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The Overlapping of Parvalbumin and Orexin Immunoreactivity in Mice Brain Regions Mediating Depressive Behaviors

Superposición de la Inmunorreactividad de Parvalbúmina y Orexina en Regiones Cerebrales de Ratones que Median Conductas Depresivas

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ZHANG, J.; ZHANG, H.; ZHAO, W. & CHU, L. The overlapping of parvalbumin and orexin immunoreactivity in mice brain regions mediating depressive behaviors. *Int. J. Morphol., 42(6)*:1618-1627, 2024.

SUMMARY: There is a significant correlation between regulatory functions of orexin system and depressive symptoms. Parvalbumin (PV) interneurons, the main subtype of inhibitory g-aminobutyric acid (GABA) interneurons, are involved in the regulation of depression-like behaviors too. To provide several lines of evidence regarding neural connection between two types of neurons in the depression associated nuclei, a double-label immunohistochemical staining technique was employed to reveal overlap between PV and orexin immunoreactivity in the mouse brain. Medial prefrontal cortex (mPFC) mainly consists of the cingulate cortex, area 1 (Cg1), prelimbic cortex (PrL) and infralimbic cortex (IL), in which regions contained similar obvious overlap between orexin fibers and PV somas with axons. In the basal forebrain (BF), overlap was observed in the lateral septal nucleus, ventral part (LSV), the nucleus accumbens shell (AcbSh), bed nucleus of the stria terminalis, ventral division (BSTV), ventral pallidum (VP), central amygdaloid nucleus (Ce). In the diencephalon, besides the lateral hypothalamic area (LH) and dorsomedial hypothalamic nucleus (DM), in which two regions contained overlap between orexin positive perikarya with fibers and PV immunoreactivity, different densities of orexin and PV fibers were closely intermingled in the paraventricular hypothalamic nucleus (PVN), paraventricular thalamic nucleus (PVT), arcuate hypothalamic nucleus (Arc). In the brainstem, densities of intermixed orexin and PV fibers were from high to low in the dorsal raphe nucleus (DR), laterodorsal tegmental nucleus (LDTg), periaqueductal gray (PAG), lateral parabrachial nucleus (LPB), median raphe nucleus (MnR), and orexin buttons were embedded within PV fibers, as for the ventral tegmental area (VTA), overlap was observed between PV somas with axons and orexin fibers. Hence, PV and orexin neurons and its projections jointly target several brain regions involved in regulating depression, which provides anatomic evidence supporting coordinated PV/orexin actions within these brain regions.

KEY WORDS: Parvalbumin; Orexin; Depression; Reward.

INTRODUCTION

Depression is a mental disorder characterized by emotional distress, the core symptoms of depression include long-lasting low mood, loss of pleasure and interest, sleep disorders, cognitive impairment seriously affecting people's physical and mental health (Cheng *et al*., 2020). Orexin neurons are specifically localized within the hypothalamus, and project throughout the rat brain, including regions are closely associated with depression (Nambu *et al*., 1999). Orexin can mediate many neurophysiological processes such as reward, cognition, emotion, sleep-wake, stress, in which depression patients usually show disordered states. Harris *et al*. (2005) found that intracerebral injection of orexin-A reduced depression-like behavior in mice. Reduced cerebrospinal fluid orexin levels were observed in suicidal patients with major depressive disorder (MDD) compared with patients suffering from adjustment disorder or dysthymia with suicide attempts (Lu *et al*., 2017). Clinical studies confirmed that dysregulation of orexin release may be involved in the pathogenesis of depression, but they found orexin-A levels were higher in depression patients than in the control group (Salomon *et al*., 2003).

A subpopulation of GABAergic neurons contains parvalbumin (PV), which is associated with regulatory inhibition, and depression is associated with functional regulation of PV neurons. Ji *et al*. (2020) found that over-inhibition mediated by PV neurons can lead to depression-like behaviors and cognitive dysfunction. Knowland *et al*.

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(2017) suggested that increased ventral pallidum PV neuronal activity is a salient feature of depression. Inhibition of PV interneurons reduced the latency of inactivity and feeding in an unfamiliar environment in mice, and improved the depression-like performance (Fogaça *et al*., 2021). However, Perova *et al*. (2015) found that inhibition of mouse medial prefrontal PV neurons promoted helpless behaviors.

So far, known brain regions related to depression mainly include the prefrontal cortex (PFC), nucleus accumbens (Acb), ventral pallidum (VP), lateral hypothalamic area (LH), hippocampus, ventral tegmental area (VTA) (Xu *et al*., 2020), lateral septal nucleus (LS), dorsomedial hypothalamic nucleus (DM) (Wang *et al*., 2023), paraventricular thalamic nucleus (PVT) (Kawatake-Kuno *et al*., 2024), arcuate hypothalamic nucleus (Arc) (Fang *et al*., 2021), bed nucleus of the stria terminalis (BST), lateral habenula (LHb), paraventricular hypothalamic nucleus (PVN), periaqueductal gray (PAG), median raphe nucleus (MnR) (Muir *et al*., 2019), amygdala, laterodorsal tegmental nucleus (LDTg), dorsal raphe nucleus (DR), parabrachial nucleus (PBN) (Cheng *et al*., 2020), *etc*. Despite extensive information regarding the distribution of orexin and PV systems, the extent to which they interact and regulate common substrates within these brain fields related to depression, remains unknown.

In the present study, a double-label immunohistochemical technique was used to investigate overlapping distributions of PV and orexin immunoreactivity in above-mentioned depression associated nuclei in the mouse brain. Orexin and PV neurons and fibers were immunohistochemically labeled and stained distinguishable colors with separate peroxidase reactions and could be directly examined in the same tissue sections. Our results provide several lines of anatomic evidence regarding coordinated orexin/PV actions within these brain regions for depression-related control.

