



# Distinct effects of IPSU and suvorexant on mouse sleep architecture

Daniel Hoyer<sup>1,2</sup>, Thomas Dürst<sup>1</sup>, Markus Fendt<sup>1,3</sup>, Laura H. Jacobson<sup>1,4</sup>, Claudia Betschart<sup>5</sup>, Samuel Hintermann<sup>5</sup>, Dirk Behnke<sup>5</sup>, Simona Cotesta<sup>5</sup>, Grit Laue<sup>6</sup>, Silvio Ofner<sup>5</sup>, Eric Legangneux<sup>7</sup> and Christine E. Gee<sup>1,8\*</sup>

<sup>1</sup> Neuroscience, Novartis Institutes for BioMedical Research, Basel, Switzerland

<sup>2</sup> Department of Pharmacology and Therapeutics, Faculty of Medicine, Dentistry and Health Sciences, The University of Melbourne, Parkville, VIC, Australia

<sup>3</sup> Center of Behavioral Brain Sciences, Institute for Pharmacology and Toxicology, Otto-von-Guericke University Magdeburg, Magdeburg, Germany

<sup>4</sup> The Florey Institute of Neuroscience and Mental Health, The University of Melbourne, Parkville, VIC, Australia

<sup>5</sup> Global Discovery Chemistry, Novartis Institutes for BioMedical Research, Basel, Switzerland

<sup>6</sup> Metabolism and Pharmacokinetics, Novartis Institutes for BioMedical Research, Basel, Switzerland

<sup>7</sup> Translational Medicine, Novartis Institutes for BioMedical Research, Basel, Switzerland

<sup>8</sup> Center for Molecular Neuroscience Hamburg, Institute for Synaptic Physiology, Hamburg, Germany

## Edited by:

Michel A. Steiner, Actelion  
Pharmaceuticals Ltd., Switzerland

## Reviewed by:

Michihiro Mieda, Kanazawa  
University, Japan  
Brock T. Shireman, Janssen, USA

## \*Correspondence:

Christine E. Gee, Center for  
Molecular Neurobiology Hamburg,  
Institute for Synaptic Physiology,  
Falkenried 94, 20251 Hamburg,  
Germany  
e-mail: christine.gee@  
zmnh.uni-hamburg.de

Dual orexin receptor (OXR) antagonists (DORAs) such as almorexant, SB-649868, suvorexant (MK-4305), and filorexant (MK-6096), have shown promise for the treatment of insomnias and sleep disorders. Whether antagonism of both OX<sub>1</sub>R and OX<sub>2</sub>R is necessary for sleep induction has been a matter of some debate. Experiments using knockout mice suggest that it may be sufficient to antagonize only OX<sub>2</sub>R. The recent identification of an orally bioavailable, brain penetrant OX<sub>2</sub>R preferring antagonist 2-((1*H*-Indol-3-yl)methyl)-9-(4-methoxypyrimidin-2-yl)-2,9-diazaspiro[5.5]undecan-1-one (IPSU) has allowed us to test whether selective antagonism of OX<sub>2</sub>R may also be a viable strategy for induction of sleep. We previously demonstrated that IPSU and suvorexant increase sleep when dosed during the mouse active phase (lights off); IPSU inducing sleep primarily by increasing NREM sleep, suvorexant primarily by increasing REM sleep. Here, our goal was to determine whether suvorexant and IPSU affect sleep architecture independently of overall sleep induction. We therefore tested suvorexant (25 mg/kg) and IPSU (50 mg/kg) in mice during the inactive phase (lights on) when sleep is naturally more prevalent and when orexin levels are normally low. Whereas IPSU was devoid of effects on the time spent in NREM or REM, suvorexant substantially disturbed the sleep architecture by selectively increasing REM during the first 4 h after dosing. At the doses tested, suvorexant significantly decreased wake only during the first hour and IPSU did not affect wake time. These data suggest that OX<sub>2</sub>R preferring antagonists may have a reduced tendency for perturbing NREM/REM architecture in comparison with DORAs. Whether this effect will prove to be a general feature of OX<sub>2</sub>R antagonists vs. DORAs remains to be seen.

**Keywords:** orexin receptor antagonist, insomnia, pharmacology, REM and NREM sleep

## INTRODUCTION

Since the link between the hypocretin/orexin system and sleep disorders was discovered (Chemelli et al., 1999; Lin et al., 1999; Nishino et al., 2000), there has been much interest in developing orexin receptor antagonists (ORAs) for the treatment of insomnia. Several dual orexin receptor antagonists (DORAs) have now been tested in the clinic and have demonstrated sleep inducing properties in healthy volunteers and/or in patients suffering from insomnia (Brisbare-Roch et al., 2007; Winrow et al., 2011; Bettica et al., 2012a). Whereas ORAs are expected to be without the side effects characteristic of currently available treatments, questions about mechanism related safety have accompanied their development. As the lack of orexin signaling causes narcolepsy with cataplexy, there is concern that DORAs may induce sudden loss of motor control or sleep attacks. Whereas the therapeutic potential

for treating insomnia with ORAs is undisputedly high, there is less consensus regarding the necessity of targeting both receptors and whether selective antagonists might reduce potential safety concerns without loss of efficacy (Mieda et al., 2013).

