

INTRACEREBROVENTRICULAR VASOPRESSIN
PRODUCES NORADRENERGIC β -RECEPTOR-DEPENDENT
POTENTIATION OF THE PERFORANT PATH-EVOKED
POTENTIAL IN THE DENTATE GYRUS *in vivo*

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**Intracerebroventricular vasopressin produces
noradrenergic β -receptor-dependent potentiation of the
perforant path-evoked potential in the dentate gyrus *in
vivo***

by

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Abstract

Long-term potentiation of a synaptic and/or spike response, which is widely accepted as a model for neuronal plasticity, was initially elicited by high frequency stimulation in the hippocampus (Bliss and Lomo, 1973), but can also be induced by neuromodulators like norepinephrine (NE; Neuman and Harley, 1983; Walling and Harley, 2004). The neuropeptide vasopressin (AVP) (Chen et al, 1993; Chepkova et al, 2001; Dubrovsky et al, 2002; Dubrovsky et al, 2003) also produces enhancement of synaptic responses and has a role in promoting memory (Bohus et al, 1982). *In vitro* studies have shown NE plays a role in AVP-initiated increases in cyclic AMP (cAMP) (Brinton, 2000; Brinton, 1993).

It has been proposed that NE, released into the dentate gyrus *in vivo* mediates AVP memory enhancement (Bohus et al, 1993). This thesis examined 1) the perforant path-dentate gyrus evoked response after intracerebroventricular (icv) AVP; 2) the β -adrenergic influence on perforant path AVP effects through the use of a double pipette procedure using the β -adrenergic antagonist, propranolol; and 3) further examined the pharmacology of the perforant path AVP response by the use of the V1a selective agonist DGAVP, an AVP fragment without peripheral effects, in the urethane anesthetized rat.

In the first experiment, AVP (1 ng; icv) produced potentiation of perforant path population spike amplitude in 15/16 animals and of the EPSP slope in 11/16 animals. AVP significantly increased the population spike amplitude with a mean increase of 50% over baseline at 30 minutes after AVP. Individual animals showed increases of up to 100% at peak potentiation. The mean increase for EPSP slope was 21% over baseline at 30 minutes after AVP. Those experiments followed beyond an hour (n=4) showed mean amplitude increases of 100% at 60 minutes and mean slope increases of 24% at 60 minutes. At 100 minutes post AVP, mean spike amplitude was at a 90% increase with the EPSP slope at a 16% increase above baseline.

In the second experiment using a two-pipette recording technique, perforant path evoked potentials were recorded using a β -adrenergic antagonist (propranolol)-filled pipette and a saline-filled pipette before and after icv AVP. AVP produced a mean increase of 50% of the baseline population spike amplitude in all 6 animals on the saline pipette at 30 minutes after AVP. AVP also produced potentiation of the EPSP slope on the saline pipette in all animals with a mean increase of 25% at 30 minutes after AVP infusion. With the propranolol pipette at 30 minutes all 6 animals showed an unchanged or decreased population spike amplitude averaging 21% of baseline and a decreased EPSP slope averaging 26% of baseline.

In the final experiment, the AVP fragment, DGAVP increased in the population spike amplitude in 3 animals with a mean amplitude of 100% above baseline at 30 minutes which remained constant at 60 minutes. DGAVP did not significantly alter EPSP slope.

Taken together, the results of these experiments demonstrate that AVP produces long-lasting potentiation of the perforant path evoked potential *in vivo* in the dentate gyrus, without tetanic stimulation. This confirms *in vitro* experiments on the effects of AVP on the perforant path evoked potential. The dependence of AVP-induced electrophysiological potentiation on NE receptor mediation in the dentate gyrus parallels the results obtained with AVP-infusion in the dentate gyrus *in vivo* on the potentiation of avoidance memory and with *in vitro* effects of AVP potentiation of NE-mediated cAMP production.

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List of Abbreviations

AVP	- arginine vasopressin
AVP-LTP	- arginine vasopressin-induced long-term potentiation
Arc	- activity-regulated cytoskeleton-associated protein
BDNF	- brain-derived neurotrophic factor
CA	- catecholamine
CREB	- cAMP-response element binding protein
DDAVP	- 1-deamino-8-D-arginine-vasopressin
DGAVP	- desglycinamide arginine vasopressin
DG	- dentate gyrus
DG-LTP	- desglycinamide-lysine vasopressin-induced long-term potentiation
cAMP	- cyclic adenosine 3', 5'- monophosphate
DA	- dopamine
EPSP	- field excitatory postsynaptic potential
FOS	- immediate early gene c-fos
IP3	- inositol triphosphate
irAVP	- immunoreactive AVP
LC	- locus coeruleus
LTD	- long-lasting depression
LTP	- frequency (tetanus) induced long-term potentiation
LVP	- lysine vasopressin
MAM	- methylazoxy-methanol acetate
MAPK	- mitogen-activated protein kinase
MPT	- alpha-methyl-p-tyrosine methylester
NE-LTP	- norepinephrine-induced long-term potentiation
NGF	- nerve growth factor
NMDA	- N-methyl-D-aspartate
NT3	- neurotrophin 3
PGI	- paragigantocellularis
PROP	- propranolol, a beta-adrenergic antagonist
PVN	- paraventricular nuclei
SON	- supraoptic nuclei
5-HT	- serotonin

Introduction

Learning and memory are essential for all animals in order to satisfy their own basic needs and to adapt to changes in the conditions of life. For every living organism, adaptation reflects behavioral modification by experience. In order to cope with these ongoing challenges throughout life, the brain is constantly reconstructing itself. Thus the cognitive, behavioural and emotional status of an organism reflects a lifelong self-adjustment and self-optimization process. Neuronal plasticity is a fundamental process by which the brain adapts to sensory, cognitive, emotional, social, and endocrine inputs or a combination of such inputs. During learning and memory, neurotransmitters and neuropeptides promote a variety of structural and molecular changes that take place throughout the brain.

Ramon Y Cajal initially proposed a theory of memory storage as involving the growth of new connections or the alteration of existing ones (Bailey et al., 2000). Donald Hebb further proposed that memories are represented as enduring changes in the functional circuitry of the brain and that the synaptic contacts between neurons serve as the pliable substrate for “memory traces” (Abraham et al., 1991). Specifically Hebb suggested the association of pre- and post- synaptic activity in two neurons elicits some change in one or both of the neurons such that the synaptic connection between them is strengthened (Hebb 1949). This model for memory formation has been intensively studied in the dentate gyrus of the hippocampus, a structure which initially provided evidence of long-lasting synaptic plasticity in the mammalian brain (Bliss and Lomo 1973). The discovery of long-lasting potentiated synaptic responses supported Hebb’s hypothesis of a cellular mechanism for learning and memory.

Long-term potentiation (LTP) of a synaptic response, which is widely accepted as a model for neuronal plasticity was initially elicited by high frequency stimulation (Bliss and Lomo, 1973), but also can be induced by a neuromodulator like norepinephrine (NE; Neuman and Harley, 1983; Walling and Harley, 2004). The

neuropeptide arginine vasopressin (AVP) (Chen et al, 1993; Chepkova et al, 2001; Dubrovsky et al, 2003) also produces enhancement of synaptic responses suggesting its possible role in memory formation. Studies have examined whether AVP and NE together play a neuromodulatory role in synaptic plasticity (Brinton, 2000; Brinton, 1993).

The present study is concerned with two hypotheses. The first is that *in vivo* AVP will induce long lasting potentiation of perforant path input to the dentate gyrus, as has been observed *in vitro* (Chen et al, 1993), without tetanic stimulation of the perforant path. The second is that the modulation of the perforant path potential by AVP is dependent upon β -adrenoceptor activation.

These hypotheses arise from earlier evidence that AVP effects on learning and memory in the dentate gyrus require NE input to be effective (Bohus et al, 1993; Kovacs et al, 1979a; 1979b). If NE mediates the effects of AVP on learning and memory, and, if long-term potentiation-like effects play a critical role in learning and memory, AVP should, through its interactions with NE, produce an AVP-LTP of perforant path input that requires β -adrenoceptor support as does the LTP effect of NE itself. Finally, the ability of a more restricted V1 activating fragment to induce perforant path LTP is assessed.

The introduction briefly reviews AVP pathways and receptor distribution, reviews evidence of the role that AVP may play in learning and memory and the evidence for memory-like changes in the dentate gyrus circuit with an emphasis on those mediated by NE and AVP. The focus first turns to the structure most studied in memory research, the hippocampus and, more specifically, the dentate gyrus.

1.1 The Hippocampal Dentate Gyrus and Memory Processes

The hippocampus is a bilaterally symmetrical structure comprised of the hippocampus proper and the dentate gyrus. These two fields each contain a densely packed sheet of cells, pyramidal and granule cells respectively, which are the principal cell type of their field and which project outside their respective fields, as well as a variety of interneurons, cells which limit their influence to the local circuit (Teyler, 1991). An important feature is the intrinsic trisynaptic circuitry of the hippocampus: the entorhinal cortex projects to dentate gyrus granule cells (the perforant path), the granule cells project onto CA3 pyramidal cells (the mossy fiber system) which project to CA1 pyramidal cells (the Shaffer collaterals) which in turn project to the subiculum. The main input into the hippocampal system is from the entorhinal cortex which receives inputs from multiple cortical regions and all sensory modalities. One of the primary projections of the entorhinal cortex is to the dentate gyrus via cells in its second cortical layer (Amaral and Witter, 1995). Thus the granule cells are in a position to control the flow of information within the hippocampus as they receive excitatory input from the entorhinal cortex via the perforant path into the molecular layer, and activate pyramidal cells in CA4 and CA3.

The hippocampal formation has been one of the most extensively studied regions in the quest for further understanding of learning and memory processes. Much of the evidence used to examine the hippocampus and its role in learning and memory was obtained through experimental studies with animals where damage was not limited to the hippocampus. Selective removal of the hippocampal region consisting of CA1-CA3 pyramidal cells and hilar and granule cells in the dentate gyrus gives a clearer picture of the extent of hippocampal involvement in learning and memory. In studies by Jarrard and colleagues (Bouffard and Jarrard, 1988; Morris et al, 1990), axon-sparing ibotenic acid lesions of the hippocampus are used to study the effects of selective removal on the acquisition of spatial and non-spatial information; complex, non-spatial representational learning; and acquisition and utilization of contextual information. These studies show

that rats with selective hippocampal removal by ibotenate lesioning exhibit severe impairments in acquisition of spatial information when tested in the radial maze (Bouffard and Jarrard, 1988), the rewarded alternation task (Jarrard and Hyko, 1994) and the Morris water maze (Morris et al., 1990). In general, these studies have shown that the performance of the rats with the hippocampus removed is impaired on tasks that require utilization of spatial and contextual information, but is like that of controls in the learning and handling of non-spatial information.

Many studies have tried to examine the specific function of the dentate gyrus and its role in learning and memory. These studies have found that intradentate colchicine, a neurotoxin that preferentially destroys granule cells and mossy fibers, disrupts the acquisition and performance of spatial reference memory in the Morris swim maze (Sutherland et al, 1983; Jeltsch et al, 2001), working memory in the radial arm maze (McLamb et al, 1988a; Jeltsch et al, 2001) and the acquisition of a two-way active avoidance response in a Y-maze (McLamb et al, 1988b). Xavier and colleagues (1999) tested rats with dentate gyrus lesions on tasks requiring spatial reference and working memory in the Morris water maze and found that their deficit was the same as rats with complete hippocampal lesions when the start location varied on each trial. From their findings, Jeltsch and colleagues (2001) speculated that both types of memory are sensitive to granule cell damage.

Rolls (1996) suggests that pattern separation may be a function of the dentate gyrus and the mossy fiber projections to CA3. Pattern separation can be described as a mechanism for separating partially overlapping patterns of activation so that one pattern may be retrieved as separate from the other pattern (Rolls 1996). Rolls' model proposes that pattern separation is facilitated by the sparse connections in the mossy fiber system, which connects granule cells in the dentate gyrus to pyramidal neurons in the CA3. The mossy fiber inputs to the CA3 from the dentate gyrus may influence which CA3 neurons will fire based on the distribution of activity in the dentate gyrus.

Lee and Kesner (2004) tested rats with selective dentate gyrus lesions on the acquisition and retrieval of contextual fear conditioning. The lesioned rats showed initial impairments in freezing behavior but eventually reached the level of freezing in controls with subsequent training. When retrieval was examined in rats with dentate gyrus lesions 24 hours after acquisition, the animals showed a significant deficit in freezing compared to controls. These studies support the hypothesis that the dentate gyrus plays a role in both the encoding and retrieval of spatial information.

Since the dentate gyrus receives inputs from all sensory modalities, it is possible that the dentate gyrus uses sensory markers to mark a spatial location. Kesner and colleagues (2004) speculate that one function of the dentate gyrus would be to encode events and separate events in space resulting in spatial pattern separation. This would ensure that new highly processed sensory information is organized efficiently in the hippocampus. The induction and maintenance of LTP in the dentate gyrus is enhanced after exposure to novel or enriched environments (Davis et al, 2004) suggesting a role in encoding new spatial environments.

1.1.1 Long-term Potentiation

In 1949, Donald Hebb published his now famous synaptic modification postulate: “When an axon of cell A is near enough to excite cell B and repeatedly or persistently takes part in firing it, some growth process or metabolic change takes place in one or both cells such that A’s efficiency, as one of the cells firing B, is increased” (Hebb, 1949).

In 1973, Bliss and Lomo first reported that brief high-frequency electrical stimulation of the perforant path input to the dentate gyrus of the hippocampus could elicit a lasting enhancement of synaptic transmission in this pathway that persisted for days and this enhancement was termed LTP. LTP is a synapse-specific enhancement of

excitatory postsynaptic responses that has been repeatedly proposed as a mechanism of memory (Douglas & Goddard, 1975; Bliss & Collingridge, 1993; Izquierdo, 1993). LTP shares several characteristics with memory including synapse specificity (Dunwiddie and Lynch, 1978), rapid acquisition, incremental nature, durability and persistence for very long periods in the absence of stimulation. The fact that it can be triggered by brief trains of high frequency stimulation is presumably analogous to the way brief, unique sensory or behavioral stimuli set the learning process in motion (Teyler and DiScenna, 1987; Bliss & Collingridge, 1993; Izquierdo & Medina, 1995). In addition, LTP is accompanied by biochemical and morphological changes which have long been proposed as a basic mechanism upon which synaptic connections are strengthened as a result of a learning experience.

Andersen and Lomo (1966) electrophysiologically examined the unique anatomy of the hippocampus and found that the extracellular population field potentials recorded at various depths directly reflect excitatory postsynaptic potentials (EPSPs) and cell body spike discharges. The evoked potentials reflect summed synaptic currents (EPSP) and unit spiking activity (population spike). The response of the population of granule cells firing action potentials to elicited perforant path stimulation is measured as the population spike. The initial slope of the evoked response is a measure of synaptic strength. Long-term potentiation, LTP, was first described in the dentate gyrus and was seen as long-lasting increases in both the population spike amplitude and in EPSP slope (Bliss and Lomo, 1973).

1.1.1.1 Dentate and Long Term Potentiation (LTP) studies

Bliss and Lomo (1973) set a precedent for studying field potentials in the dentate gyrus in awake animals for the purpose of investigating the properties and persistence of LTP. Brief high frequency stimulation of the dentate gyrus in the rabbit *in vivo* was found to induce LTP lasting as long as 3 days. Tetanization of the perforant path results in robust and enduring increases in the population spike amplitude and the EPSP slope in the dentate gyrus evoked by single pulse stimulation of this input pathway (Bliss and Lomo, 1973; Bliss and Garner-Medwin 1973; Bronzino et al., 1994). This homosynaptic LTP is presumed to involve glutamate-mediated modulation of glutamate synapses.

However some early studies established that perforant path stimulation affected the population spike and EPSP components differently. Potentiation of the population spike was found to be much greater than that of EPSP (Bliss and Gardner-Medwin, 1973). Racine and colleagues (1983) reported that the EPSP potentiation decayed at a faster rate than the population spike potentiation. Subsequent studies from several labs confirmed in rats that LTP can last from 1 hour to a day to many weeks, depending on the stimulation parameters (Barnes 1979; de Jonge and Racine 1985; Staubli and Lynch 1987; Jeffrey et al., 1990; Abraham et al., 1993; Bronzino et al, 1994). Abraham and colleagues demonstrated that in the dentate gyrus LTP could last many months (1993), even up to a year (Abraham et al, 2002), under certain conditions. Protocol variables like the number and timing of high frequency stimulus trains (Huang and Kandel, 1994; Abraham et al 2002), the time before and after stimulation that the animal is in the recording chamber (Abraham et al 2002) and the level of neuromodulatory activity at the induction time (Swanson-Park et al 1999) have been found to influence LTP duration.

Studies have reported that the unusually long duration of LTP may be due to structural synaptic modifications such as an increase in the number of synapses (Lynch et al., 1988; Lynch and Baudry, 1991; Bliss and Collingridge, 1993) and changes in dendritic spines. Many studies have reported that the early maintenance phase of LTP in the dentate gyrus is protein synthesis-dependent (Otani and Abraham, 1989; Otani et al., 1989; Nguyen et al., 1994). Kelly et al (1998) reported that nerve growth factor plays a role in potentiation of the rat perforant path-granule cell synapses.

1.1.1.2 Norepinephrine-Induced LTP

The neurotransmitter NE has been studied in the hippocampus and been found to be involved both in memory processes and in hippocampal plasticity. In behavioral studies, NE has been linked to enhanced memory retrieval, increased behavioral responses to novel objects or novel stimulation, and facilitated learning of new response contingencies in a familiar task (Sara and Bergis, 1991). Various studies have shown that NE depletion in rodents and monkeys results in increased distractability and deficits in spatial working memory while administration of NE agonists improved memory performance (Carli et al, 1983; Arnsten and Contant, 1992; Cai et al, 1993). The major source of dentate gyrus NE is the terminals of the locus coeruleus (LC) (Loy et al, 1980). The LC has been a site of activation for studies examining the physiological effects of NE release (Dahl and Winson, 1985; Harley and Milway, 1986; Harley et al, 1989; Klukowski and Harley, 1994; Walling et al, 2004). The LC is a noradrenergic nucleus of the pontine reticular formation, which sends a dense terminal projection to the dentate gyrus, as well as to neocortex and other forebrain areas (Aston-Jones et al, 1999).

