance with the potential for cognitive disruption. Several DORAs have been shown to promote sleep in humans (*26*, *27*), and the few underpowered studies characterizing the effects of DORAs on human cognitive performance have been promising (*26*, *68*). The current results further suggest that DORAs will demonstrate a larger therapeutic margin than the current standards of care for insomnia and that greater clinical assessment of these compounds is warranted.

MATERIALS AND METHODS

Subjects

Studies were conducted in accordance with Merck Institutional Animal Care and Use Committee and the National Research Council's *Guide for the Care and Use of Laboratory Animals*. Rats and monkeys were housed under standard laboratory conditions of controlled temperature, humidity, and lighting [12-hour light:12-hour dark; lights on at 0530 for rhesus monkey and 1800 for rat (that is, reverse light-dark cycle for rats)]. For all studies, compounds were administered in the active period to avoid ceiling or floor effects on sleep measures.

Rat electrocorticogram

Male Sprague-Dawley rats (Charles Rivers Laboratory; $n = 8$ to 16 per study, weight: 450 to 600 g) were singly housed in polycarbonate cages (19 inches × 10.5 inches × 8 inches; LabProducts) with free access to food and water. Before testing, rats were implanted with telemetric devices (TL10M3-F50-EEE, Data Sciences International) using a surgical procedure similar to that described previously (*69*) to assess sleep versus wake.

Rat novel object recognition test

Male Wistar Hannover rats (Charles Rivers Laboratory; $n = 10$ to 22 per group) weighing 200 to 250 g were housed two per cage under reverse

12-hour light-dark conditions (lights on 1800). One hour before testing, animals were brought to the testing room and habituated. Testing was performed during the animal's active phase under dim-light conditions.

After habituation, each rat was given compound and, 30 min later, placed into the test arena for 3 min with two identical objects (E1). The test arena consisted of a vinyl, opaque cylinder 32 inches in diameter with walls 16 inches tall. Objects used were custom-fabricated geometric shapes (a cone and sphere) similar in overall size (about 3 inches in height × 3 inches in diameter). Exploration of an object was recorded when the animal's nose was pointed in the direction of the object at a distance <1 inch. One hour later, rats were placed back into the testing arena for 3 min (E2), which now contained one object identical to that used in E1 and another object to which the animal had not been previously exposed. The amount of time animals explored the novel object relative to the familiar object was the primary measure. In addition, time spent exploring the objects during E1 was also recorded and analyzed. Objects and object locations were randomly assigned and counterbalanced across groups. Animals were included in the analysis if exploration of each object during E1 was >1 s, total E1 exploration of both objects was >4 s, and total exploration of both objects during E2 was >1 s.

Arc protein expression in hippocampus

Male Wistar Hannover rats (Charles River Laboratory) weighing 250 to 300 g were housed under conditions and treated with compound in an identical manner as in the novel object recognition studies. The same test arena and objects used in the E1 procedure from the rat novel object recognition studies were also used in these studies. Animals were divided into four groups (n = 16 per group). The first three groups were dosed with either vehicle or one of two doses of test compound 30 min before being exposed to novel object recognition arena and objects. For each compound examined, a low dose that did not affect novel object recognition and a high dose that disrupted novel object recognition were examined (doses were chosen after obtaining the results from the novel object recognition experiments). Five minutes after being exposed to the novel object recognition arena and objects, animals were placed back into their home cages. Each animal was euthanized with CO_2 90 min after receiving their injection, brains were removed, and hippocampus was dissected and prepared for Western blot analysis to measure Arc protein, as described in the Supplementary Methods. A fourth group, the home cage group received vehicle and was placed back in their home cage after dosing. These animals were euthanized 90 min after being given vehicle with the same euthanasia procedure described above.

Monkey electrocorticogram

Twenty-four adult male rhesus monkeys (*Macaca mulatta*; 8 to 15 kg) were housed singly in 33 inch × 28 inch × 36 inch cages (Allentown Caging) modified to allow cognition testing (see Supplementary Methods for caging details). Animals were fed on a calorie-controlled diet of laboratory chow supplemented with fruit and vegetables to achieve Clingerman body condition scores of 2.5 to 3 (*70*). Water was available ad libitum with the exception of those monkeys trained to perform cognitive tasks, for which access was restricted for up to 4 hours before and during cognitive testing.

