

Distribution of vasotocin- and vasoactive intestinal peptide-like immunoreactivity in the brain of penduline tit

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SUMMARY

Penduline tits are songbirds, used as model animals in numerous studies of sexual conflict. Nevertheless, the distribution of neuropeptides in the brain of this avian species remains largely unknown. Here we present some of the first results on distribution of vasotocin (AVT) and vasoactive intestinal peptide (VIP) in the brain of this songbird species, using immunohistochemical mapping. The bulk of AVT-like cells are found in the hypothalamic supraoptic, paraventricular and suprachiasmatic nuclei, medial bed nucleus of the stria terminalis, and along the lateral forebrain bundle. Most AVT-like fibres course toward the median eminence, but also in the medial bed nucleus of the stria terminalis, preoptic area and lateral septum. Further terminal fields occur in the dorsal thalamus, ventral tegmental area and pretectal area. Most VIP-like cells are in the lateral septal organ and arcuate nucleus. VIP-like fibres are distributed extensively in the hypothalamus, preoptic area, lateral septum, nucleus of the diagonal band. They are also found in the medial bed nucleus of the stria terminalis, amygdaloid nucleus of taenia, robust nucleus of the arcopallium, caudo-ventral hyperpallium, nucleus accumbens and the brainstem. The results indicate a high degree of conservatism

in both AVT and VIP neuropeptidergic systems across avian species, as well as the different vertebrate taxa. The anatomical distribution of AVT and VIP in the penduline tit supports an involvement of these peptides in reproductive behaviours and social decision making, widely studied in this wild bird species.

Key words: Vasotocin – Vasoactive intestinal peptide – Mapping – Penduline tit – Avian brain

INTRODUCTION

Parental care is costly in terms of energy (Houston et al., 2005). The parents have to optimize the amount of care they provide for a successful breeding, while saving resource for future breeding (Houston et al., 2005). Although a minimum of cooperation is required for a successful breeding, the interest of the individual parent is to reduce its investment in parental care as much as possible, leaving the greatest burden of rearing offspring to its mate (van Dijk et al., 2012). In certain bird species the resolution of sexual conflict over parental care may lead to nest desertion. The most thoroughly studied example is the Eurasian penduline tit (*Remiz pendulinus*) (Pogany et al., 2015), a small migratory passerine bird [Passerida, Remizinae (Cramp et al., 1993)]. Because of its unusual breeding pattern (sequential polygyny and polyandry followed by uniparental –maternal or paternal – care), the Eurasian penduline tit is a

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model species to study sexual conflict (van Dijk et al., 2012). From early spring, unmated males start to build their nest in willow or poplar trees while singing to attract a female. They usually finish the nest together with the female they have attracted. As egg-laying ends, one bird deserts the nest, leaving the incubation and offspring upbringing to the mate (Pogány et al., 2008). Strictly monoparental, the parental care is mostly provided by the female (in 47-65 % of the cases), but, in 6-18% of the cases, it is assumed by the male (Pogány et al., 2008, van Dijk et al., 2012). As a result of intense sexual conflict over parental care, 29-40% of all nests are being abandoned by both parents (Szentirmai et al., 2007, Pogány et al., 2012, Pogány et al., 2015). Biparental care has never been observed in this species (Pogány et al., 2012). During its remarkably long breeding season (from early April to August), these birds may have up to 5 mates.

Despite the existence of numerous studies on the behaviour, ecology and life history of this bird, only limited data are available on its neuroanatomy. Apart from our previous work, restricted to the comparison of the arginine-vasotocin-like (AVT+) and vasoactive intestinal peptide-like (VIP+) immunoreactivity in some limbic nuclei of the social brain network in the blue tit and the penduline tit (Montagnese et al., 2014), we found no data on the specific distribution of neuropeptides of behavioural relevance. These two peptides were chosen for their known implication in the modulation of reproductive and social behaviour. Briefly, in songbirds, vasotocin, together with VIP, has been shown to modulate courtship behaviour (Goodson, 1998a, b). The social organization of songbirds has been linked to the activity of the vasotocinergic neurones in the limbic circuit (Goodson, 2008, Goodson et al., 2012). In addition, VIP and AVT suppress the agonist song (directed towards conspecifics) in songbirds (Goodson, 1998a). A recent study in zebra finch underlines the activation of VIP expression during nesting behaviour (Kingsbury et al., 2015).

The present study is aimed at specifying the distribution of vasotocin and VIP systems in the brain of the penduline tit, as a prerequisite for further functional neuronatomical and behavioural investigation in this species.

MATERIALS AND METHODS

Specimen collection and processing

Five male Eurasian penduline tits (*Remiz pendulinus*), all adults, were caught at their nests, in the Dnestr Delta National Park, Ukraine, between 21 May 2009 and 26 May 2009, by using a mist net and by playing species specific songs as bait. The research was approved by the Ministry of Environmental Protection of the Ukraine and the National Park of Lower Dnestr Region. The work was car-

ried out in accordance with the Directive 2010/63/EU of the European Parliament and of the Council on the protection of animals used for scientific purpose.

After ketamine-xylazine anaesthesia, the brains were dissected out and immediately fixed by immersion in a solution of 4% paraformaldehyde in 0.1 M phosphate buffer. Samples were stored at 4°C until further processing. Fixed brains were transferred to a 20% sucrose solution for 24 hours, before being sectioned at a 60 µm thickness on a freezing microtome (Frigomobil, Zeiss). Three series of alternate sections were taken. One series was immediately mounted and stained with cresyl violet for identification of the structures. Two other series were processed for immunocytochemistry.

Immunohistochemistry

Two antisera were used for this study, an anti-AVT [kind gift from Prof. David Gray University of Witwatersrand, Johannesburg, RSA (Gray and Simon, 1983)] and an anti-VIP [gift from Dr. Tamás Görcs (Gulyas et al., 1990)]. Both were raised in rabbits and diluted in PBS-Tween 20 (anti-AVT: 1/60000, anti VIP: 1/10000). The specificity of these antibodies has been described in our previous publication (Montagnese et al., 2015).

Sections were washed in PBS. Endogenous peroxidase activity was quenched by 0.1% H₂O₂ in PBS for 15 minutes. Following several washes in PBS containing 0.1% Tween 20 (Sigma-Aldrich, Steinheim, Germany), sections were incubated for 2 hours in a solution of 1% normal goat serum in PBS-Tween 20, and then transferred overnight to the rabbit primary antiserum. Then, sections were extensively washed in PBS-Tween 20, incubated for 2 hours in a biotinylated goat anti-rabbit IgG (Vector, Burlingame, CA) at 1/100 in PBS-Tween-20, rinsed and incubated with avidin-biotin complex (Vector, Burlingame CA) diluted in PBS for 2 hours. Sections were rinsed first in PBS, then in Tris buffer (pH 8), before being incubated in a solution containing 0.015 % of diaminobenzidine tetrahydrochloride (DAB, Sigma-Aldrich, Steinheim, Germany) and 0.25% ammonium nickel sulphate hexahydrate (Fluka Chemie, Buchs, Switzerland) in Tris buffer. After 5-minute preincubation, the enzymatic reaction was initiated by adding 5 µl H₂O₂ (0.1%)/5 ml DAB solution. The reaction was stopped 10 minutes later by rinsing with Tris buffer, followed by PBS. Sections were then mounted on gelatine-subbed slides and coverslipped with DPX (Sigma-Aldrich, Steinheim, Germany). Control of specificity included omission of the primary antisera, and absorption of the antiserum with the antigen. Non-specific staining was not observed in the tissue.

Identification of the brain structures

Contour drawings of the Nissl-stained sections

served as templates on which the AVT and VIP immunoreactive cells, fibres and terminal fields were recorded. For identification of the brain regions, we used the canary atlas (Stokes et al., 1974) and the chicken atlases (Kuenzel and Mason, 1988, Puelles et al., 2007). For the septal areas, we identified the different subdivisions as defined by Goodson et al. (2004). The subgroups of the AVT+ neuronal cells were identified according to the terminology established by Berk et al., 1982. To compose the table, the relative abundance of the immunoreactive elements was visually estimated and classified as occasional (not systematically present or sporadic), few, moderate and numerous; whereas the variability of abundance between birds is represented by a "/" between the smallest and highest estimate.

RESULTS

According to the original purpose of the present study, i.e. the precise description of brain regions containing AVT+ and VIP+ structures, we did not intend here to compare peptide distribution between males and females. Previous studies, including those of our group (Montagnese et al., 2014, Montagnese et al., 2015), have already indicated that, whenever considerable intersex differences were found, these always meant deficiency or complete lack of neuropeptides in certain brain regions of female birds, especially with AVT. Thus, in order to achieve the most complete anatomical picture, the mapping of peptides in males only seemed to be the best choice.

Vasotocin-like immunoreactive neurones, fibres and terminal fields

As shown in Fig. 1 and Table 1, the great majority of the AVT-immunoreactive (AVT+) neuronal perikarya were found in the hypothalamic ventral supraoptic (Groups *L1*, *L2*), suprachiasmatic (Group *L2*), periventricular (Groups *P1-P3*) and paraventricular (Group *P3*) nuclei (Fig. 2A, B, C). Many perikarya were also dispersed amongst the fibres of the lateral forebrain bundle (Group *L1*, Figs. 1, 2E, F). Most of these neurones were multipolar, more rarely bipolar. Their axons were essentially running through the lateral hypothalamus into the hypothalamic-hypophyseal tract toward the median eminence (Figs. 1, 3K), including those originating from the paraventricular nucleus (Fig. 2A). Some fibres followed a periventricular course. A few axons were crossing through the supraoptic decussation toward the contralateral hypothalamus. Fibres were occasionally observed in the hypothalamic dorsal area and the dopaminergic A13 region.

Neurones and fibres were present in several extrahypothalamic areas, mainly in two sexually dimorphic nuclei: the medial preoptic and periventricular nuclei (group *P1/2*) and the medial

bed nucleus of the stria terminalis (Group *DD2*, Figs. 1, 3C, Table 1). In some birds, the preoptic neurones were continuous with those of the paraventricular nucleus.

Some AVT+ neurones were also present in the thalamus, on the lateral side of the lateral forebrain bundle (Group *DD1*, Figs. 1-2I). Some multipolar AVT+ neurones were also dispersed in the mesencephalic reticular formation, ventral tegmental area, substantia nigra pars compacta, raphe nuclei and nucleus linearis caudalis (Figs. 1, 2G, H, J, Table 1). The more numerous the neurones the less numerous were the fibres in the brainstem.

The main extrahypothalamic sites showing AVT+ fibres were the medial bed nucleus of the stria terminalis, lateral septal areas and the preoptic region (Table 1, Figs. 1, 3A, C). In addition to these areas, fibres were encountered in the nucleus of the diagonal band (Fig. 3B), in and around the nucleus of the pallial commissure and in the septal commissural nucleus as well as in the periventricular preoptic nucleus, nucleus accumbens and ventral pallidum (Fig. 3E) (see also Table 1 and Fig. 1). In the thalamus, many AVT+ fibres terminated in the medial part of the thalamic anterior and posterior dorsomedial nuclei (Table 1, Figs. 1, 3F, H, I).

