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Brain Research 768 (1997) 147–156

BRAIN RESEARCH

Research report

Vasopressin in the forebrain of common marmosets (*Callithrix jacchus*): studies with in situ hybridization, immunocytochemistry and receptor autoradiography

Zuoxin Wang [a,*], Diane Toloczko [b], Larry J. Young [a], Kathleen Moody [c], John D. Newman [c], Thomas R. Insel [a,b]

[a] *Department of Psychiatry and Behavioral Sciences, Emory University School of Medicine, Atlanta, GA 30322, USA*
[b] *Yerkes Regional Primate Research Center, Emory University, Atlanta, GA 30322, USA*
[c] *Laboratory of Comparative Ethology, National Institute of Child Health and Development, NIH, Poolesville, MD 20837, USA*

Accepted 6 May 1997

Abstract

The distribution of vasopressin (AVP) producing cells, their projections and AVP receptors was examined in the brain of common marmosets (*Callithrix jacchus*) using in situ hybridization, immunocytochemistry and receptor autoradiography. Clusters of cells labeled for AVP mRNA or stained for AVP immunoreactivity (AVP-ir) were found in the paraventricular (PVN), supraoptic (SON) and suprachiasmatic nuclei (SCN) of the hypothalamus. Scattered AVP producing cells were also found in the lateral hypothalamus and the bed nucleus of the stria terminalis (BST). Neither AVP mRNA-labeled nor AVP-ir cells were detected in the amygdala. Although AVP-ir fibers were evident outside of the hypothalamic-neurohypophyseal tract, a plexus of fibers in the lateral septum, as observed in the rat brain, was not detected. Receptor autoradiography using ^{125}I-linear-AVP revealed specific binding for AVP receptors in the nucleus accumbens, diagonal band, lateral septum, the BST, SCN, PVN, amygdala, anterodorsal and ventromedial nucleus of the hypothalamus, indicating sites for central AVP action in the marmoset brain. Together, these data provide a comprehensive picture of AVP pathways in the marmoset brain, demonstrating differences from rodents in the distribution of cell bodies, fibers and receptors. © 1997 Elsevier Science B.V.

Keywords: Stria terminalis; Amygdala; Lateral septum; Hypothalamus

1. Introduction

Vasopressin (AVP) is a nonapeptide synthesized in neurons of the mammalian brain and released via the posterior pituitary into the bloodstream where it influences the maintenance of fluid homeostasis and blood pressure [7,42]. AVP is also released into target areas within the central nervous system where it acts as a neurotransmitter or neuromodulator to regulate physiological and behavioral functions [4,12,16,29,32,49]. The actions of AVP are mediated by three subtypes of membrane bound receptors, namely V_{1a}, V_{1b} and V_2 receptors, with V_{1a} predominating in the central nervous system [2,23,44].

Neural pathways for AVP differ markedly across mammalian species. For example, a dense cluster of AVP-immunoreactive (AVP-ir) fibers in the lateral septum was found in the rodent [10,48] but not in the monkey [5] or human brain [18]. In voles, the distribution of brain AVP receptors shows a species-specific pattern associated with social organization and behavior [22]. Although AVP-ir or mRNA labeling has been examined in several species of non-human primates [5,21,25,40], AVP receptor binding was only examined in a few brain areas in the rhesus monkey [37].

The common marmoset (*Callithrix jacchus*) is a New World primate characterized by group care of infants, twin births and varying degrees of monogamy [35]. In a previous study, we examined AVP- and oxytocin (OT)-immunoreactive pathways in the marmoset brain [47]. This study demonstrated that AVP cells could be detected by immunocytochemistry in several hypothalamic or extrahypothalamic nuclei, but were not evident in the medial nucleus of the amygdala — a region with AVP cells in the rat brain. Although these results suggest that AVP is not

* Corresponding author. Fax: +1 (404) 7273233; E-mail: zwang@unix.cc.emory.edu

expressed in the marmoset amygdala, such a conclusion is limited by a problem inherent with AVP immunocytochemistry: AVP-ir cells in the extrahypothalamic areas may be underestimated because these cells can be reliably visualized only by in situ hybridization or by immunocytochemistry on colchicine-treated animals [30,43].

In the present study, we first examined the distribution of AVP producing cells in the marmoset brain by in situ hybridization. To compare the distribution of AVP mRNA-labeled with AVP-ir cells, we also stained a set of brain sections using AVP immunocytochemistry. Finally, we examined the distribution of AVP receptors by using an iodinated AVP ligand (^{125}I-linear-AVP) in AVP receptor autoradiography. Our goal was to systematically map the AVP system in the marmoset brain and compare results with those of previous studies in rodents and primates to further our understanding of the evolution of the brain AVP system. A comprehensive picture of the central AVP system will provide important information for further studies of the functional significance of AVP in the common marmoset.