All rhesus monkeys were implanted with subcutaneous telemetric devices (D70-EEE; Data Sciences International), typically many months before the current study, with a surgical procedure similar to that described previously (*25*, *69*). To assess sleep/wake, we used telemetric implants to simultaneously record electrocorticogram (ECoG), electrooculogram (EOG), electromyogram (EMG), and locomotor activity in 12 monkeys not trained to perform cognitive tasks, such that effects on sleep were not affected by animals' expectation of performing cognitive testing.

Delayed match to sample task in monkeys

All cognition testing occurred in the animal's home cage, which was equipped with a touch screen. Each delayed match to sample trial began with the presentation of a single "sample" image (150 × 200 pixels; 1.8 inches × 2.3 inches) in one of eight colors at the center of a touch screen. The trial was initiated when the monkey touched the sample image, at which point the screen became blank throughout a retention period. After the retention period, four choice images (150 × 200 pixels) were presented, one in each corner of the screen. One of the four choice images matched the color of the sample image, whereas the remaining three "distractor" images were drawn from the pool of remaining colors. To obtain a reinforcer, the monkey was required to touch the choice image for which the color matched that of the sample image. Incorrect choices were not reinforced and resulted in a 5-s timeout. On completion of the trial, an intertrial interval of 5 s was presented before the next trial. Failure to respond to the sample or choice within 30 s resulted in the screen turning blank for a 5-s timeout before the start of the next trial. Test sessions consisted of 96 trials, counterbalanced for sample color, retention interval, choice distractor color, and location of correct image. Sessions were terminated after completion of 96 trials or after 40 min had elapsed. Three discrete retention intervals were titrated for each subject's baseline to give performance of about 80 to 100%, 55 to 65%, and 35 to 45% of correct responses at the short, medium, and long retention interval, respectively. Retention intervals ranged from 0.25 to 0.5 s, 2.5 to 14 s, and 9 to 39 s for the short, medium, and long retention interval, respectively. Response latencies to sample and choice images were also recorded.

Serial choice reaction time task in monkeys

At the onset of each trial, 10 blue square "target" images, evenly distributed at 3-inch intervals along the perimeter of the touch screen were presented together with a centrally located circular "centering" image. The trial was initiated by the monkey touching the centering image, which turned gray. The monkey was required to continuously touch the centering image (1.4-inch diameter circle) throughout a variable delay of 0.25 to 7.5 s. On completion of the delay, one of the blue target images turned red for one of four cue durations before turning back to blue. To obtain a reinforcer, the monkey was required to touch the target image that had been "cued" red within a 5-s limited hold period. Inaccurate choices were not reinforced and resulted in a 3-s timeout. On completion of the trial, an intertrial interval of 2 s was initiated before the next trial. A failure to respond to the centering image within 60 s resulted in the screen becoming blank for a 2-s timeout before the start of the next trial. If the monkey initiated a trial but removed its hand from the touch screen before the appearance of the cue, the trial was terminated and the screen was blanked during a 2-s intertrial interval. Sessions were terminated after 15 min, and cue location and duration were pseudo-randomly chosen. Cue duration and the target size were titrated for each subject on the basis of performance during previous baseline sessions to yield a performance of 60 to 80% correct responses for trials. The briefest cue duration varied from 0.04 to 0.1 s, and the cues varied in size from 0.2 to 0.7 inches.

Compounds

DORA-22 was synthesized at Merck according to Coleman *et al.* (*31*). Diazepam and scopolamine were purchased from Sigma-Aldrich, eszopiclone was purchased from Sunovion Pharmaceuticals, and zolpidem was purchased from Teva Pharmaceuticals.