Fewer fibres were seen in the thalamic ventral periventricular nucleus and intermediate periventricular nuclei of Puelles (Puelles et al., 2007), and zona incerta (Table 1, Figs. 1, 2). Single fibres entered the lateral habenula (Table 1, Figs. 1, 3G). In the brainstem, AVT+ fibres were mostly present in the midbrain central gray (Fig. 3M), intercollicular nucleus, ventral tegmental area, and the nucleus linearis caudalis (Table 1, Fig. 1). Less frequent AVT+ fibres were observed in the mesencephalic and pontine reticular formations, both compact and reticular parts of the substantia nigra and A8 area. Occasional AVT+ fibres were observed in the pretectal areas, pedunculo-pontine tegmental nucleus, ventral subcoeruleus nucleus (Table 1, Fig. 1) and in the proximity of the abducens and angular nuclei (Fig. 2N, O).

Vasoactive Intestinal Peptide-like immunoreactive neurones, fibres and terminal fields

Numerous perikarya were immunoreactive for VIP in the lateral septal organ, the hypothalamic inferior and tuberal nuclei of all birds (Figs. 1, 2K, L, Table 1). They were rare in the lateral hypothalamic area (Fig. 4Q), midbrain central gray (Fig. 1), mesencephalic lateral and medial reticular formation (Fig. 1), intercollicular nucleus (Fig. 1), ventral tegmental area (Figs. 1, 4T), substantia nigra pars reticulata and pars compacta (Fig. 1), locus coeruleus (Fig. 1), A8, Raphe/Linearis caudalis (Figs. 1, 3M, N) and among the fibres of the lateral lemniscus.

The highest density of fibres and terminal fields were found in the subpallial and hypothalamic

structures (Fig.1 and Table1). In the subpallium, these include the lateral septum (Fig. 4N) – in particular the ventral and ventrolateral parts of the caudal lateral septum (Fig.4K) – the intermediate band of the medial septum, the pallidal septal area (Fig. 4H), the nucleus of the diagonal band (Fig. 4N), the lateral part of the bed nucleus of the stria terminalis including the lateral part of the rostral bed nucleus of the stria terminalis along the ventral part of the lateral ventricle, the nucleus accumbens (Fig. 4N)– particularly its rostral striatopallidal part (Fig 4G) – a crescent-shaped area in the ventral pallidum (Fig 4E,N), preoptic nuclei and area, in particular in the medial preoptic nucleus. Fibres were also present ventrally to the lateral forebrain bundle, in the nucleus basalis of Meynert and the striatopallidal amygdaloid area of Puelles (Puelles et al., 2007), (Fig. 4F). It should be noted that the medial striatum, including area X, lateral striatum, globus pallidus and the medial septal nucleus, is virtually devoid of VIP+ staining while only a few fibres were present in the commissural septal nucleus. The lateral hypothalamic area (Fig. 4O, Q), hypothalamic ventromedial nucleus core (Fig. 4O), paraventricular nucleus, infundibular/tuberal nuclei together with the median eminence (Fig. 4R), hypothalamic inferior nucleus, and, to a less extent, the dorsal hypothalamic area, hypothalamic anterior nucleus and hypothalamic periventricular nucleus are the hypothalamic regions with the densest VIP+ terminal field.

In the pallial telencephalon, VIP+ fibres frequently reached the medial arcopallium and the nucleus taenia (Fig. 4J) via the occipitomesencephalic tract but more rarely the intermediate, posterior and ventral arcopallia, and the extended amygdala. In males, a few fibres run along the medial surface of the arcopallial robust nucleus like a shell (Fig.4I).

Some VIP+ fibres coursed along the septomesencephalic tract (Fig. 4C) and along the lateral wall of the lateral ventricle. These fibres enter the periventricular ventromedial nidopallium immediately dorsally to the palliosubpallial lamina (Table 2), and the periventricular dorsomedial mesopallium. Some VIP+ fibres coursed along the ventral mesopallial lamina before entering the core of the dorsal mesopallium (Puelles et al., 2007). The mesopallial high vocal centre received a greater, though modest, number of VIP+ fibres. Rare VIP+ fibres coursed lateralward in the ventral telencephalon possibly toward the lateral (striatal) part of the olfactory tubercle along the ventral olfactory tract as identified in Puelles et al. (2007). In the rostral telencephalon, most VIP+ fibres branched in the medial part of the olfactory tubercle. A few VIP+ fibres run along the midline, then the lateral wall of the lateral ventricle, and enter the medial part of the rostral hyperpallium apicale. Some fibres are branching and terminating in the medial ventral mesopallium. Rare VIP+ fibres coursed through the hyperpallium apicale, and the hippocampal

formation (Fig. 4A), most of them running along the periventricular layer toward the dorsolateral corticoid area. Some VIP+ fibres were found in the dorsolateral corticoid area mainly in its medial and rostrocaudal parts (Fig. 4B). Few VIP+ fibres reached the frontal nidopallium following a ventral subpial pathway. Some VIP+ fibres in the rostral lateral nidopallium were restricted to the corticoid plate of Puelles (Puelles et al., 2007). Moving more caudally, rare VIP+ fibres run along the palliosubpallial lamina and enter the central intermediate nidopallium. More VIP+ fibres were seen in the caudal medial nidopallium at the level of the auditory fields. In the thalamus, VIP+ fibres were essentially present in the medial dorsal thalamus (thalamic anterior dorsomedial and thalamic posterior dorsomedial nuclei), a region identified as A13 by Puelles et al. (2007), and the periventricular zone (Table 1, Figs. 1, 4P).

Some of them coursed along the dorsal surface of the thalamic dorsomedial nuclei lateral ward, terminating into the posterior dorsolateral nucleus and the superficial parvicellular nucleus, in the nucleus paramedianus internus, the internal centromedial nucleus of Puelles et al. (2007), the thalamic ventrolateral nucleus, the thalamic posterior dorsointermediate nuclei, the dorsal part of the nucleus reticularis superior, the intergeniculate leaflet and the zona incerta. In the mesencephalon and brainstem, the main areas containing VIP+ fibres are the midbrain central gray (Fig. 4S), the intercollicular nucleus (Fig. 4U), the ventral tegmental area (Fig. 4T), the optic tectum (mainly its stratum griseum centrale), the substantia nigra and the pontine reticular formation (see also Table 1 and Fig.1). Fibres were less frequent but consistently observed in several pretectal nuclei (nucleus of the septomesencephalic tract, pretectal nucleus, diffuse pretectal nucleus), the perirubral and retro-rubral (dopaminergic A8) fields, the medial and lateral mesencephalic reticular formation, the pedunculopontine tegmental nucleus, the locus coeruleus, the dorsal and ventral subcoeruleus nuclei, and the nucleus linearis caudalis raphe (Table 1, Fig. 1). Scarce VIP+ fibres were observed in the dorsal raphe and trapezoid body (Table 1).

Although the density of the VIP-immunoreactive (VIP+) fibres may vary considerably between individuals, within any given subject the relative density between areas remains consistent.

DISCUSSION

We described the distribution of AVT and VIP immunoreactivity throughout the entire brain of male penduline tits. To our knowledge, this is the first comprehensive study performed in the *Remizidae* family and, within it, the *Remiz* genus.

Notes on Methodology

The appearance and precise localization of the

peptides in question might strongly depend on functional factors such as the mating and breeding stage of individuals. Since the specimens were collected in the wild at various stages of the breeding season, this would inevitably generate variability of the anatomical distribution and detectability of these peptides.

Distribution of AVT immunoreactive neurones and fibres and comparison with other avian species.

The vast majority of the AVT+ neuronal distribution is consistent with earlier observations in other bird species [blue tit (Montagnese et al., 2015), canary (Kiss et al., 1987), zebra finch (Goossens et al., 1977), dark-eyed junco (Panzica et al., 1999), starling (Goossens et al., 1977), domestic fowl (Viglietti-Panzica, 1986), Japanese quail and Peking duck (Goossens et al., 1977; Bons, 1980; Viglietti-Panzica, 1986), pigeon (Berk et al., 1982) and budgerigar (Fabris et al., 2004)]. The AVT+ neurones can be grouped in a lateral system (lateral part of the preoptic-hypothalamic region), a periventricular system (extending from the preoptic area to the tuberal hypothalamus) and a dorsal diencephalic system (dorsal to the lateral forebrain bundle and the occipitomesencephalic tract), as defined by Berk et al., 1982 and Viglietti-Panzica, 1986. Briefly, the lateral system includes the supraoptic and suprachiasmatic nuclei, as well as rostral and ventral lateral neurones in the lateral preoptic area, neurones intermingled with the lateral forebrain bundle, accessory neuronal groups in the lateral and dorsolateral hypothalamus. The periventricular system is composed of the periventricular and paraventricular nuclei, extending from the preoptic recess to posterior parts of the paraventricular nucleus, at the ventral border of the thalamic anterior dorsomedial nucleus.

Finally, the dorsal diencephalic system includes several clusters, near the lateral and dorsal borders of the lateral forebrain bundle, the medial and dorsal surface of the occipitomesencephalic tract, partially overlapping with the medial bed nucleus of the stria terminalis. The latter area was reported to contain AVT as one of its neurochemical markers in quail (Aste et al., 1998), as well as in passerine species (Panzica et al., 1999). The lateral system is more similar to that of the blue tit (Montagnese et al., 2015) than to that of the fowl, duck, pigeon and Japanese quail (Berk et al., 1982; Viglietti-Panzica, 1986).

Nevertheless, in the penduline tits, the lateral system is not as clearly separated from the dorsal diencephalic system as it is in other species (Goossens et al., 1977; Berk et al., 1982; Viglietti-Panzica, 1986). The periventricular system appears similar to that of other passerines (Goossens et al., 1977; Panzica et al., 1999; Montagnese et al., 2015), fowl, duck and pigeon (Viglietti-Panzica, 1986). As for the dorsal diencephalic system, with

a known species dependent variation (Viglietti-Panzica, 1986), it displays a notable variability between penduline tit individuals. This might be related to the reproductive stage of the bird.

We identified a few AVT+ neurones in the mid-brain of the penduline tit. The frequency of these neurones is highly variable between individuals, and is also inversely related to the density of AVT+ fibres in the same regions. Such neurones were not observed in blue tits (Montagnese et al., 2015), canaries (Kiss et al., 1987) or zebra finches (Voorhuis and de Kloet, 1992). A few AVT+ neurones were observed also in the lateral mesencephalic reticular formation of the quail (Panzica et al., 1988).

Distribution of AVT+ fibres in the hypothalamus is similar to that observed in other birds. Nevertheless, some discrepancy occurs in a few nuclei such as the hypothalamic ventromedial nucleus and the lateral mammillary and premammillary nuclei, in which fibres have been identified in a few species (Panzica et al., 1988; Panzica et al., 1999; Panzica et al., 2001; Leung et al., 2009), but these did not occur in the penduline tit.

Consistent with observation in other passerine and non-passerine bird species, numerous vasotocinergic fibres are present in several extrahypothalamic areas of penduline tits, including the lateral septum, medial bed nucleus of the stria terminalis, preoptic area, in particular medial preoptic nucleus (Kiss et al., 1987; Viglietti-Panzica et al., 1992; Voorhuis and de Kloet, 1992; Panzica et al., 1999; Fabris et al., 2004; Montagnese et al., 2015).

Contrary to the situation in blue tit (Montagnese et al., 2015), the medial arcopallium and nucleus taeniae of the amygdala were virtually devoid of staining in penduline tits. At variance with other findings obtained in canaries, zebra finches and dark-eyed juncos (Kiss et al., 1987; Voorhuis and de Kloet, 1992; Panzica et al., 1999), we found fibres in the ventral pallidum and nucleus accumbens, but none in the nucleus robustus of the arcopallium.