Several lines of evidence suggest that selective OX<sub>2</sub>R antagonists may be sufficient for sleep induction and may have a reduced tendency for induction of cataplexy and/or narcolepsy in comparison with DORAs. In knockout mice, the sleep inducing properties of the DORA almorexant require intact OX<sub>2</sub>Rs but not OX<sub>1</sub>Rs (Mang et al., 2012). Also, selective OX<sub>2</sub>R antagonists induce sleep in rats and mice, whereas OX<sub>1</sub>R selective antagonists do not [(Dugovic et al., 2009; Steiner et al., 2013); but see Morairty et al. (2012)]. Together these findings strongly suggest that antagonizing OX<sub>2</sub>R may be sufficient for sleep induction.

In humans, orexin deficiency leads to narcolepsy with cataplexy (Nishino et al., 2000). The narcolepsy/cataplexy phenotype is mimicked in mice lacking orexin peptides, lacking orexinergic neurons or mice lacking both orexin receptors (OXR) (Chemelli et al., 1999; Hara et al., 2001; Kalogiannis et al., 2011). Although mice lacking OX<sub>2</sub>R also have sleep attacks, the incidence of cataplexy in these mice is close to null (Willie et al., 2003). Thus, there may be a reduced risk of inducing cataplectic events when only OX<sub>2</sub>R are antagonized. In dogs, mutations in OX<sub>2</sub>R alone are sufficient to cause narcolepsy with cataplexy. Interestingly, sporadic narcolepsy with cataplexy in dogs is associated with orexin deficiency resulting in more severe symptoms than the OX<sub>2</sub>R mutations (Baker et al., 1982; Ripley et al., 2001). It is tempting to speculate that OX<sub>2</sub>R antagonists might be less prone to induce symptoms similar to either narcolepsy or cataplexy than DORAs even in this very sensitive species. To date, although functionally relevant polymorphisms of the OXRs have been described in humans (Thompson et al., 2004; Rainero et al., 2008; Annerbrink et al., 2011), mutations of the receptors have not been linked to narcolepsy/cataplexy. Rather loss of the orexinergic neurons or, extremely rarely, mutations resulting in loss of the peptides have been reported to be the underlying cause (Peyron et al., 2000; Thannickal et al., 2000). Together, such findings suggest it may be a general rule that loss of OX<sub>2</sub>R signaling has a reduced propensity for inducing narcolepsy and/or cataplexy as compared to loss of the entire orexin signaling pathway.

We have therefore been interested in determining whether antagonists of OX<sub>2</sub>R are comparable to DORAs in their ability to induce sleep. Recently, we identified the orally bioavailable, brain penetrating OX<sub>2</sub>R preferring antagonist IPSU that induces sleep when administered at the start of the active (dark) phase in mice (Betschart et al., 2013). The aim of the present study was to investigate whether IPSU and the DORA suvorexant affect the natural sleep architecture, by testing low doses of the two compounds during the light phase when mice are primarily inactive and spend a high proportion of the time sleeping.

## METHODS

All experiments were performed according to Swiss guidelines and law and were approved by the Veterinary Authority of Basel-Stadt, Switzerland. Every effort was made to minimize the number of animals used and to minimize any pain or discomfort. Male C57Bl/6 mice weighing 25–35 g were single or group-housed on wood shavings in type II (14 × 16 × 22 cm) and type III (15 × 22 × 37 cm) cages, respectively. Each cage contained a nest box, a piece of wood and tissue paper nesting materials, and animals had access to food and water *ad libitum*. The housing cages were placed in a temperature and humidity controlled room (20–24°C, 45% humidity) with a light/dark cycle of 12:12 (lights on at 03:00, max 80 Lux).

Suvorexant and IPSU were both synthesized and purified *in house* according to published procedures (Cox et al., 2008; Betschart et al., 2013). We selected doses that were effective at promoting sleep in mice for the first 4 h when administered at the start of the dark phase [Betschart et al. (2013) and unpublished observations]. At the mouse OXRs, IPSU has about 6.2 ×

higher affinity at OX<sub>2</sub>R than OX<sub>1</sub>R (pK<sub>d</sub> OX<sub>1</sub>R 6.34, OX<sub>2</sub>R 7.23) whereas suvorexant is about 6.5 × more potent at OX<sub>1</sub>R than OX<sub>2</sub>R [pK<sub>d</sub> OX<sub>1</sub>R 8.77, OX<sub>2</sub>R 8.06; FLIPR assay Callander et al. (2013)]. Both compounds are highly brain penetrant. One hour after oral dosing of 50 mg/kg, brain levels reached 8778 pmol/g for IPSU and 10329 pmol/g for suvorexant giving free levels of 53.6 and 67.0 pmol/g, respectively (Betschart et al., 2013). In the present study we decided to dose IPSU at the previously effective dose of 50 mg/kg and to reduce suvorexant to 25 mg/kg to better match the estimated OX<sub>2</sub>R occupancy. At 1 h following 25 mg/kg suvorexant brain levels reached 3605 pmol/g and free levels were therefore 23.4 pmol/g. Estimating the available antagonist concentrations to be 53.6 and 23.4 nM for IPSU and suvorexant, we estimated receptor occupancies according to:

$$\text{Bound} = \text{Bmax}/(1 + \text{Kd}/\text{L})$$

where Bmax is 100%, Kd the affinity from the FLIPR assay, and L the free brain concentrations at the doses tested.

