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Overlapping Distributions of Parvalbumin Immunoreactivity and Projections of Orexin Neurons in Sleep-Wake Related Brain Areas in the Mouse

Distribuciones Superpuestas de Inmunorreactividad de Parvalbúmina y Proyecciones de Neuronas de Orexina en Áreas Cerebrales Relacionadas con el sueño y la Vigilia en el Ratón

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SUMMARY: The hypothalamic peptide orexin has a prominent role in arousal-related processes. Parvalbumin (PV) positive neurons, as one of the most important subtypes of γ-aminobutyric acid (GABA) interneurons, which also participate in sleep-wake regulation. To broaden the understanding of the neural control and connection between these two types of neurons in the sleep associated nuclei, a double-label immunohistochemical method was used to investigate the overlap between PV immunopositive distributions and projections of orexin neurons in mouse brain. In the basal forebrain, overlap was observed in the nucleus accumbens shell (AcbSh), the nucleus of the horizontal limb of the diagonal band (HDB), magnocellular preoptic nucleus (MCPO), substantia innominata (SI), but avoided the nucleus accumbens core (AcbC). In the diencephalon, this dual innervation extended in the direction near the edge of the 3rd ventricle (3V) to the ventrolateral preoptic nucleus (VLPO), although the density of PV neurons there was somewhat lighter. Orexin immunoreactive neurons in several lateral hypothalamic areas (LH) were embedded within dense clusters of PV processes when the buttons of orexin were in obvious contact with PV immunoreactivity. In the remainder of hypothalamus, mainly including the ventral tuberomammillary nucleus (VTM), there were few fine orexin fibers and buttons contact with PV soma. Intermingled orexin and PV immunoreactivity were observed in the ventral tegmental area (VTA), and sparse overlap was observed in the ventrolateral periaqueductal gray (VLPAG) and laterodorsal tegmental nucleus (LDT) in the brainstem. Thus, these two different types of neurons, originating in different parts of the brain, together target several brain regions and brainstem monoaminergic nuclei involved in the roles of motivation, stress, locomotion, especially in the regulation of sleep.

KEY WORDS: parvalbumin; Orexin; Hypothalamus; Basal forebrain; Behavioral state.

INTRODUCTION

The orexin neurons, scattered across the lateral hypothalamic area (LH), strongly excite all the wake-promoting brain plus the midline thalamus and cortex and receive inputs from multiple brain areas that are involved in the regulation of sleep/wakefulness (Scammell *et al*., 2017). Orexin neurons also project axons throughout the brain and spinal cord to modulate downstream neuronal activity by orexin itself and with other co-transmitters that are synthesized in the orexin neurons and released with orexin (Scammell *et al*., 2017). Intracerebroventricular administration of synthesized orexin peptide in rodents increased wakefulness and suppressed rapid eye movement (REM) and non-rapid eye movement (NREM) sleep in a dose-dependent manner (Hagan *et al*., 1999).

Parvalbumin (PV) neurons account for about 40 %~50 % of the total number of GABA inhibitory interneurons. Activation of the projection pathway from PV positive neurons in the basal forebrain (BF) to the reticular thalamic nucleus (Rt) induces arousal and inhibits NREM sleep and spindles (Thankachan *et al*., 2019). In addition, inhibition of PV neurons in Rt do not affect the formation of spindles, but increase the awakening time (Marín, 2012). Studies have also demonstrated that the REM sleep period of mice is accompanied by the activation of PV positive neurons in the cerebral cortex, and the activity of pyramidal neurons in this period is mainly inhibited by PV neurons (Niethard *et al*., 2016). Optical stimulation of BF-PV neurons produced rapid transitions to wakefulness from NREM sleep

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but does not affect REM-wakefulness transitions (McKenna *et al*., 2020). Thus, PV neurons and their neural connection pathway may play an important role in sleep-wake regulation.

Currently, known brain regions related to sleep mainly include the ventrolateral preoptic nucleus (VLPO) (Scammell *et al*., 2017), brainstem, Rt (Scammell *et al*., 2017), and nucleus accumbens (Acb) (Lazarus *et al*., 2013), etc. The main arousal regulatory systems include BF cholinergic cell groups, dorsal raphe nucleus (DR), locus coeruleus (LC), LH, ventral tegmental area (VTA), tuberomammillary nucleus (TMN), ventral periaqueductal gray matter (vPAG), and laterodorsal tegmental nucleus (LDT) (Scammell *et al*., 2017).

The present study was undertaken to provide a detailed description of overlapping distributions of PV immunoreactivity and projections of orexin neurons in above-mentioned sleep-wake related brain areas in the mouse brain by double-labeling technique. The present data provide anatomic evidence supporting coordinated orexin/PV actions within these brain regions.

MATERIAL AND METHOD

Experimental Animals. Male SPF inbred C57BL/6J mice (Shanghai SLAC Laboratory Animal Co., Ltd., China), each weighing about 20-26 g, were used in this study. Mice were housed in the Laboratory Animal Research Centre of Zhejiang Chinese Medical University and maintained in a temperature and light controlled environment (lights on at 07:00 and off at 19:00, an ambient temperature of 21-23 ˚C, and a relative humidity of 50-60 %). Standard mouse chow and water was available *ad libitum*. All efforts were made to minimise the number of animals utilised in this study and any pain and discomfort experienced by the mice.

Ethical consideration. The protocol for animal handling was approved and followed the animal guidelines established by the Laboratory Animal Research Centre of Zhejiang Chinese Medical University, Hangzhou, in Zhejiang province. (Protocol Number: IACUC-20220627-07).