Systematic descriptions of the distribution of AVT+ fibres in thalamic areas of birds are rare. In the dorsal thalamus, the distribution of AVT+ fibres is very similar to that of the blue tit (Montagnese et al., 2015). Similarities have also been observed in the thalamic posterior dorsomedial nucleus, the intermediate periventricular nucleus and ventral periventricular nucleus, the zona incerta in songbirds (Kiss et al., 1987; Leung et al., 2009) and Japanese quails (Panzica et al., 2001). No fibres have been observed in the nucleus ovoidalis, the thalamic anterior lateral nucleus and the posterior dorsolateral nucleus, the nucleus rotundus, and the ventral lateral geniculate, unlike in a few other species (Kiss et al., 1987; Panzica et al., 1988; Fabris et al., 2004; Leung et al., 2009). To our knowledge, habenular vasotocinergic fibres have only been observed in songbirds [present data,

(Montagnese et al., 2015; Kiss et al., 1987; Voorhuis and de Kloet, 1992)]. The distribution of AVT+ fibres in mesencephalic and brainstem centres is similar to that seen in other birds. Fibres have been seen in the optic tectum, the pretectal nuclei, the midbrain central gray, the intercollicular nucleus, the lateral mesencephalic reticular formation, the ventral tegmental area, the substantia nigra, the locus coeruleus, the dorsal and ventral subcoeruleus nuclei, the raphe nuclei and the reticular pontine formation both in penduline tit and in other bird species (present data, (Kiss et al., 1987; Panzica et al., 1988; Voorhuis and de Kloet, 1992; Panzica et al., 1999; Leung et al., 2009; Montagnese et al., 2015). In these regions, differences involve the abundance of the fibres: for instance, AVT+ fibres are scarce in the locus coeruleus of tits, contrasting with their abundance in quails (see Figure 4M, N in Panzica et al., 2001). In tits, AVT+ fibres are absent in some nuclei, but present in other birds (nucleus of Edinger-Westphal, the nucleus of the basal optic root [aka nucleus ectomammillaris], dorsal nucleus of the oculomotor nerve (Panzica et al., 1999; Panzica et al., 2001; Leung et al., 2009)).

Distribution of VIP neurones and fibres and comparison with other avian species

VIP+ neurones are mainly grouped in the lateral septal organ and in the tuberal areas of the hypothalamus, as in other birds (Yamada et al., 1982; Macnamee et al., 1986; Peczely and Kiss, 1988; Cloues et al., 1990; Hof et al., 1991; Kuenzel and Blahser, 1994; Aste et al., 1995; Deviche et al., 2000; den Boer-Visser and Dubbeldam, 2002; Kosonsiriluk et al., 2008; Montagnese et al., 2015). The distribution of VIP+ neurones in the telencephalon and diencephalon of penduline tits is very similar to that of the songbirds (Deviche et al., 2000; Bottjer and Alexander, 1995; Montagnese et al., 2015). A few differences may occur: rare stained cells in the lateral hypothalamic area were observed in penduline tits but not in other avian species. VIP+ neurones have been seen in the arcopallium surrounding nucleus robustus, and in the caudal nidopallium in the song sparrow and starling (Ball et al., 1988), and in the lateral and medial striatum of zebra finches (Bottjer and Alexander, 1995), whereas such neurones did not occur in the penduline tits. It should be noted that VIP+ neurones are more widely distributed in the pallial telencephalon and diencephalon of Columbidae (Peczely and Kiss, 1988; Cloues et al., 1990; Norgren and Silver, 1990; Hof et al., 1991; den Boer-Visser and Dubbeldam, 2002). In galliform birds, VIP+ neurones do not appear in the telencephalon, but some have been observed in the hypothalamus and in the nucleus rotundus (Macnamee et al., 1986; Kuenzel and Blahser, 1994; Aste et al., 1995; Kosonsiriluk et al., 2008).

In the brainstem, scattered VIP+ neurones ap-

pear in several areas. VIP+ neurones in the midbrain central gray, substantia nigra, intercollicular nucleus and ventral tegmental area have also been observed in other bird species (Ball et al., 1988; Aste et al., 1995; Bottjer and Alexander, 1995; den Boer-Visser and Dubbeldam, 2002; Kosonsiriluk et al., 2008; Montagnese et al., 2015). VIP+ neurones present in the perirubral region, mesencephalic reticular formation and raphe nuclei of penduline tits have not yet been observed in other bird species. Conversely, we failed to identify such neurones in the interpeduncular nucleus, the ventral subcoeruleus nucleus, the locus coeruleus, and the lateral paragigantocellular reticular nuclei, which have been detected in doves (den Boer-Visser and Dubbeldam, 2002), hen (Kosonsiriluk et al., 2008) or chickens (Kuenzel and Blahser, 1994).

The extensive distribution of VIP+ fibres and terminal fields throughout the hypothalamus, (essentially in the anterior nucleus, lateral hypothalamic area, paraventricular, periventricular and tuberal and infundibular nuclei and median eminence) matches that observed in many bird species [blue tit (Montagnese et al., 2015), zebra finch (Bottjer and Alexander, 1995), pigeon and dove (Hof et al., 1991; den Boer-Visser and Dubbeldam, 2002); quail (Yamada et al., 1982; Aste et al., 1995), Bantam and Thai hen (Macnamee et al., 1986; Kosonsiriluk et al., 2008), chicken (Kuenzel and Blahser, 1994)].

In the thalamus, VIP+ fibres are commonly found in the thalamic dorsomedial and the dorsolateral nuclei, and the zona incerta, similarly to other species [blue tit (Montagnese et al., 2015), pigeon (Hof et al., 1991), quail (Aste et al., 1995), collared dove (den Boer-Visser and Dubbeldam, 2002)]. Unlike in the blue tit and zebra finch, only a few fibres were immunoreactive for VIP in the lateral habenular nucleus of the penduline tit (Bottjer and Alexander, 1995; Montagnese et al., 2015).

In the telencephalon, one of the main contingents of fibres and terminals are present in the lateral septal areas, and this is the case in all species examined [pigeon (Peczely and Kiss, 1988; Hof et al., 1991), dove (den Boer-Visser and Dubbeldam, 2002), Bantam and Thai hen (Macnamee et al., 1986; Kosonsiriluk et al., 2008), chicken (Kuenzel and Blahser, 1994), quail (Yamada et al., 1982; Aste et al., 1995), zebra finch (Bottjer and Alexander, 1995), estrildid and emberizid species (Goodson et al., 2004), blue tit (Montagnese et al., 2015)].

Another important brain centre containing many VIP+ fibres is the medial bed nucleus of the stria terminalis. VIP immunoreactivity in this region has been observed in the blue tit (Montagnese et al., 2015), zebra finch (Bottjer and Alexander, 1995) and quail (Aste et al., 1995), but not in the pigeon, dove, and chicken (Hof et al., 1991; Kuenzel and Blahser, 1994; den Boer-Visser and Dubbeldam,

2002). The VIP immunoreactivity in the preoptic area of the penduline tit resembles that found in songbirds (Bottjer and Alexander, 1995; Montagnese et al., 2015), but differences with other species do appear. In the quail, weak VIP immunostaining was detected in the medial preoptic nucleus (Aste et al., 1995), whereas in the chicken VIP+ fibres were observed only in the ventral preoptic region (Kuenzel and Blahser, 1994), and, in the pigeon, in the anterior preoptic nucleus (Hof et al., 1991). In the penduline tit, many other telencephalic areas contain some VIP+ fibres, as in some, but not all, bird species: area corticoidea dorsolateralis (blue tit, pigeon), the medial hyperpallium apicale (blue tit, zebra finch), the densocellular part of the hyperpallium (blue tit, pigeon), the hippocampal area (blue tit, collared dove, homing pigeon), the arcopallium (blue tit, zebra finch, pigeon, chicken, quail) including the amygdaloid nucleus taeniae (blue tit, quail), olfactory tubercle (blue tit, pigeon, quail), nucleus accumbens (blue tit, pigeon, quail, Thai hen), medial septal nuclei (blue tit, pigeon, Thai hen), ventral pallidum (blue tit, chicken, quail) (Yamada et al., 1982; Erichsen et al., 1991; Hof et al., 1991; Kuenzel and Blahser, 1994; Aste et al., 1995; Bottjer and Alexander, 1995; den Boer-Visser and Dubbeldam, 2002; Kosonsiriluk et al., 2008; Montagnese et al., 2015). In the penduline tit, as in the blue tit (Montagnese et al., 2015), we were unable to identify the medial magnocellular nucleus of the anterior nidopallium, and few fibres were present in the higher vocal centre of the caudal mesopallium and around arcopallial robust nucleus. Interestingly, these song nuclei contain abundant VIP+ fibres and terminals in some other song birds [zebra finch, song sparrow and starling (Ball et al., 1988; Bottjer and Alexander, 1995)].

Distribution of VIP+ fibres and terminals in the penduline tit brainstem was similar to that observed in other species. These are present mainly in the midbrain central gray, the ventral tegmental area, the intercollicular nucleus, the optic tectum and pontine reticular formation, and less frequently in the pretectal nuclei, the mesencephalic the reticular formation, the substantia nigra, the locus coeruleus, the subcoeruleus nuclei, and the raphe structures [zebra finch (Bottjer and Alexander, 1995), pigeon (Hof et al., 1991), collared dove (den Boer-Visser and Dubbeldam, 2002), chicken (Kuenzel and Blahser, 1994), Thai hen (Kosonsiriluk et al., 2008) and quail (Aste et al., 1995)]. No VIP immunoreactivity was detected in a number of structures, which are known to contain VIP+ elements in columbiform and galliform species (interpeduncular nucleus and its surrounding, dorsal tegmentum, in and around the 7th, 9th and 10th cranial nerve nuclei, parabrachial area, motor nucleus of the trigeminal nerve, subtrigeminal reticular nucleus, ventral part of the medial vestibular nucleus and nucleus of the solitary tract) (Hof et

al., 1991; Kuenzel and Blahser, 1994; Aste et al., 1995; den Boer-Visser and Dubbeldam, 2002; Kosonsiriluk et al., 2008).

Phylogenetic consideration

Vasotocinergic/vasopressinergic systems: The vasotocinergic/vasopressinergic system appears highly conserved in the vertebrates. The vasotocinergic hypothalamo-hypophyseal system is present in all classes of vertebrates, including mammals, where vasotocin is replaced by vasopressin [for review (Moore and Lowry, 1998; de Vries and Miller, 1999; Caldwell and Young, 2006; Godwin and Thompson, 2012)]. In mammals, as in birds, hypothalamic neurones are also found in a number of other centres such as the suprachiasmatic nucleus, the lateral hypothalamic and periventricular areas (for review de Vries and Miller, 1999; Moore and Lowry, 1998; Caldwell and Young, 2006). In mammals, neurones were also found in the dorsomedial hypothalamic nucleus, the posterodorsal hypothalamic area, the dorsal capsule of the ventromedial hypothalamic nucleus and the arcuate nucleus and its surroundings (for review see de Vries and Miller, 1999; Moore and Lowry, 1998; Caldwell and Young, 2006). We did not observe the equivalent of the latter hypothalamic localisation in tits. In mammals and birds, vasopressinergic or AVT+ fibres also occur in the anterior hypothalamic area, parastriatal, premammillary and supramammillary nuclei (Moore and Lowry, 1998).

