STIMULATION OF THE ANTERODORSAL THALAMIC NUCLEUS ELICITS AN EVOKED POTENTIAL IN THE DENTATE GYRUS IN THE RAT BRAIN

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Stimulation of the AnteroDorsal Thalamic Nucleus Elicits an Evoked Potential in the Dentate Gyrus in the Rat Brain

by

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ABSTRACT

The hippocampus has long been theorized to play an important role in learning, memory, and spatial navigation. An input conveying vestibular information is likely to exist if the hippocampus plays a significant role in spatial navigation. Cells that respond to the spatial location of an animal have been found in the mammalian hippocampus, and their firing is influenced by vestibular cues (O'Keefe & Dostrovsky, 1971; Wiener et al. 1995), which originate in the semicircular canals and traverse the vestibular nuclei, the mammillary nuclei, and the anterior thalamic nucleus (ADTN).

In this study, monopolar stimulation of the ADTN reliably elicited an EPSP in the dentate gyrus (DG), the first structure in the trisynaptic relay network of the hippocampus. A series of experiments designed to determine the route by which an ADTN-DG signal would travel had mixed results. While a DG depth-profile and perforant path (PP)-lesion trials suggested at least part of the signal was the result of PP activation, cross-potentiation data did not yield results entirely consistent with a lateral perforant path (LPP) or medial perforforant path (MPP) route of travel.

Norepinephrine (NE), a neuromodulator, may play a role in input attenuation and selection in the PP-DG connection. The effect of norepinephrine on the ADTN evoked potential was investigated in this study to ascertain if there is a consistent effect on this potential vestibular input, to determine its consistency with the hypothesis that such input is through the PP, and to look for evidence that NE effects are path and/or modality-specific. 500 μg/kg idazoxan had a different effect on the ADTN EPSP than a

known LPP input, suggesting a different route for ADTN input; however, the idazoxan effects on the LPP input were not consistent with norepinephrine effects on the LPP. PGi stimulation, another method of enhancing NE input, yielded mixed effects on the ADTN EPSP, even when effects on the PP EPSP were consistent.

The results suggest the ADTN EPSP may enter the DG in a mixed fashion or via a route not examined in this study. The existence of the ADTN signal in the DG is consistent with the theory that there is an ADTN input to the hippocampus that may play a role in head-direction and place-code processing.

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My stay in Newfoundland was a beloved and magical time in my life; my love to all of those who made it so.

This work is dedicated to my parents, Rod and Diane King, and to my sister, Gina.

CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

ADTN - anterodorsal thalamic nucleus
CA1 - area CA1 of Ammon's horn
CA3 - area CA3 of Ammon's horn
DG - dentate gyrus
EC - entorhinal cortex
EPSP - excitatory post-synaptic potential
GABA - gamma aminobutyric acid
GPa - glycogen phosphorylase a
HD - head direction
HPC - hipocampal place cells
IPSP - inhibitory post-synaptic potential
ISI - interstimulus interval
LC - locus coeruleus
LEC - ventrolateral entorhinal cortex
LPP - lateral perforant path
LOT - lateral olfactory tract
LTP - long term potentiation
MEC - dorsomedial entorhinal cortex
ML - molecular layer
MPP - medial perforant path
NE - norepinephrine
NMDA - n-methyl-d-aspartate
pEPSP - population excitatory post-synaptic potential
PGi - nucleus paragigantocellularis
PP - perforant path
PPD - paired pulse depression
PPF - paired pulse facilitation
PS - population spike

INTRODUCTION

VESTIBULAR INPUT AND SPATIAL NAVIGATION

Research investigating the neurobiological processes of spatial navigation has increased dramatically in the last decade, but has been of interest to neuroscientists for a long time. Since the first recording of place units (O Keefe, 1971) the hippocampus has been suggested to play a role in spatial mapping (O'Keefe & Nadel, 1978). Various studies have implicated the hippocampus as an active participant in navigation and cognitive mapping circuitry, because hippocampal lesions lead to a selective deficit in spatial learning tasks, while sparing other types of learning (Jarrard, 1993). Of interest to investigators is the type of sensory stimulus which exerts influence over an animal's ability to navigate. Two categories of influential cues have evolved in the literature: landmark-based and ideothetic. Ideothetic cues include vestibular, proprioceptive, and visual motion information. In studies in which visual and vestibular cues are put in conflict, investigators have found that rodent navigation is determined by different cues in different situations. Vestibular cues seem to be of primary importance to a basic ability to navigate, as rodents are capable of navigation in the complete absence of external sensory input, for example, in a darkened room or if the animal is rendered sightless (Quirk et al, 1990). An animal's ability to use ideothetic cues to construct knowledge of distance and bearing relative to a starting point is referred to as path integration or dead reckoning (McNaughton et. al., 1991; Gallistel, 1990). Cue conflict situations have revealed a strong vestibular contribution to this ability; for example, if a gerbil searches away from its nest in a dark

experimental arena, and the floor of the arena is rotated above vestibular threshold, the gerbil compensates for the rotation and navigates back to the nest successfully. If the arena floor is rotated below vestibular threshold, however, the gerbil does not compensate for the rotation (Mittelstaedt & Mittelstaedt, 1980). Direct evidence of vestibular involvement in spatial orientation comes from studies by Miller et al., (1983) in which rats were tested on a spatial memory task. The contribution of motor feedback to memory was minimized in this study because rats were moved from a starting point by vehicle, and had to navigate back to the starting point. Blind rats performed the task well before vestibular nucleus lesions were made, but were seriously impaired following the lesion. Matthews et al. (1988) tied this dependance on vestibular cues for navigation back to the hippocampus by demonstrating that hippocampal lesions impair the ability of rats to use vestibular information to maintain a sense of bearing in a rotating box.

