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STUDIES ON THE CENTRALLY-MEDIATED MOTOR EFFECTS OF ARGININE VASOPRESSIN

by

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ABSTRACT

Arginine vasopressin (AVP) induces motor effects when administered into the cerebral ventricles, the ventral septal area (VSA), or the vestibular cerebellum of the rat brain. These motor effects range from minor changes in motor behavior such as stereotyped grooming behaviors, often combined with lengthy periods of immobility and staring, to severe changes in motor behavior including barrel rotation and myoclonic/myotonic convulsions. These motor effects probably result from an interaction of AVP with the V_1-type of AVP receptor. They involve a sensitization process whereby an initial exposure to AVP increases the likelihood of severe motor effects upon subsequent AVP exposure. These and other data suggest a possible neurotransmitter role for AVP in motor behavior. However, the neuroanatomical substrate and the physiological and pharmacological mechanisms through which AVP might play such a role have not yet been elucidated.

Since AVP-like immunoreactivity and AVP-binding sites exist in the amygdala, and since the amygdala is reciprocally connected with the VSA and can be kindled to produce motor effects, it was suggested that the medial amygdala might play a role in AVP-induced motor effects. This hypothesis was tested by examining whether AVP would produce motor effects following injection into the medial amygdaloid nucleus (meA).

An initial injection into the meA caused minor motor effects. Subsequent injections, 24 hours later, caused severe motor effects including barrel rotations and myoclonic/myotonic convulsions. These results suggest that the meA is a sensitive site for AVP-induced motor effects and that these motor effects are sensitized by prior exposure to AVP.

A potential receptor basis for the AVP-induced motor and sensitization effects in the meA was investigated using AVP analogues. These analogues were directed against V_1 and V_2 AVP receptor subtypes. A potent and selective V_1 antagonist, $d(CH_2)_5$Tyr(Me)AVP, blocked both the motor and sensitization effects produced by meA AVP injection. Conversely, a potent and selective V_2 receptor agonist, DDAVP, did not affect motor activity upon meA injection. DDAVP did, however, sensitize animals to subsequent meA AVP injection. These results suggest that the motor effects observed after meA AVP injection are mediated via AVP receptors that resemble the V_1 type while the sensitization effect may be mediated via both V_1 and V_2 receptor subtypes.

In conclusion, this work supports the hypothesis that AVP may play a neurotransmitter role in motor behavior, and suggests that the meA may function as a CNS site for the mediation of such behavior.

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DEDICATION

To my family and to Karin who deserve special praise for their love and support.

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LIST OF ABBREVIATIONS

ANOVA	Analysis of variance
AVP	Arginine vasopressin
BST	Bed nucleus of the stria terminalis
CNS	Central nervous system
DBB	Diagonal band of Broca
DDAVP	1-desamino-8-D-arginine vasopressin
EEG	Electroencephalogram
g	Gram
h	Hour
icv	Intracerebroventricular
IP	Inositol phosphate
ip	Intraperitoneal
kg	Kilogram
LCV	Lateral cerebral ventricle
M	Molar
meA	Medial amygdaloid nucleus
mg	Milligram
min	Minute

ml	Millilitre
mm	Millimetre
ng	Nanogram
pmol	Picomole
POAH	Preoptic/anterior hypothalamic area
PVN	Paraventricular nucleus
s	Second
SCN	Suprachiasmatic nucleus
VSA	Ventral septal area
μg	Microgram
μl	Microlitre
μM	Micromolar

DEFINITIONS

Motor effects - A group of behavioral responses caused by injection of arginine vasopressin into the brain (ventricles or sensitive tissue loci). These may include one or more of the following (in order of severity):

Severity	Score	Behavior
Minor	0	Grooming
"	1	Pauses (immobility)
"	2	Prostration
"	3	Circling
"	3	Locomotor difficulties
Severe	4	Barrel Rotations
"	5	Convulsions
"	6	Death

Sensitization effect - A process whereby an initial injection of AVP causes minor motor effects such as grooming or pauses, whereas a subsequent injection 6 h to 6 days later causes severe motor effects such as barrel rotations or convulsions (Kasting et al., 1980; Poulin and Pittman, 1991, unpublished results).

V_1 receptor - Refers to a particular type of receptor for arginine vasopressin. Also called the vasopressor or simply "pressor" receptor because it mediates the effects of AVP on vascular smooth muscle (contraction) and on hepatocytes (glycogenolysis). Most CNS AVP binding sites resemble this type of receptor (for review see van Leeuwen, 1987).

V_2 receptor - Refers to a particular type of receptor for arginine vasopressin. Mediates changes in water permeability in the luminal membrane of distal portions of the nephron. Some CNS AVP binding sites resemble this type of receptor.

CHAPTER 1

INTRODUCTION

BACKGROUND

Arginine vasopressin (AVP) is a well known neuropeptide that is synthesized chiefly in two hypothalamic nuclei, the supraoptic nucleus (SON) and the paraventricular nucleus (PVN), and released into the systemic circulation in the neural lobe of the pituitary gland. Since AVP is released into the peripheral circulation and affects distant target organs it fits the classical definition of a hormone, and it has long been recognized as such (for review see Morris et al., 1987). However, in recent years, largely stimulated through the work of De Wied (1965, 1971), evidence for a neurotransmitter function in the central nervous system (CNS) has grown.

In fact, AVP has met most, if not all, of the generally accepted criteria for recognition as a CNS neurotransmitter (for review see Doris, 1984; Riphagen and Pittman, 1986). Establishing a physiological role for a neuropeptide involves several of these criteria (Kasting, 1989). These include an anatomical substrate that might mediate neurotransmission (i.e. AVP-containing somata and axonal

pathways), physiological mechanisms of action (i.e. endogenous AVP release under physiologically relevant conditions), pharmacological mechanisms of action (i.e. AVP receptors), and measurable physiological effects.

In fulfillment of these criteria, immunocytochemical studies have provided strong evidence for the presence of AVP-containing nuclei in several brain loci, including the suprachiasmatic nucleus of the hypothalamus (SCN), the bed nucleus of the stria terminalis (BST) and the medial amygdaloid nucleus (meA) (van Leeuwen et al., 1978; Baskin et al., 1983; Dorsa et al., 1983, 1984). Retrograde tracing studies combined with immunocytochemistry suggest that AVP-containing fibers from nuclei such as the BST reach diverse areas, such as the ventral septal area (VSA) and the diagonal band of Broca (DBB) (De Vries and Buijs, 1983; van Leeuwen and Caffé, 1983). Electrophysiological studies suggest that synaptic release of AVP may involve calcium-dependent potassium depolarization (Buijs and van Heerikhuze, 1982). Finally, push-pull perfusion studies have provided evidence that endogenous release may occur in areas which contain putative AVP receptors, such as the VSA (Cooper et al., 1979; Landgraf et al., 1990).

Despite strong evidence for a vasopressinergic role in CNS neurotransmission, the functional significance of this neuropeptide is unclear. AVP appears to, among other actions, facilitate memory (De Wied, 1976; Gash and Thomas, 1983), influence blood pressure (Versteeg et al., 1982; Matsuguchi

et al., 1982; Pittman et al., 1982), induce antipyresis (Kasting et al., 1978, 1979; Banet and Wieland, 1985) and cause motor effects (for review see Gash et al., 1987). These effects range from stereotyped behavioral responses such as grooming (Meisenberg, 1981; Ferris et al., 1984) to severe disturbances of motor behavior such as convulsive activity (Kasting et al., 1980; Wurpel et al., 1986). It is the ability of AVP to induce such motor effects in the amygdala that will be explored by this work.

A. BEHAVIORAL EFFECTS INDUCED BY AVP

1) Autonomic Effects

DeWied (1965) was among the first to demonstrate behavioral actions mediated by AVP with his finding that icv administration of pituitary extracts potentiates avoidance behavior in rats. Since then, AVP has been implicated in a number of autonomic behaviors (see Table 1). These effects include nociceptive, analgesic, (Warren and Gash, 1983; Kordower and Bodnar, 1984, 1985) and antipyretic effects (Kasting et al., 1978; Cooper et al., 1979). Vasopressin may influence several other autonomic behaviors as well, including sleep (Danquir, 1983), sexual behavior (Södersten et al., 1983, 1985) and feeding behavior (Aravich and Sladek, 1986).

2) Motor Effects

Arginine vasopressin elicits a variety of motor behaviors upon CNS injection. For example, stereotyped behaviors such as grooming (Meisenberg, 1981; Ferris et al., 1984), escape-like locomotion (Meisenberg, 1981), and prostration (Kasting et al., 1980) have been observed (see Table 1).

AVP can also produce an unusual pattern of spontaneous motor activity characterized by lengthy pauses (immobility), transient ataxia (loss of fine motor control), head tilt, nystagmus and body sway. This motor activity may progress to barrel rotation (repeated rolling about the body's longitudinal axis). In addition, some animals exhibit convulsive behavior, characterized by myoclonus (intermittent muscular contraction) and/or myotonus (sustained muscular contraction). These convulsions may be accompanied by dyspnea, apnea and death, often associated with pulmonary edema.

Thus, motor effects induced by exogenous application of AVP (usually icv) range in severity from normal stereotyped behaviors such as grooming, to relatively minor motor abnormalities such as ataxia, to more severe disturbances of motor behavior such as barrel rotation and myoclonic/myotonic convulsions. Furthermore, this activity involves a sensitization process, such that subsequent exposures to AVP will be more likely to cause severe motor effects than an initial exposure.

Table 1

Behavioral Effects of Arginine Vasopressin (AVP)

Behavior	Description
Avoidance behavior	
Active avoidance	Acquisition
	Resistance to extinction
Passive avoidance	Consolidation
	Retrieval
Experimental amnesia	Prevention
	Reversal
Motor behavior	
Ataxia	Locomotor difficulties
Barrel rotation	Rolling along body's long axis
Circling	
Convulsion	Myoclonus and/or myotonus
Escape efforts	
Grooming	Saliva spreading
Immobility	
Prostration	
Staring	
Rewarded behavior	
Food reward	(Problem solving)
Sexual reward	(T-maze)
Sweet preference	(Maze performance)
Thermoregulation	
Antipyresis	
Cardiovascular regulation	
Centrally-evoked pressor response	
Maternal behavior	Facilitation
Other CNS effects	
Analgesia	
Nociceptive effects	
Sexual behavior	

(Adapted from van Wimersma Greidanus, 1984)

Several peptides produce motor effects similar to those seen with AVP, when injected icv, or into discrete brain regions. Some of these peptides [and threshold doses] include somatostatin [10-20 μg] (Cohn and Cohn, 1975), substance P [40 μg] (Rondeau et al., 1978), oxytocin [1 μg] (Kruse et al., 1977), and enkephalin [10 μg] (Frenk et al., 1978). Arginine vasopressin is one of the most potent peptides for inducing severe motor effects, often a thousand times more potent than other peptides (Kasting et al., 1980).

Kasting et al. (1980) were the first to demonstrate that AVP-induced motor effects involve a sensitization process, whereby initial administration of AVP induces minor behavioral effects such as immobility, whereas subsequent administration of AVP two days later, in doses as small as 9.2 pmol (10 ng) causes severe motor effects, such as convulsions. These doses can be compared physiologically with the 200-600 ng of AVP reputedly stored in the pituitary gland (Burnard, 1985b), CSF levels of 1.25-15 pg/ml (Jenkins et al., 1980; Reppert et al., 1983), amygdalar levels of 17.3 pg/mg (Epstein et al., 1983), and interestingly, plasma levels as high as 1.2 ng/ml under conditions of acute stress (Husain et al., 1979).

In general, little is known about the mechanisms of peptide-induced motor effects. However, two of these peptides, AVP (Kasting et al., 1980) and the endogenous opiates (β-endorphin and met-enkephalin)(Urca et al., 1977; Cain and

Corcoran, 1984) may involve a sensitization process. However, only AVP sensitization has been investigated in some detail. The mechanism of AVP-induced motor effects will be explored in Section E.

2) Sensitization Effect

The potency of AVP to induce motor effects is drastically increased (more than a hundred fold) by prior exposure to a single AVP injection (Kasting et al., 1980; Burnard et al., 1983; Burnard, 1985b; Naylor et al., 1985). This has been called sensitization (Kasting et al., 1980). This sensitization is dose dependent as a greater sensitization is seen if higher sensitizing doses are employed. It can be observed about 6 hours after an initial injection and lasts approximately six days, following a single injection. Sensitization can also be observed after multiple, intermittent AVP injections, however, the shorter the interinjection period the less likely sensitization will occur (Poulin and Pittman, 1991, unpublished results). The mechanism of this sensitization will be explored in Section E.

B. PHYSIOLOGICAL SIGNIFICANCE OF THE MOTOR EFFECTS INDUCED BY AVP

The physiological significance of AVP-induced motor effects is unclear, however, several functions have been suggested. These functions include the possibility that AVP induces thermoregulatory behavior (i.e. behaviors that reduce heat gain and increase heat loss) by stimulating behaviors such as grooming, prostration and escape-like locomotion (Willcox et al., in press, 1992). Severe AVP-induced motor effects are viewed as a malfunction of the behavioral thermoregulatory system.

Another hypothesis suggests that AVP-induced motor effects form part of a behavioral repertoire used to maintain balance and posture (i.e. AVP helps maintain vestibular system integrity) (Balaban et al., 1989). Severe AVP-induced motor effects are viewed as disturbances of the vestibular system (i.e. a loss of motor coordination and postural control).

1) Behavioral Thermoregulation

Considerable evidence has accumulated suggesting that arginine vasopressin may act as an endogenous antipyretic in the brain (for review see Pittman et al., 1988). The mechanisms(s) by which AVP induces antipyresis are unknown, but the reduction of febrile body temperature towards prefebrile levels involves

multiple components (for review see Cooper, 1987). These include efferent pathways for increased heat loss and reduced heat production, including thermoregulatory behaviors, such as grooming, prostration and escape-like locomotion (Hainsworth, 1969; Roberts and Robinson, 1969; Roberts and Mooney, 1974). Since AVP induces both antipyresis (Kasting et al., 1978; Cooper et al., 1979; Kovacs and De Wied, 1983) and thermoregulatory behaviors such as grooming, escape-like locomotion (Meisenberg, 1981; Ferris et al., 1984), and prostration (Kasting et al., 1980) when injected into a lateral cerebral ventricle of the rat brain, this raises the possibility that AVP may reduce febrile body temperature, at least in part, by activating thermoregulatory behaviors.

This hypothesis is particularly attractive because the ventral septal area (an area extending from the diagonal bands of Broca to the anterior hypothalamus, of the rat basal forebrain), provides a possible tissue locus for these effects. AVP has been found to induce both antipyresis (Cooper et al., 1979; Ruwe et al., 1985) and motor behaviors (Naylor et al., 1985) in the VSA. Thus, there exists the possibility that AVP-induced motor effects may, at least in part, reflect activation of behavioral thermoregulatory responses.

Observations that AVP induces both putative antipyretic and convulsive activity resulted in the hypothesis that AVP may be involved in the aetiology or mediation of febrile convulsions (Kasting et al., 1981; Veale et al., 1984). These

convulsions might be conceptualized as a severe malfunction of the behavioral thermoregulatory system. Support for this hypothesis comes from several studies employing the hyperthermia-induced convulsions model of febrile convulsions. This model studies convulsions obtained through the elevation of body temperature of young or developing animals by external means. Evidence suggesting a role for AVP comes from several sources. For example, exposure to high ambient temperatures leads to both elevated plasma levels of AVP (Forsling et al., 1976) and convulsive activity (Burnard et al., 1986a). As well, higher levels of AVP have been found in the plasma of hyperthermic convulsing Long Evans rats than in hyperthermic non-convulsing Long Evans rats (Kasting et al., 1981).

Studies that have employed homozygous Brattleboro rats, which are AVP deficient (Valtin et al., 1965), also support the involvement of AVP in hyperthermia-induced convulsions. For example, Brattleboro rats either fail to convulse or convulse at much higher body temperatures than their "AVP normal" cousins, the Long Evans rat. Furthermore, AVP antiserum-treated Long Evans rats also fail to convulse, or convulse at much higher temperatures, than their untreated counterparts (Kasting et al., 1981), suggesting a lack of AVP raises the threshold for hyperthermia-induced convulsions. Moreover, body temperature is seen to drop during febrile convulsions which is similar to the effect of icv administered AVP (Kasting et al., 1980).

Interestingly, hyperthermia-induced convulsive activity might undergo a sensitization process similar to that observed upon repeated AVP exposure. A study by Klauenberg and Sparber (1984) showed that repeated immersion of rats in warm water (45°C) at 4-day intervals led to higher percentages of rats convulsing upon subsequent immersion.

Practical support for the AVP/febrile convulsions hypothesis comes from studies demonstrating that stimuli evoking endogenous release of AVP (e.g. hemorrhage or osmotic stimuli) can sensitize the brain to the convulsive effects of exogenously applied AVP (Burnard et al., 1983). In addition, a V_1 (vasopressor) AVP receptor antagonist ($d(CH_2)_5Tyr(Me)AVP$) will block both the antipyretic (Kasting and Wilkinson, 1986) and the convulsive effects of AVP (Naylor et al., 1985; Burnard et al., 1986). Thus, a postulated role for AVP in behavioral thermoregulation, that may under pathophysiological conditions be related to febrile convulsions, finds some support in the literature.

2) Vestibular Physiology

Balaban et al. (1989) suggested that a connected network of structures that may include vestibular nuclei, midline cerebellar cortex, the fastigial nucleus, midbrain structures near the interstitial nucleus of Cajal, basal forebrain structures (e.g. BST and amygdala) and the basal ganglia might regulate postural stability.

AVP-induced motor effects are believed to result from asymmetric activation of these circuits, which can result in a stereotypical progression of motor effects culminating in barrel rotation.

Interestingly, barrel rotation has long been recognized as a sign of unilateral damage to central or peripheral vestibular circuits (Dickinson, 1865; Muskens, 1922). This response was initially reported by Pourfour de Petit in 1710, after cerebellar damage, and was subsequently demonstrated to result from asymmetric manipulation of anterior or posterior (vertical) semicircular canals in quadrupeds (Muskens, 1904, 1914, 1922).

In this paradigm, AVP-induced motor effects in quadrupeds appear to reflect a disorder of the vertical semicircular canals. Motor effects including unstable gait, unilateral hindlimb and forelimb extension, contralateral flexion with falling toward the flexed side, head tilt toward the flexed side, ocular nystagmus and circling movements, are thought to be less severe manifestations of vestibular disorder. These motor effects are thought, under certain circumstances, to culminate in the more severe manifestation of barrel rotation (Balaban et al., 1989). Convulsive activity is also believed to represent a severe manifestation of AVP exposure and is thought to be mediated by a different mechanism, possibly related to hypoxia.

C. ANATOMICAL SUBSTRATE FOR THE MOTOR EFFECTS INDUCED BY AVP

Little is known about the precise neuroanatomical loci which are responsible for AVP-induced motor effects because most studies have employed icv injection to study these effects. Hence, the major non-endocrine vasopressinergic somata and their CNS pathways will be reviewed, with subsequent review of loci known to be sensitive to the motor effects of AVP, followed by a comment on their potential interaction.