As in some reptilian and mammalian species, extrahypothalamic AVT+ neurones of the penduline tit were accumulated in the bed nucleus of the stria terminalis (Stoll and Voorn, 1985; Wang et al., 1999; Rood and De Vries, 2011). This region belongs to the extended amygdala and is involved, among others, in the regulation of fear- and aggression-related components of social behaviour (Walker et al., 2003; Bosch and Neumann, 2012).

Both in reptiles and mammalian species, an important contingent of extrahypothalamic vasotocinergic and vasopressinergic fibres has been observed in the preoptic area and the ventral telencephalon (nucleus of the diagonal band, nucleus accumbens, and tuberculum olfactorium) and the septal nuclei (Stoll and Voorn, 1985; Propper et al., 1992; Wang et al., 1999; Caldwell and Young, 2006; Rood and De Vries, 2011; Otero-Garcia et al., 2014). In mammals, in addition to those regions, the medial thalamic nuclei, the zona incerta, the lateral habenular nucleus, the midbrain central gray, the substantia nigra, the ventral tegmental area, the subcoeruleus and raphe nuclei, and the pontine reticular formation also contain vasopressinergic fibres, similar to birds (Wang et al., 1999; Caldwell and Young, 2006; Rood and De Vries, 2011; Otero-Garcia et al., 2014).

Overall, there seem to be marked similarities between the neuroanatomical organization of AVT/vasopressin containing nuclei and fibre tracts in

the brain of birds and mammals.

Vasoactive intestinal peptidergic system

In all vertebrate classes, including birds, VIP+ neurones are present in the preoptic and tuberal regions (Lorén et al., 1979; Sims et al., 1980; Obata-Tsuto et al., 1983; Rosténe, 1984; Simerly et al., 1986; Batten et al., 1990; Mathieu et al., 1999). In mammalian hypothalamus, VIP is also localized in the suprachiasmatic nucleus (Lorén et al., 1979; Sims et al., 1980; Obata-Tsuto et al., 1983; Obata et al., 1999). The latter was not confirmed in the present study, although VIP has been reported to occur in the suprachiasmatic nucleus of other bird species (Peczely and Kiss, 1988).

Apart from the above mentioned areas, containing VIP+ perikarya, VIP+ fibres are present in many other hypothalamic and extrahypothalamic areas in all classes of vertebrates. With the evolution of the forebrain, VIP+ fibres tend to invade the cortical areas, the lateral septum, the nucleus accumbens, the anterior hypothalamus and the additional mesencephalic and pontine structures [Reptile (Petko and Ihionvien, 1989; Reiner, 1991; Hirunagi et al., 1993), mammals (Lorén et al., 1979; Sims et al., 1980)]. Common to birds and mammals, but apparently not to reptiles, is the wide distribution of VIP+ fibres over the thalamus and the premammillary/mammillary areas and the substantia nigra, raphe nuclei (Hirunagi et al., 1993; Lorén et al., 1979; Sims et al., 1980; Obata-Tsuto et al., 1983; Simerly and Swanson, 1987; Lantos et al., 1995; Ahnaou et al., 2006). In mammals, VIP elements can be grouped into four major systems: 1) an intracortical system; 2) a system centred on the amygdala and the bed nucleus of the stria terminalis, also connecting with the nucleus accumbens, septal areas and the hypothalamus; 3) a pathway originating from the suprachiasmatic nucleus, connected with hypothalamic and thalamic areas; 4) a pathway originating in the central gray of the midbrain (Lorén et al., 1979; Sims et al., 1980; Rosténe, 1984). Corresponding systems are potentially present in penduline tits and other bird species.

CONCLUSIONS

The reproductive behaviour of the penduline tit, compared to other songbirds, is characterized by multiple breeding and its extreme response to the conflict over parental care: nest desertion by one parent or both (Szentirmai et al., 2007). Nest desertion has also been observed in other species and is frequent in precocious bird species (Kosztolanyi et al., 2006). Distribution of AVT+ and VIP+ neurones and fibres in the penduline tits is not fundamentally different from those found in other non-precocious songbirds and in other bird taxa, suggesting that the basis of the various reproductive behaviours lies in subtle changes in

neuropeptide or receptor expression, rather than in the main architecture of neuropeptide systems. The candidate regions to detect such subtle changes are parts of the social brain network system and social decision-making system (Newman, 1999; O'Connell and Hofmann, 2012), including less studied regions rich in AVT and VIP cells or fibres such as the caudal brainstem nuclei. The present study defines the position and borders of these candidate regions.

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Table 1. Distribution of VIP- and AVT immunoreactive neurones, fibres and terminal fields in the brain of male penduline tits

	VIP immunoreactivity		AVT immunoreactivity	
	cells	fibres terminals	cells	fibres terminals
TELENCEPHALON				
Pallium				
Hyperpallium apicale		+		
Densocellular part of the hyperpallium/dorsal mesopallium (Jarvis et al., 2013)		-/+		
Mesopallium/ventral mesopallium (Jarvis et al., 2013)				
<i>medial periventricular</i>		+		
<i>dorsal (core)</i>		+ / ++		
<i>medial and central part</i>		+ / ++		
<i>intermediate medial</i>		- / ++		
<i>Higher vocal centre</i>		++		
Dorsolateral corticoid area		++		
Nidopallium				
<i>frontal periventricular</i>		+		
<i>frontal, corticoid plate</i>		+		
<i>medial periventricular ventral</i>		+ / ++		
<i>intermediate central part</i>		+		
<i>intermediate lateral part (island field)</i>		- / +		
<i>intermediate periventricular ventral</i>		- / ++		
<i>Field L (part of the intercalated nidopallium-Jarvis et al., 2013)</i>		+ / ++		
<i>caudal medial</i>		+ / ++		
<i>caudal lateral</i>		+		
Hippocampal formation		+		
Parahippocampal area		+		
Arcopallium				
<i>medial</i>		+ / ++		+
<i>Robust Nucleus shell</i>		++		
<i>intermediate</i>		+ / ++		
<i>posterior</i>		+ / ++		
<i>ventral</i>		+		
<i>Nucleus taeniae of the amygdala</i>		++ / +++		
<i>Extended amygdala</i>		+		
Subpallium				
Striatal subpallium				
Medial striatum		+		
Nucleus accumbens				
<i>Striatopallidal accumbens</i>		+++		
<i>rostral pole</i>		++ / +++		
<i>shell</i>		+ / +++		- / ++
<i>core</i>		++ / +++		- / ++

	VIP immunoreactivity		AVT immunoreactivity	
	cells	fibres terminals	cells	fibres terminals
Pallidal subpallium				
globus pallidus		+		
Ventral pallidum		++/+++		+ / ++
Bed nucleus of the stria terminalis				
<i>medial part</i>		++/++++		- / ++
<i>ventral part</i>		+ / ++	++	++
<i>dorsolateral part</i>		++	++/+++	++/++++
<i>magnocellular part</i>		+ / ++		+
Basal nucleus (Meynert)		++/+++		
Striatopallidal subpallium				
Olfactory tubercle		++		
<i>medial TuO</i>		+++ / +++++		
<i>lateral TuO</i>		+		
Septal subpallium				
Lateral septum				
<i>rostral "pole"</i>		++/+++		- / ++
<i>rostral SLc.v</i>		+++		+ / ++
<i>rostral SLc.vl</i>		+++		+ / ++
<i>SLr.dl</i>		+		- / ++
<i>SLr.m</i>		++/+++		
<i>SLc.d</i>		- / ++		- / +++++
<i>SLc.v</i>		+++ / +++++		+ / +++++
Medial septum				
<i>medial septal nucleus</i>		+		
<i>intermediate band</i>		++/+++		+
Nucleus of the diagonal band		++/+++		+ / +++++
Septocommissural nucleus		- / ++		+ / ++
Corticohabenular and corticoseptal tract		- / ++		
Nucleus of the pallial commissure		++/+++		
Lateral septal organ		++++		
Preoptic area		+++	- / +++++	++ / +++++
Medial preoptic nucleus		+++ / +++++	- / +++++	+ / +++++
<i>Tractus</i>				
Lamina frontalis superior/lamina mesopallialis dorsalis (Jarvis et al. 2013)		+		
Lamina pallio-subpallialis		+		
Septomesencephalic tract		- / ++		
Ventral amygdalofugal tract		++/+++		
DIENCEPHALON				
Thalamus				
Anterior dorsomedial nucleus		++/+++		++/+++
Posterior dorsolateral nucleus		+ / ++		
Posterior dorsomedial nucleus		++/+++		- / ++
Posterior dorsointermediate nucleus		+		

	VIP immunoreactivity		AVT immunoreactivity	
	cells	fibres terminals	cells	fibres terminals
Nucleus paramedianus internus		+		
Superficial parvocellular nucleus		+		
Intermediate periventricular nucleus (Puelles et al. 2007)		+ /+++		+ /++
Intermediate centromedial nucleus (Puelles et al. 2007)		+		+
Ventrolateral nucleus		+		
Ventral periventricular nucleus (Puelles et al. 2007)		+ /+++		+ /+++
Nucleus reticularis superior dorsal part		+		
Lateral geniculate		+		
Intergeniculate leaflet		+		
Nucleus of the septo-mesencephalic tract		+ /+++		
Lateral habenula		+		+
Prethalamus				
Zona incerta		+ /++++		
A13 area		+ /++++		
Hypothalamus				
Ventral supraoptic nucleus			++++	
External supraoptic nucleus			++++	
Suprachiasmatic nucleus			++++	
Hypothalamic anterior nucleus		+ /++++		+
Lateral hypothalamic area	+	+++		
Periventricular nucleus		+ /++++		+ /++++
Paraventricular nucleus		+ /++++	++++	
Ventromedial nucleus core		+ /++++		
Dorsomedial nucleus		++		+
Medial hypothalamic area				+
Dorsal hypothalamic area		++		
Nucleus subthalamicus		+ /++++		
Posterior nucleus		+ /++++		
Inferior nucleus	+++	+++		
Infundibular nucleus	++++	+ /++++		
Tuberal/Arcuate) nucleus	++++	+ /++++		
Retromammillary nucleus		+ /++++		
Dorsal supraoptic decussation		+		
BRAINSTEM				
Pretectum/mesencephalon				
Mesencephalic nucleus lentiformis		+		
Diffuse pretectal nucleus		+		
Pretectal nucleus		- /+		+
Medial pretectal nucleus		+ /++++		
Pretectal area		- /+++		
Perirubral region	+	+ /++++		+
Midbrain central gray	+	++		+ /++++
Intercollicular nucleus	+	++		+ /++++
Lateral reticular formation	+	+	+ /++	+ /++
Medial reticular formation	+	+	+ /++	+ /++
Mesencephalic nucleus lentiformis				+
Ventral tegmental area	+	+ /++++	+ /++++	+ /++++
Substantia nigra pars reticulata	+	+ /++		+ /++
Substantia nigra pars compacta	+	++	+ /++	+ /++
A8 area	+	+ /+++		+ /+++
Optic tectum		+ /+++		
Stratum griseum centrale		+ /+++		
Rhombencephalon				
Pedunculo-pontine tegmental nucleus		+ /+++		+
Locus coeruleus		+ /+++		
Dorsal nucleus subcoeruleus		+		
Ventral nucleus subcoeruleus		+		+ /+++
Nucleus linearis caudalis/Raphe	+	+ /+++	+ /++++	+ /++++
Dorsal raphe	+	+	+ /++	+ /++
Pontine reticular formation		+		+ /+++
Medial vestibular nucleus		- /+		
Trapezoid bodies		+		

