Published in final edited form as:

Anesth Analg. 2013 August ; 117(2): . doi:10.1213/ANE.0b013e318297366e.

Propofol Stimulates Noradrenalin-Inhibited Neurons in the Ventrolateral Preoptic Nucleus by Reducing GABAergic Inhibition

Yu-Wei Liu, PhD,

Department of Anaesthesiology, UMDNJ, New Jersey Medical School, Newark, New Jersey

Wanhong Zuo, MD, MS, and

Department of Anaesthesiology, UMDNJ, New Jersey Medical School, Newark, New Jersey

Jiang-Hong Ye, MS, MD

Department of Anaesthesiology, UMDNJ, New Jersey Medical School, Newark, New Jersey

Abstract

Background—The cellular mechanisms underlying the sedative effect of general anesthetics are not completely understood. Accumulating evidence indicates that the ventrolateral preoptic area (VLPO) of the hypothalamus plays a critical role. The VLPO contains two major types of neurons, the noradrenalin-inhibited GABAergic projecting neurons (NA(–) neurons), and the noradrenalin-excited interneurons (NA(+) neurons) which are probably also gamma-aminobutyric acid (GABA)-containing neurons. Our previous work suggests that NA(–) neurons are normally under the inhibitory control of NA(+) neurons. Previous studies also show that GABAergic agents including propofol activate GABAergic projecting neurons in the VLPO, which is believed to lead to the inhibition of the arousal-producing nuclei in the tuberomammillary nucleus and sedation. However, how propofol activates VLPO neurons remains unclear. We explored the possibility that propofol activates NA(–) neurons indirectly, by inhibiting GABAergic transmission including those from VLPO NA(+) neurons.

Methods—Electrophysiological activities were recorded from VLPO cells in acute brain slices of rats.

Corresponding Author: Jiang-Hong Ye, MS, MD UMDNJ, New Jersey Medical School Department of Anaesthesiology, UMDNJ, New Jersey Medical School, 185 South Orange Avenue, Newark, NJ 07103 Phone: 973-972-1866 FAX: 973-972-4172 ye@umdnj.edu.

The authors declare no conflicts of interest.

Reprints will not be available from the authors.

DISCLOSURES: Name: Yu-Wei Liu, PhD

Contribution: This author helped design the study, conduct the study, analyse the data, and write the manuscript

Attestation: Yu-Wei has seen the original study data, reviewed the analysis of the data, and approved the final manuscript

Name: Wanhong Zuo, MD, MS

Contribution: This author helped analyse the data, and write the manuscript

Attestation: Wanhong has seen the original study data, reviewed the analysis of the data, and approved the final manuscript

Name: Jiang-Hong Ye, MS, MD

Contribution: This author helped design the study, analyse the data, and write the manuscript

Attestation: Jiang-Hong has seen the original study data, reviewed the analysis of the data, approved the final manuscript, and is the author responsible for archiving the study files

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Results—Propofol facilitates the discharges of NA(–) neurons and reduces the frequency, but not the amplitude of spontaneous GABAergic inhibitory postsynaptic currents in NA(–) neurons. Conversely, propofol suppressed the discharges of NA(+) neurons.

Conclusion—Propofol excites VLPO NA(–) neurons by reducing GABAergic transmission, at least in part by inhibiting VLPO NA(+) neurons. This may be a critical mechanism contributing to propofol-induced sedation.