MATERIAL AND METHOD

Experimental Animals. Male SPF inbred C57BL/6J mice (Shanghai SLAC Laboratory Animal Co., Ltd., China), each weighing about 21-25 g, were housed in the Laboratory Animal Research Centre of Zhejiang Chinese Medical University and maintained in a room with conditions of an automatically controlled light-dark cycle, appropriate ambient temperature and humidity (lights on at 07:00 and off at 19:00, an ambient temperature of 20-25 ˚C, and a relative humidity of 55-60 %). Standard mice were provided *ad libitum* access to food and water. Every effort was made to minimize the number of animals utilized in this study and potential distress, pain, discomfort to the mice throughout all experiments.

Ethical consideration. All experimental procedures for animal handling were 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. Mice (n=3) were subjected to anesthesia using sodium pentobarbital (60mg/kg, i.p.). Upon confirmation that the mice were unresponsive to tail- or foot-pinching, they were transcardially perfused with 0.9 % saline followed by 4 % paraformaldehyde, both at room temperature, then their brains were isolated, post-fixed for 4 h at 4˚C in the same fixative, and then transferred to 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, then stored at -20 °C until immunohistochemical staining.

Immunohistochemical procedures. 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 1h at room temperature, removing solution without washing, and subsequently incubated overnight at 4 °C with the primary antiserum. 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 tissue 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) at room temperature for 2 h, 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 with PBS, placed on gelatin-coated slides, air-dried overnight, dehydrated across an ascending alcohol gradient, cleared in xylene, and coverslipped.

Microscopy. Images were captured by an Olympus IX71 microscope. Adobe Photoshop CS2 was employed 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

Medial prefrontal cortex (mPFC). The mPFC mainly consists of three subregions, namely the cingulate cortex, area 1 (Cg1), prelimbic cortex (PrL), infralimbic cortex (IL), Within the Cg1, low-to-moderate density of varicose orexin fibers with buttons were intermingled with moderate-to-high density of branching PV fibers, orexin fibers were randomly dispersed among PV immunoreactivity contained somas and fibers there (Fig. 1A, a1). Moderate density of medium-sized PV-positive cells and fibers were distributed throughout the PrL. This region contained moderate density of varicose orexin fibers with buttons which were closely contacted with PV emitting fibers mainly (Fig. 1A, a2). Extensive labeling of both orexin and PV immunoreactivity was observed in the IL. Moderate-to-high density of slender varicose orexin fibers with buttons were intermixed and contacted with PV somas and emitting fibers (Fig. 1A, a3). Distributions of orexin and PV immunoreactivity in these three subregions of mPFC were similar, overlap of orexin and PV immunoreactivity was easily observed.

Lateral septal nucleus, ventral part (LSV). The LSV was abundant with orexin fibers of varying thicknesses and lengths and boutons. They were distributed in the most lateral part and orientated along the margin of the lateral ventricle, and low density of PV thin fibers were intermingled within this region, overlap between orexin and PV fibers was slight too (Fig. 1B, b).