In the rodent studies, DORA-22, diazepam, eszopiclone, and zolpidem were administered orally in 20% vitamin E tocopherol polyethylene glycol succinate (TPGS). Scopolamine (1 mg/kg) was given intraperitoneally and served as a positive control in each novel object recognition study. For novel object recognition and Arc studies, compounds were given 30 min before placing animals in the test arena. For ECoG studies, compound was given daily on three successive days at 9 a.m., 3 hours into the active circadian phase, with discrete 3-day vehicle-compound-vehicle repeated-measures crossover designs for each treatment and dose. Between each treatment arm, animals were given a 4-day washout period.

In the rhesus monkey ECoG studies, DORA-22 (1, 3, 10, and 30 mg/kg, orally), diazepam (1, 5, and 10 mg/kg, orally), eszopiclone (1, 3, and 10 mg/kg, orally), and zolpidem (1 and 3 mg/kg, intramuscularly) were administered 5 hours into the active circadian phase. Zolpidem was administered intramuscularly to rhesus monkey because pilot experiments revealed poor absorption when given orally. Three-day vehicle-compound-vehicle or baseline-compound-baseline design protocols were used for each compound with the appropriate vehicle for each compound [DORA-22, 6% sucrose; eszopiclone, 20% vitamin E TPGS; diazepam, 0.5% carboxymethylcellulose; zolpidem, *N*-methyl-2-pyrrolidone/polyethylene glycol 200 (20:80)].

In rhesus monkey cognition studies, delayed match to sample performance was assessed at the period of maximal decrease in active wake. Diazepam (1, 5, and 10 mg/kg, orally) and eszopiclone (1, 3, and 10 mg/kg, orally) were administered 90 and 150 min before the delayed match to sample and serial choice reaction tasks, respectively. DORA-22 (3, 10, and 30 mg/kg, orally) was administered 40 and 100 min before the delayed match to sample and serial choice reaction tasks, respectively. Because of its pharmacokinetics, doses of zolpidem (0.03, 0.1, and 0.3 mg/kg, intramuscularly) were administered twice to each subject (on separate days), with delayed match to sample and serial choice reaction tasks evaluated 30 min after the treatment. A minimum of 1-week compound-free washout period was used between compounds.

Data analysis

Sleep/wake ECoG for rat and rhesus monkey. ECoG, EMG, EOG (rhesus monkey only), and locomotor activity signals were collected to characterize sleep/wake states; total sleep was used as the endpoint of interest to compare effects across compounds. Sleep was assigned and distinguished from wake with a customized version of the sleep algorithm Somnologica (Embla Systems) based on a combination of ECoG output (primarily low-voltage, high-frequency signals), EMG activity, wake characteristic EOG (rhesus monkey only), and movement within the field of the radiofrequency receiver.

For each compound and dose, each animal's cumulative time spent asleep during the first 2 hours after compound administration was expressed as a difference from its respective time in sleep after vehicle administration. Data were analyzed using one-way ANOVA with dose as a within-subjects factor. *F* values are provided in the text along with the degrees of freedom in parentheses. Significant main effects were further investigated with a one-sample *t* test to compare the amount of sleep exerted by each compound to zero (because the vehicle response was subtracted out of the compound response) plus the variability (SEM) associated with the average vehicle response.

Arc expression and cognition measures for rat and rhesus monkey. Novel object recognition and Arc data were analyzed with between-subjects ANOVA, and post hoc tests (Fisher's least significance difference) were used to compare compound-treated groups to vehicle. Delayed match to sample and serial choice reaction data were subjected to individual one- or two-way ANOVA with repeated measures, as appropriate. Significant main effects were further investigated using within-subjects *t* tests, comparing individual doses to the appropriate vehicle control.

SUPPLEMENTARY MATERIALS

www.sciencetranslationalmedicine.org/cgi/content/full/5/179/179ra44/DC1
Methods
Results
Fig. S1. Representative Western blots demonstrating Arc and actin expression in rat hippocampus.

