

### **Archive ouverte UNIGE**

https://archive-ouverte.unige.ch

Article scientifique

Article 2011

Published version

**Open Access** 

This is the published version of the publication, made available in accordance with the publisher's policy.

\_ \_ \_ \_ \_

# Sleep-deprivation regulates α-2 adrenergic responses of rat hypocretin/orexin neurons

Uschakov, Aaron; Grivel, Jérémy; Cvetkovic Lopes, Vesna; Bayer, Laurence; Bernheim, Laurent; Jones, Barbara E; Muhlethaler, Michel; Serafin, Mauro

### How to cite

USCHAKOV, Aaron et al. Sleep-deprivation regulates  $\alpha$ -2 adrenergic responses of rat hypocretin/orexin neurons. In: PloS one, 2011, vol. 6, n° 2, p. e16672. doi: 10.1371/journal.pone.0016672

This publication URL:	https://archive-ouverte.unige.ch/unige:25150
Publication DOI:	<u>10.1371/journal.pone.0016672</u>

© The author(s). This work is licensed under a Creative Commons Attribution (CC BY) <u>https://creativecommons.org/licenses/by/4.0</u>

# Sleep-Deprivation Regulates α-2 Adrenergic Responses of Rat Hypocretin/Orexin Neurons

Aaron Uschakov<sup>1</sup><sup>®</sup>, Jeremy Grivel<sup>1</sup><sup>®</sup>, Vesna Cvetkovic-Lopes<sup>1</sup>, Laurence Bayer<sup>1</sup>, Laurent Bernheim<sup>1</sup>, Barbara E. Jones<sup>2</sup>, Michel Mühlethaler<sup>1</sup>, Mauro Serafin<sup>1</sup>\*

1 Département de Neurosciences fondamentales, Centre Médical Universitaire, Genève, Switzerland, 2 Department of Neurology and Neurosurgery, McGill University, Montreal Neurological Institute, Montreal, Quebec, Canada

### Abstract

We recently demonstrated, in rat brain slices, that the usual excitation by noradrenaline (NA) of hypocretin/orexin (hcrt/orx) neurons was changed to an inhibition following sleep deprivation (SD). Here we describe that in control condition (CC), i.e. following 2 hours of natural sleep in the morning, the  $\alpha_2$ -adrenergic receptor ( $\alpha_2$ -AR) agonist, clonidine, had no effect on hcrt/orx neurons, whereas following 2 hours of SD (SDC), it hyperpolarized the neurons by activating G-protein-gated inwardly rectifying potassium (GIRK) channels. Since concentrations of clonidine up to a thousand times (100  $\mu$ M) higher than those effective in SDC (100 nM), were completely ineffective in CC, a change in the availability of G-proteins is unlikely to explain the difference between the two conditions. To test whether the absence of effect of clonidine in CC could be due to a down-regulation of GIRK channels, we applied baclofen, a GABA<sub>B</sub> agonist known to also activate GIRK channels, and found that it hyperpolarized hcrt/orx neurons in that condition. Moreover, baclofen occluded the response to clonidine in SDC, indicating that absence of effect of clonidine in CC could not be attributed to down-regulation of GIRK channels. We finally tested whether  $\alpha_2$ -ARs were still available at the membrane in CC and found that clonidine could reduce calcium currents, indicating that  $\alpha_2$ -ARs associated with calcium channels remain available in that condition. Taken together, these results suggest that a pool of  $\alpha_2$ -ARs associated with GIRK channels is normally down-regulated (or desensitized) in hcrt/orx neurons to only become available for their inhibition following sleep deprivation.

Citation: Uschakov A, Grivel J, Cvetkovic-Lopes V, Bayer L, Bernheim L, et al. (2011) Sleep-Deprivation Regulates α-2 Adrenergic Responses of Rat Hypocretin/ Orexin Neurons. PLoS ONE 6(2): e16672. doi:10.1371/journal.pone.0016672

Editor: Fabien Tell, The Research Center of Neurobiology-Neurophysiology of Marseille, France

Received November 11, 2010; Accepted December 31, 2010; Published February 8, 2011

**Copyright:** © 2011 Uschakov et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Funding: Swiss Fonds National 31003A-113996. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors have declared that no competing interests exist.