The Roles of the Hippocampus

The hippocampus has long been shown to be important to, or involved in, the processing of memory, due to its integration of multimodal inputs and to its role as a relayer of such information to regions of the cerebral cortex (Squire, 1987). Effects of hippocampal lesions are sometimes tied to the behavioural state of the animal; for example, the hippocampus' role in orienting to a novel stimulus has been shown to be modulated by behavioral state. Rats with hippocampal lesions that are already involved in a task when a novel stimulus is introduced do not orient to the new stimulus (Wickelgren & Isaacson, 1963). Harley (1972) pointed out that orientation of these rats to the second

presentation of the novel stimulus was normal; therefore, the rats were aware of the first stimulus presentation, but did not explore it. The differences seen in hippocampal vs. non-lesioned animals which become apparent in certain behavioural states is consistent with the idea that hippocampal function involves altering or attenuating putative sensory input over the perforant path (Winson, 1980 & 1981). In light of findings regarding the firing properties of hippocampal cells, the hippocampus has been included in various models of a cognitive mapping circuit, and is presumed to be a locus of a representation of an animal's physical location in its environment (O'Keefe & Nadel, 1978; Taube et al., 1996). Some cells in the primate DG respond to whole-body motion and to the direction of such motion (O'Mara et al., 1994). Cells whose firing patterns correlate with an animal's place in an environment, termed hippocampal place cells (HPC), are found in CA1 and CA3 (O'Keefe & Dostrovsky, 1971). The area in the environment in which a HPC responds (termed the place field) differs for each cell between different environments, and each HPC usually has only one place field per environment. The population of place cells is presumed to map an animal's entire local environment and to accurately encode the animal's location in that environment (Touretzky & Redish, 1996; Wilson & McNaughton, 1993).

Various studies have shown that HPCs are established in response to, and are influenced by, a variety of sensory inputs. Place fields will rotate around the centre of an environment to remain aligned with visual cues (O'Keefe & Conway, 1978; Gothard et al., 1994), including local and distant cues, and those related to local surface orientation (Muller & Kubie, 1987). However, HPCs continue to fire according to the same spatial

location in an environment after removal of all differentiating visual cues (O'Keefe & Speakman, 1987) or after the lights are turned off (McNaughton et al., 1989; Quirk et al., 1990), as long as the rat has some familiarity with the environment before visual cues are removed. In addition, blind rats have normal place cells (Hill & Best, 1981). Maintenance of HPC firing in the absence of visual cues requires abilities in path integration. Path integration requires a coherent place code, which may be found in the population of HPCs, and ideothetic information regarding the direction and extent of current motion by the animal.

Ideothetic input has been shown to directly influence HPC firing. Some researchers have published studies that show that HPCs are sensitive to vestibular cues; for example, Wiener et al. (1995) allowed a rat to explore an arena, turned the lights off, and rotated the arena floor so the rat was rotated relative to visual cues, which remained in their original location relative to the outside of the arena. When the lights were turned on, HPCs usually ignored the visual cues and rotated their preferred firing direction with the apparatus. In other experiments, visual input exerts a greater influence on HPC firing. Sharp et al. (1995) reported that a series of similar manipulations showed an interaction between visual and ideothetic cues, with different sensory modalities exerting greater influence on HPC activity in different situations. Clearly, the generation of HPC firing is dependent on a variety of sensory inputs to the hippocampus.

Sensory Input to the Hippocampus via the EC

The EC has been identified as a major region of passage of sensory input to the

hippocampus. The EC has six cell layers, one cell free lamina dessicans (layer IV) and a superficial molecular layer. The superficial layers (I, II and III) receive substantial input from the olfactory structures of the telencephalon, the adjacent perirhinal cortex and subicular complex, the medial septal complex, the amygdaloid complex, the thalamic nuclei reuniens and centralis medialis and the contralateral EC, in addition to light projections from a variety of other cortical and subcortical areas. The deep layers (IV, Va, Vb and VI) receive afferents mainly from CA1, limbic cortices (agranular insular cortex, medial prefrontal region, retrosplenial cortex), the supramammillary nucleus of the hypothalamus, and the subiculum. There appears to be more influence by the deep layers on the superficial layers than vice versa; various cell types in layers Va, Vb and VI project either directly or via substantial collateralization to layers I-III. The fibres in layers II and III give rise to the main EC input to the hippocampus, the PP; there is evidence that the deep layers may also contribute a minor PP projection (Kohler, 1985). All or nearly all of layer II cells, which are morphologically diverse, contribute to the PP (Schwartz & Coleman, 1981; Germroth et al., 1989a). The PP is comprised of a medial-lateral gradient of input such that axons from the ventrolateral EC (LEC) project to the outer one-third of the DG molecular layer, while those from the dorsomedial EC (MEC) synapse in the middle one-third (Hjorth-Simonsen & Jeune, 1972, Steward, 1976). These two anatomical divisions of the PP are termed the lateral PP (LPP) and the medial PP (MPP), respectively. Pulse stimulation of the PP evokes a population potential from the granule cells in the DG, composed of a population excitatory post-synaptic potential (pEPSP) and, if stimulation is sufficient, a population granule cell firing event, termed the population

spike, superimposed on the pEPSP. The amplitude of the population spike is maximal when recorded at the granule cell body layer and reflects the number of cells firing, as well as their synchrony of discharge (Bliss & Lomo, 1973).

