Vasopressin in the Brain of a Desert Hibernator, the Jerboa (Jaculus orientalis): Presence of Sexual Dimorphism and Seasonal Variation


Unité de Neurosciences, Département de Biologie, Faculté des Sciences, Université Mohamed V, Rabat, Morocco (N.L.-G., W.A.B.), URA-CNRS 1332 "Neurobiologie des fonctions rythmiques et saisonnières," Université Louis Pasteur, Strasbourg, France (N.L.-G., P.P.), University Medical Center, Department of Physiology, Genève, Switzerland (M.D.-D.), Neuroscience Unit, Ottawa Civic Hospital, Ontario, Canada (M.L.H.J.H.), and Netherlands Institute for Brain Research, Amsterdam, The Netherlands (R.M.B.)

ABSTRACT

The distribution of vasopressin innervation in the brain of the jerboa (Jaculus orientalis) was investigated, with special attention to sex differences and seasonal variations. Vasopressin perikarya were observed in the paraventricular and supraoptic nuclei, the suprachiasmatic nucleus, the periventricular nucleus, the medial preoptic area, the bed nucleus of the stria terminalis, and the medial amygdaloid nucleus. In addition, vasopressin cell bodies were observed in the ventral retrochiasmatic area. After treatment with colchicine, vasopressin perikarya were also observed around the organum vasculosum laminae terminalis, in the medial diagonal band of Broca, and in the dorsal medial preoptic nucleus.

Vasopressin fibers were also found to be more widespread in the jerboa brain than in other rodents. Fibers were observed in the medial diagonal band of Broca, the stria medullaris, the tuber cinerum, the area postrema, the medial vestibular nucleus, and the dorsal motor nucleus of the vagus.

Sexual dimorphism and seasonal variation in vasopressin immunoreactivity were observed in areas that not only showed a testosterone-dependent vasopressin innervation in other rodents but also in the paratenial and mediodorsal thalamic nuclei, the tuber cinerum, the supramammillary complex, the zona incerta, the interpeduncular complex, and the dorsal and medial raphe nuclei. A denser vasopressin innervation was observed in spring/summer (sexual active period) than in autumn. Numerous brain structures contained vasopressin receptors (cerebral cortex, hypothalamus, substantia nigra, dentate gyrus, thalamic nuclei, superior colliculus, dorsal cochlear nucleus, and cerebellum); no sex- or season-related differences were observed.

These data indicate a high level of vasopressin in the jerboa brain, which may reflect an adaptation to its harsh bioclimatic environment.

Indexing terms: gonadal hormones, lateral septum, sexual cycle, suprachiasmatic nuclei, vasopressin receptors

In several mammalian species, vasopressin (VP)-producing neurons consistently have been found in several structures such as the paraventricular (PVN) and supraoptic nuclei (SON), the suprachiasmatic nucleus (SCN; Swaab et al., 1975; Vandesande et al., 1975; Van Leeuwen et al., 1978), the bed nucleus of the stria terminalis (BNST), and the medial amygdaloid nucleus (MA; Caffee and Van Leeuwen, 1983; Sofroniew, 1983). With regard to their central efferent projections, VP neurons in the PVN innervate the medulla oblongata and spinal cord (Buijs, 1978; Swanson et al., 1980; Luiten et al., 1985; Sofroniew, 1985). The VP

Accepted December 15, 1994.

Address reprint requests to Dr. N. Lakhdar-Ghaizal, URA-CNRS 1332 "Neurobiologie des fonctions rythmiques et saisonnières," Université Louis Pasteur, 12 rue de l'Université, Strasbourg, France.
neurons of the SCN also send major projections to the medial preoptic nucleus (MPN), the paraventricular thalamic nuclei (PVT), the paraventricular/dorsomedial complex of the hypothalamus (PVN/DMH), and the mesencephalic central gray (Swanson and Cowan, 1975; Hoorneman and Buijs, 1982; Watts and Swanson, 1987; Buijs et al., 1993; Kalsbeek et al., 1993). A portion of these VP neurons also appears to project to the organum vasculosum laminae

