

**Neuroendocrinology:
retrospect and perspectives**

Editors: Korf,H.-W. and Usadel,K.H.

Springer Verlag, Berlin

1997

**SEXUAL DIMORPHISM, STEROID-INDUCED PLASTICITY, AND
BEHAVIORAL SIGNIFICANCE OF THE VASOTOCINERGIC
INNERVATION OF THE AVIAN BRAIN**

G.C.Panzica, N.Aste[°], C.Castagna, J.Balthazart*, and C.Viglietti-Panzica

Dept. Anatomy, Pharmacology, and Forensic Medicine, University of Torino, Torino, Italy,
*Lab. Biochemistry, University of Liège, Liège, Belgium

[°] present address: Institute of Molecular Neurobiology, Shiga University of Medical Science,
Otsu, Shiga, Japan.

Corresponding author:
Prof. GianCarlo Panzica,
Dipartimento di Anatomia, Farmacologia e Medicina Legale,
c.so M. D'Azeglio 52 I-10126 Torino (Italy)
Fax +39 11 6707732 Phone +39 11 6707729
e-mail giancarlo.panzica@unito.it

Panzica GC, Aste N, Castagna C, Balthazart J, Viglietti-Panzica C. 1997. Sexual dimorphism, steroid-induced plasticity, and behavioral significance of the vasotocinergic innervation of the avian brain. In: Korf H-W, Usadel KH(Eds.). *Neuroendocrinology: retrospect and perspectives*. Berlin: Springer Verlag. p 127-150.

ABSTRACT

This review focuses on the neuroanatomical distribution of vasotocin (the antidiuretic hormone) in the avian brain. This peptide is synthesized by a well described system of diencephalic magnocellular neurons, but, in addition, more recent studies demonstrated the presence of immunoreactive sexually dimorphic parvocellular groups or fibers in diencephalic and extradiencephalic regions of different species, including the Japanese quail. The main cluster has been detected in the male in a region considered as the avian homologue of the mammalian nucleus of the stria terminalis (nST). These cells are not visible in the female and this dimorphism has been confirmed also by in situ hybridization studies. Moreover, sexually dimorphic vasotocin-positive fibers are present in regions involved in the control of different aspects of reproduction, i.e. the nucleus preopticus medialis (copulatory behavior), the lateral septum (secretion of GnRH), and the nucleus intercollicularis (vocalization control). In the male the vasotocin-immunoreactivity in these regions is strictly testosterone-dependent: castration, or exposure to a short-day photoperiod decrease VT-immunoreactivity to female levels. Administration of estradiol-benzoate to embryos (a treatment that abolishes masculine sexual behavior) results in a dramatic decrease of the VT-immunoreactivity in all these brain regions of male quail. Behavioral experiments demonstrate that intracerebroventricular administration of vasotocin strongly inhibits male sexual behavior. These data suggest that this peptide in birds is not only the antidiuretic hormone, but also plays a central role in the control of diverse aspects of reproduction.

Key Words: Vasotocin, Japanese quail, Avian Brain, Nucleus of the Stria Terminalis, Testosterone, Vasopressin, Male Sexual Behavior

Vasotocin (VT) is a non-mammalian neurohypophysial hormone (Acher *et al.* 1993) belonging to a large family of structurally and functionally related neuropeptides that includes arginin vasopressin (VP), oxytocin, mesotocin (MT), isotocin, and hydrins (Acher, 1993). VT and VP were originally identified as neurohypophysial hormones produced by hypothalamic magnocellular elements (Leng *et al.* 1992) and regulating hydromineral balance (Acher and Chauvet, 1995). Because of the role played by these neuropeptides in osmoregulation, they were called by the general name of antidiuretic hormone, ADH. However these two nonapeptides are also secreted at the level of the median eminence and probably by a large amount of nerve terminals within several brain regions to regulate respectively the adenohypophysis (Makara *et al.* 1996) and a variety of brain functions and behaviors (Dantzer and Bluthé, 1993; Insel *et al.* 1993). In birds, the physiological functions classically attributed to VT are the regulation of water and electrolytic balance (see Simon Oppermann *et al.* 1988; Ramieri and Panzica, 1989 for reviews) and the control of blood pressure (Szczepanska-Sadowska *et al.* 1985). Like VP (and oxytocin) in mammals, VT is also involved in the control of several aspects of avian reproduction such as oviposition by causing oviduct contractions (Rzasa and Ewy, 1982; Shimada *et al.* 1986; Koike *et al.* 1988; Rice *et al.* 1985), and activating male sexual behavior (Bernroider and Leutgeb, 1994; Goodson *et al.* 1996; Kihlström and Danninge, 1972; Leutgeb, 1995), as well as vocalizations (Voorhuis *et al.* 1991).

Distribution of vasotocin in birds

a) *The magnocellular system*

The distribution of the magnocellular neurosecretory neurons in the avian hypothalamus had been at first studied by means of histochemical techniques (Gomori reaction). These investigations demonstrated a scattered pattern with local cluster-like aggregations of neurosecretory elements, but considerable species differences were observed in the pattern and cell size of the neurosecretory nuclei (for a review see: Oksche and Farner, 1974). Later the availability of antibodies to VT and MT (the analogue of the mammalian oxytocin) and the development of ICC

techniques allowed to localize both these nonapeptides and/or their respective carrier proteins (neurophysins) in the hypothalamo-neurohypophysial system of several avian species (for full review of the less recent literature see: Viglietti-Panzica and Panzica, 1991). In recent years, the development, the anatomical localization and the response to osmoregulation challenge of VT-gene expressing neurons has been studied in the chicken and quail, by means of *in situ* hybridization (ISH) techniques. These studies demonstrated an excellent correspondence between the distribution of the VT-mRNA containing elements and of the VT immunoreactive (VT-ir) neurons (Milewski *et al.* 1989; Mühlbauer *et al.* 1993; Chaturvedi *et al.* 1994; Grossmann *et al.* 1995; Aste *et al.* 1996a; Jurkevich *et al.* 1997). The more precise identification of this system obtained by means of these new techniques suggested that the distribution of magnocellular neurosecretory elements follows a similar scheme in the investigated species (including both oscine and non-oscine birds) and that this distribution can be easily compared to the distribution observed of the hypothalamo-neurohypophysial system in mammals (Sánchez *et al.* 1991).

As a rule it is very difficult to match immunocytochemically detectable peptidergic cell groups and cerebral nuclei defined by classical methods of neuroanatomy such as the Nissl stain. This explains why different terminologies have been used by different authors to describe the anatomical distribution of peptidergic systems and this confusion could result in inaccurate homologies with other vertebrates (for further discussion see: Fasolo *et al.* 1988; Viglietti-Panzica and Panzica, 1991; Sánchez *et al.* 1991; Moore and Lowry, 1997). For this reason, Berk *et al.* (1982) suggested a nomenclature for VT cell groups in the pigeon brain based exclusively on the topographic position of VT-immunoreactive (VT-ir) cell clusters. This nomenclature was subsequently used for the study of other avian species (Japanese quail, domestic mallard, domestic fowl) and has been proposed as a general rule for non-oscine birds (Viglietti-Panzica, 1986) (see Fig.1).

According to these studies three diencephalic regions contain VT-ir cells: the lateral preoptic area and lateral hypothalamus (L1-L5 groups), the periventricular preoptic area and periventricular hypothalamus (P1-P3 groups), and the dorsal diencephalon (DD1-DD2 groups). The L1 and L2 clusters are located close to the optic tract, lateral and medial respectively; the former is intermingled with the lateral forebrain bundle. The L3 group lies dorsal to L2 and medial to L1; these 3 groups correspond to the mammalian supraoptic nucleus (Sánchez *et al.* 1991). The periventricular system extends in ventro-dorsal direction. The P2 and P3 groups roughly correspond to the mammalian paraventricular nucleus (PVN) pars parvocellularis, whereas the L4 and DD2 group correspond to the magnocellular portion of the PVN (Sánchez *et al.* 1991). In general, cells in the periventricular groups have a cell size slightly smaller than in the other groups (Sánchez *et al.* 1991). The hypothalamo-neurohypyseal system of oscine birds is organized with a similar distribution pattern (Goossens *et al.* 1977; Kiss *et al.* 1987; Voorhuis and De Kloet, 1992; Deviche *et al.* 1996), even if no comparison has been performed yet with the VT-ir cell groups of these species and those identified in the pigeon.

b) The parvocellular elements and fibers' distribution

The combination of more sensitive ICC methods with newly developed ISH techniques, as well as the analysis of the system in various physiological conditions contributed to the discovery of a broader distribution of VT-ir cell bodies (smaller and less intensely labelled than the so called magnocellular neurons) and fibers, that are not directly connected to the hypothalamo-neurohypophysial system. VT-ir neurons were observed around the nucleus rotundus (RT) and in the region between this nucleus and the lateral geniculate nucleus. Larger elements were also identified in the lateral reticular formation. A small but distinct cluster was located dorso-laterally to the RT near the isthmo-optic tract (Panzica *et al.* 1988; Aste *et al.* 1996a). A rather large group of parvocellular VT-ir elements was identified in the avian nucleus of the stria terminalis (nST). This group was at first identified in oscine birds (Kiss *et al.* 1987; Voorhuis *et al.* 1988; Voorhuis and De Kloet, 1992; Deviche *et al.* 1996), and later on also in galliforms (Aste *et al.* 1995, 1996a, 1996b, 1997a; Jurkevich *et al.* 1996, 1997).