\* E-mail: mauro.serafin@unige.ch

• These authors contributed equally to this work.

### Introduction

The hypocretin/orexin (hcrt/orx) neurons of the hypothalamus play an important role in maintaining arousal, since their absence results in narcolepsy with cataplexy (for reviews, see [1–4]). The hcrt/orx neurons exert their influence during waking when they discharge selectively [5,6], and through widespread projections [7] and excitatory actions upon multiple systems including all the activating and arousal systems in the brain [8].

Using rat brain slices, we have recently shown that hcrt/orx neurons are in turn normally excited by the multiple neurotransmitters of the arousal systems, including importantly noradrenaline [9]. However, using sleep-deprivation (SD), an approach used extensively to investigate sleep regulation and homeostasis (for recent review [10]), we discovered that following two hours of gentle SD, hcrt/orx neurons changed their response to noradrenaline from an excitation to an inhibition [11]. We proposed that such a phenomenon could contribute to the sleepiness that accompanies SD since during the natural sleep-wake cycle, the hcrt/orx neurons diminish firing before and cease firing during sleep [5,6] and since their absence results in narcolepsy associated with increased daytime sleepiness (for review [12]). Our goal in the present study was to investigate further the mechanisms underlying the emergence of inhibitory responses to noradrenaline following SD in rat brain slices. Our results suggest that a pool of  $\alpha_2$ -ARs associated with GIRK channels must become available for inhibition of hcrt/orx cells with SD.

### Results

## The $\alpha_2$ agonist clonidine hyperpolarizes hcrt/orx neurons following sleep-deprivation

To investigate the mechanism underlying the hyperpolarizing response to noradrenaline following sleep deprivation [11], hcrt/ orx neurons were identified according to criteria illustrated in a previous publication [13] and tested for their response to the bath-application of the  $\alpha_2$ -AR agonist clonidine in either CC or SDC (see Fig. 1A). It was found that in CC, hcrt/orx neurons never responded to clonidine applied at either 100 nM (n=6/6, not shown), 10  $\mu$ M (n=6/6, Fig. 1B) or even 100  $\mu$ M (n=3/3, not shown). In contrast, almost all hcrt/orx neurons decreased their activity in response to the agonist in SDC (n=5/6 with clonidine at 100 nM, Fig. 1C and n=9/10 with clonidine at 10  $\mu$ M, Fig. 1D). These results indicate that the difference between the CC

and SDC conditions cannot be explained by changing availability of G-proteins (see Discussion).

That the hyperpolarizing response was mediated by  $\alpha_2$ -ARs was confirmed by using a selective  $\alpha_2$ -AR antagonist, idoxazan (10  $\mu$ M; n = 3/3, Fig. 1E), which in SDC completely blocked the response to clonidine at 10  $\mu$ M. To test whether the inhibitory action of clonidine could result from the sleep deprivation procedure, we then applied the same procedure at a time of minimal homeostatic sleep pressure, i.e. during the dark period between 8 and 10 pm (see Methods), and found that, in this case, cells never responded to clonidine (n = 4/4, not shown).

These data show that the hyperpolarizing response of hcrt/orx neurons to noradrenaline that we have previously demonstrated in SDC [11] actually depend on  $\alpha_2$ -ARs which are regulated by SD. It is noteworthy that, in contrast, the depolarizing and excitatory responses to noradrenaline in CC described previously [11], were found here to depend on  $\alpha_1$ -ARs as they were mimicked by the  $\alpha_1$ -AR agonist L-phenylephrine. These  $\alpha_1$ -AR-dependent responses were however not regulated in the same manner as the  $\alpha_2$ -AR-dependent responses, as they did not change in between CC and SDC (n = 17/26 in CC and 5/12 in SDC; p = 0.15, Fischer exact test).