**Abbreviations**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>AA</td>
<td>anterior amygdala transition zone</td>
</tr>
<tr>
<td>ACo</td>
<td>anterior cortical amygdaloid nucleus</td>
</tr>
<tr>
<td>ACh</td>
<td>accumbens nucleus</td>
</tr>
<tr>
<td>ACg</td>
<td>anterior cingulate cortex</td>
</tr>
<tr>
<td>AD</td>
<td>anterodorsal thalamic nucleus</td>
</tr>
<tr>
<td>AHi</td>
<td>amygdal hippocampal area</td>
</tr>
<tr>
<td>AHy</td>
<td>anterior hypothalamic area</td>
</tr>
<tr>
<td>Amb</td>
<td>ambiguous nucleus</td>
</tr>
<tr>
<td>AOn</td>
<td>anterior olfactory nucleus</td>
</tr>
<tr>
<td>APir</td>
<td>amygdalopiriform transition</td>
</tr>
<tr>
<td>Aqu</td>
<td>cerebral aqueduct</td>
</tr>
<tr>
<td>Arc</td>
<td>arcuate nucleus</td>
</tr>
<tr>
<td>AV</td>
<td>anteroventral thalamic nucleus</td>
</tr>
<tr>
<td>BAOT</td>
<td>bed nucleus of accessory olfactory tract</td>
</tr>
<tr>
<td>B</td>
<td>basal nucleus of Meynert</td>
</tr>
<tr>
<td>BL</td>
<td>basolateral amygdaloid nucleus</td>
</tr>
<tr>
<td>BM</td>
<td>basomedial amygdaloid nucleus</td>
</tr>
<tr>
<td>BST</td>
<td>bed nucleus of stria terminalis</td>
</tr>
<tr>
<td>BSTM</td>
<td>bed nucleus of stria terminalis, medial</td>
</tr>
<tr>
<td>CA</td>
<td>central amygdaloid nucleus</td>
</tr>
<tr>
<td>CA1, CA3</td>
<td>field CA1, CA3 of Ammon’s horn</td>
</tr>
<tr>
<td>CA1mo</td>
<td>field CA1 of Ammon’s horn, molecular layer</td>
</tr>
<tr>
<td>CC</td>
<td>corpus callosum</td>
</tr>
<tr>
<td>Cemo</td>
<td>cerebellum, molecular layer</td>
</tr>
<tr>
<td>CG</td>
<td>central gray</td>
</tr>
<tr>
<td>CGD</td>
<td>central gray, dorsal</td>
</tr>
<tr>
<td>CI</td>
<td>claustrum</td>
</tr>
<tr>
<td>ClI</td>
<td>caudal linear nucleus raphae</td>
</tr>
<tr>
<td>cp</td>
<td>cerebelum pedunculum, basal</td>
</tr>
<tr>
<td>CPu</td>
<td>caudate putamen</td>
</tr>
<tr>
<td>CxA</td>
<td>cortex amygdala transition zone</td>
</tr>
<tr>
<td>Cx5</td>
<td>cerebral cortex, layer 5</td>
</tr>
<tr>
<td>DC</td>
<td>dorsal cochlear nucleus</td>
</tr>
<tr>
<td>DG</td>
<td>dentate gyrus</td>
</tr>
<tr>
<td>DMH</td>
<td>dorsomedial thalamic nucleus</td>
</tr>
<tr>
<td>DPB</td>
<td>dorsal parabrachial nucleus</td>
</tr>
<tr>
<td>DR</td>
<td>dorsal raphe nucleus</td>
</tr>
<tr>
<td>DTg</td>
<td>dorsal tegmental nucleus</td>
</tr>
<tr>
<td>dtgs</td>
<td>dorsal tegmental decussation</td>
</tr>
<tr>
<td>En</td>
<td>endopiriform nucleus</td>
</tr>
<tr>
<td>f</td>
<td>fornix</td>
</tr>
<tr>
<td>fi</td>
<td>fimbria hippocampi</td>
</tr>
<tr>
<td>GP</td>
<td>globus pallidus</td>
</tr>
<tr>
<td>HDB</td>
<td>horizontal diagonal band of Broca</td>
</tr>
<tr>
<td>ic</td>
<td>internal capsule</td>
</tr>
<tr>
<td>ICM</td>
<td>islands of Calleja, major island</td>
</tr>
<tr>
<td>IMD</td>
<td>intermediodorsal thalamic nucleus</td>
</tr>
<tr>
<td>InG</td>
<td>intermediate gray layer, superior colliculus</td>
</tr>
<tr>
<td>IP</td>
<td>interpeduncular nucleus</td>
</tr>
<tr>
<td>IPIP</td>
<td>interpeduncular nucleus, inner post nucleus</td>
</tr>
<tr>
<td>IPOP</td>
<td>interpeduncular nucleus, outer post nucleus</td>
</tr>
<tr>
<td>La</td>
<td>lateral amygdaloid nucleus</td>
</tr>
<tr>
<td>LDTg</td>
<td>laterodorsal tegmental nucleus</td>
</tr>
<tr>
<td>LG</td>
<td>lateral geniculate nucleus</td>
</tr>
<tr>
<td>LH</td>
<td>lateral hypothalamic area</td>
</tr>
<tr>
<td>LHB</td>
<td>lateral habenular nucleus</td>
</tr>
<tr>
<td>lo</td>
<td>lateral olfactory tract</td>
</tr>
<tr>
<td>LoT</td>
<td>nucleus lateral olfactory tract</td>
</tr>
<tr>
<td>LoTv</td>
<td>nucleus lateral olfactory tract, dorsal</td>
</tr>
<tr>
<td>LPgI</td>
<td>lateral paragigantocellular nucleus</td>
</tr>
<tr>
<td>LPO</td>
<td>lateral preoptic area</td>
</tr>
<tr>
<td>LS</td>
<td>lateral septum</td>
</tr>
<tr>
<td>LSD</td>
<td>lateral septum, dorsal</td>
</tr>
<tr>
<td>LSi</td>
<td>lateral septal nucleus, intermediate</td>
</tr>
<tr>
<td>LSV</td>
<td>lateral septum, ventral</td>
</tr>
<tr>
<td>MA</td>
<td>medial amygdaloid nucleus</td>
</tr>
<tr>
<td>MG, MGN</td>
<td>medial genulate nucleus</td>
</tr>
<tr>
<td>MHB</td>
<td>medial habenular nucleus</td>
</tr>
<tr>
<td>ml</td>
<td>medial lemniscus</td>
</tr>
<tr>
<td>mlf</td>
<td>medial longitudinal fasciculus</td>
</tr>
<tr>
<td>MM</td>
<td>medial mammillary nucleus, medial</td>
</tr>
<tr>
<td>MnPO</td>
<td>median preoptic nucleus</td>
</tr>
<tr>
<td>MnR</td>
<td>median raphe nucleus</td>
</tr>
<tr>
<td>mo</td>
<td>molecular layer in the dentate gyrus</td>
</tr>
<tr>
<td>Mo5</td>
<td>motor trigeminal nucleus</td>
</tr>
<tr>
<td>MP</td>
<td>medial mammillary nucleus, posterior</td>
</tr>
<tr>
<td>MPO</td>
<td>medial preoptic area</td>
</tr>
<tr>
<td>MPT</td>
<td>medial pretectal area</td>
</tr>
<tr>
<td>MS</td>
<td>medial septum</td>
</tr>
<tr>
<td>mt</td>
<td>mammillothalamic tract</td>
</tr>
<tr>
<td>NPC</td>
<td>nucleus of the posterior commissure</td>
</tr>
<tr>
<td>opt</td>
<td>optic tract</td>
</tr>
<tr>
<td>OX</td>
<td>optic chiasma</td>
</tr>
<tr>
<td>Pa</td>
<td>paraventricular hypothalamic nucleus</td>
</tr>
<tr>
<td>PAG</td>
<td>periaqueductal gray</td>
</tr>
<tr>
<td>PAMc</td>
<td>paraventricular hypothalamic nucleus, anterior magnocellular</td>
</tr>
<tr>
<td>PaPc</td>
<td>paraventricular hypothalamic nucleus, anterior parvocellular</td>
</tr>
<tr>
<td>Pe</td>
<td>periventricular hypothalamic nucleus</td>
</tr>
<tr>
<td>PF</td>
<td>parafascicular nucleus</td>
</tr>
<tr>
<td>PGI</td>
<td>paragigantocellular reticular nucleus</td>
</tr>
<tr>
<td>PH</td>
<td>posterior hypothalamic nucleus</td>
</tr>
<tr>
<td>Pir</td>
<td>piriform complex</td>
</tr>
<tr>
<td>PM</td>
<td>premammillary nucleus</td>
</tr>
<tr>
<td>PV</td>
<td>premammillary nucleus, ventral</td>
</tr>
<tr>
<td>pn</td>
<td>pontine nucleus</td>
</tr>
<tr>
<td>po</td>
<td>polymorph layer of the dentate gyrus</td>
</tr>
<tr>
<td>Pr6</td>
<td>principal sensory trigeminal nucleus</td>
</tr>
<tr>
<td>PT</td>
<td>paratentorial thalamic nucleus</td>
</tr>
<tr>
<td>PVA</td>
<td>paraventricular thalamic nucleus</td>
</tr>
<tr>
<td>Py</td>
<td>pyramidal tract</td>
</tr>
<tr>
<td>Re</td>
<td>reuniens thalamic nucleus</td>
</tr>
<tr>
<td>RF</td>
<td>rhinal fissure</td>
</tr>
<tr>
<td>Rh</td>
<td>rhomboide nucleus</td>
</tr>
<tr>
<td>ROB</td>
<td>raphe obscurus nucleus</td>
</tr>
<tr>
<td>RPa</td>
<td>raphe pontis nucleus</td>
</tr>
<tr>
<td>RRF</td>
<td>retrolenticular field</td>
</tr>
<tr>
<td>RTf</td>
<td>reticulotegmental nucleus pons</td>
</tr>
<tr>
<td>RTG</td>
<td>reticulotegmental nucleus of the pons, pericentral part</td>
</tr>
<tr>
<td>sch</td>
<td>suprachiasmatic nucleus</td>
</tr>
<tr>
<td>SFI</td>
<td>subcommissural organ</td>
</tr>
<tr>
<td>SFO</td>
<td>subfornical organ</td>
</tr>
<tr>
<td>G</td>
<td>granule cell layer of the dentate gyrus</td>
</tr>
<tr>
<td>SM</td>
<td>stria medularis</td>
</tr>
<tr>
<td>SMT</td>
<td>subthalamic nucleus, medial</td>
</tr>
<tr>
<td>SNC</td>
<td>substantia nigra, compacta</td>
</tr>
<tr>
<td>SNL</td>
<td>substantia nigra, lateral</td>
</tr>
<tr>
<td>SNR</td>
<td>substantia nigra, reticular</td>
</tr>
<tr>
<td>so</td>
<td>stratum oriens, field CAI</td>
</tr>
<tr>
<td>SOL</td>
<td>nucleus of solitary tract</td>
</tr>
<tr>
<td>SON</td>
<td>supraoptic nucleus</td>
</tr>
<tr>
<td>Sp5</td>
<td>spinal trigeminal nucleus</td>
</tr>
<tr>
<td>st</td>
<td>stria terminalis</td>
</tr>
<tr>
<td>Subc</td>
<td>subcoeruleus nucleus</td>
</tr>
<tr>
<td>Sug</td>
<td>superficial gray layer, superior colliculus</td>
</tr>
<tr>
<td>SUM</td>
<td>supramammillary nucleus</td>
</tr>
<tr>
<td>sup</td>
<td>supramammillary decussation</td>
</tr>
<tr>
<td>TS</td>
<td>triangular septal nucleus</td>
</tr>
<tr>
<td>Tu</td>
<td>olfactory tubercle</td>
</tr>
<tr>
<td>VDB</td>
<td>ventral diagonal band of Broca</td>
</tr>
<tr>
<td>VL</td>
<td>ventrolateral thalamic nucleus</td>
</tr>
<tr>
<td>VLL</td>
<td>ventricular nucleus lateral lemniscus</td>
</tr>
<tr>
<td>VMH</td>
<td>ventromedial hypothalamic nucleus</td>
</tr>
<tr>
<td>VP</td>
<td>ventral pallidum</td>
</tr>
<tr>
<td>VPB</td>
<td>ventral parabrachial nucleus</td>
</tr>
<tr>
<td>VTA</td>
<td>ventral tegmental area</td>
</tr>
<tr>
<td>VTg</td>
<td>ventral tegmental nucleus</td>
</tr>
<tr>
<td>Xcep</td>
<td>decussation superior cerebellar peduncle</td>
</tr>
<tr>
<td>ZI</td>
<td>zona incerta</td>
</tr>
</tbody>
</table>
THE JERBOA BRAIN VASOPRESSIN SYSTEM