+: occasional; ++: few; +++: moderate; ++++ numerous

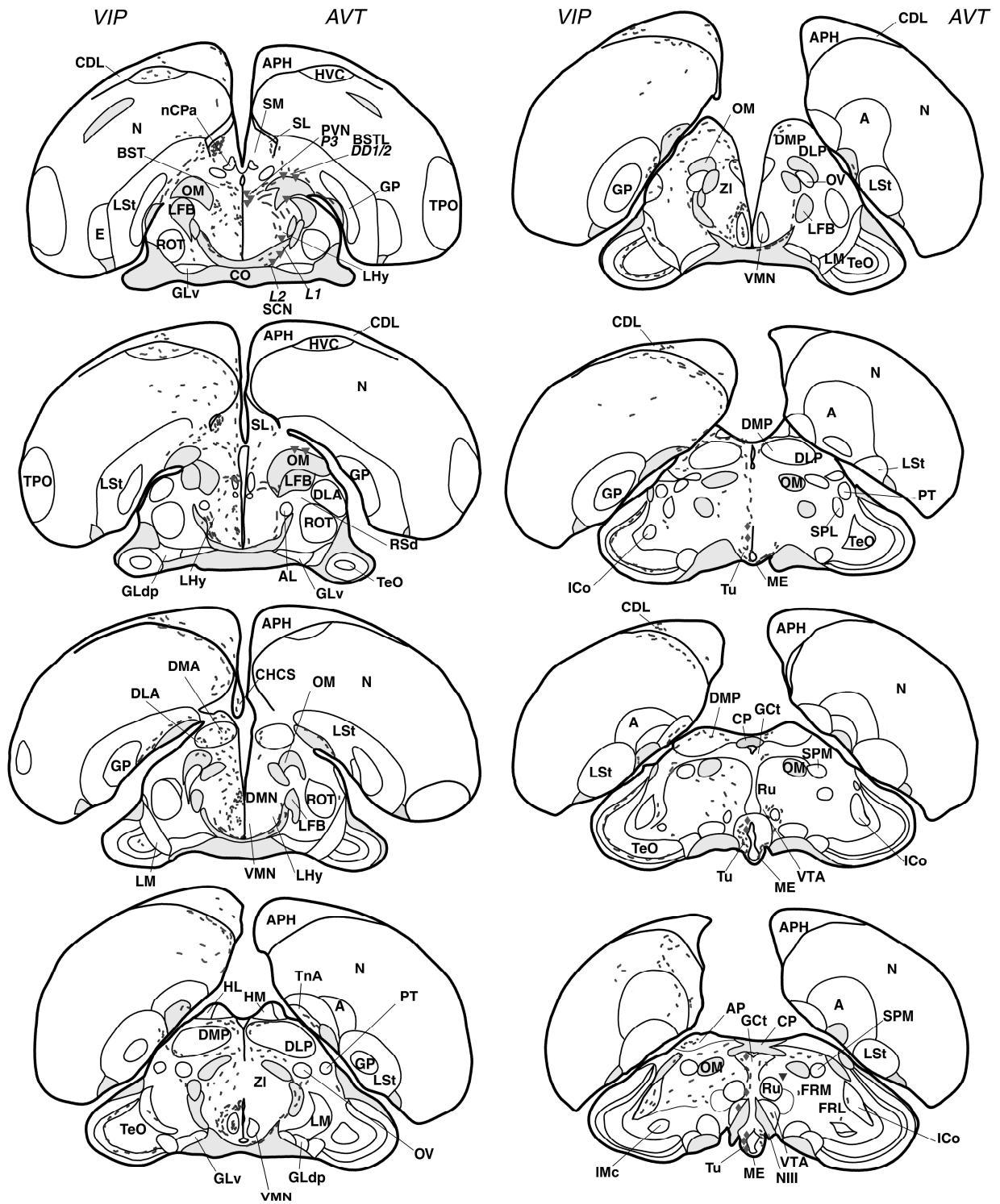


Fig. 1.

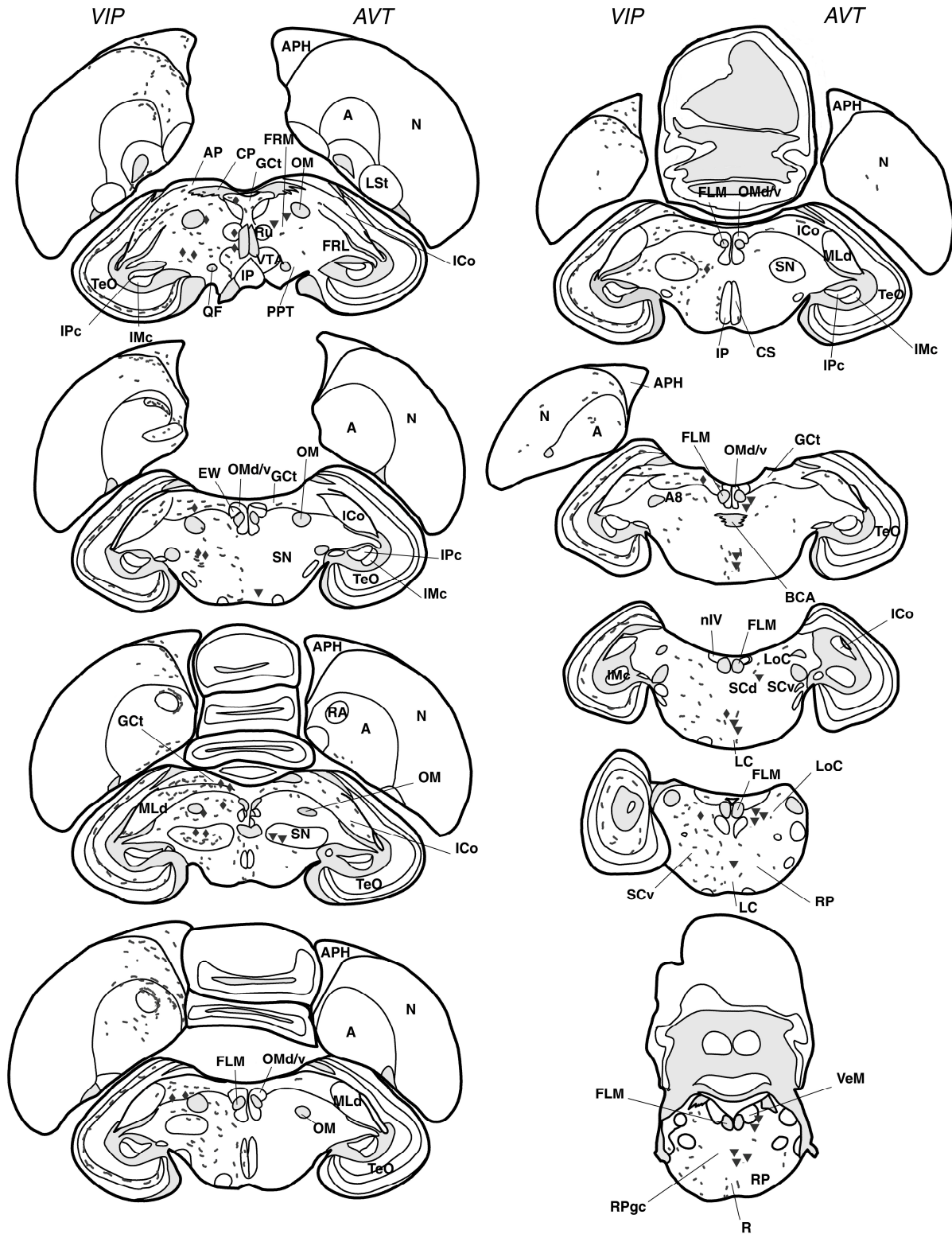


Fig. 1. Continued

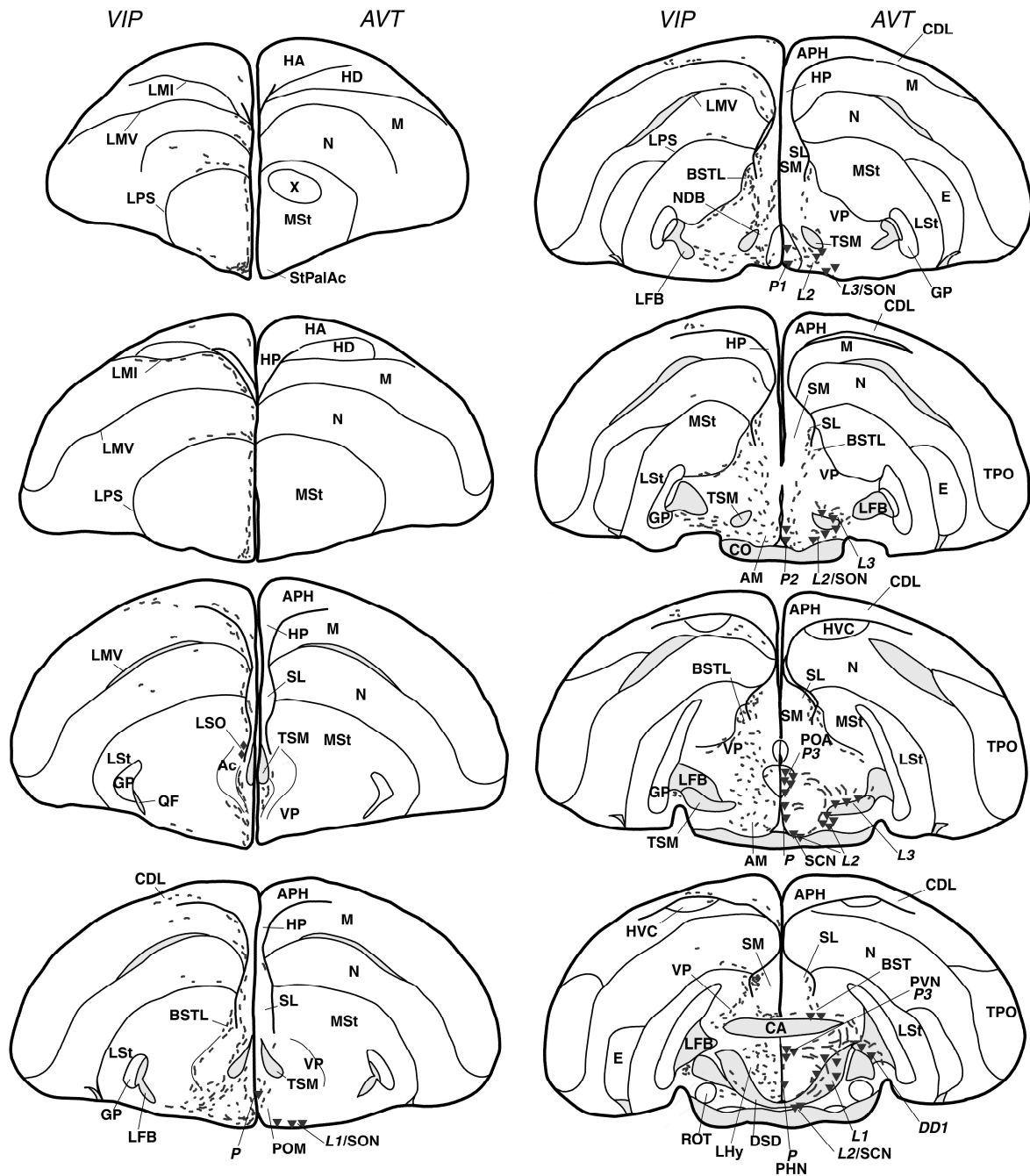


Fig.1. Continued.