### Clonidine-dependent hyperpolarization following sleepdeprivation is mediated by GIRK channels

Since the activation of  $\alpha_2$ -ARs is known to hyperpolarize neurons through the opening of GIRK channels [14], we tested whether such channels were indeed implicated in the present effects of clonidine. For that purpose we first used barium which, at a small concentration (200 µM), is known to act as a blocker of GIRK channels [15]. When clonidine at 10 µM was applied in presence of barium in SDC, it was found to have no effect (n = 4/4in current clamp, Fig. 1F, and n = 3/3 in voltage-clamp, not shown). To further demonstrate the implication of GIRK channels in SDC, clonidine effects were studied while applying voltage ramps from -150 to +10 mV. When, in CC, current-voltage relationships before (artificial cerebrospinal fluid, ACSF, see Methods) and during application of clonidine at  $10 \,\mu\text{M}$  were compared, we found that clonidine had no effect (Fig. 2A, ACSF vs clonidine, n = 5). In contrast, in SDC, current-voltage relationships before (ACSF) and during application of 10 µM clonidine revealed currents (Fig. 2B, n = 4; mean  $E_{inv} \pm$  $SEM = -54.0 \pm 2.94 \text{ mV}$  that were small and outward at potentials positive to  $E_K$  (set at -55 mV, see Methods) but strongly rectified and became inward below E<sub>K</sub>.

## GIRK channels are not down-regulated in the control condition

One possible explanation for the above results could be that GIRK channels are not available in CC and become available in SDC. To test this hypothesis, we applied baclofen, a selective GABA<sub>B</sub> receptor agonist, also known to activate GIRK channels in many CNS neurons [15]. In current-clamp, whereas clonidine had no effect in CC, baclofen at 10  $\mu$ M, hyperpolarized every cell (n = 5/5, not shown). To demonstrate that the baclofen effect was



Figure 2. Effects of clonidine on hcrt/orx neurons in control and sleep deprived conditions. (A) In CC, as evident by subtraction of the current-voltage relationships obtained at voltages from -150 to +10 mV before (ACSF) and during application of clonidine at  $10 \mu$ M, bath-application of clonidine did not elicit any current in hcrt/orx neurons. (B) In SDC, in contrast, subtraction of the currents measured in presence and absence of clonidine revealed a clonidine-dependent inward potassium current. Indeed, the inversion potential is around -55 mV which is the inversion potential of potassium in the experimental conditions (see Methods). doi:10.1371/journal.pone.0016672.q002

due to the presence of GIRK channels, we next studied the effect of baclofen using voltage-clamp ramps. As illustrated in Fig. 3A, baclofen triggered an inwardly rectifying current (n = 4), thereby suggesting that in CC, GIRK channels are still available on the membrane.

To exclude the possibility that clonidine and baclofen acted on different populations of GIRK channels, we applied clonidine in SDC, following a prior application of baclofen, and found that clonidine evoked no additional effect in that condition (Fig. 3B, n = 4; compare with Fig. 2B). Altogether these data indicate that the lack of effect of clonidine in CC cannot be explained by a down-regulation of GIRK channels.

### Clonidine reduces calcium currents in control condition

Another possibility to explain the lack of responsiveness of hcrt/ orx neurons to clonidine in CC could be that the  $\alpha_2$ -ARs become down-regulated (or desensitized) in that situation [14,16]. If this were the case, one would expect that modulation of other membrane currents by these receptors would also be impeded.