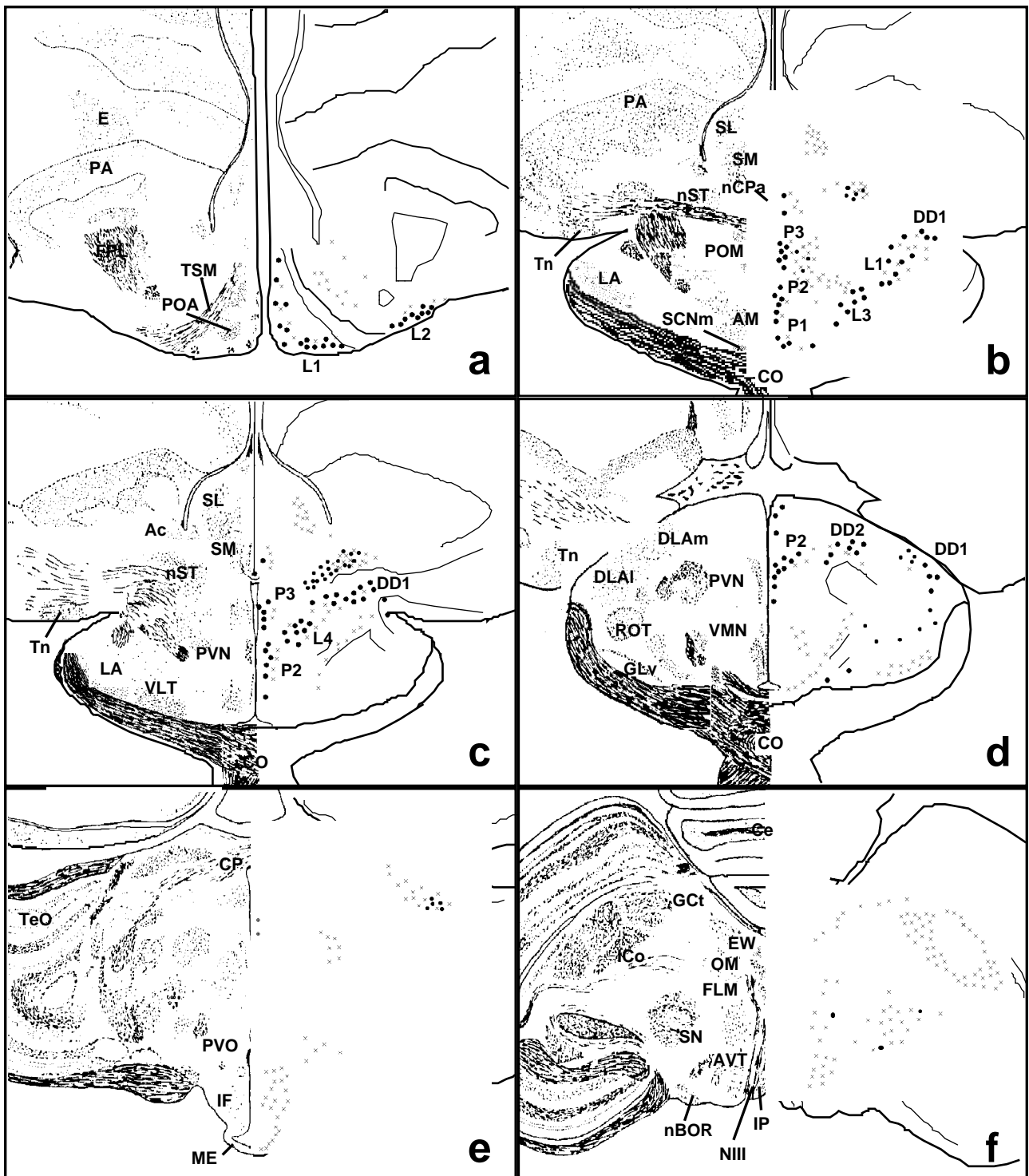


Fig. 1. Distribution of VT-ir structures in the limbic, hypothalamic, and rostral mesencephalic regions of the male Japanese quail. The drawings correspond to six different levels taken from transverse sections through the rostro-caudal extent. The left side of the drawings shows the disposition of nuclei in Nissl-stained sections. The schematic drawings on the right side show the distribution of VT-ir structures. Black dots represent immunoreactive cell bodies, and small crosses indicate positive fibers. Letters indicate different VT-ir cell groups: **L1-L4**, lateral hypothalamic groups; **P1-P3**, periventricular groups; **DD1-DD2**, dorsal diencephalic groups (Viglietti-Panzica, 1986). The density of symbols indicates the relative differences in the number of cells or fibers not their actual numbers.

Ac, nucleus accumbens; **AM**, nucleus anterior medialis hypothalami; **AVT**, area ventralis of Tsai; **Ce**, cerebellum; **CO**, optic chiasma; **CP**, commissura posterior; **DLAI**, nucleus dorsolateralis anterior, pars lateralis; **DLAm**, nucleus dorsolateralis anterior, pars medialis; **E**, ectostriatum; **EW**, nucleus of Edinger and Westphal; **FLM**, fasciculus longitudinalis medialis; **Gct**, substantia grisea centralis; **Glv**, nucleus geniculatus lateralis, pars ventralis; **ICo**, nucleus intercollicularis; **IF**, nucleus infundibularis; **IP**, nucleus interpeduncularis; **LA**, nucleus lateralis anterior thalami; **ME**, median eminence; **nBOR**, nucleus of the optic basal root; **nCPA**, nucleus commissurae pallii; **nNII**, nervus oculomotorius; **nST**, nucleus striae terminalis; **OM**, nucleus nervi oculomotorii; **PA**, paleostriatum augmentatum; **POA**, nucleus preopticus anterior; **POM**, nucleus preopticus medialis; **PVN**, nucleus paraventricularis; **PVO**, paraventricular organ; **ROT**, nucleus rotundus; **SCNm**, nucleus suprachiasmaticus, pars medialis; **SL**, nucleus septalis lateralis; **SM**, nucleus septalis medialis; **SN**, substantia nigra; **TeO**, optic tectum; **TSM**, tractus septum-mesencephalicum; **Tn**, nucleus teniae; **VLT**, nucleus ventrolateralis thalami; **VMN**, nucleus ventromedialis.

VT-ir fibers were described in several brain regions in different species. However a complete description is available only for a few species (e.g. canary: Kiss *et al.* 1987; zebra finch: Voorhuis, De Kloet, 1992; Junco hyemalis: Deviche *et al.* 1996; quail: Viglietti-Panzica *et al.* 1997). In quail, VT-ir fiber endings were observed in the telencephalon (lateral septum, nST), in the whole diencephalon, in the mesencephalon [optic tectum, nucleus intercollicularis (ICo), substantia grisea centralis, area ventralis of Tsai, and substantia nigra], in the pons (raphe nuclei, locus coeruleus, and tegmentum), and in the medulla (nucleus of the solitary tract). Isolated VT-ir fibers were observed in several other regions all over the brain. A similar distribution has been reported in the canary and in the Junco hyemalis, in addition, in song birds a large supply of VT-ir fibers was detected around the nucleus robustus archistriatalis (Kiss *et al.* 1987; Deviche *et al.* 1996). This nucleus and the ICo (which is also labelled by VT-ir fibers in both oscines and non-oscine birds) are both implicated in the control of several aspects of the song.

Sexual dimorphism of vasotocin system

In both oscine (canary) and non-oscine birds (domestic fowl, quail), a limited number of studies have revealed a robust sexual dimorphism of the VT-ir cell group in the nST. In galliforms, immunocytochemical studies showed that VT-ir neurons are present in the nST of males only and cannot be visualized in females (Jurkevich *et al.* 1997; Aste *et al.* 1997a). In the canary, this difference is less extreme and both sexes have VT-ir neurons in the nST but they are present in larger number in males than in females (Voorhuis *et al.* 1988, 1990). No sexual dimorphism was by contrast observed at this level in another oscine species, the zebra finch (Voorhuis and De Kloet, 1992). In galliforms, this sex difference has been also confirmed at the level of VT transcripts by means of ISH techniques. In the domestic fowl, positive cells were detected in the nST of males but not females (Jurkevich *et al.* 1997). In the Japanese quail however, contrary to what had been observed during the immunocytochemical studies, weakly labelled VT-gene expressing neurons are also present in the female nST (Aste *et al.* 1997a). The different sensitivity of the two employed methods and a different ratio transcription/translation of VT-gene may account for this discrepancy. In quail, a sexually dimorphic population of scattered VT-ir neurons has also been observed within the boundaries of the medial preoptic nucleus (POM). These cells are visible utilizing the ICC technique only in males. They are more numerous in the caudal than in the rostral region of the nucleus, and apparently they merge with the sexually dimorphic population of the nST (Aste *et al.* 1997a). ISH techniques essentially confirmed these observations but similar to what had been observed in the nST, weakly labelled VT-expressing elements were detected in female quail POM.

These sexually dimorphic VT-ir elements have similar characteristics in all avian species where they have been observed so far. They are smaller than the neurons belonging to the hypothalamo-neurohypophysial system, the extension of their dendritic arborization is less complex (generally bipolar), and the immunostaining is weaker than observed in the magnocellular population. This last finding could account for the fact that these smaller VT-ir cells were not observed in some studies. Their visualization critically depends on specific aspects of the immunocytochemical technique (they are not visible in paraffin-embedded material or in too thin sections). It is important to remember here that a similar population of sexually dimorphic VP-ir neurons can be clearly evidenced in mammals but only after colchicine injection (Van Leeuwen *et al.* 1985).

VT-ir fibers also present a sexually dimorphic distribution in the brain of the few species that have been investigated in detail. In the Japanese quail (Viglietti-Panzica *et al.* 1992), domestic fowl (Jurkevich *et al.* 1997), and canary (Voorhuis *et al.* 1988), the lateral septum of males contains a denser VT-ir innervation than the female septum. In other oscine species, such as the zebra finch, no sexual dimorphism has however been reported (Voorhuis and De Kloet, 1992).