Stimulation of this ascending NE pathway was initially reported to modulate the rate of spontaneous unit activity in the hippocampus (Segal & Bloom, 1976), and the spike potential amplitude evoked by stimulation of the main excitatory input to the dentate gyrus, the perforant path (Assaf et al, 1978). NE-modulated signals were first observed in the hippocampus by Segal and Bloom (1976). They reported that iontophoretically applied NE appeared to suppress all hippocampal unit firing whether spontaneous or driven by cholinergic stimulation, but natural release of NE through activation of the LC enhanced tone-induced excitation (Segal & Bloom, 1976). Also enhancement was observed when LC stimulation was paired with sensory inputs to neocortex (Berridge and Foote, 1991). These results led to the proposal that LC-NE acts to enhance the signal-to-noise ratio both in sensory areas and in the hippocampus (Harley, 1991).

In 1983, NE application in the dentate gyrus was found to result in a long-lasting potentiation of the perforant path evoked population spike amplitude in the granule cells of the dentate gyrus. Neuman and Harley (1983) observed that one to eight minutes of NE iontophoretic application in the granule cells produced a potentiation of the population spike amplitude that climbed to 40% *in vivo* and lasted at least 30 min. Such observations were reported as NE-induced long-lasting potentiation (NELLP), a heterosynaptic modulation by NE of glutamate responses to distinguish it from the tetanic stimulation-induced homosynaptic potentiation.

1.1.1.3. Norepinephrine-Induced Long-Term Potentiation: *In vitro* studies

Lacaille and Harley (1985) found that in the hippocampal slice, ten minutes of NE exposure (10 μ M) produced a 31% enhancement of spike amplitude that was long-lasting

(30 minutes) in one-quarter of the experiments. Forty-seven percent of the spike amplitude potentiation could be accounted for by the increase in EPSP slope, thus suggesting an additional increase in EPSP/spike coupling. The population spike and EPSP slope potentiation was seen in all slices exposed to the β -adrenoceptor agonist isoproterenol and was blocked with a β -adrenoceptor antagonist, timolol, arguing for β -adrenoceptor mediation. In contrast, an α -receptor agonist, phenylephrine, and antagonist, phentolamine produced only weak effects.

Stanton and Sarvey (1985) carried out a number of *in vitro* experiments to better examine NE effects in the dentate gyrus. They discovered two forms of NE-induced potentiation of population spike amplitude through the manipulation of emetine, a protein synthesis inhibitor. A short-term NE-induced potentiation was seen in the presence of emetine. This potentiation only lasted as long as NE was present. An NE-induced long-lasting potentiation occurred in the absence of emetine and lasted for the 5 hour recording time. The thirty minutes of NE superfusion produced an enhancement of spike amplitude that was 85% greater than control. In the same study, Stanton and Sarvey (1985) found forskolin, an adenylate cyclase activator, greatly enhanced the NE-potentiation dose-response curve supporting a role for adenylate cyclase generation of cAMP in NE potentiation. Both forms of NE potentiation were blocked by the β -adrenergic antagonist, propranolol, a β_1/β_2 antagonist and metoprolol, a β_1 antagonist.

In a subsequent study, Stanton and Sarvey (1985b) examined slices from normal and NE-depleted hippocampi and found high frequency LTP of the perforant path population spike was blocked in the dentate gyrus. Upon recovery from the depletion, LTP occurred in the slice. Forskolin and β -adrenergic antagonists produced the same

results as in the previous study. The final follow-up study reported that LTP in the dentate gyrus and NELLP both correlated with increased cAMP in the dentate gyrus, with NE stimulation showing increased cAMP long after washout (Stanton and Sarvey, 1985c). These studies by Stanton and Sarvey supported the existence of NELLP in the dentate gyrus *in vitro* and showed that this form of LTP depends on the activation of β -adrenergic receptors, adenylate cyclase and protein synthesis and the elevation of cAMP. They also argued that high frequency LTP in dentate gyrus normally recruits this pathway.

1.1.1.4 Norepinephrine-induced Long-Term Potentiation: *in vivo* Studies

As discussed previously, Neuman and Harley (1983) first described NELLP in the dentate gyrus *in vivo* in anesthetized animals in which the population spike of the perforant path potential was consistently potentiated by NE iontophoresis in the granule cell layer. A subsequent study conducted by Dahl and Winson (1985) *in vivo* supported this long-lasting enhancement of the population spike amplitude. In one of the earliest experiments on the modulation of perforant path evoked potential in the dentate gyrus by LC electrical stimulation, they reported that the population spike amplitude showed long-lasting potentiation without any change in the EPSP slope at the cell body level. They observed a decrease in the EPSP slope at the dendritic level.

Population spike enhancement has also been found following glutamate activation of the LC to stimulate endogenous NE in the anaesthetized rat. Harley and Milway (1986) ejected glutamate into the LC and reported a spike enhancement 40% greater than the controls and lasting more than 20 minutes in 39% of the animals tested. The EPSP slope increased briefly (2-3 minutes) in half of the experiments and decreased or was

unchanged in the remainder of experiments. The different latencies suggested that EPSP slope and spike effects were uncorrelated. Another study by Harley and Evans (1988) supported these results. Harley and Sara (1992) found the EPSP slope again did not correlate with the population spike amplitude. EPSP slope increases occurred on less than 30% of the evoked potentials with increased population spikes.

Recently, Walling and colleagues (2004) investigated the activation of the LC in the urethane anesthetized rat by the neuropeptide, orexin-A. They reported that orexin-A caused a long-lasting and gradually increasing (over 3 hours) potentiation of the population spike amplitude with no change in the EPSP slope.

Spike amplitude enhancement by glutamate activation of the LC has also been seen in the awake animal (Sara and Bergis, 1991; Klukowski and Harley, 1994; Walling and Harley, 2004). A study in the awake rat by Sara and Bergis (1991) examined effects of the drug idazoxan, which increases the release of NE, and found potentiation of the population spike amplitude, but no change in the EPSP slope. Walling and Harley (2004) studied NE release and subsequent modulation of the perforant path evoked potential in dentate gyrus at 3 hours and at 24 hours following glutamate activation of the LC. They found that LC activation resulted in immediate spike potentiation lasting 3 hours, but observed both spike and EPSP slope potentiation 24 hours after LC activation. Propranolol, a β -adrenergic antagonist, and anisomycin, a protein synthesis inhibitor, given (icv) before LC activation was found to block both slope and spike potentiation at 24 hours. Thus, LC activation seems to initiate a β -adrenergic and protein synthesis dependent long-lasting enhancement in the granule cell excitability and synaptic strength of the perforant path input to the dentate gyrus.

While *in vitro* studies concluded that α -agonists failed to mimic the effects of NE and that all potentiation depended on β -receptor activation, Chaulk and Harley (1998) in an *in vivo* study, found a possible role for both α - and β -adrenoceptors in NE potentiation. They used icv NE to mimic *in vitro* conditions *in vivo* and found that both the β -agonist isoproterenol and the α -agonist phenylephrine, produced potentiation although potentiation was more commonly long-lasting in isoproterenol treated animals. Both β - and α -antagonists attenuated NE potentiation in the icv studies. Babstock and Harley (1992) examined the role of the β -receptors *in vivo* after stimulation of the paragigantocellularis (PGI). The PGI provides a major source of glutamate excitation to the LC which is another method of activating NE release. PGI activation induced only spike potentiation which was blocked by the β -antagonist propranolol. In contrast, Munro and colleagues (2001) found that the β -antagonist, timolol, partially prevented high-frequency-induced LTP *in vivo* of the EPSP slope, but LTP of the population spike amplitude was unaffected.

The inconsistent demonstration of LTP of the EPSP slope in NE-induced potentiation studies *in vivo* stands in contrast to the previous *in vitro* data but is fairly consistent within the *in vivo* experiments.

NE-induced LTP shares important qualities with the late-phase tetanic induced LTP. β -adrenergic receptor activation was found to be required for tetanus-induced late-phase LTP at both the lateral and medial perforant pathways (Bramham et al., 1997) and at the mossy fiber-CA3 synapse (Huang and Kandel, 1996). More recently, Straube et al. (2003) have shown that novelty exploration transforms early-phase LTP (non-protein

synthesis dependent) in the dentate gyrus into late-phase LTP, which is prevented by the β -antagonist propranolol, as well as by the protein translation inhibitor, anisomycin.

NE has been reported to work with the neuropeptide AVP in the enhancement of memory (Kovacs et al, 1979a;1979b) which will be discussed shortly. The following section is a brief overview of AVP, its anatomical distribution and its historical role in learning and memory studies.

1.2 AVP

1.2.1 Structure, neural pathways and receptor distribution

AVP is a nonapeptide derived from larger precursor proteins. In peptide synthesis, the amino group of one amino acid is bonded with the carboxyl group of the adjoining amino acid. The side chains of the amino acids identify the various peptides and are responsible for their physical and functional attributes (McEwen 2004). AVP and oxytocin are synthesized in, transported within, and secreted by both magnocellular cells and parvocellular cells which are predominantly located within the paraventricular (PVN) and supraoptic (SON) nuclei. The magnocellular cells are neuroendocrine cells which project to the capillaries in the posterior pituitary lobe where they secrete their contents as hormones into the systemic circulation. Some parvocellular cells secrete releasing hormones into the portal circulation of the anterior pituitary gland, others activate, inhibit, or modulate, activity in other neurons in the brain and others synapse on blood vessels in the brain (Sofroniew, 1985; Buijs et al, 1991). Extrahypothalamic (from the bed nucleus of the stria terminalis) and hypothalamic (from PVN) AVP-ergic fibers innervate limbic areas like septum and hippocampus (Sofroniew 1985; Buijs 1991). AVP

and OT are released synaptically from axon terminals and from dendrites and somata of hypothalamic neurons (Pow and Morris, 1989).

The neuropeptide concept was originally formulated by De Wied (1965) when his findings indicated the neurogenic effects of pituitary hormones. Generally, De Wied came to recognize that AVP is a peptide that can act at both peripheral target sites and at neural structures within the brain. In both periphery and brain, metabolic alterations mediated by peptidase enzymes convert the parent peptide into smaller peptides. The neuropeptide concept proposes that in the brain the smaller active fragments generated from AVP can function as neurotransmitters or neuromodulators at brain target sites. It also suggests that some of these fragments act at relevant brain sites to influence memory consolidation and retrieval. Traditionally in De Wied's studies lysine vasopressin (LVP) has the same function as AVP but the form is found only in the pig. A number of studies carried out by De Wied and colleagues (1972) found that the entire AVP molecule, which produced other hormonal effects, was not required for effects on memory processes. Desglycinamide-lysine vasopressin (DG-LVP), produced from the parent peptide LVP with the glycine residue removed, lost its endocrine activity but retained its ability to inhibit extinction of a pole-jump avoidance response. The behavioral research of De Wied and colleagues is reviewed in a later section.

Studies have shown that AVP binding sites are widely distributed throughout the brain and the spinal cord (Tribollet et al., 1998). Tribollet and colleagues (1998) summarized the distribution of central AVP binding sites using microscopy autoradiography in the adult rat brain and compared it with distribution patterns found in other mammalian species. Receptors specific for AVP were identified within numerous areas of the brain and spinal cord in the rat and other mammals but there are marked

species related differences in their distribution, which may be because these receptors mediate species typical behaviors.

Some studies using non-radioactive selective analogues together with tritiated AVP suggested that only V1a-like vasopressin receptors are detectable within the brain (Barberis et al, 1995; Tribollet et al., 1998b). There appears also to be an absence of V2 receptor mRNA in the adult rat brain (Ostrowski et al, 1992; Saito et al, 1995), whereas it may be expressed during development (Saito et al, 1995; Croiset and De Wied, 1997). A study by Hirasawa and colleagues (1994) suggests that the hippocampus also contains V1b receptor mRNA, but expression is low which probably accounts for the failure to detect it in some autoradiographic studies (Sugimoto et al, 1983). The oxytocin receptor also has a high affinity for AVP, which has been speculated to mediate central effects of both AVP and oxytocin (Elands et al, 1988).

There are still questions concerning the central distribution of AVP-ergic cells and their fibers. In some cases, knowledge is lacking about the terminal sites of localized cell clusters, while in others the sites of the AVP-ergic fibers or terminals are known, but not their cells of origin. In particular, while the origin of the AVP-ergic fibers in the ventral hippocampus (CA1, CA3) appears to be in the medial amygdala (Caffe et al., 1987), the origin of the AVP-ergic fibers in the dentate gyrus is not clear. Immunohistochemical studies have shown AVP-ergic neurons in a variety of structures, such as the bed nucleus of stria terminalis (De Vries and Buijs, 1983; Caffe' and van Leeuwen, 1983; Sofroniew, 1985), the vertical limb of the nucleus of the horizontal diagonal band (Sofroniew, 1985), the septum (Sofroniew, 1985), the PVN (Buijs, 1978; De Vries et al., 1985), the SON (De Vries et al.,1985), the suprachiasmatic nucleus

(Buijs, 1978; Sofroniew and Weindl, 1980), the dorsomedial hypothalamus (Caffe' and van Leeuwen, 1983; De Vries et al., 1985), the medial amygdaloid nucleus (Caffe' and van Leeuwen, 1983; De Vries et al., 1985; Sofroniew, 1985), the locus coeruleus (LC) (Caffe' and van Leeuwen, 1983; Caffe' et al., 1985; De Vries et al., 1985; Sofroniew, 1985), the nucleus subcoeruleus (Caffe' et al., 1985; Sofroniew, 1985), and the spinal cord (Kai-Kai et al., 1986). In many of these neurons, AVP has a neuromodulatory effect since it produced long-lasting facilitation of the response to the excitatory neurotransmitter glutamate (Urban, 1987). De Vries and colleagues (1984) found that the distribution of AVP in the rat brain included androgen dependent pathways in which a higher density of AVP fibres in the bed nucleus of the stria terminalis, the amygdala and the LC is found only in male rats.

Since neuropeptides are often produced in small amounts and rapidly transported from the cell body, many of these studies used the axonal transport inhibitor colchicine to permit cell body staining. However, colchicine has profound effects on the ultrastructural features of neurons (Alonso, 1988) and can affect peptide production (Ceccatelli et al., 1991; Re'thelyi et al., 1991). This technique has been replaced by *in situ* hybridization that permits detection of cells possessing peptidergic mRNA. Hallbeck and colleagues (1999) used this technique to identify the distribution of neurons that may be the origin of AVP in the hippocampus of the male Sprague-Dawley rat. AVP mRNA labeling was seen in the pyramidal layer of the hippocampus throughout the CA1-CA3 fields and in the dentate gyrus. Thus hippocampal granule cells may produce AVP. AVP binding sites in the dentate gyrus are the densest within the hippocampus (Brinton et al., 1984; Phillips et al, 1988).

Studies have reported that AVP activates hippocampal neurons (Versteeg et al., 1984; Brinton and McEwen, 1989; Giri et al., 1990; Maegawa et al., 1992), which supports the possible involvement of AVP in learning and memory (Bohus, 1977; Leshner and Roche, 1977; Bohus et al, 1978; De Wied and Versteeg, 1979). Hallbeck and colleagues (1999) concluded that their findings of AVP mRNA expression throughout the hippocampus indicate that many of the AVP effects in the hippocampus could be elicited by intrinsic AVP-ergic neurons. There is also evidence that AVP is released extrajunctionally (Buijs and Swaab, 1979), which suggests that AVP may diffuse over long distances to other brain areas. Many regions containing AVP receptors lack AVP-ergic or oxytocinergic fiber innervation and some regions are devoid of receptor sites but instead show neuropeptide terminals (Barberis and Tribollet, 1996). Such observations suggest these peptides may also be involved in nonsynaptic communication.

1.2.2 AVP and learning and memory: A history of De Wied's studies

The earliest reports on AVP and its effects on learning and memory came from the classic studies by De Wied and his colleagues in the late 1960s in which he originally investigated the effect of a posterior pituitary lobectomy and the consequent loss of associated hormones on avoidance behavior. Avoidance behavior is a behavioral response which an organism uses to prevent contact with an aversive stimulus, either actively avoiding it or passively avoiding it through behavioral inhibition. Active and passive avoidance tasks were the primary paradigms used by De Wied and colleagues (De Wied and Bohus, 1966; De Wied, 1976; De Wied, 1977; Bohus, Kovacs and De Wied, 1978) in their research on AVP and oxytocin and memory processes. These consisted of multi-trial active avoidance tasks like the pole jump and the shuttlebox tasks, and the single-trial passive avoidance task. Such tests reflect the ability of the animals to handle sudden and dramatic changes in their environment. In 1965, De Wied reported that a

conditioned avoidance response is extinguished at a much more rapid rate with the removal of the posterior pituitary lobe. The lobectomy increased the rate of extinction of the shuttlebox avoidance training response while a relatively crude extract called pitressin tannate, prepared from the posterior and intermediate lobes, prevented this abnormally rapid rate of extinction and maintained the conditioned response. This extract consisted of the posterior pituitary hormones, AVP and oxytocin, and the intermediate lobe hormone melanocyte stimulating hormone. LVP was also found to inhibit this rapid extinction rate when injected peripherally (De Wied and Bohus, 1966). It was concluded from their studies that the peptides present in the posterior and intermediate lobe of the pituitary are physiologically involved in maintaining, but not acquiring the learned avoidance response.

Subsequently, De Wied and Bohus (1966) also found that when rats were treated with pitressin tannate, they exhibited a delay in the extinction of the active avoidance response and maintained the previously acquired avoidance response for a longer period consistent with the view that the peptides in pitressin were preserving and promoting long-term memory of the conditioned response. De Wied (1971) compared the effects of various peptides including LVP and angiotensin on acquisition of a pole-jump active avoidance response in intact rats. The rationale for using these particular peptides was that they were structurally or physiologically similar to AVP. They found only LVP inhibited extinction in acquisition of the pole-jump avoidance response and the critical period for administration was immediately or 1 hour after completion of the first extinction session. The authors concluded that AVP is the peptide in pitressin that improves retention and its long-term effect suggests an influence on memory consolidation. The effect did not appear to be related to the effects of AVP on blood pressure or carbohydrate metabolism since neither the pressor substance, angiotensin II, nor insulin or growth hormone had any effects on the extinction of the avoidance response.