Since activation of  $\alpha_2$ -ARs often produces a decrease in calcium currents [17], we examined whether this response to clonidine occurred in CC. For this purpose, cells had first to be identified electrophysiologically [13] using a regular electrode solution and then patched a second time with a cesium-containing electrode to decrease potassium currents. Some cells were in addition injected with neurobiotin for immunohistochemical identification (inset of Fig. 4A). Electrophysiologically identified hcrt/orx cells were then submitted, every 10 seconds, to a voltage step from -90 to 0 mV to evoke an inward calcium current (Fig. 4A). As evidenced in



Figure 3. GIRK channels are present on hcrt/orx neurons. (A) Subtraction of the current-voltage relationships obtained at voltages from -150 to +10 mV before (ACSF) and during application of baclofen at 10  $\mu$ M. revealed a baclofen-dependent inward current that reversed around -55 mV, which is the inversion potential of potassium in the experimental conditions. (B) In SDC, subtraction of the current-voltage relationships from -150 to +10 mV measured successively in presence of baclofen alone (baclofen) and following subsequent addition of clonidine.

doi:10.1371/journal.pone.0016672.g003

Fig. 4A and B, application of clonidine at 10  $\mu$ M reversibly decreased the total calcium current (n = 5). These results suggest that in CC, functional  $\alpha_2$ -ARs linked to calcium channels must still be available at the membrane.

### Discussion

This study shows that in CC, hcrt/orx neurons were insensitive to the  $\alpha_2$ -AR agonist, clonidine, whereas they were hyperpolarized, through the activation of GIRK channels, in SDC. Several lines of evidence suggest that a pool of  $\alpha_2$ -ARs associated with GIRK channels becomes available for inhibition of hcrt/orx cells with sleep deprivation.

In our original study on the consequences of SD [11], we showed that, in juvenile rat brain slices, noradrenaline usually depolarized and excited hcrt/orx neurons in CC, but hyperpolarized and inhibited the same cells in SDC. These data stood in contrast to those in mice, in which noradrenaline always hyperpolarized hcrt/orx cells [18,19]. It is noteworthy that since our own (unpublished) observations are in agreement with these data, the mouse model could not be used in the present study to further explore this process. In mice, the hyperpolarization of hcrt/orx cells depended on  $\alpha_2$ -ARs, but  $\alpha_1$ -dependent excitatory responses could also be evoked by L-phenylephrine, although only after the selective blockage by idoxazan [18], indicating that under baseline laboratory conditions in mice the  $\alpha_2$ -ARs predominate. Not surprisingly, we found that the hyperpolarizing responses to noradrenaline in SDC depended on  $\alpha_2$ -ARs since they could be mimicked by clonidine and antagonized by idoxazan. It is noteworthy, in contrast, that the excitatory action of noradrenaline observed in CC, which depends on  $\alpha_1$ -ARs, since it was mimicked by L-phenylephrine, was not similarly regulated, since it did not change significantly between CC and SDC. This indicates that the



Figure 4.  $a_2$ -ARs activation in the control condition reduces total calcium current in identified hcrt/orx neurons. (A) Total calcium current measured in response to a voltage step from -90 to 0 mV in a typical hcrt/orx neuron before (control), during (clonidine) and after (wash) bath-application of clonidine at 10  $\mu$ M. In the insets, the recorded neuron (arrowhead) is shown to be Hcrt/Orx-positive (middle inset) and neurobiotin-positive (right inset). (B) Time-dependent reduction of the total calcium current following transient application of clonidine at 10  $\mu$ M. BZX, 50  $\mu$ M D-APV, 30  $\mu$ M bicuculline, 1  $\mu$ M tetrodotoxin, 20 mM TEA, 4 mM 4-AP and 3 mM CICs in the ACSF.

inhibitory effect of noradrenaline, apparent in SDC [11], results from the emergence of an  $\alpha_2$ -AR dependent response which overrides the  $\alpha_1$ -AR dependent one. The reasons for the species differences in baseline responses to noradrenaline on the one hand and in responses to SD on the other hand, with hyperpolarizations as shown here in rats, versus depolarisations (following potentiation of glutamate responses) in mice [20] are not understood. Functionally however, with respect to the sleepiness normally accompanying SD (see final paragraph of Discussion), it must be stressed that the rat model seems more relevant than the mouse one.