Tissue preparation. Animals (n=4) were deeply anesthetized with sodium pentobarbital (60 mg/kg, i.p.). Upon confirmation that the mice were unresponsive to tail- or foot-pinching, they were perfused with saline and then fixed with 4 % paraformaldehyde. After postfixed for 4 h at 4 ˚C, and then cryoprotected in 10 %, 20 %, and 30 % sucrose in 0.1 M phosphate buffered saline (PBS) at 4 ˚C until they sank. Four sets of 30 μm sections were prepared using a Leica freezing microtome (Leica, type 820-II) at the coronal plane, mounted in a cryoprotectant solution, and the stored at -20 °C until immunohistochemical staining.

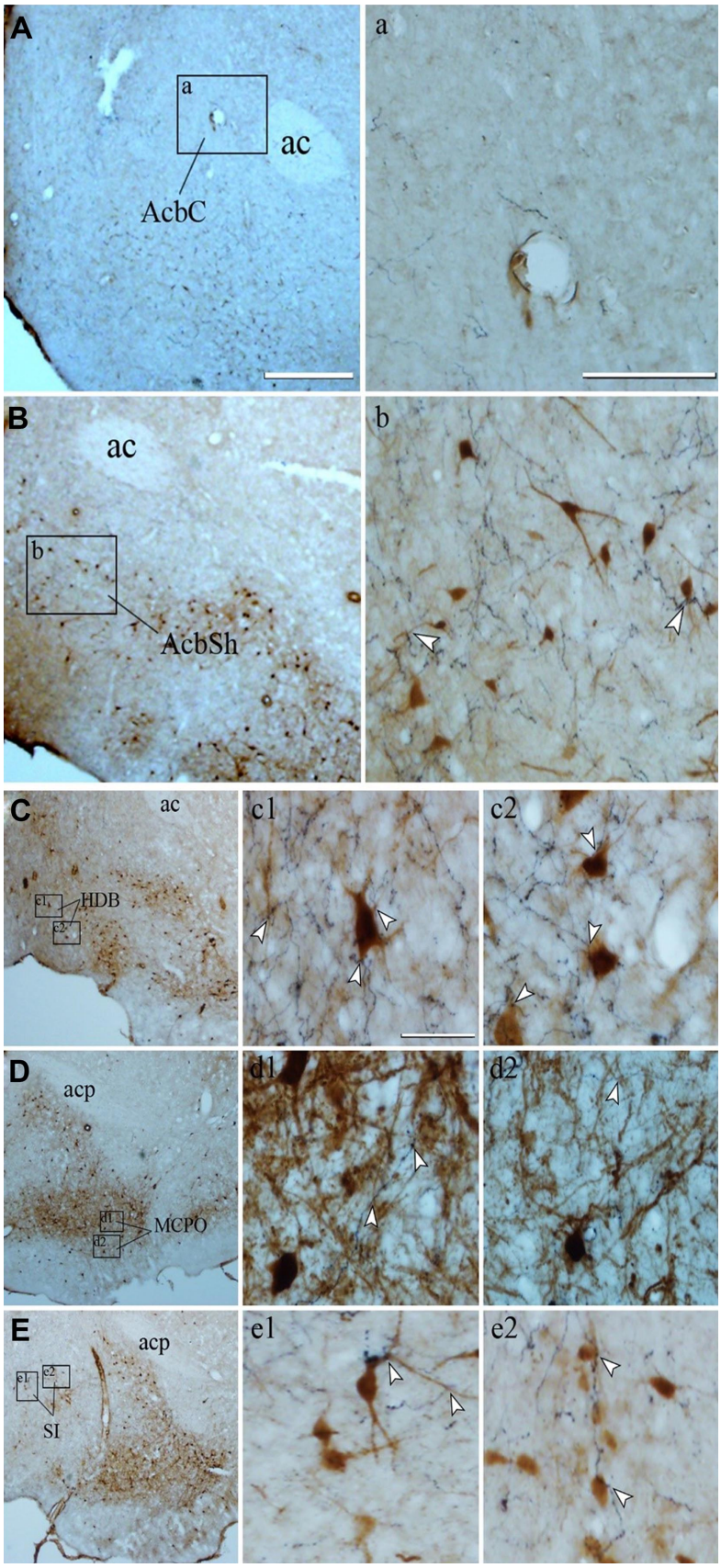
Immunohistochemistry. The first set of stored free-floating tissue sections were rinsed in PBS, washed with 3 % H_2O_2 in PBS for 30 min, blocked with 10 % goat serum (Cat. No. S-1000, Vector Laboratories, Tucson, AZ, USA) and 0.3 % Triton X-100 in PBS for 1 h at room temperature, and then incubated with the primary antiserum for 24 h at 4 °C. The primary antibodies consisted of a monoclonal mouse anti-orexin (1:1,000, Cat. No. sc-80263, Santa Cruz Biotechnology, Santa Cruz, CA, USA) to identify orexin neurons and a monoclonal rabbit anti-PV (1:1,000, Cat. No. ab181086, Abcam, Cambridge, UK) to identify PV neurons. Then the sections were incubated with secondary antibodies (a biotinylated goat anti-mouse IgG antibody (Cat. No.BA-9200, Vector Laboratories, Burlingame, CA, USA) and a biotinylated goat anti-rabbit IgG antibody (Cat. No. BA-1000; Vector Laboratories) for 2 h at room temperature, followed by avidin-biotin-horseradish peroxidase complex (ABC) solution (Vectastain Elite ABC Kit; Vector Laboratories; Cat. No. PK-4000). The sections were rinsed with PBS and then visualized with diaminobenzidine (DAB) in the presence of nickel ammonium sulphate. Then, the sections were rinsed again with PBS, placed on gelatin-coated slides, air-dried overnight, dehydrated across an ascending alcohol gradient, cleared in xylene, and coverslipped.