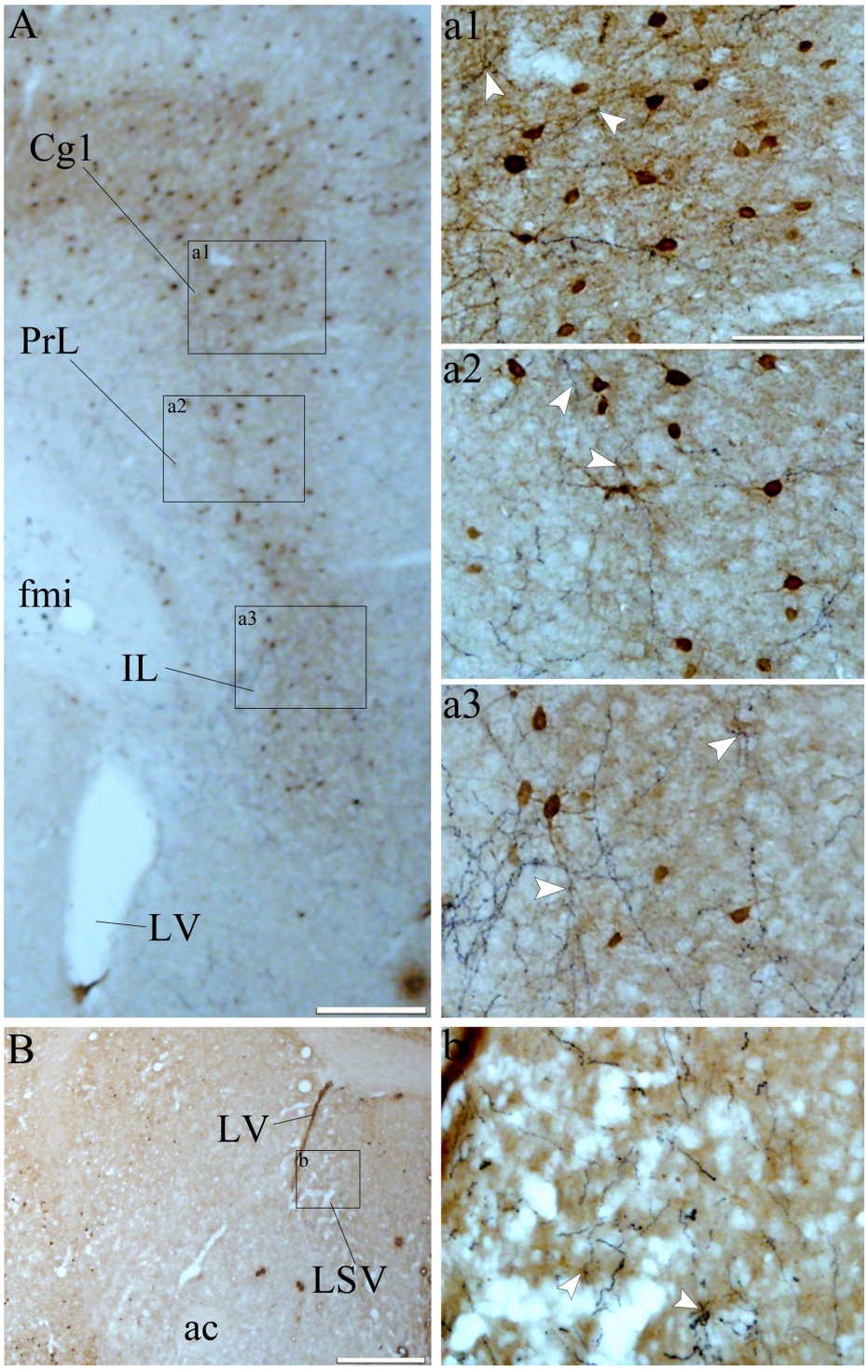

Fig. 1. Brightfield images depicting intermingled orexin and PV immunoreactivity in the cingulate cortex, area 1 (Cg1, A, a1), prelimbic cortex (PrL, A, a2), infralimbic cortex (IL, A, a3) and lateral septal nucleus, ventral part (LSV, B), a1-b are higher-magnification images of the boxed areas depicted in the two photomicrographs in the left column. PV immunoreactive elements stained with monoclonal rabbit anti-PV, appear brown; orexin immunopositive elements stained with monoclonal mouse anti-orexin, appear black. Abbreviations: fmi, forceps minor of the corpus callosum; LV, lateral ventricle; ac, anterior commissure. Scale bars=500 μm at the bottom of photomicrograph in B, 200 μm in A, 100 μm in a1 (applies to a1-b).

Accumbens nucleus (Acb). PV immunoreactivity mainly contained somas, axons and dendrites in this region, and orexin varicose fibers and buttons were mainly contacted with PV axons or dendrites, and had less contact with PV somas. Notably, overlap between two types of neurons only in the shell of nucleus accumbens (AcbSh) (Fig. 2A, a).

Bed nucleus of the stria terminalis, ventral division (BSTV). Moderate numbers of orexin fibers were observed in the BSTV, and they were thick and of vary lengths with numerous boutons. PV thin fibers were sparsely distributed in this region, two types of fibers were intermixed with each other, and many orexin buttons were embedded within PV fibers (Fig. 2B, b).

Ventral pallidum (VP). Within the VP, moderate-to-high density of PV immunoreactivity mainly contained somas, emitting slender axons and branching dendrites were dispersed over the VP, intermingled with a low-to-moderate concentration of varicose orexin fibers and tiny buttons, and orexin fibers got in apparent contact with PV fibers (Fig. 2C, c).

Central amygdaloid nucleus (Ce). In the Ce, only PV fibers and orexin fibers with buttons were noted, and densities of these immunopositive elements were somewhat lighter, PV slender fibers, orexin varicose fine fibers and tiny buttons were randomly dispersed and had contact with each other in this region, and some tiny orexin buttons were embedded within PV fibers (Fig. 2D, d1).

Paraventricular hypothalamic nucleus (PVN). Moderate density of slender, varicose orexin fibers with buttons in the PVN were intermingled with low-to-moderate density of branching slender PV fibers. Orexin varicose fibers were randomly dispersed among the PV fibers there, and considerable orexin buttons were embedded within PV fibers (Fig. 2D, d2).