This inhibitory action of clonidine in SDC is due to the opening of GIRK channels [15,21] as indicated by the voltage-dependence of the current elicited by the agonist with its characteristically marked inward rectification at potentials negative to  $E_K$  and its sensitivity to minute amounts of barium. These results are congruent with a number of studies in many different systems indicating that inhibitory actions of  $\alpha_2$ -AR activation can be mediated by activation of GIRK channels [14].

As clonidine was completely ineffective in CC, it appears that the regulation of noradrenergic responses in rat hcrt/orx cells is entirely dependent on the presence (in SDC) or absence (in CC) of  $\alpha_2$ -AR-dependent responses. To explain the absence of response in CC, several hypotheses can be evoked (for reviews [22–24]): a down-regulation of GIRK channels [24], a change in the availability of G-proteins (mediated by regulators of G-proteins, RGS [25] or guanine-nucleotide-dissociation inhibitor proteins, GDI [26]), a general desensitization of  $\alpha_2$ -ARs by clonidine [14,24], and finally a down–regulation (or heterologus desensitization) of a pool of  $\alpha_2$ -ARs associated with the GIRK channels [14,16,24].

With respect to the first hypothesis, we were able to demonstrate that down-regulation of GIRK channels could not be involved in the absence of response to clonidine in CC since GIRK-dependent responses to baclofen could still be evoked in that condition. In addition, occlusion experiments in SDC between clonidine and baclofen showed that the population of GIRK channels responding to both agonists must be the same. The second hypothesis, that a change in the availability of G-proteins under the regulatory influence of RGS or GDI proteins could explain the switch between CC and SDC, is not supported by our results either. Indeed, in studies that have tackled the question of modulation of responses to agonists by the availability of G-proteins, data suggest modest shifts in dose-responses [27-29]. In our experiments however, even at concentrations that were one thousand times higher in CC  $(100 \ \mu\text{M})$  than those efficient in SDC  $(100 \ n\text{M})$ , clonidine was completely ineffective. The third hypothesis, that the  $\alpha_2$ -ARs are desensitized in CC, is certainly not true for all  $\alpha_2$ -ARs, since clonidine was still able in that condition to decrease the total calcium current, as is the case in other preparations [17]. In addition, for those receptors responsible for the hyperpolarization in SDC, the mechanism is certainly not a homologous desensitization involving the continuous presence of the agonist [24]. Indeed, noradrenaline release during the two conditions must be exactly opposite to what would be needed for such an agonistmediated desensitization to occur. In CC, the animals have slept for 2 hours and thus noradrenaline release must have been relatively low, whereas during the waking associated with SD, noradrenaline release must certainly have been higher [30,31]. It is nonetheless during SD that the  $\alpha_2\text{-dependent}$  responses develop.

At this stage, with no evidence for either a down-regulation of GIRK channels or a change in G-protein availability, together with evidence for a persisting action of clonidine on calcium currents in CC, one is left with the hypothesis that, those  $\alpha_2$ -ARs

associated with calcium channels and those associated with GIRK channels must be differentially regulated. Those receptors associated with GIRK channels could thus form a pool that is down-regulated (or heterologously desensitized) in CC and which is up-regulated (or resensitized) selectively with sleep-deprivation to provide inhibition of hcrt/orx neurons. The signal associated with the sleep deprivation process that triggers that change remains to be investigated.

Functionally, the present data suggest that changing the response of hcrt/orx neurons to noradrenaline through the emergence of functional  $\alpha_2$ -ARs during sleep deprivation could contribute to the increased sleepiness associated with that condition. Regulation of adrenergic receptors could represent a homeostatic response to the continuous discharge by the hcrt/orx neurons that would occur during enforced waking [6]. Increasing hyperpolarization of the hcrt/orx neurons through the  $\alpha_2$ -ARs would serve to dampen their responsiveness and decrease their firing such as to indirectly facilitate sleep by removing their excitatory influence from their diverse targets, including the other arousal systems of the brain.

