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DISTRIBUTION OF VASOPRESSIN, OXYTOCIN AND VASOACTIVE INTESTINAL POLYPEPTIDE IN THE HYPOTHALAMUS AND EXTRAHYPOTHALAMIC REGIONS OF TREE SHREWS

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Abstract—Vasopressin (VP), oxytocin (OXT) and vasoactive intestinal polypeptide (VIP) in the brain modulate physiological and behavioral processes in many vertebrates. Day-active tree shrews, the closest relatives of primates, live singly or in pairs in territories that they defend vigorously against intruding conspecifics. However, anatomy concerning peptidergic neuron distribution in the tree shrew brain is less clear. Here, we examined the distribution of VP, OXT and VIP immunoreactivity in the hypothalamus and extrahypothalamic regions of tree shrews (*Tupaia belangeri chinensis*) using the immunohistochemical techniques. Most of VP and OXT immunoreactive (-ir) neurons were found in the paraventricular nucleus (PVN) and supraoptic nucleus (SON) of the hypothalamus. In addition, VP-ir or OXT-ir neurons were scattered in the preoptic area, anterior hypothalamic areas, dorsomedial hypothalamic nucleus, stria terminalis, bed nucleus of the stria terminalis and medial amygdala. Interestingly, a high density of VP-ir fibers within the ventral lateral septum was observed in males but not in females. Both VP-ir and VIP-ir neurons were found in different subdivisions of the suprachiasmatic nucleus (SCN) with partial overlap. VIP-ir cells and fibers were also scattered in the cerebral cortex, anterior olfactory nucleus, amygdala and dentate gyrus of the hippocampus. These findings provide a comprehensive description of VIP and a detailed mapping of VP and OXT in the hypothalamus and extrahypothalamic regions of tree shrews, which is an anatomical basis for the participation of these neuropeptides in the regulation of circadian behavior and social behavior.

Key words: hypothalamus, paraventricular nucleus, suprachiasmatic nucleus, amygdala, lateral septum.

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Abbreviations: 3V, third ventricle; ac, anterior commissure; Arc, arcuate hypothalamic nucleus; ACB, nucleus accumbens; AHy, anterior hypothalamus; BLA, basolateral amygdaloid nucleus; BST, bed nucleus of the stria terminalis; cc, corpus callosum; CCK, cholecystokinin; Cd, caudate; CeA, central amygdaloid nucleus; CeL, central amygdaloid nucleus, lateral division; CeM, central amygdaloid nucleus, medial division; CRF, corticotropin-releasing factor; DG, dentate gyrus of the hippocampus; DMH, dorsomedial hypothalamic nucleus; fx, fornix; Hipp, hippocampus; ic, internal capsule; -ir, immunoreactive; LA, lateral amygdaloid nucleus; LHA, lateral hypothalamic area; LHB, lateral habenular nucleus; lo, lateral olfactory tract; LPO, lateral preoptic area; LS, lateral septum; LSd, dorsal LS; LSv, ventral LS; LV, lateral ventricle; ME, median eminence; MeA, medial amygdaloid nucleus; MEex, median eminence, external zone; MEin, median eminence, internal zone; MPO, medial preoptic area; NDB, nucleus of the diagonal band of Broca; och, optic chiasm; opt, optic tract; OXT, oxytocin; PaA, anterior paraventricular hypothalamic nucleus; PaD, dorsal paraventricular hypothalamic nucleus; PaL, lateral paraventricular hypothalamic nucleus; PaM, medial paraventricular hypothalamic nucleus; PaP, posterior paraventricular hypothalamic nucleus; PaV, ventral paraventricular hypothalamic nucleus; Pe, periventricular hypothalamic nucleus; Pu, putamen; PVN, paraventricular nucleus of the hypothalamus; PVT, paraventricular nucleus of the thalamus; SCN, suprachiasmatic nucleus; SOM, somatostatin; SON, supraoptic nucleus; SPa, subparaventricular zone of the hypothalamus; st, stria terminalis; StHy, striohypothalamic nucleus; TBS, Tris-buffer saline; TBST, TBS containing 0.5% triton X-100; VH, ventral hippocampus; VIP, vasoactive intestinal polypeptide; VMH, ventromedial hypothalamic nucleus; VP, vasopressin.

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INTRODUCTION

The tree shrew (*Tupaia belangeri*), a representative of the order Scandentia, is day-active animal living in an arboreal habitat in South and Southeast Asia (Peng et al., 1991). Currently, the whole genome sequencing of Chinese tree shrew (*Tupaia belangeri chinensis*) has been completed (Fan et al., 2013). According to molecular phylogenetic studies, tree shrews are the closest relatives of primates (Janecka et al., 2007; Kriegs et al., 2007; Fan et al., 2013). Recently, there are many attempts to use tree shrews as animal models in studying hepatitis B virus infections (Yan et al., 1996a,b), hepatitis C virus (Zhao et al., 2002), and myopia (Norton et al., 2006). Tree shrews are also thought to be one promising animal model that is used to study stress-related disorders such as depression (Czeh et al., 2005; Fuchs, 2005). Neuropeptides in the mammalian central nervous system modulate many behaviors, including stress-related disorders, social behavior, and aggressive behavior (Bosch et al., 2005; Kozicz et al., 2008; Neumann and Landgraf, 2012). However, little is known about the distribution of neuropeptides in the tree shrew brain.