1) Principal Non-Endocrine Vasopressinergic Somata and their CNS Connections

The PVN, SCN and the BST are the principal sites of non-endocrine vasopressinergic somata, as indicated by nonanatomical methods as well as immunohistochemical and lesion studies (Buijs, 1978; Sofroniew and Weindl, 1978a; De Vries and Buijs, 1983; van Leeuwen and Caffé, 1983). Vasopressinergic somata have also been localized to the supraoptic nucleus, locus coeruleus, dorso-medial hypothalamus, lateral septum and the medial amygdaloid nucleus (Buijs and Swaab, 1979; Sofroniew and Weindl, 1981; Caffé and van Leeuwen, 1983; Sofroniew, 1983b). In addition, these regions stain positively for neurophysin (Sofroniew, 1978b), the precursor hormone of AVP, thus providing evidence for vasopressin synthesis (see Figure 1).

a) Paraventricular Nucleus

Vasopressinergic pathways originating in the PVN and projecting to brainstem areas have been deduced from immunocytochemical, retrograde transport, lesion and electrophysiological studies. These target areas include the nucleus of the tractus solitarius, dorsal vagal nucleus, substantia nigra, mesencephalic gray area, and raphe nuclei (Buijs, 1978; Sofroniew, 1980; De Vries and Buijs, 1983). Additional studies have provided evidence for vasopressinergic projections to the diagonal band of Broca (Swanson, 1977) and the BST (Buijs et al., 1978). The PVN may be the primary source of vasopressin to the spinal cord (Swanson, 1977; Buijs, 1978; Sofroniew, 1983b) and the amygdala (Buijs, 1978). There is also some evidence for a projection to the septum pellucidum, herein-after referred to as "septum" (Buijs, 1978; Pittman et al., 1981).

b) Suprachiasmatic Nucleus

Vasopressinergic somata originating in the SCN have been observed, using immunohistochemical and lesion studies, to project to the organum vasculosum lamina terminalis (Buijs, 1978; Hoorneman and Buijs, 1982), the periventricular thalamic nuclei (Buijs, 1978; Hoorneman and Buijs, 1982) and the mediodorsal thalamus (Sofroniew and Weindl, 1978a). Similar studies suggest vasopressinergic neurons project to the medial amygdala (Sofroniew, 1980) and lateral septum

(Sofroniew and Weindl, 1978a; Sofroniew, 1980).

Other SCN targets include strong projections to the lateral habenula and less conspicuous projections to the DBB, dorsal raphe nuclei, mesencephalic gray area, and the ventral hippocampus (Buijs, 1978; Sofroniew and Weindl, 1978a; Sofroniew, 1980).

c) Bed Nucleus of the Stria Terminalis

Vasopressin-containing cells exist in the BST and project primarily to the ventral aspect of the lateral septal area (VSA) (van Leeuwen and Caffé, 1983). Support for this projection has come from studies using immunocytochemistry (van Leeuwen and Caffé, 1983; De Vries et al., 1985), lesioning (De Vries and Buijs, 1983), electrophysiology (Disturnal et al., 1985) and retrograde transport (De Vries and Buijs, 1983).

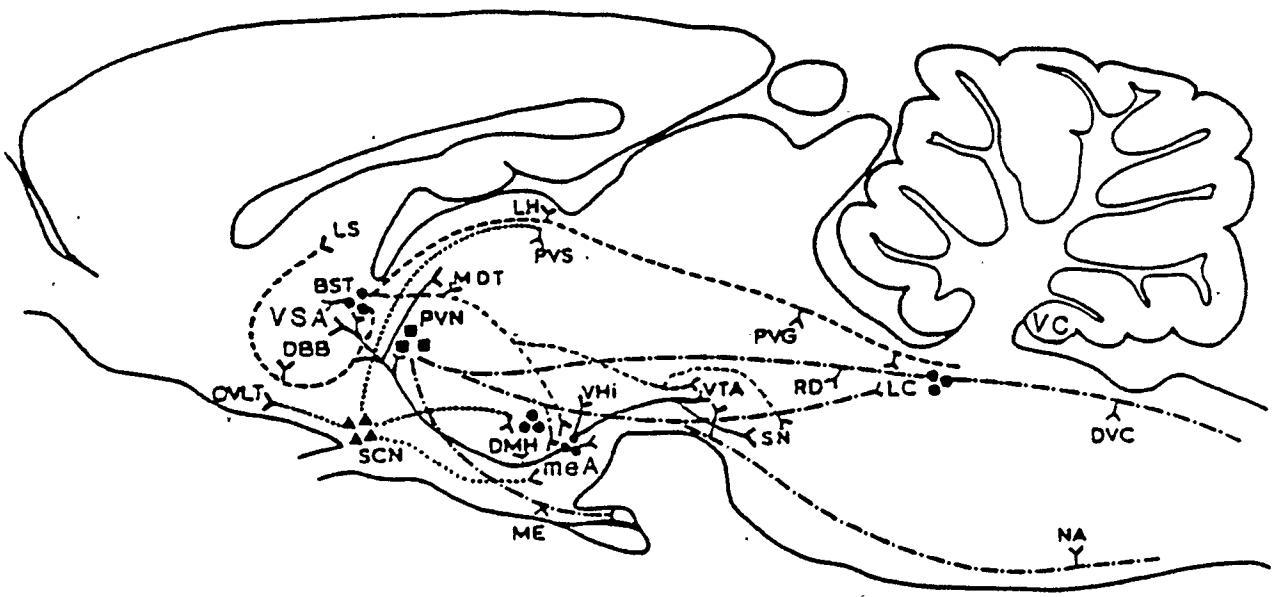
Interestingly, the BST receives its major input from the amygdala (van Leeuwen and Caffé, 1983) and sends vasopressinergic projections to the lateral habenula, dorsal and ventro-medial hypothalamus, ventral tegmental area, raphe nuclei, diagonal band of Broca and most importantly, the amygdala (Conrad and Pfaff, 1976a; De Vries and Buijs, 1983; De Vries et al., 1985).

2) AVP-Sensitive Brain Loci

Kasting et al. (1980) were the first to report that icv administration

Figure 1.

The major vasopressinergic pathways in the rat brain with their most likely origin (see text). Pathways of the paraventricular nucleus (PVN) are indicated by (-.-.-). Pathways of the suprachiasmatic nucleus (SCN; *triangles*) are indicated by (.....). Vasopressinergic cell groups seen only after colchicine treatment are indicated by *black dots*. Pathways of the bed nucleus of the stria terminalis (BST) are indicated by (- - -). Pathways of the medial amygdaloid nucleus (meA) are indicated by (—). DBB, diagonal band of Broca; DMH, dorsomedial hypothalamus; DVC, dorsal vagal complex; LC, locus coeruleus; LH, lateral habenula; LS, lateral septum; MDT, mediodorsal thalamus; ME, median eminence; NA, nucleus ambiguus; OVLT, organum vasculosum lamina terminalis; PVG, periventricular gray; PVS, periventricular nucleus; RD, dorsal raphe nucleus; SN, substantia nigra; VC, vestibular cerebellum; VHi, ventral hippocampus; VSA, ventral septal area; VTA, ventral tegmental area (adapted from De Vries et al., 1985).


LS
LH
PVS
BST
MDT
VSA
PVN
DBB
PVG
VC
OVLT
VHi
VTA
RD
LC
SCN
DMH
meA
SN
DVC
ME
NA

of AVP elicits motor effects in the rat. Nevertheless, it was not until Naylor et al.'s (1985) localization of a sensitive forebrain site in the ventral septal area, that an actual tissue locus was found for these effects.

a) Ventral Septal Area

The VSA is a non-discrete region of the septum that is located ventral to the lateral septum and lateral to the vertical limb of the diagonal band of Broca (Naylor et al., 1985). It is bordered caudally by the BST and laterally and ventrally by the vertical limb of the diagonal band of Broca. As such it also includes portions of the following nuclei: the BST, the septo-hypothalamic nucleus, preoptic-anterior hypothalamic nuclei (POAH), nucleus of the vertical limb of the diagonal band of Broca, and the medial preoptic nucleus (Bleier and Byne, 1985). Therefore, this tissue locus consists mainly of the ventral aspect of the lateral septum and several portions of bordering nuclei. Not surprisingly, binding studies indicate that the VSA exhibits very high AVP binding (i.e. greater than 100 fmoles/mg) (Biegon et al., 1984; De Kloet et al., 1985).

b) Vestibular Cerebellum

A second tissue locus for AVP-induced motor effects has been reported in the nodular cerebellum (lobule X) (Maiti et al., 1986), also known as the

vestibular cerebellum, because it receives primary vestibular afferents (Rotter et al., 1979). Rats injected with AVP in this area demonstrated almost identical responses observed with icv and VSA injections including: grooming, rearing, circling, ataxia, crouching, barrel rotation and clonic convulsions. In common with the VSA, the cerebellum exhibits dense AVP binding (approximately 200 fmoles/mg) (Pearlmutter et al., 1983).

c) Neural Network

Evidence exists for two AVP-sensitive brain loci - one in the VSA and the other in the vestibular cerebellum. However, the extent of their interaction, if any, is unknown.

The possibility of interaction between these sites may exist, however, either through a neuroanatomical substrate or through their close proximity to the ventricles. A neuroanatomical substrate that may allow communication between these sites has yet to be confirmed but a recent study by Haines et al. (1990) suggests that the VSA and vestibular cerebellum may communicate through connections each area possesses with hypothalamic nuclei. This possibility will be explored further in CH. 4.

Nevertheless, as yet the neuroanatomical substrate responsible for the motor and/or sensitization effects induced by AVP is unclear.

D. PHYSIOLOGICAL MECHANISMS FOR THE MOTOR EFFECTS INDUCED BY AVP

There is a paucity of data concerning the potential physiological mechanism(s) underlying the motor effects seen following CNS AVP injection. Most investigations of AVP-induced motor effects have employed icv injection and these studies have given little insight into the conditions which might lead to endogenous release of AVP.

Nevertheless, potential mechanisms might be inferred from push-pull perfusion studies, which suggest that endogenous AVP release may occur in the septum under febrile conditions (Cooper et al., 1979; Landgraf et al., 1990). In addition, electrophysiological data from Mathieson et al. (1989) and receptor blocking studies from Naylor et al. (1988) suggest that stimulation of the BST may result in AVP release into the VSA. This release has been associated with a drop in febrile body temperature and can be blocked by a V_1 antagonist. Kasting and Martin (1983) showed that immunoreactive vasopressin content in the septal area and the amygdala changes during endotoxin fever in rats suggesting possible AVP release into these sites.

Finally, studies on passive avoidance behavior suggest that hippocampal release of AVP may occur during passive avoidance tests (Laczi et al., 1983). Thus, there is some evidence for endogenous release of AVP into both the septum,

hippocampus and the amygdala, which might mediate different autonomic actions of AVP. Nevertheless, this evidence is indirect and has not been observed for AVP-induced motor effects.

E. PHARMACOLOGICAL MECHANISMS FOR THE MOTOR EFFECTS INDUCED BY AVP

Data for the pharmacological mechanisms underlying AVP-induced motor effects is much more clear than for the physiological mechanisms. A brief review of the evidence for the existence of CNS AVP receptors is in order before proceeding to discussion of putative pharmacological mechanisms.

1) AVP Receptors

a) Classification

Two types of vasopressin receptors have been well characterized in the periphery, based on different ligand selectivities and different effector mechanisms (Michell et al., 1979; Jard, 1983). One type is referred to as the V_1 (pressor) receptor and is characterized by high affinity AVP binding and linkage to a Ca^{2+} dependent, phosphatidyl inositol mechanism. The other type of peripheral AVP receptor is referred to as the V_2 (antidiuretic) receptor and is characterized by high affinity AVP binding and linkage to a Ca^{2+} independent, cAMP dependent

mechanism.

In the CNS, autoradiographic and homogenate binding studies suggest that three or more AVP binding site subtypes may exist (Barberis, 1983; Dorsa et al., 1983, 1984; Pearlmutter et al., 1983; Junig et al., 1985). Two subtypes resemble peripheral V_1 and V_2 AVP receptors (Dorsa et al., 1983, 1984; Pearlmutter et al., 1983; Junig et al., 1985). A third type of AVP receptor has been characterized in the rat adenohypophysis that resembles neither V_1 nor V_2 subtypes (Antoni, 1984; Jard et al., 1986). Like the V_1 receptor it is coupled to a Ca^{2+} dependent, phosphatidyl inositol mechanism but it possesses neither a vasopressor nor an antidiuretic ligand selectivity. This receptor has been classified as a V_{1b} receptor while the original V_1 (vasopressor) receptor is now referred to as V_{1a}, or simply V_1 (Jard et al., 1986). Thus, at least three subtypes of AVP receptors may exist with different binding properties and varying pharmacological profiles.

b) Distribution

In an effort to localize the central receptors of AVP, labelled peripherally active agonists and antagonists have been used. Recent autoradiographic binding studies have confirmed the presence of AVP binding sites in numerous areas of the rat brain (see Table 2). A high degree of binding has been observed in hypothalamic sites such as the paraventricular nucleus and the supraoptic nucleus.

Moderate binding was observed in extrahypothalamic sites such as the nucleus of the tractus solitarius, cerebellum, and most importantly, extrahypothalamic areas associated with AVP activity (septum, hippocampus, and amygdala) (Baskin et al., 1983; Biegon et al., 1984; Brinton et al., 1984; De Kloet et al., 1985).

For many brain areas, most notably AVP-active brain areas such as the lateral septum, the hippocampus, the ventral septal area and the amygdala, the binding sites resemble in many respects a V_1 receptor (Dorsa et al., 1984; Freund-Mercier et al., 1988a,b; Poulin et al., 1988). Several arguments favour the V_1 receptor subtype. These hinge on two approaches, the first is pharmacological and the second is physiological/behavioral.

Pharmacological studies have shown that V_1 agonists and antagonists demonstrate higher affinity for [^{3}H]AVP-binding sites in these brain areas than do V_2 and oxytocic agonists and antagonists (for review see van Leeuwen, 1987). In addition, *in vitro* application of AVP to sections from several brain regions induces V_1 receptor-associated changes (i.e. membrane phospholipid hydrolysis) (Kiraly et al., 1986; Hinko and Pearlmutter, 1987) but does not induce V_2 receptor-associated cAMP activation (Courtney and Raskind, 1983; Dorsa et al., 1983; Audigier and Barberis, 1985).

Perhaps the strongest argument for V_1 receptors comes from physiological and behavioral studies which have shown that many of these actions can be

Table 2

Distribution of Binding Sites for [^{3}H] AVP and Density of AVP-Immunoreactive Fibers in the Adult Male Rat Brain

Brain region	Amount of binding[a] (fmoles/mg)	AVP fiber density[b]
Telencephalon		
Amygdala - central nucleus	>100	+
Amygdala - medial nucleus	50-100	++
BST	>100	+++
Cortex - frontal	>100	+
Diagonal band of Broca	nd	+++
Hippocampus - entorhinal cortex	50-100	-
Hippocampus - gyrus dentatus	26-50	-
Hippocampus - parietal cortex	50-100	-
Hippocampus - ventral	>100	+
Nucleus accumbens	50-100	-
Septum - medial	26-50	-
Septum - lateral (including VSA)	>100	+++
Striatum	>100	++
Diencephalon		
Hypothalamus - d.m.n.	nb	+++
Hypothalamus - p.v.n.	>100	+++
Hypothalamus - s.o.n.	>100	+++
Neural lobe	nd	+++
Thalamus - mediodorsal nucleus	50-100	+++
Thalamus - p.v.n.	nd	+++
Mesencephalon		
Central gray area	nb	+
Medial geniculate nucleus	50-100	+
Raphe nucleus - dorsal	26-50	++
Substantia nigra	50-100	++
Tegmentum - ventral	nd	++
Rhombencephalon		
Cerebellum	>100	+
Medulla oblongata (pv)	50-100	+
Nucleus reticularis	0-25	-
Nucleus tractus solitarius	>100	++

[a] Only those brain regions were included where specific binding was assessed by the difference between total and nonspecific binding (1000-fold excess of cold AVP). Binding (in femtomoles per milligram) of tissue: 0-25, none or low density; 26-50, moderate density; 50-100, high density; >100, very high density; nd, not determined; nb, no binding.

[b] no or single fibers; +, low fiber density; ++, high fiber density; +++, very high fiber density (Pearlmutter et al., 1983; De Vries et al., 1985; van Leeuwen, 1987; Freund-Mercier et al., 1988a,b).

blocked by V_1 antagonists. V_1 antagonists block AVP-induced hippocampal neuronal firing (Tiberiis et al., 1983; Smock et al., 1990), AVP-induced antipyresis (Naylor, 1987) and AVP-induced motor disturbances (Naylor et al., 1985; Burnard et al., 1986).

Nevertheless, discretion must be used when interpreting the literature as attempts at characterizing AVP receptor subtypes with different antagonists can lead to misleading data, for the following reasons:

1) *In vitro* vs. *in vivo* studies - some analogues block *in vitro* but not *in vivo* responses to AVP (Manning and Sawyer, 1983).

2) Specificity - antagonists for V_1, V_2, or oxytocin-like receptors vary widely in their specificity. For example, some V_1 antagonists can block uterine-type oxytocin receptors to varying degrees. As well, some V_2 antagonists can block V_1 and oxytocin receptors to varying degrees (Manning and Sawyer, 1984; Sawyer and Manning, 1985).

In summary, the presence of V_1-like receptors and to a lessor degree V_2-like receptors has been implicated in the CNS. V_1-like receptors seem to exist in the septum, amygdala, cerebellum and hippocampus, among other areas.

2) Motor Effects

Induction of motor activity by AVP seems to require the intact molecule. That is, fragments of the AVP molecule (AVP is a nonapeptide) are not effective elicitors of motor effects. Poulin (1991, personal communication) found that C-terminal peptides, AVP_{5-9} and AVP_{4-9}, were not able to induce motor effects. Kruse et al. (1977) tested both ring and tail fragments of AVP (i.e. peptides from N and C terminal ends respectively) and found no motor effects. Hence, these studies imply that it is the intact AVP molecule that is responsible for the motor effects seen with AVP exposure.

AVP-induced motor effects likely result from an interaction of AVP with receptors similar to the peripheral V_1 type of AVP receptor. Evidence for V_1 involvement comes from receptor blocking studies which demonstrated that the motor disturbances resulting from icv or VSA, AVP exposure could be completely blocked by prior administration of the highly potent and selective V_1 antagonist $d(CH_2)_5$Tyr(Me)AVP (i.e. [1-(β-(mercapto-β, β-cyclopentamethylene propionic acid) 2-(0-methyl) tyrosine]AVP). Furthermore, V_1-like AVP binding sites have been characterized in the VSA (Poulin et al., 1988) and these sites seem to represent functional receptors, since AVP binding causes an increase in phosphatidyl inositol turnover (Shewey and Dorsa, 1988), an increase in the excitability of septal neurons (Raggenbass et al., 1987) and modulation of neuronal

electrical impulses to other neurotransmitters (Joëls and Urban, 1984; Disturnal et al., 1987).