terminals (OVLT; Swanson and Cowan, 1975; Hoorneman and Buijs, 1982; Kalsbeek et al., 1993). In addition, VP neurons within the BNST and MA have been shown to provide the larger part of VP innervation observed in the limbic system, such as the lateral septum (LS), the vertical limb of the diagonal band of Broca (VDB), the lateral habenula (LHb), and the ventral hippocampus (De Vries and Buijs, 1983). The multiple origins and widespread distribution of VP fibers in the central nervous system (CNS) of mammals may explain the involvement of this peptide in a variety of brain functions such as regulation of water intake, blood pressure (Ripphagen and Pittman, 1986), thermoregulation (Banet and Wieland, 1985), expression of hibernation (Buijs et al., 1986; Hermes et al., 1989), and circadian rhythms (Buijs et al., 1988, 1993; Kalsbeek and Buijs 1992).

An interesting aspect of VP immunoreactivity in cell bodies of the BNST and MA is their dependency on plasma concentrations of gonadal hormones. Castration has been shown to lead to a specific decrease in VP labeling in cell bodies in the BNST and MA and in their projecting fibers in the LS, VDB, and LHb. Subsequent testosterone replacement was able to restore the level of VP immunoreactivity (De Vries et al., 1985; Van Leeuwen et al., 1985; Buijs et al., 1986; Wang et al., 1993). Changes in immunoreactivity of a part of the central VP system have also been shown to occur naturally following seasonal changes in plasma testosterone levels. In rodent species such as the European hamster, the garden dormouse, and the Siberian hamster, the decrease in day length at the end of the summer induces gonadal regression and a consequent decrease in the plasma level of testosterone. Concomitantly, a gonadally induced disappearance of part of the central VP labeling could be observed (Buijs et al., 1986; Hermes et al., 1990; Ouarour, 1991). These findings suggest that variations in VP immunoreactivity might be actively involved in the expression of certain seasonal functions (Pétet et al., 1990; Pétet, 1991).

The jerboa (Jaculus orientalis) lives in a particular biotope of the Moroccan high continental shelf where it must adapt to extreme climatic conditions in winter by hibernating (El Hilali and Veillet, 1975) and to high temperatures and arid conditions in the summer by decreasing its water excretion. For example, in this species, basal plasma VP concentrations have been shown to be 10-fold higher than in the rat (70 vs. 6 pg/ml; Baddouri et al., 1984). Moreover, under dry diet conditions, which reflect the hydration challenges to which it is subjected in its biotope during summer, the jerboa has twice as much VP in the neurohypophysis than does the rat (Bensalah-Alaoui, 1984). As certain central functions, such as osmoregulation, show similar changes during these different environmental conditions, and as the lateral septum is implicated in osmoregulation, thermoregulation, and expression of the hibernation cycle, it was of interest to investigate whether or not VP changes in the neuroendocrine axis reflect a central adaptation and whether or not seasonal changes in central VP immunoreactivity also occur in the jerboa. A study of the distribution of VP receptors was also performed.

MATERIALS AND METHODS

Animals

Forty-six adult male and female jerboas (Jaculus orientalis) were captured in May in the Middle Atlas Mountains (region of Boulemane, Morocco) and transported to Strasbourg, France. To study possible seasonal changes in central VP labeling and the involvement of sex steroids in this phenomenon, the animals were divided into four groups: (1) sexually active males (n = 8) and sexually active females (n = 7), (2) males (n = 4) castrated during the period of sexual activity (early May) and killed 6 weeks after castration together with controls, (3) sexually inactive males (n = 8) and sexually inactive females (n = 7; September–October), and (4) sexually inactive males (n = 4) implanted with testosterone and killed 6 weeks later together with control groups.

In addition to these groups, sexually active males (n = 2) and females (n = 2) and sexually inactive males (n = 2) and females (n = 2) were treated with colchicine (5 μg/animal in 10 μl of 0.9% NaCl injected intracerebroventricularly) for 48 hours to investigate the existence of possible additional perikarya.

Animals in groups of four to eight were housed in cages maintained under natural conditions of temperature and photoperiod. Food consisted of barley grains and sunflower seeds, which were available ad libitum. The diet was supplemented with lettuce leaves once every 3 days.

To ensure that changes in climatic conditions and photoperiods following the transition of the animals from Morocco to Strasbourg did not interfere with VP innervation, we compared the central VP immunostaining from two males and two females perfused in Morocco in spring and autumn with animals killed in Strasbourg, also in spring and autumn.

The seasonal sexual cycle of male jerboas was determined by killing animals (n = 7 at each point) in the field at different periods of the year and then by weighing the testes. The testes weight was also verified after each perfusion step. Brains were sampled at the periods of maximal and minimal testes weights. No jerboa was studied at the time of decreasing testicular weight (July–August).

Immunocytochemistry

For immunocytochemistry, animals were anesthetized with sodium pentobarbital (35 mg/kg), perfused intracardially with 20 ml of 0.9% NaCl, which was followed immediately by three fixative solutions, as described by Yamada et al. (1987): the first fixative was a mixture of 4% paraformaldehyde (PF), 0.2% picric acid, and 0.5% glutaraldehyde (Gl) in 0.1 M phosphate buffer (PB) at pH 7.6; the second was 4% PF, 0.2% picric acid, and 0.2% acetic acid in PB, pH 7.6; the third solution consisted only of 4% PF in PB, pH 7.6. Brains were removed and postfixed overnight at 4°C in the last fixative. Subsequently, 50-μm sections were cut in the coronal plane by using a Vibratome (Lancer) and placed in 0.05 M Tris buffer containing 0.9% NaCl (Tris–NaCl), pH 7.6. Before immunostaining, the free-floating sections were incubated in 0.5% H2O2 in PB containing 0.5% Triton X-100 (PBTr).

The free-floating sections were then treated as follows: (1) overnight incubation at 4°C with rabbit VP antisemum (Trusel 10-4) diluted 1:1,000 in PBT, pH 7.6, under mild agitation; (2) rinsing 3 x 10 minutes in PBT, pH 7.6; (3) 1-hour incubation at room temperature in goat anti-rabbit IgG serum (Betsy) diluted 1:100 in PBT, pH 7.6; (4) rinsing 3 x 10 minutes in PBT, pH 7.6; (5) 1-hour incubation at room temperature in the peroxidase-antiperoxidase complex (PAP) diluted 1:1,000 in PBT, pH 7.6; (6) rinsing 3 x 10 minutes in PBT, pH 7.6; and (7) 15-minute development
of peroxidase activity by a solution of 0.025% 3,3'-diaminobenzidine in 0.05 M Tris–NaCl containing 0.01% H2O2.