Microscopy. The sections were assessed, and images were captured using an Olympus IX71 microscope. We employed Adobe Photoshop CS2 in post-processing the digital photomicrographs. The anatomical landmarks employed in this study were based on a mouse brain atlas. The outlines of the sections and major structures were evaluated at low magnification (4×), followed by mapping the profiles of the immunoreactive neurons using high magnification.

RESULTS

Acb. Overlap of both fiber types seemed to avoid the nucleus accumbens core (AcbC) (Fig. 1A, a). The axons or dendrite of PV were in close contact with the thicker buttons and fibers of orexin in the nucleus accumbens shell (AcbSh) while the PV soma overlapped with the orexin fibers to a less extent (Fig. 1B, b).

The nucleus of the horizontal limb of the diagonal band (HDB). HDB contained considerable overlap between orexin and PV immunoreactivity. The orexin varicose fibers were randomly dispersed among the PV neurons there. In this region, the PV neurons had larger soma, and dispersed among PV slender axons were thin orexin fibers with numerous



small boutons, and they got in apparent contact with PV soma and dendrites (Fig. 1C, c1, c2).

Magnocellular preoptic nucleus (MCPO). In the MCPO, there were many thick, branching PV fibers with large soma and buttons, intermingled with some orexin thin fibers and buttons (Fig. 1D, d1, d2).

Substantia innominata (SI). Within the SI, there was obvious overlap of PV and orexin fibers. A moderate distribution of orexin fibers was noted in this region, intermixed with a less density of PV immunopositive elements. Intermingled with the PV soma were long, thin, varicose orexin fibers and PV processes were intermingled with orexin fibers and many small boutons (Fig. 1E, e1, e2).

VLPO. This dual innervation extended in the direction near the edge of the 3rd ventricle (3V) to the VLPO, although the density of PV neurons there was somewhat lighter (Fig. 2A, a).

Ventral tuberomammillary nucleus (VTM). In the remainder of hypothalamus, mainly including the VTM, there were few fine orexin fibers and buttons contact with PV soma (Fig. 2B, b).

Fig. 1. Brightfield images depicting lack of orexin and PV fibers in the nucleus accumbens core (AcbC, A), and intermingled orexin and PV fibers in the nucleus accumbens shell (AcbSh, B), the nucleus of the horizontal limb of the diagonal band (HDB, C), magnocellular preoptic nucleus (MCPO, D), and substantia innominata (SI, E), a, b, c1, c2, d1, d2, e1 and e2 are higher-magnification images of the boxed areas depicted in the five photomicrographs in the left column. Orexin immunopositive axons, stained with monoclonal mouse anti-orexin, appear black, whereas PV immunoreactive elements, stained with monoclonal rabbit anti-PV, appear brown. Abbreviations: ac, anterior commissure; acp, anterior commissure, posterior. Scale bars=500 μm at the bottom of photomicrograph in the left column (applies to A to E), 100 μm in a and b, 50 μm in c1 (applies to c1, c2, d1, d2, e1 and e2).