Additional evidence that suggests the motor effects are mediated via receptors that resemble the V_1-type is provided by studies that show that the potent V_2 receptor agonist DDAVP (1-desamino-8-D-arginine vasopressin) does not induce motor effects upon icv or VSA injection (Burnard et al., 1986; P. Poulin, 1991, personal communication). Studies showing that AVP fails to directly stimulate cAMP accumulation (as would a V_2 receptor) in membrane preparations or homogenates from the septal area (Courtney and Raskind, 1983; Dorsa et al., 1984) suggest V_2 receptors may not play an important role in the septum.

Data from Kruse et al. (1977) suggest that AVP-induced motor effects may be a direct effect of AVP injection and not mediated by cholinergic, noradrenergic, dopaminergic or seritonergic functions, since blocking agents for all these transmitters failed to affect AVP-induced motor effects when injected icv. Conversely, Yamada and Furukawa (1981) provided evidence that dopaminergic inhibition and cholinergic activation might be involved in AVP's motor effects. For example, they found that when dopamine receptors were blocked by systemic injection of dopamine antagonists, such as fluphenazine and haloperidol, barrel rotation induced by icv AVP was enhanced. Anti-Parkinsonian drugs such as trihexyphenidyl (a muscarinic receptor blocker) reversed this effect. Other studies

have reported that cholinergic mechanisms may be involved in AVP-induced barrel rotation. For example, Wurpel et al. (1986) found that atropine produced a 50% depression in the incidence of AVP-induced barrel rotation. Hence, these data suggest the possibility of a dopaminergic and a cholinergic component to AVP-induced motor effects.

In summary, two brain loci (the VSA and the vestibular cerebellum) have thus far been implicated in AVP-induced motor effects and these motor effects are most likely to be mediated by V_1-like receptors. However, the neuropharmacological links between these areas and motor areas are unclear. Thus, the pharmacological mechanism(s) underlying AVP-induced motor effects appear to involve V_1-like receptors but may also involve cholinergic and/or dopaminergic mechanisms.

3) Sensitization Effect

AVP-induced sensitization was first reported by Kasting et al. (1980) when they found that an initial icv injection of AVP caused minor motor effects such as ataxia and prostration, whereas a subsequent AVP injection two days later, caused severe motor effects, such as barrel rotations, myoclonic/myotonic convulsions and death.

Further studies reported that this sensitization could be induced in the VSA (Naylor et al., 1985) and in the vestibular cerebellum (Maiti et al., 1986) by prior injection with AVP. Hence, both brain loci that respond to AVP's motor effects seem to undergo sensitization after an initial AVP exposure.

The pharmacological mechanism(s) of this sensitization, however, is not known. There is evidence suggesting that the cerebellum may be necessary for the sensitization effect seen with icv AVP exposure. Removal of the cerebellum completely abolished the sensitization normally observed after icv injection with AVP, but did not eliminate the motor effects observed after icv AVP injection. However, incidence of these motor effects was depressed (for review see Balaban et al., 1989).

Poulin and Pittman (1991, unpublished results) have investigated the characteristics and putative mechanism(s) responsible for AVP-induced sensitization through behavioral, [^{3}H]AVP binding, and [^{3}H] inositol phosphate assay studies. Previous behavioral data from this group suggest that a single icv injection of AVP (10 pmol) is enough to induce sensitization. This phenomenon can be observed from 6 h up to 6 days following an initial injection but is not detectable after 6 days. Daily injection (10 pmol, icv) induced and maintained this sensitization, however, hourly or weekly injections had no detectable effect.

Binding assay studies revealed that the sensitization phenomenon is more

likely associated with an enhanced, AVP-induced intracellular signal transduction rather than an upregulation (increase in number) of AVP receptors. Increases in inositol phosphate accumulation in VSA tissue were observed in animals sensitized by prior icv exposure to AVP and this IP accumulation could be blocked by prior application of a V_1 antagonist. Neither a V_2 agonist, nor oxytocin, could effectively stimulate this second messenger system, indicating that this effect may be specific to V_1 receptors. Binding studies revealed no changes in binding characteristics (i.e. dissociation constant or maximum binding) of VSA AVP receptors, hippocampal or lateral septal tissue after pretreatment with AVP, indicating that sensitization was unlikely to be due to an increase in number of VSA AVP receptors (Poulin and Pittman, 1991, unpublished results).

Behavioral studies using V_1 antagonists also suggest that the sensitization process may occur via interaction of AVP with the V_1 type of AVP receptor (Burnard et al., 1986). Nevertheless, there is also evidence for involvement of V_2 (P. Poulin, 1991, personal communication), oxytocin (Poulin and Pittman, 1988) and somatostatin (Burnard et al., 1985) receptors in the sensitization process. Interestingly, somatostatin-induced sensitization may be abolished through pretreatment with a V_1 receptor antagonist and occurs in rats genetically deficient in AVP (Burnard et al., 1985a) indicating a potential interaction of somatostatin with the vasopressinergic system. Starcevic et al. (1988) and Balaban et al. (1989)

also provide evidence for interaction between somatostatinergic and vasopressinergic systems.

Taken together, these studies imply that several peptides may induce the sensitization process, several brain loci may be involved, and that this process may occur, at least in part, through enhanced signal transduction at the V_1 receptor, possibly mediated by an increase in phosphatidyl inositol hydrolysis in AVP responsive neurons. At this point it is not known how sensitizing agents act (i.e. do they interact directly with receptors that also respond to AVP, or by potentiating AVP release, or via separate pathways, or by some other mechanism?) These questions will be addressed in subsequent sections.

In summary, AVP induces a series of motor effects. These effects might be related to behavioral thermoregulation and/or vestibular physiology. Putative anatomical, physiological and pharmacological mechanisms have been explored and data suggest the existence of at least two sites of action, and a strong role for the V_1 receptor in both the motor effects and the sensitization effect seen with CNS AVP injection. Nevertheless, it seems plausible that strong AVP binding coupled with a high density of vasopressinergic fibers in other brain areas might indicate the presence of other loci sensitive to the motor effects of AVP. Evidence suggests that one of these loci may lie in the amygdala.

F. THE AMYGDALA

1) Background

The amygdaloid complex plays an important role in the central nervous system, modulating endocrine functions, visceral effector mechanisms, and complex behavioral patterns including defense, ingestion, aggression, reproduction, learning and possibly thermoregulation (Anand and Brobeck, 1952; Kawakami et al., 1972; de Olmos et al., 1985; Federico et al., 1990). Traditionally, this modulation was thought to occur via an extensive network of projections to regions more directly implicated in these functions, especially the hypothalamus. However, recent anatomical findings have lessened the importance of amygdalo-hypothalamic connections in light of strong amygdalar connections with sensory systems, the basal ganglia, the brainstem (de Olmos et al., 1985), the basal forebrain (Gaykema et al., 1990, 1991) and the motor cortex (Sripanidkulchai et al., 1984; Kita and Kitai, 1990).

Most amygdalar connections are reciprocal, therefore it may be misleading to consider them solely on the basis of net inputs or outputs. With two-way axonal links to other brain areas, such as the prefrontal cortex or hippocampus, it appears that the amygdala may play an important associational function. However, the presence of direct connections to the motor cortex (Kita and Kitai, 1990) and to other areas that modulate motor behavior such as the striatum and basal ganglia

(Krettek and Price, 1978a; Royce, 1978) suggests that the amygdala has both associational and more direct motor functions. Thus, the amygdala may play a multifaceted role in motor behavior, including that induced by AVP.

2) Principal Amygdalar Somata and their CNS Connections

In the past, the amygdaloid complex has been conceptualized in terms of corticomedial and basolateral amygdaloid groups. However, anatomical and histochemical evidence suggests a division of this multinuclear structure into four groups - olfactory, basolateral, central, and medial (de Olmos et al., 1985). These groups will be reviewed in terms of their major anatomical connections and their potential contributions to motor behaviors in the rat.

a) *Olfactory Amygdaloid Group*

This group includes the anterior amygdaloid area, the nucleus of the lateral olfactory tract, the rostral tip of the medial amygdaloid nucleus, the anterior and posterolateral cortical amygdaloid nuclei and the amygdalopiriform transition area (de Olmos et al., 1985). The principal role of the olfactory amygdala is in olfaction (de Olmos et al., 1985). This nuclear group receives input from the mitral cells of the main olfactory bulb and connects reciprocally with other areas

receiving olfactory input, including the olfactory cortex (Haberly and Price, 1978; Luskin and Price, 1983).

In addition, extensive reciprocal connections with hippocampal-related areas, especially the entorhinal cortex, characterize the olfactory amygdala (Wyss, 1981; Luskin and Price, 1983). These interconnections suggest that the olfactory amygdala may play a role in both olfaction and learning. Other afferent connections include the medial amygdala, horizontal limb of the diagonal band of Broca, locus coeruleus and raphe nuclei (Conrad and Pfaff, 1976a; Ottersen, 1982; de Olmos et al., 1985). The main efferents of the olfactory amygdala consist of projections to the piriform cortex (primary olfactory cortex), the central amygdaloid nucleus and the lateral hypothalamus (de Olmos et al., 1985).

No evidence for connections to motor cortex or to areas mediating motor behavior has been documented (de Olmos et al., 1985).

b) *Central Amygdaloid Group*

The central amygdaloid group consists of the central nucleus of the amygdala, the dorsolateral portions of the sublenticular substantia innominata, as well as the lateral division of the BST (de Olmos et al., 1985). This group receives a variety of cortical afferents which are, for the most part, not reciprocated. Afferents come from the entorhinal cortex, agranular insular cortex,

and perirhinal cortex, all part of the rat allocortex (i.e. olfactory and limbic cortices). Little direct feedback exists from the central amygdaloid group to the allocortex (de Olmos et al., 1985).

A variety of interconnections exist with this group and lateral preoptic and lateral hypothalamic areas (Swanson et al., 1984; de Olmos et al., 1985). In addition, reciprocal connections exist with brain stem areas such as the parabrachial area and medulla (Schwaber et al., 1982; de Olmos et al., 1985). Therefore, the central amygdaloid group can potentially influence both sensory and motor aspects of autonomic function. Other projections to dopaminergic neurons in the tegmentum as well as the pedunculopontine tegmental nucleus and trigeminal motor nucleus gives this group potential influence over somatomotor events (de Olmos et al., 1985).

c) *Basolateral Amygdaloid Group*

The basolateral amygdaloid group consists of the lateral and basolateral nuclei as well as the lateral part of the basomedial nucleus. This group is characterized by a wide range of reciprocal connections with the piriform cortex (primary olfactory cortex), agranular insular cortex (olfactory/limbic cortex) and the prelimbic cortex (de Olmos et al., 1985). In addition, subcortical projections exist to the dorsal and ventral striatum. This projection is not well reciprocated

but striatal information may return to the basolateral amygdaloid group via the pallidum (Groenewegen and van Dijk, 1984; Carlsen et al., 1985). Thalamic connections include inputs from the nucleus reunions and paratenial nucleus as well as other thalamic nuclei, however, few thalamic efferents exist (Krettek and Price, 1977; Siegal et al., 1977; Herkenham, 1978). Intra-amygdalar connections include afferent projections to the olfactory amygdala and a dense projection to the central amygdaloid group, with few other connections.

The basolateral group also receives dense afferents from the ventral forebrain and sends afferents to the primary motor cortex, indicating potential direct and indirect influences over the organization of movement (Sripanidkulchai et al., 1984; Kita and Kitai, 1990).

d) *Medial Amygdaloid Group*

The medial amygdaloid group consists mainly of the nucleus of the medial amygdala (meA) and surrounding nuclei including the posteriomedial sublenticular substantia innominata, medial and intermediate BST, bed nucleus of the accessory olfactory tract, the posteriomedial cortical amygdaloid nucleus, the amygdalo-hippocampal transition area, and the basomedial nucleus. Of note is the important position of the meA among nuclei of the medial amygdaloid group. This nucleus might act as a gateway for the medial group's thalamic afferents and brainstem

efferents (de Olmos et al., 1985). Also important are neuroanatomical and neurochemical data suggesting that the meA might be considered part of a larger anatomical and functional entity that includes the BST and central amygdaloid nucleus; in essence, a single nucleus split in two parts by fibers of the internal capsule (de Olmos et al., 1985; Holstege et al., 1985; Caffé et al., 1987).

The meA has reciprocal connections with the BST (Swanson and Cowan, 1979; Ottersen, 1980; Weller and Smith, 1982; Caffé et al., 1987) and PVN (Pittman et al., 1981; Sawchenko and Swanson, 1983) both of which are putative suppliers of vasopressin to the VSA, the anterior hypothalamus and the entire amygdaloid complex.

Other important reciprocal connections of the meA include hypothalamic areas such as the ventromedial hypothalamus (Saper et al., 1976; Krettek and Price, 1978a; Luiten et al., 1983) and lateral hypothalamus (implicated in behavioral thermoregulation) (Veening, 1978; Ottersen, 1980) as well as connections to the dorsomedial hypothalamus (Ter Horst and Luiten, 1986), VSA (Disturnal et al., 1985; Caffé et al., 1987), POAH (Swanson, 1976; Conrad and Pfaff, 1976; Ottersen, 1980; Caffé et al., 1987), and ventral hippocampus (Caffé et al., 1987; Albeck et al., 1990).

In addition, noteworthy connections are the meA's efferents to the mediodorsal thalamus, the peripeduncular nucleus, and to targets in the ventral

tegmental area, reticular formation, and mesencephalic central gray matter (de Olmos et al., 1985). Particularly dense projections to the mediodorsal thalamus indirectly link the meA to frontal area 2 (rat motor cortex) providing the meA with the potential to influence motor behavior (Siegel et al., 1977; Young et al., 1984; McDonald, 1987). In addition, projections to dopaminergic neurons in the ventral tegmentum connect the meA to an area that has been implicated in numerous motor disorders, including Parkinson's disease, Huntington's chorea, and tardive dyskinesia. Interestingly, pathophysiology of this area has been associated with homeostatic imbalances in thirst and temperature regulation (for review see Fallon and Loughlin, 1985) and both overhydration (for review see Andrew, 1991) and fever (for review see Pittman et al., 1988) have been associated with convulsive activity. Finally, portions of the medial amygdaloid group project to the medial prefrontal cortex (Sarter and Markowitsch, 1984) providing a more direct link to areas capable of influencing motor behavior.

The medial amygdaloid group receives afferent projections from both the basolateral (Krettek and Price, 1978b; Nitecka et al., 1981; Ottersen, 1982) and central amygdaloid groups (de Olmos, 1972; Krettek and Price, 1978b), sends sparse projections back to the basolateral group and slightly more to the central group (Krettek and Price, 1978b; Nitecka et al., 1981; Ottersen, 1982; Weller and Smith, 1982). In summary, the medial amygdaloid group (and especially the

meA) lies in an important position from where it may potentially influence motor behavior, especially AVP-induced motor effects.

3) Amygdalar AVP Innervation

The entire amygdaloid complex is innervated by vasopressinergic fibers. The medial amygdaloid nucleus has the most dense supply of vasopressinergic nerve terminals (Buijs and Swaab, 1979; Sofroniew, 1983a, 1983b; Zimmerman et al., 1984; De Vries et al., 1985). So far, the principal nuclei that have been identified as putative sources of vasopressin to the amygdaloid complex, are the SCN, PVN and the BST. In addition, the meA itself may supply AVP to the entire amygdaloid complex.

Some evidence suggests that SCN vasopressinergic neurons project principally to the medial amygdala (Sofroniew, 1980). However, lesion studies do not support this projection, as SCN lesioning leaves amygdalar vasopressinergic fibers unaffected (Hoorneman and Buijs, 1982). In contrast, the PVN appears to supply the entire amygdaloid complex with vasopressin (Buijs, 1978; Sofroniew, 1980).

Likewise, lesion, dye injection and castration studies support vasopressinergic projections from the BST to most of the amygdaloid complex (De

Vries and Buijs, 1983; van Leeuwen and Caffé, 1983; De Vries et al., 1985). The meA receives especially strong vasopressinergic projections from the BST (De Vries et al., 1985).

4) Amygdalar AVP Release

To establish a physiological role for AVP-induced motor effects in the amygdala (or in any potential brain locus) it is necessary to demonstrate endogenous release of this neuropeptide under physiologically relevant conditions. To date, this has not been accomplished, although there is some evidence suggesting that endogenous release of AVP in the brain can sensitize rats to AVP's motor effects. Burnard et al. (1983) used hemorrhage and hypertonic saline, both of which are believed to trigger AVP release in the brain, to sensitize rats to the motor effects of subsequent icv injections with AVP. Interestingly, endogenous release of AVP in the brain may be implicated in other AVP-induced behavioral (autonomic) effects. For example, Kasting and Martin (1983) have shown decreased amygdalar AVP levels during endotoxin fever in rats, which may suggest increased AVP release. It is conceivable endogenous release of amygdalar AVP might be involved in AVP-induced motor effects although, as yet, no clear evidence exists for this possibility.

5) Amygdalar AVP Receptors

Evidence for the existence of vasopressin receptors in the amygdala can be inferred from autoradiographic and radioligand binding studies. The strongest binding of vasopressin occurs in the central amygdaloid nucleus and surrounding areas (Biegon et al., 1984; Freund-Mercier et al., 1988a,b; Tribollet et al., 1988) whereas moderate binding occurs in the medial and basolateral amygdaloid nuclei (Freund-Mercier et al., 1988a,b). Binding kinetics and ligand selectivity of amygdalar binding sites closely resemble that of the V_1 type of AVP receptor (Biegon et al., 1984; Dorsa et al., 1984; Lawrence et al., 1988; Tribollet et al., 1988).

G. RATIONALE FOR RESEARCH

There is evidence to suggest that the amygdala (specifically the meA) may be a sensitive site for AVP-induced motor effects. However, this requires further investigation. Therefore, experiments were designed to meet the following goals:

1) To determine whether AVP induces motor effects (and a sensitization process) when injected into the meA.
2) To characterize the putative receptor(s) mediating AVP-induced motor effects and the sensitization process in the meA.

CHAPTER 2

AVP-INDUCED MOTOR EFFECTS IN THE MEDIAL AMYGDALA: LOCALIZATION OF A SENSITIVE SITE AND ESTABLISHMENT OF AVP-SPECIFIC EFFECTS

A. INTRODUCTION

Numerous studies suggest that the neuropeptide arginine vasopressin causes motor effects when injected into the VSA or the vestibular cerebellum of the rat brain (for review see Cooper, 1987; Balaban et al., 1989). These effects range from stereotyped motor behaviors such as grooming, to severe motor effects such as myoclonic/myotonic convulsions, and can even lead to death. In the VSA, a sensitization process has been observed such that a subsequent exposure to AVP, from 6 hours to 6 days after an initial exposure, elicits much more severe motor effects than an initial exposure (Kasting et al., 1980; Naylor et al., 1985; Poulin and Pittman, 1991, unpublished results). AVP-induced sensitization has also been reported in the vestibular cerebellum (Maiti et al., 1986). Evidence has been presented in CH. 1. that implicates the amygdala as a potential site for AVP-induced motor effects. For example, AVP-like immunoreactivity as well as AVP binding sites exist in the amygdala (Dorsa et al., 1984; Lawrence et al., 1988). In addition, the amygdala is a locus for the kindling of a variety of motor effects,

including myoclonic/myotonic convulsions (for review see Goddard, 1980).