Fig.1. Representative drawings of the distribution of Vasotocin-like immunoreactive (AVT, right half of the brain) and Vasoactive intestinal peptide-like immunoreactive (VIP, left half) perikarya (triangles and diamonds, respectively) and fibres (short lines) throughout the brain of the penduline tit (*Remiz pendulinus*). (A arcopallium, A8 dopaminergic cell group, Ac nucleus accumbens, AL ansa lenticularis, AM hypothalamic anterior medial nucleus, AP pretectal area, APH parahippocampal area, BCA brachium conjunctivum ascendens, BST bed nucleus of the stria terminalis, BSTL lateral part of the bed nucleus of the stria terminalis, CA anterior commissure, CDL dorsolateral corticoid area, CHCS cortico-habenular and corticoseptal tract, CO optic chiasm, CP posterior commissure, CS superior central nucleus, DD1-DD2 dorsal diencephalic vasotocinergic cells groups (Berk et al., 1982), DHA dorsal hypothalamic area, DLA anterior dorso-lateral thalamic nucleus, DLP posterior dorso-lateral thalamic nucleus, DMA anterior dorsomedial thalamic nucleus, DMN medial dorsal hypothalamic nucleus, DMP posterior dorsomedial thalamic nucleus, DSD dorsal supraoptic decussation, E entopallium, EW nucleus of Edinger-Westphal, FLM medial longitudinal fascicle, FRL lateral mesencephalic reticular formation, FRM medial mesencephalic reticular formation, GCt central gray, GLdp posterodorsal part of the lateral geniculate nucleus, GLv ventral part of the lateral geniculate nucleus, GP globus pallidus, HA apical part of the hyperpallium, HD densocellular part of the hyperpallium, HL lateral habenular nucleus, HM medial habenular nucleus, HP hippocampal formation, HVC higher vocal centre, ICo intercollicular nucleus, IMc magnocellular part of the

isthmus nucleus, IP interpeduncular nucleus, IPc parvocellular part of the isthmus nucleus, L1-L4 hypothalamic vasotocinergic cells groups (Berk et al., 1982), LC nucleus linearis caudalis, LFB lateral forebrain bundle, LM mesencephalic nucleus lentiformis, LMI intermediate mesopallial lamina, LMV ventral mesopallial lamina, LoC locus coeruleus, LPS pallio-subpallial lamina, LSO lateral septal organ, LSt lateral striatum, LHy lateral hypothalamic area, M mesopallium, ME median eminence, MLd dorsal part of the lateral mesencephalic nucleus, MSt medial striatum, N nidopallium, NDB nucleus of the diagonal band of Broca, NIII oculomotor nerve, nCPa nucleus of the pallial commissure, nIV nucleus of the trochlear nerve, OM occipitomesencephalic tract, OMd/v oculomotor nuclei, Ov nucleus ovoidalis, P-P1-P3 hypothalamic periventricular vasotocinergic cells groups (Berk et al., 1982), PHN periventricular hypothalamic nucleus, POA preoptic area, POM magnocellular preoptic nucleus, PPT pedunculo-pontine tegmental nucleus, PT pretectal nucleus, PTM medial pretectal nucleus, PVN paraventricular hypothalamic nucleus, QF quincifrontal tract, R raphe nuclei, RA robust nucleus of the arcopallium, ROT nucleus rotundus, RP pontine reticular formation, RPgc gigantocellular part of the caudal pontine reticular nucleus, RSd dorsal part of the superior reticular nucleus, Ru red nucleus, SCN suprachiasmatic nucleus, SCd dorsal subcoeruleus nucleus, SCv ventral subcoeruleus nucleus, SL lateral septum, SM medial septum, SN substantia nigra, SON supraoptic nucleus, SPL lateral spiriform nucleus, SPM medial spiriform nucleus, StPaAc striatopallidal area of the nucleus accumbens of Puelles et al. (2007), TeO optic tectum, TnA nucleus taeniae of the amygdala, TPO temporo-parieto-occipital area, TSM septopallial mesencephalic tract, Tu tuberal/arcuate nucleus, VeM medial vestibular nucleus, VLT ventrolateral thalamic nucleus, VMN ventromedial hypothalamic nucleus, VP ventral pallidum, VTA ventral tegmental area, X area X, ZI zona incerta).

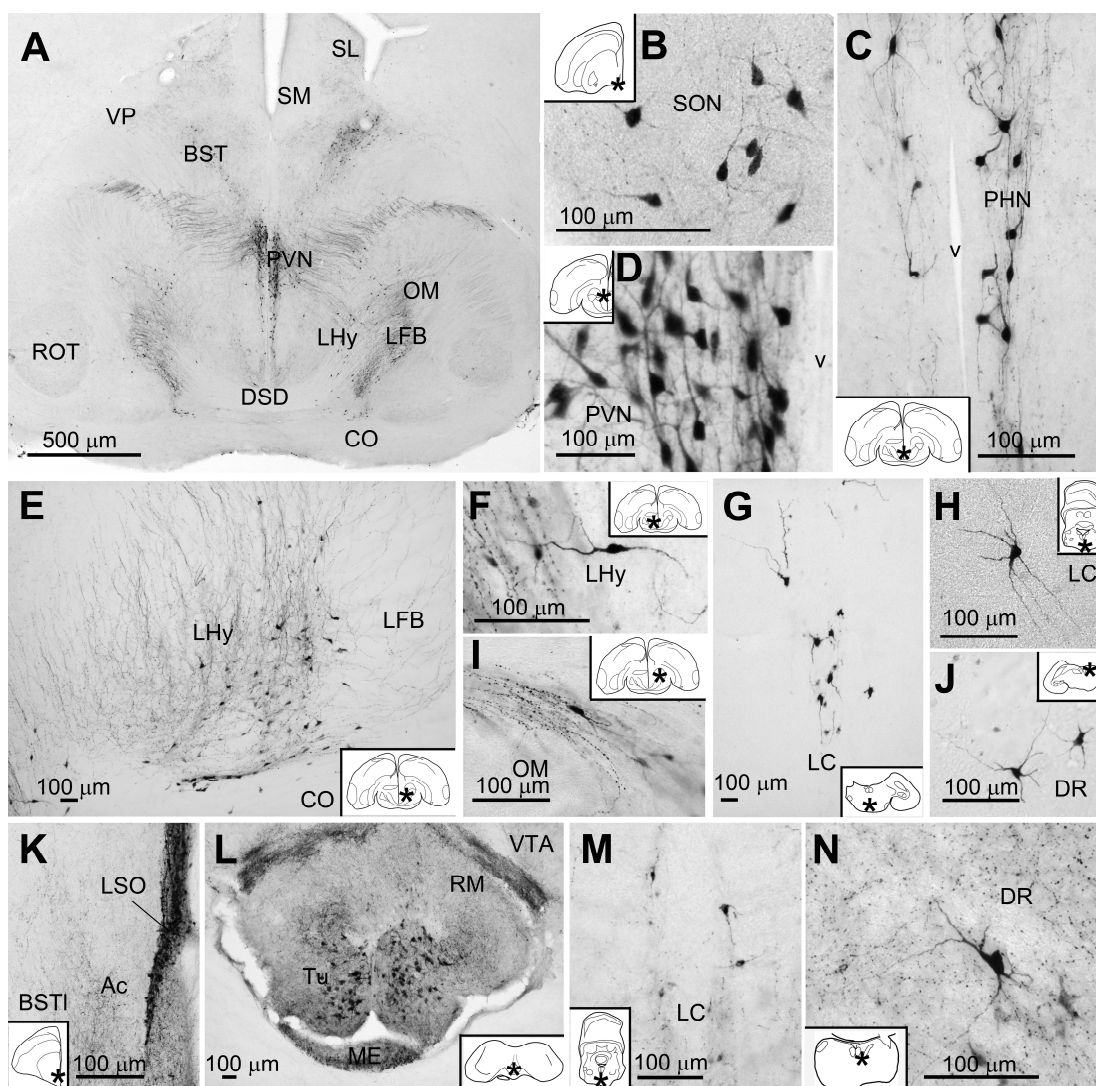


Fig. 2. Microscopic images of vasotocin-like and VIP-like immunoreactive neurones and fibres in the brain of penduline tit. (A) Low power photomicrograph showing the distribution of vasotocin-like immunoreactive neurones and fibres in the diencephalon of penduline tit. (B-J) High power photomicrographs of vasotocin-like immunoreactive perikarya in the brain of penduline tits. (B) supraoptic nucleus, (C) hypothalamic periventricular nucleus, (D) hypothalamic paraventricular nucleus, (E, F) lateral hypothalamic area, (G, H) raphe/nucleus linearis caudalis. (I) *DD2* group, lateral part of the bed nucleus of the stria terminalis, (J) dorsal raphe. (K-N) Photomicrographs of VIP-like immunoreactive perikarya in the brain of penduline tits. (K) lateral septal organ, (L) arcuate nucleus, (M) nucleus linearis caudalis, (N) dorsal raphe. Inset: the star indicates the location of the photographed neurones on the topogram. (Abbreviations in addition to those listed in the legend to Fig. 1: DR dorsal raphe). Calibration bar 100 µm except (A): 500 µm.