Introduction

Propofol, a newer di-isopropylphenol compound, is the most widely used intravenous drug for induction of general anesthesia. The cellular mechanisms underlying its sedative effect are not completely defined. Accumulating evidence, however, indicates that the ventrolateral preoptic area (VLPO) of the hypothalamus in the endogenous sleep pathway plays a critical role.¹ The VLPO contains two major types of neurons: the majority are γ -Aminobutyric acid (GABA)-containing projecting neurons targeting the arousal-producing nuclei in the tuberomammillary nucleus (TMN).² This type of neuron is inhibited by noradrenalin (NA(–) neuron). Conversely, the minority are excited by noradrenalin (NA(+) neurons),³ which are probably interneuron and may contain GABA as well.⁴ Importantly, NA(–) neurons may normally be under the inhibitory control of NA(+) neurons.^{3,4} Using c-Fos expression, a previous study demonstrates that GABAergic agents, including propofol, activate the VLPO neurons.¹ These investigators interpret their data to indicate that by activating VLPO neurons, and the release of GABA into the TMN, propofol inhibits the activity of the arousal-producing nucleus, and consequently induces sedation.¹ However, it remains unclear how propofol activates VLPO neurons.

General anesthetics have marked effects on synaptic transmission. Most previous studies found that propofol enhances the function of GABA_A receptors (GABA_ARs)^{5–7} and may depress the release of glutamate, the major excitatory neurotransmitter.⁸

In the present study by using a combination of pharmacological and electrophysiological approaches we show that there are functional GABA_ARs on the NA(+) neurons in the VLPO. By activating these GABA_ARs, propofol suppresses NA(+) neurons and the release of GABA onto the NA(–) neurons, which results in the excitation of NA(–) neurons.

Materials and Methods

Brain Slice Preparation

All procedures were performed in accordance with National Institutes of Health guidelines and with the approval of the Animal Care and Utilization Committee of University of Medicine and Dentistry of New Jersey. All efforts were made to minimize the number of animals used and to minimize their suffering. We studied 40 Sprague-Dawley rats at postnatal days 20–30. Brain slices were prepared as described previously.⁴ Rats were anesthetized and then decapitated. Coronal slices (230–250 μ m thick) containing the VLPO were cut with a Compressstome VF-200 slicer (Precisionary Instruments Inc., Greenville, NC, USA), then immediately transferred to a holding chamber and incubated for at least 1 hour at room temperature (24°–25°C) in oxygenated (95% O₂/5% CO₂) artificial cerebrospinal fluid (ACSF) before the electrophysiological recording. The ACSF contained the following (in mM): 126 NaCl, 2.5 KCl, 1.25 NaH₂PO₄, 1 MgCl₂, 2 CaCl₂, 25 NaHCO₃, 1 L-ascorbate, and 11 glucose, and oxygenated with 95% O₂ / 5% CO₂ (carbogen).

Electrophysiology

Electrophysiological recordings were obtained with an Axon 200B amplifier, a Digidata 1440A A/D converter, and Clampfit 10.3 software (Molecular Devices Co., Union City, CA, USA). Data were filtered at 2 kHz and sampled at 5 kHz. All electrophysiological recordings were obtained at 32.6°C. ACSF was perfused at a rate of 1.5–2 ml/min. Patch pipettes had a resistance of 6–8 M Ω when filled with internal solution containing (in mM)¹ 140 cesium methanesulfonate, 5 KCl, 2 MgCl₂, 10 Hepes, 2 MgATP, 0.2 GTP for voltage clamp recordings, or 140 potassium gluconate, 5 KCl, 2 MgCl₂, 10 Hepes, 2 MgATP, and 0.2 GTP for current clamp recordings. The pH of both pipette solutions was adjusted to 7.2 with Tris-base and the osmolality to 310 mOsmol/L with sucrose. In voltage-clamp experiments, spontaneous inhibitory postsynaptic currents (sIPSCs) were examined in whole-cell configuration at a holding potential of 0 mV in the presence of AP5 (50 μ M), 6, 7-dinitroquinoxaline-2, 3-dione (DNQX) (20 μ M) and strychnine (1 μ M) to block glutamate and glycine receptors, respectively. Spontaneous discharges of VLPO neurons were recorded by the loose-patch cell-attached technique or in whole-cell mode.

Chemicals and application

We purchased DNQX, DL-2-amino-5-phosphono-valeric acid (DL-APV), strychnine, gabazine, 2, 6-diisopropylphenol (propofol), noradrenaline (NA) from Sigma (St Louis, MO). All chemicals were applied to the cell by bath perfusion.