2. Materials and methods

2.1. Subjects

Subjects in this study were laboratory born common marmosets (*Callithrix jacchus*) from a colony in the National Institute of Child Health and Human Development, Poolesville, MD. The colony was maintained according to the NIH guidelines for animal research. Subjects lived in family groups where food and water were provided ad libitum.

2.2. In situ hybridization

Two adult female and one adult male marmosets that were 2 to 3.5 years old were anesthetized with sodium pentobarbital (60 mg per 1000 g body weight) and decapitated. Brains were removed, frozen on dry ice, and stored at −80°C until they were cut into 20-μm sections with a cryostat. The sections were thaw-mounted onto subbed and gel-coated slides and stored at −80°C until they were processed for AVP in situ hybridization according to the procedure used previously in rodents [45]. Briefly, sections were postfixed in 4% paraformaldehyde and rinsed in phosphate-buffered saline (PBS) at 4°C. Thereafter, sections were rinsed in 1.5% triethanolamine (TEA), treated in 0.25% acetic anhydride in TEA, defatted in chloroform and dehydrated in a series of graded concentrations of ethanol. 150 μl of hybridization solution which contained approximately 1×10^6 dpm of the probe was placed on each slide. Slides were covered and placed in an incubator for 14 h at 37°C. After incubation, slides were washed, dehydrated, and air-dried. The 48mer oligonucleotide probe was complementary to the rat AVP mRNA sequence coding for amino acids 129–144, which are located at the carboxyl-end of the glycopeptide region of the AVP precursor peptide. The probe was labeled at the 3′-end with [^{35}S]dATP (New England Nuclear) by using terminal deoxynucleotidyl transferase (Life Technologies), purified on a Nen-sorb column (New England Nuclear), and mixed with the hybridization buffer. The hybridization buffer contained 50% formamide, 4 × SSC, 1 × Denhardt's solution, 10% dextran sulfate, 0.3 M NaCl, 10 mM Tris, and 10 mM dithiothreitol (DTT). To locate the probe, slides were dipped in Kodak NTB-2 track emulsion (1:1 with 0.6 M ammonium acetate, pH 3.5) under safelight and stored desiccated in light-tight boxes at 4°C for 4 weeks before they were developed. AVP mRNA labeled cells were identified in dark-field illumination under a microscope. Four to five sections at 380-μm intervals for each brain area were examined for each animal. Adjacent sections from the same animals treated with a sense probe did not produce any labeling, confirming the specificity of the hybridization reaction.

2.3. Immunocytochemistry

AVP-ir staining was performed on a set of brain sections from two adult male marmosets (4 years old). Alternate brain sections from those animals were previously used for oxytocin immunoreactive staining [47]. The animals were anaesthetized with sodium pentobarbital and perfused through the ascending aorta with 0.9% saline, followed by 5% acrolein in 0.1 M phosphate buffer (PBS), pH 7.6. Transverse brain sections (50 μm) were cut with a microtome. Floating sections were rinsed in 0.05 M Tris-HCl containing 0.9% NaCl (Tris-NaCl, pH 7.6), incubated 10 min in Tris-NaCl with 0.5% Triton (Tris-Triton) and 20% goat serum and 90 min in rabbit-anti-AVP serum (ICN, Lisle, IL) 1:4000 in Tris-Triton containing 2% goat serum (Tritrigo) at 37°C. The sections were then incubated in biotinylated goat-anti-rabbit 1:300 in Tritrigo at room temperature for 60 min, and in ABC complex for 60 min. Floating sections were stained with 0.05% 3,3′-diaminobenzidine (DAB) with 0.003% H_2O_2. Sections were mounted on slides, air dried and coverslipped. AVP-ir cells or fibers in each brain area were examined on 4–5 sections at 150-μm intervals for each animal. Specificity control of antiserum included staining sections with anti-AVP serum that was pretreated with 50 μM AVP which eliminated specific staining.