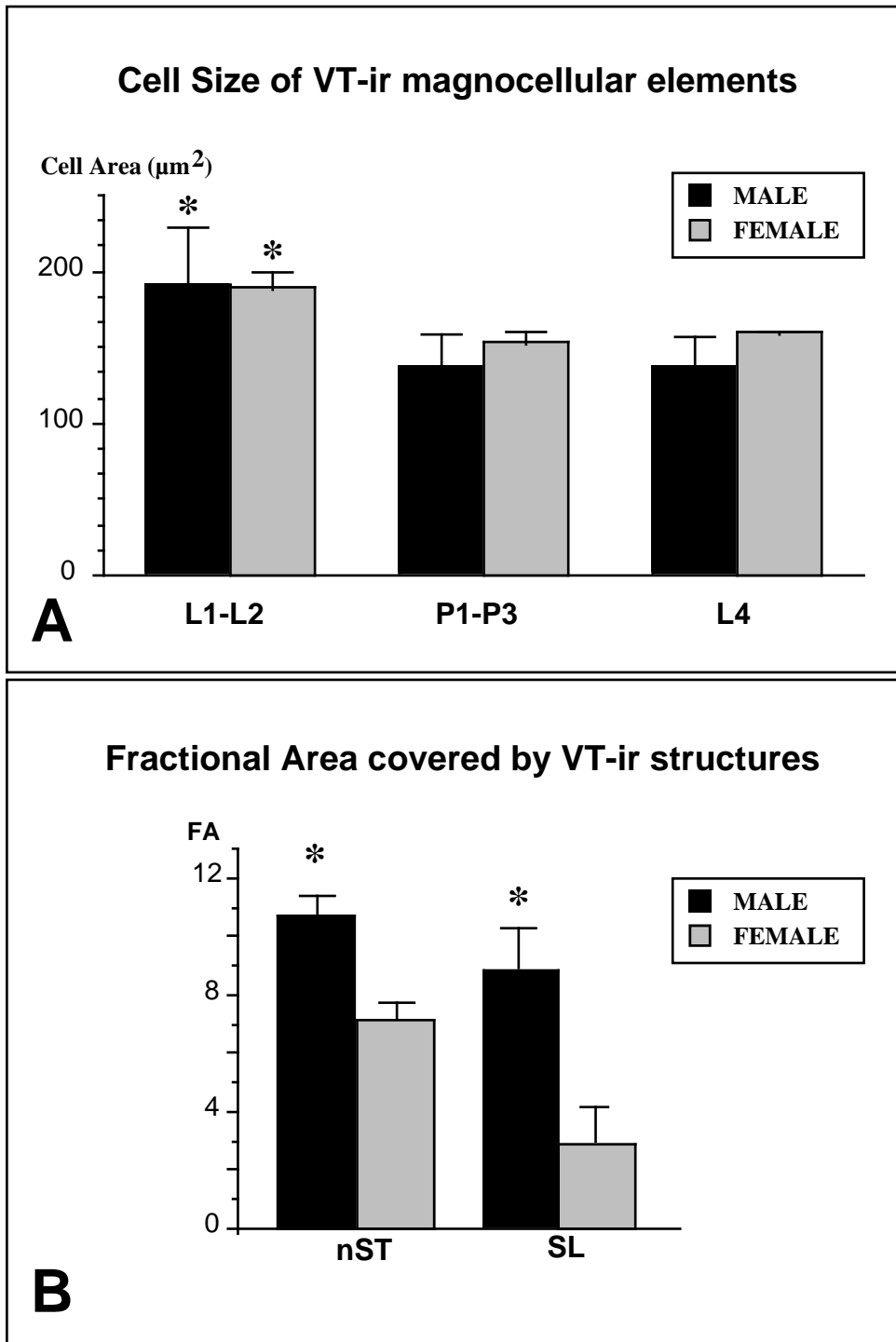


Fig. 2. Sex differences in the VT-ir cells and fibers in the quail brain

A. Cross sectional cell area of VT-ir magnocellular elements in adult quail of both sexes. Measurements were performed on Bouin fixed, paraffin embedded material. The values are the mean cell area (μm^2) \pm standard error of the mean (number of birds each group = 4). A minimum of 100 cells per bird were measured. A two-way ANOVA was performed with sex and position as factor. The results indicated a significant effect of position ($F=3.943$, $p=0.038$), whereas no effect was detected for the sex ($F=0.866$, $p=0.3645$) or for the interaction of sex and position ($F=0.279$, $p=0.76$). Subsequent Fisher PLSD test confirmed that the cell sizes in the L1-L2 groups are significantly larger than in the other two groups in both sexes (asterisk, $p<0.05$).

B. Fractional area (FA) covered by VT-ir structures in the nucleus of the stria terminalis (nST) and in the lateral septum (SL). FA was measured on SUSA fixed, paraffin embedded material as described in Viglietti Panzica *et al.*, 1994. In both nST and SL there is a significant (asterisk, $p<0.01$) sexual difference of VT-ir structures that cover a larger area in male than in female quail. Data from Jurkevitz *et al.*, 1996.

We completed more recently a study on the entire brain of the Japanese quail (Viglietti-Panzica *et al.* 1997) and observed sex differences in the density of VT-ir fibers (higher density in males than in females) in the lateral septum, the nST (Fig.2), the POM, the periventricular hypothalamus, the ICo, and the locus coeruleus. These results demonstrate the existence of a widespread sexual dimorphism in the VT innervation of the quail brain. This dimorphism is not only restricted to regions involved in the control of sexual behavior (lateral septum, nST, and POM), but involves several other regions related to other functions, such as the control of vocalizations (ICo).

In summary, the sex differences evidenced so far in the avian brain involve at least part of the parvocellular elements (those located in the nST which is the equivalent of the mammalian bed nucleus of the stria terminalis) and the fibers systems located mainly in the prosencephalon. Until now, no sexual dimorphism of the hypothalamo-neurohypophysial system has been described. No evident difference in cell distribution or number has been reported. Lastly, cell size measurements evidenced significant differences among the groups, but no difference among sexes (Fig. 2).

In contrast to these findings that indicate only limited sex differences in the VT circuits, the quantification of VT mRNA in homogenates of whole brains in the domestic fowl has revealed that the concentration of VT transcripts is twice higher in cockerels compared to hens (Barth *et al.* 1995). Recent data have also confirmed the presence of a similar sex difference in homogenates of whole quail brain (Muhlbauer, Aste, Grossmann, Panzica, unpublished). Taking into account the small number of sexually dimorphic parvocellular VT neurons, these data suggest that the observed sex differences in brain VT mRNA content can not be exclusively related to the parvocellular cell groups. It is hence possible that there is a less obvious sex difference in the number of magnocellular hypothalamic neurons or in their peptide content. Alternatively, it is also possible that the presence of relatively large differences in VT mRNA concentrations in conjunction with only limited differences in the protein as indicated by ICC studies is an indication of sex specific controls in the translation of the VT transcripts or in the turnover rate of VT mRNA (Jurkevich *et al.* 1996).

Seasonal and hormone-dependent plasticity of the vasotocin system

Recent investigations on the avian vasotocin system have also been devoted to variations that could occur as a function of the season or the hormonal status of the bird. It is well known that many species of birds are highly photodependents (photoperiodic) and that their circulating testosterone (T) levels fall to very low values when the animals are exposed to short days (during the fall and the winter in natural environment). In these conditions the amount of VT-ir fibers in the lateral septum, nST, and POM, as well as the number of cells in the nST, dramatically decreases both in oscines (canary: Voorhuis *et al.* 1988; junco: Deviche *et al.* 1996) and in non-oscine birds (quail: Viglietti-Panzica *et al.* 1992; Panzica *et al.* 1996a).

In male quail changes in VT innervation of these brain regions are apparently controlled by T: the amount of ir fibers was strongly reduced or completely disappeared in gonadectomized birds, whereas T-treatment of castrated males restored the innervation to a density that is typically seen in sexually mature males (Viglietti-Panzica *et al.* 1992, 1994) (Fig. 3).

The T-dependence of the VT-ir circuitries is also reflected by the drop of the ir fibers in the POM and septum observed during aging (when circulating T levels spontaneously decrease to very low values) and by the restoration of VT innervation which was observed in aged male quail treated with exogenous T (Panzica *et al.* 1996a). Interestingly, when ovariectomized females are treated with doses of T that are sufficient to induce a full restoration of the innervation in castrated males no detectable changes in the septal innervation and in the nST can be detected (Viglietti-Panzica *et al.* 1992). The insensitivity of female vasotocinergic circuits to T has been recently confirmed by an independent experiment. T-treated gonadectomized female quail have very rare or absent VT-ir fibers also in the POM and the nST (Castagna *et al.* 1997b).