Data Analysis and Statistics

Electrophysiological events were analyzed using Clampfit 10.3 (Molecular Devices Co). Drug-induced changes in the frequency and amplitude of sIPSCs and the frequency of firings in a given cell were calculated by averaging the changes induced by drug administration (a 3-min period at the peak of a drug response from each cell) and normalized to the pre-drug baseline. The data from the same treatment were pooled. All experimental values are presented as the mean $\pm$ se of the mean (S.E.M.) while n indicated the number of neurons tested. Changes in the firing/ membrane potential/ sIPSC within groups induced by propofol were analyzed by one-way analysis of variance (ANOVA) followed by the Bonferroni's *post hoc* test using corrected *P*-values. A paired *t*-test was used to analyze differences between control and drug groups (i.e., 10 μ M gabazine on firing rate). Dose-response data were fitted to the logistic equation: $y = 100x^a/(x^a + x_0^a)$, where *y* is the percentage change, *x* is the concentration of propofol, the slope parameter, and *x*₀ the propofol concentration which induces a half-maximal change. Two-tailed unpaired Student's *t*-tests were used to compare the change in firing rate/ membrane potential induced by propofol in the absence and presence of gabazine. To compare the frequency or amplitude of sIPSCs between control and propofol conditions, cumulative interevent-interval/ amplitude distributions were constructed, and the Kolmogorov–Smirnov (K-S test) statistic was applied to detect statistically important differences between interevent-interval/ amplitude distributions. Cumulative distributions of events were analyzed using the Kolmogorov–Smirnov test.) Significance for all analyses was determined at *P* < 0.05.

Results

Identification of VLPO neurons

Experiments were conducted in acute brain slices containing the VLPO. In keeping with previous reports,^{3,4,9} we found two major types of cells within the VLPO: the majority were triangular and multipolar in shape (Fig. 1A1), characterized by a potent low-threshold spike (LTS) (not shown) and inhibited by NA (100 μ M, 15 s) (NA(–) neurons, Fig. 1B1). Conversely, the minority was fusiform and bipolar in shape (Fig. 1A2), had no LTS (not

shown) and excited by NA (NA(+) neurons, Fig. 1B2). In the following experiments, we identified the cell type based on their pharmacological and morphological characteristics.

Propofol stimulates NA(–) neurons involving GABA_ARs

We first examined the effects of propofol on the spontaneous firing of NA(–) neurons under current clamp (Fig. 2A). Initially, these neurons spontaneously fired at 3.6 ± 0.5 Hz under control conditions and had a resting potential of -59.2 ± 3.1 mV (range: -53.7 to -64.3 mV, $n = 32$). The propofol-induced membrane depolarization depended on its concentration with an EC₅₀ of 70 nM ($F(5,32) = 16.5$, $P < 0.0001$, one-way ANOVA; Fig. 2B). Specifically, 1 nM, 100 nM, and 10 μ M propofol induced depolarization by 0.4 ± 0.05 mV ($n = 6$, $P = 1$), 3.2 ± 0.5 mV ($n = 9$, $P < 0.0001$), and 5.6 ± 1.2 mV ($n = 4$, $P < 0.0001$) (Bonferroni's posttest, Fig. 2A1 and 2B), respectively. Likewise, propofol concentration-dependently induced an acceleration of firing rate ($F(5,32) = 65.3$, $P < 0.0001$, one-way ANOVA, Fig. 2C): between 1 and 100 nM, propofol-induced acceleration increases with its concentrations (1 nM: 3.3 ± 1.8 %, $n = 6$, $P = 1$; 10 nM: 23.5 ± 3.6 %, $n = 7$, $P < 0.0001$; 100 nM: 47.3 ± 4.5 %, $n = 9$, $P < 0.0001$, Bonferroni's posttest; Fig. 2A and 2C). The effects of propofol were reversible after washout of propofol. Conversely, at 1 μ M, propofol had no significant effect (2.5 ± 1.6 %, $n = 6$, $P = 1$), and at 10 μ M, propofol significantly reduced the firing rate (-12.8 ± 2.6 %, $n = 4$, $P = 0.026$, Bonferroni's posttest; Fig. 2C).