The potentials evoked via the LPP and MPP differ in small but characteristic ways in their pharmacological and electrophysiological properties, such as paired pulse effects and LTP (McNaughton, 1980). Both divisions of the PP use glutamate as the principal neurotransmitter (Storm-Mathisen et al., 1983); the terminal zone also stains for GABA (Germroth et all., 1989b) and neuropeptides such as enkephalin (outer one-third only) and cholecystokinin (middle one-third only) (Stengaard et al., 1993). The LEC and MEC project via the PP to the full septotemporal axis of the DG, such that the lateral to medial topography of the EC is projected as a septo-temporal gradient in the DG (Witter & Amaral, 1991). Collaterals from this PP projection synapse in the subiculum, CA2, CA3, and on inhibitory neurons in the hilus, which provide inhibitory feedback to the molecular layer of the DG (Tamamaki & Nojyo, 1993).

That at least two different sensory modalities are conveyed to the DG via the PP has been shown in literature that focuses on two types of sensory input: olfactory and auditory. A summary of such data is presented following a review of data describing the electrophysiology of the PP.

ELECTROPHYSIOLOGY OF THE LPP AND MPP

Although the PP was originally described as a homogenous system (Blackstad, 1958), refinements in biochemical and electrophysiological techniques led to the

distinction of LPP and MPP used at present. Waveform characteristics of PP-evoked potentials were found to correspond with the portion of the molecular layer in which stimulated fibres were observed to terminate (marked by anterograde degeneration; McNaughton & Barnes, 1977); it was found that the square root of the rise time of the EPSP (χ) is approximately proportional to the distance from the granule cell layer to the activated synapses in the molecular layer. McNaughton and Barnes also found that post-activation facilitation of EPSPs increased with increasing χ and that there was a step-like transition in this regard at the middle of the molecular layer. The laminar nature of the DG is such that the profile of the EPSP at various distances from the granule cell layer reflects the level of the activated fibres (Gloor et al., 1963; Lomo, 1971) and the level of maximal extracellular negativity reflects the site of the activated synaptic population; such profiles show a distinct difference between stimulation of the LEC and the MEC. This was confirmed directly in subsequent hippocampal slice experiments. In support of the hypothesis of two distinct PP systems was the finding that activation of the MPP has no effect on the LPP EPSP at interstimulus intervals in which paired-pulse effects are observed in either pathway alone. In a subsequent study, McNaughton (1980) demonstrated that paired pulse effects in the LPP and MPP differ. In paired pulse experiments, a first conditioning pulse is delivered 10-1000 ms prior to a second test pulse of equal intensity. The test pulse is often potentiated or depressed compared to the conditioning pulse. McNaughton showed that at interpulse intervals of 20 ms, LPP EPSPs exhibited facilitation of approximately 60%, while MPP EPSPs showed much less facilitation of only 10%, which gave way to depression by 100 ms that was maximal

(12%) at 310 ms (the longest interval tested); alternatively, LPP EPSPs were still facilitated at an interpulse interval of 100 ms and were unaffected by a conditioning pulse more than 310 ms previous. This finding has been confirmed in subsequent studies (Riche-Bennett et al. 1993; Andreasen & Hablitz, 1994). Paired-pulse facilitation (PPF) is thought to result from increased neurotransmitter release, as biochemical presynaptic alterations can enhance or reduce PPF, and post-synaptic changes via second messengers are too slow a process to account for short-interval PPF (Andreasen & Hablitz, 1994). The time course and amount of PPF is unaffected by bicuculline, ruling out the possibility of a decrease in GABAergic inhibition as a PPF mechanism. The mechanism of paired-pulse depression remains unclear. One hypothesis is that the conditioning pulse activates a GABA-mediated IPSP that inhibits the second response (Rausche et al. 1989); however, findings that GABA antagonist application in the slice did not alter PPD in the MPP do not support this (Kahle & Cotman, 1993). An alternative hypothesis is that the amount of transmitter released during the second pulse is decreased (McNaughton, 1980), but further study is required to determine if this is correct.

Olfactory Input to the DG via the PP

Although the hippocampus was associated with the olfactory system by early neuroscientists (Broca, 1878; Elliot-Smith, 1903) and LOT-evoked potentials were recorded in the DG by Wilson and Steward in 1978, clear anatomical evidence for a disynaptic connection was not established until 1990 (Schwerdtfeger et al.). Neurons of the lateral olfactory bulb degenerated to the dendrites of the same neurons that were

labeled when Fast Blue was injected into the molecular layer of the DG. The LOT-evoked potential has a latency of 14-20 ms and laminar profiles in the DG confirm that its point of arrival is in the DG molecular layer, specifically the outer molecular layer, which is the site of termination of the LPP. The EPSP is abolished by LEC, but not MEC, lesions, providing further evidence that olfactory information travels via the LPP. Wilson & Steward also showed that a conditioning pulse delivered to the LOT resulted in potentiation of a test pulse to the LEC, but not the MEC, providing evidence that the LOT and LEC are part of a common pathway.

Recent in-vivo studies also show an olfactory-DG connection. Noxious aromatics cause a burst of rhythmical 15-30Hz waves in the hilus of the DG that is not observed in response to visual, auditory, tactile (Vanderwolf, 1992) or gustatory stimuli (Heale & Vanderwolf, 1994). The fast waves can be blocked with the muscarinic antagonists scopolamine and atropine, while the LOT EPSP remains intact, indicating that the fast waves are somehow dependent on cholinergic transmission.