The expected occupancies of suvorexant are therefore 93% at OX<sub>1</sub>R, 73% at OX<sub>2</sub>R and for IPSU 11% at OX<sub>1</sub>R and 48% at OX<sub>2</sub>R. These values are similar to the effective values for sleep induction at OX<sub>2</sub>R reported by Gotter et al. (2013).

## IMPLANTATION OF ELECTROCORTICOGRAM/ ELECTROENCEPHALOGRAM (EEG) AND ELECTROMYOGRAM (EMG) ELECTRODES

Mice were administered buprenorphine (0.05 mg/kg s.c.) 1 h before surgery, anesthetized with ketamine/xylazine (110 mg/kg, 10:1, i.p.) and placed in a stereotaxic frame. The skull was exposed and four miniature stainless steel screws (SS-5/TA Science Products GmbH, Hofheim, Germany) attached to 36-gauge, Teflon-coated solid silver wires were placed in contact with the frontal and parietal cortex (3 mm posterior to bregma, ±2 mm from the sagittal suture) through bore holes. The frontal electrodes served as reference. The wires were crimped to a small 6-channel connector (CRISTEK Micro Strip Connector) that was affixed to the skull with dental acrylic. Electromyograph (EMG) signals were acquired by a pair of multistranded stainless steel wires (7SS-1T, Science Products GmbH, Hofheim, Germany) inserted into the neck muscles and also crimped to the headmount. After surgery, mice were kept singly in cages and allowed to recover on a heating pad. Buprenorphine, 0.05 mg/kg, s.c. was given 8 and 16 h after surgery to control pain. After 24 h, the mice were housed with their former cage mates and allowed to recover for 2 weeks.

## SLEEP STUDIES

Mice were habituated to individual cages in a sound-attenuated recording chamber for 6–10 days (lights on 03:00, lights off 15:00, max 80 lux) at a temperature of about 23°C. During the studies, mice had access to food and water *ad libitum*, to one sheet of nesting paper and a piece of wood but no nesting box. Mice were weighed and attached to recording cables that connected their headmounts to a commutator (G-4-E, Gauschi) allowing free movement in the experiment boxes, 1 day before beginning

the experiment. The recording chamber was opened each day during the light period between 08:00 and 09:00 to care for the mice and all experimental manipulations and oral applications were performed in a time window of 5–15 min before the start of the recordings at 09:00, exactly 6 h after lights on. Day 1, the mice were manipulated and habituated to the oral application syringe. Day 2, they received vehicle (methylcellulose 0.5%, 10 mL/kg, per os). Day 3, 50 mg/kg IPSU or 25 mg/kg suvorexant was administered *per os*. Recordings began at 09:00 (hour 0) and continued for 23 h. The experimental chamber was secured about 5 min prior to start of the recordings and the mice remained undisturbed for the next 23 h. On Day 4, the mice were returned to their normal housing cages for at least 2 weeks before returning to the experiment.

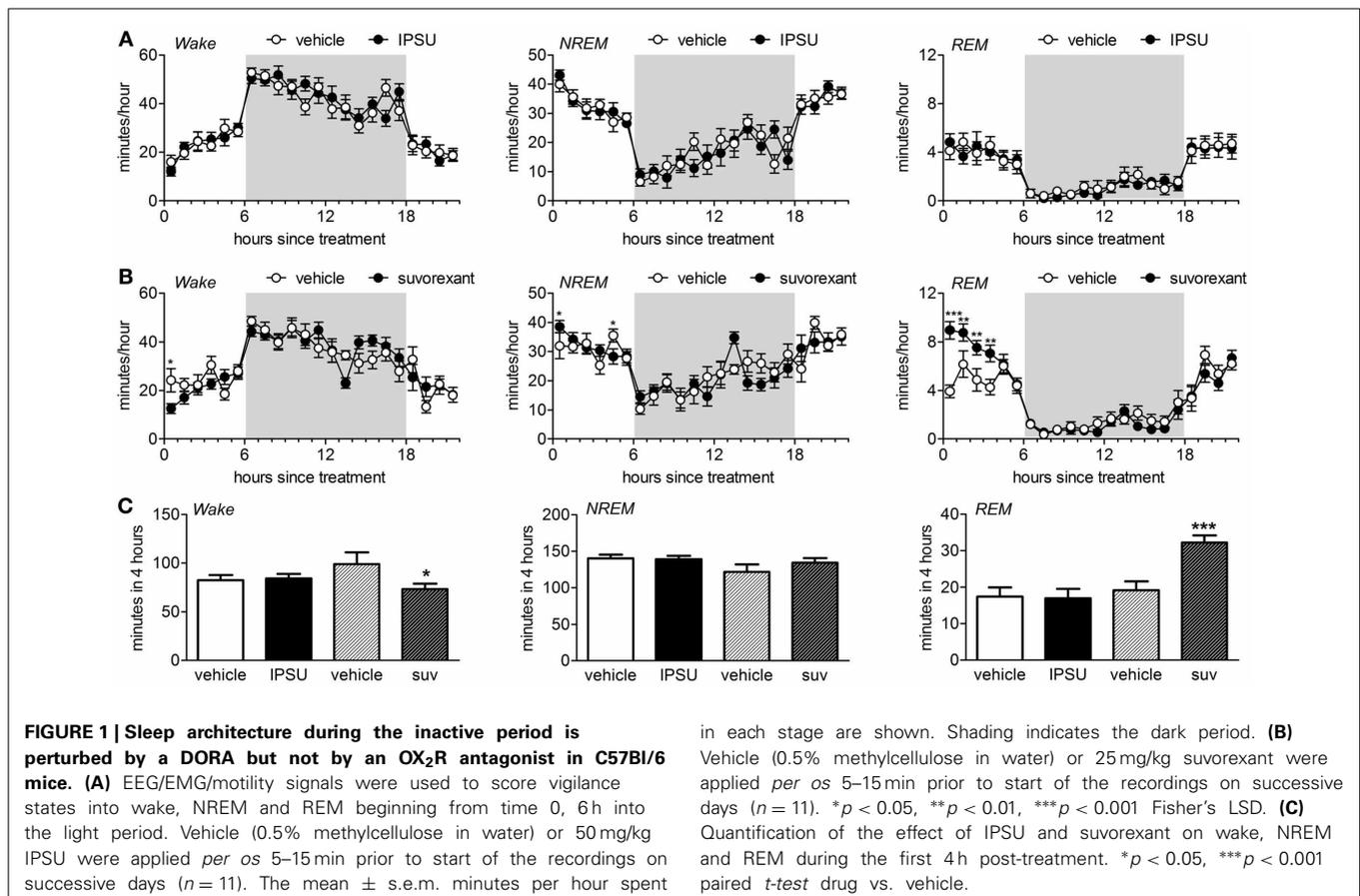
EEG/EMG signals were amplified using a Grass Model 78D amplifier (Grass Instrument CO., Quincy, MA, USA), analog filtered (EEG: 0.3–30 Hz, EMG: 5–30 Hz) and acquired using Harmonie V5.2 (acquisition frequency: 200 Hz with calibration the first day, record duration: 23h). Animals were video recorded during data collection, using an infrared video camera and locomotor activity was detected using infrared sensors (InfraMot Infrared Activity Sensor 30-2015 SENS, TSE Systems) placed in the roof of the boxes. Activity signals were acquired in 10 s intervals by the software Labmaster V2.4.4. EEG/EMG and activity recordings were imported into and scored in 10 s epochs using the rodent scoring module of Somnologia into wake, NREM