REFERENCES AND NOTES

1. J. K. Walsh, C. L. Engelhardt, The direct economic costs of insomnia in the United States for 1995. *Sleep* **22** (Suppl. 2), S386–S393 (1999).
2. M. M. Ohayon, Prevalence of DSM-IV diagnostic criteria of insomnia: Distinguishing insomnia related to mental disorders from sleep disorders. *J. Psychiatr. Res.* **31**, 333–346 (1997).
3. M. M. Ohayon, P. Lemoine, V. Arnaud-Briant, M. Dreyfus, Prevalence and consequences of sleep disorders in a shift worker population. *J. Psychosom. Res.* **53**, 577–583 (2002).
4. S. Weyerer, H. Dilling, Prevalence and treatment of insomnia in the community: Results from the Upper Bavarian Field Study. *Sleep* **14**, 392–398 (1991).
5. G. E. Simon, M. VonKorff, Prevalence, burden, and treatment of insomnia in primary care. *Am. J. Psychiatry* **154**, 1417–1423 (1997).
6. I. Berlin, D. Warot, T. Hergueta, P. Molinier, C. Bagot, A. J. Puech, Comparison of the effects of zolpidem and triazolam on memory functions, psychomotor performances, and postural sway in healthy subjects. *J. Clin. Psychopharmacol.* **13**, 100–106 (1993).
7. P. Danjou, I. Paty, R. Fruncillo, P. Worthington, M. Unruh, W. Cevallos, P. Martin, A comparison of the residual effects of zaleplon and zolpidem following administration 5 to 2 h before awakening. *Br. J. Clin. Pharmacol.* **48**, 367–374 (1999).
8. I. Hindmarch, A. Patat, N. Stanley, I. Paty, U. Rigney, Residual effects of zaleplon and zolpidem following middle of the night administration five hours to one hour before awakening. *Hum. Psychopharmacol.* **16**, 159–167 (2001).
9. S. Otmani, A. Demazières, C. Staner, N. Jacob, T. Nir, N. Zisapel, L. Staner, Effects of prolonged-release melatonin, zolpidem, and their combination on psychomotor functions, memory recall, and driving skills in healthy middle aged and elderly volunteers. *Hum. Psychopharmacol.* **23**, 693–705 (2008).
10. C. R. Rush, D. L. Armstrong, J. A. Ali, P. J. Pazzaglia, Benzodiazepine-receptor ligands in humans: Acute performance-impairing, subject-rated and observer-rated effects. *J. Clin. Psychopharmacol.* **18**, 154–165 (1998).
11. T. Roth, K. M. Hartse, F. J. Zorick, M. E. Kaffeman, The differential effects of short- and long-acting benzodiazepines upon nocturnal sleep and daytime performance. *Arzneimittelforschung* **30**, 891–894 (1980).
12. G. Darcourt, D. Pringuey, D. Sallière, J. Lavoisy, The safety and tolerability of zolpidem—An update. *J. Psychopharmacol.* **13**, 81–93 (1999).
13. H. Allain, D. Bentué-Ferrer, S. L. Breton, E. Polard, J. M. Gandon, Preference of insomniac patients between a single dose of zolpidem 10 mg versus zaleplon 10 mg. *Hum. Psychopharmacol.* **18**, 369–374 (2003).
14. I. Hindmarch, E. Legangneux, N. Stanley, S. Emegbo, J. Dawson, A double-blind, placebo-controlled investigation of the residual psychomotor and cognitive effects of zolpidem-MR in healthy elderly volunteers. *Br. J. Clin. Pharmacol.* **62**, 538–545 (2006).
15. E. Weinling, S. McDougall, F. Andre, G. Bianchetti, C. Dubruc, Pharmacokinetic profile of a new modified release formulation of zolpidem designed to improve sleep maintenance. *Fundam. Clin. Pharmacol.* **20**, 397–403 (2006).
16. F. Barbone, A. D. McMahon, P. G. Davey, A. D. Morris, I. C. Reid, D. G. McDevitt, T. M. MacDonald, Association of road-traffic accidents with benzodiazepine use. *Lancet* **352**, 1331–1336 (1998).

