

Extrahypothalamic distribution of vasotocin-immunoreactive fibers and perikarya in the avian central nervous system.*

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SUMMARY

Immunohistochemical analysis of the extrahypothalamic distribution of vasotocin-like immunoreactive elements within the central nervous system of the domestic fowl and Japanese quail, revealed several mesencephalic, pontine and bulbar target areas topographically identifiable. Extrahypothalamic immunopositive perikarya were observed in diencephalic and mesencephalic locations after glutaraldehyde fixation.

INTRODUCTION

An increasing amount of observations point to the presence of vasopressin (VP)-immunoreactive cell bodies and fibers outside the hypothalamic region of the mammalian brain in several mesencephalic, rhomboencephalic and spinal areas (Sofroniew 1983, 1985; De Vries *et al.*, 1985). Observations on the extrahypothalamic distribution of vasotocin (VT) and/or mesotocin in non-mammalian vertebrates are to date sparse (Weindl *et al.*, 1980; Blähser 1983, Blähser 1984) and only recently partly presented systematically for the mesencephalic and pontine regions of the domestic fowl (Panzica *et al.*, 1986).

* Dedicated to Prof. Guido Filogamo

MATERIALS AND METHODS

In the present study we used conventionally fixed (Bouin or SUSA) and paraffin embedded brains of adult Japanese quails (6) and forty-five day-old chickens (8). In addition, 4 adult Japanese quails were perfused according to De Vries *et al.* (1985) with highly concentrated glutaraldehyde (5%). After a brief postfixation (1-2 hours) the dissected brains were washed in phosphate buffer overnight. Subsequently, they were serially sectioned at 50-60 μm by means of an Oxford Vibratome. The immunocytochemical procedure and controls for paraffin embedded material were previously described (Panzica 1985; Panzica *et al.*, 1986; Viglietti-Panzica 1986). Essentially, the same procedure was applied also to the Vibratome sections. Endogenous peroxidase activity was blocked by pretreatment with H_2O_2 . The primary antisera used (anti-VT, 1:4000; anti-neurophysins, 1:2000) were kindly provided by D.A. Gray (Bad Nauheim, FRG) and M.V. Sofroniew (Oxford, UK). Antibodies specifications are reported by Gray and Simon (1983) and Sofroniew *et al.* (1978).

RESULTS AND DISCUSSION

Extrahypothalamic fiber distribution

The pattern of distribution of the VT-immunoreactive fibers was similar both in quail and chicken. Adjacent sections stained to detect neurophysin (NPH) immunoreactivity showed a similar distribution. According to previous observations (*pigeon*: Weindl and Sofroniew, 1982) positive fibers were observed in circumventricular organs of quail and chicken (organum vasculosum laminae terminalis, median eminence, subcommissural organ, subfornical organ). Several extrahypothalamic districts (encephalic, mesencephalic, pontine and bulbar nuclei) showed a variable amount of VT and NPH fibers. Their semidiagrammatic distribution is reported in Fig. 1A-E (see legend for abbreviations of nuclei). At diencephalic levels, positive fibers were observed in GL, ML, PM and ECM nuclei. In the mesencephalon, VT-and NPH-immunoreactive fibers were distributed in Gct, TP, AVT, and in the FRL. VT and NPH fibers were observed also in superficial layers of the optic tecta. At pontine levels, immunopositive terminals and fibers were detected in the rostral part of the LoC. A larger amount of positive fibers was noted in the ScD and ScV. Scattered fibers were also observed in TTD and RP. Positive fibers ran in the raphe region, in the lemniscus spinalis and in the region near the medial ventral incisure. No fibers or endings were observed onto the motoneurons contributing to the cranial nerves. Caudally, VT-and NPH-immunopositive fibers are restricted to the ventral region near the median incisure, the raphe region and the lemniscus spinalis. At bulbar levels positive fibers were detected in S, GC and IM, surrounding the nX. A few scattered fibers were also seen in the raphe ventralis. The described pattern of distribution was rather constant in different conditions of fixation, but after the glutaraldehyde fixation, the VT-positive fibers showed a wide distribution and a stronger immunoreactivity.

In all the regions in which immunopositive fibers and terminals were detected, part of them ran in close association with large or small blood vessels, sometimes ending