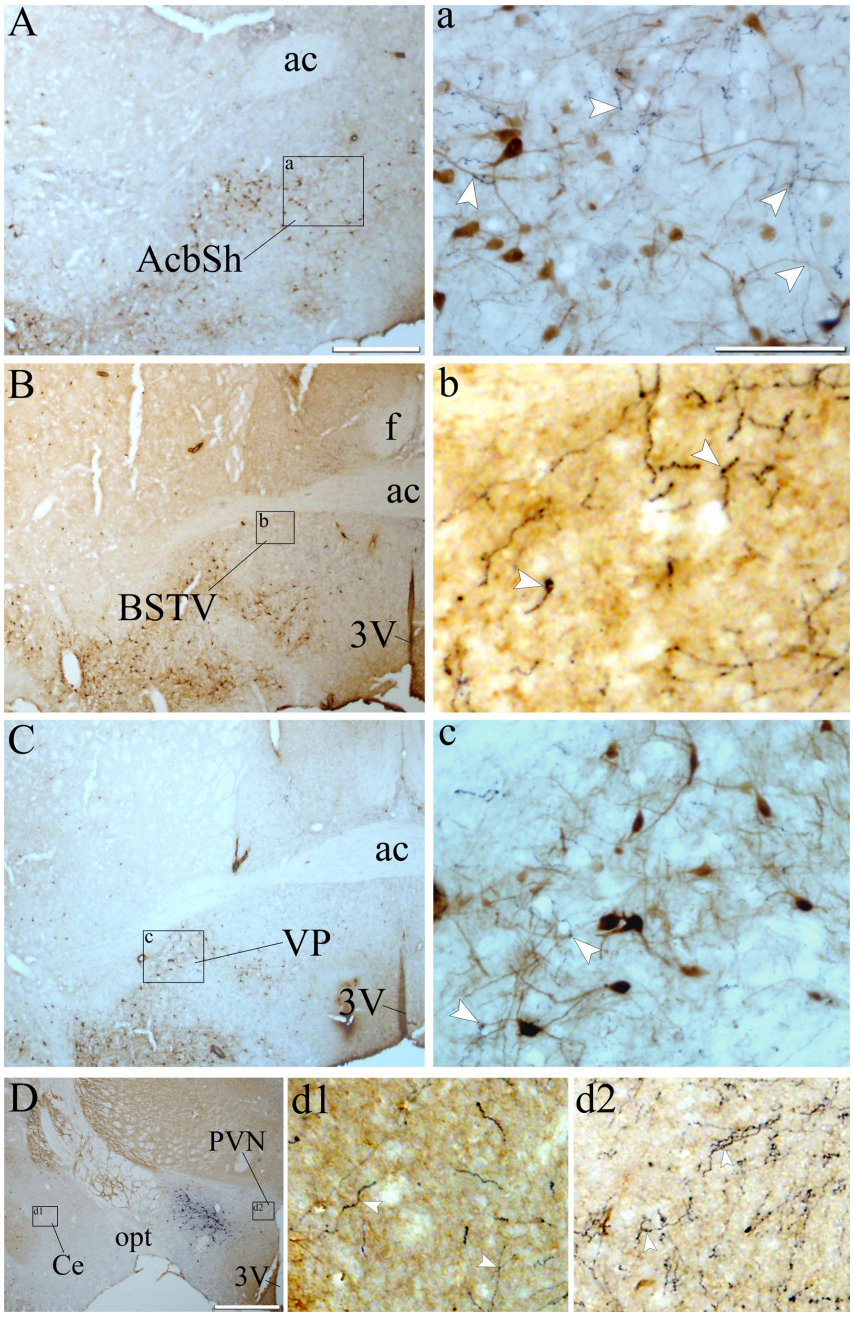

Fig. 2. Brightfield images depicting intermingled orexin and PV immunoreactivity in the shell of nucleus accumbens (AcbSh, A), bed nucleus of the stria terminalis, ventral division (BSTV, B), ventral pallidum (VP, C), central amygdaloid nucleus (Ce, D, d1), paraventricular hypothalamic nucleus (PVN, D, d2), a-d2 are higher-magnification images of the boxed areas depicted in the four photomicrographs in the left column. PV immunoreactive elements stained with monoclonal rabbit anti-PV, appear brown; orexin immunopositive elements stained with monoclonal mouse anti-orexin, appear black. Abbreviations: ac, anterior commissure; f, fornix; 3V, 3rd ventricle; opt, optic tract. Scale bars=500 μm at the bottom of photomicrograph in the left column (applies to A-D), 100 μm in a (applies to a-d2).

Paraventricular thalamic nucleus (PVT). Moderate overlap between orexin and PV fibers was observed in the PVT, which contained a moderate concentration of varicose orexin fibers and large buttons, with moderate density of branching slender PV fibers intermingled in between, and some orexin thick buttons were embedded within PV fibers (Fig. 3A, a).

Lateral hypothalamic area (LH). In the LH, PV and orexin neurons had dense distributions with complete neuronal morphology, and overlap between two types of neurons was relatively more, there was a high degree of contact between somas with emitting thick axons of varying lengths and elongated arborized dendrites of PV and orexin immunoreactivity (Fig. 3B, b).

Dorsomedial hypothalamic nucleus (DM). A few stained orexin cells were scattered lateral to the DM, and moderate thin orexin fibers were also detected in the diffuse part of the DM at the medial part. PV thin fibers randomly spread over this region, and some of them got in apparent contact with orexin fibers (Fig. 3C, c).

Arcuate hypothalamic nucleus (Arc). Within the Arc, the density of orexin immunoreactivity was homogeneous, which just contained some short fibers with buttons, randomly dispersed among the PV fibers there (Fig. 3D, d).

Periaqueductal gray (PAG). In this region, many orexin fibers with varicose structures and numerous buttons were observed, with moderate-to-high density of branching PV fine fibers intermingled in between, and considerable orexin buttons were embedded within PV fibers (Fig. 4A, a).

Ventral tegmental area (VTA). Within the VTA, low density of PV neurons with somas and fine axons were noted, as for orexin neurons, just sparse thin fibers and tiny buttons were observed.