17. M. Partinen, K. Hirvonen, C. Hublin, M. Halavaara, H. Hiltunen, Effects of after-midnight intake of zolpidem and temazepam on driving ability in women with non-organic insomnia. *Sleep Med.* **4**, 553–561 (2003).
18. J. C. Verster, E. R. Volkerts, A. H. Schreuder, E. J. Eijken, J. H. van Heuckelum, D. S. Veldhuijzen, M. N. Verbaten, I. Paty, M. Darwish, P. Danjou, A. Patat, Residual effects of middle-of-the-night administration of zaleplon and zolpidem on driving ability, memory functions, and psychomotor performance. *J. Clin. Psychopharmacol.* **22**, 576–583 (2002).
19. J. C. Verster, D. S. Veldhuijzen, E. R. Volkerts, Residual effects of sleep medication on driving ability. *Sleep Med. Rev.* **8**, 309–325 (2004).
20. J. C. Verster, D. S. Veldhuijzen, A. Patat, B. Olivier, E. R. Volkerts, Hypnotics and driving safety: Meta-analyses of randomized controlled trials applying the on-the-road driving test. *Curr. Drug Saf.* **1**, 63–71 (2006).
21. J. C. Verster, E. R. Volkerts, B. Olivier, W. Johnson, L. Liddicoat, Zolpidem and traffic safety—The importance of treatment compliance. *Curr. Drug Saf.* **2**, 220–226 (2007).
22. R. A. España, T. E. Scammell, Sleep neurobiology from a clinical perspective. *Sleep* **34**, 845–858 (2011).
23. T. E. Scammell, C. J. Winrow, Orexin receptors: Pharmacology and therapeutic opportunities. *Annu. Rev. Pharmacol. Toxicol.* **51**, 243–266 (2011).
24. D. B. Whitman, C. D. Cox, M. J. Breslin, K. M. Brashear, J. D. Schreier, M. J. Bogusky, R. A. Bednar, W. Lemaire, J. G. Bruno, G. D. Hartman, D. R. Reiss, C. M. Harrell, R. L. Kraus, Y. Li, S. L. Garson, S. M. Doran, T. Prueksaritanont, C. Li, C. J. Winrow, K. S. Koblan, J. J. Renger, P. J. Coleman, Discovery of a potent, CNS-penetrant orexin receptor antagonist based on an *N,N*-disubstituted-1,4-diazepane scaffold that promotes sleep in rats. *ChemMedChem* **4**, 1069–1074 (2009).
25. C. J. Winrow, A. L. Gotter, C. D. Cox, S. M. Doran, P. L. Tannenbaum, M. J. Breslin, S. L. Garson, S. V. Fox, C. M. Harrell, J. Stevens, D. R. Reiss, D. Cui, P. J. Coleman, J. J. Renger, Promotion of sleep by suvorexant—A novel dual orexin receptor antagonist. *J. Neurogenet.* **25**, 52–61 (2011).
26. P. Bettica, G. Nucci, C. Pyke, L. Squassante, S. Zamuner, E. Ratti, R. Gomeni, R. Alexander, Phase I studies on the safety, tolerability, pharmacokinetics and pharmacodynamics of SB-649868, a novel dual orexin receptor antagonist. *J. Psychopharmacol.* **26**, 1058–1070 (2012).
27. P. Bettica, L. Squassante, J. A. Groeger, B. Gennery, R. Winsky-Sommerer, D. J. Dijk, Differential effects of a dual orexin receptor antagonist (SB-649868) and zolpidem on sleep initiation and consolidation, SWS, REM sleep, and EEG power spectra in a model of situational insomnia. *Neuropsychopharmacology* **37**, 1224–1233 (2012).
28. L. de Lecea, T. S. Kilduff, C. Peyron, X. Gao, P. E. Foye, P. E. Danielson, C. Fukuhara, E. L. Battenberg, V. T. Gautvik, F. S. Bartlett II, W. N. Frankel, A. N. van den Pol, F. E. Bloom, K. M. Gautvik, J. G. Sutcliffe, The hypocretins: Hypothalamus-specific peptides with neuroexcitatory activity. *Proc. Natl. Acad. Sci. U.S.A.* **95**, 322–327 (1998).
29. T. Sakurai, A. Amemiya, M. Ishii, I. Matsuzaki, R. M. Chemelli, H. Tanaka, S. C. Williams, J. A. Richardson, G. P. Kozlowski, S. Wilson, J. R. Arch, R. E. Buckingham, A. C. Haynes, S. A. Carr, R. S. Annan, D. E. McNulty, W. S. Liu, J. A. Terrett, N. A. Elshourbagy, D. J. Bergsma, M. Yanagisawa, Orexins and orexin receptors: A family of hypothalamic neuropeptides and G protein-coupled receptors that regulate feeding behavior. *Cell* **92**, 573–585 (1998).
30. C. Peyron, D. K. Tighe, A. N. van den Pol, L. de Lecea, H. C. Heller, J. G. Sutcliffe, T. S. Kilduff, Neurons containing hypocretin (orexin) project to multiple neuronal systems. *J. Neurosci.* **18**, 9996–10015 (1998).