and REM sleep. Epochs during which there were state transitions were scored as the state present for at least 50% of the epoch. The time in each state per hour was calculated and mean  $\pm$  s.e.m. is shown. Restricted maximum likelihood analysis (REML) was applied to the data from the first 6 h to determine if there was a statistically significant effect of treatment or a significant interaction between treatment and hour. When either treatment or the interaction was significant ( $p < 0.05$ ), *post-hoc* Fisher's Least Significant Difference (LSD) tests were applied to determine hour by hour where there were significant differences between the vehicle and compound days.

The first 2 h following drug treatment were, in addition, manually scored to assess sleep-wake transitions including very short awakenings i.e., those with durations of 1–5 s that are often seen in mice and which would not be detected using 10 s epochs.

## RESULTS

The ORA IPSU (50 mg/kg) had no effect on the sleep architecture of mice when administered during the middle of the light phase (Figures 1A,C). The amount of time spent in wake, NREM and REM were unchanged following administration of IPSU relative to the previous day when vehicle was administered [treatment: wake  $F_{(1, 120)} = 0.002$ ,  $p = 0.96$ , NREM  $F_{(1, 120)} = 0.002$ ,  $p = 0.97$ , REM  $F_{(1, 120)} = 0.001$ ,  $p = 0.97$ , treatment  $\times$  hour: wake  $F_{(5, 120)} = 0.59$ ,  $p = 0.71$ , NREM  $F_{(5, 120)} = 0.56$ ,  $p = 0.73$ , REM  $F_{(5, 120)} = 0.44$ ,  $p = 0.82$ , REML].



In contrast, the DORA suvorexant (25 mg/kg) slightly decreased time spent in wake, had weak effects on time in NREM but strongly increased the amount of time spent in REM sleep during the first 4 h immediately following application [Figures 1B,C, treatment: wake  $F_{(1, 120)} = 3.4, p = 0.066$ , NREM  $F_{(1, 120)} = 0.44, p = 0.51$ , REM  $F_{(1, 120)} = 28.9, p < 0.001$ , treatment  $\times$  hour: wake  $F_{(5, 120)} = 2.31, p = 0.048$ , NREM  $F_{(5, 120)} = 1.76, p = 0.13$ , REM  $F_{(5, 120)} = 3.37, p = 0.007$ , REML].

Manual rescoring of the first 2 h after vehicle or drug application confirmed that the automatic scoring was in excellent agreement when amount of time in each stage was compared, better than 90% as previously reported by us and others (Pick et al., 2011; Mang et al., 2012). The number of awakenings was, however, different. Very short awakenings were found within epochs scored as NREM (Léna et al., 2004). We therefore used the results from the manual scoring to quantify the latency to NREM or REM and the sleep-wake transitions (Table 1).

Both IPSU and suvorexant showed a tendency to shorten the latency to NREM and REM sleep but only the shortening of latency to REM by suvorexant was significantly different from the vehicle. Awakenings from NREM were unaffected by either compound. Whereas awakenings from REM were significantly increased by suvorexant, the OX<sub>2</sub>R antagonist IPSU had no effect.