2.4. Receptor autoradiography

An alternate set of brain sections from the animals used for AVP mRNA labeling was processed for AVP receptor binding. Sections were thawed at room temperature, preincubated in 50 mM Tris-HCl buffer (pH 7.4) for 5 min × 2, and then placed in the incubation buffer consisting of 50 mM Tris-HCl (pH 7.4) with 10 mM $MgCl_2$, 0.1% BSA,

0.05% bacitracin, and 50 pM tracer for 60 min at room temperature. The tracer was ^{125}I-linear-AVP ligand (Phenylacetyl–dTyr(Me)–Phe–Gln–Asn–Arg–Pro–Arg–Tyr–NH_2; New England Nuclear-Dupont, Boston, MA) that was previously characterized to have a high affinity and selectivity for V_{1a} receptors in rodents [24,33]. Following incubation, sections were lightly fixed in 0.1% paraformaldehyde, washed in 50 mM Tris-HCl with 10 mM $MgCl_2$ for 5 min $\times$ 4 at 4°C and 30 min in room temperature with stirring. After being dipped in water, sections were dried immediately under a stream of cool air and then exposed to a BioMax MR film (Kodak) for 3 days. In order to define non-specific binding, adjacent sections from each subject were incubated in buffer with 50 μM of the selective V_{1a} ligand d(CH_2)$_5$[Tyr(Me)]AVP. In addition, to characterize the specificity of ^{125}I-linear-AVP in marmosets, in some brain sections, 50 μM selective V_{1a} ligand d(CH_2)$_5$[Tyr(Me)]AVP, V_2 ligand [d(CH_2)$_5$,D-Phe^2,Ile^4,Ala^9-NH_2]-AVP, or selective OT ligand [Thr^{4}Gly^7]OT were used as competitors. Brightfield autoradiograms were compared to plates in Stephan et al. [34] for anatomic designations.