31. P. J. Coleman, J. D. Schreier, C. D. Cox, M. J. Breslin, D. B. Whitman, M. J. Bogusky, G. B. McGaughey, R. A. Bednar, W. Lemaire, S. M. Doran, S. V. Fox, S. L. Garson, A. L. Gotter, C. M. Harrell, D. R. Reiss, T. D. Cabalu, D. Cui, T. Prueksaritanont, J. Stevens, P. L. Tannenbaum, R. G. Ball, J. Stellabott, S. D. Young, G. D. Hartman, C. J. Winrow, J. J. Renger, Discovery of [(2*R*,5*R*)-5-{[(5-fluoropyridin-2-yl)oxy]methyl}-2-methylpiperidin-1-yl][5-methyl-2-(pyrimidin-2-yl)phenyl]methanone (MK-6096): A dual orexin receptor antagonist with potent sleep-promoting properties. *ChemMedChem* **7**, 415–424 (2012).
32. J. F. Guzowski, B. L. McNaughton, C. A. Barnes, P. F. Worley, Environment-specific expression of the immediate-early gene *Arc* in hippocampal neuronal ensembles. *Nat. Neurosci.* **2**, 1120–1124 (1999).
33. J. F. Guzowski, G. L. Lyford, G. D. Stevenson, F. P. Houston, J. L. McGaugh, P. F. Worley, C. A. Barnes, Inhibition of activity-dependent arc protein expression in the rat hippocampus impairs the maintenance of long-term potentiation and the consolidation of long-term memory. *J. Neurosci.* **20**, 3993–4001 (2000).
34. D. J. Sanger, The pharmacology and mechanisms of action of new generation, non-benzodiazepine hypnotic agents. *CNS Drugs* **18** (Suppl. 1), 9–15 (2004).
35. N. J. Wesensten, T. J. Balkin, G. L. Belenky, Effects of daytime administration of zolpidem versus triazolam on memory. *Eur. J. Clin. Pharmacol.* **48**, 115–122 (1995).
36. D. Warot, G. Bensimon, P. Danjou, A. J. Puech, Comparative effects of zopiclone, triazolam and placebo on memory and psychomotor performance in healthy volunteers. *Fundam. Clin. Pharmacol.* **1**, 145–152 (1987).
37. T. Kuitunen, M. J. Mattila, T. Seppälä, K. Aranko, M. E. Mattila, Actions of zopiclone and carbamazepine, alone and in combination, on human skilled performance in laboratory and clinical tests. *Br. J. Clin. Pharmacol.* **30**, 453–461 (1990).
38. T. J. Balkin, V. M. O'Donnell, N. Wesensten, U. McCann, G. Belenky, Comparison of the daytime sleep and performance effects of zolpidem versus triazolam. *Psychopharmacology* **107**, 83–88 (1992).
39. H. Allain, A. Patat, A. Lieury, F. Le Coz, C. Janus, G. Menard, J. Gandon, Comparative study of the effects of zopiclone (7.5 mg), zolpidem, flunitrazepam and a placebo on nocturnal cognitive performance in healthy subjects, in relation to pharmacokinetics. *Eur. Psychiatry* **10** (Suppl. 3), 129s–135s (1995).
40. T. R. Leufkens, J. S. Lund, A. Vermeeren, Highway driving performance and cognitive functioning the morning after bedtime and middle-of-the-night use of gaboxadol, zopiclone and zolpidem. *J. Sleep Res.* **18**, 387–396 (2009).
41. T. Roehrs, L. Merlotti, F. Zorick, T. Roth, Sedative, memory, and performance effects of hypnotics. *Psychopharmacology* **116**, 130–134 (1994).
42. C. R. Rush, R. R. Griffiths, Zolpidem, triazolam, and temazepam: Behavioral and subject-rated effects in normal volunteers. *J. Clin. Psychopharmacol.* **16**, 146–157 (1996).
43. N. J. Wesensten, T. J. Balkin, G. L. Belenky, Effects of daytime administration of zolpidem and triazolam on performance. *Aviat. Space Environ. Med.* **67**, 115–120 (1996).
44. U. Rudolph, F. Crestani, D. Benke, I. Brünig, J. A. Benson, J. M. Fritschy, J. R. Martin, H. Bluethmann, H. Möhler, Benzodiazepine actions mediated by specific γ-aminobutyric $acid_A$ receptor subtypes. *Nature* **401**, 796–800 (1999).
45. M. M. Savic, D. I. Obradović, N. D. Ugresic, J. M. Cook, P. V. Sarma, D. R. Bokonjić, Bi-directional effects of benzodiazepine binding site ligands on active avoidance acquisition and retention: Differential antagonism by flumazenil and β-CCt. *Psychopharmacology* **180**, 455–465 (2005).