Researchers also found long-term behavioral effects of AVP in studies involving passive avoidance responses in which the animal's motor activity is decreased in order to avoid the aversive stimulus. The passive avoidance test consists of an acquisition trial in which a rat receives a footshock after entering a box, and a test trial, in which latency time to enter the box again is measured (Ader and De Wied, 1972). The stronger the association of the box with the footshock, the longer the latency will be. The acquisition trial and the test trial are often separated by a 24-hour period. The passive avoidance test allows measurement of drug effects on consolidation, by administering the drug before or just after the acquisition trial, and retrieval, by administration before the test trial (Reijmers et al., 1998). Ader and De Wied (1972) used this passive avoidance task to test the effects of LVP (subcutaneous) and were able to demonstrate that this AVP analog not only induces strengthened maintenance of a conditioned avoidance response in a multi-trial active avoidance paradigm, but also in the single-trial passive avoidance paradigm.

In a 1976 study by De Wied, examining the effects of AVP, intraventricular administration of AVP (1 ng/ μ l) was given immediately (0 hours), 3 hours, 6 hours or 23 hours after a passive avoidance learning trial. Rats given AVP exhibited longer latencies to enter the shock compartment relative to controls, when AVP was given immediately after the learning trial and 23 hours after the trial. Kovacs and colleagues (1978) tested LVP effects on acquisition and retention of a passive avoidance response in which rats were trained to escape footshock from a grid floor by jumping onto a bench; stepping down to the floor produced more footshock. LVP given 10 minutes before the passive avoidance retention session did not influence acquisition, but facilitated retention since the step-down latency increased.

1.2.2.1 AVP's effects on memory acquisition, consolidation and retrieval

Memory processes involve consolidation and retrieval of acquired information. Studies have examined the role of AVP on both consolidation and retrieval memory processes as well as acquisition in an effort to distinguish its specific role.

Bohus and colleagues (1978) studied time gradient effects of icv administration of AVP on retention of a passive avoidance response. AVP significantly prolonged reentry latencies relative to the controls and demonstrated a time dependent facilitation. AVP was most effective when injected immediately posttraining (consolidation effect) or 1 hour prior to the 24-hour retention test (retrieval effect). These results were similar to those of the 1976 study previously described. Bohus and colleagues (1978b) investigated both peripheral and centrally administered AVP and found no effect on acquisition, but significantly delayed extinction on both active and passive avoidance tasks. The observation that the retention effects occur at a much lower dose centrally than peripherally is consistent with the notion that AVP exerts its effect by influencing central rather than peripheral receptor sites.

Other researchers studied the effects of AVP on retrograde amnesia to examine effects on memory retrieval. Retrograde amnesia refers to the inability to remember experiences that occurred just before a temporary but severe disturbance of the normal physiological activity of the brain (McEwen 2004). The amnesia can be produced by postlearning or preretention application of a variety of treatments that disrupt neural activity, such as carbon dioxide (Rigter et al, 1974; Sato et al, 2004), electroconvulsive shock or pentylenetetrazole (Bookin and Pfeifer,1978) or by inhibiting protein synthesis using puromycin. There is a debate about whether the amnesia produced is due to disruption of memory consolidation, in which short-term memories are changed into permanent long-term memories, or due to an impairment of memory retrieval because of the demonstrations of spontaneous recovery from this amnesia (Davis et al., 1971; Miller

and Springer, 1973). Rigter and colleagues (1974) tested the ability of DG-LVP to reverse retrograde amnesia induced by carbon dioxide treatment using a single trial step-through passive avoidance task. They found that the amnesia was reduced when DG-LVP was given before the training trial, the test trial, or both. Such results seem to indicate that the peptide protected memory consolidation, because of the peptide's anti-amnesic effect when given before training, and memory retrieval because of its anti-amnesic effect when injected before the test trial.

Bohus and colleagues (1982) found an AVP-induced reversal of retrograde amnesia when AVP was microinjected into the dentate gyrus of the hippocampus or into the central amygdala. They also found that icv AVP (10 ng) or subcutaneously (2 µg) injected AVP resulted in an anti-amnesic effect on pentylenetetrazole-induced amnesia in a single-trial passive avoidance task. Such findings together suggest that the dentate gyrus or amygdala could mediate the consolidation and/or the retrieval effects of AVP.

1.2.2.2. Peripheral and Central effects of AVP on Memory

The pioneering work by De Wied and colleagues produced some controversy mainly due to the difficulty in separating peripheral from central effects. It was suggested that changes in arousal from the action of peripheral AVP on blood pressure might act as an additional reinforcement in aversive memory tasks so the peripheral effects may mediate the apparently positive effects of AVP in the modulation of memory processes. Thus studies were needed to determine whether the behavioral effects of AVP could be produced without the pressor or peripheral effects. Studying AVP fragments provided one way to address this issue.

These investigations revealed that the more powerful peptide effects on conditioned avoidance behavior were found with C-terminal fragments of AVP (De Wied et al., 1987). The AVP fragment desglycinamide (DGAVP) and AVP (4-8), in particular, exhibited almost no peripheral effects, but potentiated the consolidation and retrieval

processes in an aversive memory task (De Wied et al., 1984; Gaffori et al., 1985). AVP (4-8) appears to be the active sequence of the DGAVP molecule.

Gaffori and De Wied (1986) investigated the time periods for the modulating effects of AVP and several analogues with a sc injection (3 µg) in rats in a passive avoidance paradigm. AVP 4-8 and AVP 5-8 were most active when given immediately after the learning trial; DGAVP and AVP 5-9 were most active when given 23 hours after the learning trial while 1-deamino-8-D-arginine-vasopressin (DDAVP) and AVP 4-9 were most active when given immediately and 23 hours after the learning trial. So the analogues seem to have different time-related effects on memory.

Car and Murtazina (1994) investigated the effects of AVP and its analogue ([d(CH₂)^{1/5},Tyr(Me)²]AVP) in a lever-touch autoshaping model of learning and memory. The analogue lacks endocrine and pressor activity. ICV injection of 1 µg AVP and 2 µg of the analogue 24 hours prior to extinction did not alter the response in extinction sessions compared to the saline controls, but in the retention test, a higher level of correct responses in analogue-treated rats was found. The behavioral processes involved in the extinction of an operant response are not clear. During the early stages of extinction, the level of responding may possibly reflect the strength of retention of the conditioned response, so extinction could be considered as a measure of memory for the original task. The delay in extinction, seen in other studies, does suggest a retention-enhancing effect of AVP. But as extinction continues, the decline in response over a period of time can itself be considered as a form of learning.

In a study by Kumar and Karanth (1995) animals conditioned in a T-maze with appetitive (10% sucrose) and aversive (2.0 mA footshock) events were administered (icv) a single dose of 2.5, 5, 10 or 20 ng AVP 20 minutes before testing. In the retention test conducted with the same training apparatus 72 hours after conditioning, the peptide treated rats showed a dose-dependent increase in latencies to enter the previously shocked goal arm with the absence of such a difference in responding to the non-shocked goal

arm. This differential response was not observed in saline-treated rats. The ICV studies and analogous studies are the main evidence that peripheral effects are not necessary for memory improvement.

The physiological significance of central AVP modulation has also been demonstrated using specific antisera. An antiserum temporarily blocks the biological activity of AVP in the brain when given icv and results in a time-dependent decline in memory processes (Van Wimersma Greidanus & De Wied, 1976; Van Wimersma Greidanus et al., 1975). Wimersma Greidanus et al. (1975) found that icv administration of AVP antiserum either 30 minutes before or immediately after the learning trial induces a marked deficit in a one-trial passive avoidance task, when tested 24 or 48 hours later, but not when tested 2 minutes or 1 hour after the learning trial. This suggests that memory rather than learning processes are disrupted by AVP inactivity. Kovacs and colleagues (1982) examined the effect of intraventricular anti-AVP serum and also microinjections into the dentate gyrus on passive avoidance behavior. When injected immediately after the learning trial the anti-AVP serum resulted in marked impairment of the behavioral performance for the dentate gyrus group. Injection into the cerebral ventricles only affected passive avoidance retention when given at a higher dosage than that given in the dentate gyrus group. This indicates that the effects of the hippocampal application cannot be explained by leakage to the brain ventricular system and the neutralization of the AVP must have occurred within the hippocampus.

Collectively, it seems that under normal circumstances AVP is primarily involved in memory processes of consolidation and retrieval and not in the initial phases of memory processing like attention, arousal and motivation. However, under certain conditions, AVP does influence learning through peripheral effects. Gaffori and De Wied, (1985) found enhanced avoidance learning on a pole-jump task when AVP (3 μ g sc) was given at a high dose immediately before the training. Such enhancing effects were not seen with a high dose of the fragment DG-AVP which is devoid of endocrine

effects. Arousal level effects of the peptide have also been found to enhance an other AVP effect on learning (Skopkova et al,1991). Acquisition of a shuttlebox avoidance task was facilitated in low-activity rats with the lowest dose of DG-AVP (0.1 μg sc) but not in high activity rats as prerated on the basis of exploratory behavior. AVP also improves acquisition of a new behaviour when the subject's ability to process information and learning is impaired, as in completely hypophysectomized rats (De Wied 1965; Bohus et al, 1973; De Wied and Gispen, 1977),.

1.2.3 Brain sites involved in the AVP influence on memory

Lesion and microinjection techniques have provided insight into the localization of the brain sites involved in mediating the effects of AVP on memory storage and retrieval. In a study by Van Wimersma Greidanus et al. (1974), the mediodorsal thalamus and the parafascicular nucleus were lesioned resulting in an impairment in acquisition of a pole-jump avoidance task. LVP (sc) reversed this impairment. When the parafascicular nucleus alone was lesioned, there was no effect. Thus it was concluded that the parafascicular region and the mediodorsal thalamus were not essential for the AVP effects to occur. On the other hand, limbic system sites such as hippocampus (Van Wimersma Greidanus et al., 1976), septal region (Van Wimersma Greidanus et al., 1975b), and amygdala (Van Wimersma Greidanus et al., 1979b) do play a role in mediating the effects of AVP on retention behavior. LVP (3 μg and 9 μg sc) given before extinction failed to maintain avoidance responding in rostral septal lesioned subjects, which involved complete destruction of the medial septal nucleus and partial destruction of the lateral septal nucleus and nucleus accumbens (Van Wimersma Greidanus et al., 1975b). An anterodorsal hippocampal lesion accelerated the rate of extinction in a pole-jump shock avoidance response which was unaffected by LVP (Van Wimersma Greidanus et al, 1976). Lesions to the central and basolateral amygdaloid complex also prevented the inhibitory effects of DG-LVP on extinction of a pole-jump avoidance task (Van

Wimersma Greidanus et al, 1979b). These results suggest that an intact septal region and anterodorsal hippocampal region is important for mediating the AVP-induced prolonged extinction in the pole-jump avoidance task.

Ibragimov (1990) also found enhanced acquisition of an active avoidance reflex response after the hippocampal administration of AVP agonists 60 minutes before each session.

In a study examining immunoreactive AVP (irAVP) content in various midbrain- limbic system sites following footshock, Laczi and colleagues (1983a,b) found that irAVP levels increased in both the LC and the central amygdala, but decreased in the lateral septum and hippocampus. They also found that the hippocampal irAVP content was related to avoidance performance in that good avoiders exhibited reduced hippocampal irAVP content.

Kovacs and colleagues (Kovacs et al., 1979; 1982; and 1986) found that postlearning and preretention microinjection of 20-25 pg AVP or AVP fragments into the dorsal or ventral hippocampus resulted in an improvement of retention of passive avoidance behavior. When 50 pg AVP was microinjected into midline dorsal septal nuclei or dorsal raphe nucleus, retention was enhanced by increased reentry latency at the 24 hr retention test but not at 48 hr. AVP (25pg) microinjected in either the central nucleus of the amygdala or the LC failed to have any effect on consolidation. Such doses are behaviorally ineffective when injected into the lateral ventricle (Bohus et al, 1978b). The results of these experiments as well as those of Bohus et al. (1982) suggest that selective brain sites may mediate both consolidation and retrieval effects of AVP while other sites may mediate either consolidation or retrieval. As previously discussed, Bohus and colleagues (1982) examined the ability of AVP to reverse retrograde amnesia in a step-through passive avoidance task when injected into specific brain structures. The rationale for this is that the amnesia is seen as an impairment of retrieval. AVP (10 ng icv) reversed the severe amnesia, induced by penetylenetetrazole, when injected into

either the central amygdaloid complex or the hippocampal dentate gyrus. AVP injection into the dorsal septum and dorsal raphe nucleus failed to reverse the amnesia. The findings suggest that the hippocampal dentate gyrus mediates the effects of AVP in both memory consolidation and retrieval, but the dorsal septal nucleus and dorsal raphe nucleus are involved in the effects of AVP on consolidation, but not retrieval. The amygdala is suggested to be involved in the retrieval process and not consolidation.

1.2.3.1 FOS protein and AVP

One of the consequences of neuronal excitation by AVP, acting through V1 receptors, is the activation of the immediate early gene *c-fos*. Studies have shown that the icv injection of AVP stimulates *c-fos* mRNA expression in the hippocampus and lateral septum (Andreae and Herbert, 1993; Giri et al., 1990). This provides evidence that the central action of AVP must take place in these structures. The increase in *c-fos* message is rapid and short term, thus it became more practical to measure the gene product, Fos protein, since protein expression often is of longer duration.

Studies using Fos protein confirm the differential sensitivity of the hippocampus. Fos protein was measured following an icv AVP injection in unconditioned and conditioned mice (Paban et al., 1999). The conditioned mice learned a visual discrimination task and were then given icv AVP (2 ng) while the unconditioned mice just received the icv AVP injection. The unconditioned mice showed an increase in Fos protein expression in the dentate gyrus over the entire septotemporal area, the CA1 and CA3 hippocampal fields, also through the lateral septum, the bed nucleus of the stria terminalis, and the basolateral and central amygdaloid nuclei. In contrast, in the conditioned mice, the increase in Fos expression was specifically detected in the dentate gyrus along its septotemporal axis, the ventral CA3 hippocampal field and the lateral septum. Such specific Fos protein activated sites following post-training icv AVP supports the view that these specific areas (septum and hippocampus) are involved in the

enhancing effect of AVP on memory consolidation in visual discrimination learning. The conditioned mice tended to have specific Fos expression only in areas specific to hippocampus, a central target structure of the effects of AVP on memory processes. The authors hypothesized that the Fos expression seen in the lateral septum is a consequence of the hippocampal activation based on the neural connections between these two structures (Paban et al., 1999)

1.3 AVP *in vitro* and *in vivo* studies

Studies conducted by Joels, Urban, De Wied and other colleagues have used electrophysiological techniques in both *in vivo* and *in vitro* paradigms to investigate the putative neurotransmitter-neuromodulator functions of AVP. Joels and Urban (1982) investigated the actions of AVP on single cells in the lateral septal complex and the dorsal hippocampus. The iontophoretically applied AVP induced significant excitation of the cells of lateral septal complex and the dorsal hippocampus. Such excitation of hippocampal cells was also seen in a study by Muhlethaler and Dreifuss (1982) and also reported in ventral hippocampal cells (Urban and Killian, 1990). AVP enhanced responsivity of glutamate-induced activity in both structures, especially in those cells which did not respond to AVP on its own. This suggests a neuromodulatory role of AVP. This modulatory influence on lateral septal neuronal responsivity was found to persist up to 15 minutes after termination of AVP treatment in a follow-up study by Joels and Urban (1984).

Chepkova et al. (1995) carried out CA1 neuronal recording in slices for 15 minutes in a 0.1 mM solution of either AVP or the AVP fragment (4-8) both of which resulted in an increase in the amplitude and slope of the Schaffer collateral EPSP in 21

neurons tested. The peptide-induced increase in EPSP slope reached a maximum 30-45 minutes after peptide application. In 14 of these neurons the increase in EPSP slope lasted throughout the 60-120 minute washout period. In 7 of these, the increase was followed by a gradual decline to the pre-administration level.

AVP also increases the perforant path evoked EPSP elicited in the granule cell body layer of the dentate gyrus *in vitro* (Chen et al., 1993). In this study AVP was studied in the presence of varying concentrations of calcium. The effect was dose-dependent and peptide specific since there was no effect with oxytocin infusion (Chen et al, 1993). This effect of AVP was blocked by a V1 receptor antagonist demonstrating that the long-term enhancement induced by AVP is receptor- specific (Chen et al, 1993).

In the presence of 1.5 mM calcium, 100-500 nM AVP applied for 15 minutes increased the amplitude and slope of the EPSP which persisted for the 60 minute period of exposure. This enhancement also lasted in the absence of AVP for 60 minutes. In the presence of 2.5 mM calcium (a supraphysiological concentration) similar concentrations of AVP decreased the amplitude and slope of the EPSP. Thus the potentiation occurred only at physiological levels of calcium. This implicated dependency of long-term AVP-induced potentiation on extracellular calcium concentration was also observed in the expression of AVP-induced modulation of NE effects, where in the presence of 0.8 mM calcium AVP potentiated noradrenergic-induced cAMP formation, while in 2.5 mM calcium AVP induced a depression of noradrenergic-induced cAMP formation (Brinton & McEwen, 1989). Neuromodulation of NE and AVP is further discussed in the following section.

Calcium is critical in the development of the CNS (Berridge, 1993) and the various types of calcium channels play a critical role in neurite growth, gene expression, and modulation of learning and memory (Bliss and Collingridge, 1993; Goelet et al, 1986). Calcium is a critical component of LTP expression and maintenance (Dunwiddie and Lynch, 1979) and because calcium regulates expression of long lasting AVP potentiation or depression (depending on the concentration) we can speculate that the mechanism is a calcium-dependent mechanism that is common to many forms of LTP previously studied (Dunwiddie and Lynch 1979; Malenka, 1991; Son and Brinton, 1998; Son and Brinton, 2001).