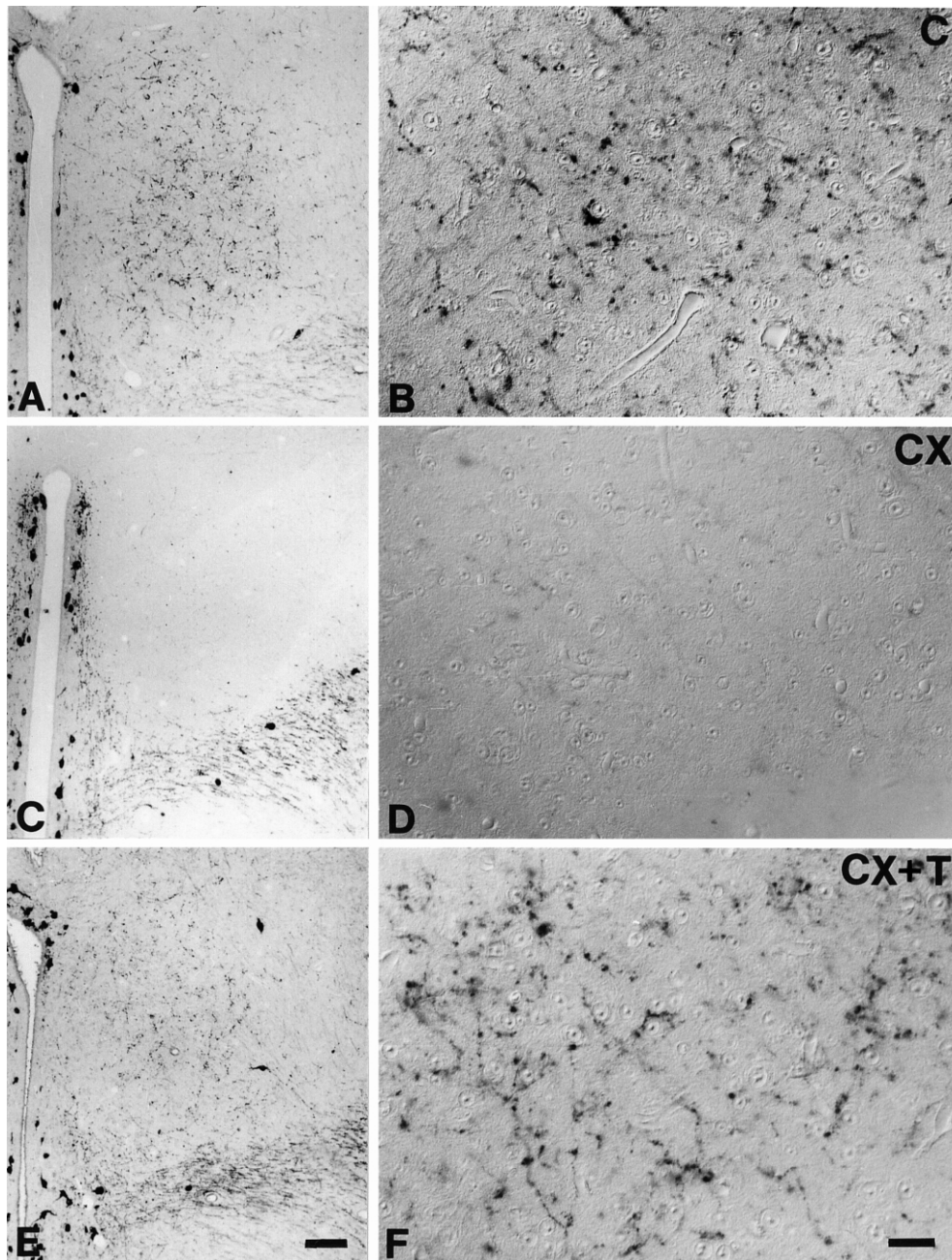


Fig. 3. Effects of testosterone on VT-ir structures in the POM of male quail. Top: control sham-operated male (C); Middle: castrated male (CX); Bottom: castrated male treated with testosterone (CX+T). Note the almost total loss of immunoreactivity in CX male quail. On the left low power enlargements (Bar = 100 μ m), on the right higher enlargements showing close contacts between VT-ir fibers and negative cell bodies (Bar = 25 μ m).

These data taken together suggest that in quail the sexual dimorphism in the VT innervation does not depend on a differential activation by T in the two sexes (activational effect) but it originates during the embryonic period (organizational effect). These sex differences and controls by T are substantially different from those previously demonstrated in the canary brain (Voorhuis *et al.* 1988). In this species T-treatment of females enhances the VT immunoreactivity in the lateral septum to a male-typical level. It therefore appears that in the canary the sex dimorphism observed in adult birds only reflects an activational role of T.

A clear anatomical specificity was thus observed in the sexually dimorphic features and T sensitivity of the male quail VT system which was affected at the level of the limbic and preoptic region, but not at the level of the magnocellular neurons or of the hypothalamo-neurohypophysial tract which were considered as controls (Viglietti-Panzica *et al.* 1992, 1994; Panzica *et al.* 1996a).

The dimorphism of the quail vasotocinergic elements in the septum and in the nST is in some sense qualitatively similar to the situation observed in adult rats (De Vries and Al Shamma, 1990). In this species there is also a sexual dimorphism in the induction by T of the VP innervation of the septum: a denser innervation is still observed in males than in females after a treatment with identical doses of T. Moreover like in the rat (De Vries *et al.* 1985), the VT-ergic system of quail consists of elements that are sensitive and elements that are not sensitive to steroids.

There is no specific information on the origin of the steroid-sensitive VT-ir fibres in birds. Tracing studies in ducks (Korf, 1984) and quail (Balthazart *et al.* 1994, Balthazart and Absil, 1997) have shown that the periventricular region projects respectively to the septum and to the POM, but there is no demonstration that the VT-ir cell bodies are implicated in these projections. Whether the VT-ir and VT mRNA expressing neurons in quail POM and nST (Aste *et al.* 1997a) are the origin of the immunoreactive fibers that outline these two nuclei and the lateral septum remains to be experimentally analyzed. In rat, a sexually dimorphic T-sensitive population of VP-ir neurons has been evidenced in the bed nucleus of the stria terminalis and in the medial amygdaloid nucleus (De Vries, 1990). Recent studies testing the effects of various hormonal manipulations during the rat development demonstrated similar changes in the VP immunostaining of cells of the bed nucleus of the stria terminalis and the medial amygdaloid nucleus and of fibers in the lateral septum, suggesting these two nuclei are responsible of the sexually dimorphic VP-ir projection to the lateral septum (Wang *et al.* 1993). This conclusion has not been tested experimentally however.

The results summarized above indicate that the sexually dimorphic innervation of the lateral septum and POM could be organizational in nature. To test this hypothesis, we injected quail embryos on day 8 of incubation either with estradiol benzoate (EB) or with the aromatase inhibitor, R76713, or with the solvent as a control. All subjects (males and females) were gonadectomized at 4 weeks post-hatching and implanted with Silastic capsules filled with T in order to place the adult birds in similar endocrine conditions. Major qualitative differences were still observed in the VT-ir innervation of the POM, lateral septum, and nST in the different experimental groups (see table 1). In agreement with our previous studies, dense fibers networks were observed in control males but not females. EB-treated males had completely lost this immunoreactivity and had lost the capacity to display copulatory behavior as previously shown in many experimental studies (see for a review Adkins Regan, 1990). Conversely, R76713-treated females displayed a male-typical VT-ir innervation of these two brain regions and they also displayed high levels of male-like copulatory behavior, as previously reported (Balthazart *et al.* 1992b).

No effect of the experimental treatments was detected at the level of the hypothalamo-hypophyseal tract (Castagna *et al.* 1997b). These results indicate that the sensitivity to T of the male VT circuits ending in the POM, lateral septum, and nST is organized during the embryonic period and becomes sexually differentiated following exposure of the female brain to estrogens. The sexual dimorphism observed in the adults is hence truly organizational in its origin. Because this embryonic organization of the innervation of the POM by VT is controlled by mechanisms that are very similar to the mechanisms that organize the sex difference in male typical copulatory behavior (Balthazart and Ball, 1995), these results also suggest a very close relationship between the VT innervation of POM and the control of male sexual behavior.

	MC	MR76	MEB	FC	FR76	FEB
POM	++++	++++	+	+	+++	+
NST	++++	++++	+	+	+++	+
SL	++++	++++	+	+	+++	+
Behavior	+	+	-	-	+	-

Table 1. Semiquantitative analysis of the distribution of VT-ir structures in the medial preoptic nucleus (POM), in the nucleus of the stria terminalis (nST), and in the lateral septum (SL) of male and female Japanese quail treated *in ovo* with estradiol benzoate (EB) or with the aromatase inhibitor, R76713. The last row indicates the presence or the absence of male copulatory behavior after each treatment. Details on the experimental procedure are given in the text. MC= male controls, FC= female controls, MR76= male treated with aromatase inhibitor R76713, FR76= female treated with aromatase inhibitor R76713, MEB= male treated with EB, FEB= female treated with EB. The number of + in each group is proportional to the area covered by VT-ir structures, + = very low and ++++ = very high density of positive fibers.

Effects of vasotocin on reproductive behavior in male quail

Taken together, the anatomical data reviewed above were demonstrating a number of correlations between the brain vasotocinergic innervation and specific aspects of male reproductive behavior in quail. Specifically, it had been established that VT specifically innervates the sexually dimorphic medial preoptic nucleus, a structure that plays a key role in the activation of male sexual behavior (Panzica *et al.* 1996b), that this innervation is T-sensitive and that it is sexually dimorphic in the organizational sense (irreversibly differentiated by early estrogen action; Castagna *et al.* 1997b). It had also been shown that VT-ir fibers are observed in close contact with aromatase-ir cells in the preoptic area and hypothalamus which supported the idea that VT could be implicated in the control of reproduction (aromatase is a limiting step in the activation of copulatory behavior by T; Balthazart *et al.* 1997a). VT and its mammalian homologue, VP had previously been observed to have powerful effects on a variety of behaviors used in a reproductive context by amphibians, mammals and a few species of birds including the domestic fowl, pigeon, zebra finch and canary (see Castagna *et al.* 1997a for the full list of references). However, no data were available to assess the possible significance of VT for the control of reproductive behavior in quail.

The possible role of VT on quail behavior was first tested by peripherally injecting castrated male quail that were chronically treated with exogenous T (subcutaneous Silastic implants) with various doses of either [Arg⁸]-vasotocin (VT; Sigma V0130) or the potent V1 receptor antagonist ([Deamino-Pen¹, Tyr(Me)², Arg⁸]-Vasopressin or dPTyr(Me)AVP; Bachem H-5340) and measuring the effects of these injections on appetitive sexual behavior measured by a learned social proximity response (time spent near, and frequency of looks through a window separating two adjacent cages, one containing the stimulus female and one containing the experimental male; see Balthazart *et al.* 1995, 1997b) and on consummatory sexual behavior (mount attempts and cloacal contact movements) as well as on crowing, a T-dependent vocalization.