After a final rinse in buffer (0.1 M Tris–NaCl, pH 7.6), sections were mounted on gelatin-coated slides, air dried for several hours, dehydrated, and coverslipped in Eukitt (Labonord, France).

The specificity of the staining was checked by staining alternate sections with (1) VP antiserum (1:500) preadsorbed on Sepharose beads coupled to VP, (2) oxytocin (OT) antiserum (O-I-V) (1:1,000), and (3) VP antiserum (1:500) preadsorbed on Sepharose beads coupled to OT (VP/OT), 1:500. Additional specific tests were done on colchined animals with VP/OT antiserum (1:5000).

In the absence of a stereotaxic atlas of the jerboa brain, the rat atlases of Paxinos and Watson (1982) and of Swan-son (1992) were used to identify structures on cryostat sections of the jerboa brain, according to a frontal plane with reference to Bregma. Sections were stained with cresyl violet.

After adsorption of VP antiserum onto Sepharose beads coupled to VP, no VP staining was observed. Because VP antiserum (Truus) can stain magnocellular OT cell bodies in the hypothalamus, it was preadsorbed with OT, resulting in disappearance of the OT cross reaction. OT cross reaction was observed in the brainstem structures, where VP and OT immunoreactivity are present. With the VP antiserum preadsorbed with OT, VP staining persisted in these structures. VP staining also persisted in the structures where additional VP immunoreactive neurons appeared after colchicine treatment.
No difference in VP immunoreactivity was observed between animals perfused in Morocco with those perfused in Strasbourg.

**Autoradiography**

Sexually active (two males, two females) and sexually inactive (two males, two females) jerboas were anesthetized with pentobarbital. After decapitation, the brain was removed and frozen in isopentane cooled to -25°C with dry ice. Coronal sections, 16-µm thick, were cut in a cryostat, laid on chromatolium gelatin-coated slides, air dried, and processed for light microscopic autoradiography.

Autoradiography of VP binding sites was performed as described elsewhere (Dubois-Dauphin et al., 1994) by using 0.05–0.07 nM of [125I]-labeled vasopressin antagonist [Pheα1, D-Tyr(4)β2, Argβ6, Tyr-NH₂β9]AVP ([125I]-VPA; see Schmidt et al., 1991). Hydroxy[Thrβ4, Glyβ8]oxytocin, a selective oxytocin agonist (Manning and Sawyer, 1985), was added to the incubation medium to reduce the binding of radioactive ligands to oxytocin receptors (Audigier and Barberis, 1985). To assess nonspecific binding, adjacent sections were treated under the same conditions except that the incubation medium contained 2 µM of nonradioactive VP. Sections were then placed in contact with Hyperfilm-β max (American Shlam) for 1–3 days. Films were developed in Kodak D19. Sections were counterstained with cresyl violet, and structures were identified by comparing them with the rat brain sections from the atlases cited earlier.

**RESULTS**

**VP immunoreactivity in the jerboa brain**

**Cell bodies.** All the results illustrated correspond to those obtained in noncolchicinized animals except for the result presented in Figure 3b.

In all portions of the PVN (Fig. 1: −1.5, −1.8) and in the accessory cell bodies in the lateral hypothalamic areas (Fig. 2b), immunoreactive neurons were present in large numbers and were generally densely packed. In the rostral PVN, some perikarya sent projections directly toward the third ventricle (Fig. 3a), and others were seen quite close to the wall of the third ventricle in all planes of the periventricular layer (Fig. 2b). Some of the accessory VP cells localized between the fornix and the ventral border of the stria medullaris sent their axons dorsolaterally toward the internal capsule or ventrally toward the hypothalamohypophyseal tract (Fig. 1: −1.5). VP-labeled accessory peri-
Fig. 2.  

a: Some VP neurons in the rostral paraventricular nucleus (PVN) seem to send projections toward the third ventricle layer (arrowhead). ×45.4. 

b: VP cell bodies are located in the ventromedial, dorsomedial, dorsal, and dorsolateral subdivisions of the suprachiasmatic nucleus (SCN). Note the tearlike shape of this nucleus and the densely packed neurons in the lateral hypothalamic area. ×94.5. 

c: VP immunoreaction in the SON shows axonal projections of the cell bodies situated in the lateral extremities of the optic chiasma. Note the dorsal trajectory of these fibers (arrowheads). ×94.5. 

d: Parvocellular neurons (arrowheads) are seen in the bed nucleus of the stria terminalis (BSTN). Fibers are dense from the ventricular border to the most medial area of this structure. ×19.3.
Fig. 3. a: Two layers of VP cells are localized in the ventral posterior hypothalamus (arrows). RCh, retrochiasmatic area of the posterior hypothalamus; ot, optic tract. ×113.3. b: Cell bodies are observed around the organum vasculosum laminae terminalis (OVLT; arrowheads) after colchicine treatment. This vascular organ also contains VP neurons and fibers without colchicine treatment. ×45.4. c: VP neurons localized in the median preoptic nucleus (MnPO; arrowheads) and in the periventricular areas. ×45.4. d: A large number of VP cell bodies are seen in the medial preoptic nucleus (MPO). ×45.4. e–f: A population of VP-immunoreactive perikarya (arrowheads) is observed quite close to the blood vessel (bv; arrow) in the rostral optic chiasma (ox). e: ×45.4; f: ×113.5.
Figure 4
karya were scattered in the ventral part of the internal capsule.

In the anterior SON, immunopositive perikarya were localized in the lateral part of the nucleus, which projects dorsolaterally (Fig. 2c) toward the internal capsule.

The SCN, which have a tearlike shape, showed a strong VP immunocytochemical reaction. Immunopositive cell bodies were densely packed in the dorsomedial and ventromedial subdivisions of the SCN and were also present in the dorsal and dorsolateral subdivisions in the periphery of the nucleus (Fig. 2b). A small number of VP-positive cells could also be seen in the ventral SCN, just above the optic chiasma.

The VP cells of the BNST (Fig. 2d) and the MA were clearly labeled. These parvocellular neurons sent out thin neuronal processes.

Posteriorly, VP neurons were observed in the two external layers of the retrochiasmatic portion of the ventral posterior hypothalamus (Fig. 3a). In addition, a small number of neurons were seen around the OVLT (Fig. 3b) and around a blood vessel in the vicinity of the rostral optic chiasma (Fig. 3e–f). A population of magnocellular and parvocellular neurons appeared in the anterior periventricular hypothalamic nucleus, in the ventral part of the median preoptic nucleus, under the decussation of the anterior commissure (Fig. 3c), and in the medial preoptic area (MPO; Fig. 3d). No VP cell bodies were observed in the locus coeruleus, with or without colchicine.

Fibers and nerve terminals. In the LS, along the lateral edge (Fig. 6d) and in the vertical and horizontal limbs of the diagonal VDB, very dense VP innervation was observed (Fig. 6g). In the LS, many fibers surrounded nonlabeled cell bodies. Heavily and densely packed fibers appeared in the medial VDB.

In the dorsolateral hypothalamus, VP-immunolabeled fibers appeared in the stria medullaris (Fig. 4a). In the retrochiasmatic hypothalamic areas, VP-immunoreactive fibers were seen in the tuber cinereum (TC), around the ventromedial hypothalamic nucleus (VMH), and in the DMH (Fig. 7a).