46. B. J. Wiltgen, B. P. Godsil, Z. Peng, F. Saab, H. L. June, M. L. Linn, J. M. Cook, C. R. Houser, T. J. O'Dell, G. E. Homanics, M. S. Fanselow, The α1 subunit of the GABA(A) receptor modulates fear learning and plasticity in the lateral amygdala. *Front. Behav. Neurosci.* **3**, 37 (2009).
47. E. R. Korpi, G. Gründer, H. Lüddens, Drug interactions at $GABA_A$ receptors. *Prog. Neurobiol.* **67**, 113–159 (2002).
48. U. Rudolph, H. Möhler, GABA-based therapeutic approaches: $GABA_A$ receptor subtype functions. *Curr. Opin. Pharmacol.* **6**, 18–23 (2006).
49. R. W. Olsen, W. Sieghart, International Union of Pharmacology. LXX. Subtypes of γ-aminobutyric $acid_A$ receptors: Classification on the basis of subunit composition, pharmacology, and function. Update. *Pharmacol. Rev.* **60**, 243–260 (2008).
50. I. Tobler, C. Kopp, T. Deboer, U. Rudolph, Diazepam-induced changes in sleep: Role of the α 1 $GABA_A$ receptor subtype. *Proc. Natl. Acad. Sci. U.S.A.* **98**, 6464–6469 (2001).
51. R. M. McKernan, P. J. Whiting, Which $GABA_A$-receptor subtypes really occur in the brain? *Trends Neurosci.* **19**, 139–143 (1996).
52. P. J. Whiting, GABA-A receptor subtypes in the brain: A paradigm for CNS drug discovery? *Drug Discov. Today* **8**, 445–450 (2003).
53. S. Akbarian, M. M. Huntsman, J. J. Kim, A. Tafazzoli, S. G. Potkin, W. E. Bunney Jr., E. G. Jones, $GABA_A$ receptor subunit gene expression in human prefrontal cortex: Comparison of schizophrenics and controls. *Cereb. Cortex* **5**, 550–560 (1995).
54. J. M. Fritschy, H. Mohler, $GABA_A$-receptor heterogeneity in the adult rat brain: Differential regional and cellular distribution of seven major subunits. *J. Comp. Neurol.* **359**, 154–194 (1995).
55. T. Ohnuma, S. J. Augood, H. Arai, P. J. McKenna, P. C. Emson, Measurement of GABAergic parameters in the prefrontal cortex in schizophrenia: Focus on GABA content, $GABA_A$ receptor α-1 subunit messenger RNA and human GABA transporter-1 (HGAT-1) messenger RNA expression. *Neuroscience* **93**, 441–448 (1999).
56. J. N. Marcus, C. J. Aschkenasi, C. E. Lee, R. M. Chemelli, C. B. Saper, M. Yanagisawa, J. K. Elmquist, Differential expression of orexin receptors 1 and 2 in the rat brain. *J. Comp. Neurol.* **435**, 6–25 (2001).
57. H. Dietrich, F. Jenck, Intact learning and memory in rats following treatment with the dual orexin receptor antagonist almorexant. *Psychopharmacology* **212**, 145–154 (2010).
58. C. Brisbare-Roch, J. Dingemanse, R. Koberstein, P. Hoever, H. Aissaoui, S. Flores, C. Mueller, O. Nayler, J. van Gerven, S. L. de Haas, P. Hess, C. Qiu, S. Buchmann, M. Scherz, T. Weller, W. Fischli, M. Clozel, F. Jenck, Promotion of sleep by targeting the orexin system in rats, dogs and humans. *Nat. Med.* **13**, 150–155 (2007).
59. E. Akbari, N. Naghdi, F. Motamedi, Functional inactivation of orexin 1 receptors in CA1 region impairs acquisition, consolidation and retrieval in Morris water maze task. *Behav. Brain Res.* **173**, 47–52 (2006).
60. E. Akbari, N. Naghdi, F. Motamedi, The selective orexin 1 receptor antagonist SB-334867-A impairs acquisition and consolidation but not retrieval of spatial memory in Morris water maze. *Peptides* **28**, 650–656 (2007).
61. E. Akbari, F. Motamedi, N. Naghdi, M. Noorbakhshnia, The effect of antagonization of orexin 1 receptors in CA_1 and dentate gyrus regions on memory processing in passive avoidance task. *Behav. Brain Res.* **187**, 172–177 (2008).
62. K. E. Boschen, J. R. Fadel, J. A. Burk, Systemic and intrabasalis administration of the orexin-1 receptor antagonist, SB-334867, disrupts attentional performance in rats. *Psychopharmacology* **206**, 205–213 (2009).
63. J. Fadel, R. Pasumarthi, L. R. Reznikov, Stimulation of cortical acetylcholine release by orexin A. *Neuroscience* **130**, 541–547 (2005).