VT inhibited in a dose-dependent manner (doses ranging from 2 to 20 µg/bird) both the appetitive and consummatory aspects of male sexual behavior. All aspects of sexual behavior were inhibited by the highest dose of VT (20 µg) when tested immediately after the injection but only measures of appetitive behavior were decreased if the test was delayed by 30 min. Mount attempts and cloacal contacts were not affected in these conditions (Castagna and Balthazart, 1996). During independent experiments, crowing occurrences were counted by repeated 5 min.-long observations of the birds in their home cage. The occurrence frequency of crowing was sharply decreased by the systemic injection of 2 or 10 µg VT per bird and this effect lasted one to two hours. Injections of the V1 receptor antagonist, dPTyr(Me)AVP (50-100 µg/bird) on the contrary increased crowing frequency for periods up to 4 hours suggesting that the behavioral

effects of VT are receptor mediated and relatively specific (See Fig 4, A-B).

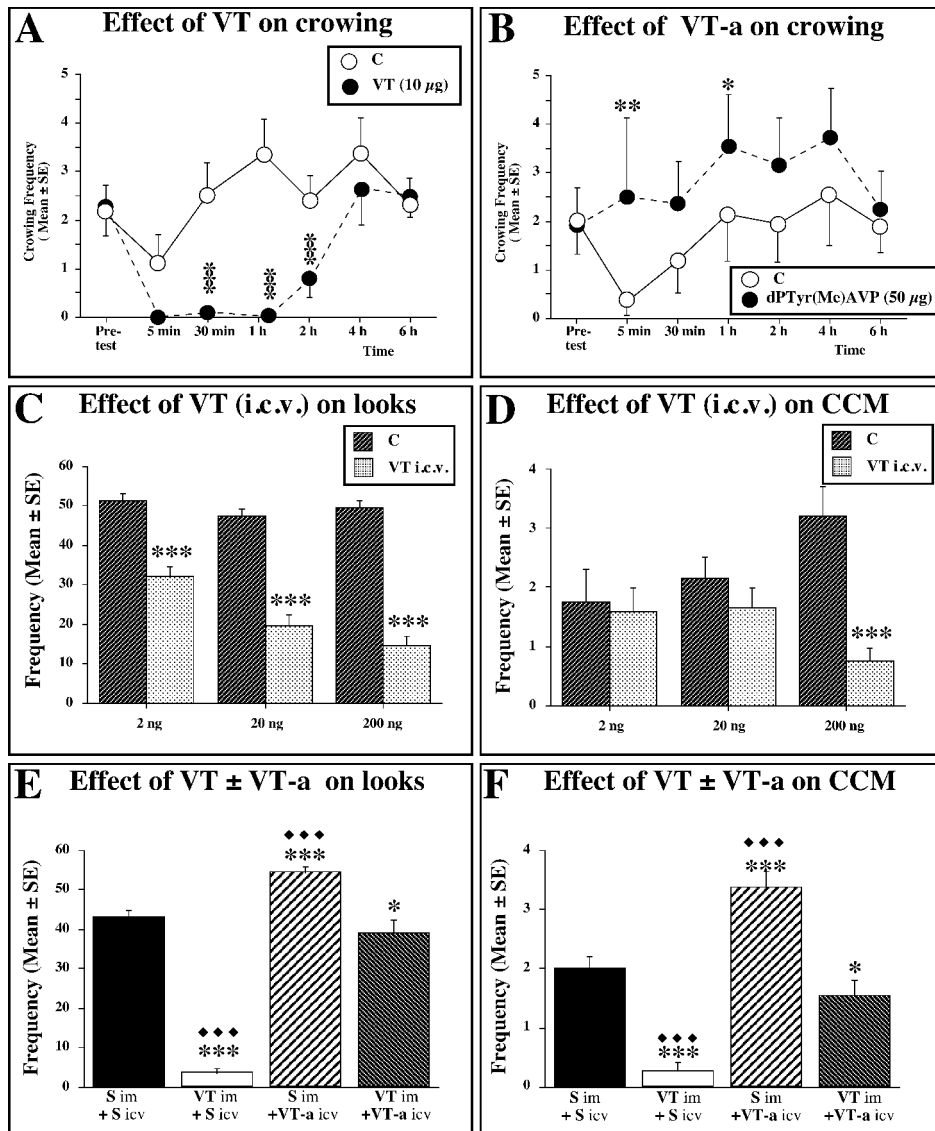


Fig. 4. Effects of VT and of a V1 receptor antagonist (VT-a) on the appetitive (Looks) and consummatory (cloacal contact movements; CCM) aspects of male sexual behavior as well as on crowing in castrated male Japanese quail chronically treated with testosterone.

A-B Effects of i.m. injections of VT (10µ g/bird) or of the VT-a (50 µ g/bird) on crowing occurrence frequency. Birds were observed after different intervals between the injection and the observations (5 and 30 min, 1,2,4 and 6 hours; pretest is a test done as control before the injections). After the demonstration of a significant overall effect by analyses of variance (ANOVA), data were analyzed by post-hoc t tests for matched samples to compare control and VT or VT-a-injected birds at each time point. * = p<.05; ** = p<.01 and *** = p<.001.

C-D Effects of i.c.v. injections of VT (2-200 ng/bird) on appetitive and consummatory aspects of male sexual behavior. Data were first analyzed by ANOVA with two repeated variables that demonstrated significant effects of the treatments. At each dose level, the control and VT data were compared then by post-hoc t-tests and the results of these tests are indicated by asterisks at the top of the bars. * = p<.05; ** = p<.01 and *** = p<.001.

E-F Effects of i.m. injections of vasotocin (VT; 20 µ g/bird), of i.c.v. injections of the VT-a (500 ng/bird), and of the combined treatment with VT and VT-a on appetitive and consummatory aspects of male sexual behavior. Injections of the vehicle (Saline [S]) were always made to control for non specific effects of i.m. or i.c.v. injections. Data were analyzed by one way ANOVA that demonstrated significant overall effects of the treatments. The 4 treatments were then compared by post-hoc t-tests and their results are indicated by symbols at the top of the bars. * = p<.05, ** = p<.01 and *** = p<.001 for comparisons with the control group (S im+S icv); p<.01 and = p<.001 for comparisons with the VT im+ VT-a icv group.

These data therefore suggested that VT plays an inhibitory role on many aspects of male sexual behavior in quail. To test the hypothesis that the behavioral effects of VT were centrally mediated (as suggested by the dense T-sensitive vasotocineric innervation of the POM, a key

center in the control of male reproductive behavior; Panzica *et al.* 1996b) VT was injected in the third ventricle of castrated male quail that were chronically treated with exogenous T. Increasing doses of VT in the range 2 to 200 ng per subject inhibited both appetitive and consummatory components of male sexual behavior in a dose-dependent manner when injected centrally into the third ventricle. Appetitive behavior was, however, more sensitive to the treatment than consummatory behavior that was significantly depressed only by the highest dose (See Fig 4, C-D). At low doses, appetitive aspects of male sexual behavior only were inhibited. The highest VT dose (200 ng) that produced strong behavioral effects when injected i.c.v. had no effect when injected systemically (smallest active dose was about 2 μ g) indicating that the minimal effective dose was 10 to 100 times larger in systemic compared to central injection. This observation already provided some indication that the behavioral effects of VT were centrally mediated (Castagna *et al.* 1996).

This was further confirmed in subsequent experiments in which behavioral effects of a systemic VT injection could be blocked by a central injection of the V1 receptor antagonist dPTyr(Me)AVP. This study was carried out with one group of birds that had learned the social proximity response used to measure appetitive sexual behavior. Before each behavioral test, each bird received one i.c.v. injection followed 5 min later by one systemic (i.m) injection. The central injection contained either the VT-antagonist (VT-a) or the saline 9 ‰ solution. The i.m. injection contained either VT or saline 9 ‰. Behavioral tests were initiated immediately after the i.m. injection. The combination of the two central and two peripheral treatments therefore defined four separate experimental conditions. Quail were injected with 1) saline i.m and saline i.c.v., or 2) VT i.m and saline i.c.v., or 3) the VT-a i.c.v. and saline i.m., or finally 4) VT i.m and the VT-a i.c.v. Each subject was tested in these 4 conditions and could therefore be used as his own control.

This experiment first confirmed that a central injection of VT very efficiently blocks both appetitive and consummatory aspects of male sexual behavior (Fig 4, E-F). In contrast, the VT antagonist when injected alone enhanced these aspects of the behavior clearly indicating that in physiological conditions, endogenous VT exerts a tonic inhibition on these behaviors. Finally the experiment also showed that the central injection of the antagonist was able to inhibit the behavioral effects of a systemic injection of VT, indicating that systemically injected VT really exerts its effects at the central level (Castagna *et al.* 1997a).

Conclusions

According to the literature reviewed above, four main groups of vasopressinergic neurons can be identified in the mammalian brain, namely the magnocellular and parvocellular neurons located in the PVN and SON, and the parvocellular elements identified (normally after colchicine injection) in the SCN, bed nucleus of the stria terminalis, and medial amygdala (De Vries *et al.* 1994). Magnocellular neurons of the PVN and SON are at the origin of the thick fibers that project to the neurohypophysis through the hypothalamo-hypophysial tract. The parvocellular neurons of the PVN project to the hindbrain and spinal chord probably participating in a pathway that controls neurovegetative functions. There is no evidence at present that these specific vasopressinergic projections are controlled by steroids.

The other three VP-ir cell groups could hence be at the origin of the steroid-sensitive vasopressinergic projections that have been described in mammals (see for a review: De Vries, 1995). However, available data suggest that the VP-ir cells of the SCN do not contain steroid receptors and do not have a sexually dimorphic distribution. It has therefore been suggested that all steroid-sensitive vasopressinergic fibers in the mammalian brain originate from the sexually dimorphic VP-ir cell populations located in the bed nucleus of the stria terminalis and medial amygdala (De Vries *et al.* 1992).