Dubrovsky and colleagues (2003) examined icv AVP (1 μ g) in the anesthetized rat with tetanic stimulation and found that icv AVP produced a significant enhancement of tetanus-induced potentiation in the amplitude of both EPSP slope and population spikes of the perforant path-evoked potentials in the dentate gyrus. The effects were evident 1 minute after tetanization. The population spike amplitude increased continuously for the 2 hour recording time, reaching values 100% above baseline level. Without tetanization, dentate gyrus-evoked potentials were often of higher amplitude than baseline, but these values did not reach statistical significance.

1.4 Neuromodulation of NE and AVP

A neuromodulatory action of a neurotransmitter enhances or diminishes the effects of another neurotransmitter on a given target neuron by means of an interaction between the receptors for the two neurotransmitters. A neuromodulatory action is mediated by metabotropic receptors. It occurs when G-protein coupled transmitter effects produce long-lasting neuronal changes that influence the normal responses to a fast ionotropic

neurotransmitter. A neuromodulatory effect is of slow onset and results in a longer lasting metabolic and structural effect that alters the transmitter output or responsivity of the target neuron to other transmitter inputs (McEwen, 2004). This provides a mechanism by which biochemical association could occur. A neuromodulatory interaction that has been linked to memory function and which has been the subject of biochemical investigation is the interaction between NE and AVP (Church, 1983; Brinton, 1990). This is also the focus of the present electrophysiological study.

There has been extensive research on the possibility that the three brainstem-telencephalic monoaminergic projection systems, NE, dopamine (DA) and serotonin (5-HT) modulate memory processes (Hasselmo, 1994; Myhrer, 2003). Of particular importance to this thesis, is the noradrenergic projection system that originates in the cell populations localized in the LC of the pons and the LC fibres projecting to telencephalic structures implicated in memory processing for example, the hippocampus and the septum. These same structures receive terminals from extrahypothalamic AVP-containing fiber projections or contain AVP receptors. As outlined previously, there is support for the propositions that noradrenergic projections facilitate memory processing (Borrell et al, 1983, Ellis, 1985; Sara, 1985; Lee and Ma, 1995), at least in some types of learning and memory. Studies by De Wied and Kovacs and colleagues have investigated the possibility that the central neurohypophysial peptidergic fiber systems modulate memory processes by influencing neurotransmission in these brainstem-telencephalic monoaminergic projections. Their research has specifically focused on the fiber projections containing NE or DA.

Kovacs et al. (1977) carried out behavioral and biochemical experiments to investigate an AVP and catecholamine interaction in memory processing. In the behavioral experiments, the catecholamine synthesizing enzyme inhibitor, alpha-methyl-p-tyrosine methylester (MPT) was injected alone or in combination with LVP and learning and retention of a bench-jump passive avoidance task was assessed. The

treatment had no effect on learning but the MPT and LVP combination prevented the LVP-retention effect. The biochemical experiments tested if LVP influenced NE through the study of NE levels after LVP injection in different areas of the brain. The NE turnover rate was measured in these structures. LVP was not found to affect NE content in the hypothalamus, septum or striatum but it increased the MPT-induced disappearance of NE in the hippocampus. This study supports the findings that AVP plays a role in retention and also suggests that NE, or catecholamines in general, may be involved in mediating AVP's effect since inhibiting catecholamine synthesis prevented AVP enhancement of retention.

Kovacs et al. (1979a) examined the effects of AVP (not LVP) on memory consolidation in a passive avoidance task and on NE neurotransmission. AVP injection into the dentate gyrus of the hippocampus facilitated passive avoidance memory consolidation and increased utilization of NE in that structure and also in the dorsal septal nuclei. Kovacs, Bohus and Versteeg (1979b) reported that intra-dentate injections of 25 pg of AVP were effective in improving task retention of passive avoidance behaviour and that NE depletion, using 6-OHDA lesions of the dorsal noradrenergic bundle, blocked this effect. The results of this study led the authors to conclude that the dorsal noradrenergic bundle-LC-NE fiber pathway is important for the expression of AVP-induced facilitation of passive avoidance memory consolidation and also that the interaction between AVP and the dorsal noradrenergic bundle pathway-NE fiber system occurs in the region of the fiber terminals of this pathway in the dentate gyrus.

Church (1983) examined the influence of LVP, NE and their combination on the stimulation of cAMP accumulation in the hippocampal slice. He found that NE produced a 4-5 fold increase in cAMP concentration, but no effect was seen with LVP alone. However, LVP potentiated the cAMP stimulative effect of NE twice as much as NE alone. In support of these findings, another study found that anti-AVP serum, which reduces endogenous AVP, injected into the hippocampus and septal area attenuated

retrieval and consolidation and also showed that AVP enhances NE turnover in these structures (Veldhuis et al., 1987).

Tanaka et al. (1977) also studied icv AVP effects on catecholaminergic rates of utilization in various brain sites. Their study supports the lesion and microinjection studies described previously in that the same regions showed increased AVP-induced catecholaminergic utilization. These regions included the hippocampus, parafaciscular thalamus, dorsal septal nucleus, LC and dorsal raphe nucleus.

In a later biochemical investigation of this AVP dependency on NE, Brinton and McEwen (1989) found that AVP not only significantly potentiated NE-induced cAMP formation but the potentiation was selective for β -adrenoceptor-stimulated adenylate cyclase. It was later found that AVP-induced potentiation was a Ca^{2+} -dependent process and could be blocked by an antagonist to the calcium-binding protein, calmodulin (Brinton and Brownson, 1993). So it has been proposed that NE release into the dentate gyrus mediates the AVP memory enhancement.

The microinjection, lesion and biochemical studies provide evidence for the theory that the memory-modulating effects of AVP involve brainstem and forebrain limbic system structures that are implicated in memory processing. These studies support the hypothesis that the interaction of AVP and central catecholamine neurotransmitters, in particular, NE, mediate AVP's influence on memory processing with targeted studies identifying the dentate gyrus as a critical structure in this interaction.

The purpose of the present study is to ask if AVP plays a role in the potentiation of glutamatergic perforant path input to the dentate gyrus *in vivo* and to determine whether that potentiation is dependent on local noradrenergic β -receptor activation. In the first experiment, urethane-anaesthetized rats are given icv infusions of AVP while

responses to perforant path input are monitored in the dorsal dentate gyrus. In the second experiment the procedure is repeated but two micropipettes, one filled with saline and a second filled with the β -receptor antagonist, propranolol, are used to monitor the perforant path evoked potential responses before and following icv infusion of AVP. A third experiment examined icv infusion of the AVP fragment, DGAVP which selectively activates the V1A receptor, while responses to perforant path stimulation were again monitored in the dorsal dentate gyrus.

METHODS

All procedures and methods were conducted in accordance with the guidelines and procedures of the Canadian Council on Animal Care and were approved by the Memorial University of Newfoundland Institutional Animal Care Committee.

Experiment 1

Subjects:

Twenty male Sprague-Dawley rats weighing 250-300 g; Memorial University vivarium, were anesthetized with urethane (1.5 g/kg; i.p.).

Surgical procedure:

When tail pinch no longer caused a response, the head fur was shaved, and the animal was placed into the earbars in a stereotaxic frame, in the skull flat position. Temperature was maintained at 37.5 degrees Celsius with the use of a rectal probe and heating pad. Skin was cut along the midline and held back with forceps. Coordinates were mapped as follows: the cannula was targeted to the lateral ventricle at 0.8 mm posterior to bregma and 1.5 mm lateral; the recording electrode was targeted to the dentate gyrus at 3.5 mm posterior to bregma and 2.0 mm lateral and the stimulating

electrode was targeted to the perforant path at 7.2 mm posterior to bregma and 4.1 mm lateral. A stainless steel guide cannula (28 ga; Plastics One, Roanoke VA) was lowered into the lateral ventricle to a depth of 3.2 mm below skull surface and secured with dental cement to a jeweller's screw implanted into the skull.

Recording and stimulating procedure :

A single saline-filled glass micropipette was lowered 2.5-3 mm into the dentate gyrus, and a single bipolar stimulating electrode (NE-100; Kopf) lowered 3.0 mm into the perforant path. Stimulation consisted of a single .2 ms square wave pulse delivered with a 10 second interstimulus interval (0.1 Hz). Depths were optimized to produce a maximal, positive-going population spike in the dentate granule cell layer. Recordings were amplified by a Grass P5 series amplifier (filters 1 Hz - 3 kHz). The signals were digitized (10 kHz) and stored on a computer using the Datawave Technologies "Brainwave" software package. After 23-30 minutes of baseline recording, 1 ng (in 2 μ l saline) AVP was given icv via an internal injection cannula (40 μ m tip) placed into the guide cannula in the lateral ventricle. Infusion occurred at a rate of 2 μ l/min. Responses were recorded for a minimum period of 30 minutes post-injection.

Upon completion of the experiment, methylene blue (1%; 2 μ l) was delivered into the lateral ventricle to confirm ventricular placements. The animal was then sacrificed and the brain fresh frozen in -72 degrees Celsius methylbutane. Cannula and electrode placements were confirmed histologically. Brains were sectioned in a cryostat in the coronal plane (40 μ m thickness) and alternate sections were taken for dye verification (methylene blue) and electrode placements (Nissl stain). Data were included if methylene blue was located within the lateral ventricle.

Experiment 2

Subjects:

Six male Sprague-Dawley rats weighing 250-300 g; Memorial University vivarium) were anesthetized with urethane (1.5 g/kg; i.p.).

Surgical and recording procedure:

This experiment followed the same surgical procedure for cannula and recording procedures as the first experiment, but used two recording micropipettes (40 μm tips) in which one recording pipette was filled with saline (0.9%) and the other filled with the norepinephrine β -antagonist, propranolol (50 μM , mixed in 0.9% saline). This methodology has been used previously to study local pharmacological effects in the dentate gyrus (see Munro et al, 2001). The pipettes were placed 0.5 mm apart in a 2 electrode holder with each optimized for a positive population spike. Typically the posterior pipette was more medial and deeper than the anterior one. The pipettes were adjusted until similar population spikes were recorded on each so as to be comparable prior to AVP infusion.

Experiment 3

Three male Sprague-Dawley rats weighing 250-300 g; Memorial University vivarium, were anesthetized with urethane (1.5 g/kg; i.p.).

Surgical and recording procedure:

This experiment followed the procedures of experiment 1 with the exception that the AVP fragment DGAVP (1 ng/2 μl saline), was injected into the lateral ventricle instead of AVP.

Data Analysis:

The Brainware Software allowed for parameter extraction to be performed to measure 2 characteristics of the response: (1) the excitatory post-synaptic potential (EPSP) slope - average rate of change (rise/run; mV/ms) of the rising segment of the

EPSP, (2) the population spike - average voltage difference (mV) between the peak and trough of the downward deflecting spike event. Following measurement extraction of each waveform, data were transferred to an Excel spreadsheet program. The final graphs were prepared in Prism (for percentages, graphing and statistics). Paired samples t-tests were performed on the average 30 minutes baseline compared to the average of 30 minutes post injection.

RESULTS

Experiment 1: Response to AVP

The effect of intracerebroventricular (icv) application of AVP on the population spike amplitude and the EPSP slope of the perforant path-dentate gyrus evoked potential was examined post AVP infusion in 16 rats.

1.1 Population spike changes

AVP reliably produced potentiation of the population spike amplitude in 15 out of 16 animals at the 1 ng dose (Table 1-1 and Figure 1-1). Fifteen of the animals infused with AVP demonstrated potentiation of the dentate gyrus evoked response as potentiation of the amplitude of the population spike. Eleven rats showed potentiation of EPSP slope. Table 1-1 shows the effect of 1 ng AVP icv on the population spike amplitude and the EPSP slope in the dentate gyrus in each animal (n=16).

In the fifteen animals, AVP increased the population spike amplitude with a mean increase of 94% averaged over 30 minute post AVP. This increase typically occurred within 3-5 minutes and potentiation continued to rise over the 30-35 minutes post-injection period. Individually the increase ranged from 10% to 250% at the peak in some experiments. The baseline mean increased from an amplitude of 2.67 mV to 3.66 mV 30 minutes post AVP application ($t = 5.57$; $df = 14$; $p < 0.05$). One animal showed a significant decrease of 28% in population spike amplitude and showed no change in EPSP slope.

In four experiments the potentiation was followed beyond 30 minutes and lasted more than 1 hour in all cases. The longest monitoring occurred in experiment 12 (Table 1-1 or #4 in Table 1-2) which had a mean baseline (30 minutes) of 2.17 mV of the population spike amplitude, the 30 minutes post mean was 3.28 mV, in the next 30 minutes block (1 hour post) it increased to 3.84 mV and in the last 30 minutes block (2

hours after AVP administration) the amplitude was at a mean of 3.95 mV. The group that was followed beyond an hour (n = 4) showed a mean increase of 80% (in the 30-60 minute block) across animals with individual increases ranging from 41% to 246%.

Table 1-1. Effects of intracerebroventricular (icv) application of AVP on the population spike amplitude and the EPSP slope of the perforant path-dentate gyrus evoked potential in the urethane anesthetized rat (n = 16).

Single Pipette Experiments N=16	Population Spike Amplitude (mV)				EPSP Slope (mV/mS)			
	Pre AVP Mean	Std. Dev.	Post AVP Mean	Std. Dev.	Pre AVP Mean	Std. Dev.	Post AVP Mean	Std. Dev.
1	0.28	0.063	0.79*	0.300	1.73	0.056	2.19*	0.203
2	0.64	0.138	3.10*	0.757	2.07	0.026	2.47*	0.124
3	5.46	0.302	5.18**	0.414	2.63	0.086	2.66 ns	0.129
6	5.90	0.269	6.32*	0.396	4.65	0.055	4.74*	0.068
7	3.02	0.196	3.61*	0.358	3.33	0.054	3.59*	0.099
8	0.56	0.051	1.28*	0.374	2.21	0.095	1.94 ns	0.253
9	2.36	0.075	3.51*	0.337	3.18	0.029	3.46*	0.101
10	4.38	0.039	5.01*	0.339	3.16	0.082	2.78**	0.125
11	1.25	0.344	2.32*	0.263	3.71	0.125	3.64 ns	0.071
12	2.17	0.072	3.28*	0.626	2.34	0.060	3.18*	0.505
13	1.13	0.061	1.83*	0.463	2.86	0.085	2.85 ns	0.089
14	3.42	0.214	5.63*	0.883	4.51	0.126	6.06*	0.644
15	2.67	0.163	3.96*	0.578	2.09	0.046	2.30*	0.044
16	4.55	0.299	5.64*	0.844	5.71	0.064	6.10*	0.132
17	2.46	0.430	2.87*	0.544	2.89	0.104	2.98*	0.133
18	2.55	0.138	3.25*	0.715	5.27	0.108	5.86*	0.342
Group Mean N= 16	2.67	1.72	3.66*	1.55	3.27	1.20	3.55*	1.39

Data represent means in millivolts (mV) for the 25 minutes pre AVP mean and an average of 30 minutes post AVP (1 ng) infusion. * indicates an increase at a minimum of $p < 0.05$ between pre and post means as a result of two-tailed paired t-test on raw data. ** indicates a significant decrease between pre and post mean responses.

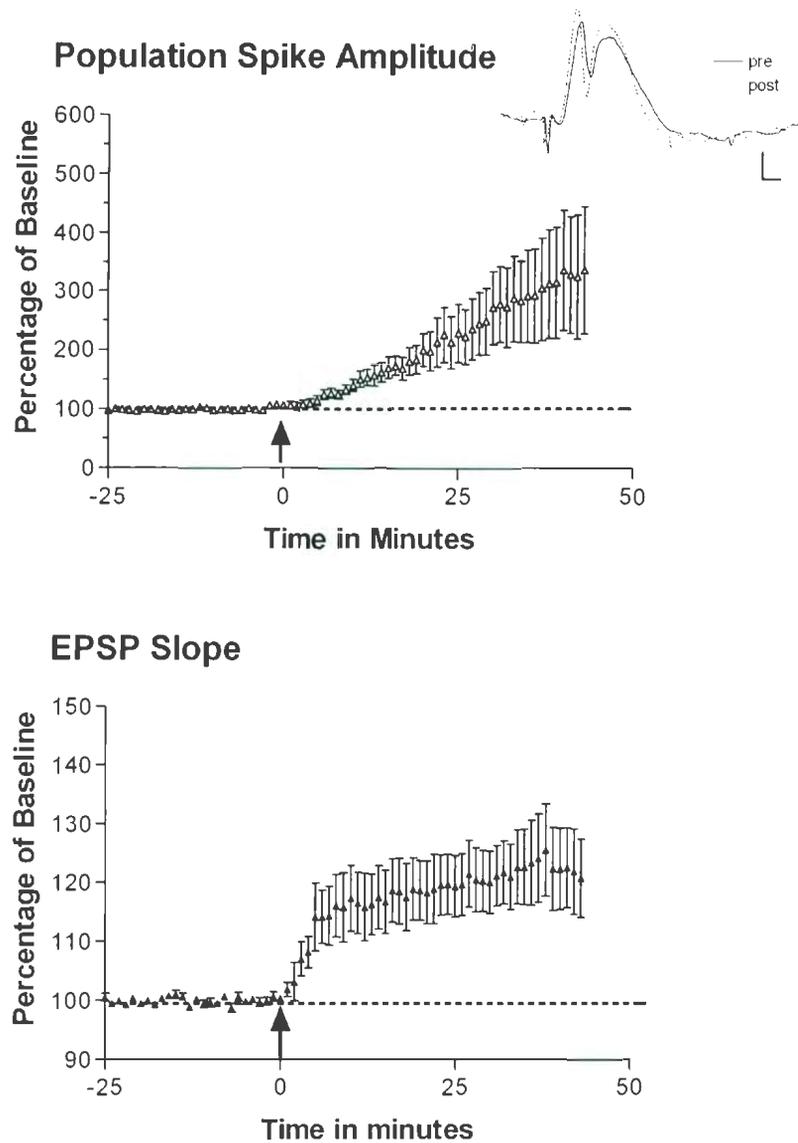


Figure 1-1: Intracerebroventricular application of AVP induces a potentiation of the population spike amplitude and field EPSP slope in the urethane anesthetized rat. A) i.c.v AVP (arrow) induced a long-term potentiation of the dentate gyrus evoked population spike in the anesthetized rat ($n = 15$). *Inset*: Sample waveforms with parameter measurements of the dentate gyrus evoked potential before (solid line) and after (dashed) infusion of AVP into the lateral ventricle. Scale bar = 2 mV and 2 ms. B) Intracerebroventricular AVP also produced a long-term potentiation of the EPSP ($n = 11$).