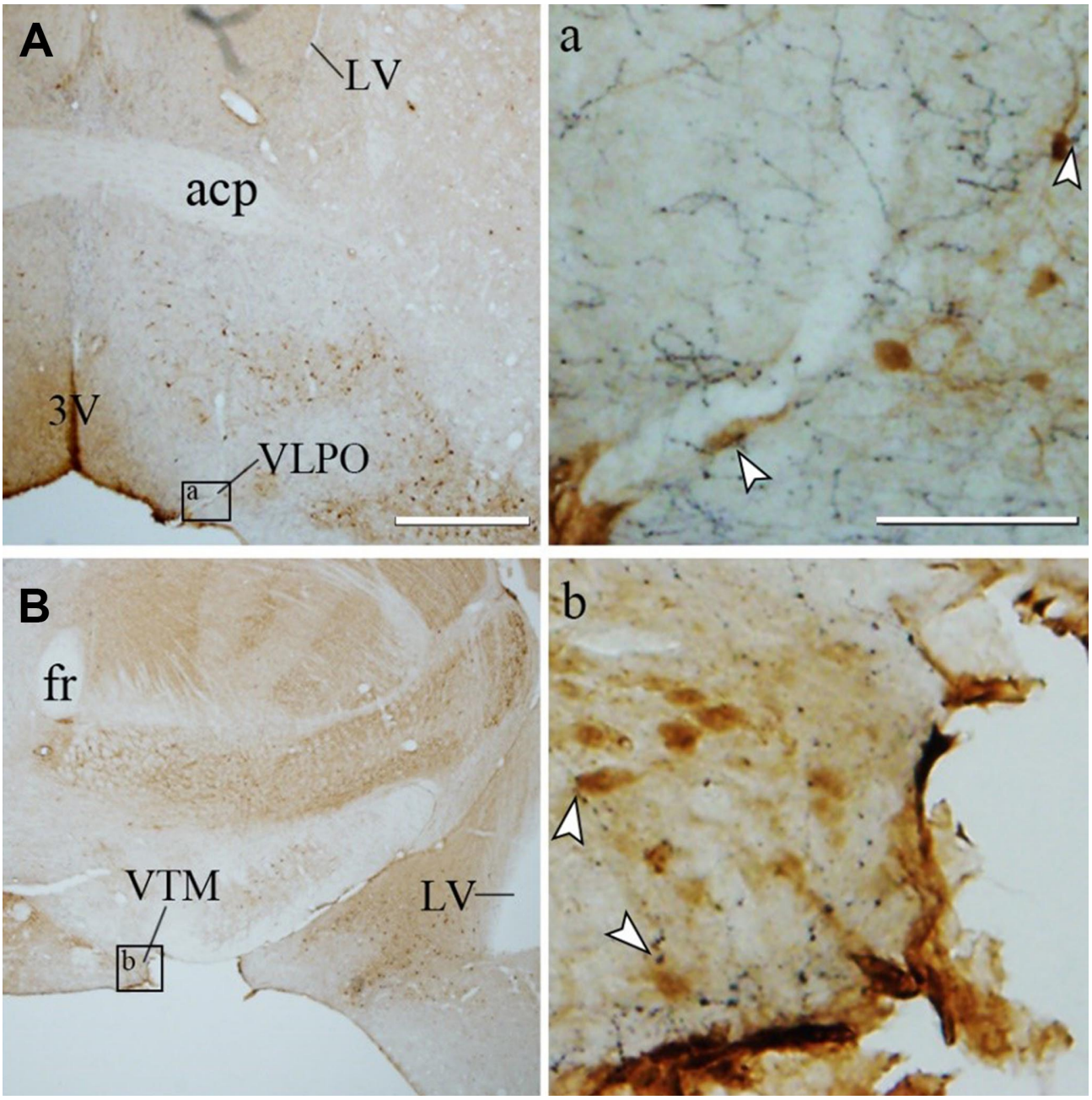


Fig. 2. Brightfield images depicting intermingled orexin and PV fibers in the ventrolateral preoptic nucleus (VLPO, A) and ventral tuberomammillary nucleus (VTM, B), a and b are higher-magnification images of the boxed areas depicted in the two photomicrographs in the left column. Orexin immunopositive axons, stained with monoclonal mouse anti-orexin, appear black, whereas PV immunoreactive elements, stained with monoclonal rabbit anti-PV, appear brown. Abbreviations: acp, anterior commissure, posterior; LV, lateral ventricle; fr, fasciculus retroflexus. Scale bars=500 μm at the bottom of photomicrograph in the left column (applies to A and B), 100 μm in a and b.

LH. Moderate density of thick, varicose orexin fibers and small buttons in the anterior part of lateral hypothalamus (LHa) were in close contact with the soma and slender axons of PV (Fig. 3A, a1, a2). A few PV soma and elongated axons around the perifornical area and in the tuberal part of lateral hypothalamus (LHt) were embedded within a dense collection of orexin soma and thick axons of varying lengths, including some thick orexin buttons (Fig. 3B, b1, b2). In the mammillary part of lateral hypothalamus (LHm), orexin fibers were thick, long and contained many large boutons, with a few PV soma and axons intermingled in between (Fig. 3C, c1, c2).