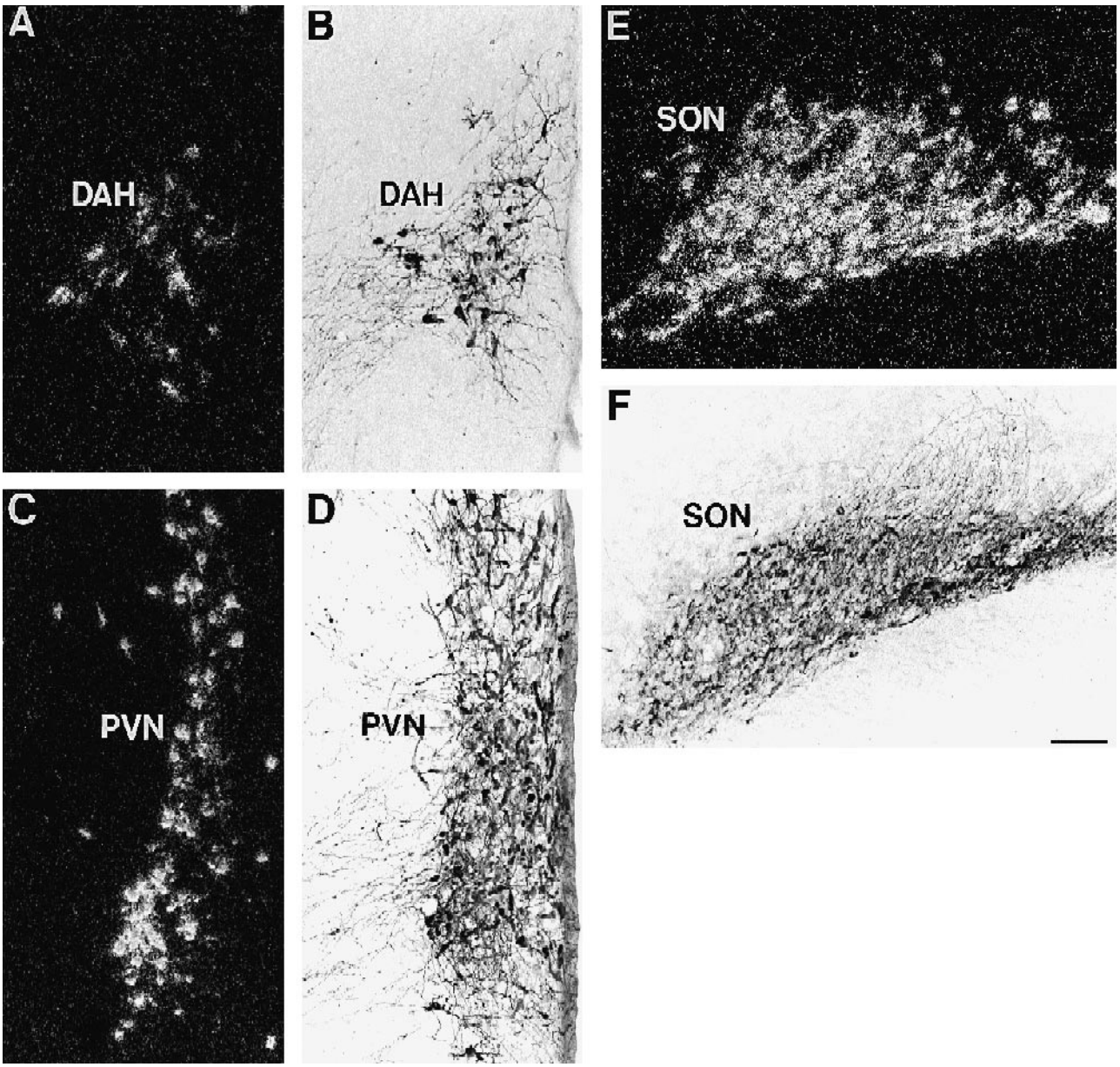


Fig. 1. Photomicrographs displaying AVP mRNA-labeled (dark-field illumination) or AVP-ir cells (bright-field illumination) in the area of anterodorsal (DAH; A and B), paraventricular (PVN; C and D) and supraoptic nuclei (SON; E and F) of the hypothalamus of a common marmoset. Scale bar = 100 μm.

3. Results

3.1. AVP producing cells

A dense cluster of AVP mRNA-labeled or AVP-ir cells was found in the paraventricular (PVN; Fig. 1C and D) and supraoptic nuclei (SON; Fig. 1E and F) of the hypothalamus. A moderate number of AVP producing cells was found in the dorsal anterior hypothalamus, especially in the rostralmost part dorsal to the preoptic periventricular nucleus (DAH; Fig. 1A and B). A few AVP cells were also found in the suprachiasmatic nucleus of the hypothalamus (SCN). In addition, scattered AVP mRNA-labeled or AVP-ir cells were found in the bed nucleus of the stria terminalis (BST; Fig. 2A and B) and in the area of lateral hypothalamus which is lateral to the PVN and dorsal to the optic tract and amygdala (ALH; Fig. 2C and D). In all brain regions, the in situ hybridization and immunocytochemistry resulted in matched distribution patterns of AVP cells. No AVP cells were found in any aspects of the amygdala by either technique.