## DISCUSSION

Whereas IPSU did not perturb the normal sleep pattern of mice during the inactive period, suvorexant profoundly altered the sleep pattern, doubling the time spent in REM. The latency to REM and number of awakenings from REM was also selectively increased by suvorexant but not by IPSU. This pattern is similar to that found when these compounds were dosed at the start of the dark (active) period in mice (Betschart et al., 2013). Suvorexant promoted sleep primarily by increasing REM with small effects on NREM and IPSU promoted sleep primarily by promoting NREM and to a lesser degree REM (Betschart et al., 2013). The stronger enhancement of REM vs. NREM by suvorexant has also been seen in rats and in both healthy humans and humans suffering from insomnia (Winrow et al., 2011; Herring et al., 2012; Sun et al., 2013). Interestingly, our findings indicate that this DORA influences REM independently of the circadian dosing time, increasing the time in REM during both the light (active) phase and dark (active) phase whereas, at the dose tested, the OX<sub>2</sub>R preferring

antagonist IPSU influences sleep only during the active phase in mice.

Influencing the balance between REM and NREM is an area of potential differentiation between OX<sub>2</sub>R preferring antagonists and DORAs. Classic benzodiazepines, “Z drugs” such as zolpidem, and antidepressants are well known for suppressing REM sleep whereas ORAs certainly lack this property. Although IPSU is not highly selective for OX<sub>2</sub>R vs. OX<sub>1</sub>R at mouse receptors ( $\sim 6.2\times$ ), the opposite is true for suvorexant, which prefers mouse OX<sub>1</sub>R vs. OX<sub>2</sub>R ( $\sim 6.5\times$ , Betschart et al., 2013; Callander et al., 2013). Thus, the balance between antagonism of OX<sub>1</sub>R and OX<sub>2</sub>R may contribute to the differential effects of ORAs on sleep architecture. Our findings suggest that reducing the level of OX<sub>1</sub>R antagonism shifts the sleep balance toward NREM. Supporting this hypothesis, almorexant induces a greater REM increase in OX<sub>1</sub>R<sup>-/-</sup> than in wildtype mice (Mang et al., 2012). Likewise, whereas both almorexant and the OX<sub>2</sub>R antagonist JNJ-10397049 increased NREM during the light phase in rats, only almorexant also increased REM, and co-application of an OX<sub>1</sub>R antagonist significantly reduced the NREM induced by the OX<sub>2</sub>R antagonist (Dugovic et al., 2009). For the most part, DORAs increase REM more than NREM in rodent studies when % increase is considered (Brisbare-Roch et al., 2007; Winrow et al., 2011; Betschart et al., 2013; Black et al., 2013). However, the contribution of REM as a proportion of total sleep time varies for different compounds. For example, in mice almorexant-induced increases in REM remain within the proportion seen during normal sleep, even at high doses (Mang et al., 2012), whereas suvorexant increases REM proportion much above that seen during normal sleep (Betschart et al., 2013). Almorexant is unusual among the DORAs in that it appears to become a somewhat OX<sub>2</sub>R preferring antagonist *in vivo*. The *ex vivo* occupancy of almorexant was found to be about 2x higher and much longer lasting at OX<sub>2</sub>Rs (>12 h) vs. OX<sub>1</sub>Rs (Morairty et al., 2012). This preference is most likely driven by the unusual kinetics (Malherbe et al., 2009; Mang et al., 2012; Callander et al., 2013) so that with short exposures the compound may act as a DORA, and when equilibrium is allowed to be reached almorexant has functional selectivity for OX<sub>2</sub>Rs. Evidence against our hypothesis includes the description of a newer DORA that increases NREM preferentially in rats (Sifferlen et al., 2013). The structure of this compound is quite similar to that of almorexant but whether the kinetics also bias it toward OX<sub>2</sub>R selectivity when equilibrated

**Table 1 | Effect of the OX<sub>2</sub>R preferring antagonist IPSU and the DORA, suvorexant on latency to sleep and sleep to wake transitions during the first 2 h after administration during the inactive period.**

	Vehicle	IPSU	Diff	Vehicle	Suvorexant	Diff
Latency to NREM (minutes)	9.5 ± 3.0	2.6 ± 1.0	-7.0 ± 3.3	13.1 ± 5.9	4.8 ± 1.9	-8.3 ± 6.3
Latency to REM (minutes)	22.1 ± 3.4	14.5 ± 2.3	-7.6 ± 4.5	37.0 ± 10.1	8.4 ± 2.3*	-28.6 ± 8.8
Awakenings from NREM	25.1 ± 2.6	25.3 ± 2.0	0.2 ± 2.4	19.4 ± 1.6	18.6 ± 1.9	-0.8 ± 2.4
Awakenings from REM	10.2 ± 0.9	10.8 ± 1.1	0.6 ± 1.1	8.2 ± 1.4	15.6 ± 1.2**	7.4 ± 1.6
Short awakenings from NREM (<5s)	23.55 ± 2.8	26.5 ± 4.3	2.9 ± 1.9	25.7 ± 4.0	26.6 ± 3.1	0.9 ± 2.1
Total awakenings	58.8 ± 4.7	62.6 ± 5.1	3.7 ± 2.7	53.3 ± 4.9	60.8 ± 3.8***	7.5 ± 1.9

\* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ , paired *t*-test.

is unknown. Interestingly, species differences also exist in the effects of DORAs on sleep architecture. In dogs, suvorexant predominantly increased NREM (Winrow et al., 2011). The effects of DORAs on REM in humans appear to more closely mimic the effects in rodents rather than dogs as in the clinical settings, REM was preferentially enhanced with SB-649868 (Bettica et al., 2012a,b,c) and suvorexant (Herring et al., 2012; Sun et al., 2013). With the limited data available, whether the differential effects of the DORAs and OX<sub>2</sub>R antagonists on sleep architecture are in fact due to differences in receptor affinity/occupancy or are due to other factors such as compound class and species, remains to be seen as more compounds from different chemical classes are developed.

