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

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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 cinereum, 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 cinereum, 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. © 1995 Wiley-Liss, Inc.

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; Caffé 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

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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 mesence-

phalic 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

AA	anterior amygdala transition zone	MnPO	median preoptic nucleus
ACo	anterior cortical amygdaloid nucleus	MnR	median raphe nucleus
Acb	accumbens nucleus	mo	molecular layer in the dentate gyrus
ACg	anterior cingulate cortex	Mo5	motor trigeminal nucleus
AD	anterodorsal thalamic nucleus	MP	medial mammillary nucleus, posterior
AHi	amygdalohippocampal area	MPO	medial preoptic area
AHy	anterior hypothalamic area	MPT	medial pretectal area
Amb	ambiguous nucleus	MS	medial septum
AOn	anterior olfactory nucleus	mt	mammillothalamic tract
APir	amygdalopiriform transition	NPC	nucleus of the posterior commissure
Aq	cerebral aqueduct	opt	optic tract
Arc	arcuate nucleus	Ox	optic chiasma
AV	anteroventral thalamic nucleus	Pa	paraventricular hypothalamic nucleus
BAOT	bed nucleus of accessory olfactory tract	PAG	periaqueductal gray
B	basal nucleus of Meynert	PaMc	paraventricular hypothalamic nucleus, anterior magno-cellular
BL	basolateral amygdaloid nucleus	PaPc	paraventricular hypothalamic nucleus, anterior parvo-cellular
BM	basomedial amygdaloid nucleus	Pc	posterior commissure
BST	bed nucleus of stria terminalis	Pe	periventricular hypothalamic nucleus
BSTM	bed nucleus of stria terminalis, medial	PF	parafascicular nucleus
CA	central amygdaloid nucleus	PGi	paragigantocellular reticular nucleus
CA1, CA3	field CA1, CA3 of Ammon's horn	PH	posterior hypothalamic nucleus
CA1mo	field CA1 of Ammon's horn, molecular layer	Pir	piriform complex
CC	corpus callosum	PM	premmammillary nucleus
Cemo	cerebellum, molecular layer	PMV	premmammillary nucleus, ventral
CG	central gray	pn	pontine nucleus
CGD	central gray, dorsal	po	polymorph layer of the dentate gyrus
Cl	claustrum	Pr5	principal sensory trigeminal nucleus
CLi	caudal linear nucleus raphe	PT	paratenial thalamic nucleus
cp	cerebral peduncle, basal	PVA	paraventricular thalamus nucleus
CPu	caudate putamen	Py	pyramidal tract
CxA	cortex amygdala transition zone	Re	reuniens thalamic nucleus
Cx5	cerebral cortex, layer 5	Rf	rhinal fissure
DC	dorsal cochlear nucleus	Rh	rhomboid nucleus
DG	dentate gyrus	ROb	raphe obscurus nucleus
DMH	dorsomedial thalamic nucleus	RPn	raphe pontis nucleus
DPB	dorsal parabrachial nucleus	RRF	retrotrubral field
DR	dorsal raphe nucleus	RtT	reticulotegmental nucleus pons
DTg	dorsal tegmental nucleus	RtTG	reticulotegmental nucleus of the pons, pericentral part
dtgx	dorsal tegmental decussation	sch	suprachiasmatic nucleus
En	endopiriform nucleus	sco	subcommissural organ
f	fornix	SFi	septohippocampal nucleus
fi	fimbria hippocampus	SFO	subfornical organ
GP	globus pallidus	sg	granule cell layer of the dentate gyrus
HDB	horizontal diagonal band of Broca	sm	stria medullaris
ic	internal capsule	SMT	subthalamic nucleus, medial
ICjM	islands of Calleja, major island	SNC	substantia nigra, compacta
IMD	intermediodorsal thalamic nucleus	SNL	substantia nigra, lateral
InG	intermediate gray layer, superior colliculus	SNR	substantia nigra, reticular
IP	interpeduncular nucleus	so	stratum oriens, field CA1
IPIP	interpeduncular nucleus, inner post subnucleus	Sol	nucleus of solitary tract
IPOP	interpeduncular nucleus, outer post subnucleus	SON	supraoptic nucleus
La	lateral amygdaloid nucleus	Sp5	spinal trigeminal nucleus
LDTg	laterodorsal tegmental nucleus	st	stria terminalis
LG	lateral geniculate nucleus	Subc	subcoeruleus nucleus
LH	lateral hypothalamic area	SuG	superficial gray layer, superior colliculus
LHB	lateral habenular nucleus	SUM	supramammillary nucleus
lo	lateral olfactory tract	sumx	supramammillary decussation
LoT	nucleus lateral olfactory tract	TS	triangular septal nucleus
LoTd	nucleus lateral olfactory tract, dorsal	Tu	olfactory tubercle
LPGi	lateral paragigantocellular nucleus	VDB	ventral diagonal band of Broca
LPO	lateral preoptic area	VL	ventrolateral thalamic nucleus
LS	lateral septum	VLL	ventricular nucleus lateral lemniscus
LSD	lateral septum, dorsal	VMH	ventromedial hypothalamic nucleus
LSi	lateral septal nucleus, intermediate	VP	ventral pallidum
LSV	lateral septum, ventral	VPB	ventral parabrachial nucleus
MA	medial amygdaloid nucleus	VTA	ventral tegmental area
MG, MGN	medial geniculate nucleus	VTg	ventral tegmental nucleus
MHB	medial habenular nucleus	xscp	decussation superior cerebellar peduncle
ml	medial lemniscus	ZI	zona incerta
mlf	medial longitudinal fasciculus		
MM	medial mammillary nucleus, medial		