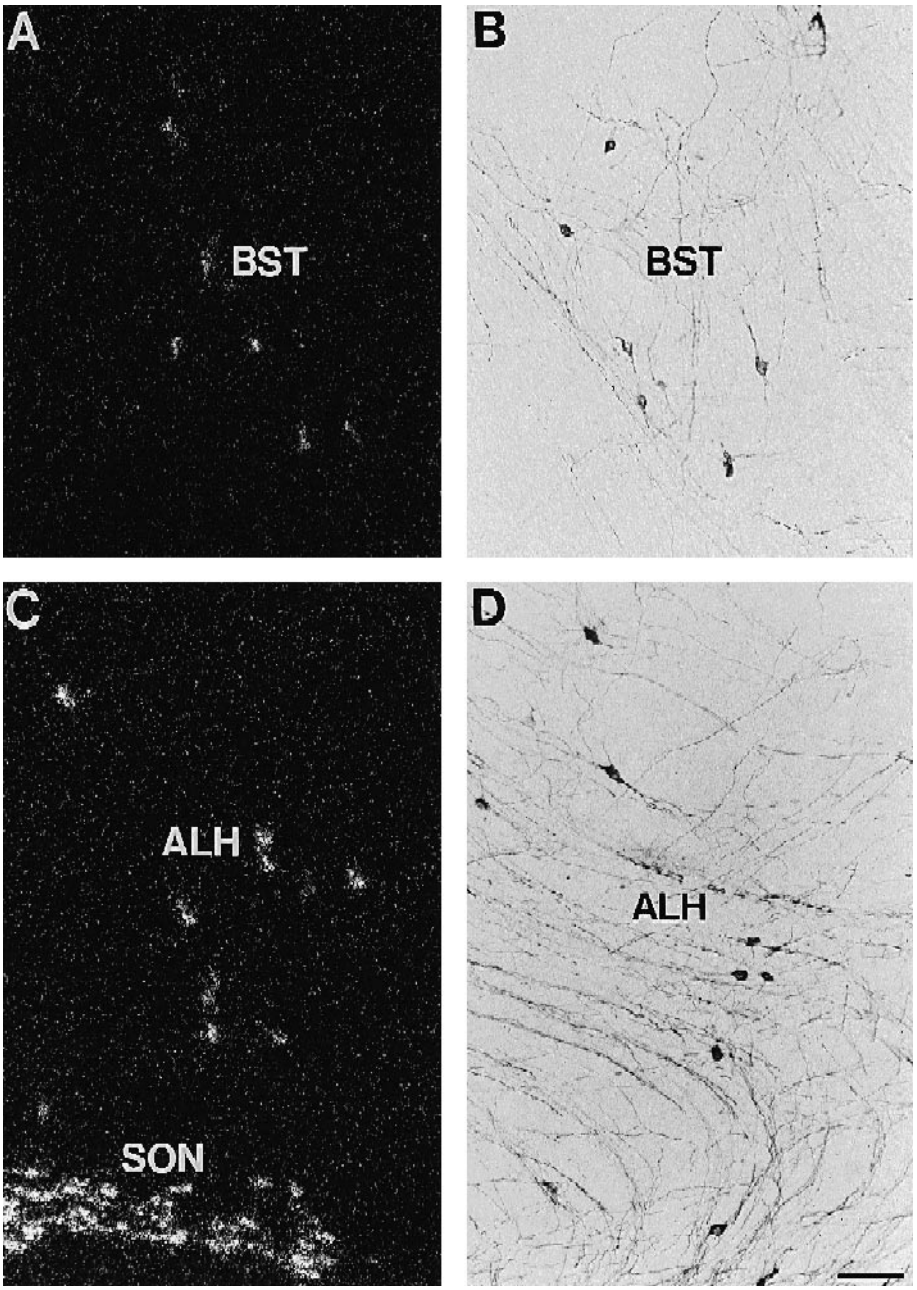


Fig. 2. Photomicrographs displaying AVP mRNA-labeled (dark-field illumination) or AVP-ir cells (bright-field illumination) in the bed nucleus of the stria terminalis (BST; A and B) and the area of lateral hypothalamus (ALH) of a common marmoset. SON, supraoptic nucleus of the hypothalamus. Scale bar = 100 μm.

3.2. AVP-ir fibers

Heavily stained AVP-ir fibers formed a paraventriculo-supraoptico-neurohypophyseal tract, as found in a previous study [47]. In addition, AVP-ir fibers from the PVN projected ventrolaterally to the SON and dorsally to the BST. AVP-ir fibers were also found between the SON and the lateral hypothalamus. Scattered AVP-ir fibers were found in the BST (Fig. 3A), septum (Fig. 3B), nucleus accumbens (AC; Fig. 3C), and diagonal band (DB; Fig. 3D). In the septum, a few AVP-ir fibers was found in the lateral area but these fibers did not form a dense plexus as found in rodents. In the medial septum, scattered AVP-ir fibers extended ventrally to the DB. No AVP-ir fibers were detected in any aspects of the amygdala.

3.3. AVP receptor binding

^{125}I-linear-AVP specific binding was found in several areas in the marmoset brain (Fig. 4). Intense binding was found in the lateral septum predominantly in the ventral part, and in the BST, extending ventrally to the dorsal hypothalamus (DAH), and to some extent, to the PVN. Intense binding was also detected in the SCN and the stria longitudinalis (SL). Moderate binding was found in the substantia innominata (SI), diagonal band (DB), lateral hypothalamus (ALH), and ventromedial nucleus of the hypothalamus (VMH) extending ventrally to the infundibulum. In the amygdala, moderate binding was found in the medial (MA), central (CE) and cortical nucleus (CA). Weak but consistent binding was found in the fibrae