Anatomical and electrophysiological evidence suggests that the medial amygdaloid nucleus (meA) may be involved in AVP-induced motor effects. For example, vasopressinergic nerve terminals have been localized to the meA (Buijs and Swaab, 1979; De Vries et al., 1985) as well as putative receptors for AVP that resemble the V_1 subtype (Dorsa et al., 1984; Freund-Mercier et al., 1988a,b). In addition, reciprocal connections have been found with sites important in AVP-induced motor effects such as the VSA (Caffé et al., 1987).

Hence, there is evidence that implicates the meA in AVP-induced motor effects. Therefore, experiments were undertaken to determine whether microinjection of AVP into the meA of the rat brain would cause motor effects (possibly involving a sensitization process).

B. METHODS

Forty-three male, Sprague-Dawley rats weighing 250-300 g at the time of surgery were used as subjects. All rats were housed in a colony room on a 12 h light-dark cycle (lights on: 0:700 h) at an environmental temperature of 22 $\pm$ 1°C. Animals had ad libitum access to standard laboratory rat chow and water.

Rats were anaesthetized with sodium pentobarbital (Somnotol; 65 mg/kg ip) and bilateral stainless-steel, 23-gauge guide cannulae were implanted

stereotaxically (2.8 mm posterior to bregma, 3.5 mm lateral to lamda) according to the atlas of Paxinos and Watson (1982), such that the tips rested 5 mm above the meA area. Four stainless-steel anchor screws were inserted into the skull and the cannulae were secured with cranioplast cement. Twenty-seven gauge stainless-steel stylets were inserted to protect the cannulae from contamination when not in use. Animals recovered from surgery for 7-10 days before experimentation.

Animals were assigned randomly to one of four groups (see below):

Group	n	Day 1	Day 2
Experimental	12	AVP	AVP
Control 1	9	AVP	NaCl
Control 2	13	NaCl	AVP
Control 3	9	NaCl	NaCl

In the experimental group, AVP [92 pmol (100 ng) in 1.0 μl vehicle, Bachem, Inc.] was infused twice, 24 hours apart, to test for motor effects and to test for a sensitization effect. Control trial 1 examined nonspecific motor effects of the vehicle [1.0 μL sterile, pyrogen-free, 0.9% physiological saline]. Control trial 2 tested nonspecific sensitization effects of the vehicle. Control trial 3 controlled for nonspecific effects of the injection procedure.

All experiments were performed between 0800-1600 h, to control for circadian changes in endogenous AVP levels (Reppert et al., 1981, 1982, 1987).

One hour prior to experimentation, rats were placed individually into large plexiglass cages. Two 27-gauge stainless-steel needles were connected via sterile PE-20 tubing, to gas tight 10 μl glass syringes mounted on a Harvard infusion pump, which were filled subsequently with either AVP (in vehicle) or vehicle alone. Separate needles and tubing were used for AVP and vehicle injection and each was sterilized between injections. Stock AVP solution (10^{-3} M) was stored at 4°C and was diluted with vehicle to the appropriate concentration immediately prior to injection. AVP or vehicle was injected bilaterally into unrestrained rats, over a 60 second period, after lowering the needles through the guide cannulae to the level of the meA (i.e. 5 mm below guide cannulae). Following injection, needles were left in place for 60 additional seconds to prevent solution from being drawn up the needle tracts upon removal of the needles.

Motor effects were recorded and scored according to a predetermined behavioral code which is a reliable, repeatable, and quantifiable means of ranking the severity of departure from normal motor activity (Goddard et al., 1969; Racine, 1972; Kasting et al., 1980; Burnard et al., 1985b). Motor effects were scored after injection, for 10 min at 1-min intervals (for behaviors observed during the interval) by the following scale:

Score	Behavior
0	Normal motor activity (or stereotyped behaviors such as grooming)
1	Pauses (immobility) - absence of activity (10 sec or more)
2	Prostration - sprawled out posture
3	Locomotor difficulties, head swaying, complete loss of balance
4	Barrel rotations - spinning along the longitudinal axis of the body
5	Myoclonic/myotonic convulsions
6	Death

Results were presented as the highest score that each animal received throughout the 10-min period.

To control for potential experimenter bias when scoring the motor effects, 12 of the 43 animals used for this experiment were scored without prior knowledge of the solutions injected (i.e. blind trial). These results were compared with the results obtained from animals who received equivalent treatments but were scored with knowledge of the solutions injected.

To ensure the reliability and repeatability of the behavioral scoring procedure, a separate trial was conducted that involved icv injection of AVP into a group of five animals, on two consecutive days. The resultant behaviors were scored independently by two different examiners. Data were tested for differences between examiners on both days.

When all trials had been completed, animals were overdosed with sodium pentobarbital anaesthetic and a few minutes later tissue injection sites were marked by 1 μl injections of Pelican Special black ink. Rat brains were perfused with saline followed by neutral formalin via aortic injection. Subsequently, their brains were removed and sectioned into coronal slices, at 60 μm, on a sledge microtome. Relevant sections were stained with neutral red dye. The injections were localized histologically by light microscopy, before comparison of behavioral scores, to ensure an unbiased verification of sites.

To test the site specificity of any potential motor and sensitization effects observed upon AVP injection, animals from the experimental group (AVP//AVP) (// - indicates 24 h separation) were separated into two groups: those which received injections within the meA on at least one side of the brain (n=6), and those which received injections in sites surrounding, but not impinging upon the meA, on both sides of the brain (n=6). Differences in behavioral responses between groups on Day 1 and on Day 2 (i.e. motor effects), were analyzed by the Mann-Whitney U test (i.e. Wilcoxon rank sum test). Differences in behavioral responses within groups from Day 1 to Day 2 (i.e. sensitization effect) were analyzed by the Wilcoxon matched-pairs signed-ranks test. These tests are the nonparametric equivalents of the unpaired and paired Student's t-tests and are appropriate for ranked data (Dawson-Saunders and Trapp, 1990). Differences

were considered statistically significant at $\alpha \leq 0.05$.

The experimental group was compared with each of the control groups to examine whether AVP was specific in its ability to induce motor effects and the sensitization effect in the meA. Only data from animals whose injections occurred within or impinged upon the meA (on at least one side of the brain) were considered for these comparisons. Results from each experimental day (Day 1 or Day 2), were presented in raw form and groups were compared according to the Kruskal-Wallis one-way ANOVA by ranks (the nonparametric equivalent of the one-way ANOVA). If significant differences were found ($\alpha \leq 0.05$), these differences were further examined by post-hoc group comparisons using the Mann-Whitney U test, with a downward adjustment of the α level to compensate for the increased probability of a type 1 error, common to post-hoc multiple comparisons (Dawson-Saunders and Trapp, 1990). α was divided by the number of comparisons (c) in order to maintain the overall probability of a type 1 error at 0.05 (i.e. each comparison must be significant at α/c to be declared statistically significant).

C. RESULTS

Results from the blind trial were not significantly different from the results obtained from the other experimental trials on either Day 1 ($p > 0.55$; Mann-

Whitney U test) or Day 2 ($p > 0.51$; Mann-Whitney U test). Results from the inter-examiner reliability and repeatability comparisons revealed that there were no significant differences between the scoring of each examiner on either Day 1 ($p > 0.50$; Mann-Whitney U test) or Day 2 ($p > 0.41$; Mann-Whitney U test) (Fig. 2).

In order to test the site specificity of the motor effects observed with amygdalar AVP injection the behavioral scores of rats following an initial (Day 1) AVP (92 pmol/1.0 μl saline) injection within the meA were compared to the behavioral scores of rats who received similar AVP injections but outside of the meA. Fig. 3 displays injection sites for animals used in this comparison while Fig. 4.A presents their behavioral scores. After an initial exposure to AVP, most rats were unaffected but a few rats, in both groups, displayed minor (i.e. score < 3) motor effects consisting of grooming and/or long pauses. However, no significant difference was noted between groups ($p > 0.47$; Mann-Whitney U test). Following AVP injection into sites within the meA some animals also displayed mouth movements similar to those seen in Stage 1 (i.e. least severe stage) amygdaloid kindled seizures (see Racine, 1972).

When the animals were retested one day later (Day 2), the motor responses of the animals injected within the meA were much greater than the motor responses elicited from injection of animals in sites surrounding but not impinging

upon the meA ($p < 0.006$, Mann-Whitney U test) (see Fig. 4.B). Most animals in the group that received meA injections of AVP on Day 2 exhibited a stereotypical pattern of behavioral changes. This was characterized by locomotor difficulties, swaying of the head and complete loss of balance, which usually progressed to barrel rotation and myoclonic/myotonic convulsions. Some animals exhibited dyspnea, apnea and death, possibly associated with pulmonary edema.

The sensitization phenomenon is exhibited in Fig. 5.A. The animals who received AVP injections within the meA had much stronger behavioral responses to the peptide 24 hours after (Day 2) the initial (Day 1) AVP injection ($p < 0.03$; Wilcoxon matched-pairs signed-ranks test). In contrast, behavioral scores of animals whose injections were outside the meA were not significantly different from Day 1 ($p > 0.20$; Wilcoxon matched-pairs signed-ranks test) (Fig. 5.B.).

Histological examination of injection sites localized to the meA from the animals in the Experimental group and Control groups 1-3 is represented in Fig. 6. Most injections occurred within the meA or areas slightly caudal to the meA. Only data from animals that received injections within the meA (on at least one side of the brain) were considered for subsequent comparisons (see below).

Group	n	Day 1	Day 2
Experimental	6	AVP	AVP
Control 1	6	AVP	NaCl
Control 2	10	NaCl	AVP
Control 3	6	NaCl	NaCl

The results from Control trials 1-3, which examined non-specific motor effects of the vehicle, nonspecific sensitization effects of the vehicle and nonspecific effects of the injection procedure respectively, are summarized in Fig. 7. No significant differences in motor responses between groups were seen with an initial injection (Day 1) of AVP (Experimental group) within the meA or an initial injection of any of the control solutions (Fig. 7.A) ($p > 0.79$; Kruskal-Wallis one-way ANOVA). In contrast, Day 2 experiments revealed highly significant differences between the different groups ($p < 0.006$; Kruskal-Wallis one-way ANOVA) (Fig. 7.B). Post-hoc comparisons between the Experimental group (AVP//AVP) and each treatment group revealed differences between these groups that ranged in significance from $p < 0.02$ to $p < 0.002$. Since three post-hoc comparisons were made, the α level was adjusted downward so that a minimum of $\alpha \leq 0.02$ was required for a difference to be considered statistically significant (i.e. $\alpha/c = 0.05/3 = 0.02$) for any multiple comparisons.

The first comparison controlled for nonspecific motor effects of the vehicle (Experimental group vs Control 1). Comparison of the behavioral responses of sensitized animals (i.e. received AVP on Day 1) to a subsequent injection of either AVP (Exp. group) or vehicle (Control 1) demonstrated that animals exhibited greater motor responses after AVP injection than after injection of the vehicle alone ($p < 0.004$; Mann-Whitney U test) (Fig. 7.B). The second comparison

controlled for nonspecific sensitizing effects of the vehicle (Experimental group vs Control 2). This comparison revealed that pretreatment with AVP induced greater sensitization than did pretreatment with the vehicle ($p < 0.02$; Mann-Whitney U test) (Fig. 7.B). The third comparison controlled for nonspecific motor effects due to the injection procedure (Experimental group vs Control 3). Comparison of these two groups revealed that the Experimental group (AVP//AVP) had a much greater ability to induce motor effects than consecutive treatments with vehicle ($p < 0.002$; Mann-Whitney U test) (Fig. 7.B).

Interestingly, the vehicle demonstrated a sensitizing ability, as animals injected with AVP after pretreatment with saline, exhibited stronger motor effects upon a subsequent (Day 2) injection than an initial injection (Day 1). However, this effect was not as great as that observed with AVP pretreatment (Fig. 7.B).

D. DISCUSSION

This study investigated whether AVP induces motor effects when injected into the meA. In addition, these experiments investigated whether these motor effects might undergo a sensitization process. As well, effects of a control solution (physiological saline) on both of these processes were examined. Finally, potential nonspecific effects of the injection procedure were assessed.

Data from the blind trial and from the inter-examiner reliability and

repeatability trials indicate that neither experimenter bias nor the reliability/repeatability of the scoring procedure were significant confounding factors in these experiments. Results from these investigations suggest that AVP causes motor effects when injected into the meA. The motor effects increase in severity via a sensitization process after an initial injection. These effects seem to be localized within the meA since AVP injected into sites outside the meA did not induce motor activity.

The curious observation of a saline-induced sensitization (Control group 2 - NaCl//AVP) merits further comment. The most likely explanation may be that the initial injection (Day 1) of a comparatively large volume of liquid into a small area of tissue, and the lowering of the injection cannulae into the brain, resulted in tissue damage, causing endogenous release of AVP. This endogenous release may have been enough to induce the sensitization process, such that subsequent injection (Day 2) of a pharmacological dose of AVP (92 pmol) had severe motor effects. Another plausible explanation also rests upon endogenous release of AVP. That is, the injection procedure involved touching the animals, and sometimes light restraint, in order to lower the injection cannulae into the brain successfully. Stressful conditions such as these have been associated with up to two hundred-fold increases in plasma AVP concentrations (Husain et al., 1979) and stressful conditions also produce large increases in CSF AVP content (Wang et al., 1981).

This may have contributed to the sensitization of these animals.

The question naturally arises as to why such postulated increases in CNS AVP would not cause motor effects, in addition to the sensitization effect (i.e. in Control group 3). The answer may lie in dosage. Endogenous release is unlikely to result in amygdalar levels of AVP that match the pharmacological doses employed in this study. Perhaps the induction of the sensitization process requires smaller concentrations of AVP than the induction of motor effects. Indeed, data from Burnard et al., (1983) would seem to corroborate this. This group found that stimuli that evoke endogenous release of AVP into the CNS (e.g. hemorrhage or hypertonic saline) could sensitize rats to the motor effects of subsequent AVP infusion but could not induce motor effects in sensitized animals.

Previous studies involving VSA injection of AVP have demonstrated that severe motor effects can result from injections with smaller doses of AVP than those involved in this study. Doses of 10 pmol AVP, injected icv or into the VSA, can cause severe motor effects in sensitized animals (Naylor et al., 1985; Poulin and Pittman, 1991, unpublished results). Although in preliminary trials, doses of 10 pmol AVP were injected into the meA of animals sensitized with a prior injection of AVP, the motor effects seen at these doses were minor (data not shown). This suggests that the meA may not be as important a locus for AVP-induced motor effects as the VSA. These findings were consistent with those by

Federico (1990), who found that antipyresis in the meA required doses approximately 80 times as great as the threshold doses for similar effects in the VSA.

An alternative explanation for AVP's weaker effects in the meA is that the meA is not an active site for AVP-induced motor effects, and that such effects are the result of diffusion of the neuropeptide to the VSA, either through tissue or through leakage into the ventricles. However, the fact that AVP injections closer to the VSA (the VSA extends approximately -1.8 mm (posterior) from bregma), for example, injections located at -2.3 mm did not elicit greater motor effects than injections in the meA argues against this suggestion. Similarly, injections occurring closer to the ventricles did not elicit greater motor effects than meA injections of AVP.

Thus, these data suggest that the meA may be a site at which AVP induces motor effects. This raises the possibility that the meA may form part of a complex neural network that includes the VSA and the vestibular cerebellum, where AVP has multiple behavioral roles. However, anatomical, physiological and pharmacological mechanisms through which these functions might be mediated remain obscure and merit further investigation.

Figure 2.

Comparison of scores attributed to the same group of rats (n=5) by two different examiners. Rats received unilateral injections of AVP on 2 consecutive days (10 pmol icv in 1.0 μl pyrogen-free, physiological saline). No significant differences were observed between groups scored by both examiners on either Day 1 ($p > 0.50$; Mann-Whitney U test) or Day 2 ($p > 0.41$; Mann-Whitney U test).

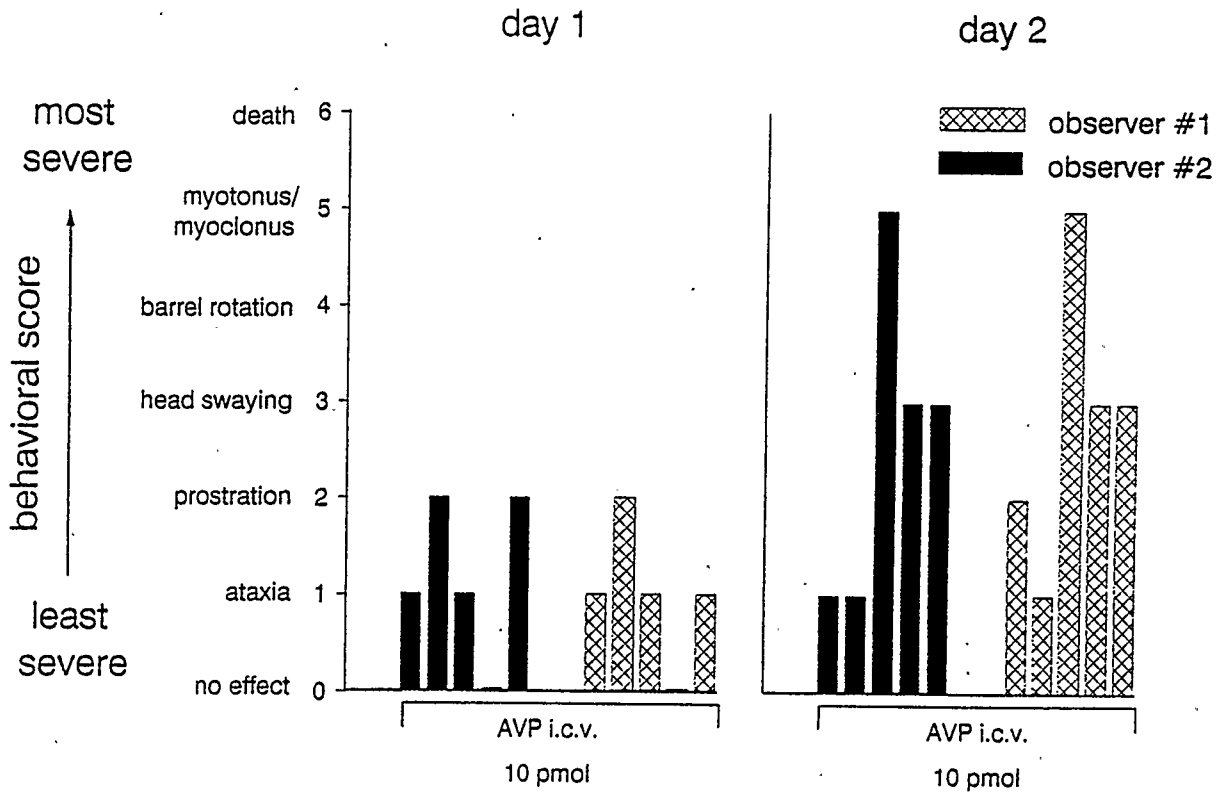
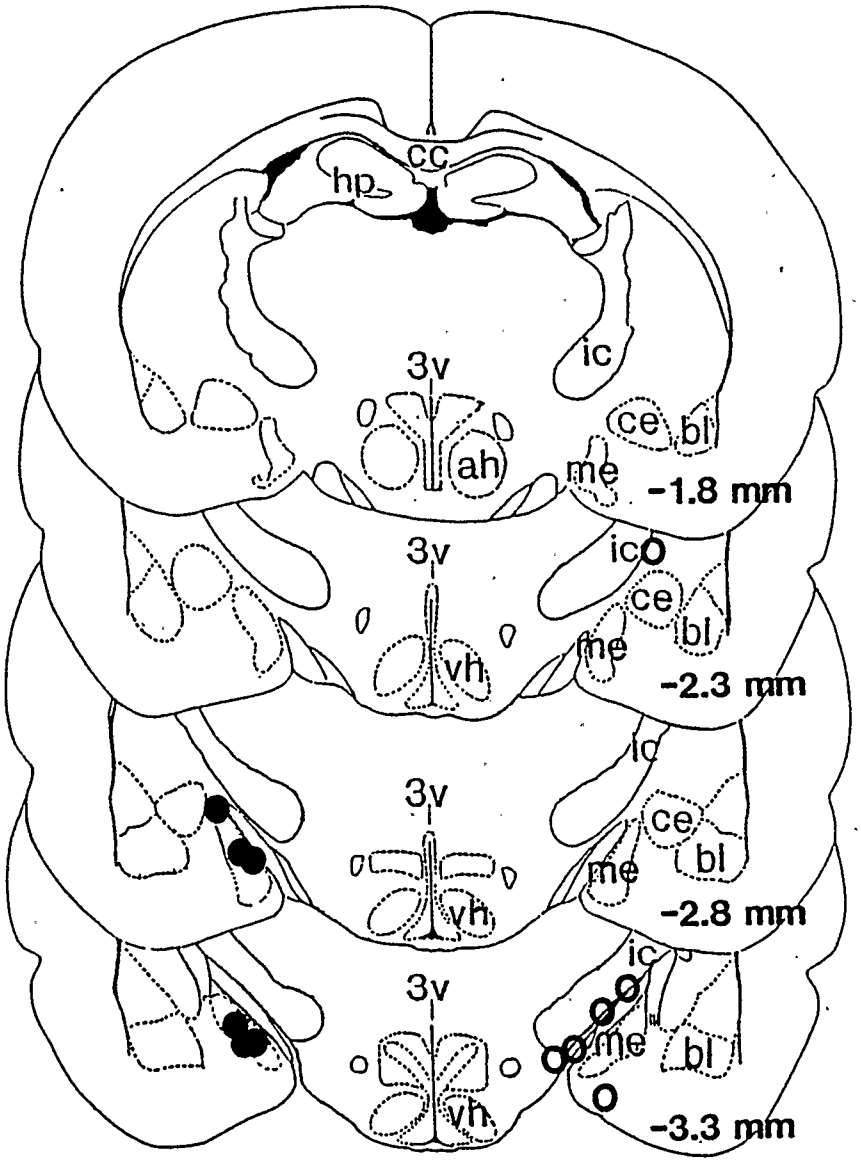