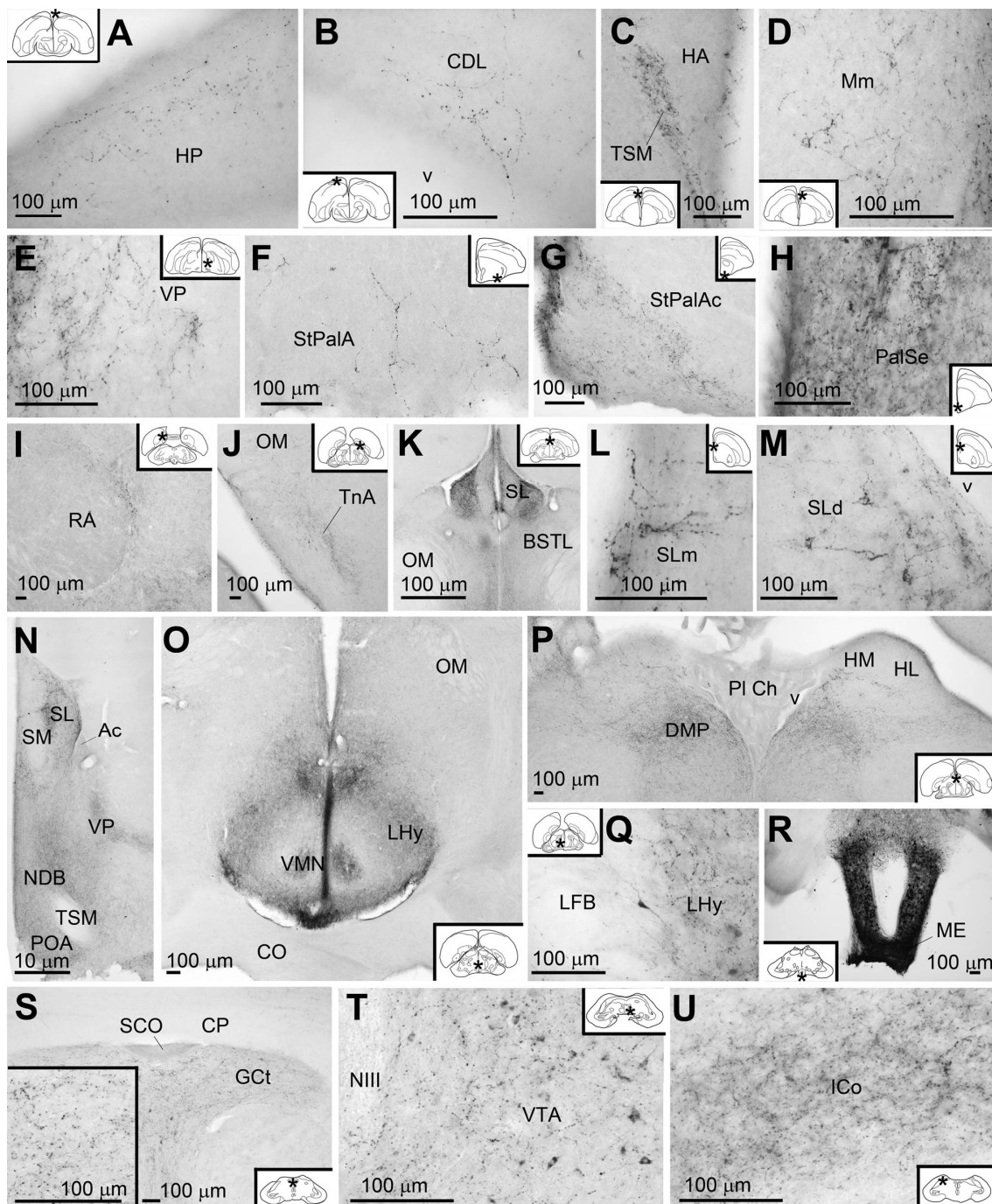


Fig. 4. Photomicrographs of Vasoactive intestinal peptide-like immunoreactive fibres and terminal fields in the brain of the penduline tit. (A) dorsal hippocampal formation, (B) ventral dorsolateral corticoid area, (C) rostral septomesencephalic tract, (D) ventral medial mesopallium, (E) ventral pallidum, (F) striatopallidal amygdoid area, (G) striatopallidal area of the accumbens nucleus, (H) pallidoseptal transition, (I) robust nucleus of the arcopallium, (J) nucleus taeniae of the amygdala, (K) overview of the septal areas at caudal level (L,M) rostral lateral septum in its medial and lateral-dorsal subdivisions respectively, (N) low magnification overview of the rostral septal and preoptic areas, (O) low magnification overview of hypothalamic terminal fields, (P) medial dorsal thalamic terminal field, (Q) neurones and terminal field in the lateral hypothalamic area, (R) median eminence, (S) mesencephalic central gray, (T) ventral tegmental area, (U) intercollicular nucleus. Inset: stars indicate the location of the photographed fields in the topogram. (Abbreviations in addition to those listed in the legend to Fig. 1: Mm medial mesopallium, PalSe pallidoseptal transition, PICh choroid plexus, SCO subcommissural organ, SLd laterodorsal subdivision of the lateral septum SLm medial subdivision of the lateral septum, StPalA striatopallidal amygdoid area, v ventricle). Calibration bar 100 μ m, except in (N):10 μ m.

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REFERENCES

- AHNAOU A, YON L, ARLUISON M, VAUDRY H, HANNIBAL J, HAMON M, ADRIEN J, BOURGIN P (2006) Immunocytochemical distribution of VIP and PACAP in the rat brain stem: implications for REM sleep physiology. *Ann N Y Acad Sci*, 1070: 135-142.
- ASTE N, BALTHAZART J, ABSIL P, GROSSMANN R, MULHBAUER E, VIGLIETTI-PANZICA C, PANZICA GC (1998) Anatomical and neurochemical definition of the nucleus of the stria terminalis in Japanese quail (*Coturnix japonica*). *J Comp Neurol*, 396: 141-157.
- ASTE N, VIGLIETTI-PANZICA C, FASOLO A, PANZICA GC (1995) Mapping of neurochemical markers in quail central nervous system: VIP- and SP-like immunoreactivity. *J Chem Neuroanat*, 8: 87-102.
- BALL GF, FARIS PL, HARTMAN BK, WINGFIELD JC (1988) Immunohistochemical localization of neuropeptides in the vocal control regions of two songbird species. *J Comp Neurol*, 268: 171-180.
- BATTEN TF, CAMBRE ML, MOONS L, VANDESANDE F (1990) Comparative distribution of neuropeptide-immunoreactive systems in the brain of the green molly, *Poecilia latipinna*. *J Comp Neurol*, 302: 893-919.
- BERK ML, REAVES TA, JR., HAYWARD JN, FINKELSTEIN JA (1982) The localization of vasotocin and neurophysin neurons in the diencephalon of the pigeon, *Columba livia*. *J Comp Neurol*, 204: 392-406.
- BONS N (1980) The topography of mesotocin and vasotocin systems in the brain of the domestic mallard and Japanese quail: immunocytochemical identification. *Cell Tissue Res*, 213: 37-51.
- BOSCH OJ, NEUMANN ID (2012) Both oxytocin and vasopressin are mediators of maternal care and aggression in rodents: from central release to sites of action. *Horm Behav*, 61: 293-303.
- BOTTJER SW, ALEXANDER G (1995) Localization of met-enkephalin and vasoactive intestinal polypeptide in the brains of male zebra finches. *Brain Behav Evol*, 45: 153-177.
- CALDWELL HK, YOUNG WS, III (2006) Oxytocin and Vasopressin: Genetics and Behavioral Implications. In: Lajtha A, Lim R (Eds.) *Handbook of Neurochemistry and Molecular Neurobiology*. Springer US, pp 573-607.
- CLOUES R, RAMOS C, SILVER R (1990) Vasoactive intestinal polypeptide-like immunoreactivity during reproduction in doves: influence of experience and number of offspring. *Horm Behav*, 24: 215-231.
- CRAMP S, PERRINS CM, BROOKS DJE (1993) Handbook of the birds of Europe, the Middle East and North Africa. Birds of the western Palearctic. *Oxford University Press, Oxford*, Vol. 7.
- DE VRIES GJ, MILLER MA (1999) Anatomy and function of extrahypothalamic vasopressin systems in the brain. In: I.J.A. Urban JPHB, Wed DD (Eds.) *Prog Brain Res*. Elsevier, pp 3-20.
- DEN BOER-VISSER AM, DUBBELDAM JL (2002) The distribution of dopamine, substance P, vasoactive intestinal polypeptide and neuropeptide Y immunoreactivity in the brain of the collared dove, *Streptopelia decaocto*. *J Chem Neuroanat*, 23: 1-27.
- DEVICHE P, SALDANHA CJ, SILVER R (2000) Changes in brain gonadotropin-releasing hormone- and vasoactive intestinal polypeptide-like immunoreactivity accompanying reestablishment of photosensitivity in male dark-eyed juncos (*Junco hyemalis*). *Gen Comp Endocrinol*, 117: 8-19.
- ERICHSEN JT, BINGMAN VP, KREBS JR (1991) The distribution of neuropeptides in the dorsomedial telencephalon of the pigeon (*Columba livia*): a basis for regional subdivisions. *J Comp Neurol*, 314: 478-492.
- FABRIS C, BALLARIN C, MASSA R, GRANATO A, FABIANI O, PANZICA GC, COZZI B (2004) The vasotocinergic system in the hypothalamus and limbic region of the budgerigar (*Melopsittacus undulatus*). *Eur J Histochem*, 48: 367-372.
- GODWIN J, THOMPSON R (2012) Nonapeptides and social behavior in fishes. *Horm Behav*, 61: 230-238.
- GOODSON JL (1998a) Territorial aggression and dawn song are modulated by septal vasotocin and vasoactive intestinal polypeptide in male field sparrows (*Spizella pusilla*). *Horm Behav*, 34: 67-77.
- GOODSON JL (1998b) Vasotocin and vasoactive intestinal polypeptide modulate aggression in a territorial songbird, the violet-eared waxbill (Estrildidae: *Uraeginthus granatina*). *Gen Comp Endocrinol*, 111: 233-244.
- GOODSON JL (2008) Nonapeptides and the evolutionary patterning of sociality. In: Inga DN, Rainer L (Eds.) *Prog Brain Res*. Elsevier, pp 3-15.
- GOODSON JL, KELLY AM, KINGSBURY MA (2012) Evolving nonapeptide mechanisms of gregariousness and social diversity in birds. *Horm Behav*, 61: 239-250.
- GOODSON JL, LINDBERG L, JOHNSON P (2004) Effects of central vasotocin and mesotocin manipulations on social behavior in male and female zebra finches. *Horm Behav*, 45: 136-143.
- GOOSSENS N, BLAHSER S, OKSCHE A, VANDESANDE F, DIERICKX K (1977) Immunocytochemical investigation of the hypothalamo-neurohypophysial system in birds. *Cell Tissue Res*, 184: 1-13.
- GRAY DA, SIMON E (1983) Mammalian and avian antidiuretic hormone: Studies related to possible species variation in osmoregulatory systems. *J Comp Physiol*, 151: 241-246.
- GULYAS AI, GORCS TJ, FREUND TF (1990) Innervation of different peptide-containing neurons in the hippocampus by GABAergic septal afferents. *Neuroscience*, 37: 31-44.
- HIRUNAGI K, ROMMEL E, OKSCHE A, KORF HW (1993) Vasoactive intestinal peptide-immunoreactive cerebrospinal fluid-contacting neurons in the reptilian lateral septum/nucleus accumbens. *Cell Tissue Res*, 274: 79-90.
- HOF PR, DIETL MM, CHARNAY Y, MARTIN JL, BOURAS C, PALACIOS JM, MAGISTRETTI PJ (1991) Vasoactive intestinal peptide binding sites and fibers in the brain of the pigeon *Columba livia*: an autoradiographic and immunohistochemical study. *J Comp Neurol*, 305:

- 393-411.
- HOUSTON AI, SZÉKELY T, MCNAMARA JM (2005) Conflict between parents over care. *Trends Ecol Evol*, 20: 33-38.
- IBATA Y, OKAMURA H, TANAKA M, TAMADA Y, HAYASHI S, IJIMA N, MATSUDA T, MUNEKAWA K, TAKAMATSU T, HISA Y, SHIGEYOSHI Y, AMAYA F (1999) Functional morphology of the suprachiasmatic nucleus. *Front Neuroendocrinol*, 20: 241-268.
- JARVIS ED, YU J, RIVAS MV, HORITA H, FEENDERS G, WHITNEY O, JARVIS SC, JARVIS ER, KUBIKOVA L, PUCK AE, SIANG-BAKSHI C, MARTIN S, MCELROY M, HARA E, HOWARD J, PFENNING A, MOURITSEN H, CHEN CC, WADA K (2013) Global view of the functional molecular organization of the avian cerebrum: mirror images and functional columns. *J Comp Neurol*, 521: 3614-3665.
- KINGSBURY MA, JAN N, KLATT JD, GOODSON JL (2015) Nesting behavior is associated with VIP expression and VIP-Fos colocalization in a network-wide manner. *Horm Behav*, 69: 68-81.
- KISS JZ, VOORHUIS TA, VAN EEKELEN JA, DE KLOET ER, DE WIED D (1987) Organization of vasotocin-immunoreactive cells and fibers in the canary brain. *J Comp Neurol*, 263: 347-364.
- KOSONSIRILUK S, SARTSOONGNOEN N, CHAIYACHET OA, PRAKOBSAENG N, SONGSERM T, ROZENBOIM I, EL HALAWANI M, CHAISEHA Y (2008) Vasoactive intestinal peptide and its role in continuous and seasonal reproduction in birds. *Gen Comp Endocrinol*, 159: 88-97.
- KOSZTOLANYI A, SZEKELY T, CUTHILL IC, YILMAZ KT, BERBEROGLU S (2006) Ecological constraints on breeding system evolution: the influence of habitat on brood desertion in Kentish plover. *J Anim Ecol*, 75: 257-265.
- KUENZEL WJ, BLAHSER S (1994) Vasoactive intestinal polypeptide (VIP)-containing neurons: distribution throughout the brain of the chick (*Gallus domesticus*) with focus upon the lateral septal organ. *Cell Tissue Res*, 275: 91-107.
- KUENZEL WJ, MASSON M (1988) *A Stereotaxic Atlas of the Brain of the Chick (Gallus Domesticus)*: Johns Hopkins University Press.
- LANTOS T, GÖRCS TJ, PALKOVITS M (1995) Immunohistochemical mapping of neuropeptides in the premammillary region of the hypothalamus in rats. *Brain Res Rev*, 20: 209-249.
- LEUNG CH, GOODE CT, YOUNG LJ, MANEY DL (2009) Neural distribution of nonapeptide binding sites in two species of songbird. *J Comp Neurol*, 513: 197-208.
- LORÉN I, EMSON PC, FAHRENKRUG J, BJÖRKLUND A, ALUMETS J, HÅKANSON R, SUNDLER F (1979) Distribution of vasoactive intestinal polypeptide in the rat and mouse brain. *Neuroscience*, 4: 1953-1976.
- MACNAMEE MC, SHARP PJ, LEA RW, STERLING RJ, HARVEY S (1986) Evidence that vasoactive intestinal polypeptide is a physiological prolactin-releasing factor in the bantam hen. *Gen Comp Endocrinol*, 62: 470-478.
- MATHIEU M, TRABUCCHI M, VALLARINO M, PINELLI C, RASTOGI RK (1999) Distribution of vasoactive intestinal peptide-like immunoreactivity in the brain and pituitary of the frog (*Rana esculenta*) during development. *Brain Res*, 851: 105-115.
- MONTAGNESE CM, SZEKELY T, CSILLAG A, ZACHAR G (2015) Distribution of vasotocin- and vasoactive intestinal peptide-like immunoreactivity in the brain of blue tit (*Cyanistes coeruleus*). *Frontiers in Neuroanatomy*, 9: 90.
- MONTAGNESE CM, SZEKELY T, GRAY D, BALAZSA T, ZACHAR G (2014) Immunoreactivity distribution of vasotocin and vasoactive intestinal peptide in brain nuclei of two songbird species with different breeding systems. *Brain Behav Evol*, 83: 140-149.
- MOORE FL, LOWRY CA (1998) Comparative neuroanatomy of vasotocin and vasopressin in amphibians and other vertebrates. *Comp Biochem Physiol C Pharmacol Toxicol Endocrinol*, 119: 251-260.
- NEWMAN SW (1999) The medial extended amygdala in male reproductive behavior a node in the mammalian social behavior network. *Ann N Y Acad Sci*, 877: 242-257.
- NORGREN RB, JR., SILVER R (1990) Distribution of vasoactive intestinal peptide-like and neurophysin-like immunoreactive neurons and acetylcholinesterase staining in the ring dove hypothalamus with emphasis on the question of an avian suprachiasmatic nucleus. *Cell Tissue Res*, 259: 331-339.
- O'CONNELL LA, HOFMANN HA (2012) Evolution of a vertebrate social decision-making network. *Science*, 336: 1154-1157.
- OBATA-TSUTO HL, OKAMURA H, TSUTO T, TERUBAYASHI H, FUKUI K, YANAIHARA N, IBATA Y (1983) Distribution of the VIP-like immunoreactive neurons in the cat central nervous system. *Brain Res Bull*, 10: 653-660.
- OTERO-GARCIA M, MARTIN-SANCHEZ A, FORTES-MARCO L, MARTINEZ-RICOS J, AGUSTIN-PAVON C, LANUZA E, MARTINEZ-GARCIA F (2014) Extending the socio-sexual brain: arginine-vasopressin immunoreactive circuits in the telencephalon of mice. *Brain Struct Funct*, 219: 1055-1081.
- PANZICA GC, ASTE N, CASTAGNA C, VIGLIETTI-PANZICA C, BALTHAZART J (2001) Steroid-induced plasticity in the sexually dimorphic vasotocinergic innervation of the avian brain: behavioral implications. *Brain Res Brain Res Rev*, 37: 178-200.
- PANZICA GC, CALCAGNI M, RAMIERI G, VIGLIETTI-PANZICA C (1988) Extrahypothalamic distribution of vasotocin-immunoreactive fibers and perikarya in the avian central nervous system. *Basic Appl Histochem*, 32: 89-94.
- PANZICA GC, PLUMARI L, GARCÍA-OJEDA E, DEVICHE P (1999) Central vasotocin-immunoreactive system in a male passerine bird (*Junco hyemalis*). *J Comp Neurol*, 409: 105-117.
- PECZELY P, KISS JZ (1988) Immunoreactivity to vasoactive intestinal polypeptide (VIP) and thyrotropin-releasing hormone (TRH) in hypothalamic neurons of the domesticated pigeon (*Columba livia*). Alterations following lactation and exposure to cold. *Cell Tissue*

- Res, 251: 485-494.
- PETKO M, IHIONVIEN M (1989) Distribution of substance P, vasoactive intestinal polypeptide and serotonin immunoreactive structures in the central nervous system of the lizard, *Lacerta agilis*. *J Hirnforsch*, 30: 415-423.
- POGÁNY Á, ALTBÄCKER V, SZÉKELY T (2008) Male signalling and dominance in the penduline tit *Remiz pendulinus*. *Acta Zoologica Academiae Scientiarum Hungaricae*, 54: 191-199.
- POGÁNY Á, DIJK REV, HORVÁTH P, SZÉKELY T (2012) Parental behavior and reproductive output in male-only cared and female-only cared clutches in the Eurasian penduline tit (*Remiz pendulinus*). *The Auk*, 129: 773-781.
- POGANY A, KOSZTOLANYI A, MIKLOSI A, KOMDEUR J, SZEKELY T (2015) Biparentally deserted offspring are viable in a species with intense sexual conflict over care. *Behav Processes*, 116: 28-32.
- POGANY A, SZENTIRMAI I, KOMDEUR J, SZEKELY T (2008) Sexual conflict and consistency of offspring desertion in Eurasian penduline tit *Remiz pendulinus*. *BMC Evol Biol*, 8: 242.
- PROPPER CR, JONES RE, LOPEZ KH (1992) Distribution of arginine vasotocin in the brain of the lizard *Anolis carolinensis*. *Cell Tissue Res*, 267: 391-398.
- PUELLES L, MARTINEZ-DE-LA-TORRE M, PAXINOS G, C. W, S. M (2007) *The Chick Brain in Stereotaxic Coordinates: An Atlas Featuring Neuromeric Subdivisions and Mammalian Homologies*: Academic Press.
- REINER A (1991) A comparison of neurotransmitter-specific and neuropeptide-specific neuronal cell types present in the dorsal cortex in turtles with those present in the isocortex in mammals: implications for the evolution of isocortex. *Brain Behav Evol*, 38: 53-91.
- ROOD BD, DE VRIES GJ (2011) Vasopressin innervation of the mouse (*Mus musculus*) brain and spinal cord. *J Comp Neurol*, 519: 2434-2474.
- ROSTÉNE WH (1984) Neurobiological and neuroendocrine functions of the vasoactive intestinal peptide (vip). *Prog Neurobiol*, 22: 103-129.
- SIMERLY RB, GORSKI RA, SWANSON LW (1986) Neurotransmitter specificity of cells and fibers in the medial preoptic nucleus: an immunohistochemical study in the rat. *J Comp Neurol*, 246: 343-363.
- SIMERLY RB, SWANSON LW (1987) The distribution of neurotransmitter-specific cells and fibers in the anteroventral periventricular nucleus: implications for the control of gonadotropin secretion in the rat. *Brain Res*, 400: 11-34.
- SIMS KB, HOFFMAN DL, SAID SI, ZIMMERMAN EA (1980) Vasoactive intestinal polypeptide (VIP) in mouse and rat brain: An immunocytochemical study. *Brain Research*, 186: 165-183.
- STOKES TM, LEONARD CM, NOTTEBOHM F (1974) The telencephalon, diencephalon, and mesencephalon of the canary, *Serinus canaria*, in stereotaxic coordinates. *J Comp Neurol*, 156: 337-374.
- STOLL CJ, VOORN P (1985) The distribution of hypothalamic and extrahypothalamic vasotocinergic cells and fibers in the brain of a lizard, *Gekko gekko*: presence of a sex difference. *J Comp Neurol*, 239: 193-204.
- SZENTIRMAI I, SZEKELY T, KOMDEUR J (2007) Sexual conflict over care: antagonistic effects of clutch desertion on reproductive success of male and female penduline tits. *J Evol Biol*, 20: 1739-1744.
- VAN DIJK RE, SZEKELY T, KOMDEUR J, POGANY A, FAWCETT TW, WEISSING FJ (2012) Individual variation and the resolution of conflict over parental care in penduline tits. *Proc Biol Sci*, 279: 1927-1936.
- VIGLIETTI-PANZICA C (1986) Immunohistochemical study of the distribution of vasotocin reacting neurons in avian diencephalon. *J Hirnforsch*, 27: 559-566.
- VIGLIETTI-PANZICA C, ANSELMETTI GC, BALTHAZART J, ASTE N, PANZICA GC (1992) Vasotocinergic innervation of the septal region in the Japanese quail: sexual differences and the influence of testosterone. *Cell Tissue Res*, 267: 261-265.
- VOORHUIS TA, DE KLOET ER (1992) Immunoreactive vasotocin in the zebra finch brain (*Taeniopygia guttata*). *Brain Res Dev Brain Res*, 69: 1-10.
- WALKER DL, TOUFEXIS DJ, DAVIS M (2003) Role of the bed nucleus of the stria terminalis versus the amygdala in fear, stress, and anxiety. *Eur J Pharmacol*, 463: 199-216.
- WANG Z, YOUNG LJ, DE VRIES GJR, INSEL TR (1999) Voles and vasopressin: A review of molecular, cellular, and behavioral studies of pair bonding and paternal behaviors. In: I.J.A. Urban JPHB, Wed DD (Eds.) *Prog Brain Res*. Elsevier, pp 483-499.
- YAMADA S, MIKAMI S, YANAIHARA N (1982) Immunohistochemical localization of vasoactive intestinal polypeptide (VIP)-containing neurons in the hypothalamus of the Japanese quail, *Coturnix coturnix*. *Cell Tissue Res*, 226: 13-26.