### **Materials and Methods**

### Experimental conditions

Young (17–21 days) Sprague-Dawley rats (Laboratoires Charles River, France) were maintained in a 12 h light/dark cycle (lights on from 8:00 am to 8:00 pm) and treated according to the regulations of the Swiss Federal Veterinary Office. The study was reviewed and approved by the "office vétérinaire cantonal" (*approval ID: 31.1.1007/3248/0*). As previously described, after a normal cycle of sleep and waking, rats were either allowed to sleep for 2 h from 8:00 to 10:00 am or kept fully awake; being gently sleep deprived (for details see [11,32] for the same 2 hours interval. Then, neurons were recorded between 11 am and 1 pm in control condition (CC) and sleep deprived condition (SDC), respectively. Some experiments were also conducted at night (between 8 and 10 pm), in which case night-vision goggles were used.

### Slice preparation

250 μm-thick hypothalamic coronal slices containing the hcrt/ orx neurons were obtained at 10 am from CC or SD rats. Slices were maintained *in vitro* for only 3 hours following sacrifice since EEG studies and our own previous study demonstrated that the consequences of sleep deprivation can be observed during the three hours following the deprivation protocol [11,33]. Tissue was left to recover for 1 hour at room temperature in artificial cerebrospinal fluid (ACSF) containing (in mM): 130 NaCl, 5 KCl, 1.25 KH<sub>2</sub>PO<sub>4</sub>, 1.3 MgSO<sub>4</sub>, 20 NaHCO<sub>3</sub>, 10 glucose and 2.4 CaCl<sub>2</sub>, and bubbled with 95% O<sub>2</sub> and 5% CO<sub>2</sub>. Subsequently, individual slices were transferred to a thermoregulated (32°C) recording chamber mounted on an upright microscope (Axioskop; Zeiss, Oberkochen, Germany) equipped with an infrared camera and continuously superfused at 4–5 ml/min with ACSF.

### Electrophysiology

Patch pipettes (4 to 7 M $\Omega$ ) were pulled on a DMZ universal puller (Zeitz-Instrumente, Munich, Germany) from borosilicate glass capillaries (GC150F-10; Harvard Apparatus, France) and filled with a solution containing (in mM) 126 KMeSO<sub>4</sub>, 4 KCl, 5 MgCl<sub>2</sub>, 0.1 BAPTA, 10 HEPES, 8 phosphocreatine, 3 ATP, 0.1 GTP (pH 7.3, 285–300 mOsm) and 2% neurobiotin (Vector Labs, REACTOLAB, S.A., Switzerland) to allow subsequent immuno-histochemical identification as previously described [13]. Whole-

cell recordings were performed between 11 am and 1 pm in the current-clamp or voltage-clamp mode using Axopatch 200A or 200B amplifiers (Molecular devices, Sunnywale, CA, USA). The membrane potential values were not compensated for junction potential (estimated at -9.6 mV). Voltage-clamp data were accepted when series resistance was  $<20 \text{ M}\Omega$  and changed <15% during recording, and analysed with Clampfit 10.0 (Molecular devices).

In voltage-clamp experiments designed to reveal a baclofen or clonidine-dependent activation of GIRK currents, the external potassium concentration was changed from 6.25 to 16 mM to raise the driving force for this ion. In these experimental conditions the equilibrium potential for potassium was estimated from the Nernst equation as  $E_{\rm K} = {\rm RT/F*ln}([{\rm K}^+]_{\rm out}/[{\rm K}^+]_{\rm in}) = -55$  mV. 100 ms-long ramps from -150 to +10 mV were repeated every 10 seconds before and following addition of clonidine or baclofen, respectively. Experiments were done in presence of 1  $\mu$ M tetrodotoxin (TTX, Latoxan, Rosans, France), 10  $\mu$ M NBQX, 50  $\mu$ M D-APV, 30  $\mu$ M bicuculline (all from Tocris) to block sodium action potentials and synaptic activity. Current traces are signals averaged from three traces for ACSF, baclofen and clonidine, respectively.