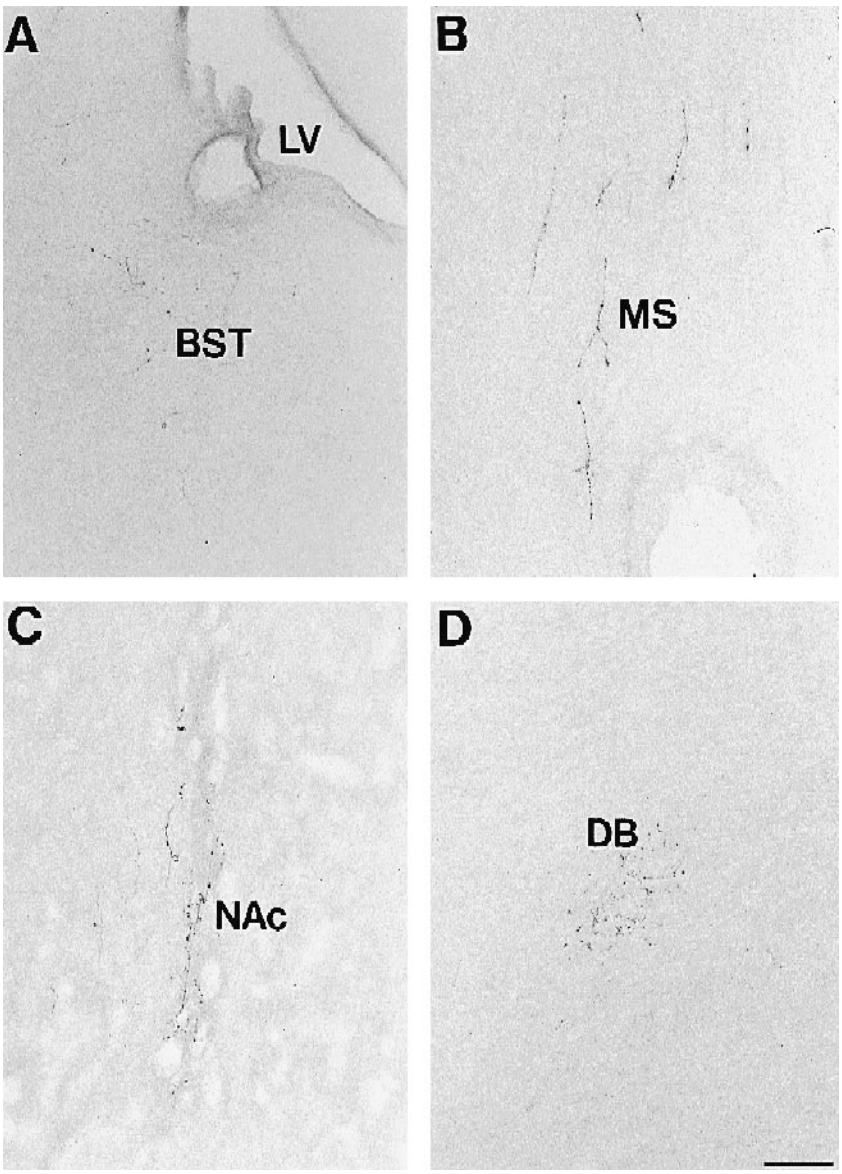
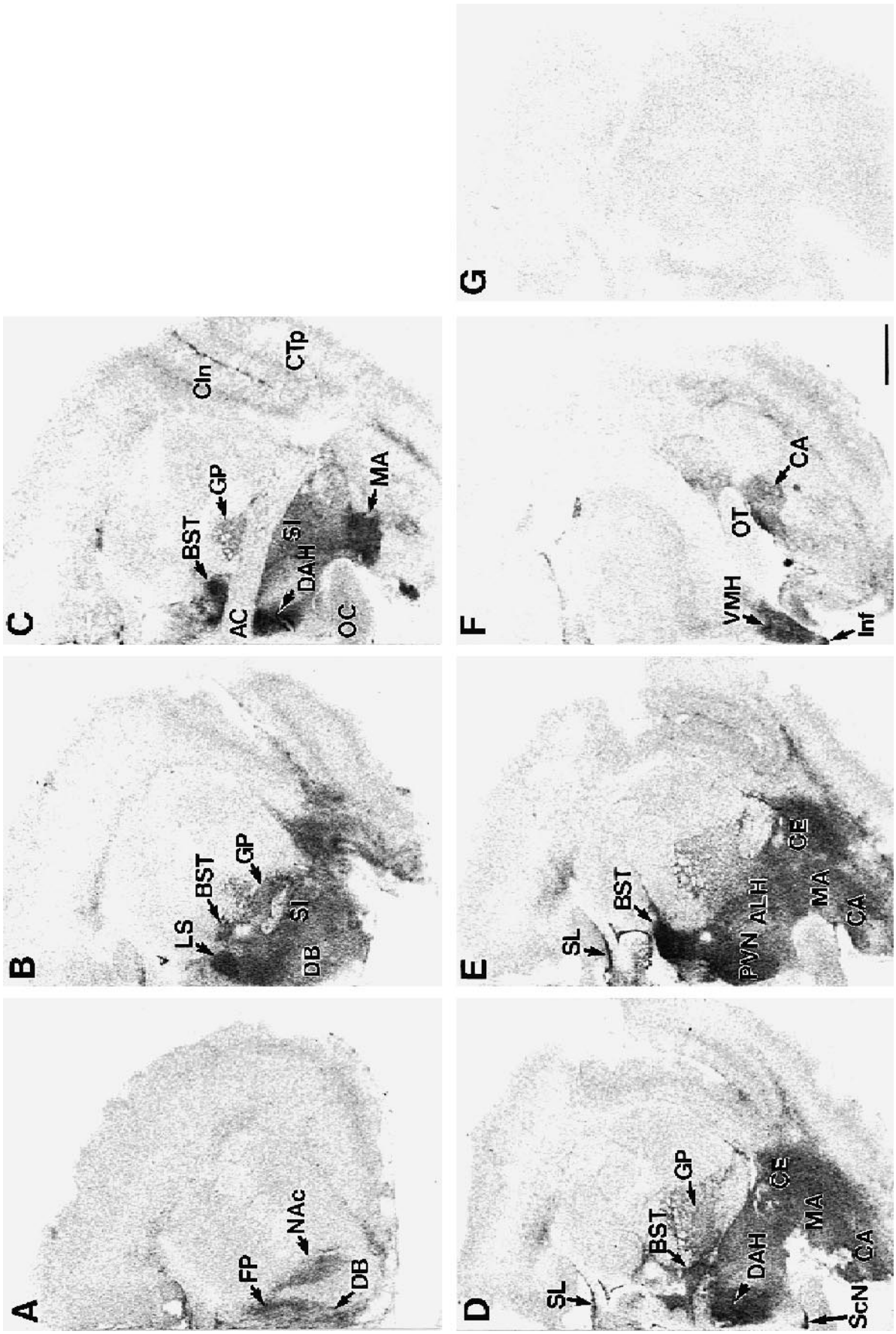