General anesthetics including propofol have marked effects on synaptic transmissions. Most previous studies found that propofol enhances the function of GABA_ARs (¹⁰⁻¹³ from ⁹). To determine whether propofol-induced acceleration of firing of NA(–) neurons involves GABA_ARs, we compared propofol's action in the absence and presence of the specific GABA_AR antagonist gabazine. The application of gabazine (10 μ M) alone substantially accelerated the ongoing firing rate (by 35.7 ± 4.5 %, $n = 6$, $P < 0.0001$) and induced membrane depolarization (by 3.1 ± 0.3 mV, $n = 6$, $P < 0.0001$, paired *t*-test; Fig. 2D), indicating that NA(–) neurons are normally under strong tonic inhibition, mediated by GABA_ARs.⁴ After a new stable baseline was established in the continuous presence of gabazine, adding propofol (100 nM) only slightly changed the firing rate (5.2 ± 0.3 %, $n = 5$), or the membrane potential (0.3 ± 0.1 mV, $n = 5$; Fig. 2E), which are much less than the increases produced by 100 nM propofol in the absence of gabazine (firing rate: $P < 0.0001$; membrane potential: $P = 0.001$; unpaired *t*-test). When GABA_ARs are blocked, the stimulating effect of propofol may be underestimated due to nonlinear summation of excitation. That is, propofol's effect may be less in neurons with a higher firing rate. To test this possibility, we compared the effect of propofol on NA(–) neurons of different basic firing rates. As illustrated in Fig. 2F, propofol exerted similar acceleration on firing of NA(–) neurons regardless their basic firing rates.

Propofol decreases the frequency of spontaneous inhibitory postsynaptic currents (sIPSCs) on NA(–) neurons

We next recorded sIPSCs from NA(–) neurons in the presence of AP5 (50 μ M), DNQX (20 μ M) and strychnine (1 μ M) and at a holding potential of 0 mV. Under these conditions, the spontaneous events were eliminated by 10 μ M gabazine (data not shown), indicating that they were mediated by GABA_ARs. Propofol (100 nM) significantly decreased sIPSC frequency (by 57.5 ± 5.5 %, from 2.6 ± 0.3 to 1.1 ± 0.2 Hz, $n = 7$, $P < 0.0001$, Fig. 3A-B) and increase the mean amplitude (by 6.4 ± 0.8 %, from 42.4 ± 3.8 pA in control to 45.3 ± 4.5 pA in propofol, $n = 7$, $P < 0.0001$; paired *t*-test). These were further illustrated by cumulative plots of the incidence of various intervals (K-S = 59.2%, $P < 0.0001$) and amplitudes (K-S = 25.3%, $P < 0.0001$) between successive sIPSCs (K-S test, Fig. 3C).

Propofol suppresses the firing of NA(+) neurons

A major GABAergic input to the NA(−) neuron is from the local GABAergic interneurons: the NA(+) neurons. To determine the role of NA(+) neurons in propofol-induced reduction of sIPSCs in NA(−) neurons, we examined the effects of propofol (1–10,000 nM) on the spontaneous firing of NA(+) neurons (Fig. 4A). As expected, propofol suppressed the ongoing firing. The concentration dependence was well fitted by a logistic equation ($r^2 = 0.99$), giving an IC_{50} of 120 nM ($F(5,30) = 12.5$, $P < 0.0001$, one-way ANOVA; Fig. 4B), and the maximal inhibition was $-78.2 \pm 14.8\%$ of baseline ($n = 6$, $P < 0.0001$; Bonferroni's posttest). This effect of propofol was recovered to control levels ($97.8 \pm 3.1\%$ for firing rate, $n = 6$ and $98.3 \pm 3.3\%$ for membrane potential, $n = 6$) after washout of propofol.