The amygdaloid complex contained VP-immunopositive fibers. A large number of VP fibers were localized along the optic tract in the stria terminais. In the dorsal thalamus, immunopositive fibers appeared in the anterior PVN and in the paratenial nuclei (Fig. 8a). This VP network caudally extended toward the mediadorsal thalamic nuclei, where the fiber density was higher (Fig. 8d). Immunopositive fibers appeared in the posterior PVT and in the LHb (Fig. 8g). Here, the fibers reached the habenular commissure.

In the mesencephalon, a heavy concentration of labeled fibers was observed in the supramamillary nuclei and their decusation. In the ventral hippocampus, immunoreactive fibers were dense in the CA1–CA3 cell layers, whereas no fibers were observed in the dentate gyrus. The immunocytochemical reaction was also present in fibers of the ventral tegmental zone, in the medial regions of the central interpeduncular nucleus (IP), where they extended to its inner (IPi) and outer (IPO) postsubnuclei (Fig. 4b).

In the brainstem, VP innervation was particularly dense in the dorsal raphe (DR; Fig. 9d). Fibers were also observed in the medial, pontis, magnus, and obscurus subnuclei. In the laterodorsal tegmental nucleus, a VP fiber field between the locus coeruleus and subcoeruleus was observed. Lateral to these structures, labeled fibers and terminals appeared in the medial and lateral parabrachial nucleus and along the trigeminal motor nucleus. Fibers were also localized in the medial vestibular nucleus (Fig. 4c), and a small number were present in the dorsal part of the prepositus hypoglossal nucleus. More caudally, immunostained VP fibers were seen transversing through the nucleus of the solitary tract (Fig. 4d), in the area postrema (Fig. 4e), and in the dorsal motor nucleus of the vagus in the vicinity of the central canal (Fig. 4f).

A number of immunoreactive fibers could also be observed perivascularly in the metencephalon and in the myelencephalon. All of the periventricular zones contained immunoreactive fibers that reached the supraependymal layer, particularly in the septal and hypothalamic areas.

Sexual dimorphism and seasonal variation

Testicular weights showed that in the jerboa the period of sexual activity begins in early spring and lasts until the middle of July (Fig. 5). During this period, the mean testicular weight was 3,583 ± 307 mg. In late summer and autumn, gonadal weight fell to 352 ± 52 mg, indicating the start of the period of sexual quiescence. A similar range of testicular weights was obtained in animals perfused in the laboratory in May–June and in September–October.

The distribution and the relative intensity of VP immunoreactivity in cell bodies and fibers in the jerboa brain is presented in Table 1.

Cell bodies. The number of immunoreactive perikarya observed in the BNST was lower in females (Fig. 6b) and in sexually inactive males (Fig. 6c) than in sexually active males (Fig. 6a). These variations were also observed in the cell bodies of the MA. Following colchicine treatment, no additional VP cells appeared in the BNST and the MA of sexually active females and sexually inactive males and females as opposed to sexually active males (data not shown).

Fibers and nerve terminals. Within the limbic system, sexual dimorphism and seasonal variation in VP immunoreaction was observed in the BNST (Fig. 6a–c), the LS (Fig. 6d–f), along the edge of the lateral ventricle, and in the vertical limbs of the VDB (Fig. 6g–i). In these structures, fewer fibers and nerve terminals were present in females and in sexually inactive males than in sexually active male jerboas. Sexual dimorphism and seasonal variations were also seen in the horizontal limb of the VDB, in the medial BNST, and posteriorly in the medial amygdaloid complex. In these structures, densely packed immunoreactive fibers were observed in sexually active males. In sexually active females, only scattered immunoreactive fibers were observed; in sexually inactive males, there was a total absence of fibers.
Fig. 5. Diagram illustrates the course of testicular weight (expressed in mg/100 g of body weight) in jerboas killed in the field over a 2-year period. Each point represents the mean testes weight of at least five animals. High testicular weights are observed during the period of sexual activity, beginning in spring. A decrease in testes weight begins in July, with complete regression occurring in August.

TABLE 1. Relative Distribution of Vasopressin Immunoreactivity in Different Brain Areas of Jerboas Studied Under Different Conditions

<table>
<thead>
<tr>
<th>Structures</th>
<th>SM</th>
<th>SF</th>
<th>IM</th>
<th>CM</th>
<th>IMT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bed nucleus of the stria terminalis</td>
<td>+++</td>
<td>+</td>
<td>+</td>
<td>O</td>
<td>+++</td>
</tr>
<tr>
<td>cell bodies</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>fibers</td>
<td>+++</td>
<td>+</td>
<td>+</td>
<td>O</td>
<td>+++</td>
</tr>
<tr>
<td>Median amygdaloid nucleus</td>
<td>++</td>
<td>—</td>
<td>O</td>
<td>O</td>
<td>+</td>
</tr>
<tr>
<td>cell bodies</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>fibers</td>
<td>+++</td>
<td>+</td>
<td>O</td>
<td>O</td>
<td>+</td>
</tr>
<tr>
<td>Lateral septum</td>
<td>+++</td>
<td>+</td>
<td>—</td>
<td>—</td>
<td>+++</td>
</tr>
<tr>
<td>Vertical diagonal band of Broca</td>
<td>+++</td>
<td>+</td>
<td>—</td>
<td>—</td>
<td>+++</td>
</tr>
<tr>
<td>Medial diagonal band of Broca</td>
<td>+++</td>
<td>O</td>
<td>O</td>
<td>—</td>
<td>+++</td>
</tr>
<tr>
<td>Horizontal diagonal band of Broca</td>
<td>++</td>
<td>+</td>
<td>—</td>
<td>—</td>
<td>++</td>
</tr>
<tr>
<td>Paratenial thalamic nucleus</td>
<td>+++</td>
<td>+</td>
<td>+</td>
<td>—</td>
<td>++</td>
</tr>
<tr>
<td>Mediodorsal thalamic nucleus</td>
<td>+++</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>Lateral habenula nucleus</td>
<td>+++</td>
<td>+</td>
<td>—</td>
<td>—</td>
<td>++</td>
</tr>
<tr>
<td>Tuber cinerum</td>
<td>++</td>
<td>—</td>
<td>O</td>
<td>O</td>
<td>+</td>
</tr>
<tr>
<td>Stria medullaris</td>
<td>+++</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>Stria terminalis</td>
<td>+</td>
<td>+</td>
<td>—</td>
<td>—</td>
<td>+</td>
</tr>
<tr>
<td>Supramammillary nucleus</td>
<td>+++</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+++</td>
</tr>
<tr>
<td>Periaqueductal gray substance</td>
<td>++</td>
<td>+</td>
<td>—</td>
<td>—</td>
<td>++</td>
</tr>
<tr>
<td>Interpeduncular complex</td>
<td>++</td>
<td>+</td>
<td>—</td>
<td>—</td>
<td>+</td>
</tr>
<tr>
<td>Dorsal raphe nucleus</td>
<td>+++</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>Medial raphe nucleus</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

1 Each entry of + signs represents the VP fibers' density observed in all jerboas used in different conditions: O, almost no fibers or cell bodies; —, only scattered fibers; +, a low fiber or cell body density; ++, a medium fiber or cell body density; ++++, a high fiber or cell body density; +++++, a very high fiber density. SM: sexually active males; SF: sexually active females; IM: sexually inactive males; CM: castrated males; IMT: sexually inactive males with testosterone implant.

The stria medullaris, the stria terminalis, and the TC around the VMH (Fig. 7a–c) showed sexual dimorphism and seasonal variation in the fiber density, with a greater number of VP-immunoreactive fibers in sexually active males than in sexually active females or sexually inactive males.