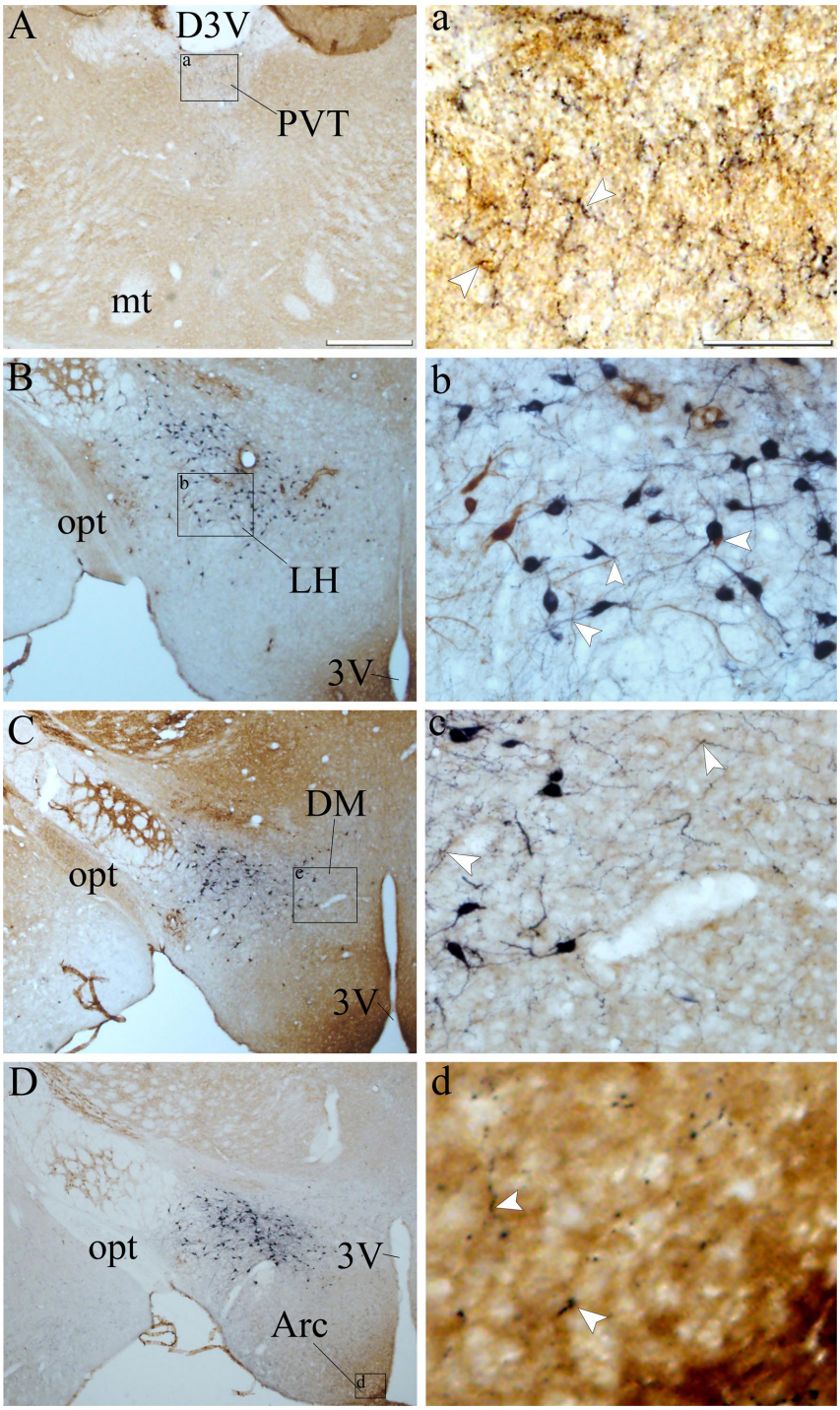

Fig. 3. Brightfield images depicting intermingled orexin and PV immunoreactivity in the paraventricular thalamic nucleus (PVT, A), lateral hypothalamic area (LH, B), dorsomedial hypothalamic nucleus (DM, C) and arcuate hypothalamic nucleus (Arc, D). a-d are higher-magnification images of the boxed areas depicted in the four photomicrographs in the left column. PV immunoreactive elements stained with monoclonal rabbit anti-PV, appear brown; orexin immunopositive elements stained with monoclonal mouse anti-orexin, appear black. Abbreviations: D3V, dorsal 3rd ventricle; mt, mammillothalamic tract; opt, optic tract; 3V, 3rd ventricle. Scale bars=500 μm at the bottom of photomicrograph in the left column (applies to A-D), 100 μm in a (applies to a-d).