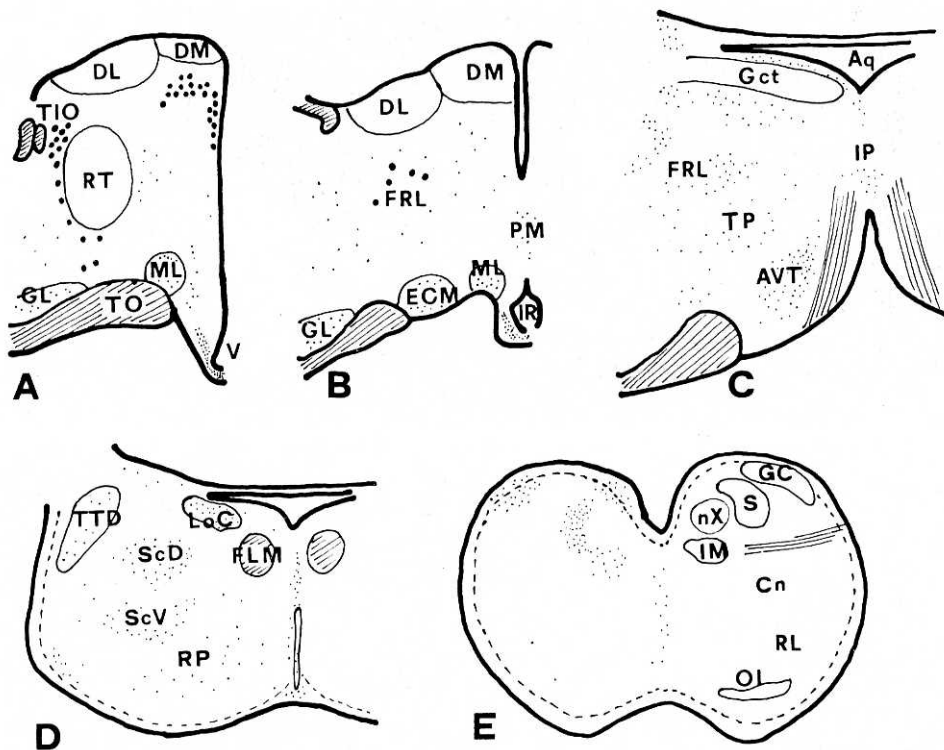


Fig. 1A-E - Schematic drawings of the diencephalon (A-B), mesencephalon (C), pons (D) and medulla oblongata (E) of 2 months-old Japanese quail. Larger dots represent VT-immunopositive cell bodies. Small dots represent the distribution of VT-fibers. List of abbreviations (Baylé *et al.*, 1974, and Panzica *et al.*, 1986): AVT, area ventralis tegmentalis; Cn, n. centralis medullae oblongatae; DL, n. dorsolateralis thalami; DM, n. dorsomedialis thalami; ECM, n. ectomamillaris; FLM, fasciculus longitudinalis medialis; FRL, formatio reticularis lateralis; GC, n. gracilis et cuneatus; Gct, substantia grisea centralis; GL, n. geniculatus lateralis; IM, n. intermedius; IP, n. interpedicularis; IR, infundibular recess; IM, n. intermedius; IP, n. interpedicularis; IR, infundibular recess; LoC, locus coeruleus; ML, n. mammillaris lateralis; nX, n. motorius nervi vagi; OI, n. olivaris inferior; RL, n. reticularis lateralis; RP, n. reticulari pontis caudalis; RT, n. rotundus thalami; S, n. tractus solitarius; ScD, n. subcoeruleus dorsalis; ScV, n. subcoeruleus ventralis; TIO, tractus isthmo-opticus; TO, tractus opticus; TP, n. tegmenti pedunculo-pontinus; TTD, n. et tractus descendens nervi trigemini; V, 3rd ventricle.

directly in their surroundings. These spatial relationships between VT fibers and blood vessels suggest a functional role in regulating properties of brain vessels. Supporting this hypothesis, Kretzschman *et al.* (1986) demonstrated a specific binding of Arg-VP to hippocampal microvessels.

In the described neural target areas, immunohistochemically stained fibers or terminals can often be seen contacting neuronal cell bodies, sometimes lining negative perikarya or dendrites (Fig. 2B). Association between VT-immunopositive thin-beaded fibers or endings and cell bodies were particularly evident in the FRL, SCv, and AVT. The presence of close contacts between VT-immunoreactive endings and cell bodies, observed also in the mammalian brain (Sofroniew 1983), as well as the wide