In the dorsal thalamus of sexually active females (Fig. 8b) and sexually inactive males versus sexually active males (Fig. 8c), a reduction of VP-immunoreactive fibers and terminals in the paratenial nucleus was observed (Fig. 8a). In the mediodorsal thalamic nuclei (Fig. 8d–f) and in the LHb (Fig. 8g–i), VP innervation was also sexually dimorphic and showed a seasonal variation. No difference was observed in the PVT between the different groups of animals studied.

In the supramammillary nuclei and their decussation and in the ventral tegmental zone, sexually active males showed more VP-immunoreactive fibers than sexually active females and sexually inactive males. In the hippocampus and in the periaqueductal gray, a dense VP network was observed in sexually active males. In these structures, VP fibers were absent in inactive males and sparse in sexually active females. In the interpeduncular complex, VP immunoreactivity was much lower in sexually active females (Fig. 9b) than in sexually active males (Fig. 9a), whereas only a few fibers were stained in sexually inactive animals (Fig. 9c). In the brainstem of this last group, the fiber density was also reduced in the DR (Fig. 9f) as compared with sexually active females (Fig. 9e) and sexually active males.
Fig. 6. Sexual dimorphism and seasonal variation are detected in the immunoreactivity of neurons (arrowheads) in the bed nucleus of the stria terminalis (BST; a–c), the lateral septum (LS; d–f), and the vertical limb of the diagonal band of Broca (VDB; g–i). In these structures, more VP immunostaining is observed in sexually active males (a,d,g) than in sexually active females (b,e,h) and sexually inactive males (c,f,i): a–c: ×105, d–i: ×154.
(Fig. 9d). Similar variations were also observed in the medial raphe, in the laterodorsal tegmental nucleus, and in its myelencephalic extension. In the pons, no variations were observed in VP immunoreactivity with respect to season or sex of the animal.

In male jerboas castrated during the period of sexual activity and killed 6 weeks later, VP immunolabeling in the perikarya and fibers was identical to that observed in sexually inactive male animals. In sexually inactive males, testosterone implants completely restored VP immunoreactivity to that observed in sexually active animals.

**Distribution of vasopressin binding sites**

The distribution of vasopressin binding sites is shown on autoradiograms obtained from sections incubated with $[^{125}\text{I}]$-VPA (Figs. 10–11). Binding sites were uncovered in the olfactory system (the mitral cell and external plexiform layers of the olfactory bulb, the olfactory nucleus, and the olfactory tubercle; Fig. 10A), in the cerebral cortex (layer 5, from the rostral to the most caudal pole of the brain [Fig. 10A–E], and the piriform cortex; Fig. 10C–D), and in the limbic system (the Calleja islands, the ventral pallidum, the VDB, the intermedial and dorsal lateral septal nuclei [Fig. 10B], the BNST [lateral and ventral], the amygdaloid nuclei [basolateral anterior, cortical, and posteromedian], the amygdalohippocampal area, and the amygdalopiriform transition zone; Figs. 10C–E, 11A–B). Specific binding sites were observed in the molecular and polymorph layers of the dentate gyrus and in the polymorph layer of the CA3 region (Figs. 10C–E, 11D). Posteriorly, binding sites were present in the ventral CA1 molecular layer and in the stratum oriens (Fig. 10D). The hippocampal pyramidal layers and dentate gyrus granule cell layers were devoid of specific binding.

In the thalamus, $[^{125}\text{I}]$-VPA binding sites were present in the rhomboid nucleus (Fig. 10C), in the parafascicular nucleus, in the medial pretectal area, in the nucleus of the posterior commissure, in the periaqueductal gray, in the median and lateral geniculate complex, in the substantia nigra (Fig. 10D), in the substantia nigra pars compacta (Fig. 11C), and in the superior colliculus (superficial and intermediate gray layers; Fig. 10E).

In the hypothalamus, binding sites were present in the anterior hypothalamic area, the zona incerta, the SCN (Figs. 10C, 11A–B). Caudally, specific binding was observed in the lateral hypothalamic area surrounding the dorsomedian and ventromedian hypothalamic nuclei (Fig. 11B), the ventral tegmental area (Fig. 11C), the premammillary nucleus (Fig. 10D), and the interpeduncular nucleus (Fig. 10E). No binding was detected in the PVN and SON.

In the brainstem, binding sites were present in the dorsal cochlear nucleus, the spinal nucleus of the trigeminal nerve, and the paragigantocellular nucleus (Fig. 10F). More caudally, binding sites were present in the inferior olive, the nucleus of the solitary tract, and the area postrema. In the cerebellum, specific binding sites were detected in the molecular layer (Fig. 11F).

No difference in the distribution of $[^{125}\text{I}]$-VPA binding sites was observed between males and females or between the sexually active and sexually inactive animals.

The $[^{125}\text{I}]$-VPA binding sites detected were specific; the binding was suppressed in the presence of an excess of...
Fig. 8. VP immunostaining is observed in several thalamic areas. The density of fibers is higher in sexually active males (a,d,g) than in sexually active females (b,e,h) or in sexually inactive males (c,f,i) in the paratenial (PT) and mediodorsal nuclei of the thalamus (MD) and in the lateral habenular nucleus (LHB). a-f: ×68.9, g-i: ×95.8.
unlabeled VP. The use of an iodinated VP antagonist, specific for the V₁ subtype of receptors, confirms our previous claim that the hypothalamic VP receptors are of the V₁ type in Jaculus orientalis and in others species (Tribollet et al., 1988; Dubois-Dauphin et al., 1990).

**DISCUSSION**

**VP immunoreactivity in the brain of the jerboa**

Central VP is known to play a major role in the homeostasis of water balance and cardiovascular control (Palkovits...
and Zaborsky, 1977; Loewy and McKellar, 1980). Dehydration induces an increase in VP mRNA gene expression and VP plasma concentrations (Burbach et al., 1986; Sherman et al., 1986). VP is also involved in the regulation of the reproductive axis and seasonal functions such as hibernation (Buijs et al., 1986; De Vries, 1990; Hermes et al., 1990).

In the limbic system, VP content is sexually dimorphic and varied under the influence of steroid hormones (De Vries et al., 1986). These specific regulatory roles of VP find a morphological correlation in the present data because VP immunoreactivity is more widespread in the brain of the desert jerboa than in all other rodents studied to date (Sofroniew, 1983, 1985; Ju et al., 1986; Buijs, 1990). Furthermore, in the jerboa, a direct correlation between seasonal sexual activity and VP immunoreactivity has been observed in a larger number of central structures than in other studied seasonal breeders (Buijs et al., 1986; Hermes et al., 1990, Ouraour, 1991; Dubois-Dauphin et al., 1994).

In the jerboa, the central structures thought to be involved in cardiovascular regulation, osmotic regulation,
and blood pressure (Crofton et al., 1979; Swanson, 1977, 1987) contain a large population of VP neurons and fibers. These structures not only include the PVN and SON, as in other mammals (Rhodes et al., 1981; De Vries et al., 1985; Ju et al., 1986), but also the ventral retrochiasmatic area of the posterior hypothalamus, the tissue adjacent to the OVLT; the periventricular nucleus, the MnPO, the MPO, the LS and in the brainstem, the area postrema and the dorsal vagal/solitary nucleus complex (Miselis et al., 1984; Sofroniew, 1985). These results suggest that more VP may be synthesized and released in the jerrboa brain, which in turn could explain the high VP plasma concentrations detected in this species (Baddouri et al., 1984). Moreover, the LS, which is known to have neuronal connections with the neurosecretory neurons of the hypothalamus in the rat (Epstein et al., 1990), and which is known to play a crucial physiological role in water intake behavior, osmoregulation (Poulain et al., 1980; Epstein et al., 1990), and thermoregulation (Cooper et al., 1979), exhibited a denser VP innervation in the jerrboa than in the rat and European hamster (personal observations) and Siberian hamster (Dubois-Dauphin et al., 1994). The results obtained may have several explanations. In the biotope of the jerrboa, the period of acute dryness and high external temperatures starts in late spring and early summer (June–July). The denser VP labeling observed in the LS during this period suggests that VP may participate with the neuroendocrine system in the maintenance of hydroelectrolytic and temperature homeostasis under these local, harsh environmental conditions. Besides this regulatory role, the dense VP immunolabeling in the LS in spring and early summer may also be correlated with the onset of the period of sexual activity, as VP has been implicated in sexual behavior (De Vries, 1990; Bamshad et al., 1993).