Auditory Input to the DG via the PP

Auditory-evoked potentials are somewhat more complex than olfactory EPSPs. Deadwyler et al. (1981) found that tones elicit two waves in the outer molecular layer of the DG; N1, with an onset latency of 20 ms, and N2, which has an onset latency of 45-55 ms. Deadwyler et al. found evidence, in the form of depth profiles and EC lesion studies, that N1, but not N2, appears to enter the DG via the PP. Interestingly, the appearance of both N1 and N2 depends on the conditioning state of the animal; N1 was present in naive

animals, unfamiliar with tones, while N2 was small or absent. In animals conditioned to respond to a tone, however, N1 was small or absent and N2 was consistently present. These results provide a clue as to the functional organization of sensory input to the DG; in that a fast, PP-carried response appeared to reflect the unfamiliarity of a sensory stimulus.

Vestibular Input to the Hippocampus

Although researchers have investigated potentials in the hippocampus that are evoked by olfactory and auditory inputs, the vestibular system has yet to be studied in this way. Consistent with cognitive mapping theories, there is an abundance of evidence that hippocampal cells and spatial navigation are influenced by vestibular input (see previous description of cue-conflict studies).

As there is no direct input to the hippocampus from the vestibular system, vestibular information reaching the DG is likely to be highly processed. Taube et al. (1996) have suggested that vestibular input to the PP may arrive via the anterodorsal thalamic nucleus (ADTN). Both electrophysiological and anatomical data support this assertion. Cells which respond to an animal's directional heading in the horizontal plane, called head direction (HD) cells, can be found in the ADTN (Taube, 1995). The firing of these cells seems to be dependent on vestibular input (Stackman & Taube, 1997); Taube and Burton (1995) found that head direction (HD) cells maintained stable directional activity when the rat was moved into a novel chamber. Since there were no familiar visual cues with which to orient HD cell firing in the novel environment, the researchers

postulated that the cells were receiving ideothetic cues, including vestibular cues, to keep track of their directional heading. As with the HPCs, conflicting results about the relative contribution of ideothetic and visual cues to HD cell firing have been found. While Goodridge & Taube (1997) found that HD cells' preferred firing directions shifted with visual cues when they were in conflict with vestibular cues, Blair and Sharp (1996) found that both vestibular and visual cues were important. Both studies agree, however, that vestibular cues contribute significantly to the generation of the HD cell signal. This hypothesis is strongly supported by the research of Stackman & Taube (1995), who found that neurochemical lesions to the rat vestibular apparatus resulted in an absence of ADTN HD cell firing.

The Anterodorsal Thalamic Nucleus

The ADTN receives afferents from three primary sources: the lateral mammillary nuclei (which respond to input from the horizontal semi-circular canal via the medial vestibular nuclei and, subsequently, the dorsal tegmental nucleus), the posterior cingulate cortex (retrospenial cortex), and the postsubiculum (van Groen & Wyss, 1995). The ADTN projects primarily to the parietal cortex and to the subicular complex, with strong projections to layers I, IV and V of the postsubiculum, layers I and III-VI of the presubiculum, and layer VI of the EC (Shibata, 1993).

Although the ADTN has no direct projection to the hippocampus, its reciprocal connections with the cingulate cortex and the subicular complex provide many possible routes for indirect communication with the hippocampus and DG. Taube et. al. (1996)

suggest that vestibular information from the medial vestibular nuclei continues through the dorsal tegmentum and the lateral mammillary nuclei to the ADTN, and that this information is conveyed to the PP via the postsubiculum and the EC. Previously, investigators believed that vestibular information reached the hippocampus from the somatosensory and motor areas of the thalamus via the parietal cortex, and subsequently through the perirhinal and entorhinal cortices (Wiener & Berthoz, 1993). Vestibular information may reach the hippocampus by either or both of these routes.

HD cells were first identified in the postsubiculum (dorsal presubiculum)(Taube et al., 1990) and have been reported in other brain regions: the lateral dorsal thalamus (Mizumori & Williams, 1993), the retrosplenial cortex (Chen et al., 1994), the striatum (Wiener 1993) and the lateral mammillary nuclei (Stackman & Taube, 1998), all of which are presumed to play a part in path integration and cognitive mapping circuits. Although all of these structures are connected to vestibular information by way of their reciprocal connections, the ADTN has the most direct connection to the vestibular system. There is evidence that the ADTN may lie early in the pathway in which HD firing patterns are generated; electrolytic and neurotoxic lesion studies have shown that ADTN HD cell firing is necessary for postsubiculum HD cell firing, but that the reverse is not true (Goodridge & Taube, 1997). Therefore, the HD signal may be generated in or close to (in terms of neural connectivity) the ADTN and then be processed further in other parts of the path integration system. In experiments where vestibular and visual cues were in conflict, the adjustments made by ADTN HD cells and HPCs were tightly coupled (Kneirim et al., 1995), in that, when place code rotation did occur, HPC place fields and HD cell

preferred firing direction rotated by the same amount. This is consistent with the theory that vestibular information directly influences ADTN HD cell firing, which in turn informs HPC firing activity. If HPC maintenance of a place code is continually updated by information from the ADTN, one would expect to find evidence of an electrophysiological pathway by which ADTN signals can enter the hippocampus.

NOREPINEPHRINE

Norepinephrine is a biogenic amine transmitter that exerts widespread modulating effects throughout the cerebellar and cerebral cortices. Converted from dopamine by dopamine-β-hydroxylase, norepinephrine is the transmitter of locus coeruleus (LC) neurons, which project diffusely throughout the cortex and cerebellum. The LC is a small group of neurons in the dorsolateral pons and is the predominant source of NE to the forebrain. The DG receives a prominent projection from the LC via the dorsal noradrenergic bundle which terminates primarily in the hilar region (Jones & Yang, 1985); NE synapses are also located in the molecular and granule cell layers (Loy et al., 1980).