1.2 EPSP slope changes

AVP reliably produced potentiation of the EPSP slope in 11 out of the 16 animals at a dose of 1 ng icv. Table 1-1 and Figure 1-1 show the effect of AVP on EPSP slope in the dentate gyrus across all animals (n=16). EPSP slope increased stepwise starting within 2-4 minutes, peaked between 10-20 minutes and continued to be elevated over the typical 30 minute recording period. The baseline mean increased from 3.27 to 3.55 mV/ms with 30 minute blocks pre and post AVP ($t = 2.45$; $df = 15$; $p < 0.05$). The increase was an average of 20% at the peak overall from baseline with a range of a 2% increase to a 46% increase in individual animals. Out of the fifteen animals that showed an increased population spike, 11 animals or 73% showed increased slope as well. In 3 of the animals AVP produced a 10% decrease from baseline in EPSP slope and no change in the EPSP slope occurred in 2 animals. Figure 1- 3 illustrates the 5 animals that showed a decrease or slight change in the EPSP slope.

The increases in EPSP slope were further followed from 75 min. to 125 min. in four experiments. Table 1-2 shows a mean increase of 21% in the 30-60 minute block, but individual animals varied, and ranged from a 5% increase to an increase of 45% 60 minutes after AVP. With reference again to experiment 12 (Table 1-1 or #4 in Table 1-2), a significant increase is seen in the EPSP slope which had a 30 minute baseline of 2.34 mV/ms, a mean of 3.18 mV/ms at 30 minutes, a mean of 3.56 mV/ms at 1 hour and a mean of 3.74 mV/ms 2 hours post AVP.

Table1-2: Effects of intracerebroventricular application of AVP on the population spike amplitude and the EPSP slope of the perforant path-dentate gyrus evoked potential in 4 urethane anesthetized rats in which the evoked response was monitored for periods extending past 60 minutes post- AVP injection (n=4).

Population Spike Amplitude (mV)					EPSP Slope (mV/ms)			
	Pre AVP (30mins)		Post AVP (60mins)		Pre AVP (30mins)		Post AVP (60 mins)	
	Mean	StdDev.	Mean	Std Dev.	Mean	Std Dev.	Mean	Std Dev.
Exp. 9	2.36	0.075	3.51*	0.699	3.18	0.029	3.37*	0.101
12	2.17	0.072	3.63*	0.626	2.34	0.060	3.56*	0.147
13	1.13	0.061	3.02*	0.898	2.86	0.085	2.90	0.089
14	3.42	0.214	5.82*	0.887	4.51	0.126	6.09*	0.645
Grp	2.27	0.106	4.00*	0.778	3.22	0.075	3.98	0.246
% mean	80% increase				21% increase			

Data represent means in millivolts (mV) at 25 minutes of baseline and at 60 minutes post AVP (1 ng) infusion.* indicates increase at a minimum of $p < 0.05$ between pre and post AVP means as a result of two-tailed paired t-test on raw data.

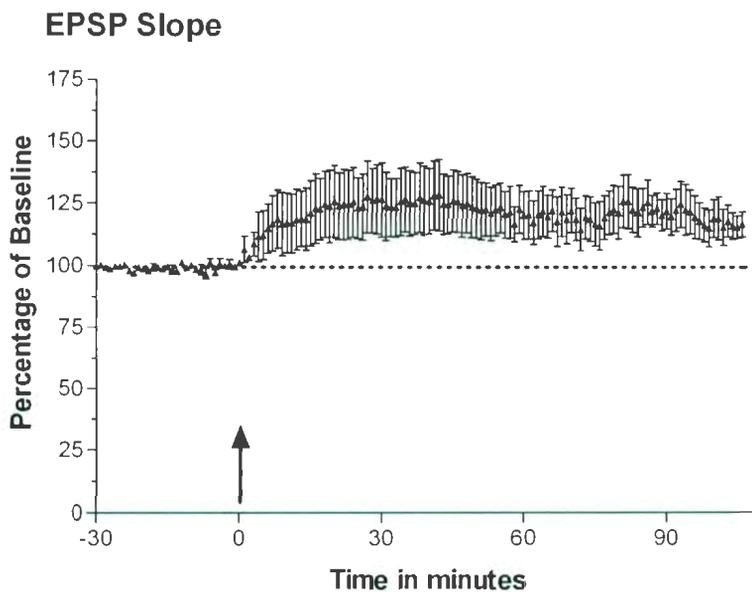
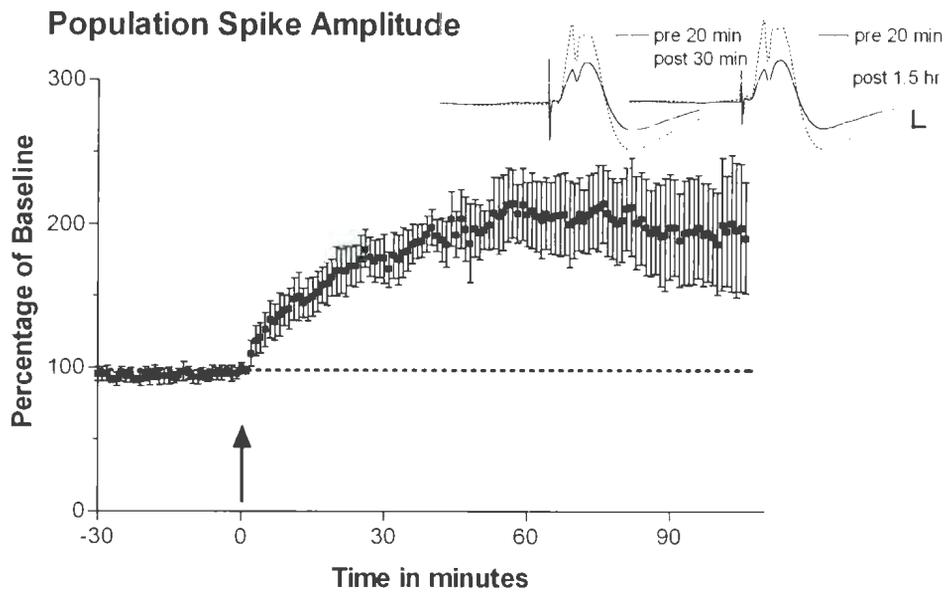


Figure 1-2 : Intracerebroventricular application of AVP induces a potentiation of the population spike amplitude and field EPSP slope in the urethane anesthetized rat that lasted beyond an hour. *Inset:* Sample waveforms (exp. 12) with parameter measurements of the dentate gyrus evoked potential before (solid line) and 30 minutes, and 1.5 hour after (dashed) infusion of AVP into the lateral ventricle. Scale bar = 2 mV and 2 ms. A) i.c.v AVP (arrow) induced a long-term potentiation of the dentate gyrus evoked population spike in the anesthetized rat and EPSP slope that lasted beyond an hour (n = 4)

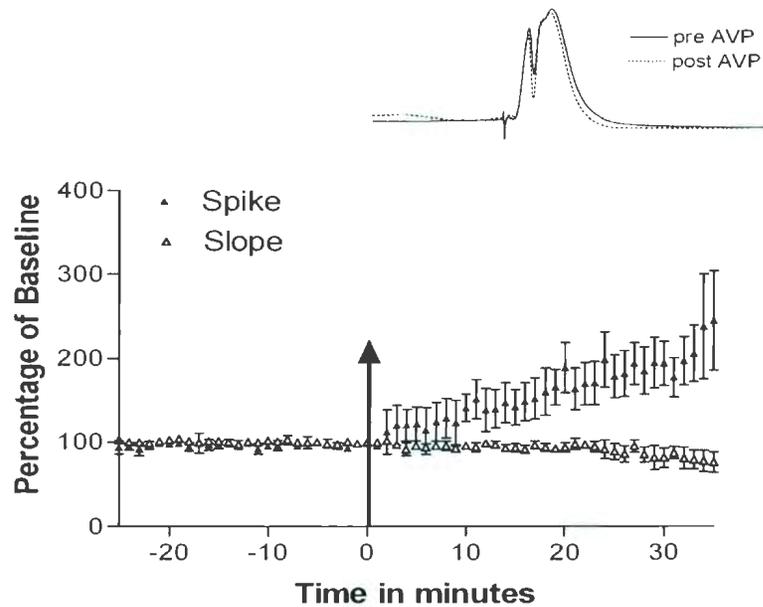


Figure 1-3 Intracerebroventricular application of AVP decreased the field EPSP slope in the urethane anesthetized rat (n=3) or produced little change (n=2). Inset: Sample waveforms for experiment 10 (Table 1-1) with parameter measurements of the dentate gyrus evoked potential before (solid line) and after (dashed) infusion of AVP into the lateral ventricle. Scale bar = 1 mV and 2 ms.

1.3 Population spike / EPSP slope relationships

An overlay of spike and slope (n = 16) is seen in Figure 1-4 to illustrate the profile of slope and spike at 20 minutes of baseline and 40 minutes after AVP.

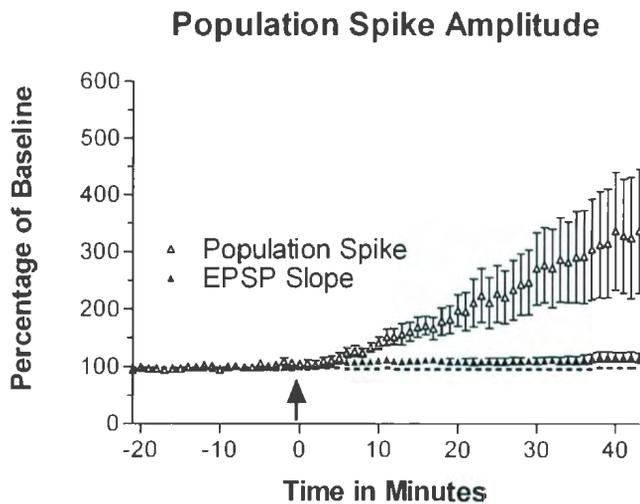


Figure 1-4: A closer look at an overlay of population spike amplitude effects against EPSP slope effects (n=16).

Since AVP was found to increase the population spike amplitude and the EPSP slope, correlations were conducted to determine if the increase in granule cell firing (the population spike) was the result of postsynaptic depolarization (EPSP slope). These analyses were examined to determine if the effects of AVP on the population spike amplitude could be accounted for by the effects of AVP on the EPSP slope.

Table 1-2 shows the correlations of population spike amplitude and EPSP slope in all 16 single pipette experiments. Prior to AVP, only two rats showed a significant increase in slope/spike correlation. Following AVP, nine rats showed significant correlations with all rats having significantly strong correlations for slope to account for more than 30% of the variance in spike.

Thus the slope/spike relationship was variable with slope accounting for some of the spike change in about a third of the experiments, but not contributing to the spike change in the majority in others. Temporal dissociations between slope and spike changes can be seen in Figures 1-1, 1-2 and 1-4.

Table 1-3. Correlations (r^2) of population spike amplitude and EPSP slope in all 16 single pipette experiments.

Single Pipette Experiments N=16	Correlation (r^2)	
	Pre AVP	Post AVP
1	0.0214	0.7152*
2	0.0055	0.4499*
3	0.1201	0.7809*
6	0.0107	0.1093
7	0.0003	0.1239
8	0.0093	0.0088
9	0.0140	0.6452*
10	0.0002	0.2409*
11	0.2007*	0.0016
12	0.9652*	0.8584*
13	0.0809	0.0453
14	0.0877	0.7814*
15	0.0480	0.1398
16	0.0731	0.3152*
17	0.1213	0.0039
18	0.1194	0.6296*
Group mean	0.1173	0.3655*

Data represent correlations of populations spike amplitude and EPSP slope

* $p < 0.05$

Experiment 2: Response to NE antagonist and AVP

2.1 Evoked potential changes

The effect of 1 ng AVP icv was examined on the population spike amplitude and EPSP slope in the dentate gyrus at both a saline and propranolol-filled (50 mM propranolol dissolved in saline) micropipette across 6 animals. Results are shown in Table 2-1 and Figure 2-1. Prior to AVP, the size of the EPSP slope, with a mean of 3.38 mV/mS for the saline pipette and 2.26 mV/mS for the propranolol pipette, and the population spike, with a mean of 2.64 mV for saline and 1.95 mV for the propranolol pipette were not significantly different ($t(\text{slope}) = 2.072, df = 5, p > 0.05$) and ($t(\text{spike}) = 1.091, df = 5, p > 0.05$).

2.2 Population spike changes on the saline pipette

AVP reliably produced potentiation of the population amplitude in all six animals at the 1 ng dose on the saline pipette. The mean increase was 50% from baseline. The increase typically occurred within 2-5 minutes and continued after 30-35 minutes post-injection. As seen in Table 2-1, the saline filled pipette showed a population spike amplitude increase from 2.64 mV to 3.59 mV post AVP ($t = 5.150; df = 5; p < 0.05$). Increases ranged from 35% to 160%.

2.3 EPSP slope changes on the saline pipette

AVP also produced a significant potentiation of the EPSP slope in four of the six animals at the 1 ng dose on the saline pipette. The mean increase was 20%. This increase typically occurred within 5 minutes and peaked at between 10-15 minutes post-injection. The saline filled pipette showed a baseline mean increase of 3.38 mV/mS to 3.80 mV/ms post AVP ($t = 2.699; df = 5; p < 0.05$). Individual increases ranged from 1% to 96%.

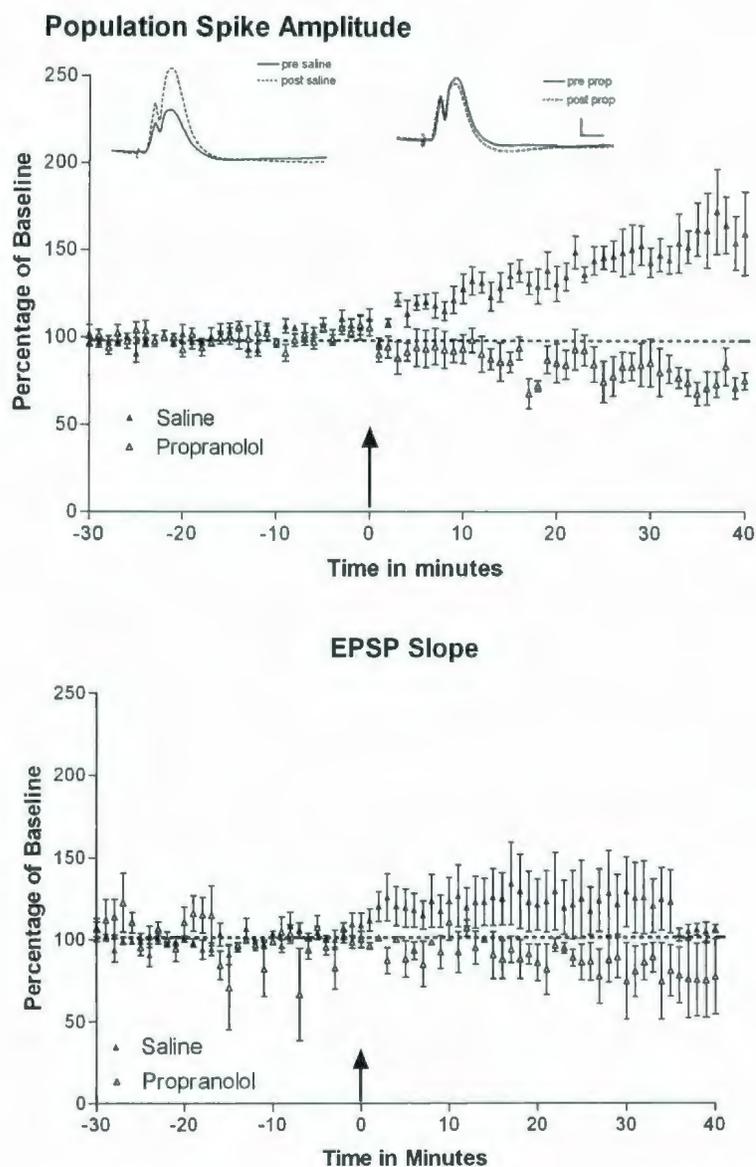


Figure 2-1: Effects of saline or propranolol infusion on the perforant path-dentate gyrus evoked potential following intracerebroventricular application of AVP. A) i.c.v. AVP induced a long-term potentiation of the dentate gyrus evoked population spike (50%) on the saline pipette in contrast to a modest decrease (17%) on the propranolol pipette ($n = 6$). *Inset*: Sample waveforms with parameter measurements of the dentate gyrus evoked potential before (solid line) and 40 minutes (dashed) infusion of AVP into the lateral ventricle. Scale bar = 1 mV and 2 ms. B) i.c.v. AVP produced potentiation of the EPSP slope (20%) in the saline pipette but showed slightly decreased EPSP slope (9%) on the propranolol pipette ($n = 6$). *Inset*: Sample waveforms with parameter measurements of the dentate gyrus evoked potential before (solid line) and 40 minutes (dashed) infusion of AVP into the lateral ventricle. Scale bar = 1 mV and 2 ms.

2.4 Population spike and EPSP slope changes in propranolol pipette

Employing the propranolol-filled micropipette, two of the six experiments showed decreased population spike amplitude and four showed no significant change. Overall the population spike amplitude decreased from 1.95 mV to 1.68 mV, but this was not significant ($t= 2.225$; $df=5$; $p<0.05$) post AVP. Overall EPSP slope in four experiments decreased a small and significant amount in the 30 minute block post AVP, from 2.26 mV/ms to 2.04 mV/ms post AVP ($t=2.954$; $df=5$; $p<0.05$). In two experiments, there was no change. The overall decrease was significant. The mean decrease in EPSP slope was 9% from baseline but animals ranged from a 9% to 27% decrease on the propranolol pipette.

Table 2-1. Effects of saline or propranolol infusion on the perforant path-dentate gyrus evoked potential using the double pipette procedure after icv AVP administration in the urethane anesthetized rat.