Finally, to determine whether propofol-induced suppression of firing of NA(+) neurons involves GABA_ARs, we compared propofol's action in the absence and presence of gabazine. The application of gabazine (10 μ M) alone substantially accelerated the ongoing firing rate (by $37.8 \pm 3.9\%$, $n = 5$, $P < 0.0001$); and induced membrane depolarization (by 3.0 ± 0.4 mV, $n = 5$, $P < 0.0001$, paired t -test; Fig. 4C), indicating that NA(+) neurons are normally under GABA_AR-mediated tonic inhibition. After a new stable baseline was established in the continuous presence of gabazine, adding propofol (100 nM) slightly inhibit the firing rate ($0.3 \pm 0.1\%$, $n = 5$).

Discussion

Our major finding is that propofol excites VLPO NA(−) neurons. This is probably mediated by GABA_ARs, and by suppressing presynaptic GABA release, which is at least partly due to the inhibition of local GABAergic NA(+) neurons. Since NA(−) neurons send inhibitory projections to TMN histaminergic neurons, NA(−) neuron excitation will suppress TMN neurons; less histamine will be released and sedation may occur. This may contribute to propofol-induced sedation.

Propofol, one of the most potent IV anesthetics, is effective at submicromolar concentrations,¹⁰ but the underlying cellular and molecular mechanisms have not been well explored. Accumulating evidence, however, indicates that the VLPO of the hypothalamus in the endogenous sleep pathway plays a critical role.¹ The VLPO is a brain structure that plays a pivotal role in sleep regulation and rhythmicity.² Neurons in the VLPO were identified as sleep-active and involved in promoting and maintaining natural sleep, which provides inhibitory control of many of the arousal nuclei, including the TMN.²

Intracellular recordings in slices revealed only two cell types within VLPO: the NA(−) neurons, also named as LTS, and the NA(+) neurons, also named as non-LTS neurons.^{3,11} Most NA(−) neurons release GABA, but some also release the small inhibitory peptide galanin.^{1,10,12} We have previously shown that VLPO NA(−) neurons may normally be under the inhibitory control of NA(+) neurons.² In the current study, we showed that gabazine significantly stimulated NA(−) neurons, indicating that NA(−) cells are tonically inhibited by GABA, which might at least in part be released from NA(+) neurons. Gabazine eliminated propofol-induced stimulation of NA(−) neurons, suggesting that propofol's effect is mediated by GABA_ARs. However, in the presence of gabazine, the inhibitory effect of the NA(+) cell on the NA(−) cell should be suppressed (Fig. 5), and the effect of propofol on the NA(+) cell is difficult to evaluate. To get around this, we examined the effect of propofol on sIPSCs in NA(−) neurons. Propofol decreased the frequency without significantly changing the mean amplitude of sIPSCs, indicating a reduction of presynaptic release of GABA. We further showed that propofol suppressed the firing of NA(+) neurons, indicating that propofol's suppression of sIPSCs in NA(−) neurons may be due at least in part by suppressing NA(+) firing. Taken together, these results support the hypothesis that propofol

accelerates the firing of NA(−) neurons by the reduction of GABA, which might be released from NA(+) neurons.