In birds, as reviewed in the introduction, ICC techniques revealed a distribution pattern for the VT-ir cell bodies belonging to the magnocellular system that can easily be superimposed to the pattern observed in mammals (Sánchez *et al.* 1991). These neurons also appear to project to the

neurohypophysis (Mikami *et al.* 1978). This projection is not modulated by steroids (Viglietti-Panzica *et al.* 1994) despite the fact that estrogen and androgen receptors are present in the paraventricular and supraoptic regions of the quail brain (Watson and Adkins-Regan, 1988; Balthazart *et al.* 1989, 1992a; Foidart *et al.* 1995). No study has, however, analyzed the potential colocalization of steroid receptors with VT in these areas.

Like in mammals, sexually dimorphic parvocellular VT-ir neurons are present in the avian brain; they are mainly clustered in a region above and caudal to the anterior commissure (nST, recently identified as the avian homologue of the mammalian bed nucleus of the stria terminalis, Aste *et al.* 1997a), and in the POM. The nST and the POM also contain both estrogen and androgen receptors (Watson and Adkins-Regan, 1988; Balthazart *et al.* 1989, 1992a; Foidart *et al.* 1995) and in analogy to what has been proposed for mammals these neurons could be the origin of the sexually dimorphic steroid-sensitive VT projections observed in the POM, the nST and the lateral septum. This is consistent with recent tracing studies that have identified connections between these areas (Balthazart *et al.* 1994; Balthazart and Absil, 1997) but specific studies combining retrograde tracing with ICC for VT should be performed to confirm the anatomical organization of these projections. No VT-ir or VT mRNA-expressing cells have been identified in the areas of the quail brain that are believed to be the structures homologous to the mammalian amygdala (archistriatum and in particular the nucleus taeniae; Aste *et al.* 1996a). The organization of the parvocellular vasotocinergic system may therefore be quite different in quail and in rat.

In addition, small to medium VT-ir cells can be observed above the optic chiasma (Panzica, 1985) in a position that could be considered homologous to the mammalian SCN, but this comparison is difficult to make given the uncertainty about the location of this nucleus in birds in general (Shimizu *et al.* 1994; Norgren and Silver, 1990) and in quail in particular. No additional information is available at present for the other mesencephalic groups of parvocellular vasotocinergic neurons that have been described in quail both with ISH (Aste *et al.* 1996a) and ICC (Viglietti-Panzica *et al.* 1997) techniques.

The sexually dimorphic parvocellular VT neurons of the limbic/preoptic region are presumably the origin of the widespread innervation of large brain areas that are namely implicated in the control of reproductive activities. These projections are steroid-sensitive and sexually differentiated. Taken together, these anatomical data suggest that VT may play a critical role in the control of reproductive behavior.

Because data in mammals (Smock *et al.* 1992; Wang *et al.* 1994; Ferris *et al.* 1994, 1996) and amphibians (Moore *et al.* 1992, 1994; Boyd, 1997; Moore and Lowry, 1997) indicated that indeed VP and VT control reproductive behavior in a variety of species, experiments were carried out to test this idea directly in quail. These studies showed that VT exerts a powerful inhibitory effect on both appetitive and consummatory components of male sexual behavior as well as on the crowing vocalization. Additional studies demonstrated that the effect of VT is centrally mediated and can be dissociated from the general stress reaction induced by systemic injections of the peptide. Finally studies using a powerful V1 antagonist also showed that endogenous VT is likely to exert tonic inhibitory effects on these behaviors because blockade of these receptors leads to a significant stimulation of all these behavioral responses. The behavioral effects of VT injections mimic brain mechanisms that are part of the normal physiology of the species because the V1 receptor antagonist was able to produce opposite effects. In mammals, the V1 receptor subtype is broadly distributed in the brain where it is supposed to mediate most if not all behavioral effects of this neuropeptide. Our results suggest that a similar situation occurs in birds although additional studies would be required to test the receptor specificity of the behavioral effects that have been described.

A number of studies on a variety of vertebrate species have previously demonstrated that VP or VT have a diversity of actions on behaviors ranging from the spawning reflex in some species of fishes to the mating call of amphibians and the flank marking response in mammals (Moore, 1992; De Wied *et al.* 1988). Many of these studies suggest that VT or VP increase the occurrence frequency of the behaviors but some inhibitory effects have also been demonstrated (e.g. inhibition

of release call in *Rana pipiens*, of spontaneous locomotion in *Rana catesbiana*, of lordosis in female rat: Diakow, 1978; Södersten *et al.* 1983; Moore, 1992). Little experimental work has unfortunately been carried out in birds. One early study showed that the injection of VT to intact sexually mature pigeons or cocks produces a short term increase in the frequency of copulatory acts (Kihlström and Danninge, 1972) but more recently, Bernroider and Leutgeb (1994) presented data in abstract form suggesting that VT decreases motivational aspects of sexual learning in quail. Two abstracts also recently reported that central VT injections stimulate courtship behavior and aggression in zebra finches, *Taeniopygia guttata* (Goodson *et al.* 1996) or singing in female white-crowned sparrow, *Zonotrichia leucophrys gambelii* (Maney *et al.* 1997).

Some of these experiments, contrary to the present set of data point to a stimulatory role of VT on reproductive behavior in birds. It is however difficult to make direct comparisons between the different studies because they were carried out in different species and in very different experimental conditions (different seasons, endocrine conditions, etc). One study has for example suggested that seasonal variations might explain these changes in VT action because injections of a VT analog activated singing in canaries in the early fall but inhibited this behavior in the winter (Voorhuis *et al.* 1991). No general theory can be offered at present to explain why VT stimulates aspects of reproductive behavior in some species but inhibits the same or similar behaviors in other species. Additional studies on a variety of animal models will be needed to answer this question.

The identification of inhibitory effects of VT on sexual behavior in male quail was somewhat unexpected because the anatomical studies that have been reviewed above had demonstrated that VT-immunoreactive fibers in the POM (Viglietti-Panzica *et al.* 1994) and septal region/nST (Viglietti-Panzica *et al.* 1992; Aste *et al.* 1997b) increase after treatment of castrates with T which at the same time activates male sexual behavior (Panzica *et al.* 1996b; Balthazart *et al.* 1996). The most obvious explanation was therefore that the increase in VT production is part of a cascade of biochemical events triggered by T in the brain that result in the activation of male sexual behavior. The behavioral studies make this interpretation impossible but alternative explanations can be offered.

Based on a number of studies carried out mostly in mammals (De Vries, 1995), it is unlikely that increases in the density of VT-ir fibers after T treatment reflect a blockade of the peptide release resulting in its accumulation in fibers and terminals (see Castagna *et al.* 1997a for further discussions). Alternatively, one could postulate that a single injection of exogenous VT decreases rather than increases the VT activity in specific brain areas because the injected compound has a short half-life and is quickly metabolized but at the same time strongly and persistently inhibits the secretion of the endogenous peptide. This interpretation however does not take into account the fact that inhibitions of sexual behavior were observed almost immediately after the injection of VT and disappeared in approximately 30 min. We therefore do not favor this possibility and suggest that the apparent contradiction between anatomical and behavioral data indicates that the T-induced increase in VT immunoreactivity should not be considered as one of the central consequence of the T action that leads to the activation of male sexual behavior but rather reflects the development of a mechanism that is implicated in the maintenance of behavioral homeostasis.

When the male sexual behavior is activated (high levels of circulating T), a negative control mechanism may need to be established in order to organize the distribution of behavioral occurrences on a short-term basis (birds should not be sexually active all the time). This mechanism is obviously not needed in castrates that are behaviorally inactive. A steroid-sensitive neuropeptidergic innervation would provide an adequate support for such a control that could be directly sensitive to environmental stimuli. On a long-term basis, changes in steroid levels would establish the anatomical substrate of this behavioral control (control of the synthesis of VT and possibly of the growth of VT-containing fibers) while on short-term basis, environmental and social stimuli could regulate the release of VT from its terminals and in this way switch off behavior for limited periods of time. Additional work is obviously required to test this hypothesis, including the identification of the brain areas (POM, septum, nST,...) where VT exerts its behavioral effects and the analysis of environmental stimuli that are able potentially to modulate

VT release at these specific anatomical sites.

In conclusion, the data collected so far indicate that the steroid-sensitive vasotocinergetic innervation of the medial preoptic area and/or of other regions such as the nST in the quail brain plays a significant role in the modulation of reproductive behavior. Steroids could therefore affect reproductive behavior both directly, by acting on steroid-sensitive neurons in the preoptic area, and indirectly, by modulating peptidergic (specifically vasotocinergetic) inputs to this area. Additional work will be needed to evaluate the full significance of the indirect effects mediated by vasotocinergetic inputs.

Acknowledgments

The collaboration between the Laboratories of Torino and Liège was supported by grants from the European Community (grant: CT94-0472), the Italian Ministero degli Esteri, and the European Science Foundation (RG95/203). This work was also supported by grants from the NIMH (R01 MH50388), the Belgian FRFC (Nbr. 9.4565.96F), the University of Liège (Fonds Spéciaux pour la Recherche) and Government of the French Community of Belgium (Action Concertée #93/98-171) to JB, and by grants from MURST (60 and 40%) to GCP and CVP, and from CNR to CVP. The European Science Foundation supported with specific grants the stay of C.Castagna in Liège and of N.Aste in Celle (Germany).