#### References

- Sakurai T (2007) The neural circuit of orexin (hypocretin): maintaining sleep and wakefulness. Nat Rev Neurosci 8: 171–81.
- 2. de Lecea L, Sutcliffe JG (2005) The hypocretins and sleep. Febs J 272: 5675–88.
- 3. Siegel JM (2004) The neurotransmitters of sleep. J Clin Psychiatry 65(Suppl 16): 4–7.
- Zeitzer JM, Nishino S, Mignot E (2006) The neurobiology of hypocretins (orexins), narcolepsy and related therapeutic interventions. Trends Pharmacol Sci 27: 368–74.
- Mileykovskiy BY, Kiyashchenko LI, Siegel JM (2005) Behavioral correlates of activity in identified hypocretin/orexin neurons. Neuron 46: 787–98.
- Lee MG, Hassani OK, Jones BE (2005) Discharge of identified orexin/ hypocretin neurons across the sleep-waking cycle. J Neurosci 25: 6716–20.
- Peyron C, Tighe DK, van den Pol AN, de Lecca L, Heller HC, et al. (1998) Neurons containing hypocretin (orexin) project to multiple neuronal systems. J Neurosci 18: 9996–10015.
- Jones BE, Muhlethaler M (2005) in The Hypocretins: Integrators of Physiological Systems, de Lecea L, Sutcliffe JG, eds. New-York: Springer. pp 289-301.
- Bayer L, Eggermann E, Serafin M, Grivel J, Machard D, et al. (2005) Opposite effects of noradrenaline and acetylcholine upon hypocretin/orexin versus melanin concentrating hormone neurons in rat hypothalamic slices. Neuroscience 130: 807–11.
- Andretic R, Franken P, Tafti M (2008) Genetics of Sleep. Annual Review of Genetics 42: 361–388.
- Grivel J, Cvetkovic V, Bayer L, Machard D, Tobler I, et al. (2005) The Wake-Promoting Hypocretin/Orexin Neurons Change Their Response to Noradrenaline after Sleep Deprivation. J Neurosci 25: 4127–4130.
- Dauvilliers Y, Arnulf I, Mignot E (2007) Narcolepsy with cataplexy. Lancet 369: 499–511.
- Eggermann E, Bayer L, Serafin M, Saint-Mleux B, Bernheim L, et al. (2003) The wake-promoting hypocretin-orexin neurons are in an intrinsic state of membrane depolarization. J Neurosci 23: 1557–62.
- Hein L (2006) Adrenoceptors and signal transduction in neurons. Cell Tissue Res 326: 541–51.
- Cruz HG, Ivanova T, Lunn M-L, Stoffel M, Slesinger PA, et al. (2004) Bidirectional effects of GABAB receptor agonists on the mesolimbic dopamine system. Nat Neurosci 7: 153–159.
- Saunders C, Limbird LE (1999) Localization and trafficking of alpha2adrenergic receptor subtypes in cells and tissues. Pharmacol Ther 84: 193–205.
- Bernheim L, Beech DJ, Hille B (1991) A diffusible second messenger mediates one of the pathways coupling receptors to calcium channels in rat sympathetic neurons. Neuron 6: 859–867.

In voltage-clamp experiments designed to investigate a clonidine-dependent effect on the total calcium current, the cells were first identified electrophysiologically with the above intrapipette solution. Then, after 20 minutes, the same electrophysiologically identified hcrt/orx cell was re-patched with a cesiumcontaining intrapipette solution (in mM): CsMeSulfonate 140, CsCl 10, Hepes 10, MgCl<sub>2</sub> 4, BAPTA 0.1, phosphocreatine 8, Na<sub>2</sub>-ATP 2, Na<sub>2</sub>-GTP 0.4 and neurobiotin 2% (pH = 7.4, 290-310 mOsm) whereby blocking potassium currents allowed the isolation of the total calcium current. Experiments were done in presence of 1 µM TTX to block sodium channels; 20 mM TEA, 4 mM 4-AP, 3 mM ClCs (all from Sigma) to block potassium channels externally and 10 µM NBQX, 50 µM D-APV, 30 µM bicuculline (all from Tocris) to block synaptic activity. A 100 mslong voltage step from -90 to 0 mV was repeated every 10 seconds allowing total calcium current to be recorded before, during and following transient application of clonidine (Sigma).