day 1
day 2
most severe
behavioral score
least severe
death 6
myotonus/ myoclonus 5
barrel rotation 4
head swaying 3
prostration 2
ataxia 1
no effect 0
observer #1
observer #2
AVP i.c.v.
10 pmol
AVP i.c.v.
10 pmol

Figure 3.

Schematic histological sections of the rat forebrain illustrating injection sites that were localized to the meA (filled circles, n=6) or to areas surrounding but not impinging upon the meA (unfilled circles, n=6) for the Experimental group (AVP//AVP). For clarity, each animal is represented by a single injection site. Injection sites that were localized to the meA are illustrated on the left side of the appropriate histological sections while injection sites outside the meA are illustrated on the right side of the appropriate sections. Abbreviations: 3V, third ventricle; ah, anterior hypothalamus; bl, basolateral amygdaloid nucleus; cc, corpus callosum; ce, central amygdaloid nucleus; hp, hippocampal area; ic, internal capsule; me, medial amygdaloid nucleus; vh, ventromedial hypothalamus. Note: Figs. 4 and 5 refer to data obtained from this group of animals.


cc
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me
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-2.8 mm
ic
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me
bl
vh
-3.3 mm

Figure 4.

A. Behavioral responses of rats to an initial bilateral injection (Day 1) of AVP (92 pmol in 1.0 μl pyrogen-free, physiological saline) within the meA compared to behavioral responses of a different group of rats to the same treatment but outside of the meA. Each bar represents the most severe behavioral score an individual animal (n=6, within meA; n=6, outside meA) received during the 10 min observation period following AVP injection. No significant difference was noted between groups ($p > 0.47$; Mann-Whitney U test).

B. Behavioral responses of rats to a second bilateral injection (Day 2) of AVP (92 pmol in 1.0 μl pyrogen free, physiological saline) within the meA (n=6) compared to behavioral responses of a different group of rats to the same treatment but outside of the meA (n=6). The group receiving injections within the meA exhibited stronger responses than the group receiving injections outside the meA ($p < 0.006$; Mann-Whitney U test).

SITE SPECIFICITY

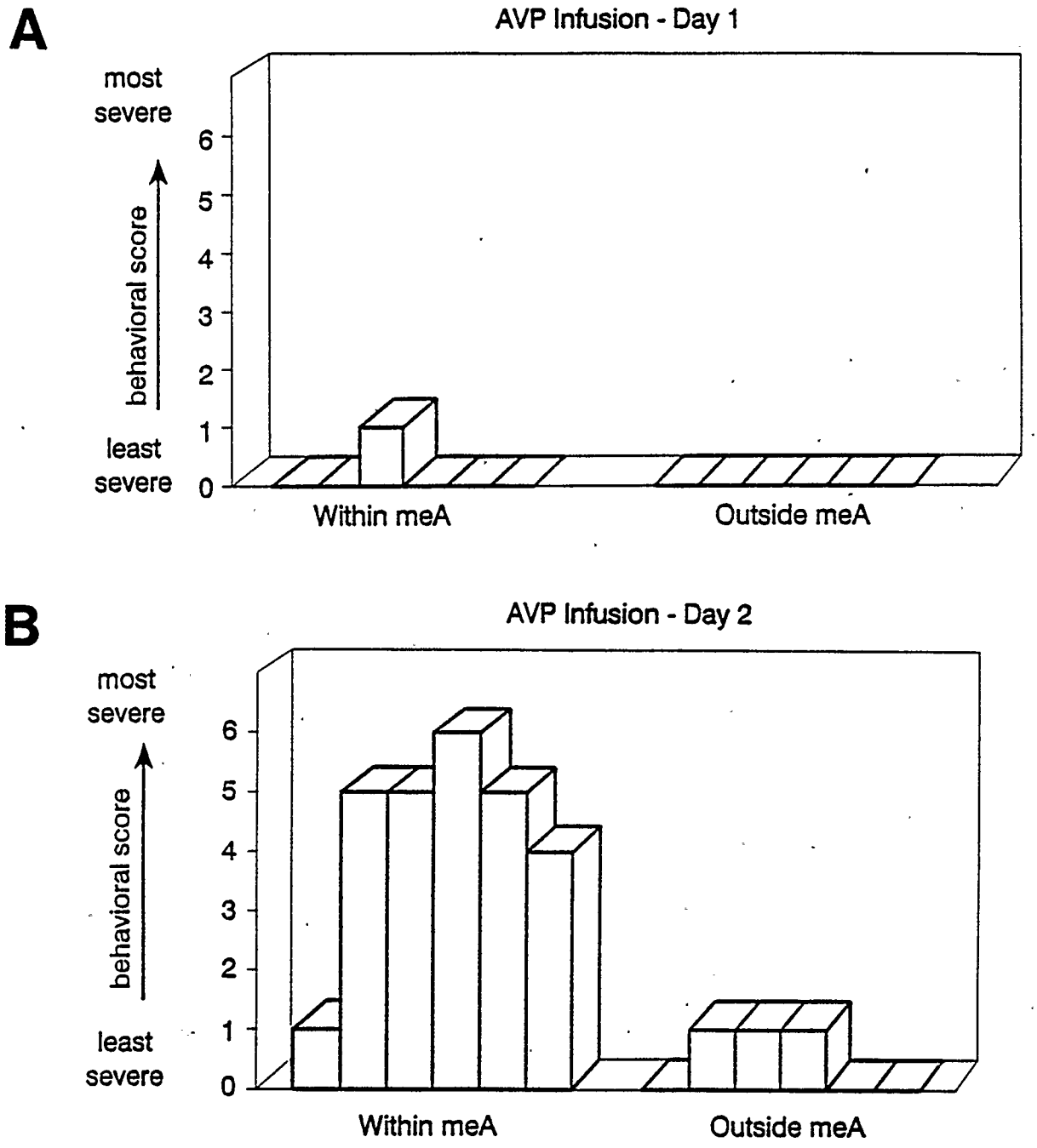


Figure 5.

A. Behavioral responses of rats (n = 6) to bilateral injections of AVP (92 pmol in 1.0 μl pyrogen-free, physiological saline) on 2 consecutive days within the meA. The responses were greater to a second injection ($p < 0.03$; Wilcoxon matched-pairs signed-ranks test).

B. Behavioral responses of rats (n = 6) to bilateral injections of AVP (92 pmol in 1.0 μl pyrogen-free, physiological saline) on 2 consecutive days outside the meA. No significant difference was observed between groups ($p > 0.20$; Wilcoxon matched-pairs signed-ranks test).

SENSITIZATION

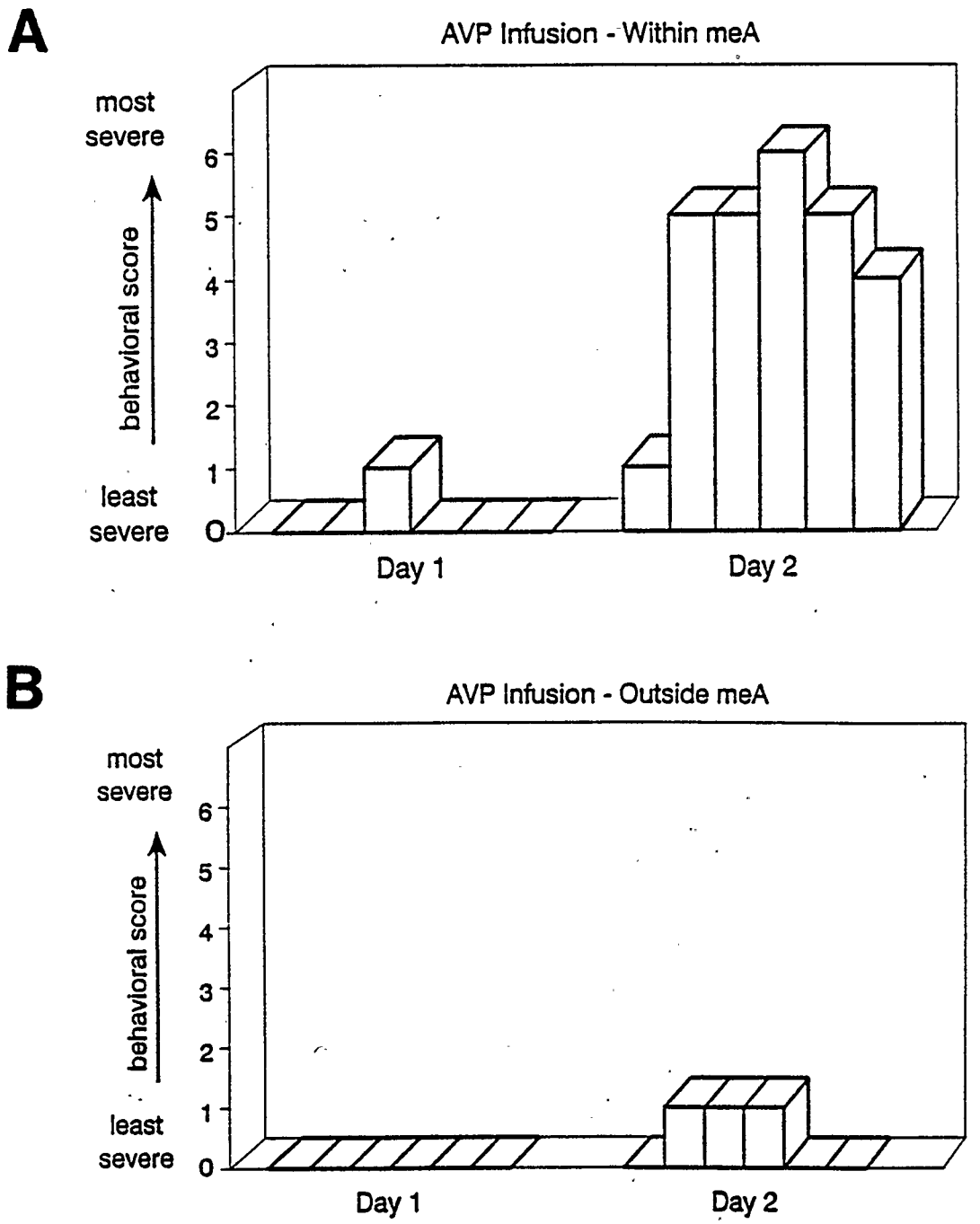
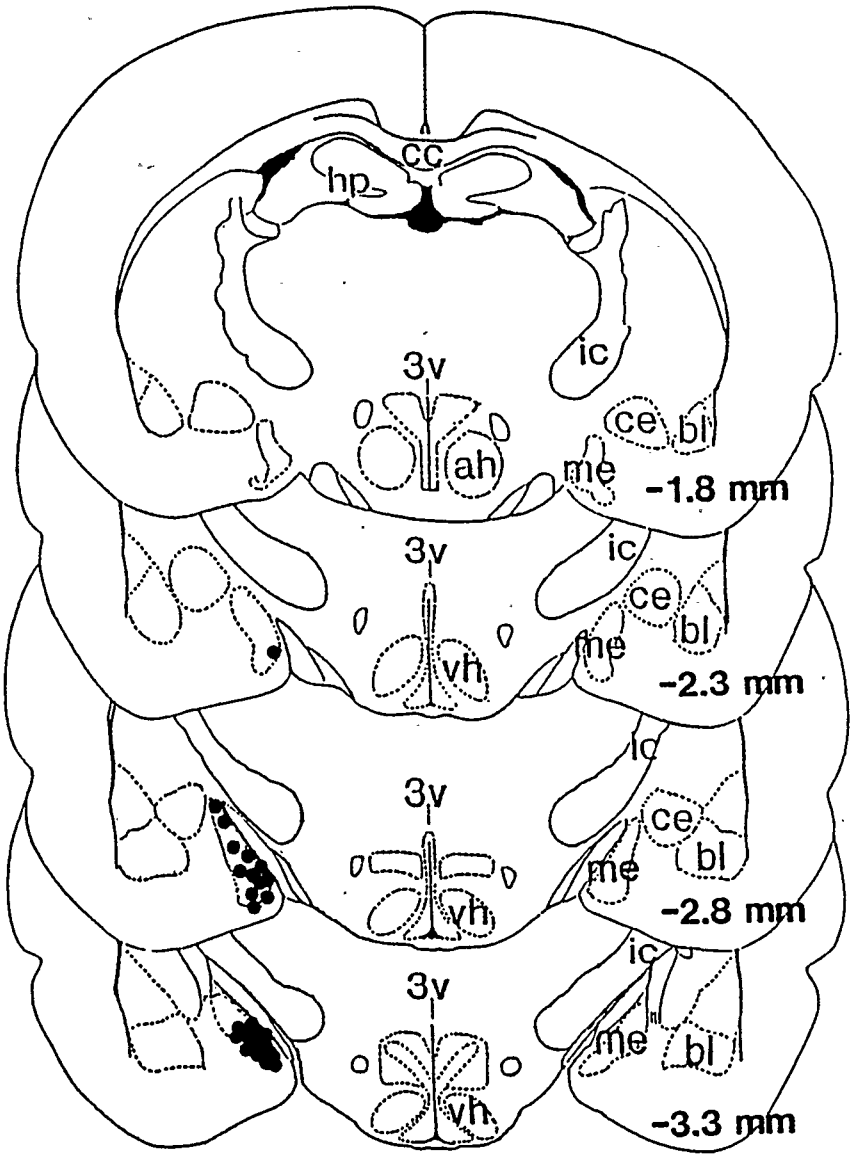


Figure 6.