64. M. Wu, L. Zaborszky, T. Hajszan, A. N. van den Pol, M. Alreja, Hypocretin/orexin innervation and excitation of identified septohippocampal cholinergic neurons. *J. Neurosci.* **24**, 3527–3536 (2004).
65. G. Telegdy, A. Adamik, The action of orexin A on passive avoidance learning. Involvement of transmitters. *Regul. Pept.* **104**, 105–110 (2002).
66. S. A. Deadwyler, L. Porrino, J. M. Siegel, R. E. Hampson, Systemic and nasal delivery of orexin-A (Hypocretin-1) reduces the effects of sleep deprivation on cognitive performance in nonhuman primates. *J. Neurosci.* **27**, 14239–14247 (2007).
67. S. Aou, X. L. Li, A. J. Li, Y. Oomura, T. Shiraishi, K. Sasaki, T. Imamura, M. J. Wayner, Orexin-A (hypocretin-1) impairs Morris water maze performance and CA1-Schaffer collateral long-term potentiation in rats. *Neuroscience* **119**, 1221–1228 (2003).
68. P. Hoever, S. de Haas, J. Winkler, R. C. Schoemaker, E. Chiossi, J. van Gerven, J. Dingemanse, Orexin receptor antagonism, a new sleep-promoting paradigm: An ascending single-dose study with almorexant. *Clin. Pharmacol. Ther.* **87**, 593–600 (2010).
69. J. J. Renger, S. L. Dunn, S. L. Motzel, C. Johnson, K. S. Koblan, Sub-chronic administration of zolpidem affects modifications to rat sleep architecture. *Brain Res.* **1010**, 45–54 (2004).
70. K. J. Clingerman, L. Summers, Development of a body condition scoring system for non-human primates using *Macaca mulatta* as a model. *Lab. Anim.* **34**, 31–36 (2005).

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