Double Pipette Experiments	Population Spike Amplitude (mV)				EPSP Slope (mV/ms)			
	Pre Saline	Post Saline	Pre Prop	Post Prop	Pre Saline	Post Saline	Pre Prop	Post Prop
3	0.54	1.40*	2.43	2.21	4.16	4.17	2.55	2.45*
5	2.71	3.63*	0.46	0.44	6.65	7.61*	3.42	3.33*
6	3.39	5.00*	2.24	2.10*	3.18	3.56*	2.05	1.78*
7	3.40	4.05*	2.30	2.19	1.61	1.76	2.36	2.15*
8	3.88	5.18*	1.84	1.02*	1.53	2.32*	0.42	0.33
9	1.94	2.29*	2.36	2.11*	3.15	3.36*	2.77	2.21
Group mean	2.64	3.59*	1.95	1.68	3.38	3.80*	2.26	2.04*

Data represent means in millivolts (mV) in the 30 minute baseline block and 30 minutes post AVP infusion and recorded by the saline or propranolol (50mM) pipettes. * minimum of $p<0.05$ compared between post and baseline mean measures as a result of two tailed paired t-tests on individual raw data.

2.5 Population spike and EPSP slope relationship

Correlational analyses of population spike and slope were performed on all 6 animals, seen in Table 2-2. There were no significant correlations between slope and spike prior to AVP on the saline pipette. Four animals showed a significant increase in correlation from baseline AVP/Sal to post AVP/Sal. In experiment 3 and 6, the AVP/Sal pipette showed no correlation between the spike and slope pre or post AVP. The significant correlations after AVP accounted for less than 20% of the variance suggesting slope contributed only moderately to spike amplitude increases. There was one small, but significant slope/spike relationship pre AVP on the propranolol pipette, this was not seen after AVP. Correlations became significant in 2 other experiments but less than 20% of the variance was attributable to slope.

Table 2-2. Correlations (r^2) of spike and slope in all 6 double pipette experiments.

Double pipette experiments	Pre	Post	Pre	Post
	AVP/Sal	AVP/Sal	AVP/Prop	AVP/Prop
3	0.0055	0.0377	0.1718*	0.0234
5	0.0363	0.2105*	0.0155	0.0068
6	0.0019	0.0438	0.0015	0.0004
7	0.0125	0.2736*	0.0565	0.0131
8	0.0046	0.3383*	0.0120	0.4775*
9	0.0992	0.4296*	0.0022	0.1861*
Group means	0.027	0.222	0.043	0.118

Group means suggest a weak positive increase in correlation after AVP between slope and spike whether there was an increase (AVP/Sal) or a decrease (AVP/Prop) in spike amplitude. These weak correlational effects may relate to the greater range of spike and slope responses after AVP.

Experiment 3: Response to an AVP Fragment, DGAVP

In order to assess the specificity of the original AVP actions, DGAVP, the selective V1a agonist was infused into the ventricles of 3 animals.

3.1. Population spike changes

DGAVP produced increases in the population spike amplitude in all 3 animals. Figure 3-1 and Table 3-1 show the effects of the AVP fragment (DGAVP) on the population spike and the EPSP slope.

The population spike amplitude increased with a total mean of 1.95 mV on the baseline 30 minute block to 3.19 mV in the 30 minute block post DGAVP. This was significant in a one-tailed t-test ($t = 3.148$; $df = 2$; $p < 0.05$). DGAVP caused a potentiation of the population spike amplitude after 2-3 minutes rising to 100% above baseline within 25 minutes post infusion. Individual increases ranged from 73% to 200%.

Table 3-1. Effects of the AVP fragment DGAVP on the population spike amplitude and EPSP slope of the perforant path-dentate gyrus evoked potential in the urethane anesthetized rat.

Fragment experiment	Population spike amplitude (mV)				EPSP slope (mV/ms)			
	Pre DGAVP Means	Std Dev.	Post DGAVP Means	Std Dev	Pre DGAVP Means	Std Dev	Post DGAVP Means	Std Dev
1	1.82	0.395	2.80*	0.408	2.15	0.067	2.11	0.072
2	1.84	0.103	3.86*	0.886	1.35	0.042	1.64*	0.078
3	2.19	0.662	2.92*	0.122	3.27	0.505	2.55*	0.535
Group mean	1.95	0.208	3.19*	0.580	2.26	0.964	2.10	0.455

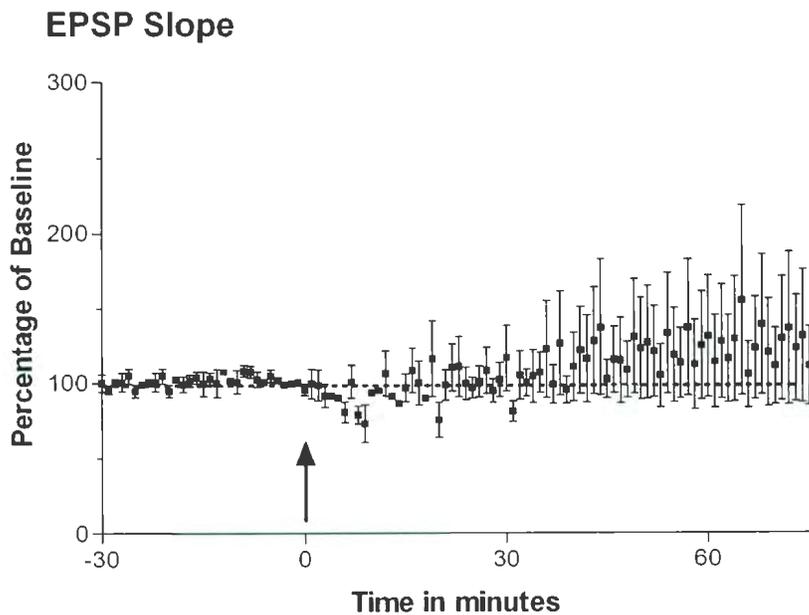
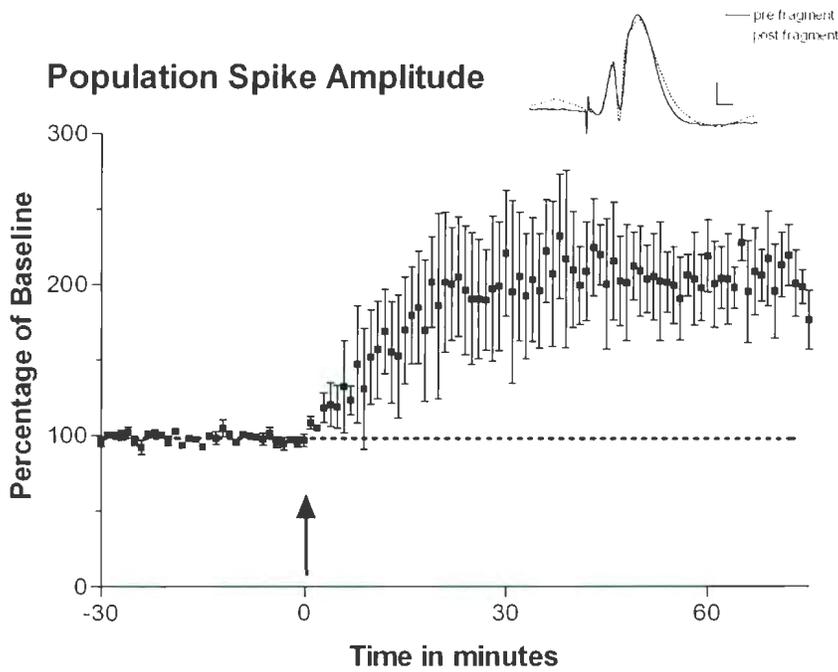


Figure 3-1: Intracerebroventricular application of DGAVP induces a potentiation of the population spike amplitude but has varied effects on the field EPSP slope in the urethane anesthetized rat. *Inset*: Sample waveforms with parameter measurements of the dentate gyrus evoked potential before (solid line) and 30 minutes after (dashed) infusion of DGAVP into the lateral ventricle. Scale bar = 2 mV and 2 ms.

3.2. EPSP slope changes

DGAVP did not alter EPSP slope overall post DGAVP ($t = 0.5269$; $df = 2$; $p > 0.05$; see Table 3-1 and Fig. 3-1). One animal showed a significant increase, one a decrease and one, no change. The percentage effects ranged from a 72% decrease to a 29% increase.

3.3. Population spike/EPSP slope relationship

There was no significant slope/spike correlation prior to DGAVP. After DGAVP, a significant slope/spike effect was seen in the rat with a decrease in EPSP slope, although spike had increased significantly overall in this experiment. Spike increases did not depend on slope changes in this experiment. Table 3-2 summarizes the correlations of the fragment experiments.

Table 3-2: Correlations (r^2) of spike and slope of fragment experiments (n=3)

Fragment Experiments	Correlation (R^2)	
	Pre DGAVP	Post DGAVP
1	0.0084	0.0058
2	0.0103	0.0110
3	0.0812	0.2836*
Group Mean (n=3)	0.0333	0.1001

4.1 Histology for Experiments 1-3

All animals showed clear methylene blue in the lateral ventricles while waveforms were indicative of proper placement with the dentate gyrus and perforant path electrodes. The

placement of the dentate gyrus recording electrodes was also reconstructed from the Paxino atlas (Paxinos and Watson, 2004). Placements for each experiment are presented in figures 4-1 to 4-3. While it was difficult to visualize the exact tips of the glass pipettes, the tracks and waveforms taken together suggest placement in or slightly below the dorsal blade granule cell layer. In Figure 4-2 both propranolol and saline pipette tips are shown with the more posterior placements being propranolol.

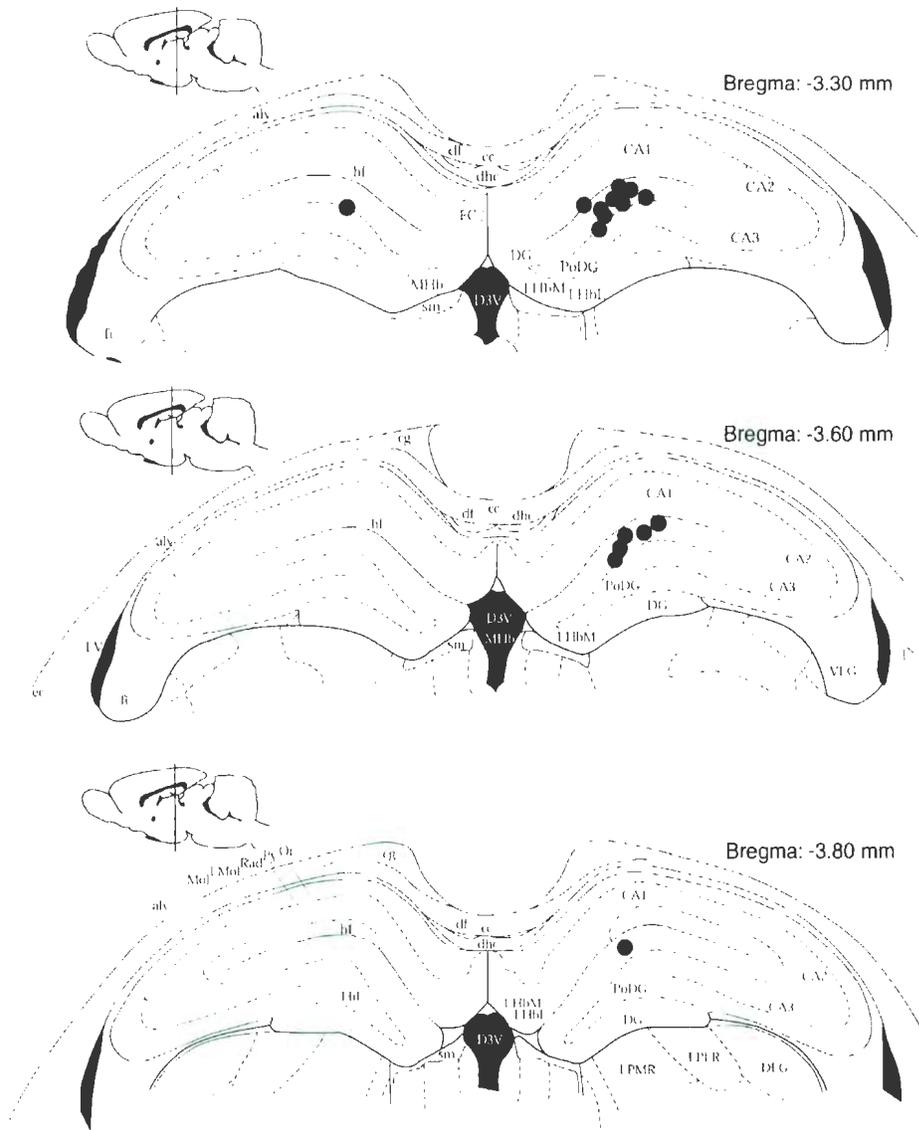


Figure 4-1: Placement of recording electrode in areas of dentate gyrus in experiment 1 showing placements of all 16 animals.

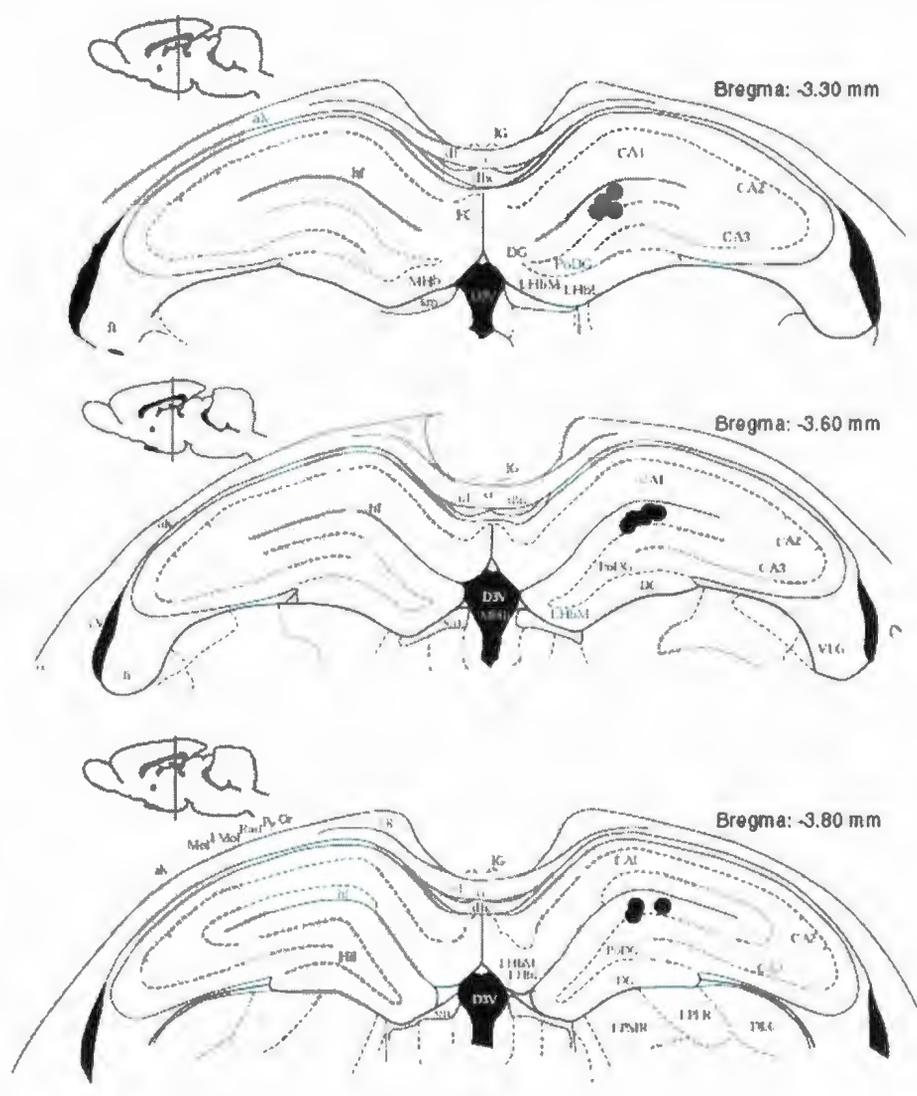


Figure 4-2: Placement of recording electrode in areas of dentate gyrus in Experiment 2, double pipette experiments, showing placements of all 6 animals.

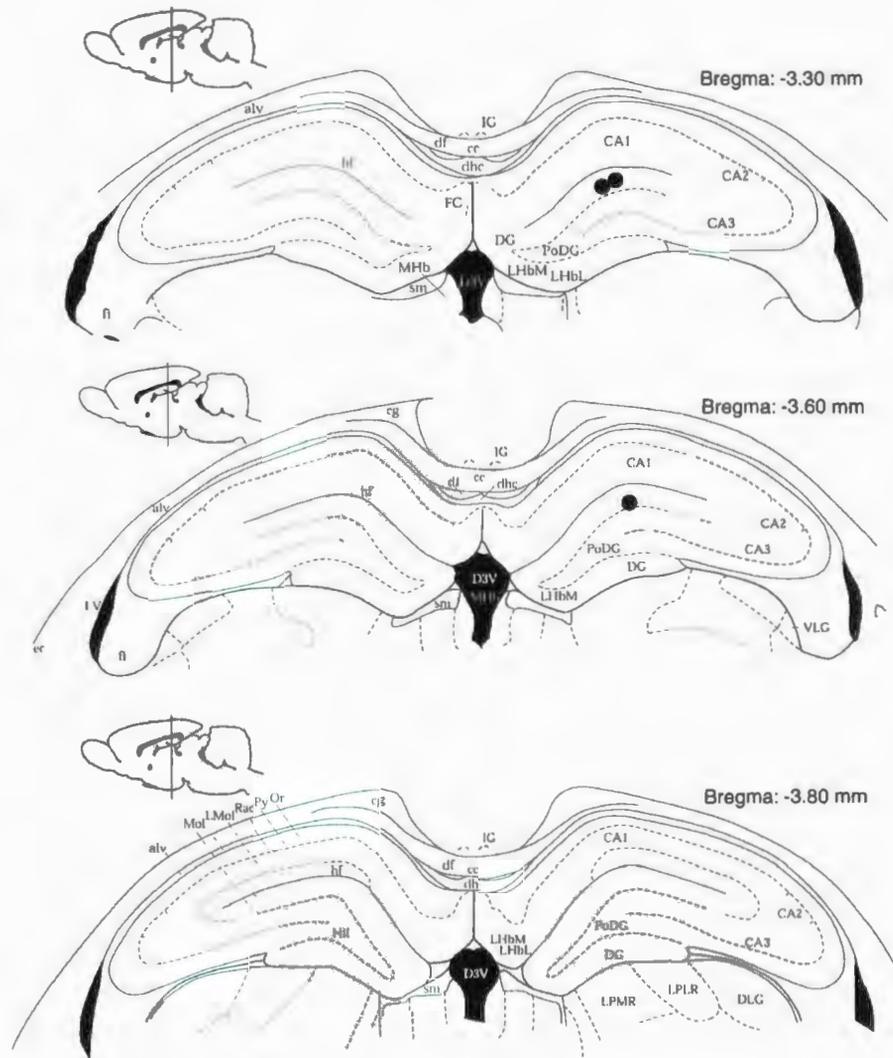


Figure 4-3: Placement of recording electrode in areas of dentate gyrus in experiment 3 showing placements of 3 animals.