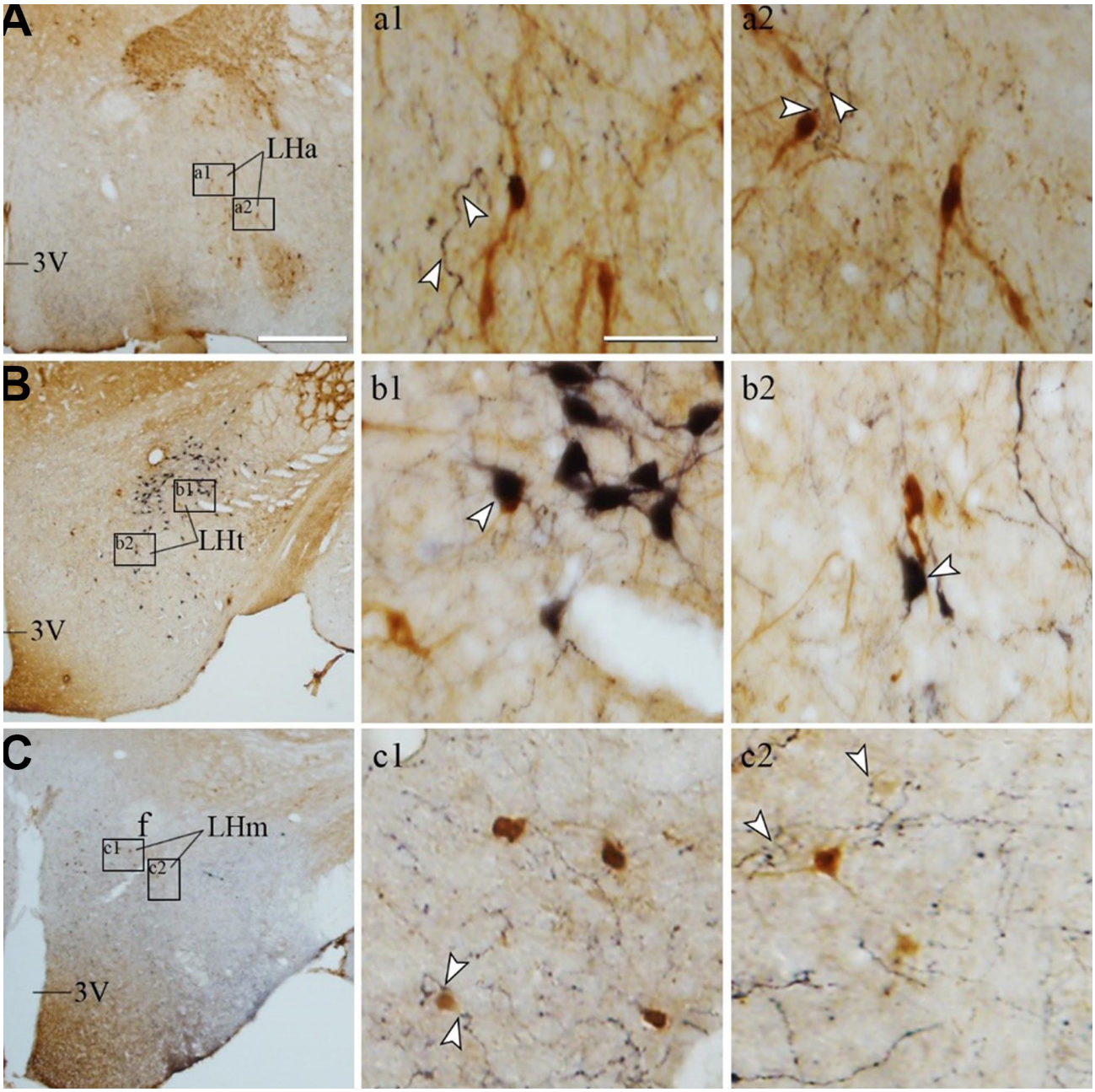


Fig. 3. Brightfield images depicting intermingled orexin and PV fibers in the anterior part of lateral hypothalamus (LHa, A), the tuberal part of lateral hypothalamus (LHt, B), and the mammillary part of lateral hypothalamus (LHm, C), a1, a2, b1, b2, c1 and c2 are higher-magnification images of the boxed areas depicted in the three photomicrographs in the left column. Orexin immunopositive axons, stained with monoclonal mouse anti-orexin, appear black, whereas PV immunoreactive elements, stained with monoclonal rabbit anti-PV, appear brown. Abbreviations: 3V, 3rd ventricle; f, fronix. Scale bars=500 μm at the bottom of photomicrograph in the left column (applies to all three photomicrographs in the left column), 50 μm in a1 (applies to a1, a2, b1, b2, c1 and c2).

Brainstem. Moderate overlap between orexin fibers and PV immunoreactivity was also observed in the VTA, which contained a moderate concentration of fine, varicose orexin fibers and buttons intermingled with a few large PV soma, emitting slender and arborized axons (Fig. 4A, a). At more caudal levels, the thick, varicose orexin immunoreactive axons and buttons were intermingled with a slight-to-moderate density of branching PV fibers in ventrolateral periaqueductal gray (VLPAG) (Fig. 4B, b). In the LDT could also be observed moderate overlap of these two fiber types, in which the thicker and highly varicose orexin fibers and buttons were intermingled with PV processes (Fig. 4C, c).