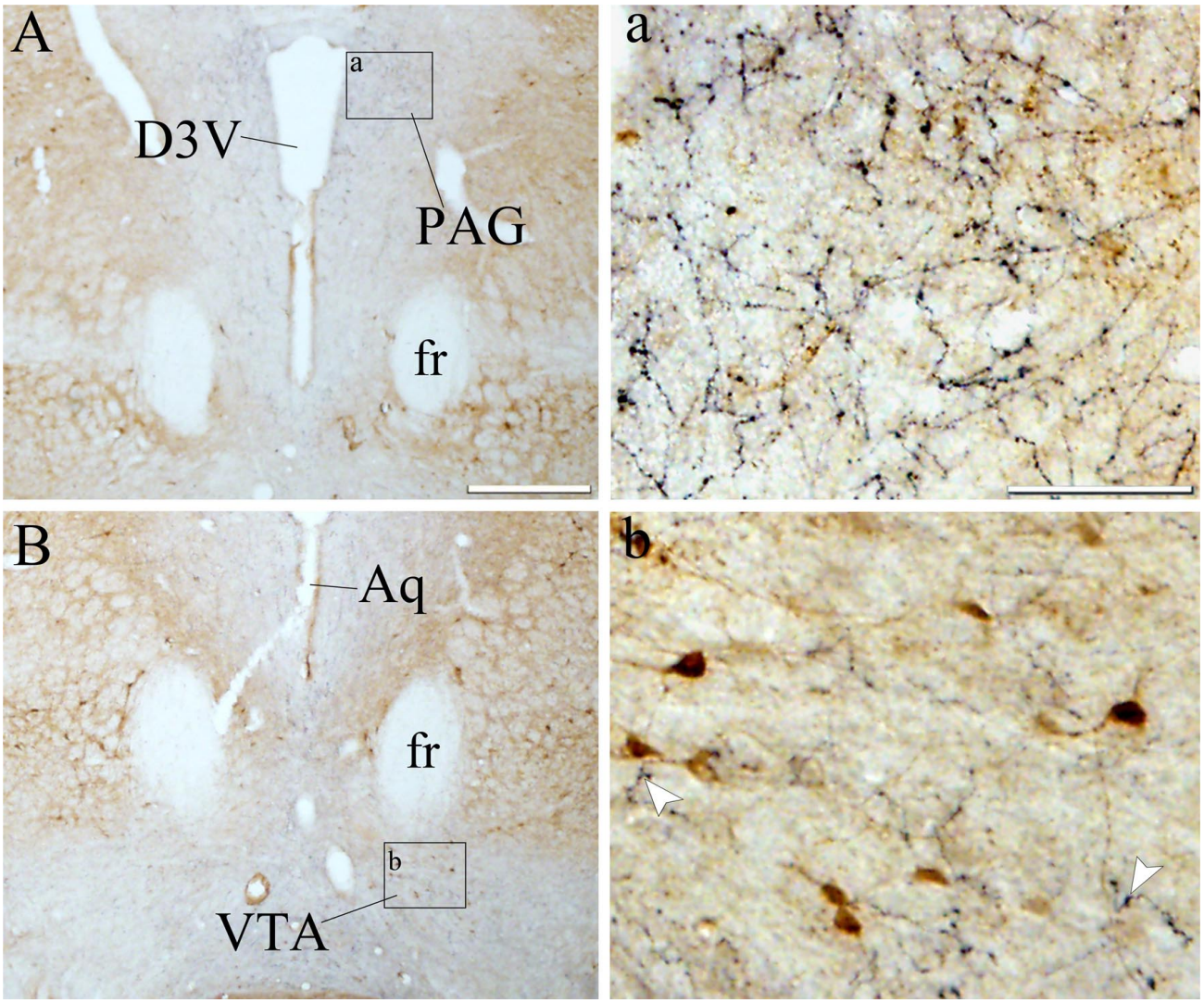

Fig. 4. Brightfield images depicting intermingled orexin and PV fibers in the periaqueductal gray (PAG, A) and ventral tegmental area (VTA, B). a-b are higher-magnification images of the boxed areas depicted in the two photomicrographs in the left column. PV immunoreactive elements stained with monoclonal rabbit anti-PV, appear brown; orexin immunopositive elements stained with monoclonal mouse anti-orexin, appear black. Abbreviations: D3V, dorsal 3rd ventricle; Aq, aqueduct; fr, fasciculus retroflexus. Scale bars=500 μm at the bottom of photomicrograph in the left column (applies to A-B), 100 μm in a (applies to a-b).

And overlap between orexin and PV immunoreactivity was slight, orexin fibers or buttons were contact with a few PV emitting slender axons (Fig.4B, b).

Laterodorsal tegmental nucleus (LDTg). Intense and extensive labeling of PV and orexin fibers was observed in the LDTg. They were intermixed with each other, and numerous orexin buttons were embedded with PV branching fibers (Fig. 5A, a1).

Dorsal raphe nucleus (DR). Long, thick orexin fibers with numerous boutons innervated the DR, with moderate-to-high density of PV slender fibers intermingled in between, and considerable orexin buttons were embedded with PV branching fibers (Fig. 5A, a2).

Median raphe nucleus (MnR). Orexin fibers were much shorter with numerous buttons in the MnR, but they were abundant in this region. Low density PV fibers got in apparent contact with these orexin fibers (Fig. 5A, a3).

Lateral parabrachial nucleus (LPB). Two types of fibers were both moderate in this region, many orexin fibers with varicose structures and numerous buttons were observed, they intermixed with slender PV fibers, and many small orexin buttons were embedded within PV fibers (Fig. 5B, b).