terminalis (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 (Riphagen 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 (Pévet et al., 1990; Pévet, 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 Veillat, 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 (9 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 (Glu) 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% H_2O_2 in PB containing 0.5% Triton X-100 (PBT).

The free-floating sections were then treated as follows: (1) overnight incubation at 4°C with rabbit VP antiserum (Truus 10-4) diluted 1:1,000 in PBT, pH 7.6, under mild agitation; (2) rinsing 3×10 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 3×10 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 3×10 minutes in PBT, pH 7.6; and (7) 15-minute development

Fig. 1. Line drawings show the distribution of VP-immunoreactive cell bodies (dots) and fibers (lines) in the brain of the jerboa (left side of the sections). The right side of the sections shows the brain structures where a sexual dimorphism (females) or a seasonal variation (sexually inactive males) was observed.

of peroxidase activity by a solution of 0.025% 3,3'-diaminobenzidine in 0.05 M Tris-NaCl containing 0.01% H_2O_2 .

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 colchicized animals with VP/OT antiserum (1:500).

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.

Figure 1 (Continued.)

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 chromalum 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 ^{125}I -labeled vasopressin antagonist [Phaa¹, D-Tyr(Et)², Arg⁶, Tyr-NH₂⁹]AVP ([^{125}I]-VPA; see Schmidt et al., 1991). Hydroxy[Thr⁴, Gly⁷]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 (Amer-

sham) 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 noncolchicized 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). $\times 45.4$. **b:** VP cell bodies are localized 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. $\times 94.5$. **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). $\times 94.5$. **d:** Parvocellular neurons (arrowheads) are seen in the bed nucleus of the stria terminalis (BSTM). Fibers are dense from the ventricular border to the most medial area of this structure. $\times 19.3$.

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. $\times 113.3$. **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. $\times 45.4$. **c:** VP neurons

localized in the median preoptic nucleus (MnPO; arrowheads) and in the periventricular areas. $\times 45.4$. **d:** A large number of VP cell bodies are seen in the medial preoptic nucleus (MPO). $\times 45.4$. **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:** $\times 45.4$; **f:** $\times 113.5$.

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 OVL (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 terminalis. 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 mediodorsal 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 supramammillary nuclei and their decussation. 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 nuclei (IP), where they extended to its inner (IPIP) and outer (IPOP) post subnuclei (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 subnucleus. 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 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 $3,583 \pm 307$ 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. 4. **a:** Frontal section of the hypothalamus shows immunopositive cell bodies in the PVN that project ventrally and dorsally to the fornix. Immunoreactive fibers are localized in the stria medullaris (arrowhead). $\times 46$. **b:** Immunopositive fibers innervate the interpeduncular complex with its inner and outer post subnuclei. $\times 86.2$. **c:** VP innervation of the medial vestibular nucleus: some fibers course in the dorsal part of the prepositus hypoglossal nucleus. MVe, medial vestibular nucleus; PrH, prepositus hypoglossal nucleus; 4v, fourth ventricle. $\times 59$. **d:** Frontal section throughout the medial nucleus of the solitary tract shows VP immunolabeling. SolM: medial nucleus of the solitary tract. $\times 63.3$. **e:** VP fibers are observed in the area postrema, the nucleus of the solitary tract, and ventrally to this nucleus in the dorsal motor nucleus of vagus. AP, area postrema; 10, dorsal motor nucleus of vagus; cc, central canal. $\times 44.8$. **f:** Persistence of VP immunolabeling in the caudal part of the dorsal motor nucleus of the vagus. Gr, gracile nucleus; dpy, pyramidal decussation. $\times 44.8$.