Fig. 3. Photomicrographs displaying AVP-ir fibers in the bed nucleus of the stria terminalis (BST; A), the medial septum (MS; B), nucleus accumbens (NAc; C), and diagonal band (DB; D) of a common marmoset. LV, lateral ventricle. Scale bar = 100 μm.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praecommissurales fornicis (FP), nucleus accumbens (NAc) and globus pallidus (GP). Weak binding was also found in the cortex, especially in the insular (CIn) and temporal (CTp) cortex. The binding by ^{125}I-linear-AVP was displaced by the V_{1a} antagonist (Fig. 4G) but not by the V_2 or the OT ligand, suggesting that ^{125}I-linear-AVP bound selectively to V_{1a} receptors in the marmoset brain.

4. Discussion

In the current study using in situ hybridization, immunocytochemistry and receptor autoradiography, we mapped the distribution of AVP both pre- and postsynaptically in the brain of the marmoset. The distribution of AVP cells in the PVN, SON and SCN was similar to the pattern found in a variety of mammalian species such as rodents [1,3,11,20] monkeys [5,21,25] and humans [13,28], supporting the assumption that hypothalamic AVP is highly conserved among mammalian species. AVP producing cells in the DAH in the present study were located in a region probably homologous to the anterior hypothalamus of the monkey [5] and the medial preoptic area of the rodent brain [1]. Our data indicate at least three differences in the pattern of extrahypothalamic AVP cells and fibers in the marmoset in comparison to rodents.

First, AVP cells were not detected in any aspect of the amygdala in the marmoset brain. In rodents, AVP mRNA-labeled or AVP-ir cells are found in the medial nucleus of the amygdala, and these cells project into the forebrain areas such as the lateral septum [6,10,45]. Although an early study reported AVP-ir cells in the medial amygdala in the macaque monkey [5], the number of these cells were far less compared to rats, which may coincide with the absence of AVP cells in the human amygdala [18]. Failure to detect AVP cells in the amygdala by in situ hybridization in the present study confirms our early finding that AVP cells are absent in the marmoset amygdala [47].

Secondly, a plexus of AVP-ir fibers was not found in the lateral septum in the marmoset. In rats, the lateral septum receives projections from AVP cells in the medial amygdala and the BST [2,6,10]. These AVP-ir projections form a plexus with a higher density in males than in females [11]. Although such a septal AVP-ir fiber plexus has been detected in several species of rodents [3,8,20,48], it has not been found in primates [5,18]. In the marmoset, lack of AVP-ir fiber plexus in the lateral septum might at least partially result from the absence of AVP cells in the amygdala. In addition, AVP cells in the BST in the marmoset may project into other brain areas. Septal AVP is found to regulate male parental care in a monogamous rodent [46]. The marmoset is also monogamous displaying bi-parental care of infants [35]. Lack of AVP-ir fibers in the septum may indicate that different mechanisms are involved in regulating male parental care in marmosets.

The third difference between the marmoset and rodents was noticed on the immuno-visualization of AVP cells in the BST. In rats, these cells usually cannot be reliably visualized by immunocytochemistry unless animals are treated with colchicine [20]. In the marmoset, however, the number of AVP-ir cells in the BST in the present and a previous study [47] was similar to the number of AVP mRNA-labeled cells, suggesting that virtually all AVP cells in the BST were immunostained although the marmoset was not treated with colchicine. This discrepancy between rats and marmosets can only be hypothesized. It may reflect species differences in the speed or efficiency of AVP transport or precursor processing in the BST cells. Alternately, AVP cells in the BST in the marmoset may be large neurons which do not require colchicine treatment for staining [17]. In fact, there is no apparent distinction between magnocellular and parvocellular AVP neurons in the hypothalamic and extrahypothalamic areas in the marmoset [47]. In addition, the number of AVP-ir cells in the BST was not significantly increased by colchicine treatment in hamsters that possess large AVP cells in the BST [17].