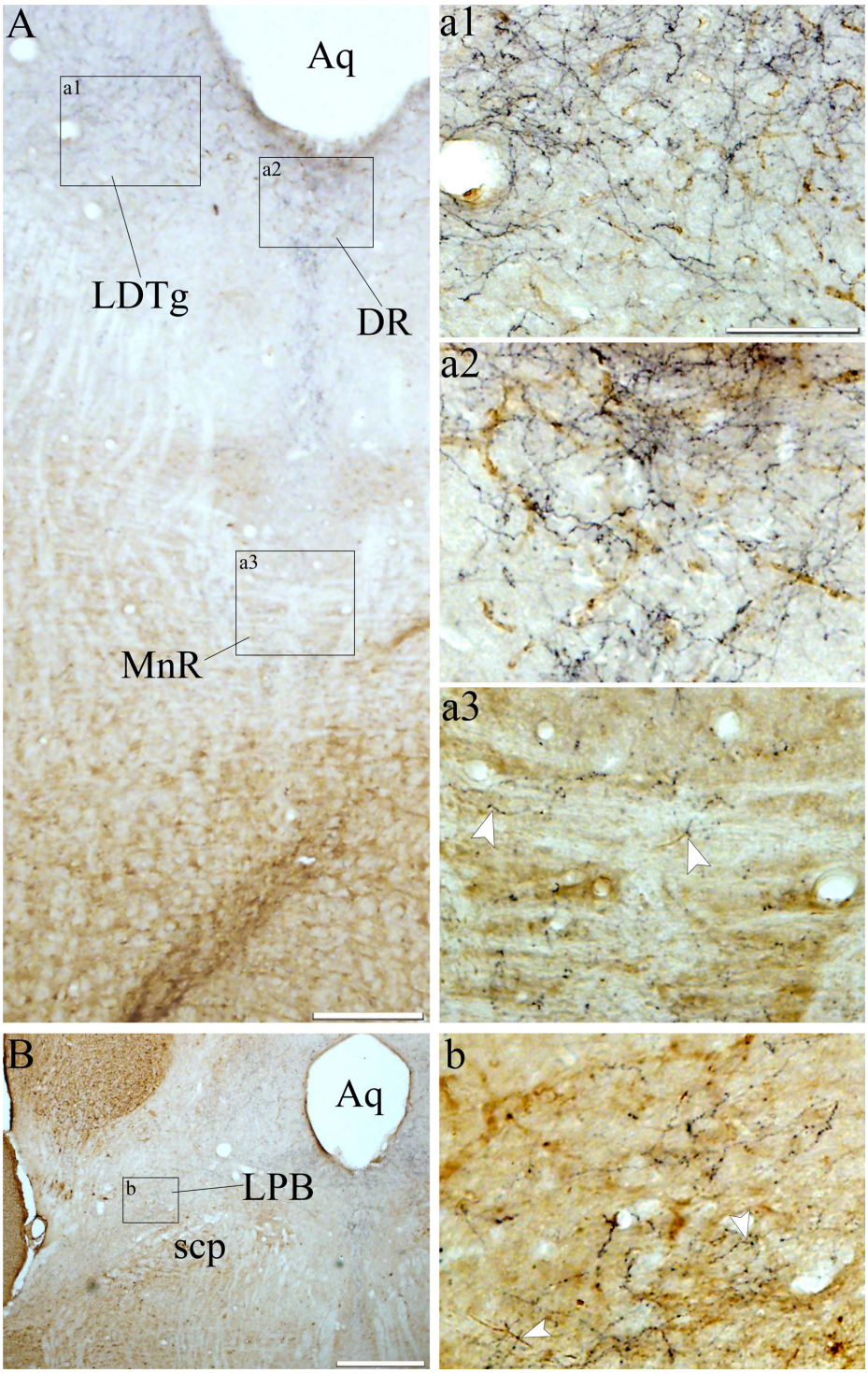

Fig. 5. Brightfield images depicting intermingled orexin and PV fibers in the laterodorsal tegmental nucleus (LDTg, A, a1), dorsal raphe nucleus (DR, A, a2), median raphe nucleus (MnR, A, a3) and lateral parabrachial nucleus (LPB, B), a1-b are higher-magnification images of the boxed areas depicted in the two photomicrographs in the left column. PV immunoreactive elements stained with monoclonal rabbit anti-PV, appear brown; orexin immunopositive elements stained with monoclonal mouse anti-orexin, appear black. Abbreviations: Aq, aqueduct; scp, superior cerebellar peduncle (brachium conjunctivum). Scale bars=500 μm at the bottom of photomicrograph in B, 200 μm in A, 100 μm in a1 (applies to a1-b).

DISCUSSION

In the present study, we employed a double-label immunohistochemical technique to detect overlapping distributions of orexin and PV immunoreactivity in depression related nuclei in the mice. Although orexin-containing neurons represent a relatively small number of cells which are specifically localized within the hypothalamus, their projections are widely distributed throughout the rat brain including the depression associated nuclei which were described above (Peyron *et al*., 1998). Our results about distributions of orexin immunoreactivity in several depression related nuclei in the mouse showed a striking similarity with previous studies (Peyron *et al*., 1998; Nambu *et al*., 1999).

PV-immunoreactive neurons are scattered throughout the brain, Hontanilla *et al*. (1998) provided a map of the distribution of PV immunoreactivity in the different nuclei of the rodent basal ganglia of the rat. In the BF, the highest density of PV neurons was found in the magnocellular preoptic nucleus (MCPO), and lower densities were found in the rostral BF when compared to medial and caudal BF (McKenna *et al*., 2013). In the adult rat thalamus, neurons of the reticular nucleus display PV-immunostaining and PV-positive fibers densely innervate most of the dorsal thalamic domains (Frassoni *et al*., 1991). A solitary cluster of PV-positive neurons was observed in the lateral hypothalamus of rodents (Celio *et al*., 2013), and the numerical density of cells was higher in the rostral than in the caudal part (Mészár *et al*., 2012). Our observation of the distributions of PV immunoreactivity in several depression related brain areas was consistent with these findings.

Moreover, overlapping distributions of orexin and PV immunoreactivity in different depression related nuclei in the mouse were observed through a double-label immunohistochemical technique in the present study (Fig. 1-5): mPFC mainly consists of the Cg1, PrL and IL, in which regions contained similar obvious overlap between orexin fibers and PV somas with axons. In the BF, overlap was observed in the LSV, AcbSh, BSTV, VP, Ce. In the diencephalon, besides the LH and DM, in which two regions contained overlap between orexin positive perikarya with fibers and PV immunoreactivity, different densities of orexin and PV fibers were closely Intermingled in the PVN, PVT, Arc. In the brainstem, densities of intermixed orexin and PV fibers were from high to low in the DR, LDTg, PAG, LPB and MnR, and orexin buttons were embedded within PV fibers, as for VTA, overlap was observed between PV somas with axons and orexin fibers. The neuroanatomical overlap between orexinergic elements and PV immunoreactivity at multiple sites of depression-related nuclei suggests that their functional co-actions are likely to be involved in modulating depression-related behaviors.

Anhedonia, a typical symptom of MDD, dysfunction of the brain reward system is thought to be the neural basis of anhedonia (Zhang *et al*., 2013). The mesolimbic dopamine reward system is mainly composed of VTA and its dopamine projections to the Acb, PFC, amygdala, hippocampus (Kumar *et al*., 2008). Orexin neurons project to the VTA, increase VTA dopamine cell activity, and concomitant dopamine release is necessary and sufficient for reward seeking which related to antidepression-like behaviors (Mahler *et al*., 2013). It is known that 10 % of inhibitory neurons in the VTA are PV neurons (Yu *et al*., 2022), dopaminergic neurons in VTA can be inhibited by projection from VTA GABA neurons (Cheng *et al*., 2020), and inhibition of VTA dopaminergic neurons resulted in depressant-like behaviors (Tye *et al*., 2013). However, Wang *et al*. (2018) found that inhibition of PV neuron signaling in the Acb prevented the development of conditioned place preference (CPP) for amphetamine and aggravated depression, and Perova *et al*. (2015) suggested that inhibition of mouse medial prefrontal PV neurons promoted helpless behaviors. In the present study we observed the overlap between orexin and PV immunoreactivity in the VTA (Fig. 4B), AcbSh (Fig. 2A), mPFC (Fig. 1A), according to these findings, we speculated that PV neurons may improve depression via disinhibition of dopaminergic neurons in the VTA and VTA dopamine projection to the Acb, PFC, thereby increasing reward effect, improving depression, therefore PV and orexin systems may have consistent effects on reward and depression.