Interestingly, the dose-dependent relationship of propofol's action on NA(−) neurons firing is a reversed U shape: while propofol at a lower concentration range facilitates the firing, at a higher concentration, propofol suppresses it. While the mechanism underlying this observation warrants further investigation, we can speculate that although propofol may exert its effect through enhancing the function of inhibitory GABA_ARs, these receptors are present on both the NA(−) and NA(+) neurons. The GABA_ARs in the NA(+) neurons may be more sensitive to propofol than those in NA(−) neurons; probably due to variations in subunit compositions, density of the receptors and their distributions, or a combination of these. In support of this, previous studies using point mutation indicated the sites on the α -subunit of the GABA_AR are relevant for the action of propofol,^{14,15} and propofol may selectively activate extrasynaptic GABA_ARs, which have subunits different from the synaptic counterpart.⁶ Furthermore, a previous study using positron emission tomography data from volunteers found that propofol's action negatively correlated most significantly with the regional distribution of [3H]diazepam and [3H]flunitrazepam (benzodiazepine) binding site densities.¹⁶ Therefore, while propofol's enhancement of GABA_ARs may inhibit both the NA(−) and NA(+) neurons, at lower concentrations, propofol-induced enhancement of the GABA_ARs in the NA(+) neurons or their terminals which make synapses on NA(−) neurons is dominated, which leads to the suppression of NA(+) neurons and the reduction in GABA release onto the NA(−) neurons. This will result in the excitation of NA(−) neurons by a mechanism of disinhibition. Conversely, at higher concentrations, the direct inhibitory effect of propofol on NA(−) neurons may become dominant and thus a suppression of NA(−) neurons occurs. Remarkably, in contrast to the reversed U shape dose-dependent relationship of propofol's effect on firing, the dose dependence of propofol-induced depolarization of the NA(−) cells is sigmoid. One plausible mechanism for this difference is that although depolarization induced by lower concentrations of propofol could increase firing of NA(−) neurons, stronger depolarization induced by higher concentrations of propofol could lead to reduced firing by the activation of the M type K⁺ channels¹⁷ or by inactivation of the voltage-gated Na⁺ channels on the NA(−) neurons.⁹

Previously, we have shown that propofol can facilitate glutamatergic excitatory postsynaptic currents to VLPO neurons, probably to the NA(−) neurons.⁹ Together, our work demonstrates that propofol can work in at least two ways to stimulate VLPO NA(−) neurons: by increasing excitatory postsynaptic currents and reducing IPSCs. Since NA(−) neurons send their inhibitory GABAergic projections to TMN histaminergic neurons, the excitation of NA(−) neurons will lead to the inhibition of TMN histaminergic neurons, resulting in sedation.

In conclusion, the present study shows that propofol at nanomolar concentrations enhanced discharges of NA(−) neurons by reducing axonal GABA release; at least in part by inhibiting VLPO NA(+) neurons. This may be a critical mechanism underlying propofol-induced sedation.

Acknowledgments

We thank Dr. Hong Nie for consulting on statistical data analysis.

Funding: This work is made possible by National Institutes of Health Grants AA15925, AA016964 to J.-H. Ye and National Natural Science Foundation of China 31371090, Foundation of Science and Technology Bureau of Wuhan, China 201250499145-27 to Y.-W. Liu.