Schematic histological sections of the rat forebrain illustrating injection sites that were localized to the meA (filled circles) for the Experimental group and Control groups 1-3 (n=28). For clarity, each animal is represented by a single injection site, on the left side of the appropriate histological section. Abbreviations: 3V, third ventricle; ah, anterior hypothalamus; bl, basolateral amygdaloid nucleus; cc, corpus callosum; ce, central amygdaloid nucleus; hp, hippocampal area; ic, internal capsule; me, medial amygdaloid nucleus; vh, ventromedial hypothalamus. Note: Fig. 7 refers to data obtained from this group of animals.


cc
hp
ic
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-1.8 mm
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me
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-2.3 mm
ic
3v
ce
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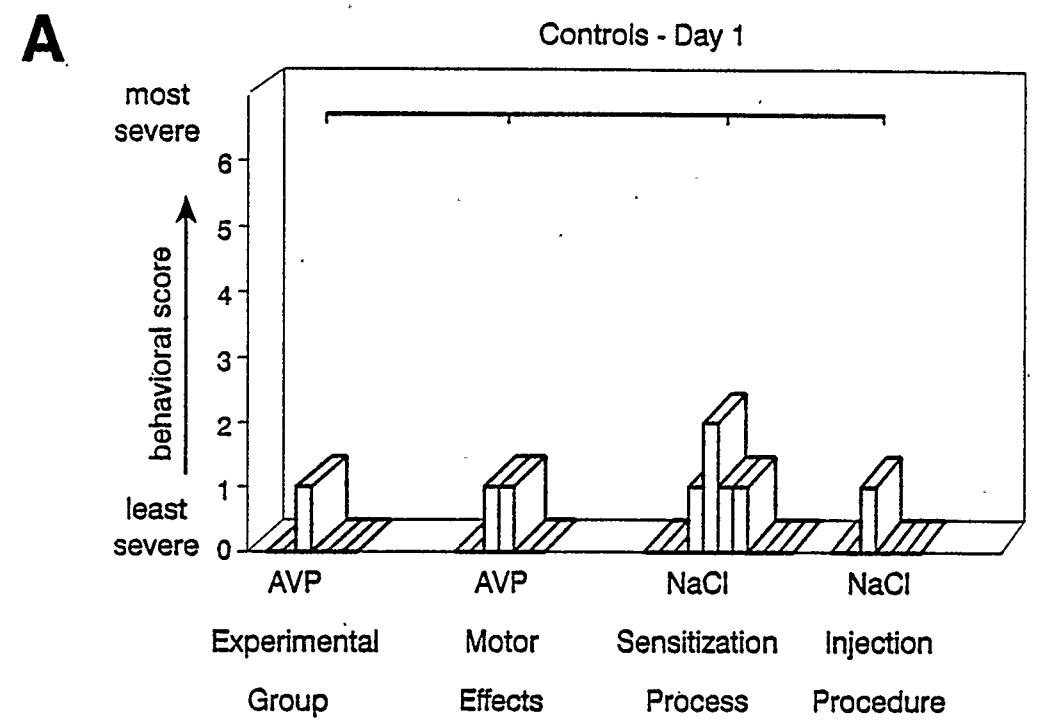
me
vh
-2.8 mm
ic
3v
me
bl
vh
-3.3 mm

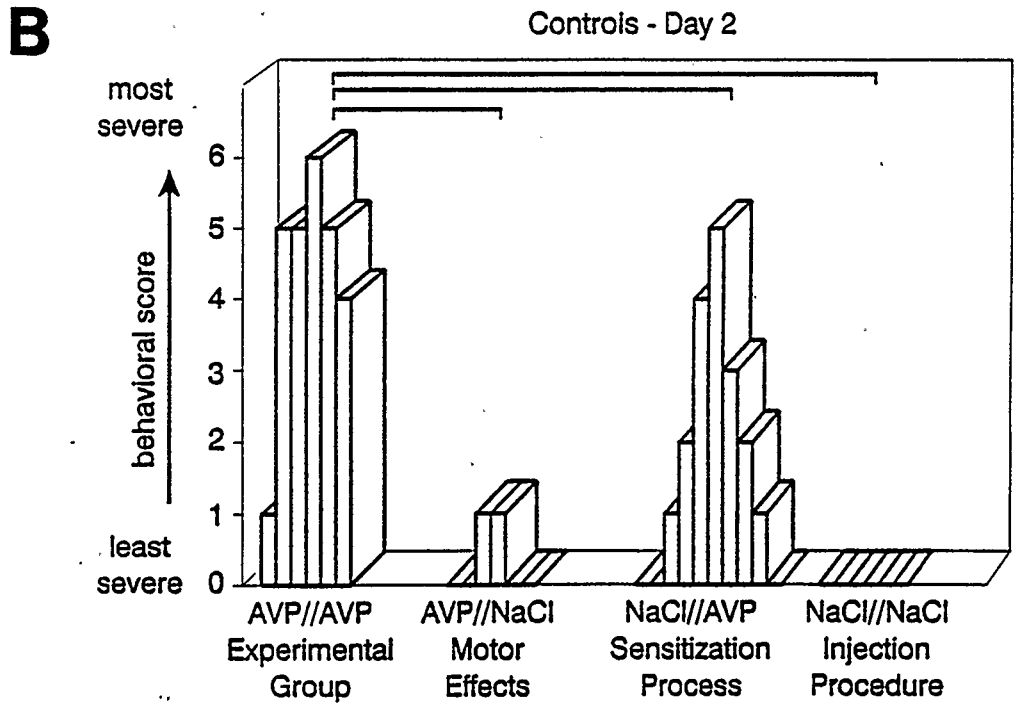
Figure 7.

A. Behavioral responses of different groups of rats to an initial (Day 1) bilateral injection of AVP (92 pmol in 1.0 μl pyrogen-free, physiological saline) or vehicle alone (1.0 μl pyrogen-free, physiological saline) such that rats received one of the following treatments: Exp. - AVP (n = 6); C_1 - AVP (n = 6); C_2 - NaCl (n = 10); C_3 - NaCl (n = 6). No significant differences were observed between groups ($p > 0.79$; Kruskal-Wallis one-way ANOVA).

B. Behavioral responses of different groups of rats to a second bilateral injection (Day 2) of either AVP (92 pmol in 1.0 μl pyrogen-free, physiological saline) or vehicle alone (1.0 μl pyrogen-free, physiological saline) such that rats received one of the following 2 day treatments: Exp. - AVP//AVP (n = 6); C_1 -AVP//NaCl (n = 6); C_2 - NaCl//AVP (n = 10); C_3 - NaCl//NaCl (n = 6) (// - indicates 24 h interval). Significant differences were observed in the behavioral responses between groups ($p < 0.006$; Kruskal-Wallis one-way ANOVA) (Exp. > C_1, $p < 0.004$; Exp. > C_2, $p < 0.02$; Exp. > C_3, $p < 0.002$; Mann-Whitney post-hoc comparisons).

Note: The bars indicate which groups were compared.


A
Controls - Day 1
most severe
behavioral score
least severe
0
1
2
3
4
5
6
AVP
AVP
NaCl
NaCl
Experimental Group
Motor Effects
Sensitization Process
Injection Procedure


B
Controls - Day 2
most severe
behavioral score
least severe
0
1
2
3
4
5
6
AVP//AVP
AVP//NaCl
NaCl//AVP
NaCl//NaCl
Experimental Group
Motor Effects
Sensitization Process
Injection Procedure

CHAPTER 3

AVP-INDUCED MOTOR EFFECTS IN THE MEDIAL AMYGDALA: CHARACTERIZATION OF THE RECEPTOR(S) MEDIATING THE MOTOR AND SENSITIZATION EFFECTS

A. INTRODUCTION

Presently, the mechanism(s) mediating the putative motor and sensitization effects of AVP in the meA are unknown. Previous studies that investigated similar effects icv or in the VSA suggest that these effects may be receptor mediated (Burnard et al., 1986; P. Poulin, 1991, personal communication). These studies support a role for receptors that resemble the V_1-type (vasopressor) of AVP receptor in both the motor and the sensitization effects observed following icv or VSA injection of AVP. The sensitization, however, is not limited to V_1-like receptors as V_2 (antidiuretic) (P. Poulin, 1991, personal communication), oxytocin (Poulin and Pittman, 1988) and somatostatin (Burnard et al., 1985) receptors have also been implicated.

Therefore, since AVP binding sites exist in the amygdala (Caffé and van Leeuwen, 1983; Dorsa et al., 1984; De Kloet et al., 1985) and since experimental evidence suggests at least some of these sites may represent AVP receptors of the V_1 type (Dorsa et al., 1984; Lawrence et al., 1988), the possibility exists that AVP may be exerting its motor and sensitization effects in the meA through a receptor

that resembles the V_1 type.

Hence, experiments were undertaken to investigate the possible receptor basis for AVP's motor and sensitization effects in the meA. Specifically, investigations focused on whether these processes could be altered by meA injection with a relatively selective antagonist of the V_1 receptor ([1-(β-mercapto-β, β-cyclopentamethylenepropionicacid),2-(O-methyl)tyrosine]arginine-vasopressin; $d(CH_2)_5$Tyr(Me)AVP; Kruszynski et al., 1980) or by meA injection with a relatively selective agonist of the V_2 subtype of vasopressin receptor (1-desamino-8-D-arginine vasopressin; DD AVP; Sawyer et al., 1974).

B. METHODS

Thirty-six male, Sprague-Dawley rats were used as subjects. Each weighed 250-300 g at the time of surgery. All rats underwent the same experimental protocol described in Chapter 2.B with the exception of the following:

Animals were assigned randomly to one of five groups (see below).

Group	n	Day 1	Day 2
Control	10	AVP	AVP
Experimental 1	6	AVP	V_1 antagonist/AVP
Experimental 2	6	AVP	V_2 agonist
Experimental 3	8	V_1 antagonist/AVP	AVP
Experimental 4	6	V_2 agonist	AVP

/ indicates 5 min separation

Trials were designed with the following objectives: one, to assess whether a V_1 AVP antagonist [$d(CH_2)_5$Tyr(Me)AVP] (Bachem) could block both the motor effects and the sensitization effect observed following meA AVP injection, suggesting a role for the V_1 receptor in these processes; two, to assess whether a V_2 AVP agonist [DD AVP] could mimic the motor effects and the sensitization effect observed following meA AVP injection, suggesting a role for the V_2 receptor in these processes.

As in Chapter 2.B all doses of AVP were 92 pmol in 1 μl pyrogen-free, physiological saline. The V_1 antagonist and the V_2 agonist were administered in doses of 100 pmol in 1 μl pyrogen-free, physiological saline.

At the end of all experimental trials, animals were separated into two groups: those who received injections within the meA on at least one side of the brain and those who received injections in sites surrounding, but not impinging upon the meA, on both sides of the brain. Only data from animals who received injections within the meA (on at least one side of the brain) were analyzed. Results were expressed as the highest score each animal received throughout the 10-min observation period following injection. Results from each experimental day (Day 1 or Day 2), were presented in raw form and groups were compared according to the Kruskal-Wallis one-way ANOVA by ranks. If significant differences were found ($\alpha \leq 0.05$), the control group was compared with the

experimental groups using the Mann-Whitney U test, with a downward adjustment of the α level, to compensate for inflation of the α value, common to multiple comparison procedures (Dawson-Saunders and Trapp, 1990). α was divided by the number of comparisons (c) in order to maintain the overall probability of a type 1 error at 0.05 (i.e. each comparison must be significant at α/c to be declared statistically significant). As four post-hoc comparisons were merited (Control vs Exp. 1-4) a p-value of 0.01 was considered a significant difference between experimental and control groups.

C. RESULTS

Histological verification of injection sites revealed that most injections occurred within the meA and surrounding areas. Twenty-six of an initial thirty-six rats were considered to have received injections within the meA on at least one side of the brain (see below). Only data from animals that received injections within the meA were considered for analysis (Fig. 8).

Group	n	Day 1	Day 2
Control	6	AVP	AVP
Experimental 1	5	AVP	V_1 antagonist/AVP
Experimental 2	5	AVP	V_2 agonist
Experimental 3	5	V_1 antagonist/AVP	AVP
Experimental 4	5	V_2 agonist	AVP

/ indicates 5 min separation

Although the design of this study consisted of trials involving all five groups concurrently, and data were analyzed in accordance with this protocol (i.e. all groups were compared with each other using the Kruskal-Wallis one-way ANOVA), the groups used to investigate the potential receptor basis for AVP's motor effects (Exp. 1 and Exp. 2) were separated from the groups used to investigate the potential receptor basis for the sensitization process (Exp. 3 and Exp. 4) for illustrative purposes.

Figure 9 displays the injection sites from the animals used to investigate the potential receptor basis for AVP's motor effects (Control group and Experimental groups 1 and 2).

Figure 10.A illustrates the results obtained from animals in the Control group (AVP//AVP), Experimental group 1 (AVP// V_1 antagonist/AVP) and Experimental group 2 (AVP//V_2 agonist). These data indicate that the groups did not differ significantly in behavioral responses to an initial (Day 1) meA injection with AVP ($p > 0.30$; Kruskal-Wallis one-way ANOVA).

Conversely, when the same groups of animals (now "sensitized") were subjected to a second infusion, 24 h after the first, significant differences in behavioral responses were noted between groups ($p < 0.001$; Kruskal-Wallis one-way ANOVA). Post-hoc comparison of Control vs Experimental group 1 revealed that the group which received the V_1 antagonist ($d(CH_2)_5Tyr(Me)AVP$) 5 min prior

to AVP (i.e. Experimental group 1) exhibited much weaker behavioral responses to AVP than the Control group ($p < 0.008$; Mann-Whitney U test). In addition, the group which received the V_2 agonist (i.e. Experimental group 2) instead of AVP had much weaker behavioral responses than the Control group ($p < 0.008$; Mann-Whitney U test) (Fig. 10.B).

Figure 11 illustrates injection sites from animals used to investigate the potential receptor basis for AVP's sensitization effects. No significant differences in behavioral responses were found between groups who received either AVP (Control), the V_1 antagonist ($d(CH_2)_5Tyr(Me)AVP$) (Exp. 3) followed 5 min later by AVP, or the V_2 agonist (DD AVP) (Exp. 4) ($p > 0.30$; Kruskal-Wallis one-way ANOVA) on Day 1 (Fig. 12.A).

Conversely, Day 2 experiments revealed significant differences in behavioral responses between groups ($p < 0.001$; Kruskal-Wallis one-way ANOVA). Post-hoc comparison revealed that rats who were treated with AVP on 2 consecutive days responded with greater behavioral scores on Day 2 than those who were treated with a V_1 antagonist 5 min prior to AVP infusion (Day 1), and then treated with AVP (Day 2) ($p < 0.01$; Mann-Whitney U test). However, no significant differences were noted in behavioral responses of rats to Day 2 treatment with AVP when they had received prior treatment (Day 1) with either AVP or V_2 agonist (Fig. 12.B) ($p > 0.17$; Mann-Whitney U test).

D. DISCUSSION

Several studies have provided evidence for the existence of AVP receptors in the meA. For example, autoradiographic and radioligand binding studies demonstrate putative receptors for AVP in the meA (Caffé and van Leeuwen, 1983; Dorsa et al., 1984). Behavioral and physiological experiments suggest that many CNS binding sites may represent receptors of the V_1 type because AVP, administered icv, has effects that are reversibly blocked by the same V_1 antagonist ($d(CH_2)_5Tyr(Me)AVP$) that was used in these experiments (Matsuguchi et al., 1982; De Wied et al., 1984; Burnard et al., 1986). In light of this, experiments were undertaken to investigate the potential receptor basis for the motor effects and for the sensitization process observed with amygdalar AVP injection.

Data from these experiments indicate that the motor and sensitization effects seen with amygdalar AVP injection were abolished by V_1 receptor blockade. These data agree with previous experiments that suggest a role for the V_1 receptor as a mediator of many peptide-induced motor effects in the CNS. For example, a systematic survey of 33 neurohypophyseal peptide hormones (and analogues) by Meisenberg and Simmons (1982) provided evidence that motor effects similar to those observed in this study were well correlated with their V_1 receptor potency.

Putative AVP receptors in the meA may be similar to those at which AVP exhibits its motor and sensitization effects in the VSA, as AVP-induced effects

were also abolished in the VSA by prior exposure to the V_1 antagonist [$d(CH_2)_5$Tyr(Me)AVP] (P. Poulin, 1991, personal communication). V_1-AVP binding sites have been characterized in the VSA and these sites seem to represent functional receptors. Support for this comes from studies that demonstrate an increase in phosphatidyl inositol turnover (Shewey and Dorsa, 1988) and modulation of neuronal electrical impulses to other neurotransmitters upon AVP binding (Joëls and Urban, 1984; Disturnal et al., 1987; Raggenbass et al., 1987).

The data from this work suggest that the V_2 receptor is unlikely to be involved with the motor effects induced by AVP, since the potent V_2 agonist [DD AVP] had no significant motor effects upon meA injection. This is not surprising in light of negative results obtained from studies which utilized the same protocol in the VSA (P. Poulin, 1991, personal communication). In other studies, the V_2 agonist [DD AVP] was only about 5% as potent in stimulating other AVP-induced behavioral effects (e.g. avoidance behavior)(Walter et al., 1978), lending support to this conclusion. Also in agreement with these data are studies that fail to detect cAMP (V_2 second messenger) accumulation after AVP application to membrane preparations or homogenates from various brain regions (Courtney and Raskind, 1983; Dorsa et al., 1983), which may suggest little V_2-mediated brain activity.

Interestingly, the V_2 agonist was found to have some sensitizing ability, since Day 2 injection with AVP resulted in a significant increase in motor activity

after prior (Day 1) exposure to this agonist. This was not significantly different from AVP's ability to induce sensitization. The V_2 agonist [DD AVP] does have some V_1 agonist ability (approximately 5% as potent as AVP) which may be enough to induce the sensitization process. In agreement with this are results from Poulin (P. Poulin, 1991, personal communication) that indicate a role for V_2-mediated sensitization in the VSA.

In conclusion, these data support a role for AVP in the induction and sensitization of motor effects in the meA. Data indicate that the V_1 receptor may, at least in part, mediate the AVP-induced motor and sensitization effects in the meA. In addition, a role for the V_2 receptor in the sensitization process may be indicated.

Figure 8. Schematic histological sections of the rat forebrain illustrating injection sites that were localized to the meA (filled circles) for the Control group and Experimental groups 1-4 (n=26). For clarity, each animal is represented by single injection site, on the left side of the appropriate histological section. Abbreviations: 3V, third ventricle; ah, anterior hypothalamus; bl, basolateral amygdaloid nucleus; cc, corpus callosum; ce, central amygdaloid nucleus; hp, hippocampal area; ic, internal capsule; me, medial amygdaloid nucleus; vh, ventromedial hypothalamus.

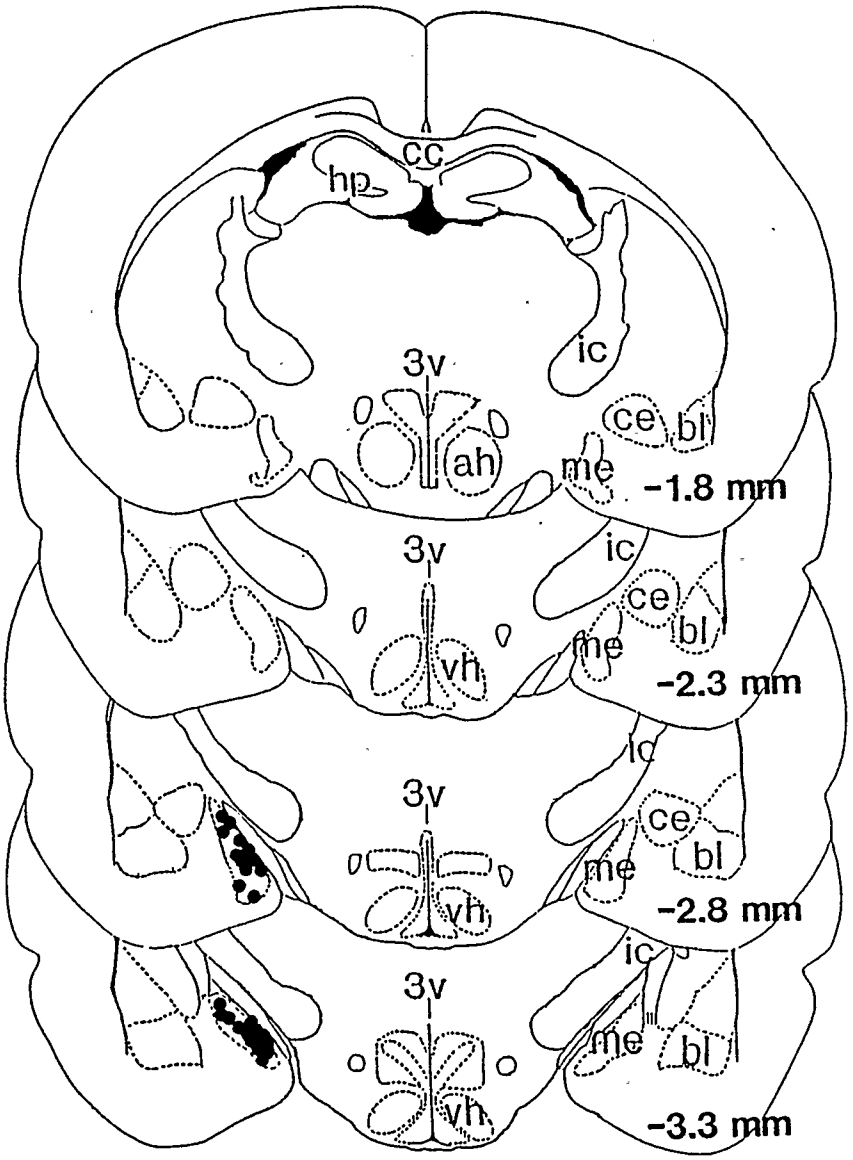

cc
hp
ic
3v
ah
ce
bl
me
-1.8 mm
3v
ic
ce
bl
me
vh
-2.3 mm
ic
3v
ce
bl
me
vh
-2.8 mm
ic
3v
me
bl
vh
-3.3 mm

Figure 9. Schematic histological sections of the rat forebrain illustrating injection sites that were localized to the meA (filled circles) for the Control group and Experimental groups 1 and 2 (n=16). For clarity, each animal is represented by a single injection site, on the left side of the appropriate histological section. Abbreviations: 3V, third ventricle; ah, anterior hypothalamus; bl, basolateral amygdaloid nucleus; cc, corpus callosum; ce, central amygdaloid nucleus; hp, hippocampal area; ic, internal capsule; me, medial amygdaloid nucleus; vh, ventromedial hypothalamus. Note: Fig. 10 refers to data obtained from this group of animals.