In the DG, norepinephrine acts as a neuromodulator; that is, its effects are superimposed on the action of the transmitter whose action it modulates. Neurotransmitters promote fast ionic currents while neuromodulators, like NE, activate intracellular second messenger systems leading to an effect with a slower time course. These systems, through cascades of protein phosphorylation, may change a variety of cellular properties over the short or long term, including ion channel properties, vesicle proteins, enzymes, and gene expression. NE exerts its effects via binding with various

types of adrenoceptors, including β_2, β_1, α_2 and α_1 receptors; beta receptors are positively coupled, and alpha receptors negatively coupled, to adenylate cyclase, which catalyses the conversion of ATP to cAMP, a common second messenger.

Modulation of the DG Evoked Response

In 1983 Neuman and Harley reported that NE iontophoresed for 2-5 min at the granule cell body layer produced significant potentiation of the DG evoked response in 80% of sites tested. This potentiation (20-400% of control) lasted for more than 30 min in 40% of the potentiated sites. This was confirmed in brain slice experiments(Lacaille & Harley, 1985; Stanton & Sarvey, 1985) which have implicated the β-receptor as the mediator of this potentiation. Potentiation was achieved with application of isoproterenol, a β-agonist, and the effect was blocked by a variety of β -antagonists, including timolol (Lacaille & Harley, 1985), propranolol, and metoprolol (Stanton & Sarvey, 1986), but not by the α-antagonist phentolamine (Lacaille & Harley, 1985). Blocking was also achieved by an NMDA blocker and protein synthesis inhibitors (Stanton & Sarvey, 1985), revealing some similarity between NE-induced LTP and frequency-induced LTP, which is blocked by similar treatments.

Effects of NE in the DG that have been reported include an increase in K^+-evoked glutamate release (Lynch & Bliss, 1986), increased Ca^{2+} entry into granule cell bodies (Stanton and Heinemann, 1986), and prolonged, mild depolarization of granule cells accompanied by an increase in membrane resistance (Lacaille & Schwarzkroin, 1988). These effects suggest that NE would increase the probability of NMDA receptor

activation in response to PP stimulation. However, further experiments could not show that NE potentiation was specific to synapses that were active when NE or its effects were present; NE and β -agonist potentiation of PP activation occurs even when there is no PP activation while NE is present or during a washout period (Lacaille & Harley, 1985; Dahl & Sarvey, 1990). These results suggest that the long-lasting potentiating effects of NE are not due solely to NMDA-r activation; this is supported by studies showing that NE-induced potentiation in CA3 is not NMDA-r dependent (Hopkins & Johnston, 1984).

In vivo investigation suggests the characterization of adrenergic effects in the DG may be more complex than in vitro results suggest. The mode of action of NE in the DG is not made clearer by in vivo results, many of which are inconsistent. Various excitatory and inhibitory systems are active in an in vivo preparation that may not be present in a slice; hence, results from the two preparations can be expected to differ. Bliss et al. reported in 1983 that high frequency LTP of the PS was not prevented by NE depletion, although the increase in EPSP slope was. Winson and Dahl (1985) found that, when iontophoresed into the cell body layer of the DG in vivo, an α-agonist (phenylephrine) and β -antagonist (sotalol) produced PS increases while the β-agonist isoproterenol produced decreases, although the effects of NE itself were not reliable and seemed to vary with exact locus of release. NE application to the mid-third of the ML resulted in decreased EPSP slope and amplitude. If NE was iontophoresed just above the cell layer, the effects on the EPSP were reversed. These results were the first to suggest that the effects of NE on PP stimulation of the DG may depend on which layer of the ML is exposed to NE; from these results, one could hypothesize that NE effects on DG responses to stimulation

of the MPP may be different than potentials elicited by the LPP, since they terminate at different levels in the ML. Dahl & Sarvey (1989) studied this possibility in the brain slice; they provided evidence that NE potentiates the MPP-evoked potential and significantly depresses those evoked by the LPP. These effects could be replicated by a β-agonist and were blocked by propranolol or an NMDA blocker.

Modes of Release in the DG

In attempts to show the effects of NE in the DG in vivo, it has been important to show that normal physiological release of NE can exert the same effects as NE iontophoresis. Several strategies have been used to achieve NE release in the DG, all involving LC stimulation.

i) Electrical stimulation of the LC

A train of pulses directed at the LC is effective in producing potentiation of PP-evoked responses in the DG. PS amplitude is consistently increased, while effects on EPSP slope are not consistent (Bliss & Wendlandt, 1977; Assaf et al. 1979). In 1985 Dahl and Winson found LC effects in the DG using a train of 6-12 Hz pulses which ended 50 ms before the PP pulse; Harley et al. (1989) found LC effects using a brief 333 Hz train with an optimal LC-PP stimulation interval of 40 ms, or using a more sustained 10 Hz stimulation. Repeated stimulation of the LC and PP resulted in LTP of the PP-evoked potential. None of the short term potentiating effects could be blocked with propranolol. Washburn and Moises (1989) reported successful blocking of LC effects as produced by

electrical stimulation in the DG by propranolol and clonidine (α-agonist); they used LC currents which only produced half-maximal potentiation of the PS, at a frequency of 333 Hz (15 ms train). Stronger electrical stimulation may result in release of peptides as well as NE in the DG (Lundberg et al., 1986); these peptides may themselves have a potentiating effect.

ii) Glutamate/pharmacological activation of the LC

Harley and Milway (1986) used micropipette ejections of glutamate in the vicinity of the LC to effect NE release in the DG in an attempt to activate LC neurons more selectively than with electrical stimulation. Such activation produced evoked-potential potentiation, long-lasting in some subjects, that could be blocked by β-antagonists delivered systemically or by cannula into the DG (Harley & Evans, 1988).

iii) Paragigantocellularis (PGi) activation of the LC

Babstock and Harley (1992) activated the LC by electrically stimulating the PGi region of the medulla, which has excitatory inputs to the LC (Ennis & Ashton-Jones, 1986). Four 0.5 ms monophasic square-wave pulses at a frequency of 333 Hz and with an intensity of 25-35 V potentiated the DG PS at 20-50 ms ISIs. This potentiation was attenuated significantly by systemic propranolol. Consistent with other LC stimulation methods, EPSP slope changes were variable. This finding is significant in that the PGi is a much easier target to successfully stimulate than the small LC, providing a technically preferable alternative method of releasing norepinephrine in the DG.