References

1. Nelson LE, Guo TZ, Lu J, Saper CB, Franks NP, Maze M. The sedative component of anesthesia is mediated by GABA(A) receptors in an endogenous sleep pathway. *Nat Neurosci.* 2002; 5:979–84. [PubMed: 12195434]
2. Gallopin T, Fort P, Eggermann E, Cauli B, Luppi PH, Rossier J, Audinat E, Muhlethaler M, Serafin M. Identification of sleep-promoting neurons in vitro. *Nature.* 2000; 404(6781):992–995. [PubMed: 10801127]
3. Gallopin T, Fort P, Eggermann E, Cauli B, Luppi PH, Rossier J, Audinat E, Muhlethaler M, Serafin M. Identification of sleep-promoting neurons in vitro. *Nature.* 2000; 404:992–5. [PubMed: 10801127]
4. Liu YW, Li J, Ye JH. Histamine regulates activities of neurons in the ventrolateral preoptic nucleus. *J Physiol.* 2010; 588:4103–16. [PubMed: 20724362]
5. Trapani G, Altomare C, Liso G, Sanna E, Biggio G. Propofol in anesthesia. Mechanism of action, structure-activity relationships, and drug delivery. *Curr Med Chem.* 2000; 7:249–71. [PubMed: 10637364]
6. Bieda MC, MacIver MB. Major role for tonic GABAA conductances in anesthetic suppression of intrinsic neuronal excitability. *J Neurophysiol.* 2004; 92:1658–67. [PubMed: 15140905]
7. Kotani Y, Shimazawa M, Yoshimura S, Iwama T, Hara H. The experimental and clinical pharmacology of propofol, an anesthetic agent with neuroprotective properties. *CNS Neurosci Ther.* 2008; 14:95–106. [PubMed: 18482023]
8. Lingamaneni R, Birch ML, Hemmings HC Jr. Widespread inhibition of sodium channel-dependent glutamate release from isolated nerve terminals by isoflurane and propofol. *Anesthesiology.* 2001; 95:1460–6. [PubMed: 11748406]
9. Li KY, Guan YZ, Krnjevic K, Ye JH. Propofol facilitates glutamatergic transmission to neurons of the ventrolateral preoptic nucleus. *Anesthesiology.* 2009; 111:1271–8. [PubMed: 19934872]
10. Franks NP. General anaesthesia: from molecular targets to neuronal pathways of sleep and arousal. *Nat Rev Neurosci.* 2008; 9:370–86. [PubMed: 18425091]
11. Gallopin T, Luppi PH, Rambert FA, Frydman A, Fort P. Effect of the wake-promoting agent modafinil on sleep-promoting neurons from the ventrolateral preoptic nucleus: an in vitro pharmacologic study. *Sleep.* 2004; 27:19–25. [PubMed: 14998233]
12. Haas H, Panula P. The role of histamine and the tuberomammillary nucleus in the nervous system. *Nat Rev Neurosci.* 2003; 4:121–30. [PubMed: 12563283]
13. Gredell JA, Turnquist PA, Maciver MB, Pearce RA. Determination of diffusion and partition coefficients of propofol in rat brain tissue: implications for studies of drug action in vitro. *Br J Anaesth.* 2004; 93:810–7. [PubMed: 15377586]
14. Krasowski MD, Koltchine VV, Rick CE, Ye Q, Finn SE, Harrison NL. Propofol and other intravenous anesthetics have sites of action on the gamma-aminobutyric acid type A receptor distinct from that for isoflurane. *Mol Pharmacol.* 1998; 53:530–8. [PubMed: 9495821]
15. Jurd R, Arras M, Lambert S, Drexler B, Siegwart R, Crestani F, Zaugg M, Vogt KE, Ledermann B, Antkowiak B, Rudolph U. General anesthetic actions in vivo strongly attenuated by a point mutation in the GABA(A) receptor beta3 subunit. *FASEB J.* 2003; 17:250–2. [PubMed: 12475885]
16. Alkire MT, Haier RJ. Correlating in vivo anaesthetic effects with ex vivo receptor density data supports a GABAergic mechanism of action for propofol, but not for isoflurane. *Br J Anaesth.* 2001; 86:618–26. [PubMed: 11575335]
17. Peretz A, Sheinin A, Yue C, Degani-Katzav N, Gibor G, Nachman R, Gopin A, Tam E, Shabat D, Yaari Y, Attali B. Pre- and postsynaptic activation of M-channels by a novel opener dampens neuronal firing and transmitter release. *J Neurophysiol.* 2007; 97:283–95. [PubMed: 17050829]

Figure 1. Noradrenaline inhibited NA(-) and excited NA(+) neurons in VLPO

A multipolar neuron (**A1**), and a fusiform and bipolar neuron (**A2**), scale bar 10 μ m. **B1**, A brief application of noradrenaline (NA, 100 μ M, 15 s) suppressed the ongoing discharges of a multipolar ventrolateral preoptic area (VLPO) neuron. Upper panel: exemplar current traces taken from the time points indicated in the lower panel. **B2**, A brief application of noradrenaline (NA, 100 μ M, 15 s) robustly accelerated ongoing discharges of a fusiform bipolar neuron. Upper panel: exemplar current traces taken from the time points indicated in the lower panel.