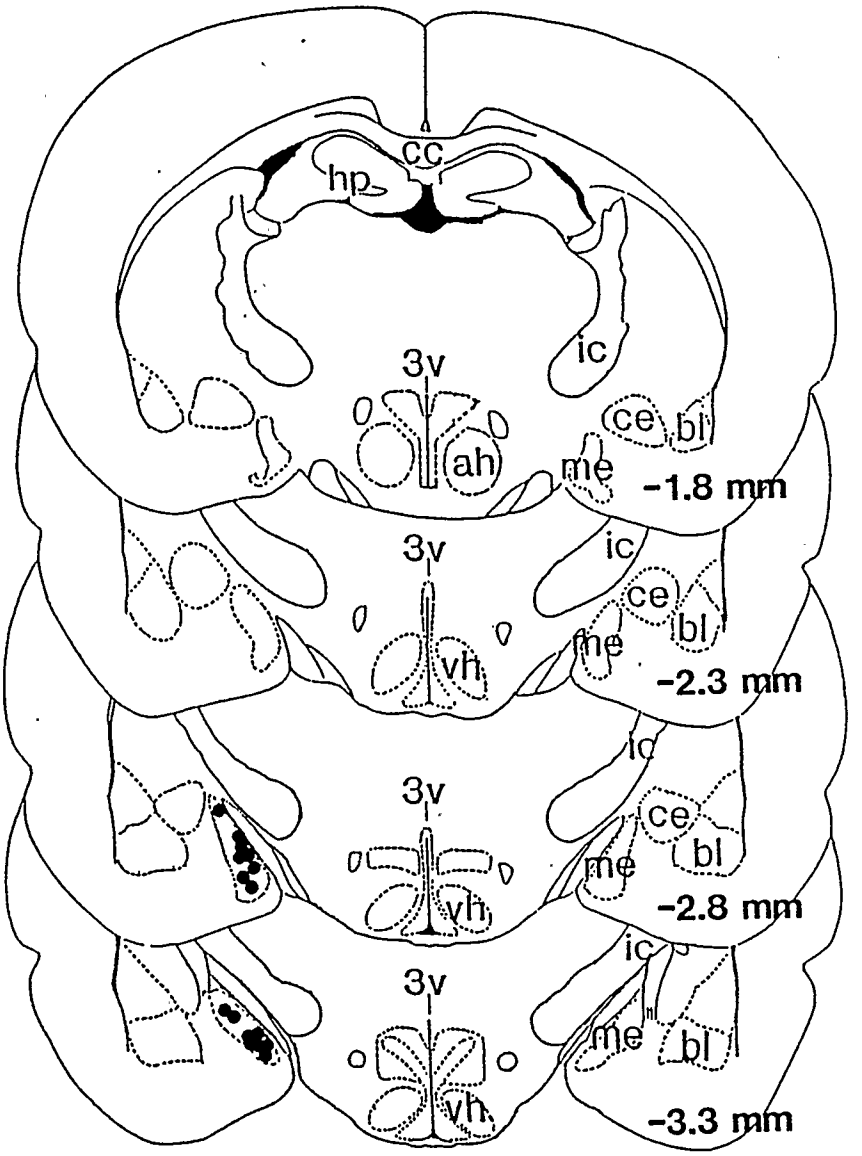

cc
hp
ic
3v
ce
bl
ah
me
-1.8 mm
3v
ic
ce
bl
me
vh
-2.3 mm
ic
3v
ce
bl
me
vh
-2.8 mm
ic
3v
me
bl
vh
-3.3 mm

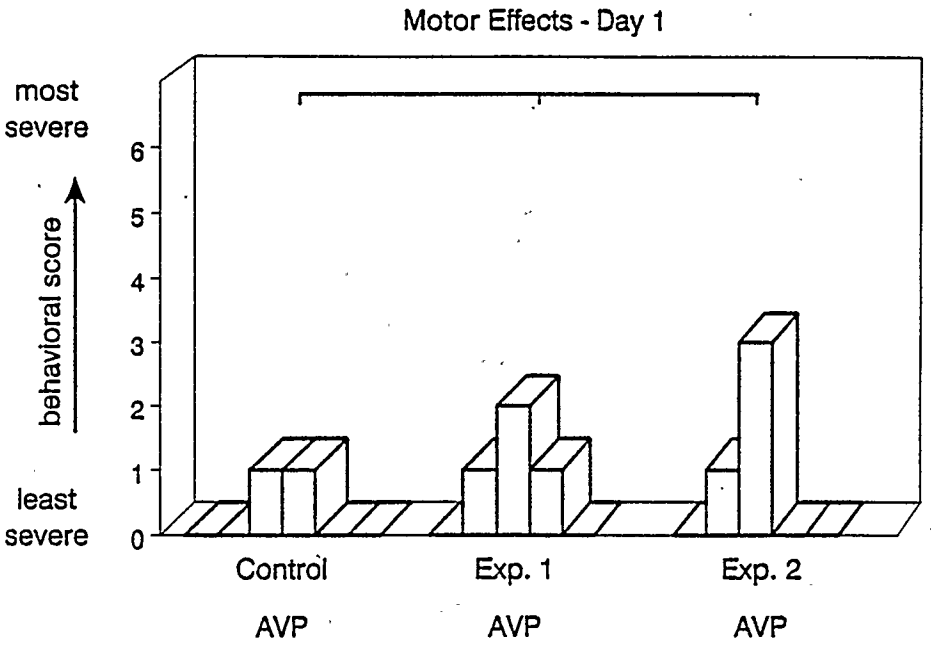
Figure 10

A. Behavioral responses of rats to an initial (Day 1) bilateral injection of AVP (92 pmol in 1.0 μl pyrogen-free, physiological saline) (Control group, n=6; Experimental group 1, n=5; Experimental group 2, n=5). No significant differences were observed in behavioral responses between groups ($p > 0.30$, Kruskal-Wallis one-way ANOVA).

B. Behavioral responses of rats to a second (Day 2) bilateral meA injection. Rats received one of the following 2 day treatments: Control - AVP//AVP (n=6); Exp. 1 - AVP//V_1 antagonist/AVP (n=5); Exp. 2 - AVP//V_2 agonist (n=5) . (// - indicates 24 h interval; / - indicates 5 min interval). V_1 antagonist - d$(CH_2)_5$Tyr(Me)AVP; V_2 agonist - DD AVP. Doses were 100 pmol in 1.0 μl pyrogen-free, physiological saline (except AVP - 92 pmol). Significant differences were observed in the behavioral responses between groups ($p < 0.001$; Kruskal-Wallis one-way ANOVA) (Control > Exp. 1 @ $p < 0.008$; Control > Exp. 2 @ $p < 0.008$; Mann-Whitney post-hoc comparisons).

Note: The bars indicate which groups were compared.

A


Motor Effects - Day 1
most severe
behavioral score
least severe
0
1
2
3
4
5
6
Control
AVP
Exp. 1
AVP
Exp. 2
AVP

B

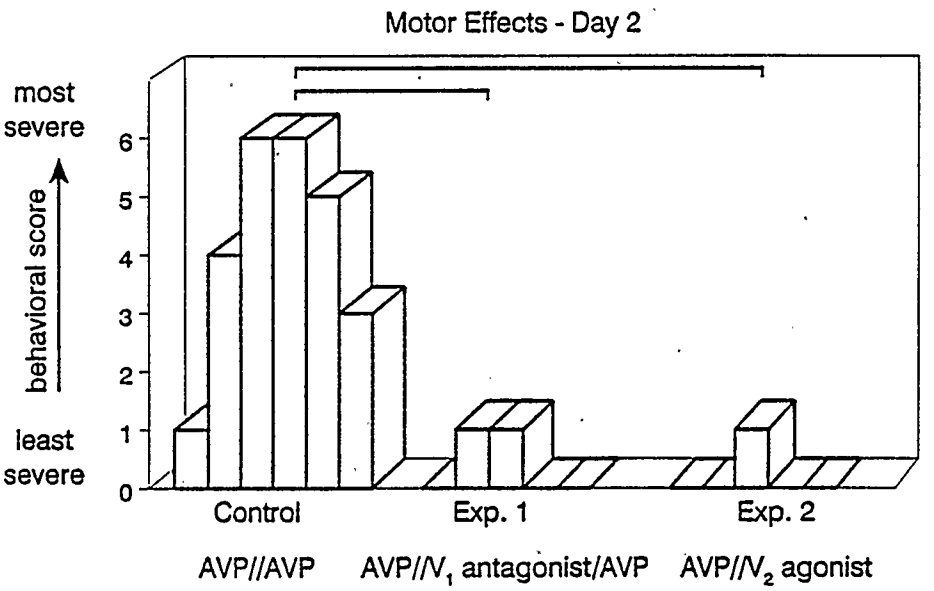

Motor Effects - Day 2
most severe
behavioral score
least severe
0
1
2
3
4
5
6
Control
AVP//AVP
Exp. 1
AVP//V1 antagonist/AVP
Exp. 2
AVP//V2 agonist

Figure 11.

Schematic histological sections of the rat forebrain illustrating injection sites that were localized to the meA (filled circles) for the Control group and Experimental groups 3 and 4 (n=16). For clarity, each animal is represented by a single injection site, on the left side of the appropriate histological section. Abbreviations: 3V, third ventricle; ah, anterior hypothalamus; bl, basolateral amygdaloid nucleus; cc, corpus callosum; ce, central amygdaloid nucleus; hp, hippocampal area; ic, internal capsule; me, medial amygdaloid nucleus; vh, ventromedial hypothalamus. Note: Fig. 12 refers to data obtained from this group of animals.

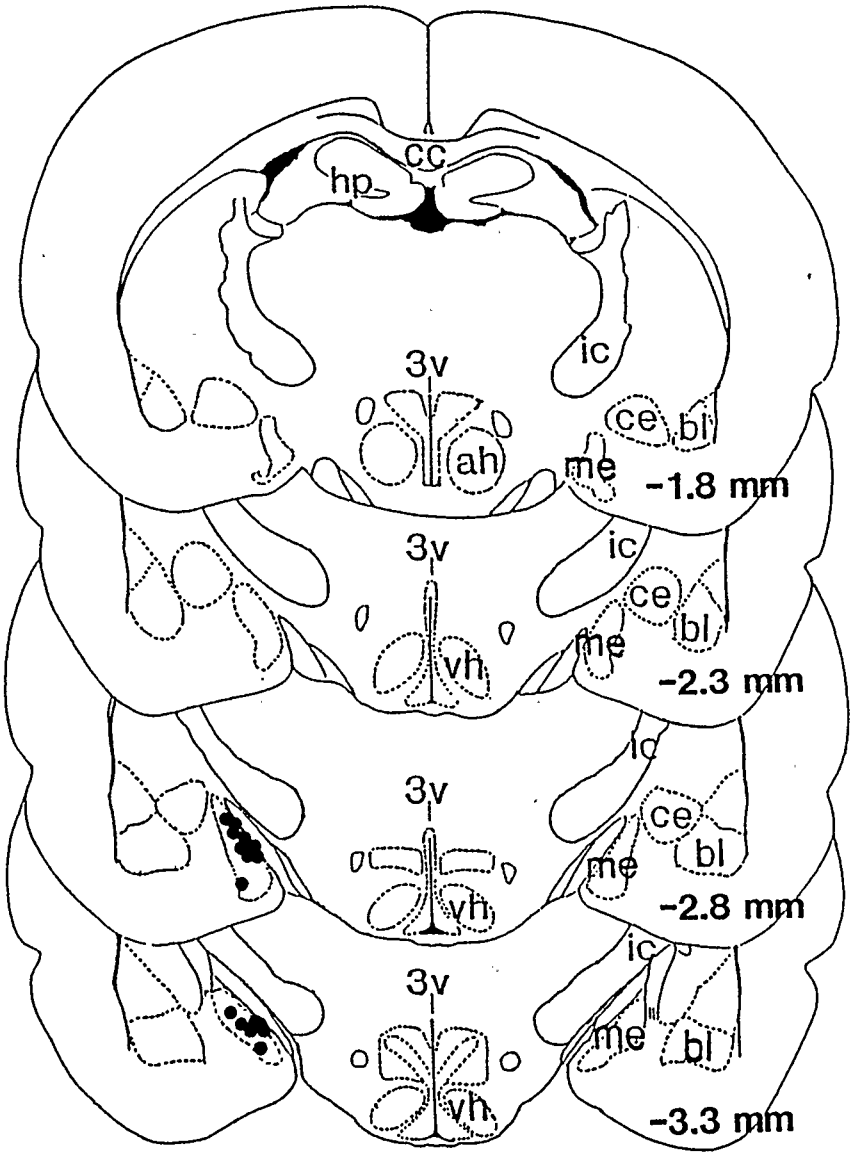
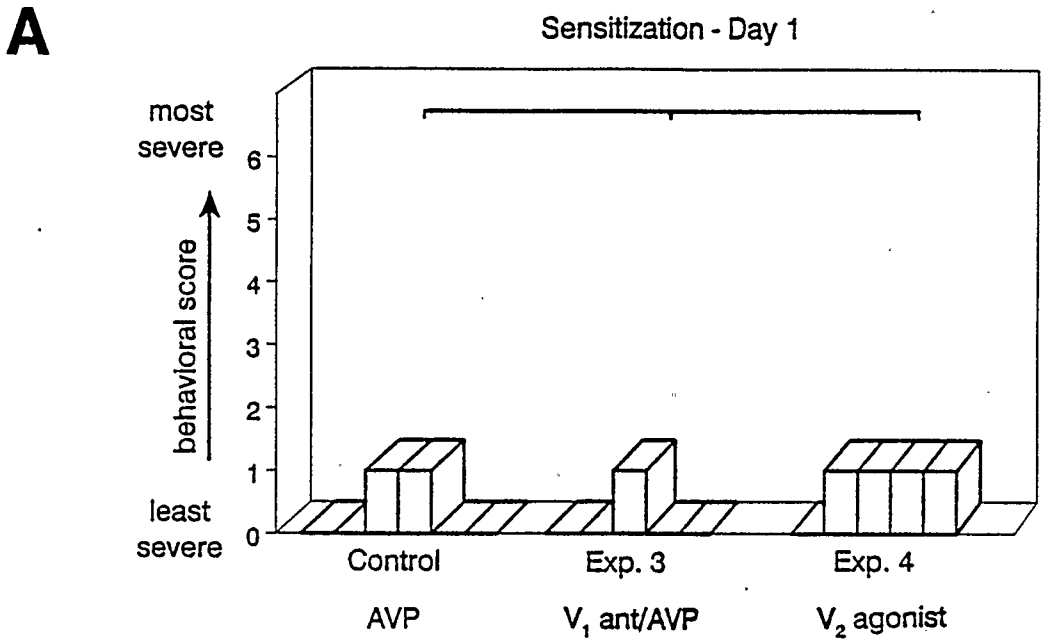

cc
hp
3v
ah
ic
ce
bl
me
-1.8 mm
3v
vh
ic
ce
bl
me
-2.3 mm
3v
vh
ic
ce
bl
me
-2.8 mm
3v
vh
ic
me
bl
-3.3 mm

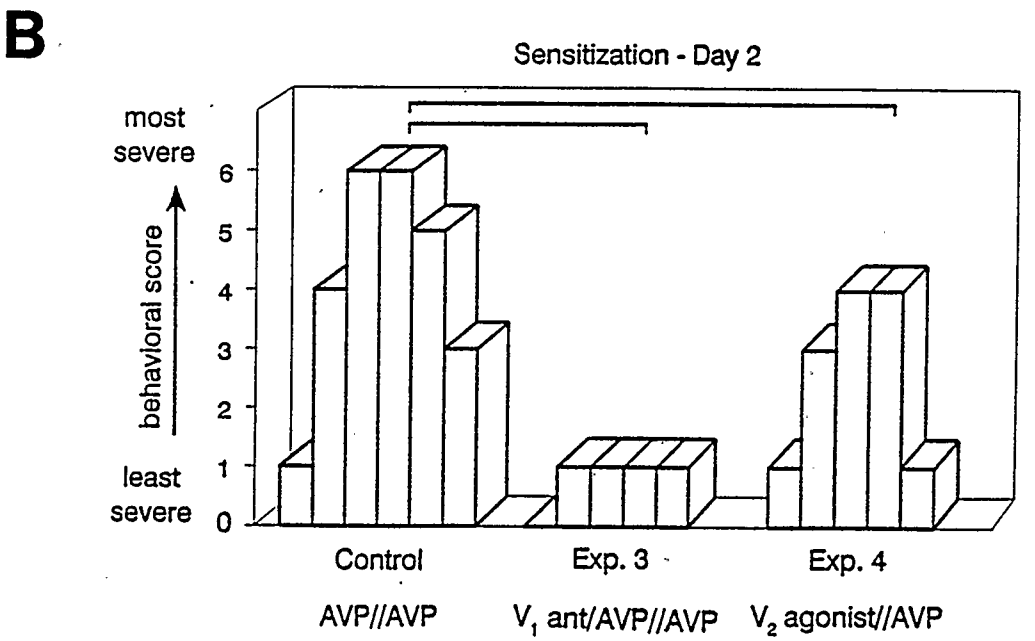
Figure 12

A. Behavioral responses of rats to an initial (Day 1) bilateral meA injection of: Control - AVP (n=6); Exp. 3 - V_1 antagonist/AVP (n=5); Exp. 4 - V_2 agonist (n=5). V_1 antagonist - $d(CH_2)_5Tyr(Me)AVP$; V_2 agonist - DD AVP. Doses were 100 pmol in 1.0 μl pyrogen-free, physiological saline (except AVP - 92 pmol). No significant differences were observed in behavioral responses between groups ($p > 0.30$; Kruskal-Wallis one-way ANOVA).

B. Behavioral responses of rats to a second (Day 2) bilateral meA injection. Rats received one of the following 2 day treatments: Control - AVP//AVP (n=6); Exp. 3 - V_1 antagonist/AVP//AVP (n=5); Exp. 4 - V_2 agonist//AVP (n=5) . (// - indicates 24 h interval; / - indicates 5 min interval). V_1 antagonist - $d(CH_2)_5Tyr(Me)AVP$; V_2 agonist - DD AVP. Doses were 100 pmol in 1.0 μl pyrogen-free, physiological saline (except AVP - 92 pmol). Significant differences were observed in the behavioral responses between groups ($p < 0.001$; Kruskal-Wallis one-way ANOVA) (Control > Exp. 3 @ $p < 0.01$; Control not sig. diff. from Exp. 4 @ $p > 0.17$; Mann-Whitney post-hoc comparisons).