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 Conditions¹

Structures	SM	SF	IM	CM	IMT
Bed nucleus of the stria terminalis					
cell bodies	+++	+	+	○	+++
fibers	++++	+	+	+	+++
Median amygdaloid nucleus					
cell bodies	++	—	○	○	+
fibers	+++	+	○	○	+++
Lateral septum	++++	++	—	—	+++
Vertical diagonal band of Broca	++++	+	—	—	+++
Medial diagonal band of Broca	+++	○	○	○	+++
Horizontal diagonal band of Broca	++	+	—	—	++
Paratenial thalamic nucleus	++++	++	+	+	+++
Mediodorsal thalamic nucleus	++++	++	+	+	+++
Lateral habenula nucleus	++++	+	—	—	+++
Tuber cinereum	++	—	○	○	++
Stria medullaris	+++	+	+	+	+++
Stria terminalis	++	+	—	—	++
Supramammillary nucleus	+++	++	+	+	+++
Periaqueductal gray substance	++	+	—	—	++
Interpeduncular complex	++	+	—	—	++
Dorsal raphe nucleus	++++	++	—	—	+++
Medial raphe nucleus	++	+	+	+	++

¹Each entry of + signs represents the VP fibers' density observed in all jerboas used in different conditions: ○, 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**: $\times 103$, **d-i**: $\times 134$.

Fig. 7. Frontal section of the tuber cinereum (arrowheads) in the dorsomedial hypothalamic nucleus (DMH) shows VP immunoreactivity in a sexually active male (a), a sexually active female (b), and a sexually inactive male (c). $\times 15$.

(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 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 tubercles; 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 I]-VPA binding sites were present in the rhomboid nucleus (Fig. 10C), in the parafascicular nucleus, in the medial prefrontal area, in the nucleus of the posterior commissure, in the periaqueductal gray, in the median and lateral geniculate complexes, in the subthalamic nucleus (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 I]-VPA binding sites was observed between males and females or between the sexually active and sexually inactive animals.

The [125 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: $\times 68.9$, g-i: $\times 95.8$.

Fig. 9. Frontal sections after immunocytochemical development show VP staining in the inner (IPIP) and outer (IPOP) interpeduncular post subnuclei (**a**: sexually active males; **b**: sexually active females; **c**: sexually inactive males). $\times 46.7$. Sexual dimorphism and seasonal

variation are also observed in the dorsal raphe nucleus (DR), with a higher density of fibers in sexually active males (**d**) than in sexually active females (**e**) or in sexually inactive males (**f**). $\times 57.6$.

unlabeled VP. The use of an iodinated VP antagonist, specific for the V_1 subtype of receptors, confirms our previous claim that the hypothalamic VP receptors are of the V_1 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

Fig. 10. A-F: Overview shows [^{125}I]-VPA binding sites in the brain of the male jerboa. Autoradiograms obtained from coronal sections are arranged from the rostral (A) to the caudal (F) poles of the brain. See list for abbreviations. Scale bar = 2 mm in A-E, 1.3 mm in F.

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., 1985). 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; Ouarour, 1991; Dubois-Dauphin et al., 1994).

In the jerboa, the central structures thought to be involved in cardiovascular regulation, osmotic regulation,

Fig. 11. $[^{125}\text{I}]$ -VPA binding sites are seen in the hypothalamus (A–C) and hippocampus (D) of the male jerboa. Scale bar = 100 μm in A–C, 70 μm in D.

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 jerboa 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 jerboa 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 jerboa, 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 jerboa, 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 (Hoorneman 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 jerboa. 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 jerboa. In the jerboa, 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 jerboa 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.

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 (Mühlethaler 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 (Hösli and Hösli, 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 bioelectrical 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.

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 anterograde 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 terminalis, the paratenial and mediodorsal thalamic nuclei, the interpeduncular complex, and the dorsal and medial raphe nuclei.

Vasopressin 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 paratenial and mediodorsal 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 immunolabeling has been described in the SCN of a desert sahalian rodent, *Taterillus petteri* (Fuminier et al., 1993). In the jerboa, the densely packed VP-immunoreactive fibers observed in the paratenial and mediodorsal 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 specie, these results suggest that a part of the VP innervation of these

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.

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