Discussion

AVP effects on the perforant path evoked potential in the dentate gyrus:

Experiment 1

Intracerebroventricular administration of 1 ng of AVP produced long-lasting potentiation of the perforant path evoked population spike in the dentate gyrus of the urethane-anesthetized rat. Potentiation of the perforant path evoked EPSP slope was also observed in a majority of experiments. This is the first report of AVP-induced long-lasting potentiation *in vivo*. In addition, the results provide a plasticity mechanism to support earlier observations of icv and direct dentate gyrus AVP infusion enhancement of the retention for avoidance learning first reported by De Wied's group and recent observations of spatial learning enhancement (Paban et al, 2003). De Wied (1976) found 1 ng of AVP given icv immediately after a passive avoidance learning trial resulted in longer latencies to enter the shock compartment relative to controls the next day. Vasopressin injected directly into the dentate gyrus (25 pg), immediately after a learning trial also improved passive avoidance behaviour (Kovacs et al, 1979). A study examining AVP in mice in the Hebb-Williams maze also found improved spatial memory following 1 ng i.c.v. and dorsal hippocampal administration, but not ventral hippocampal administration (Paban et al, 2003).

While both population spike and EPSP slope were potentiated, only slightly more than 50% of subjects showed a significant contribution of EPSP slope to the spike increases after AVP based on correlational analyses. The distinct time courses of EPSP slope and population spike increases, the failure to see EPSP slope increases in nearly a third of the experiments and the lack of significant slope/spike correlations in a substantial proportion of subjects suggests two distinct plasticity processes are engaged by AVP: EPSP slope increases and population spike or excitability increases.

EPSP slope increases in the dentate gyrus were first reported by Chen et al. (1993) in an *in vitro* study. The present study confirms their effect *in vivo*. In their dose

response study they found that at 100 nM potentiation was 18% over baseline while at 500 nM it was 50% over baseline. Our result with 2 μ l of 500 nM AVP (the concentration produced by our 1 ng solution) in the ventricles was 20% over baseline suggesting the concentration reaching the dentate gyrus was likely less than 500 nM, but above threshold, since Chen and colleagues (1993) found no response at 50 nM. Their potentiation was only observed at physiological calcium concentrations and became depression at higher calcium concentrations. Both EPSP slope increases and decreases appeared within 5 minutes of application and lasted for the entire 60 minute recording time, similar to what was found in the present study. The effect was dose-dependent and also peptide specific since there was no effect with oxytocin infusion. This demonstrates that AVP is not working through oxytocin receptors in the dentate gyrus. From the combined *in vivo* and *in vitro* evidence we conclude that AVP increases the EPSP slope or synaptic strength of perforant path input. Chen et al. (1993) suggested that the most likely mechanism for this effect is postsynaptic, but that remains to be proven.

As reviewed above, in the present study, the increase in the population spike amplitude is not fully accounted for by the EPSP slope increase. This is consistent with an increase in the coupling of the EPSP to spike generation or increased cell firing occurring independent of changes in synaptic strength. Though studies on LTP mechanisms have mainly focused on synaptic strength changes, there are other determinants of neuronal plasticity, learning, and memory which include a wide array of ion channels expressed in the neuronal dendrites. Neuronal excitability can be defined as a propensity of the neuron to generate, beyond a particular threshold, an output signal (the action potential) from a given input signal (an EPSP). This process involves the opening of voltage gated ion channels in the neuronal membrane after the synapse is activated. This coupling concept invokes complex processes involving the voltage gated Ca^{2+} , Na^{+} , and K^{+} channels in the dendrites (Reyes, 2002). These channels have been reported to either amplify or attenuate the EPSP amplitude (Reyes 2002). Ultimately to

understand excitability increases such as those observed here, it will be necessary to identify the type of ion channel properties engaged. These channels can have a powerful influence over the spread of neuronal information. Persistent changes in intrinsic excitability have been discovered in various learning tasks (Daoudal and Debanne, 2003; Zhang and Linden, 2003; Alkon et al, 1985) and examined in numerous brain areas including cortical, hippocampal and cerebellar regions (Frick et al, 2004; Doudal and Debanne, 2003; Brons and Woody, 1980). Local changes in dendritic excitability have been identified as a second Hebbian mechanism underlying long-term associative plasticity (Shrader et al., 2002; Zhang and Linden, 2003). Persistent increases in excitability, as seen in the present study, may enhance memory and retrieval of specific information.

An alternative view of the EPSP slope and spike discrepancies may relate to the complexities of the perforant path evoked potential and will be considered in the next section discussing the role of NE in the observed effects of AVP.

Two other studies have examined AVP effects *in vivo* in the dentate gyrus. Wang et al (2001) examined whether the impairment of synaptic plasticity induced by aluminum could be reversed by peripheral LVP (which has actions similar to AVP) treatment combined with tetanic stimulation. It was found that aluminum significantly reduced the amplitudes of both EPSP and population spike LTP in the dentate gyrus. After the application of LVP, the range of synaptic plasticity (which they identified with population spike LTP or LTD; EPSP slope data were not provided for the LVP treatment) in aluminum-exposed rats increased from 38% to 174%, which was similar to that in control rats (161%). It was suggested that AVP could reverse aluminum-induced impairment of synaptic plasticity. Most importantly for the present study was the observation that both the EPSP slope and the population spike were enhanced by LVP in control rats when given an input output curve of single pulse stimuli. Thus, although the investigators did not follow the effects of LVP over time they found a similar potentiation

of EPSP slope (~50% maximal) and spike (~150% maximal) as found here in Experiment 1.

Somewhat in contrast is an *in vivo* study by Dubrovsky and colleagues (2002), also using tetanic stimulation, but in combination with 1 µg of icv AVP. They reported a potentiation of the perforant path-evoked EPSP slope and spike amplitude beyond control LTP levels when tetanization was given in the presence of icv AVP (1 µg/µl). Both EPSP and population spike enhancement increased steadily within 15 minutes of application and continued to increase for 2 hours of recording time reaching 100% above baseline. Thus, AVP enhanced the LTP effect as also reported for spike amplitude by Wang et al.(2001). Without tetanization, AVP infusion was associated with higher amplitudes than baseline, but the changes were not statistically significant. The lack of AVP effects without tetanization may be due to the much higher dose (1000 fold) used by Dubrovsky as compared to both Chen's *in vitro* study (100-500 nM) and our *in vivo* study (500 nM or 1 ng/2 µl).

Inverted U curve effects have been found for AVP, both in terms of modulating cAMP and in terms of behaviour. An inverted U-shaped dose-response relationship was observed for heart rate, core temperature, gross activity, locomotion and rearing behaviour which all increased from low to moderate dose (1–10 ng) and declined after higher doses of AVP (30-100 ng) (Diamant and De Wied, 1993). A similar relationship is also seen in a study of the effects of lysine vasopressin and response prevention on the shuttlebox avoidance paradigm (Hagan et al, 1982). Both high and low doses caused an inhibitory effect while moderate doses facilitated avoidance behavior. AVP neuromodulation of NE-induced cAMP accumulation *in vitro* resulted in an inverted U shape within a concentration range of 10-1000 nM. Lower concentrations (50 – 250 nM) of AVP potentiated NE-induced cAMP accumulation while higher (1000 nM) did not potentiate. Brinton and colleagues (1994) examined AVP activation of calcium signalling pathways in cultured hippocampal neurons and found that exposure of hippocampal

neurons to AVP induced inositol-1-phosphate accumulation which was concentration dependent and exhibited a steep inverted U curve that included stimulation and inhibition of inositol -1-phosphate accumulation. The biphasic characteristic of vasopressin is not unique to its neuromodulatory effects. Lower concentrations of AVP facilitated neurite growth in cultured embryonic neurons while higher concentrations inhibited neurite outgrowth (Brinton and Gruener, 1987). Such a bell-shaped dose-response curve is characteristic of other substances that affect memory consolidation and retrieval such as NE which also favors memory enhancement at low to medium doses but depresses memory at high doses (Izquierdo 1989).

Evidence for an inverted U curve in electrophysiological potentiation effects with dosage of a vasopressin agonist comes from a CA1 study by Chepkova, Kapai, and Skrebetskii (2001). They reported enhanced population spike amplitude with an AVP fragment, AVP 4-9 in the Schaffer collateral-pyramidal CA1 cell system. Here they studied the effects of treatment with AVP 4-9, at varying dosages, on LTP induced by short- and long-term high frequency stimulation. Short term LTP became long-term and was maximal (100%) in the presence of 1 μ M AVP 4-9, but decreased in magnitude at a lower concentration (1 μ M) and at a higher concentration of AVP 4-9 (5 μ M). The modulating effect of AVP 4-9 was also found to depend on the intensity of the high frequency stimulus where it only significantly facilitated weak high frequency stimulation. This suggests that AVP 4-9 facilitates LTP but also modulates its amplitude depending on the intensity of the presynaptic activation at least in the CA1 region.

There was no facilitation of spike amplitude without LTP at the dosage of the AVP fragment used, but the authors had reported earlier that both AVP and AVP 4-8 produced potentiation of EPSPs to Schaffer collateral input and also lowered the threshold for firing and increased cell excitability in CA1 pyramidal cells. These effects were not blocked by NMDA blockers or GABA A blockers. The EPSP enhancement and cell excitability effects were separable in that not all cells that showed enhanced EPSPs

showed increased cell excitability and the time course of the two effects in a given cell could differ. A similar pattern of AVP effects on intracellularly recorded EPSPs and cell excitability has also been reported to fimbria fornix input in the lateral septum (Urban and DeWied, 1986).

The effects seen here in the dentate gyrus at the population evoked potential level parallel these earlier intracellular observations in related septohippocampal areas and suggest that AVP promotes an enhanced synaptic response to glutamate input and in parallel lowers the threshold for cell excitability or enhances slope/spike coupling.

The present observation of electrophysiological AVP enhancement effects in the dentate gyrus is also consistent with reports of cfos activation in the dentate gyrus with 2 ng AVP administered icv in either unconditioned or conditioned mice (Paban et al, 1999).

A direct test of the difference between the negative Dubrovsky result with AVP alone and the present study would require a systematic dose-response investigation. In the present study 1 ng icv was selected as it is the dose DeWied originally found to be effective in learning and memory studies as noted above. A dose response study examining AVP icv (1 ng- 200 ng) and differential effects on abnormal behaviours such as barrel rotation and crouching (Boakes et al,1985), found that doses of 10 ng and higher resulted in abnormal behavior with convulsions starting at 100 ng. These results argue against the use of high doses in assessing synaptic plasticity promotion in normal learning and memory by AVP.

β-Adrenoceptor mediation of AVP effects: Experiment 2

In the second experiment we tested the hypothesis that NE β-receptors in the dentate gyrus are critical for EPSP and spike potentiation effects by using the double pipette technique with propranolol (50 mM) and saline filled recording pipettes together with the icv administration of 1 ng AVP. Our study showed that 6/6 experiments significantly increased in population spike amplitude on the saline filled pipette post AVP. There was no significant increase in spike amplitude on the propranolol pipettes in

the same experiment and in two cases there was a small, but significant, decrease in population spike size. Thus, population spike increases by AVP require β -adrenoceptor activation. The observation of decreases is consistent with an earlier report (Harley and Evans, 1988) using locus coeruleus activation in the presence of intra-dentate propranolol and finding a decrease in spike size that was related to activation of α adrenoceptors. The possibility that AVP affects the release of norepinephrine so that an imbalance of α and β adrenoceptor activation could be occurring will be discussed later in this section.

Four of the six rats showed a significant potentiation of EPSP slope on the saline pipette, while two others did not. Three of the four that increased also had a significant but small (.2-.4) positive correlation between slope and spike after AVP. This pattern of slope and spike results on the saline pipette replicates that of Experiment 1. AVP can induce increases in both the EPSP slope and the population spike. The increases in spike size are the most consistent. EPSP slope increases only partially account for the spike increases observed supporting the initial hypothesis that there may be two mechanisms for AVP effects in dentate gyrus.

EPSP slope increases did not occur on the propranolol pipette and in three experiments EPSP slope showed a significant decrease. Slope spike correlations were rarely seen on the propranolol pipette. In one experiment both slope and spike decreased on the propranolol pipette, while both increased on the saline pipette, but the only significant slope/spike correlation occurred on the saline pipette. In only one case was there a significant increase in the slope/spike correlation after AVP on a propranolol pipette suggesting slope was contributing to the decrease in spike size.

Thus, NE β -adrenoceptor activation is required for both the EPSP slope and spike potentiation effects seen in the dentate gyrus with central AVP activation. The weak correlations between the EPSP slope and population spike effects suggest NE also operates on synaptic plasticity through two separate β -adrenergic dependent mechanisms.

Church (1983), Brinton and McEwen (1989) and Brinton, Thompson and Brownson (2000) have reported that AVP significantly potentiates NE β -adrenergic receptor action by increasing the induction of cAMP accumulation in hippocampal slices. This AVP-induced cAMP increase was found not to occur in the absence of NE. Propranolol blocked both the NE-induced accumulation of cAMP and the cAMP enhancement effect of AVP. The adenylate cyclase activator, forskolin, was found to produce a 2-fold increase in cAMP accumulation, but there was no change in the presence of AVP. This provides evidence that AVP is not modulating adenylate cyclase directly, but suggests that it is likely a receptor-coupled event. Petit, Barveris and Jard (1988) examined AVP in the superior cervical ganglion and found that AVP potentiates cAMP accumulation when induced by the specific β -agonist isoproterenol. Brinton and McEwen (1989) also found AVP-induced neuromodulation of cAMP in the dentate gyrus was a Ca^{2+} -dependent process. The AVP-induced potentiation of cAMP was blocked by an antagonist to the Ca^{2+} -binding protein, calmodulin. Interestingly, the AVP4-9 fragment did not enhance cAMP, a point I will return to in considering the effects of DGAVP.

If AVP and NE directly interact, the receptor systems may be functionally or structurally coupled in select regions within the hippocampus. Brinton (1998) examined this and found that high concentrations of AVP in postnatal animals resulted in a down-regulation of NE-induced cAMP formation, but no change in vasopressin potentiation. They also found that a site-specific upregulation of vasopressin receptors occurred following lesions to the dorsal noradrenergic bundle. This lesion also resulted in the upregulation of the vasopressin receptor system that modulates adrenergic stimulated adenylate cyclase (Brinton 1990;1998). This presence of AVP receptors after a DNB lesion indicates that the AVP receptors may be postsynaptic to noradrenergic terminals. Such results suggest that vasopressin and adrenergic receptors are an adaptive and interactive complex.

In the dentate gyrus, the present experiment argues that electrophysiological AVP-induced potentiation in the dentate gyrus requires NE and β -adrenoceptor activation. Studies by Stanton and Sarvey (1985a;1985b;1985c) also argue that high frequency-induced LTP depends on NE and β -adrenoceptor activation in the dentate gyrus and that both frequency-induced LTP and NE-induced LTP initiate increases in cAMP (Stanton and Sarvey, 1985b;1985c). LTP of the perforant path evoked potential *in vitro* could not be induced with NE depletion, but recovered after application of NE. Consistent with a requirement for cAMP, β -adrenoceptor antagonists blocked LTP in the dentate gyrus (Stanton and Sarvey,1985a;1985c). *In vivo* there is partial, but less complete, dependence of high frequency LTP effects, specifically potentiation of the EPSP slope, on β -adrenoceptor activation in dentate gyrus (Munro et al. 2001). Thus, NE appears to be an important contributor to long-term enhancement of functional connectivity in the perforant path input to dentate gyrus.

In an attempt to localize the site(s) where the interaction between NE and AVP may occur, Brinton (2000) found that there are multiple sites within the hippocampus including especially the rostral dendritic zone of the dentate gyrus and the hilar or polymorph region, as well as the CA3 subfield. In these areas there is a strong parallel between β 1 and AVP receptor distribution. Brinton (2000) also examined temporal properties of adrenergic and vasopressin receptor interactions and found that pre-activation (for 1 minute) of the vasopressin V1 receptor prior to NE activation of β -adrenergic receptors resulted in a significantly enhanced generation of cAMP. This synergy was found in both neurons and glia (Son and Brinton, 1998). This suggests that temporal priming by vasopressin may have a special role in the enhancement effect of NE-induced potentiation. Such a prediction remains to be tested in electrophysiological and/or behavioural experiments. Brinton and McEwen argued the properties of AVP-NE neuromodulation paralleled the associative learning requirements of spatial and temporal

coupling. The calcium dependency of AVP potentiation of cAMP is also in line with the calcium dependency for induction of associative long-term potentiation (Brinton 1990).

NE and AVP dissociation of slope and spike effects

One explanation for the weak correlations between slope and spike effects, which are seen with *in vivo* NE studies as well as described here for AVP, is the differential effect of NE on the medial and lateral perforant path input to the dentate gyrus.