Why might a DORA be expected to influence REM more strongly than an OX<sub>2</sub>R preferring antagonist? Intracerebroventricular or local application of orexin-A in the highly OX<sub>1</sub>R expressing locus coeruleus reduces REM sleep, an effect that is blocked by the OX<sub>1</sub>R antagonist SB-334867 (Smith et al., 2003; Mieda et al., 2011). Additionally, knock-down of OX<sub>1</sub>R receptors in the locus coeruleus selectively increases REM, without affecting NREM during the active phase (Chen et al., 2010). Interestingly, we did not see a similar dependence of circadian time on the REM enhancement by suvorexant. REM sleep is not however, exclusively modulated by OX<sub>1</sub>Rs. OX<sub>2</sub>R knockdown in the lateral pontomesencephalic tegmentum increased REM both during the active and inactive phases (Chen et al., 2013) and while OX<sub>1</sub>R antagonists alone generally do not induce REM (Steiner et al., 2013), they may increase REM on top of the effects of OX<sub>2</sub>R antagonists (Dugovic et al., 2009). Overall, both OX<sub>1</sub>R and OX<sub>2</sub>R when activated or down regulated in the appropriate regions appear to be able to modulate REM sleep. However, the modulatory role of OX<sub>1</sub>R on REM may be greater than that of OX<sub>2</sub>R.

In conclusion, we hypothesize that selective OX<sub>2</sub>R antagonists have potential for the treatment of insomnia and may prove to perturb sleep architecture to a lesser degree than some of the DORAs. More highly selective antagonists from different chemical classes will be required to test this hypothesis further.

## AUTHOR CONTRIBUTIONS

All authors read and commented on the manuscript. Daniel Hoyer and Claudia Betschart led the team and participated in writing the manuscript. Thomas Dürst planned and performed experiments and analyzed data. Claudia Betschart, Samuel Hintermann, Dirk Behnke, Silvio Ofner, and Simona Cotesta synthesized and/or designed compounds. Grit Laue performed *in vivo* analyses related to pharmacokinetics. Markus Fendt, Laura H. Jacobson, and Eric Legangneux contributed to experimental design and writing the manuscript. Christine E. Gee designed the study, performed analysis, and wrote the manuscript.

## ACKNOWLEDGMENTS

This study was entirely funded by the Novartis Institutes for BioMedical Research, Novartis AG.