The mammalian SCN, thought to be the major pacemaker for the generation of circadian rhythms (Moore, 1982), consistently shows VP neurons. In several mammalian species, SCN-derived VP produces a circadian rhythm in cerebrospinal fluid (CSF) VP content (Reppert et al., 1987). Under extreme experimental conditions, when osmotic control of VP becomes important, VP concentrations in the CSF have been shown to increase (Reppert et al., 1987). In the jerrboa, these nuclei exhibit more immunoreactive VP neurons than other rodents (De Vries et al., 1985; Sofroniew, 1985; Schimchowitsch et al., 1989; Dubois-Dauphin et al., 1990). The structures known to receive a VP innervation from the SCN in the rat (Hooranen and Buijs, 1982; Watts et al., 1987) and golden hamster (Kalsbeek et al., 1993), namely the periventricular nucleus and MPO, also contain high levels of VP-immunoreactive fibers in the jerrboa. In addition, these structures contain VP neurons localized in the vicinity of the ventricle wall or send direct projections toward the third ventricle. These observations suggest that VP might also be released in the CSF of the jerrboa. In the jerrboa, as in other rodents (De Vries et al., 1985; Castel and Morris, 1988; Kalsbeek et al., 1993), the OVLT contains numerous VP immunoreactive fibers. A part of this VP innervation, which in jerrboa could also originate from the SCN, might be of a great importance in
the regulation of both circadian functions and osmotic challenges in this desert specie.

Sexual dimorphism and seasonal variation

Our results show that in the jerboa, as in other rodents (De Vries et al., 1985; Buijs et al., 1986; Hermes et al., 1990; Ouarour, 1991), the LS, the VDB, and the LHb exhibit a sexual dimorphism and seasonal variation in VP labeling. The lower density of VP-immunoreactive fibers observed in females versus males in the spring–summer period may be explained by differences in their osmoregulatory and thermoregulatory capacities. In jerboa, the sexual dimorphism and seasonal variation observed may also be explained by a gonadal steroid control of the capacity of neurons of the BNST and/or MA to synthesize VP, as has been shown in the rat (Miller et al., 1989; De Vries et al., 1992, 1994). Indeed, this may be the case because no additional VP-immunoreactive neurons has been observed in sexually active females and sexually inactive males after the blockage of the antergrade neuronal transport. Compared with others species, VP innervation in the jerboa brain seems to be influenced by seasonal variations in gonadal hormones in a larger number of central structures (Table 1), such as the tuber cinereum, the stria medullaris and terminals, the paratendol and mediiodorsal thalamic nuclei, the interpeduncular complex, and the dorsal and medial raphe nuclei.

VP in the LS of the jerboa may be involved in the regulatory mechanisms of water balance during the arid season. In late summer (August), however, although the arid season persisted, disappearance of VP labeling occurred in the LS and other limbic structures (e.g., hippocampus and LHb). This disappearance of VP innervation is a consequence of the sexual quiescence that occurs in this period, indicating the beginning of the preparatory period of hibernation. In the hibernating species studied so far, it appears that gonadal regression and a decrease in VP content in the LS are two prerequisites for the onset of hibernation (Pévet, 1991). In the European hamster, infusion of VP into the LS in early winter prevents hibernation (Hermes et al., 1989) and is able to induce arousal from hibernation (Hermes et al., 1993). The exact mechanism by which VP in the LS regulates the temporal organization of the hibernation cycle remains to be elucidated. It appears, however, that absence of VP in autumn is necessary for the expression of hibernation (Pévet, 1991). This remains to be demonstrated in the jerboa. It also remains to be shown that, in the other brain structures where a decrease in VP content during sexual inactivity was observed, VP is involved in the control of seasonal functions.

The SCN is responsible for the VP innervation of the anterior paratendol and mediiodorsal thalamic nuclei in the rat (Hoorneman and Buijs, 1982; Watts and Swanson, 1987). In these structures, the amount of VP seems to be unaffected by seasonal changes in the activity of the sexual axis in two seasonal breeders, the European hamster (Buijs et al., 1986) and the garden dormouse (Hermes et al., 1990), whereas a considerable seasonal variation in VP immunoreactivity has been described in the SCN of a desert saharian rodent, Taterillus petteri (Fuminier et al., 1993). In the jerboa, the densely packed VP-immunoreactive fibers observed in the paratendol and mediiodorsal thalamic nuclei were influenced by the seasonal changes in the plasma concentration of gonadal steroids. However, because no sex-related differences or seasonal variations were seen in VP immunoreactivity in the SCN in this species, these results suggest that a part of the VP innervation of these

Vasopressin receptors

The distribution of vasopressin binding sites in the jerboa is generally similar to the distribution reported in other species (Dubois-Dauphin et al., 1990, 1991; Insel et al., 1991; Tribollet et al., 1992). There are, however, some new features. Vasopressin binding sites were localized in the molecular and polymorphic layers of the hippocampal formation (CA3 and dentate gyrus), whereas the pyramidal and granule cell layers were devoid of binding sites. Electrophysiological studies have reported that interneurons in the pyramidal layer of the hippocampus are sensitive to oxytocin rather than to VP (Mühlthaler et al., 1984; Raggenbass et al., 1989a). The even and dense distribution of the VP binding strongly suggests that the autoradiographic labeling is supported not only by the interneurons’ or granule cells’ dendritic arborization present in these hippocampal layers (Buhl et al., 1994) but also by other cellular elements such as glial cells. Because the compatibility between autoradiographic labeling and immunocytochemistry cannot be achieved on tissue sections with the ligand we used, there is no possibility to demonstrate which cellular type these hippocampal binding sites endow. However, an in vitro study using cell culture and another ligand have indicated that VP receptors are present on glial cells in the rat nervous system (Hösl and Hösl, 1992). Similarly, the even distribution of VP binding sites in the molecular layer of the cerebellum suggests that they could endow cell types other than neuronal ones. The presence of VP binding sites in the dorsal cochlear nucleus was not observed in other species. However, a VP innervation was reported in this structure in the guinea pig, where VP modified the bioelectric activity of cochlear neurons (Charpak et al., 1989).

In contrast with observations in the Siberian hamster (Dubois-Dauphin et al., 1991, 1994), we observed neither a sexually dimorphic expression nor a seasonal variation in the distribution of VP receptors in the jerboa. This situation is reminiscent of previous observations in the golden hamster (Dubois-Dauphin et al., 1990), which suggests that the seasonal variation of the vasopressin content of certain neurons is sufficient to control seasonal functions such as hibernation (Hermes et al., 1989).

The presence of binding sites in structures where VP immunoreactivity was detected suggests that in the jerboa, as in other species, VP could act as a neuromodulator of neuronal activity (Raggenbass et al., 1989b). However, the coexistence of VP detected by immunocytochemistry and of VP binding sites was limited to a few structures, namely the diagonal band of Broca, the SCN, the hypothalamic region surrounding the ventromedian nucleus, the ventral tegmental area, the interpeduncular nucleus, the trigeminal nucleus, and the nucleus of the solitary tract. Furthermore, the density of VP axons was often inversely related to the density of autoradiographic labeling in, for example, the dorsal septal nucleus, the thalamic lateral habenular nucleus, the periaqueductal gray, or the superficial gray layer of the superior colliculus. More striking was the absence of VP innervation in the dentate gyrus, where the strongest autoradiographic labeling was observed. Similar mismatches have been reported for other species (Dubois-Dauphin et al., 1991, 1994), which questions their physiological significance or the technical limitations of the techniques used.
structures may be originate in the BNST and/or MA. This, however, remains to be demonstrated.