NE modulation preferentially potentiates input from the medial perforant path in the middle molecular layer synapses of the dentate gyrus while depressing synaptic input from the lateral perforant path in the outer molecular layer (Dahl and Sarvey, 1989). The medial/lateral pathways not only terminate in different parts of the dentate gyrus molecular layer, but also arise from different parts of the entorhinal cortex: the medial and lateral cortices (Hjorth-Simonsen and Jeune, 1972). However the dentate gyrus and the CA3 receive projections from both medial and lateral entorhinal cortex and the two pathways show interactive effects (McNaughton and Barnes, 1977; Abraham and McNaughton, 1984). For example, when LTP is induced in one pathway, there is a concurrent long-lasting depression (LTD) of responses evoked by activation of the other pathway (Abraham and Goddard, 1983). LTD entails a decrease in synaptic efficacy at potentiated synapses which is hypothesized to be necessary to prevent oversaturation of inputs and to increase the storage capacity of the hippocampus network (Doyere et al, 1997). In the Dahl and Sarvey (1989) *in vitro* study, they reported that NE induces pathway specific potentiation and depression from stimulation of medial and lateral perforant paths respectively, as recorded in the dentate gyrus, which were both blocked by the β -adrenergic antagonist, propranolol. The NE effect, then, is asymmetrical with only the medial pathway showing potentiation. It is the medial population spike that was investigated in these experiments as judged by latency, while the EPSP was a mixture of both medial and lateral components and might not be consistently potentiated depending on the relative ratio of the two synaptic inputs.

In comparing AVP results to electrophysiological results reported for NE, typically, slope increases are seen more with NE *in vitro*. Lacaille and Harley (1985) reported *in vitro* that NE increased both amplitude and EPSP slope with 47% of the spike amplitude accounted for by the increase in EPSP slope. Another study (Stanton and Sarvey; 1987) examined dendritic EPSPs and found that NE resulted in increased dendritic EPSPs and increased population spike amplitude. The greater consistency of EPSP slope potentiation *in vitro* may relate to the easier isolation of the medial perforant path fibers in that preparation.

It will be interesting to examine whether or not AVP potentiation effects show the same pathway selectivity as NE effects. Such a selectivity would be strongly predicted by the present findings and would argue that AVP and NE cause the network to process some inputs at the expense of others. Nonetheless, NE and the activation of β -adrenoceptors is reported to be essential for both medial and lateral perforant path high frequency LTP in the perforant pathway (Bramham et al, 1997) and AVP could also contribute to potentiation of both pathways.

A recent finding shows that NE can induce a delayed EPSP slope potentiation that is not observable in the first 3 hours of recording in an awake rat (Walling and Harley, 2004). Walling and Harley found infusion of glutamate into the LC produced an initial increase in population spike with no effect on EPSP slope. Potentiation was seen 24 hours later in both the spike and EPSP slope. The β -antagonist, propranolol and the protein synthesis inhibitor, anisomycin, blocked the long-term effects on EPSP slope and spike. In the Walling and Harley experiments slope completely accounted for spike increases 24 hours after NE release, but did not account for the initial spike increases. It will be of interest to examine AVP potentiation in awake rats to see if a similarly delayed slope potentiation occurs.

To summarize Experiment 2, briefly, AVP potentiation of EPSP slope and population spike amplitude of perforant path input depends on β -adrenoceptor activation.

The dissociation of slope and spike seen in the weak or absent correlative relationship suggests AVP effects *in vivo* are like NE effects *in vivo*. This dissociation may relate to selective modulation of medial and lateral perforant path inputs or to distinct temporal and/or mechanistic modulations for the EPSP slope and spike components.

AVP Fragment Effects: Experiment 3

In the present study, the fragment DGAVP, a V1a receptor agonist, resulted in increases in population spike amplitude across all three animals while showing varied and inconsistent effects on EPSP slope including decrease, increase and no change. This variable EPSP slope pattern is also reported with NE *in vivo* (Klukowski and Harley, 1994; Harley et al, 1996; Walling and Harley, 2004) as well as in low dose *in vitro* isoproterenol studies (Dahl and Li, 1994). Population spike increases occur independently of slope increases when NE activation is increased *in vivo*, using a variety of NE release paradigms.

While using the full AVP molecule produced both slope and spike changes, the pattern of effects argued for separate mechanisms. The spike, but not slope, potentiation produced by DGAVP strengthens the case for multiple mechanisms of AVP action.

Metabolite fragments of AVP, such as DGAVP (Burbach et al., 1983), show several differences from the parent molecule. Fragments bind preferentially to the polymorph region of the dentate gyrus, whereas the full molecule binds to the molecular layer as well (Brinton 1984; 1986; Du et al, 1994). The AVP4-9 fragment was reported *not* to synergistically elevate NE-induced cAMP in *in vitro* experiments (Brinton & McEwen, 1994), although it is effective behaviourally. DGAVP, an AVP 4-8 fragment, was not tested in the cAMP study, although an earlier experiment reported DGAVP given intraperitoneally increased cAMP for up to 24 hours in several brain regions including hippocampus (Schneider et al, 1982). However the inverted U-shaped dose-response relationship for passive avoidance memory with AVP4-9 was not apparent for the AVP4-

8 derivative (Burbach et al, 1983). In this early study pg quantities of AVP4-8 were effective at all doses tried. It will be of interest in future *in vivo* electrophysiological studies to try pg doses, but the lack of an inverted U curve in the earlier memory study suggests the pattern of spike potentiation would not be altered by lower doses.

Since the spike potentiation induced by the parent molecule here was blocked by propranolol it is likely that cAMP is involved in spike potentiation, but an alternative mechanism could be a synergistic calcium action, which will be treated in more detail in a later section. NE itself influences both molecular layer and the polymorph interneuron region (Brown et al, 2005), so there could be two distinct spatial targets of modulation by AVP and its metabolites which would involve NE.

As previously mentioned in the introduction, evidence derived from studies of DGAVP suggests that this peptide analog is virtually devoid of vasopressor effects, having little antidiuretic, corticotrophin-releasing activity, cardiovascular or endocrine effects (De Wied et al, 1972; Van Wimersma Greidanus et al, 1979). Administration of DGAVP has been found to result in resistance to extinction of active avoidance behaviour (De Wied and Bohus, 1966; Burbach et al, 1983; De Wied et al, 1984; Gaffori and De Wied, 1985). Such effects on extinction suggest that the peptide tends to work by strengthening previously learned responses. In these studies, they found that both AVP and DGAVP resulted in increased resistance, but DGAVP was more potent in facilitating passive avoidance behaviour. This supports the proposal that AVP promotes the activity in the dentate gyrus directly and not through pressor effects. Our experiments show that both AVP and DGAVP increased the perforant path evoked potential in the dentate gyrus with DGAVP causing increased cell excitability. DGAVP, like AVP, was also found to improve acquisition of an autoshaped behaviour thus demonstrating the specificity of the fragment for learning and memory processes (Messing and Sparber, 1983).

De Wied and colleagues (1984) found that AVP produced a pressor effect and facilitated passive avoidance retention when administered peripherally while it only

resulted in enhanced passive avoidance response when centrally (icv) administered. AVP 4-8, the more active DGAVP molecule, produced the facilitation in the passive avoidance retention when administered either centrally or peripherally. Thus it seems the receptors mediating the pressor effects are in the periphery and not in the brain.

In the present study, the fragment molecule is working differently than in the first experiments such that it is more similar to the effects of *in vivo* NE (increased population spike amplitude and decreased or no change in the EPSP). Naturally, it is hard to draw a conclusion from the response of 3 subjects and so a larger study is needed for a more complete examination of specific vasopressin fragments. But if this outcome is an accurate reflection of DGAVP effects, then the larger vasopressin molecule appears to recruit more mechanisms than the specific targeted fragment. The larger molecule may interact more with other transmitters and pathways. It would be interesting to have done the double pipette study with the fragment and propranolol to assess the importance of β -adrenoceptors in the fragment effect. .

AVP and NE Release

Vasopressin and NE appear to coexist in the rat LC (Caffe and colleagues, 1985; 1988). High concentrations of AVP have been found in the rat and the human LC (Jenkins et al, 1984; Hawthorn et al, 1984) which also contains AVP receptors (Phillips et al, 1988). In human brain, radioimmunoassay has shown that substantial amounts of vasopressin-like immunoreactivity are present in the LC (Rossor et al, 1980). Olpe and Baltzer (1981) found that microiontophoretic administration of AVP or LVP excites noradrenergic neurons in the LC of the rat. This was also found in the LC of the cat (Andre and colleagues, 1992). Thus LC is a possible site for the interaction of vasopressin and NE containing neurons which indicates an additional site by which vasopressin could affect NE release when it is given intracerebroventricularly. AVP has been found to increase NE levels in the hypothalamus, hippocampus and tuberculum

olfactorium when given intraperitoneally (Szadowska et al, 1982) and intraventricularly (Schwarzberg et al, 1981). Since vasopressin-induced NE release has also been proposed via an intrahippocampal route, as mentioned in earlier papers (Metzger et al, 1994; Brinton et al, 2000), there may be several routes for synergy.

In this study, AVP and DGAVP were infused into the lateral ventricles with the expectation that it would be distributed easily throughout the brain to the target neurons via diffusion within the CSF and the extracellular fluid. A disadvantage of this technique is the likelihood of interaction with targets other than the area of interest. As just described, the LC itself is likely to be one source of influence in the present study. Some studies promote the use of microdialysis techniques to avoid these extra interactional effects and to mimic more closely the natural release patterns in the brain (Engelmann et al, 1992;1994;1996). A microdialysis approach would be of interest for future studies looking at AVP modulation effects restricted to the dentate gyrus.

Mechanisms of AVP Effects

In addition to the ability of AVP to enhance the β -adrenoceptor initiated rise in cAMP discussed above, AVP has important and independent actions on intracellular calcium modulation. Son and Brinton (2001) found that activation of the vasopressin V1 receptor produced a rise in intracellular Ca^{2+} through the activation of the phosphatidylinositol signalling pathway and L-type Ca^{2+} channels. Fragments such as AVP 4-9 (Nakayama et al., 2000) also have this action. AVP thus causes intracellular Ca^{2+} concentration increases via release from intracellular Ca^{2+} stores and via influx from extracellular Ca^{2+} (Nakayama et al, 2000; Yibchok et al, 2000). The rise of the intracellular Ca^{2+} concentration results in increased Ca^{2+} -dependent neurotransmitter release (Son and Brinton, 1998) and NMDA-activated Ca^{2+} currents. This suggests that AVP actions on the IP3/intracellular Ca^{2+} concentration pathway in the rat hippocampus, might be important in enhancing the range of synaptic plasticity. AVP4-8 also stimulates

IP3 metabolism in the hippocampal slice in the presence of GTP (Gu and Du, 1991a). Studies have found that among the vasopressin analogs, AVP 4-8 was a hundred times higher in potency than the parent molecule AVP in facilitating the retention of passive avoidance learning (Gaffori and De Wied, 1986; De Wied et al, 1991).

However, AVP appears to require adrenergic innervation to the hippocampus to promote synaptic plasticity (as seen here with propranolol) and learning since lesions to the dorsal noradrenergic bundle abolished the behavioral effects of AVP (Kovacs et al, 1979b). Thus, if the calcium effects are relevant they must interact in some way with NE. NE itself promotes the opening of calcium L+ channels in the dentate gyrus granule cells (Gray and Johnston,1987) and calcium effects of the two modulators may be additive or synergistic in promoting plasticity. This remains to be investigated directly.

Another possible mechanism of the AVP effects on synaptic plasticity is AVP's activation of the receptor mediated signaling pathway involving mitogen-activated protein kinase (MAPK). This pathway is also recruited by NE (Chen et al, 2007). Administration of AVP 4-8 (sc) resulted in a significant increase of MAPK activity in the hippocampus after 2 hours, but the protein levels had not increased, indicating that the MAPK increase stimulated by AVP4-8 was caused by a short-term activation process through protein phosphorylation and not by protein expression (Qiao and Du,1998).

Trophic factors are also promoted by the 4-8 AVP fragment. Zhou et al (1995) found that nerve growth factor (NGF) mRNA expression in the hippocampus was significantly enhanced 12 hours after administration of AVP4-8 (sc). They concluded that the NGF gene was one of the target genes responsible for memory-enhancing responses induced by AVP4-8 and that the enhancement of NGF gene expression may be involved in the signaling pathway mediated by the AVP4-8 receptor. In a subsequent study by Zhou and colleagues (1996), memory impaired rats prepared by prenatal hypoxia showed that NGF expression increased from a low level to a normal level when given an AVP4-8 agonist and the acquisition and maintenance of behavioural responses significantly

improved. Such studies confirm the role of both AVP4-8 and NGF in the learning and memory process. Zhou and colleagues (1997) then studied the *in vivo* expression of BDNF and neurotrophin 3 (NT3) genes as well as oxytocin and reported that only BDNF was significantly enhanced by AVP4-8 administration in the hippocampus. Although BDNF facilitates both short- and long-term memory, the increase of BDNF mRNA transcription 12 hours after AVP 4-8 indicates a long-term process. Whether any of the trophic factors could contribute to the synaptic plasticity and cell excitability changes seen here remains to be determined. They are more likely to play a role in late phases of plasticity than earlier ones such as those studied here.

Vasopressin in real life

Many of the behavioural studies on AVP have involved using an aversive situation for learning paradigm. Some studies have shown that AVP influences memory of emotional or stressful events, much like NE. Cahill's study (Cahill et al, 1994) suggested that memory is enhanced around stressful times or experiences because of a surge in NE. The participants are better able to recall a traumatic portion of a story or movie and this recall is diminished when participants are given beta blockers which block the effects of the NE surge. Some studies have reported that novelty of a stimulus or event and emotional stimuli are also associated with an AVP enhancement in processing (Naumann et al, 1991; Pietrowski et al, 1996). In Naumann et al (1991), subjects treated with AVP showed enhanced memory performance, but also showed a higher performance with emotional content as compared to neutral stimuli. A study by Inder et al (1998) found that prolonged exercise, which serves as a form of stress, increases peripheral AVP in male athletes. NE is also increased with muscular stress and has been shown to enhance memory under those conditions (Neilson and Jensen, 1994; Neilson et al, 1996). AVP may prime the information pathways under the same circumstances as NE, the

release of which has been related to novel and emotional events as well as muscular stress. AVP may normally work with NE to enhance processing of significant stimuli.

In a clinical vein one of my own interests is the neural basis of autism and recently AVP has been linked to this disorder. Autism or autism spectrum disorder is a neurological developmental disorder characterized by fundamental deficits in social behavior, social communication, language and cognitive development, excessive anxiety or hyperreactivity to stressful experiences and a tendency toward compulsive repetitive behavior. Such deficits vary in severity within the spectrum. Autism is also male biased afflicting males 4-5 times more than females (Chakrabarti and Fombonne, 2005; Fombonne, 2003). There is considerable evidence that autism is highly heritable and likely to be determined by genetic and polygenic mechanisms (Folstein et al, 2003). Given the complexity of the symptoms of autism, it is not unexpected that multiple sites of anatomical abnormalities have been hypothesized to underlie the different characteristics of this disorder. Abnormalities have been found throughout the brain including the cerebellum, amygdala, and the hippocampus (Bauman and Kemper, 1994; Courchesne,1997; Kemper and Bauman,1998; Courchesne et al, 1999; Nicolson et al, 2006). There is growing evidence that an abnormality in AVP neurotransmission may account for certain features of autism (Insel et al, 1999; Nelson et al, 2001; Jentsch et al, 2003; Schuman et al, 2004; Wassink et al, 2004; Emanuele 2006).

Many studies implicating AVP's connection with autism involve the examination of the processing of social cues, social recognition or social memory which is thought to depend on septal AVP (Thor and Holloway, 1982; Dantzer et al, 1987; 1988; Popik et al, 1992). Social memory tests are a relatively natural test of memory in rodents. The test of social memory originally proposed by Thor and Holloway (1982) was based on the fact that adult male rats spend a great amount of time investigating novel juveniles and less time investigating ones to which they have already been exposed. The rats exposed to the same juvenile 30 minutes after the initial exposure display less investigation behavior

which is interpreted as the adult forming a memory of the juvenile. But if the re-exposure occurs 2 hours later, the juvenile is not recognized and is thoroughly investigated which consists of sniffing, nosing, following and grooming. This form of memory is thought to be based on the olfactory characteristics of the juvenile conspecific.

Centrally injected AVP (0.5 - 2.0 ng icv) has been found to facilitate social memory in rats. AVP given immediately after investigation of the juvenile resulted in decreased social investigation of the same juvenile at the long (120 minute) interexposure interval (Lemoal et al, 1987). Thus the memory of that particular juvenile lasted longer with the administration of AVP. Social memory studies with AVP have focused on the lateral septum as the crucial mediating structure, but it would be of interest to examine AVP effects in dentate gyrus on this memory as well.

My own experience leads me to suggest an overactivity of the AVP system rather than a deficit as a possible abnormality in autism. Because of evidence for an inverted U curve function in the effects of AVP, even on cAMP activation, too much AVP could lead to abnormalities that would be characterized as 'overdrive' in some domains and 'losses' in others depending on the dose-response relationship. While these ideas are highly speculative, the results of the present study lead me to suggest that treatment with propranolol might be useful in reducing some autistic symptomatology. Such treatment has been tried successfully with anxiety disorders and post-traumatic stress disorders (Pitman et al, 2002; Raskind et al, 2007; Strawn et al, 2007).

Conclusion

AVP potentiates perforant path-induced EPSP slope and population spike amplitude and this potentiation appears to be long-lasting. Potentiation of the two components are dissociable suggesting distinct mechanisms. The ability of a β -adrenergic antagonist in the dentate gyrus to prevent both EPSP slope and population spike potentiation argues that these effects are mediated via a synergistic interaction with β -adrenoceptor activation by NE. A critical dependence of AVP promotion of learning and

memory in the dentate gyrus on NE has previously been demonstrated (Kovacs et al, 1979).

The present results provide a model for the way in which AVP might promote memory through the promotion of synaptic plasticity and cell excitability to glutamatergic inputs. The pharmacological block of that plasticity by blocking the post-synaptic effects of NE provides a clear counterpart to the dependence of memory enhancement on the presence of NE fibers shown earlier (Metzger et al, 1994; Alescio-Lautier and Soumireu-Morat,1998). Surprisingly, a behaviourally highly effective metabolite of AVP, DGAVP only produced long-lasting increases in cell excitability. This further supports the dissociation of the two plasticity mechanisms seen with AVP and provides a tool for their dissection in future studies. The possibility that cell excitability increases are another component of associative learning changes deserves further investigation.

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