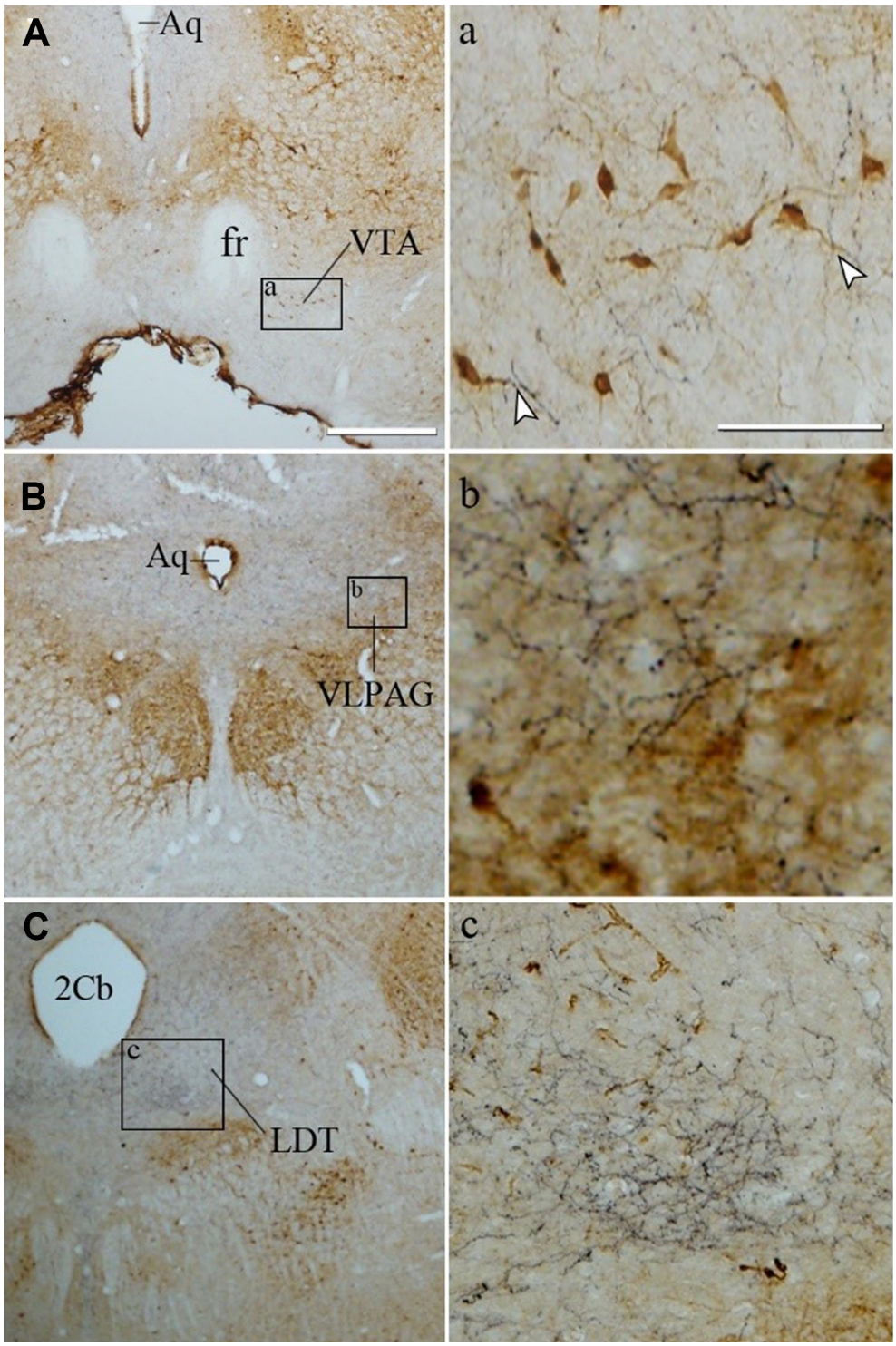


Fig. 4. Brightfield images depicting intermingled orexin and PV fibers in the ventral tegmental area (VTA, A), the ventrolateral periaqueductal gray (VLPAG, B), and the laterodorsal tegmental nucleus (LDT, C), a–c are higher-magnification images of the boxed areas depicted in the three photomicrographs in the left column. Orexin immunopositive axons, stained with monoclonal mouse anti-orexin, appear black, whereas PV immunoreactive elements, stained with monoclonal rabbit anti-PV, appear brown. Abbreviations: 2Cb, 2nd cerebellar lobule; Aq, aqueduct; fr, fasciculus retroflexus. Scale bars=500 μm at the bottom of photomicrograph in the left column (applies to A-C), 100 μm in a (applies to a-c).

DISCUSSION

The present study used a double-label immunohistochemical study to investigate overlap between PV immunoreactivity and projections of orexin neurons in sleep-wake related brain areas in the mouse. Peyron *et al.* (1998) and Nambu *et al*. (1999) observed orexin immunoreactivity fiber are distributed throughout the rat brain including the sleep-wake brain regions described above. Our current research showed a similar result with these previous studies about the distribution of orexin neurons and the fibers projections in several sleep-wake related brain areas in the mouse.

As for the distribution of PV immunoreactivity, Hontanilla *et al*. (1998) showed the distribution of PV was described in the striatum (caudate putamen complex, CPu), globus pallidus (GP), entopeduncular nucleus (EP), subthalamic nucleus (STh), and substantia nigra (SN) of the rat. In the BF, McKenna *et al*. (2013). confirmed the highest density of PV neurons was found in MCPO, followed by ventral pallidum (VP) and HDB, with the lowest density in SI, and PV neurons were predominantly of medium (15-20 μm long-axis diameter) and large (>20 μm long-axis diameter) size. In the LH, PV-immunoreactive perikarya were either small (15-20 μm in diameter) or middle-sized (25-30 μm in diameter), and the numerical density of cells was higher in the rostral than in the caudal part, which were intermingled with coarse axons of the medial forebrain bundle in mouse (Mészár *et al*., 2012). The distribution of PV immunoreactivity in the above studies were also observed in the present study.

Furthermore, overlap distributions between the projections of hypothalamic orexin neurons and PV immunoreactivity in sleep-wake related brain areas in the mouse was showed by a double-label immunohistochemical study in the present study (Fig. 1-4): In the BF, overlap was observed in the AcbSh, HDB, MCPO, SI, but avoided the AcbC. In the diencephalon, this dual innervation extended in the direction near the edge of the 3V to the VLPO, although the density of PV neurons there was somewhat lighter. Orexin immunoreactive neurons in several LH were embedded within dense clusters of PV processes when the buttons of orexin were in obvious contact with PV immunoreactivity. In the remainder of hypothalamus, mainly including the VTM, there were few fine orexin fibers and buttons contact with PV soma. Intermingled orexin and PV immunoreactivity were observed in the VTA, and sparse overlap was observed in the VLPAG and LDT in the brainstem. Perhaps the most straightforward implication of the present data is that orexin and PV may interact functionally within the brain regions containing overlapping distributions from both systems. There is a strong emphasis on the roles of orexin and PV in sleep-wake in the present study, which is discussed in the following content.

Acb as the ventral striatum contains GABAergic medium spiny neurons (MSNs, 95 %) and interneurons (5 %) (Kreitzer, 2009). Acb MSNs express the adenosine A_{2A} receptors (A_{2A}Rs), which play a predominant role in sleep induction (Huang *et al*., 2005; Lazarus *et al*., 2012). Yuan *et al*. (2017) confirm striatal A_{2A}R neurons control active-period sleep via PV neurons. Mukai *et al*. (2009) report that orexin depolarizes AcbSh neurons, which are involved in the cellular mechanisms through which orexin participate in the regulation of arousal. The present data showed that overlapping between orexin immunopositive projections and PV- immunoreactivity was observed in the AcbSh (Fig. 1B). Thus, it is speculated that PV neurons can effectively inhibit the activity of orexin neurons, thereby reducing the depolarization of orexin neurons on AcbSh neurons, which contributes to promoting sleep. Zhang *et al*. (2013) map projections of Acb A_{2A}R neurons to several brain sites (VTM, LH, VTA, and VLPAG) related with the function of sleep-wake controlled by A_{2A}R neurons. Our study showed that overlapping between orexin immunopositive fibers projections and PV-immunoreactivity was observed in the VTM (Fig. 2B), LH (Fig. 3), VTA (Fig. 4A), and VLPAG (Fig. 4B), within which PV may inhibit orexin neuron activity to promote sleep.