Although there is a large body of research characterizing AVP receptors in rodents (see review by Barberis and Tribollet [2]), among non-human primates, AVP receptors were examined only in a few brain areas of the rhesus monkey [37]. In the present study, we mapped AVP receptor binding in the marmoset brain. The ^{125}I-linear-AVP binding was displaced by the V_{1a} antagonist but not the V_2 or the oxytocin antagonist, suggesting that ^{125}I-linear-AVP bound specifically to V_{1a} receptors in the marmoset brain, as it does in rodents [24,33]. Intense binding was detected in the septum, BST, DAH, PVN and SCN whereas moderate or weak binding was found in the DB, amygdala, ALH, VMH, NAc and GP. These data indicate target sites in the brain on which AVP may act to influence physiological and/or behavioral functions in the marmoset.

Fig. 4. Photomicrographs displaying ^{125}I-linear-AVP binding in marmoset brain sections from rostral (A) to caudal (F). Intense binding was found in the lateral septum (LS), stria longitudinal (SL), the bed nucleus of the stria terminalis (BST), dorsal (DAH), paraventricular (PVN) and suprachiasmatic nucleus (SCN) of the hypothalamus. Moderate binding was found in the diagonal band (DB), substantia innominata (SI), lateral (ALH) and ventromedial (VMH) hypothalamus, as well as in the medial (MA), central (CE) and cortical nucleus (CA) of the amygdala. Weak binding was found in the fibrae praecommissurales fornicis (FP), nucleus accumbens (NAc), globus pallidus (GP), insular (CIn) and temporal (CTp) cortex. Panel G shows a brain section that was adjacent to the section in Panel E, and was incubated with ^{125}I-linear-AVP ligand with 1 μM V_{1a} antagonist, which eliminated specific binding. AC, anterior commissure; Inf, infundibulum; OC, optic chiasma; OT, optic tract. Scale bar = 2 mm.

Table 1
Vasopressin receptor binding in selected brain areas in rodents and primates

	NAc	DB	LS	BST	SCN	PVN	VMH	VLH	AMYG	DG	Ref.
Rat	+ [a]		+	+	+	+	+		+	+	[31,39]
Gerbil		+	+	+					+	+	[41]
Golden hamster			+	+				+	+		[9,36]
Siberian hamster			+	+		+	+		+		[15]
Prairie vole	+	+	+	+			+	+	+	+	[22]
California mouse	+		+	+			+		+	+	[b]
Marmoset	+	+	+	+	+	+	+		+		Current study
Rhesus monkey			+	+	+				+	+	[37]
Human		+	+	+					+	+	[27]

AMYG, amygdala; BST, bed nucleus of the stria terminalis; DB, diagonal band; DG, dentate gyrus; LS, lateral septum; NAc, nucleus accumbens; PVN, paraventricular nucleus of the hypothalamus; SCN, suprachiasmatic nucleus of the hypothalamus; VLH, ventrolateral hypothalamus; VMH, ventromedial hypothalamus.
[a] Brain regions in which AVP receptor binding was detected.
[b] Wang and Gubernick, unpublished data.

The distribution of AVP receptor binding in the marmoset brain shows similarities as well as differences compared to that of rodents and other primates (Table 1). The binding in the lateral septum, the BST and amygdala, although not necessarily in the same subdivisions or with the same density, is consistent with that found in rodents and other primates including humans. These data suggest that AVP actions on these targeting sites are conserved among mammalian species. In other brain areas, however, presence of AVP receptors show remarkable species differences not only between primates and rodents but also among rodent species. Such species differences may indicate an adaptation or reorganization of brain AVP receptors favoring species-specific physiological/behavioral functions.

The analysis of AVP producing cells, fibers and binding sites in a single study provides an opportunity to investigate the spatial relationship between pre- and postsynaptic AVP pathways. In the present study, although most of the brain regions which revealed AVP receptor binding also contained AVP cells and/or fibers, mismatches between receptors and endogenous AVP indeed existed. For example, moderate AVP receptor binding was found in various regions of the amygdala where no AVP cells or fibers were detected in the marmoset brain. These data provide further evidence to support the assumption that the presence of AVP receptor sites is independent of the presence of AVP itself. Similar mismatches between endogenous AVP and receptors in the brain are also found in rats [38], hamsters [14] and humans [27], and several explanations have been proposed [19,26].

Central AVP has been implicated in several aspects of social interaction such as memory, territory marking, partner preference, parental behavior and aggression especially in highly social animals [12,16,46,49]. Common marmosets live in groups in which social interaction plays an important role in maintenance of their social hierarchy and in their reproductive success [35]. We know little of the significance of central AVP for the social structure and behavior of marmosets, but the present study provides a comprehensive picture of the central AVP system for pursuing such functional studies.

Acknowledgements

We thank Mr. Frank Kiernan for his professional assistance in preparing photomicrographs. This research was partially supported by NIMH 54554 to Z.X.W. and 56538 to T.R.I..

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