Figure 2. Propofol excites NA(-) neurons in VLPO

A1, Spontaneous firings obtained at times indicated by lowercase letters in **A2**. The dotted lines in this and in the following figures indicate the resting membrane potentials. The distance of two dotted lines between two arrows represents the depolarization induced by propofol. **A2**, Time course of propofol (PRO)-induced increase in spontaneous firing rate. **B**, Concentration-response relationship of PRO-induced membrane depolarization ($F(5, 32) = 16.5$, $P < 0.0001$, PRO application vs. PRO-free). In all the figures, filled circles and error bars are means $\pm$ S.E.M. Numbers of cells tested in every group are indicated above the curve. The smooth curve is the best fit of the data to the logistic equation ($r^2 = 0.99$). **C**, % changes of NA(-) firing rate induced by different concentrations of PRO ($F(5, 32) = 65.3$,

$P < 0.0001$). **D1**, Spontaneous firings obtained at times indicated by lowercase letters in **D2**. **D2**, Time course of gabazine (GAZ)-induced increase in spontaneous firing rate. **E1**, Spontaneous firings obtained at times indicated by lowercase letters in **E2**. **E2**, Time course of PRO-induced increase in spontaneous firing rate in the presence of 10 μ M gabazine. **F**, PRO, 100 nM) accelerates NA(-) neuron firing with different basic firing rates. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, one-way analysis of variance (ANOVA) followed by Bonferroni's *post hoc* test, propofol application *vs.* control.

Figure 3. Propofol decreases sIPSCs in NA(-) neurons

A, Recorded at a holding potential of 0 mV and in the presence of AP5 (50 μ M), 6, 7-dinitroquinoxaline-2, 3-dione (DNQX) (20 μ M) and strychnine (1 μ M), spontaneous inhibitory postsynaptic currents (sIPSCs) traces from time points indicated in **B**. **B**, Time course of propofol-induced decrease in sIPSC frequency. **C**, Cumulative probability plots of the incidence of various inter-event intervals and amplitudes, recorded in control conditions and during the application of propofol (PRO) to the same neuron, were analyzed with the Kolmogorov–Smirnov (K-S) test. Representative plots show decreased incidence of short intervals (*left*, K-S test, $P < 0.0001$), and increase the amplitudes (*right*, K-S test, $P < 0.0001$). Summary of % changes in sIPSCs frequency or amplitude are listed. The number of cells is indicated.

Figure 4. Propofol inhibits NA(+) neurons in VLPO

A1, Spontaneous firings obtained at times indicated by lowercase letters in **A2**. **A2**, Time course of propofol (PRO)-induced decrease in spontaneous firing rate. **B**, the reduction in firing rate depends on the concentrations of PRO ($r^2 = 0.99$, $F(5, 30) = 12.5$, $P < 0.0001$). $*P < 0.05$, $***P < 0.001$, one-way ANOVA followed by Bonferroni's *post hoc* test with collected *P*-value, propofol application vs. control. **C1**, Spontaneous firings obtained at times indicated by lowercase letters in **C2**. **C2**, Time course of changes in spontaneous firing rate in the presence of gabazine (GAZ, 10 μ M) and propofol (PRO, 100 nM).

Figure 5. Working model of how propofol excites VLPO NA(-) neurons and induces sedation
 Propofol enhances the inhibitory gamma-aminobutyric acid receptors (GABA_ARs) in the NA(+) neurons, thus reduces the activity of these neurons and the release of GABA onto the NA(-) neurons. The NA(-) neurons will be excited as a result of disinhibition, which will result in: (1) more GABA being released onto the tuberomammillary nucleus, (2) less histamine being released onto the cortex and (3) sedation.