Anatomical data suggest that information from the different sensory modalities may reach the DG via specific divisions of the PP; visual and auditory information seem to be carried by the MPP while the LPP has been shown to carry olfactory input (Swanson et. al., 1987). The aforementioned study which demonstrated that norepinephrine depresses potentials evoked through the LPP and enhances those evoked via the MPP (Dahl & Sarvey, 1989) has important implications, then, about how norepinephrine may differentially affect the various types of sensory information entering the hippocampus. Babstock & Harley showed that PGi stimulation depresses the LOT evoked potential in the DG in the anesthetized rat. When subcortical inputs to the hippocampus, including those of the LC, are disrupted, hippocampal place cell responding changes to become dominated by olfactory cues (Shapiro, 1989). Taken together, these studies suggest that the contribution of different types of sensory information to the spatially-related functions of the hippocampus may be at least partially controlled by noradrenergic activity. The influence of norepinephrine release on other types of sensory information entering the DG has yet to be determined.

RATIONALE FOR PRESENT EXPERIMENT

If HPCs are strongly influenced by processed vestibular information from the ADTN, one would predict that evidence of a functional, indirect connection between the ADTN and the hippocampus can be found. Information about other sensory modalities (olfaction, audition) has been shown to enter the hippocampus via the EC-PP connection to the DG. There is anatomical evidence to suggest that ADTN output may travel via the postsubiculum to the EC and PP. If a potential is generated in the DG following ADTN stimulation, the hypothesis that ADTN activity, presumably reflecting some vestibular input, has the potential to influence HPC activity, would have stronger grounds. If the theory that norepinephrine modifies sensory input entering the hippocampus via the DG is to gain support, two aspects of hippocampal electrophysiology require further investigation: first, it is necessary to determine what types of sensory information are conveyed via the LPP and the MPP, and, second, the manner in which norepinephrine release modifies such input must be determined. The present study is concerned with determining a) the characteristics of any potentials that can be evoked in the DG of the hippocampus from electrical stimulation of the ADTN in the anesthetised rat; b) whether or not such potentials enter the hippocampus via the PP; and c) how endogenous norepinephrine release modifies these ADTN-evoked potentials.

METHODS

SUBJECTS

Thirty male Sprague-Dawley rats weighing from 260-380 grams served as subjects in these experiments. Each animal was anaesthetized with 1.5 g/kg urethane administered i.p. and had a jugular catheter surgically implanted before being fixed skull flat in a stereotaxic apparatus. Additional doses of urethane were given until foot and tail pinch responses were abolished. Body temperature was monitored by rectal probe and maintained at 37°C by a d.c. electric heating blanket and temperature control unit. Horizontal position between lambda and bregma was checked as well as their alignment with the stereotaxic apparatus. Holes were drilled in the skull using a dental drill and electrodes were lowered into the DG, PP, ADTN and either the LOT or the PGi.

ELECTRODE PLACEMENTS

A bipolar stimulating electrode (Rhodes SNE 100) was aimed at the PP (7.2 mm posterior, 4.1 mm lateral to bregma and 2.5-3.5 mm below brain surface). A monopolar insect-pin (0.4 mm tip diameter) stimulating electrode was placed in the ADTN (1.5 mm posterior, 1.35 mm lateral to bregma and 4.4-4.7 mm below brain surface). A glass micropipette of 20-40 μm tip diameter served as the recording pipette, and was positioned near the cell body layer of the DG (3.5 mm posterior and 2.0 mm lateral to bregma, 2.5-3 mm below brain surface). The pipette was filled with 0.9% physiological saline (pH 7.2). Final depth of placement of the recording pipette was adjusted to maximize the peak

amplitude of the PP-evoked potential. In animals in which the LOT was stimulated, a bipolar stimulating electrode (Rhodes SNE 100) was lowered into the LOT (5 mm anterior, 1.5 mm lateral to bregma and 5.5-6 mm below brain surface). Final depth of placement was adjusted to elicit a reliable LOT potential as described by Wilson & Steward (1978).

In animals in which the effect of PGi stimulation on the ADTN evoked potential was investigated, a bipolar stimulating electrode (Rhodes SNE 100) was lowered into the PGi (11.8 mm posterior, 1.25 mm lateral to bregma and 9.3-9.8 mm below brain surface). Final depth of placement of the PGi electrode was adjusted to provide reliable potentiation of the PP-evoked population spike at an interstimulus interval of 45 ms before the commencement of any PGi - ADTN pairings.