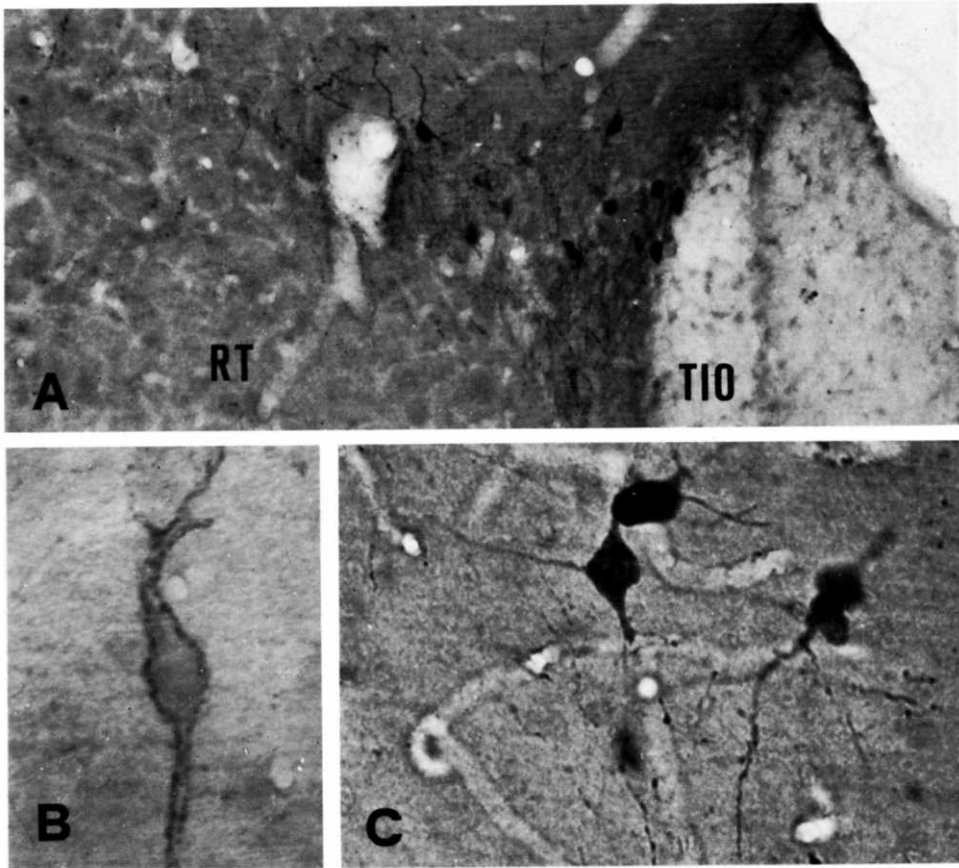


Fig. 2-A - VT-immunoreactive cell bodies in the region of the *n. lentiformis mesencephali*, between the tractus isthmo-opticus (TIO) and the *n. rotundus thalami* (RT). X 200. **B**: Close contacts between VT-immunopositive fibers and a negative perikaryon in the region of *n. subcoeruleus ventralis*. X 640. **C**: Scattered medium-sized VT-immunoreactive neurons in the formatio reticularis lateralis mesencephali. X 500.

innervation of cerebral structures by VP- (or related peptides) immunoreactive fibers (Sofroniew 1983, 1985; De Vries *et al.*, 1985) strongly support the hypothesis of a direct involvement of these neuropeptides in neurotransmission or neuromodulation (for a review see Buijs 1983).

Extrahypothalamic cell bodies

In previous reports (Weindl *et al.*, 1980; Bлахser 1983, 1984; Korf 1984; Panzica *et al.*, 1986) no VT-positive perikarya have been observed outside the classical diencephalic locations (for details of the system see: Viglietti-Panzica, 1986). In the present immunohistochemical study, done in normal animals, small immunopositive nerve cells were clearly observed in quail only after glutaraldehyde fixation. These elements, easily distinguishable from the classic magnocellular elements, were distribu-

ted around the nucleus RT, as well as ventrally in a region between this nucleus and the GL. The main group of immunopositive elements is located dorso-laterally to the RT in close association with the isthmo-optic tract (Fig. 1A, 2A). According to the atlas of Baylé *et al.* (1974), this group could correspond to the nucleus lentiformis mesencephali. Scattered, larger elements were observed also in the FRL (Fig. 2C). A careful re-evaluation of paraffin-embedded specimens resulted in the detection of very low immunopositive elements in these locations also after conventional fixations (Bouin's or SUSA fluids). We have not yet performed an immunostaining with anti-NPH of glutaraldehyde-fixed and vibratome sectioned specimens, in order to confirm that the immunoreaction was not the result of cross-reactivity due to the fixation method. However, one should consider that this kind of fixation has been proved in mammals as one of the best ways to visualize extrahypothalamic VP-ergic elements in normal animals. In fact, according to De Vries *et al.* (1985), after short-time glutaraldehyde fixation, extrahypothalamic VP-ergic clusters previously observed only in colchicine-injected rats (Sofroniew, 1985) can be visualized without any drug treatment. As previously discussed (Panzica *et al.*, 1986), some of the extrahypothalamic VT-immunoreactive fields (median raphe, LoC, ScD, SCv, and optic tecta) have not yet been demonstrated directly connected with the avian PVN (tracer studies, *pigeon*: Berk and Finkelstein, 1983; *duck*: Korf, 1984) considered as the main source of central VT-ergic projections. Till now no experimental work has been done to clarify the projections of VT-positive mesencephalic elements. In mammals (De Vries *et al.*, 1985), as well as in birds (Panzica *et al.*, 1985), some sex-steroid dependent or sexually dimorphic extrahypothalamic patterns of VP- or VT-ergic fibers were demonstrated. Further studies need to discover if in birds some extrahypothalamic clusters of perikarya could be involved in this kind of innervation.

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