### **Author Contributions**

Conceived and designed the experiments: AU JG MM MS. Performed the experiments: AU JG VC-L L. Bayer MS. Analyzed the data: AU JG L. Bernheim MM MS. Wrote the paper: AU BEJ MM MS.

- Yamanaka A, Muraki Y, Ichiki K, Tsujino N, Kilduff TS, et al. (2006) Orexin neurons are directly and indirectly regulated by catecholamines in a complex manner. J Neurophysiol 96: 284–98.
- Li Y, van den Pol AN (2005) Direct and indirect inhibition by catecholamines of hypocretin/orexin neurons. J Neurosci 25: 173–83.
- Rao Y, Liu ZW, Borok E, Rabenstein RL, Shanabrough M, et al. (2007) Prolonged wakefulness induces experience-dependent synaptic plasticity in mouse hypocretin/orexin neurons. J Clin Invest 117: 4022–33.
- Stanfield PR, Nakajima S, Nakajima Y (2002) Constitutively active and Gprotein coupled inward rectifier K+ channels: Kir2.0 and Kir3.0. Rev Physiol Biochem Pharmacol 145: 47–179.
- Lomazzi M, Slesinger PA, Luscher C (2008) Addictive drugs modulate GIRKchannel signaling by regulating RGS proteins. Trends Pharmacol Sci 29: 544–9.
- Hanyaloglu AC, von Zastrow M (2008) Regulation of GPCRs by endocytic membrane trafficking and its potential implications. Annu Rev Pharmacol Toxicol 48: 537–68.
- Kelly E, Bailey CP, Henderson G (2008) Agonist-selective mechanisms of GPCR desensitization. Br J Pharmacol 153(Suppl 1): S379–88.
- Abramow-Newerly M, Roy AA, Nunn C, Chidiac P (2006) RGS proteins have a signalling complex: Interactions between RGS proteins and GPCRs, effectors, and auxiliary proteins. Cellular Signalling 18: 579–591.
- Willard FS, Kimple RJ, Siderovski DP (2004) RETURN OF THE GDI: The GoLoco Motif in Cell Division. Annual Review of Biochemistry 73: 925–951.
- Hank PJ, Stephen JG, Guillermo G-B, Alan FS, Ben JT, et al. (2008) Chronic cold exposure increases RGS7 expression and decreases α<sub>2</ sub>-autoreceptor-mediated inhibition of noradrenergic locus coeruleus neurons. European Journal of Neuroscience 27: 2433–2443.
- Labouebe G, Lomazzi M, Cruz HG, Creton C, Lujan R, et al. (2007) RGS2 modulates coupling between GABAB receptors and GIRK channels in dopamine neurons of the ventral tegmental area. Nat Neurosci 10: 1559–1568.
- Wiser O, Qian X, Ehlers M, Ja WW, Roberts RW, et al. (2006) Modulation of Basal and Receptor-Induced GIRK Potassium Channel Activity and Neuronal Excitability by the Mammalian PINS Homolog LGN. Neuron 50: 561–573.
- 30. Jones BE (2003) Arousal systems. Front Biosci 8: S438-51.
- Tononi G, Pompeiano M, Cirelli C (1994) The locus coeruleus and immediateearly genes in spontaneous and forced wakefulness. Brain Res Bull 35: 589–96.
- 32. Vyazovskiy Vea, Welker E, Fritschy J, Tobler I (2004) Regional pattern of metabolic activation is reflected in the sleep EEG after sleep deprivation combined with unilateral whisker stimulation in mice. Eur J Neurosci in press.
- Alfoldi P, Tobler I, Borbely AA (1990) Sleep regulation in rats during early development. Am J Physiol 258: R634–44.