Stimulation Parameters

A 0.2 ms monophasic square wave pulse was delivered to the PP, ADTN or LOT at 0.1 Hz via a Neuro Data SIU 90 Isolated Current Source stimulator. PP stimulation strength varied from 150 to 350 μA among animals producing a population spike of 2-5 mV amplitude. ADTN stimulation strength varied from 200-550 μA, and LOT stimulation from 300-600 μA, among animals producing EPSPs of 0.5-2 mV amplitude. Signals from the recording pipette were coupled to a Grass H1 Z probe (model HIP 5) and then amplified using a bandwidth of 0.1 Hz-3 KHz (Grass P5 series preamplifiers, model P511K). The resulting signals were displayed on a Tektronix S013 differential amplifier oscilloscope and digitized on an IBM-PC compatible computer (1 point/0.1 ms). The data

acquisition program was Datawave version 5.3. In animals in which the PGi was to be activated, stimulation was initiated by the experimenter after a baseline of at least 50 evoked potentials at 0.1 Hz were collected each time in each animal. A 10 ms 333 Hz train consisting of 0.5 ms monophasic square wave pulses in the PGi was paired with PP or ADTN stimulation for 6 stimuli (0.1 Hz); PGi stimulation preceded PP or ADTN stimulation by an interval of 35-70 ms. PGi stimulation was delivered using a stimulator as described above.

EXPERIMENT PHASES

A. Determining the route of the ADTN EPSP into the DG:

After a reliable ADTN-evoked potential was established in the DG, several steps were taken to determine if the ADTN EPSP was transmitted via the LPP or the MPP. These steps included:

i) a DG depth profile was recorded in two animals in an attempt to determine a reversal point in the DG molecular layer for the ADTN-evoked potential. Starting at the granule cell layer (indicated by maximal positive PP-evoked EPSP amplitude) the DG recording electrode was moved upward through the molecular layer in 50 or 100 μm steps. Six PP-evoked EPSPs, followed by six ADTN-EPSPs were recorded at each step. Average amplitudes were calculated for each group of six EPSPs.

ii) recordings were made simultaneously in the PP and the DG during ADTN stimulation in order to see if the arrival of the signal in the DG was preceded by a field potential in the PP (n=4).

iii) paired-pulse stimulation of the ADTN was applied to assemble an interpulse-interval paired-pulse profile of the ADTN EPSP (n=6). A conditioning pulse followed by a test pulse was delivered to the ADTN at each of nine interpulse intervals ranging from 20 to 100 ms. The pulses were delivered every 10 s. In this manner at least 25 pulse pairs were delivered consecutively at each interstimulus interval.

iv) Paired-stimulation was applied to the PP, LOT and ADTN to test for cross-potentiating effects between the PP and ADTN, and the LOT and ADTN. Single pulse stimulation was applied every 8 sec alternately to the PP and ADTN (n= 4-6) to establish an EPSP baseline of at least 20 pulses for each. The test was conducted by delivering a conditioning pulse to one pathway followed 35 ms later by a test pulse to either the same or the other pathway. In this manner each pathway was tested with a conditioning pulse delivered to either itself or the other pathway. The tests were repeated with ISIs of 50 and 100 ms, and the entire procedure repeated with the LOT and ADTN (n = 3-5).

v) after all steps in an experiment were completed, the PP stimulating electrode was removed from the brain and placed into a Narishige holder adjacent to an injection cannula connected via plastic tubing to a 2 µl syringe . The injection cannula was adjusted in the holder such that tip was just dorsal and anterior to the tip of the PP stimulating electrode, and the cannula and electrode were aimed at the PP and positioned to elicit the maximum amplitude EPSP in the DG. Thirty 0.1 Hz pulses were delivered to the ADTN to establish a pre-Lidocaine injection baseline. 2 µl of Lidocaine were then injected by freehand injection over a period of one minute. PP EPSPs were observed for change for 5

min post-injection. If a change in EPSP amplitude was observed (n=3), 0.1 Hz EPSPs were evoked by ADTN stimulation for 10-30 minutes. If no changes were observed in the PP EPSP for 5 min post injection, the injection cannula was raised slightly and the injection process repeated. If no changes in PP EPSP amplitude were observed within 10 min of this injection, an ablating lesion of the PP was attempted via electrical current passed through the electrode tip for 6 s (n=2). Twenty minutes was allowed to pass after the lesion was made to allow any possible seizure activity to diminish (Wilson & Steward, 1978), and then the ADTN and PP EPSPs were sampled at 0.1 Hz for at least 10 min.

B. Assessment of the effects of NE on the ADTN EPSP:

In subjects in which placement of a stimulating electrode into the PGi consistently elicited strong (>30%) potentiation of the PP-evoked population spike, the effect of PGi stimulation on the ADTN EPSP was assessed as follows:

i) a series of at least 3 trial blocks of the PGi and PP stimulation pairings was performed. 0.1 Hz PP stimulation was delivered for at least 6 minutes between each of the trial blocks, each of which consisted of 6 pairings of PGi and ADTN stimulation at one interstimulus interval, ranging from 35 - 100 ms. The order of interstimulus intervals was random in each experiment (n=1-4).

ii) in animals in which the PGi electrode did not elicit consistent potentiation of the PP EPSP, idazoxan (350 or 500 μg/kg) in 0.2 ml 0.9% saline was administered via a jugular i.v. by freehand injection over a period of one minute after 60 baseline ADTN EPSPs were recorded. 0.1 Hz ADTN EPSPs were sampled for 12 to 30 min post-injection (n=4-5). Systemic administration of an α_2-receptor antagonist has been

shown to increase NE efflux in the DG as measured by microdialysis perfusion (Abercrombie et al., 1988).

Data Collection and Analysis

Parameters extracted from ADTN, PP and LOT -evoked potentials varied between experiment phases and included EPSP amplitude (vertical change from the baseline immediately preceding the stimulus artifact to the peak of the EPSP), EPSP slope, latency to the peak of the EPSP from initiation of the stimulus artifact, half-amplitude width and rise time from onset. These measures were taken from EPSPs that reflected an average of six ADTN-evoked EPSPs, calculated on-line.