References

- Acher R (1993) Neuroendocrine control of water homeostasis: the vasopressin/vasotocin/hydrin regulatory system. In: Mornex R, Jaffiol C, Leclere J (eds) *Progress in Endocrinology*. Parthenon,
- Acher R, Chauvet J (1995) The neurohypophysial endocrine regulatory cascade: precursors, mediators, receptors, and effectors. *Front Neuroendocrinol* 16: 237-289
- Acher R, Chauvet J, Chauvet MT, Michel G (1993) The avian neurohypophysial hormone-neurophysin precursors: structure, post-translational processing and evolution. In: Sharp PJ (ed) *Avian endocrinology*. Journal of Endocrinology, Ltd, Bristol, pp 149-160
- Adkins Regan EK (1990) Hormonal Bases of Sexual Differentiation in Birds. In: Balthazart J (ed) *Hormones, Brain and Behaviour in Vertebrates. Vol.1 Sexual differentiation, neuroanatomical aspects, neuropeptides and neurotransmitters*. Karger, Basel, New York, pp 1-14
- Aste N, Panzica GC, Viglietti-Panzica C, Absil P, Balthazart J, Mühlbauer E, Grossmann R (1995) The vasotocin system of the nucleus of the stria terminalis in the Japanese quail. *Soc Neurosci Abstr* 21: 357(Abstract)
- Aste N, Mühlbauer E, Grossmann R (1996a) Distribution of AVT gene expressing neurons in the prosencephalon of Japanese quail and chicken. *Cell Tissue Res* 286: 365-373
- Aste N, Viglietti-Panzica C, Mühlbauer E, Grossmann R, Panzica GC (1996b) Sexual dimorphism of vasotocinergetic structures in quail nucleus of stria terminalis. *Italian J Anatomy Embriol* 101, Suppl.1: 136-137(Abstract)
- Aste N, Balthazart J, Absil P, Grossmann R, Mühlbauer E, Viglietti-Panzica C, Panzica GC (1997a) Anatomical, neurochemical, and hodological definition of the nucleus of the stria terminalis in Japanese quail (*Coturnix japonica*). Submitted
- Aste N, Viglietti-Panzica C, Balthazart J, Panzica GC (1997b) Testosterone modulation of peptidergic pathways in the septo-preoptic region of male Japanese quail. *Poultry and Avian Biology Reviews* (in press)
- Balthazart J, Absil P (1997) Identification of catecholaminergic inputs to and outputs from aromatase-containing brain areas of the Japanese quail by tract tracing combined with tyrosine hydroxylase immunocytochemistry. *J Comp Neurol* 382: 401-428
- Balthazart J, Ball GF (1995) Sexual differentiation of brain and behavior in birds. *Trends*

Endocrinol Metab 6: 21-29

- Balthazart J, Gahr M, Surlémont C (1989) Distribution of estrogen receptors in the brain of the Japanese quail: an immunocytochemical study. *Brain Res* 501: 205-214
- Balthazart J., De Clerck A., Foidart A. (1992a) Behavioral demasculinization of female quail is induced by estrogens: Studies with the new aromatase inhibitor, R76713. *Horm.Behav.* 26: 179-203
- Balthazart J, Foidart A, Wilson EM, Ball GF (1992b) Immunocytochemical localization of androgen receptors in the male songbird and quail brain. *J Comp Neurol* 317: 407-420
- Balthazart J, Dupiereux V, Aste N, Viglietti-Panzica C, Barrese M, Panzica GC (1994) Afferent and efferent connections of the sexually dimorphic medial preoptic nucleus of the male quail revealed by *in vitro* transport of DiI. *Cell Tissue Res* 276: 455-475
- Balthazart J, Reid J, Absil P, Foidart A, Ball GF (1995) Appetitive as well as consummatory aspects of male sexual behavior in quail are activated by androgens and estrogens. *Behav Neurosci* 109: 485-501
- Balthazart J, Tlemçani O, Ball GF (1996) Do sex differences in the brain explain sex differences in the hormonal induction of reproductive behavior? What 25 years of research on the Japanese quail tells us. *Horm Behav* 30: 627-661
- Balthazart J, Absil P, Viglietti-Panzica C, Panzica GC (1997a) Vasotocinergic innervation of areas containing aromatase-immunoreactive cells in the quail forebrain. *J Neurobiol* (in press)
- Balthazart J, Castagna C, Ball GF (1997b) Aromatase inhibition blocks the activation and sexual differentiation of appetitive male sexual behavior in Japanese quail. *Behav Neurosci* 111: 381-397
- Barth SW, Jurkevich A, Grossmann R (1995) Sexual dimorphism in the expression of Arg-vasotocin in the chicken brain. *Poultry and Avian Biology Reviews* 6: 279(abstract)
- Berk ML, Reaves TA, 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
- Bernroider G, Leutgeb S (1994) V1-receptor mediated effects of vasotocin on motivational, mnemonic and aversive components of sexual learning in quail. *Soc Neurosci Abstr* 20: 1602(abstract)
- Boyd SK (1997) Brain vasotocin pathways and the control of sexual behaviors in the bullfrog. *Brain Res Bull* (in press)
- Castagna C, Balthazart J (1996) Effects of vasotocin on sexual behavior and crowing in male Japanese quail. *Italian J Anatomy Embriol* 101, Suppl.1: 148-149(abstract)
- Castagna C, Absil P, Balthazart J (1996) Central effects of vasotocin on appetitive and consummatory sexual behavior in male quail. *Soc Neurosci Abstr* 22: 2068(abstract)
- Castagna C, Absil P, Foidart A, Balthazart J (1997a) Systemic and intracerebroventricular injections of vasotocin inhibit appetitive and consummatory components of male sexual behavior in Japanese quail. *Behav Neurosci* (in press)
- Castagna C, Panzica GC, Russo C, Tlemçani O, Balthazart J (1997b) Preoptic vasotocin: a neurochemical marker of the brain circuits mediating sexually differentiated copulatory behavior in quail. *Soc Neurosci Abstr* 23: in press (abstract)
- Chaturvedi CM, Newton BW, Cornett LE, Koike TI (1994) An *in situ* hybridization and immunohistochemical study of vasotocin neurons in the hypothalamus of water-deprived chickens. *Peptides* 15: 1179-1187
- Dantzer R, Bluthé RM (1993) Vasopressin and behavior - from memory to olfaction. *Regul Pept* 45: 121-125
- De Vries GJ (1990) Sex differences in neurotransmitter systems. *J Neuroendocrinol* 2: 1-13
- De Vries GJ (1995) Studying neurotransmitter systems to understand the development and function of sex differences in the brain: the case of vasopressin. In: Micevich P, Hammer RP (eds) *Neurobiological effects of sex steroid hormones*. Cambridge Univ.Press, Cambridge, Ma, pp 254-278

- De Vries GJ, Al Shamma HA (1990) Sex differences in hormonal responses of vasopressin pathways in the rat brain. *J Neurobiol* 21: 686-693
- De Vries GJ, Buijs RM, Van Leeuwen FW, Caffé AR, Swaab DF (1985) The vasopressinergic innervation of the brain in normal and castrated rats. *J Comp Neurol* 233: 236-254
- De Vries GJ, Crenshaw BJ, Al Shamma HA (1992) Gonadal steroid modulation of vasopressin pathways. *Ann. NY Acad. Sci.* 652: 387-396. *Ann NY Acad Sci* 652: 387-396
- De Vries GJ, Al Shamma HA, Zhou L (1994) The sexually dimorphic vasopressin innervation of the brain as a model for steroid modulation of neuropeptide transmission. In: Luine VN, Harding CF (eds) *Hormonal restructuring of the adult brain. Basic and clinical perspectives.* Ann. New York Acad. Sciences. Vol. 743. New York Acad. Sciences, New York, pp 95-120
- De Wied D, Joëls M, Burbach JPH, De Jong W, De Kloet ER, Gaffori HD, Urban IJA, Van Ree JM, Van Vimersma Greidanus TB, Veldhuis HD, Versteeg DHG, Wiegant VM (1988) Vasopressin effects on the central nervous system. In: Negro-Vilar A, Conn PM (eds) *Peptide hormones: effects and mechanisms of action.* CRC Press, Boca Raton, pp 97-140
- Deviche P, García-Ojeda E, Plumari L, Panzica GC (1996) Vasotocinergic innervation in a male passerine bird (*Junco hyemalis*): effect of photoperiodic condition. *Soc Neurosci Abstr* 22: 1551 (Abstract)
- Diakow C (1978) Hormonal basis of breeding behavior in female frogs: vasotocin inhibits the release call of *Rana pipiens*. *Science* 199: 1456-1457
- Fasolo A, Panzica GC, Viglietti-Panzica C, Renda T, D'Este L (1988) Comparative chemical anatomy of the brain: concepts and methods. *Basic Appl Histochem* 32: 15-30
- Ferris CF, Delville Y, Irvin RW, Potegal M (1994) Septo-hypothalamic organization of a stereotyped behavior controlled by vasopressin in golden hamsters. *Physiol Behav* 55: 755-759
- Ferris CF, Delville Y, Brewer JA, Mansour K, Yules B, Melloni RH (1996) Vasopressin and Developmental Onset of Flank Marking Behavior in Golden Hamsters. *J Neurobiol* 30,: 192-204
- Foidart A, Houbart M, Prins GS, Balthazart J (1995) Distribution and neurochemical characterization of androgen receptor-containing cells in the quail brain. *Soc Neurosci Abstr* 21: 101 (Abstract)
- Goodson JL, Greenwood VR, Adkins-Regan E (1996) The central control of courtship and aggression in male zebra finches (*Taeniopygia guttata*): effect of vasotocin infusions. *Soc Neurosci Abstr* 22: 2068(Abstract)
- Goossens N, Blähser S, Oksche A, Vandesande F, Dierickx K (1977) Immunocytochemical investigation of the hypothalamo-neurohypophysial system in birds. *Cell Tissue Res* 184: 1-13
- Grossmann R, Kisliuk S, Xu B, Mühlbauer E (1995) The hypothalamo-neurohypophysial system in birds. *Adv Exp Med Biol* 395: 657-666
- Insel TR, Winslow JT, Williams JR, Hastings N, Shapiro LE, Carter CS (1993) The role of neurohypophysial peptides in the central mediation of complex social processes - evidence from comparative studies. *Regul Pept* 45: 127-131
- Jurkevich A, Barth SW, Aste N, Panzica GC, Grossmann R (1996) Intracerebral sex differences in the vasotocin system in birds: possible implication on behavioral and autonomic functions. *Horm Behav* 30: 673-681
- Jurkevich A, Barth SW, Grossmann R (1997) Sexual dimorphism of arg-vasotocin gene expressing neurons in the telencephalon and dorsal diencephalon of the domestic fowl. An immunocytochemical and *in situ* hybridization study. *Cell Tissue Res* 287: 69-77
- Kihlström JE, Danninge I (1972) Neurohypophysial hormones and sexual behavior in males of the domestic fowl (*Gallus domesticus*) and the pigeon (*Columba livia* Gmel.). *Gen Comp Endocrinol* 18: 115-120
- Kiss JZ, Voorhuis TAM, Van Eekelen JAM, De Kloet ER, De Wied D (1987) Organization of vasotocin-immunoreactive cells and fibers in the canary brain. *J Comp Neurol* 263: 347-364
- Koike TI, Shimada K, Cornett LE (1988) Plasma levels of immunoreactive mesotocin and vasotocin during oviposition in chickens: relationship to oxytocic action of the peptides in vitro and peptide interaction with myometrial membrane binding sites. *Gen Comp Endocrinol* 70:

- Korf HW (1984) Neuronal organization of the avian paraventricular nucleus: intrinsic, afferent, and efferent connections. *J Exp Zool* 232: 387-395
- Leng G, Dyball REJ, Luckman SM (1992) Mechanisms of vasopressin secretion. *Horm Res* 37: 33-38
- Leutgeb S (1995) Social preference in the Japanese quail (*Coturnix coturnix japonica*): hormonal modulation. Thesis, Univ. Salzburg,
- Makara GB, Kiss A, Lolait SJ, Aguilera G (1996) Hypothalamic-Pituitary Corticotroph Function After Shunting of Magnocellular Vasopressin and Oxytocin to the Hypophyseal Portal Circulation. *Endocrinol* 137: 580-586
- Maney DL, Goode CT, Wingfield JC (1997) Intraventricular infusion of arginine vasotocin induces singing in a female songbird . SBN-CRB meeting, Baltimore, MD Abstracts: 91(Abstract)
- Mikami SI, Tokado H, Farner DS (1978) The hypothalamic neurosecretory systems of the Japanese quail as revealed by retrograde transport of horseradish peroxidase. *Cell Tissue Res* 194: 1-15
- Milewski N, Ivell R, Grossmann R, Ellendorff F (1989) Embryonal development of arginine vasotocin/mesotocin gene expression in the chicken brain. *J Neuroendocrinol* 1: 473-484
- Moore FL (1992) Evolutionary precedents for behavioral actions of oxytocin and vasopressin. In: Pedersen CA, Caldwell JD (eds) *Oxytocin in maternal, sexual, and social behaviors*. New York Acad.Sci., Annals, Vol. 652, New York, pp 156-165
- Moore FL, Lowry CA (1997) Comparative neuroanatomy of vasotocin and vasopressin in the vertebrate brain. In: V.L.Trudeau (ed) *Comparative neuroendocrinology of vertebrate reproduction and growth: organismal, cellular and molecular aspects..* Comp Biochem Physiol (in press)
- Moore FL, Wood RE, Boyd SK (1992) Sex Steroids and Vasotocin Interact in a Female Amphibian (*Taricha granulosa*) to Elicit Female-Like Egg-Laying Behavior or Male-Like Courtship. *Horm Behav* 26: 156-166
- Moore FL, Lowry CA, Rose JD (1994) Steroid-neuropeptide interactions that control reproductive behaviors in an amphibian. *Psychoneuroendocrinol* 19: 581-592
- Mühlbauer E, Hamann D, Xu B, Ivell R, Udovic B, Ellendorff F, Grossmann R (1993) Arginine vasotocin gene expression and hormone synthesis during ontogeny of the chicken embryo and the newborn chick. *J Neuroendocrinol* 5: 281-288
- Norgren RB, 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
- Oksche A, Farner DS (1974) Neurohistological studies of the hypothalamo-hypophysial system of *Zonotrichia leucophrys gambelii* (Aves, Passeriformes) with special attention to its role in the control of reproduction. *Adv Anat Embryol Cell Biol* 48: 1-136
- Panzica GC (1985) Vasotocin-immunoreactive elements and neuronal typology in the suprachiasmatic nucleus of the chicken and Japanese quail. *Cell Tissue Res* 242: 371-376
- 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, García-Ojeda E, Viglietti Panzica C, Thompson NE, Ottinger MA (1996a) Testosterone effects on vasotocinergic innervation of sexually dimorphic medial preoptic nucleus and lateral septum during aging in male quail. *Brain Res* 712: 190-198
- Panzica GC, Viglietti-Panzica C, Balthazart J (1996b) The sexually dimorphic medial preoptic nucleus of quail: a key brain area mediating steroid action on male sexual behavior. *Front Neuroendocrinol* 17: 1-75
- Ramieri G, Panzica GC (1989) Comparative neuroanatomical aspects of the salt and water balance

- in birds and mammals. *J Endocrinol Invest* 12: 59-74
- Rice GE, Arnason SS, Arad Z, Skadhauge E (1985) Plasma concentrations of arginine vasotocin, prolactin, aldosterone, and corticosterone in relation to ovoposition and dietary NaCl in the domestic fowl. *Comp Biochem Physiol* 81A: 769-777
- Rzasa J, Ewy Z (1982) The effect of ovarian steroids in the response of the hen uterus to the neurohypophysial hormones. *Acta Physiol Pol* 33: 249-255
- Shimada K, Neldon H, Koike TI (1986) Arginine vasotocin (AVT) release in relation to uterine contractility in the hen. *Gen Comp Endocrinol* 64: 362-367
- Shimizu T, Cox K, Karten HJ, Britto LR (1994) Cholera toxin mapping of retinal projections in pigeon (*Columba livia*), with emphasis on retinohypothalamic connections. *Visual Neurosci* 11: 441-446
- Simon Oppermann C, Simon E, Gray DA (1988) Central and systemic antidiuretic hormone and angiotensin II in salt and fluid balance of birds as compared to mammals. *Comp Biochem Physiol* 90A: 789-803
- Smock T, Arnold S, Albeck D, Emerson P, Garritano J, Burrows K, Derber W, Sanson C, Marrs K, Weatherly H, Kruse K (1992) A peptidergic circuit for reproductive behavior. *Brain Res* 598: 138-142
- Szczepanska-Sadowska E, Simon-Oppermann C, Gray DA, Simon E (1985) Blood pressure and arginine vasotocin in normonatremic and hypernatremic ducks. *Basic Res Cardiol* 80: 116-125
- Sánchez F, Panzica GC, Viglietti-Panzica C, Aste N, Carretero J, Vázquez R (1991) A comparative analysis of the vasotocin and vasopressin systems in the chicken and rat hypothalamus. An immunocytochemical study. *J Hirnforsch* 32: 27-37
- Södersten P, Henning M, Melin P, Ludin S (1983) Vasopressin alters female sexual behaviour by acting on the brain independently of alterations in blood pressure. *Nature* 301: 608-610
- Van Leeuwen FW, Caffé AR, De Vries GJ (1985) Vasopressin cells in the bed nucleus of the stria terminalis of the rat: sex differences and the influence of androgens. *Brain Res* 325: 391-394
- Viglietti-Panzica C (1986) Immunohistochemical study of the distribution of vasotocin reacting neurons in avian diencephalon. *J Hirnforsch* 27: 559-566
- Viglietti-Panzica C, Panzica GC (1991) Peptidergic neurons in the avian brain. *Ann Sci Nat Zool*, Paris 12: 137-155
- Viglietti-Panzica C, Anselmetti GC, Balthazart J, Aste N, Panzica GC (1992) Vasotocinergetic innervation of the septal region in the Japanese quail: sexual differences and the influence of testosterone. *Cell Tissue Res* 267: 261-265
- Viglietti-Panzica C, Aste N, Balthazart J, Panzica GC (1994) Vasotocinergetic innervation of sexually dimorphic medial preoptic nucleus of the male Japanese quail: influence of testosterone. *Brain Res* 657: 171-184
- Viglietti-Panzica C, García-Ojeda E, Aste N, Castagna C, Panzica GC (1997) Sexual dimorphism of vasotocin system in the Japanese quail. *Soc Neurosci Abstr* 23: in press (Abstract)
- Voorhuis TAM, De Kloet ER (1992) Immunoreactive vasotocin in the zebra finch brain (*Taeniopigia guttata*). *Dev Brain Res* 69: 1-10
- Voorhuis TAM, Kiss JZ, De Kloet ER, De Wied D (1988) Testosterone-sensitive vasotocin-immunoreactive cells and fibers in the canary brain. *Brain Res* 442: 139-146
- Voorhuis TAM, De Kloet ER, De Wied D (1990) The vasotocin system in the canary brain. In: Balthazart J (ed) *Hormones, Brain and Behaviour in Vertebrates. Vol.1 Sexual differentiation, neuroanatomical aspects, neurotransmitters and neuropeptides*. Karger, Basel, New York, pp 168-179
- Voorhuis TAM, De Kloet ER, De Wied D (1991) Effect of a vasotocin analog on singing behavior in the canary. *Horm Behav* 25: 549-559
- Wang Z, Bullock NA, De Vries GJ (1993) Sexual differentiation of vasopressin projections of the bed nucleus of the stria terminalis and medial amygdaloid nucleus in rats. *Endocrinol* 132: 2299-2306
- Wang Z, Ferris CF, De Vries GJ (1994) Role of septal vasopressin innervation in paternal

behavior in prairie voles (*Microtus ochrogaster*). Proc Natl Acad Sci USA 91: 400-404

Watson JT, Adkins Regan EK (1988) Neuroanatomical localization of sex steroid-concentrating cells in the Japanese quail (*Coturnix japonica*): autoradiography with (³H)-testosterone, (³H)-estradiol, and (³H)-dihydrotestosterone. Neuroendocrinology 49: 51-64