In conclusion, it appears that the jerboa has several characteristics that make it an interesting biological model for investigating the physiological role of central VP in the regulation of the seasonal control of water balance and hibernation.

ACKNOWLEDGMENTS

We thank Dr. M.M. Manning (Toledo, OH, USA) for the gift of the vasopressin and oxytocin structural analogs, Dr. C. Barberis (Montpellier, France) for iodination of VPA, Ms. J. Ebener for excellent technical assistance, R. Wilhelm for taking care of the animals, M. Atras and D. Heitz for their assistance in preparing the illustrations, Dr. J. Stehle for helpful discussion of the results, and Dr. D.J. Skene for correcting the English. This study was supported by the “Comité mixte inter-universitaire franco-marocain” (actions intégrées nos. 88/321 and 94/765) and by the Swiss National Foundation (grant 31-28624.90 1). M. Dubois-Dauphin gratefully acknowledges receipt of a Career Development Award from the Prof. Dr. Max Cloëtta Foundation.

LITERATURE CITED


THE JERBOA BRAIN VASOPRESSIN SYSTEM

tions of the supramammillary nucleus in the golden hamster (Mesocricet-


of paraventricular hypothalamic eff erents to autonomic structures in

York: Raven Press, pp. 131–144.

Miller, M.A., L. Vician, D.R. Clifton, and D.M. Dorsa (1989) Sex differences
in vasopressin neurons in the bed nucleus of stria terminals by in situ

Miselis, R.R., T.M. Hyde, and R.E. Shapiro (1984) Area postrema and
43:2969–2971.

Mühlthaler, M., S. Charpak, and J.J. Dreifuss (1984) Contrasting effects of
neurohypophysial peptides on pyramidal and non-pyramidal neurones in


Ouarour, A. (1991) Analyse des mécanismes nerveux et endocrines impli-
qués dans le contrôle de la torpue diurne chez le hamster Sibérien,

Palkovits, M., and L. Zaborszky (1977) Neuroanatomy of central cardiovas-
cular control. Nucleus tractus solitarius: Aff erent and efferent neural
connections in relation to the baroreceptor reflex arc. In W. De Jong, A.P.
Provoost, and A.P. Shapiro (eds): Hypertension and Brain Mechanisms.


Pevet, P. (1991) Importance of sex steroids and neuropeptides in the pineal
control of seasonal rhythms. In J. Arendt and P. Pevet (eds): Advances in

Pevet, P., M. Masson-Pevet, M.L.H.J. Hermes, R.M. Buja, and B. Cangui-
hem (1990) How the pineal times the different seasonal functions. In D.
Gupta, P. Wollmann, and D. Ranke (eds): Neuroendocrinology. New

Pouliain, D.A., F. Ellendorf, and J.D. Vincent (1960) Septal connections with
identified oxytocin and vasopressin neurones in the supraoptic nucleus
of the rat. An electrophysiological investigation. Neuroscience 5:379–
383.

Raggenbass, M., M. Tribollet, M. Dubois-Dauphin, and J.J. Dreifuss (1989a)
Correlation between oxytocin neuronal sensitivity and oxytocin receptor
binding: An electrophysiological and autoradiographical study compar-
ing rat and guinea pig hippocampus. Proc. Natl. Acad. Sci. USA
86:750–754.

Raggenbass, M., E. Tribollet, M. Dubois-Dauphin, and J.J. Dreifuss (1989b)
Vasopressin receptors of the vasopressor (V1) types in the nucleus of the
solitary tract of the rat mediate direct neuronal excitation. J. Neurosci.
9:3929–3936.


analysis of magnocellular elements in rat hypothalamus: Distribution and
numbers of cells containing neurophysin, oxytocin, and vasopressin. J.
Comp. Neurol. 198:45–64.

Ripphagen, C.L., and J.G. Pittman (1986) Arginine vasopressin as a central

Distribution and morphometric characteristics of oxytocin and vasopres-
sin-immunoreactive neurons in rabbit hypothalamus. J. Comp. Neurol.

Sherman, T.G., O. Civelli, J. Douglass, E. Herbert, S. Burke, and S.J. Watson
(1986) Hypothalamic dynorphin and vasopressin mRNA expression in

Schmidt, A., B. Audigier, C. Barberis, S. Jard, M. Manning, K. Kolodziejczyk,
and W.H. Sawyer (1991) A radioiodinated linear vaspressin antagonist:
Ligand with high affinity and specificity for V1 receptors. FEBS Lett.
282:77–81.

Sofroniew, M.V. (1983) Morphology of vasopressin and oxytocin neurons and
their central and vascular projections. In B.A. Cross and G. Leng (eds):
The Neurohypophysis: Structure, Function and Control. Progress in Brain

Sofroniew, M.V. (1985) Vasopressin, oxytocin and their related neurophysi-
ology. In A. Björklund and T. Hökfelt (eds): Handbook of Chemical Neuro-
anatomy: GABA and Neuropeptides in the CNS, vol. 4, part 1. New York:
Elsevier, pp. 93–164.

Swaab, D.F., C.W. Pool, and F. Nijveldt (1975) Immunofluorescence of
vasopressin and oxytocin in the rat hypothalamo-neurohypophyseal

Swanson, L.W. (1977) Immunohistochemical evidence for a neurophilin-
containing autonomic pathway arising in the paraventricular nucleus of

Amsterdam: Elsevier, pp. 1–104.

Swanson, L.W. (1992) Brain Maps: Structure of the Brain. Amsterdam:
Elsevier.

Swanson, L.W., and W.M. Cowan (1975) The efferent connections of the
suprachiasmatic nucleus of the hypothalamus. J. Comp. Neurol. 160:1–
12.

Swanson, L.W., P.E. Sawchenko, S.J. Wiggard, and J.L. Price (1980)
Separate neurons in the paraventricular nucleus project to the medial
eminence and the medulla or spinal cord. Brain Res. 198:190–195.

Tribollet, E., C. Barberis, S. Jard, M. Dubois-Dauphin, and J.J. Dreifuss
(1988) Localization and pharmacological characterization of high affinity
binding sites for vasopressin and oxytocin in the rat brain by light
microscopic autoradiography. Brain Res. 442:105–118.

Tribollet, E., C. Barberis, S. Jard, M. Dubois-Dauphin, and J.J. Dreifuss
(1992) Localization and characterization of binding sites for vasopressin
and oxytocin in the brain of the guinea pig. Brain Res. 589:15–23.

in the bed nucleus of the stria terminals of the rat: Sex differences and the

Van Leeuwen, F., D.F. Swaab, and C. De Raay (1978) Immunoelectronmicro-
scopic localization of vasopressin in the rat suprachiasmatic nuclei.

Vandesande, F., K. Dierickx, and J. De Mey (1975) Identification of
vasopressin-neurophysin producing neurons of the rat suprachiasmatic

Wang, Z., N.A. Bullock, and G.J. De Vries (1993) Sexual differentiation of
vasopressin projections of the bed nucleus of the stria terminals and

smatic nucleus: II. Studies using retrograde transport of fluorescent
dyes and simultaneous peptide immunocytochemistry in the rat. J.

Watts, A.G., L.W. Swanson, and G. Sanchez-Watts (1987) Efferent projec-
tions of the suprachiasmatic nucleus: I. Studies using anterograde
transport of phaseolus vulgaris leucoagglutinin in the rat. J. Comp.
Neurol. 258:204–229.

of technique of immunohistochemical demonstration of bioactive sub-