Orexin induces wakefulness within the BF. Thakkar *et al*. (2001) found that the microdialysis perfusion of orexin into the BF (including HDB, MCPO and SI) increases during wakefulness in freely behaving rats. Eggermann *et al*. (2001) have recently shown, in the rat brain slice preparation, that orexin has a strong, direct excitatory effect on cholinergic neurons in the MCPO in the BF. Moreover, Infusions of orexin into the general region of SI consistently elicited substantial increases in waking compared to vehicle-treated rats (España *et al*., 2001). Optical stimulation of PV neurons in the BF produced rapid transitions to wakefulness from NREM sleep but did not affect REM-wakefulness transitions (McKenna *et al*., 2020). Cholinergic neurons in the BF can also activate PV neurons, thus producing arousal-promoting effects (Yang *et al*., 2014). In the present study, overlap was observed in several BF areas, including the HDB (Fig. 1C), MCPO (Fig. 1D), and SI (Fig. 1E). Therefore, coordinated orexin/PV actions promote wakefulness within the BF.

Currently, we also found the overlap within the VLPO (Fig. 2A) and LDT (Fig. 4C). De Luca *et al*. (2022) confirm that orexin neurons promote wakefulness by inhibiting sleep-promoting GABA/galanin-containing VLPO neurons via feed-forward inhibition. Orexin peptides can excite both

cholinergic and noncholinergic neurons of the LDT by both direct actions and actions on their afferents, which has implications for the role of the orexin system in the control of wakefulness and for the neural mechanisms. Although the role of PV in regulating sleep-wake in the VLPO and LDT is rarely reported, coordinated orexin/PV actions within the two brain regions cannot be excluded.

CONCLUSIONS

Both orexin and PV systems target structures implicated in an array of state-dependent physiological, cognitive, and affective processes, including those associated with sleep or arousal states. Within these brain regions, orexin and PV immunoreactivity somas and fibers are closely intermingled and, in several areas, respect precisely the same boundaries. While difference in methodologies and antibodies may influence the intensity and specificity of staining, and thus influence results, among the studies. There are also numerous orexin fibers and boutons in close apposition to PV cell bodies and fibers, in a manner suggestive of synaptic contacts. Thus, the present results provide anatomic evidence supporting coordinated orexin/PV actions within the brain.

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RESUMEN: El péptido hipotalámico orexina tiene un papel destacado en los procesos relacionados con la excitación. Neuronas positivas para parvalbúmina (PV), como uno de los subtipos más importantes de interneuronas de ácido g-aminobutírico (GABA), que también participan en la regulación del sueño-vigilia. Para ampliar la comprensión del control neuronal y la conexión entre estos dos tipos de neuronas en los núcleos asociados al sueño, se utilizó un método inmunohistoquímico de doble marcado para investigar la superposición entre las distribuciones inmunopositivas de PV y las proyecciones de las neuronas de orexina en el cerebro de un ratón. En el prosencéfalo basal, se observó superposición en la capa del núcleo accumbens (AcbSh), el núcleo de la rama horizontal de la banda diagonal (HDB), el núcleo preóptico magnocelular (MCPO), la sustancia innominada (SI), pero se evitó el núcleo del núcleo accumbens. (AcbC). En el diencéfalo, esta inervación dual se extendía en dirección cercana al margen del tercer ventrículo (3V) hasta el núcleo preóptico ventrolateral (VLPO), aunque la densidad de neuronas PV allí era algo más ligera. Las neuronas inmunorreactivas de orexina en varias áreas hipotalámicas laterales (LH) estaban incrustadas dentro de densos grupos de procesos PV cuando los botones de orexina estaban en contacto obvio con la inmunorreactividad de PV. En el resto del hipotálamo, incluido principalmente el núcleo tuberomamilar ventral (VTM), había pocas fibras finas de orexina y botones en contacto con el soma PV. Se observó inmunorreactividad de orexina y PV entremezcladas en el área tegmental ventral (VTA), y se observó una escasa superposición en el núcleo gris periacueductal ventrolateral (VLPAG) y el núcleo tegmental laterodorsal (LDT) en el tronco encefálico. Así, estos dos tipos diferentes de neuronas, que se originan en diferentes partes del cerebro, se dirigen juntas a varias regiones del cerebro y a núcleos monoaminérgicos del tronco encefálico implicados en las funciones de motivación, estrés, locomoción y especialmente en la regulación del sueño.

PALABRAS CLAVE: parvalbúmina; orexina; Hipotálamo; Prosencéfalo basal; Estado de comportamiento.

REFERENCES

De Luca, R.; Nardone, S.; Grace, K. P.; Venner, A.; Cristofolini, M.; Bandaru, S. S.; Sohn, L. T.; Kong, D.; Mochizuki, T.; Viberti, B.; *et al*. Orexin neurons inhibit sleep to promote arousal. *Nat. Commun., 13(1)*:4163, 2022.

Eggermann, E.; Serafin, M.; Bayer, L.; Machard, D.; Saint-Mleux, B.; Jones, B. E. & Mühlethaler, M. Orexins/hypocretins excite basal forebrain cholinergic neurones. *Neuroscience, 108(2)*:177-81, 2001.

España, R. A.; Baldo, B. A.; Kelley, A. E. & Berridge, C. W. Wake-promoting and sleep-suppressing actions of hypocretin (orexin): basal forebrain sites of action. *Neuroscience, 106(4)*:699-715, 2001.

Hagan, J. J.; Leslie, R. A.; Patel, S.; Evans, M. L.; Wattam, T. A.; Holmes, S.; Benham, C. D.; Taylor, S. G.; Routledge, C.; Hemmati, P.; *et al*. Orexin A activates locus coeruleus cell firing and increases arousal in the rat. *Proc. Natl. Acad. Sci. U. S. A., 96(19)*:10911-6, 1999.