Knowland *et al*. (2017) suggested that increased PV neuronal activity in the VP was a salient feature of depression, but Ji *et al*. (2019) found that pharmacological blockade or genetic knockdown of orexin receptors in VP increased depressive-like behaviors. Activation of PV axonal projections in the LH to the LHb triggered aversion which related to depressive-like behaviors (Siemian *et al*., 2021), whereas photoactivation of orexinergic cell bodies in the LH relieved anxiety-like behaviors induced by chronic social defeat stress (Wang *et al*., 2021). In the present study, overlap was noted in the VP (Fig. 2C) and LH (Fig. 3B), according to these previous studies, PV and orexin have opposite effects on depression within the VP and LH, which may be related to the heterogeneity and complex pathophysiological mechanism of depression. But our findings still suggested co-actions of orexin and PV systems on depression in these regions.

Excessive and persistent anxiety is one of the most common symptoms of depression. Lungwitz *et al*. (2012) suggested that orexin-A induced anxiety-like behaviors through interactions with glutamatergic receptors in the BST of rats. Orexin-A participated in anxiety-like behaviors by modulating the spontaneous firing activity of Ce neurons (Pan *et al*., 2020). Endogenously released orexins act on the PVT to mediate anxiety-like responses in rats (Li *et al*., 2010). Nollet *et al*. (2011) suggested that orexin neurons in the dorsomedial and perifornical hypothalamic area (DM-PFA) may contribute to the pathophysiology of depressive disorders. Although the role of PV in regulating depression in the BSTV (Fig. 2B), Ce (Fig. 2D, d1), PVT (Fig. 3A) and DM (Fig. 3C) is rarely reported, overlap within these nuclei was observed in the present study, and coordinated orexin/PV actions within these nuclei for depression-related control cannot be excluded.

In the present study, we also observed overlap within the LSV (Fig. 1B), PVN (Fig. 2D, d2), Arc (Fig. 3D), PAG (Fig. 4A), LDTg (Fig. 5A, a1), DRN (Fig. 5A, a2), MnR (Fig. 5A, a3), LPB (Fig. 5B), though functional regulations of PV and orexin systems on depression in these regions are not clear, our results provide a morphological basis for further research.

CONCLUSIONS

PV and orexin systems, originating in distinctly different parts of the brain, jointly target several brain regions involved in the regulation of depression. Within these brain regions, orexin- and PV-containing fibers are closely intermingled and, in several areas, respect precisely the same boundaries. There are also numerous orexin-containing fibers and boutons in close apposition to PV-containing cell bodies, in a manner suggestive of synaptic contacts. Conversely, PV-containing axons contact the orexin cells of the LH. Hence, our data provide several lines of anatomic evidence regarding coordinated orexin/PV actions within these brain regions for depression-related control.

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RESUMEN: Existe una correlación significativa entre las funciones reguladoras del sistema de orexina y los síntomas depresivos. Las interneuronas de parvalbúmina (PV), el principal subtipo de interneuronas inhibidoras del ácido g-aminobutírico (GABA), también están implicadas en la regulación de conductas similares a la depresión. Para proporcionar varias líneas de evidencia con respecto a la conexión neuronal entre dos tipos de neuronas en los núcleos asociados a la depresión, se empleó una técnica de tinción inmunohistoquímica de doble marcación para revelar la superposición entre la interneurona de PV y la inmunorreactividad de la orexina en el cerebro del ratón. La corteza prefrontal medial (mPFC) está formada principalmente por la corteza cingulada, área 1 (Cg1), corteza prelímbica (PrL) y corteza infralímbica (IL), en las que las regiones contenían una superposición similar entre las fibras de orexina y los somas de las interneuronas PV y sus axones. En el prosencéfalo basal (BF), se observó superposición en el núcleo septal lateral, parte ventral (LSV), la superficie del núcleo accumbens (AcbSh), el núcleo del lecho de la estría terminal, división ventral (BSTV), núcleo pálido ventral (VP) y núcleo amigdaloide central (Ce). En el diencéfalo, además del área hipotalámica lateral (LH) y el núcleo hipotalámico dorsomedial (DM), en los que dos regiones contenían superposición entre pericario positivo para orexina con fibras e inmunorreactividad de PV, se observaron que diferentes densidades de fibras de orexina e interneuronas PV estaban estrechamente entremezcladas en el núcleo hipotalámico paraventricular (PVN), núcleo talámico paraventricular (PVT) y núcleo hipotalámico arqueado (Arc). En el tronco encefálico, las densidades de fibras de orexina e interneuronas PV entremezcladas fueron altas a bajas en el núcleo del rafe dorsal (DR), núcleo tegmental laterodorsal (LDTg), sustancia gris periacueductal (PAG), núcleo parabranquial lateral (LPB) y núcleo del rafe medio (MnR). La orexina estaba incrustada dentro de las fibras de interneuronas PV. Respecto al área tegmental ventral (VTA), se observó superposición entre somas PV con axones y fibras de orexina. Por lo tanto, las neuronas PV y orexina y, sus proyecciones, actúan conjuntamente sobre varias regiones cerebrales implicadas en la regulación de la depresión, lo que proporciona evidencia anatómica que respalda las acciones coordinadas PV/orexina dentro de estas regiones cerebrales.

PALABRAS CLAVE: Parvalbúmina; Orexina; Depresión; Recompensa.

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