The distribution of neuropeptides such as vasopressin (VP) and oxytocin (OXT) has been extensively studied in

primates and rodents (Buijs et al., 1978; Sofroniew et al., 1981; Zhou and Swaab, 1999). VP and OXT are synthesized in the paraventricular nucleus (PVN) and supraoptic nucleus (SON) of the hypothalamus (George, 1978; Fliers et al., 1985). VP and OXT-immunoreactive (-ir) fibers projecting from these nuclei through the median eminence (ME) to the posterior pituitary have been reported in many mammals (Dierickx and Vandesande, 1977; Sofroniew et al., 1979; Caffe et al., 1989; Rosen et al., 2006, 2008). In addition, VP-ir cells and fibers are found in the suprachiasmatic nucleus (SCN) (Swaab et al., 1985). VP-ir fibers in the SCN project through the subparaventricular zone of the hypothalamus (SPa) and then toward the PVN of the hypothalamus (Kalsbeek et al., 1993; Dai et al., 1997; Rosen et al., 2006). These anatomical connections between SCN and PVN are considered to be the structural bases for the rhythmicity of a number of hormones. However, it is noted that human beings are day-active creatures and the experimental data collected from the nocturnal rats should be validated in the day-active animals (Bao et al., 2008). VP and OXT are also produced in the bed nucleus of the stria terminalis (BST) (van Eerdenburg et al., 1992), amygdala (Wang et al., 1997; Rosen et al., 2006), and preoptic and anterior hypothalamic areas (Wang et al., 1997). In the rat brain, the VP projections from the BST and medial amygdaloid nucleus (MeA) to the lateral septum (LS) were confirmed (De Vries and Buijs, 1983; Caffe et al., 1987). It is one of the most consistent sex differences in vertebrate brains that the LS in males has a higher VP innervation of fibers than in females (Goodson and Bass, 2001; De Vries and Panzica, 2006). VP and OXT play an important role in parental care, pair-bonding, sexual behavior, dominance–subordinance, and emotional-related behavior (Donaldson and Young, 2008; Ebstein et al., 2009; Stein, 2009; Neumann and Landgraf, 2012).

Vasoactive intestinal polypeptide (VIP), a 28-amino acid neuropeptide, was originally isolated from the porcine gastrointestinal tract and its sequence was subsequently identified in many mammals (Said and Mutt, 1970; Dimaline et al., 1984; Bodner et al., 1985). This peptide is widely distributed in the anterior olfactory nuclei, cerebral cortex, amygdala, BST, and SCN of mammals (Loren et al., 1979; Zhou et al., 1995b; Dai et al., 1997). Several functions have been attributed to VIP including circadian rhythmicity and synchrony (Aton et al., 2005; Vosko et al., 2007), maintenance of prolactin secretion (Sharp et al., 1989), modulation of territorial aggression (Goodson, 1998; Goodson et al., 2012), and regulation of VP and OXT release (Ottesen et al., 1984; Bardrum et al., 1988).

Previous reports showed that there are VP and OXT neurons in the PVN and SON of tree shrews (Sofroniew et al., 1981; Luo et al., 1995). Besides, VP cells are also found in the accessory SON, hypothalamic lateral nucleus, perifornical nucleus and ansa peduncularis (Luo et al., 1995). However, the anatomical characteristics of VP-ir, OXT-ir and VIP-ir cells and fibers in the hypothalamus and extrahypothalamic regions of tree shrews (*T. b. chinensis*) have not been entirely explored. In the present study, we aimed to provide a detailed analysis of distribution of VP, OXT and VIP in the hypothalamus and extrahypothalamic regions by using immunohistochemistry.

EXPERIMENTAL PROCEDURES

Animals

Four adult male and five adult female tree shrews (*T. b. chinensis*) (from the breeding colony at the Animal House Center of the Kunming Institute of Zoology, Kunming, PR China) were used. Tree shrews were housed individually in animal facilities under a 12-h light/dark cycle (lights on at 8:00 a.m.), with food and water available *ad libitum*. The use and care of animals in the present study was in accordance with international guidelines and protocols approved by the Animal Care and Use Committee of the University of Science and Technology of China. All efforts were made to minimize animal suffering as well as to reduce the number of animals used.

Tissue preparation

In this study, adult tree shrews were deeply anesthetized with pentobarbital sodium (80 mg/kg, i.p.). Six tree shrews were perfused with 0.9% saline followed by 4% paraformaldehyde in phosphate-buffer (0.1 M; pH 7.4). After perfusion, brains were removed and post-fixed by immersion in the same fixative overnight, at 4 °C. The others were not perfused and directly post-fixed within 4% paraformaldehyde in phosphate-buffer (0.1 M; pH 7.4), at 4 °C. Then the brains were transferred to 15% sucrose in phosphate-buffer (0.1 M; pH 7.4), until the tissues sank, followed by 30% sucrose in phosphate-buffer (0.1 M; pH 7.4). Tissues were frozen and sectioned at 40 μm on a Leica microtome (Leica CM1950, Germany) and the sections stored at 4 °C until use.

Antibody characterization

The primary antibodies rabbit anti-VP (Truus, 23/06/86, at 1:1000 dilution), rabbit anti-OXT (O-2-T, 04/04/75, at 1:400 dilution), and rabbit anti-VIP (Viper, 18-09-86, at 1:1000 dilution) were generous gifts from Dr. D. F. Swaab (Netherlands Institute for Brain Research, Amsterdam, the Netherlands) and their specificity had been tested extensively (Swaab and Pool, 1975; Dai et al., 1997; Goncharuk et al., 2001; Xu et al., 2003).

As a specificity control, the primary antibody (rabbit anti-VP, rabbit anti-OXT or rabbit anti-VIP, respectively) was omitted or preadsorbed with 100 μM VP, 100 μM OXT or 50 μM VIP (Sigma Aldrich, St. Louis, MO, USA), respectively, at room temperature for 1 h prior to overnight tissue incubation. In control sections, immunoreactive cells were seldom seen in both situations. Immunostaining was rarely reduced in sections exposed to VP antiserum preadsorbed with OXT or OXT antiserum preadsorbed with VP, which