## REFERENCES

- Annerbrink, K., Westberg, L., Olsson, M., Andersch, S., Sjödin, I., Holm, G., et al. (2011). Panic disorder is associated with the Val308Ile polymorphism in the hypocretin receptor gene. *Psychiatr. Genet.* 21, 85–89. doi: 10.1097/YPG.0b013e328341a3db
- Baker, T. L., Foutz, A. S., McNERney, V., Mitler, M. M., and Dement, W. C. (1982). Canine model of narcolepsy: genetic and developmental determinants. *Exp. Neurol.* 75, 729–742. doi: 10.1016/0014-4886(82)90038-3
- Betschart, C., Hintermann, S., Behnke, D., Cotesta, S., Fendt, M., Gee, C. E., et al. (2013). Identification of a novel series of orexin receptor antagonists with a distinct effect on sleep architecture for the treatment of insomnia. *J. Med. Chem.* 56, 7590–7607. doi: 10.1021/jm4007627
- Bettica, P., Nucci, G., Pyke, C., Squassante, L., Zamuner, S., Ratti, E., et al. (2012a). Phase I studies on the safety, tolerability, pharmacokinetics and pharmacodynamics of SB-649868, a novel dual orexin receptor antagonist. *J. Psychopharmacol.* 26, 1058–1070. doi: 10.1177/0269881111408954
- Bettica, P., Squassante, L., Groeger, J. A., Gennery, B., Winsky-Sommerer, R., and Dijk, D.-J. (2012b). 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. doi: 10.1038/npp.2011.310
- Bettica, P., Squassante, L., Zamuner, S., Nucci, G., Danker-Hopfe, H., and Ratti, E. (2012c). The orexin antagonist SB-649868 promotes and maintains sleep in men with primary insomnia. *Sleep* 35, 1097–1104. doi: 10.5665/sleep.1996
- Black, S. W., Morairty, S. R., Fisher, S. P., Chen, T.-M., Warrior, D. R., and Kilduff, T. S. (2013). Almorexant promotes sleep and exacerbates cataplexy in a murine model of narcolepsy. *Sleep* 36, 325–336. doi: 10.5665/sleep.2442
- Brisbare-Roch, C., Dingemans, J., Koberstein, R., Hoever, P., Aissaoui, H., Flores, S., et al. (2007). Promotion of sleep by targeting the orexin system in rats, dogs and humans. *Nat. Med.* 13, 150–155. doi: 10.1038/nm1544
- Callander, G. E., Olorunda, M., Monna, D., Schuepbach, E., Langenegger, D., Betschart, C., et al. (2013). Kinetic properties of “dual” orexin receptor antagonists at OX<sub>1</sub>R and OX<sub>2</sub>R orexin receptors. *Front. Neuropharmacol. Rev.* 7:230. doi: 10.3389/fnins.2013.00230
- Chemelli, R. M., Willie, J. T., Sinton, C. M., Elmquist, J. K., Scammell, T., Lee, C., et al. (1999). Narcolepsy in orexin knockout mice: molecular genetics of sleep regulation. *Cell* 98, 437–451. doi: 10.1016/S0092-8674(00)81973-X
- Chen, L., McKenna, J. T., Bolortuya, Y., Brown, R. E., and McCarley, R. W. (2013). Knockdown of orexin type 2 receptor in the lateral pontomesencephalic tegmentum of rats increases REM sleep. *Eur. J. Neurosci.* 37, 957–963. doi: 10.1111/ejn.12101
- Chen, L., McKenna, J. T., Bolortuya, Y., Winston, S., Thakkar, M. M., Basheer, R., et al. (2010). Knockdown of orexin type 1 receptor in rat locus coeruleus increases REM sleep during the dark period. *Eur. J. Neurosci.* 32, 1528–1536. doi: 10.1111/j.1460-9568.2010.07401.x
- Cox, C. D., Breslin, M. J., and Coleman, P. J. (2008). Substituted diazepam orexin receptor antagonists. Patent No. WO2008/008158.
- Dugovic, C., Shelton, J. E., Aluisio, L. E., Fraser, I. C., Jiang, X., Sutton, S. W., et al. (2009). Blockade of orexin-1 receptors attenuates orexin-2 receptor antagonism-induced sleep promotion in the rat. *J. Pharmacol. Exp. Ther.* 330, 142–151. doi: 10.1124/jpet.109.152009
- Gotter, A. L., Winrow, C. J., Brunner, J., Garson, S. L., Fox, S. V., Binns, J., et al. (2013). The duration of sleep promoting efficacy by dual orexin receptor antagonists is dependent upon receptor occupancy threshold. *BMC Neurosci.* 14:90. doi: 10.1186/1471-2202-14-90
- Hara, J., Beuckmann, C. T., Nambu, T., Willie, J. T., Chemelli, R. M., Sinton, C. M., et al. (2001). Genetic ablation of orexin neurons in mice results in narcolepsy, hypophagia, and obesity. *Neuron* 30, 345–354. doi: 10.1016/S0896-6273(01)00293-8
- Herring, W. J., Snyder, E., Budd, K., Hutzelmann, J., Snavely, D., Liu, K., et al. (2012). Orexin receptor antagonism for treatment of insomnia: a randomized clinical trial of suvorexant. *Neurology* 79, 2265–2274. doi: 10.1212/WNL.0b013e31827688ee
- Kalogiannis, M., Hsu, E., Willie, J. T., Chemelli, R. M., Kisanuki, Y. Y., Yanagisawa, M., et al. (2011). Cholinergic modulation of narcoleptic attacks in double orexin receptor knockout mice. *PLoS ONE* 6:e18697. doi: 10.1371/journal.pone.0018697
- Léna, C., Popa, D., Grailhe, R., Escourrou, P., Changeux, J.-P., and Adrien, J. (2004). Beta2-containing nicotinic receptors contribute to the organization of sleep