Note: The bars indicate which groups were compared.


A
Sensitization - Day 1
most severe
behavioral score
least severe
0
1
2
3
4
5
6
Control
AVP
Exp. 3
V1 ant/AVP
Exp. 4
V2 agonist


B
Sensitization - Day 2
most severe
behavioral score
least severe
0
1
2
3
4
5
6
Control
AVP//AVP
Exp. 3
V1 ant/AVP//AVP
Exp. 4
V2 agonist//AVP

CHAPTER 4

GENERAL DISCUSSION AND CONCLUSIONS

Arginine vasopressin has been recognized as a neurotransmitter candidate in the central nervous system. Several CNS functions for this neuropeptide have been suggested including putative roles in motor behavior, possibly related to behavioral thermoregulation or vestibular physiology. The aim of this work has been to investigate a potential role for the meA in the mediation of such AVP-induced motor behavior.

A. WHAT IS KNOWN

CNS-administered peptides can produce a variety of motor effects. These include stereotyped behaviors such as grooming (Meisenberg, 1981; Ferris et al., 1984) and escape-like locomotion (Meisenberg, 1981), and as well as motor effects that range from minor disturbances of motor behavior such as ataxia and prostration, to severe disturbances, such as barrel rotations and convulsive activity (Kruse et al., 1977; Abood et al., 1980; Kasting et al., 1980). Many of these peptide-induced effects have been correlated closely with their V_1 receptor potency (Meisenberg and Simmons, 1982).

AVP is one of the most potent peptides for producing motor effects upon

CNS administration (for review see Burnard, 1985b; Balaban et al., 1989). AVP-induced motor effects have been investigated in some detail and have been observed upon ventricular injection (Kasting et al., 1980) or after infusion of tissue loci in the vestibular cerebellum (Maiti et al., 1986) or ventral septal area (Naylor et al., 1985). Previous studies suggest a role for the V_1-type (vasopressor) of AVP receptor as a mediator of AVP's motor effects. In addition, evidence suggests that these motor effects may be sensitized via the V_1 receptor (Burnard et al., 1986), as well as via V_2 (antidiuretic) (P. Poulin, 1991, personal communication), oxytocin (Poulin and Pittman, 1988) and somatostatin (Burnard et al., 1985) receptors, and may involve post-receptor processes (P. Poulin, 1991, personal communication).

Hence, the evidence suggests that AVP induces motor effects through multiple sites of action, a sensitization process is involved, these effects appear to be receptor mediated and may involve some post-receptor processes.

B. WHAT THIS WORK HAS CONTRIBUTED

The experiments presented as part of this work were intended to investigate whether arginine vasopressin might induce motor effects, including severe motor behaviors, upon medial amygdalar injection. Therefore, the following studies were performed:

First, experiments were performed that investigated AVP's motor effects in the meA. These investigations demonstrated that AVP induces various motor effects upon meA injection and that these effects involve a sensitization process. Thus, a sensitive tissue locus was established in the meA for AVP-induced motor and sensitization effects.

Second, a potential receptor basis for the motor and sensitization effects was investigated. These investigations demonstrated that the motor and sensitization effects could be blocked via prior injection with a V_1 antagonist; in addition, a V_2 agonist had no motor effects in sensitized animals, but appeared to trigger the sensitization process. Therefore, it was concluded that AVP-induced motor and sensitization effects might be mediated, at least in part, through V_1-like AVP receptors. In addition, the sensitization process might be mediated via additional receptor types, including V_2 AVP receptors.

Thus, these findings support the hypothesis that AVP has CNS-mediated motor effects. The function of these motor effects are, as yet, unclear but may be related to behavioral thermoregulation and/or vestibular physiology. The next section will explore these possibilities.

C. WHAT IS CONTROVERSIAL

Although several studies have investigated AVP-induced motor effects and the sensitization process, the mechanisms for these processes have yet to be fully elucidated and their functions have not yet been established. Thus far, explorations of potential mechanisms of action have centered on three main approaches - anatomical, physiological and pharmacological.

Anatomical investigations have revealed AVP-specific motor effects in basal forebrain (VSA and meA) and cerebellar loci. Each of these sites also exhibits a sensitization effect. Whether these loci are separate and redundant systems or form part of an interactive neural network is not known. Direct projections from the amygdala to the motor cortex have recently been suggested by the PHA-L anterograde tracing technique (Kita and Kitai, 1990) as have amygdalar projections to the extrapyramidal motor system (Gonzales and Chesselet, 1990). In addition, cerebellar influence over motor activity has been well documented (Bloedel, 1973; Ito, 1984; Andersson et al., 1987). Thus, there exists the potential for these loci to affect motor behavior independent of one another.

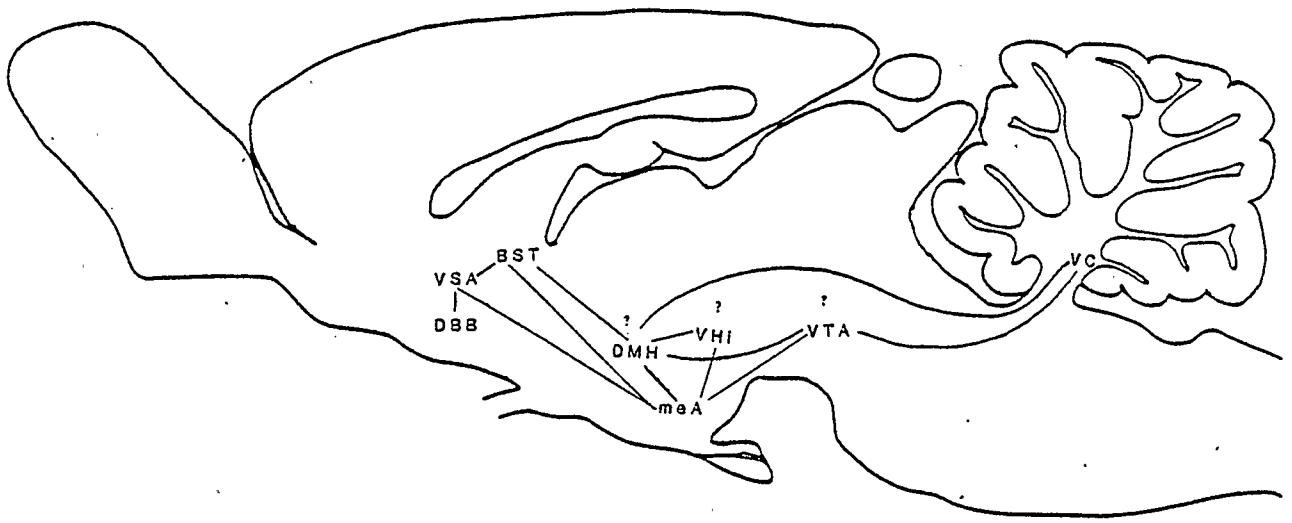
However, each locus also exhibits at least moderate AVP binding, varying densities of AVP fiber innervation and a close proximity to the ventricles. Therefore, communication between these loci might occur through fiber connections and/or ventricular release of AVP.

The evidence for direct connections between loci is sparse. Nevertheless, the potential exists for communication through a third party, such as hypothalamic (e.g. dorsomedial, ventromedial or lateral hypothalamic nuclei) or thalamic nuclei. Interestingly, Haines et al. (1990) have recently shown direct reciprocal connections between hypothalamic and cerebellar nuclei. These hypothalamic nuclei also project to CNS sites including the VSA, meA, hippocampus and basal ganglia, as well as to brainstem centers involved in a wide variety of autonomic and motor functions (Veazey et al., 1982; Shen, 1983; Ter Horst and Luiten, 1986). Of particular note is that stimulation of the cerebellar nuclei involved with this pathway leads to vasopressor responses, suggesting a potential role for AVP (Miura and Reis, 1969, 1970; Nisimaru and Kawaguchi, 1984; Chida et al., 1986) (see Fig. 13).

In addition, Royce et al. (1991) provide evidence for another potential site of interaction between brain loci sensitive to AVP's motor effects and other potentially related brain areas, including areas implicated in motor behavior. This site may occur in the centromedian thalamic nuclei. For example, the amygdala, POAH, dorsal, lateral and posterior hypothalamic nuclei, vestibular cerebellum, V-L thalamus, ventral tegmental area, vestibular nuclei, among other nuclei have recently been shown by WGA-HRP and rhodamine tracing methods to possess

Figure 13

A postulated neural network for AVP-induced motor effects. The neural connectivity of these sites has been reviewed in the text of this work. Question marks indicate that it is not known whether the site in question is a sensitive site for AVP-induced motor effects. BST, bed nucleus of the stria terminalis; DBB, diagonal band of Broca; DMH, dorsomedial hypothalamus; meA, medial amygdaloid nucleus; VC, vestibular cerebellum; VHi, ventral hippocampus; VSA, ventral septal area; VTA, ventral tegmental area.


VSA
BST
DBB
?
DMH
?
VHl
?
VTA
meA
VC

direct afferent connections to these nuclei.

Hence, there is some evidence for a neuroanatomical substrate which might connect the amygdala, VSA, and vestibular cerebellum with each other and motor areas. However, it is ill defined and as yet it has not been shown capable of mediating AVP-induced motor effects.

The potential also exists for interaction of the vasopressinergic system with other neurotransmitter systems. There is evidence to suggest that vasopressin may, at least in part, exert its motor effects via interaction with catecholaminergic nerve terminals. For example, AVP increases norepinephrine and dopamine turnover in several brain areas (Tanaka et al., 1977; Kovacs et al., 1977; Versteeg et al., 1979) and interference with catecholaminergic systems prevents many of the behavioral effects of vasopressin (Kovacs et al., 1977, 1980).

To identify possible interactions between sites and systems it would be useful to employ receptor blocking studies that involve AVP injection in one active locus and injection of receptor antagonists in other active loci. For example, one study might involve injecting a V_1 antagonist into the VSA prior to injection of the meA with AVP. Numerous combinations and permutations of this theme could be contrived.

A criticism that must be addressed in these studies is peptide diffusion. In the past, it has been difficult to attribute the effects of peptide injection to discrete

brain loci because of potential diffusion to distant areas such as the ventricles. Injection with equipment that allows for reliable delivery of nanoliter volumes may help with unwanted diffusion.

Lesioning studies that employ substances that lesion cell bodies but spare fibers of passage (e.g. kainic or ibotenic acid) combined with peptide injection may also be useful in assessing the relative contribution of each locus to the motor/sensitization effects. In addition, electrophysiological studies may help elucidate potential interactions between sites such as the vestibular cerebellum, the VSA and the meA. These studies may also help to clarify the extent that nuclei bordering the VSA (such as the BST) contribute to the motor and sensitization effects seen with AVP injection. Anatomical connectivity and density of AVP binding suggest that other potentially active loci might include the central amygdala, ventral hippocampus, several basal ganglia sites (e.g. VTA), and possibly the dorsomedial, ventromedial or lateral hypothalamus.

The physiological mechanisms responsible for AVP-induced motor and sensitization effects remain elusive. This is due, in part, to the lack of strong experimental evidence implicating endogenous AVP release in either the motor or sensitization effects. Some evidence exists that implicates endogenous AVP release in the sensitization process (Burnard et al., 1983), however, the mechanisms used to induce this release (osmotic stimulation through hemorrhage

or administration of hypertonic saline) are also potent stimuli for oxytocin release (Dyball, 1971; Brimble et al., 1978). Therefore, whether such endogenous sensitization is due to AVP's effects or those of oxytocin or another, as yet undetermined agent, is controversial. Nevertheless, this experimental paradigm represents the most successful approach to date for studying the effects of endogenous AVP release.

Despite the limited success with these experimental paradigms, there is evidence that implicates endogenous release of AVP in several disorders of motor behavior, especially certain convulsive disorders. For example, tumors that cause excessive release of AVP have been linked to convulsive activity, and disorders of water regulation which lead to overhydration also induce convulsive activity in humans (for review see Andrew, 1991). In addition, exposure to high ambient temperatures (associated with elevated plasma levels of AVP) (Forsling et al., 1976) can also lead to convulsions (see review see Burnard et al., 1986a). Furthermore, plasma vasopressin increases after certain tonic-clonic seizures in man as well as after kindling of seizure activity in rats (Aminoff et al., 1984; Greenwood et al., 1991). Finally, evidence suggests amygdalar kindling results in a long term alteration in genomic expression, resulting in enhanced AVP mRNA translation in several areas of the rat brain (Greenwood et al., 1989), which may be an indication of increased endogenous AVP release. Thus, there is some

physiological evidence for AVP's involvement in motor behavior, especially disorders of motor behavior, however most of this evidence is correlative and requires more direct experimental investigation.

Pharmacological investigations of mechanisms for AVP-induced motor and sensitization effects have yielded some interesting results. As outlined earlier in this work, the vast majority of investigations have implicated the V_1 receptor in AVP's motor effects and in the sensitization process, although other receptors have also been implicated (i.e. V_2, oxytocin, somatostatin) in the sensitization process.

Interestingly, Poulin and Pittman (1991, unpublished results) found no evidence for AVP receptor upregulation in the sensitization process. This suggests that the sensitization effect might be mediated via some other process, for example, a post-receptor process involving altered protein synthesis. Perhaps this might result in more efficient coupling between the AVP receptor and aspects of the signal transduction process. In support of this, Poulin and Pittman (1991, unpublished results) found increases in inositol phosphate levels in the VSA of rats 24 h after a septal injection with AVP. There is also some evidence to suggest altered protein synthesis may enhance signal transduction in peripheral V_1 receptors (Bielinski et al., 1988).

It is not known whether different agonists induce their sensitization effects via similar mechanisms or whether they utilize separate mechanisms, perhaps

involving separate pathways, that may converge in a common sensitization locus. In agreement with the latter suggestion is data from Balaban et al., (1989) indicating that lesioning of the AVP-sensitive site in the vestibular cerebellum abolishes AVP's ability to induce the sensitization process, via icv exposure. A useful study might involve similar lesioning of the cerebellum and subsequent VSA or meA injections of AVP (or other putative sensitizing agents) to examine whether sensitization previously initiated in these areas might also be abolished.

Finally, the issue of putative roles for AVP-induced motor and sensitization effects in physiology and pathophysiology must be addressed. Although the physiological significance of AVP-induced motor effects has been questioned (Gash et al., 1987), experimental evidence has accumulated for postulated roles in behavioral thermoregulation and vestibular function.

There is considerable evidence that AVP may be involved with central thermoregulation (for review see Cooper, 1987). Since a pivotal study by Kasting et al. (1978), many studies have suggested that AVP may act as an endogenous antipyretic in the brain (for review see Cooper, 1987; Pittman et al., 1988). Studies have also shown that the POAH and lateral hypothalamic areas are major thermoregulatory centers of the brain and may play a role in behavioral thermoregulation (Hainsworth and Epstein, 1966; Roberts and Robinson, 1969; Satinoff and Shan, 1971; Roberts and Mooney, 1974). Interestingly, both the

VSA and the meA have been shown to have extensive vasopressinergic connections with these areas (Zeisberger et al., 1983; de Olmos et al., 1985; Bleier and Byne, 1985). In addition, immunocytochemical work has shown increased AVP reactivity in fibers connecting these areas during fever (Zeisberger et al., 1983, 1986). Thus, it is possible that AVP, acting in the VSA and in the meA, may reduce febrile body temperature through interaction with these brain loci perhaps, in part, through activating behavioral heat loss mechanisms.

Similarly, these sites are indirectly connected through the stria terminalis and the ventral amygdalofugal pathway to areas implicated in motor behavior (for review see de Olmos et al., 1985) and the amygdala itself has recently been shown to have direct projections to the motor cortex (Kita and Kitai, 1990). These pathways might provide an anatomical substrate for AVP's motor effects, including thermoregulatory behaviors, and if overstimulated might induce motor disturbances.

As demonstrated in this work, the motor effects in response to medial amygdalar AVP injection increase in severity after an initial exposure to AVP, demonstrating a sensitization effect. Sensitization of the motor effects of AVP also occurs after repeated AVP injection into the ventral septal area (for review see Pittman et al., 1988). As well, repeated icv administration of AVP sensitizes both the motor effects of AVP and AVP-induced antipyresis (P. Poulin, 1991, personal

communication). Since the sites of action for the antipyretic and motor effects of AVP are similar, and since these effects can be sensitized by repeated exposure to AVP, it is possible that they may be correlated. This would further support a role for AVP in behavioral thermoregulation. In addition, because repeated administration of AVP can result in severe motor behaviors such as myoclonic and/or myotonic convulsions, it is possible that AVP-induced sensitization might provide a mechanism through which AVP might induce or mediate patho-physiological thermoregulatory behaviors, such as febrile convulsions.

Thus, this raises the possibility that tissue loci sensitive to the motor and sensitization effects of AVP are part of a complex neural network that may induce antipyresis, in part through behavioral heat loss mechanisms, and may be involved in the pathogenesis of febrile convulsions. The role of AVP in such a postulated network merits further investigation.

Equally attractive is the putative role AVP plays in vestibular physiology and pathophysiology. AVP is hypothesized to be one of an important group of peptides that controls central vestibular circuits that are specialized for motor and sensory functions, and are involved in the maintenance of postural and ocular stability. Balaban et al. (1989) have suggested that AVP-induced motor disturbances represent asymmetric activation of central structures that process vestibular information and mediate postural stability. Postural stability is thought

to be regulated through an interconnected neural network that includes vestibular nuclei, midline cerebellar cortex, the fastigial nucleus, midbrain structures near the interstitial nucleus of Cajal and basal forebrain structures such as the BST and the amygdala.

A convincing array of studies has been gathered to support this hypothesis including anatomical, physiological and pharmacological evidence. They propose a four stage model with several peptide (AVP or somatostatin) sensitive sites and one or more peptide insensitive sites that may summate the effects of the peptide-sensitive sites. In addition, the cerebellum is seen as playing a key role in adjusting the sensitivity of the system, hence it is postulated as crucial to AVP-induced behavioral sensitization.

However attractive the model may seem its workings are hypothetical at this point and it is not yet clear which neuronal populations might underlie the putative stages. Nevertheless, the hypothetical sites of action and the internal dynamics of the model itself are open to experimental verification with peptide injection studies. Electrophysiological and tract tracing studies may also prove useful, if only to verify the neuroanatomical connectivity of this postulated model.

In conclusion, investigations were undertaken to explore the potential for AVP to induce motor and sensitization effects upon meA injection, and to investigate the potential receptor basis for such effects. Results of these

investigations support a neurotransmitter role for AVP in CNS-mediated motor behavior. Current data suggest that arginine vasopressin may induce motor activity through a complex, perhaps inter-related neural network, of which the meA may be part. A possible function for these motor behaviors may be in behavioral thermoregulation and/or vestibular physiology. Further investigations are required to elucidate the potential role AVP may play in motor behavior.

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