Hontanilla, B.; Parent, A.; de las Heras, S. & Giménez-Amaya, J. M. Distribution of calbindin D-28k and parvalbumin neurons and fibers in the rat basal ganglia. *Brain Res. Bull., 47(2)*:107-16, 1998.

Huang, Z. L.; Qu, W. M.; Eguchi, N.; Chen, J. F.; Schwarzschild, M. A.; Fredholm, B. B.; Urade, Y. & Hayaishi, O. Adenosine A2A, but not A1, receptors mediate the arousal effect of caffeine. *Nat. Neurosci., 8(7)*:858-9, 2005.

Kreitzer, A. C. Physiology and pharmacology of striatal neurons. *Annu. Rev. Neurosci., 32*:127-47, 2009.

Lazarus, M.; Chen, J. F.; Urade, Y. & Huang, Z. L. Role of the basal ganglia in the control of sleep and wakefulness. *Curr. Opin. Neurobiol., 23(5)*:780-5, 2013.

Lazarus, M.; Huang, Z. L.; Lu, J.; Urade, Y. & Chen, J. F. How do the basal ganglia regulate sleep-wake behavior? *Trends Neurosci., 35(12)*:723-32, 2012.

Marín, O. Interneuron dysfunction in psychiatric disorders. *Nat. Rev. Neurosci., 13(2)*:107-20, 2012.

McKenna, J. T.; Thankachan, S.; Uygun, D. S.; Shukla, C.; McNally, J. M.; Schiffino, F. L.; Cordeira, J.; Katsuki, F.; Zant, J. C.; Gamble, M. C.; *et al*. Basal forebrain parvalbumin neurons mediate arousals from sleep induced by hypercarbia or auditory stimuli. *Curr. Biol., 30(12)*:2379-2385.e4, 2020.

McKenna, J. T.; Yang, C.; Franciosi, S.; Winston, S.; Abarr, K. K.; Rigby, M. S.; Yanagawa, Y.; McCarley, R. W. & Brown, R. E. Distribution and intrinsic membrane properties of basal forebrain GABAergic and parvalbumin neurons in the mouse. *J. Comp. Neurol., 521(6)*:1225-50, 2013.

Mészár, Z.; Girard, F.; Saper, C. B. & Celio, M. R. The lateral hypothalamic parvalbumin-immunoreactive (PV1) nucleus in rodents. *J. Comp. Neurol., 520(4)*:798-815, 2012.

Mukai, K.; Kim, J.; Nakajima, K.; Oomura, Y.; Wayner, M. J. & Sasaki, K. Electrophysiological effects of orexin/hypocretin on nucleus accumbens shell neurons in rats: an in vitro study. *Peptides, 30(8)*:1487-96, 2009.

Nambu, T.; Sakurai, T.; Mizukami, K.; Hosoya, Y.; Yanagisawa, M. & Goto, K. Distribution of orexin neurons in the adult rat brain. *Brain Res., 827(1-2)*:243-60, 1999.

Niethard, N.; Hasegawa, M.; Itokazu, T.; Oyanedel, C. N.; Born, J. & Sato, T. R. Sleep-stage-specific regulation of cortical excitation and inhibition. *Curr. Biol., 26(20)*:2739-49, 2016.

Peyron, C.; Tighe, D. K.; van den Pol, A. N.; de Lecea, L.; Heller, H. C.; Sutcliffe, J. G. & Kilduff, T. S. Neurons containing hypocretin (orexin) project to multiple neuronal systems. *J. Neurosci., 18(23)*:9996-10015, 1998.

Scammell, T. E.; Arrigoni, E. &Lipton, J. O. Neural circuitry of wakefulness and sleep. *Neuron, 93(4)*:747-65, 2017.

Thakkar, M. M.; Ramesh, V.; Strecker, R. E. & McCarley, R. W. Microdialysis perfusion of orexin-A in the basal forebrain increases wakefulness in freely behaving rats. *Arch. Ital. Biol., 139(3)*:313-28, 2001.

Thankachan, S.; Katsuki, F.; McKenna, J. T.; Yang, C.; Shukla, C.; Deisseroth, K.; Uygun, D. S.; Strecker, R. E.; Brown, R. E.; McNally, J. M.; *et al*. Thalamic reticular nucleus parvalbumin neurons regulate sleep spindles and electrophysiological aspects of schizophrenia in mice. *Sci. Rep., 9(1)*:3607, 2019.

Yang, C.; McKenna, J. T.; Zant, J. C.; Winston, S.; Basheer, R. & Brown, R. E. Cholinergic neurons excite cortically projecting basal forebrain GABAergic neurons. *J. Neurosci., 34(8)*:2832-44, 2014.

Yuan, X. S.; Wang, L.; Dong, H.; Qu, W. M.; Yang, S. R.; Cherasse, Y.; Lazarus, M.; Schiffmann, S. N.; d'Exaerde, A. K.; Li, R. X.; *et al*. Striatal adenosine A2A receptor neurons control active-period sleep via parvalbumin neurons in external globus pallidus. *Elife, 6*:e29055, 2017.

Zhang, J. P.; Xu, Q.; Yuan, X. S.; Cherasse, Y.; Schiffmann, S. N.; de Kerchove d'Exaerde, A.; Qu, W. M.; Urade, Y.; Lazarus, M.; Huang, Z. L.; *et al*. Projections of nucleus accumbens adenosine A2A receptor neurons in the mouse brain and their implications in mediating sleep-wake regulation. *Front. Neuroanat., 7*:43, 2013.

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