- and regulate putative micro-arousals in mice. *J. Neurosci.* 24, 5711–5718. doi: 10.1523/JNEUROSCI.3882-03.2004
- Lin, L., Faraco, J., Li, R., Kadotani, H., Rogers, W., Lin, X., et al. (1999). The sleep disorder canine narcolepsy is caused by a mutation in the hypocretin (orexin) receptor 2 gene. *Cell* 98, 365–376. doi: 10.1016/S0092-8674(00)81965-0
- Malherbe, P., Borroni, E., Pinar, E., Wettstein, J. G., and Knoflach, F. (2009). Biochemical and electrophysiological characterization of almorexant, a dual orexin 1 receptor (OX1)/orexin 2 receptor (OX2) antagonist: comparison with selective OX1 and OX2 antagonists. *Mol. Pharmacol.* 76, 618–631. doi: 10.1124/mol.109.055152
- Mang, G. M., Dürst, T., Bürki, H., Imobersteg, S., Abramowski, D., Schuepbach, E., et al. (2012). The dual orexin receptor antagonist almorexant induces sleep and decreases orexin-induced locomotion by blocking orexin 2 receptors. *Sleep* 35, 1625–1635. doi: 10.5665/sleep.2232
- Mieda, M., Hasegawa, E., Kisanuki, Y. Y., Sinton, C. M., Yanagisawa, M., and Sakurai, T. (2011). Differential roles of orexin receptor-1 and -2 in the regulation of non-REM and REM sleep. *J. Neurosci.* 31, 6518–6526. doi: 10.1523/JNEUROSCI.6506-10.2011
- Mieda, M., Tsujino, N., and Sakurai, T. (2013). Differential roles of orexin receptors in the regulation of sleep/wakefulness. *Front. Endocrinol. (Lausanne)*. 4:57. doi: 10.3389/fendo.2013.00057
- Morairty, S. R., Revel, F. G., Malherbe, P., Moreau, J.-L., Valladao, D., Wettstein, J. G., et al. (2012). Dual hypocretin receptor antagonism is more effective for sleep promotion than antagonism of either receptor alone. *PLoS ONE* 7:e39131. doi: 10.1371/journal.pone.0039131
- Nishino, S., Ripley, B., Overeem, S., Lammers, G. J., and Mignot, E. (2000). Hypocretin (orexin) deficiency in human narcolepsy. *Lancet* 355, 39–40. doi: 10.1016/S0140-6736(99)05582-8
- Peyron, C., Faraco, J., Rogers, W., Ripley, B., Overeem, S., Charnay, Y., et al. (2000). A mutation in a case of early onset narcolepsy and a generalized absence of hypocretin peptides in human narcoleptic brains. *Nat. Med.* 6, 991–997. doi: 10.1038/79690
- Pick, J., Chen, Y., Moore, J. T., Sun, Y., Wyner, A. J., Friedman, E. B., et al. (2011). Rapid eye movement sleep debt accrues in mice exposed to volatile anesthetics. *Anesthesiology* 115, 702–712. doi: 10.1097/ALN.0b013e31822ddd72
- Rainero, I., Gallone, S., Rubino, E., Ponzio, P., Valfre, W., Binello, E., et al. (2008). Haplotype analysis confirms the association between the HCRTR2 gene and cluster headache. *Headache* 48, 1108–1114. doi: 10.1111/j.1526-4610.2008.01080.x
- Ripley, B., Fujiki, N., Okura, M., Mignot, E., and Nishino, S. (2001). Hypocretin levels in sporadic and familial cases of canine narcolepsy. *Neurobiol. Dis.* 8, 525–534. doi: 10.1006/nbdi.2001.0389
- Sifferlen, T., Koberstein, R., Cottreel, E., Boller, A., Weller, T., Gatzfeld, J., et al. (2013). Structure-activity relationship studies and sleep-promoting activity of novel 1-chloro-5,6,7,8-tetrahydroimidazo[1,5-a]pyrazine derivatives as dual orexin receptor antagonists. Part 2. *Bioorg. Med. Chem. Lett.* 23, 3857–3863. doi: 10.1016/j.bmcl.2013.04.071
- Smith, M. I., Piper, D. C., Duxon, M. S., and Upton, N. (2003). Evidence implicating a role for orexin-1 receptor modulation of paradoxical sleep in the rat. *Neurosci. Lett.* 341, 256–258. doi: 10.1016/S0304-3940(03)00066-1
- Steiner, M. A., Gatzfeld, J., Brisbare-Roch, C., Dietrich, H., Treiber, A., Jenck, F., et al. (2013). Discovery and characterization of ACT-335827, an orally available, brain penetrant orexin receptor type 1 selective antagonist. *ChemMedChem* 8, 898–903. doi: 10.1002/cmdc.201300003
- Sun, H., Kennedy, W. P., Wilbraham, D., Lewis, N., Calder, N., Li, X., et al. (2013). Effects of suvorexant, an orexin receptor antagonist, on sleep parameters as measured by polysomnography in healthy men. *Sleep* 36, 259–267. doi: 10.5665/sleep.2386
- Thannickal, T. C., Moore, R. Y., Nienhuis, R., Ramanathan, L., Gulyani, S., Aldrich, M., et al. (2000). Reduced number of hypocretin neurons in human narcolepsy. *Neuron* 27, 469–474. doi: 10.1016/S0896-6273(00)00058-1
- Thompson, M. D., Comings, D. E., Abu-Ghazalah, R., Jereseh, Y., Lin, L., Wade, J., et al. (2004). Variants of the orexin2/hcrt2 receptor gene identified in patients with excessive daytime sleepiness and patients with Tourette's syndrome comorbidity. *Am. J. Med. Genet. B Neuropsychiatr. Genet.* 129B, 69–75. doi: 10.1002/ajmg.b.30047
- Willie, J. T., Chemelli, R. M., Sinton, C. M., Tokita, S., Williams, S. C., Kisanuki, Y. Y., et al. (2003). Distinct narcolepsy syndromes in Orexin receptor-2 and Orexin null mice: molecular genetic dissection of Non-REM and REM sleep regulatory processes. *Neuron* 38, 715–730. doi: 10.1016/S0896-6273(03)00330-1
- Winrow, C. J., Gotter, A. L., Cox, C. D., Doran, S. M., Tannenbaum, P. L., Breslin, M. J., et al. (2011). Promotion of sleep by suvorexant—a novel dual orexin receptor antagonist. *J. Neurogenet.* 25, 52–61. doi: 10.3109/01677063.2011.566953

**Conflict of Interest Statement:** All authors were or are employees of Novartis AG and may own stock in the company.

Received: 30 August 2013; accepted: 20 November 2013; published online: 10 December 2013.

Citation: Hoyer D, Dürst T, Fendt M, Jacobson LH, Betschart C, Hintermann S, Behnke D, Cotesta S, Laue G, Ofner S, Legangneux E and Gee CE (2013) Distinct effects of IPSU and suvorexant on mouse sleep architecture. *Front. Neurosci.* 7:235. doi: 10.3389/fnins.2013.00235

This article was submitted to *Neuropharmacology*, a section of the journal *Frontiers in Neuroscience*.

Copyright © 2013 Hoyer, Dürst, Fendt, Jacobson, Betschart, Hintermann, Behnke, Cotesta, Laue, Ofner, Legangneux and Gee. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) or licensor are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.