When evaluating data, one-tailed t-tests were used unless otherwise indicated.

Histology

At the conclusion of the recording session, electrolytic lesions were made at each stimulating site (0.5 mA for 1.0 s). The rat was sacrificed by decapitation and the brain removed and frozen. 30 μm sections of tissue were sliced coronally on a cryostat microtome. Slides used for verification of stimulating sites were subjected to differentiated cresyl violet and glycogen phosphorylase a staining (Harley & Bielajew, 1992). Glycogen phosphorylase a is a metabolic enzyme for which staining provides easily distinguished, fine detail of brain structure as related to metabolic activity. Slides used for verification of the recording sites were subjected to active glycogen phosphorylase staining only.

RESULTS

HISTOLOGICAL VERIFICATION OF ELECTRODE PLACEMENT

Sixteen animals were included in the data analysis, based on satisfaction of ADTN histological criteria (see Fig. 12). Animals in which the majority of the electrolytic lesion was inside the borders of the ADTN were included. PGi data from animals whose results were included in other parts of the analysis were not always included because of inaccurate placement of the PGi stimulating electrode. The use of glycogen phosphorylase a staining allowed for easy identification of the borders of the ADTN and allowed the DG recording electrode tip placements to be visible in five animals, due to its sensitivity to fine tissue damage. The cresyl violet staining was useful for identifying the PGi placement and the extent of the PP lesions, as it provides more distinct structural information than the GPa stain in those brain areas. Figure 12 shows the placements of the ADTN stimulating electrode. Figure 13 shows the placements of the PGi stimulating electrodes that were included in the analysis.

CHARACTERISTICS OF THE ADTN EPSP

A reliable EPSP can be elicited from electrical stimulation of the ADTN; typical averaged evoked potentials, recorded in the hilus or granular layer of the suprapyramidal blade of the DG, are shown in Figure 1. The EPSP was consistently preceded by a small depolarization with a very stable within-subject latency-to-peak relative to the EPSP proper. The average latency-to-peak of the ADTN-evoked potential was 10.63 ms (range

8.72 - 12.08 ms) and the average rise-time was 5.89 ms (range 5.3 - 6.35ms) for EPSPs with an average amplitude of 1.69 mV (range 0.78 B 2.64 mV) and average slope of 0.37 mV/ms (range 0.12 B 0.76 mV/ms). The EPSP did not follow stimuli of 50 Hz or greater.

Recording electrode 50 μm-increment depth profiles were obtained in two animals. Stimulation of the ADTN elicited a positive-going wave in the hilus and granular layer which became increasingly negative as the recording electrode passed from the granular layer up through the molecular layer. The depth profiles were also recorded for the PP EPSP. When the intensity of stimulation was increased before or after the depth profile was completed, the average latency to initiation of the population spike in both PP EPSP profiles was 4.2 ms. The apparent polarity inversion of the ADTN EPSP occurred approximately 50 μm closer to the granular layer than the corresponding PP-evoked potential. The latency to peak measured on the profiles was taken from the stimulus artifact to the point on the response that was furthest from the baseline immediately previous to stimulation. The average latency to peak for the PP EPSP was 4.7 ms at a depth of 0 mm (proximal to medial molecular layer) and 3.15 ms (negative peak) at 0.6 mm. The respective latencies for the ADTN EPSPs were 9 ms and 5.4 ms. The ratio of the positive peak latency to negative peak latency is therefore similar for both points of stimulation (PP: 1.5, ADTN: 1.6). Figure 2 shows a depth profile taken from one animal.

Figure 3 shows sample evoked potentials recorded simultaneously from the PP and DG following stimulation of the ADTN. A field potential was recorded in the PP with an average onset latency of 5.9 ms and average latency to peak of 6.8 ms (N=4; corresponding values for the DG-recorded potential were 5.6 and 9.4 ms, respectively).

PAIRED PULSE EFFECTS

Paired-pulse stimulation of the ADTN results in reliable and significant facilitation of both the amplitude and slope of the ADTN EPSP at ISIs from 30 to 100 ms (Table 1). This potentiation reached a maximum at an ISI of 50 ms but was still significant at an ISI of 100 ms, the longest ISI tested. See Figure 4 for an EPSP amplitude/slope vs. ISI profile and sample evoked potentials.

Figures 5 and 6 illustrate the cross-potentiating effects of paired-pulse stimulation delivered to the PP and ADTN. Paired-pulse stimulation of the PP alone resulted in 12% and 4% increases in EPSP amplitude at ISIs of 35 and 50 ms, respectively, which gave way to significant depression of 12% at an ISI of 100 ms. When the conditioning pulse was delivered to the ADTN, however, the PP amplitude was reduced from baseline an average of 16.5% at all three ISIs. When paired-pulse stimulation was applied to the ADTN alone, test pulse EPSP amplitude was, on average across all three ISIs, twice that of baseline, with the largest increase observed at an ISI of 50 ms. A conditioning pulse delivered to the PP resulted in almost no change (<4%) in the ADTN test pulse amplitude at all three ISIs (Table 2). Figures 7 and 8 show the effects of paired-pulse stimulation on the LOT and ADTN EPSPs. Paired pulse stimulation of the LOT alone resulted in a significant potentiation of 233% at an ISI of 35 ms but little change (-13% and +8%) at ISIs of 50 and 100 ms, respectively. Preceding the LOT test stimulus with a conditioning stimulus in the ADTN resulted in a significant LOT EPSP amplitude increase of 24% at an ISI of 35 ms, but smaller changes at the other ISIs (average increase of 9.5 %). A conditioning pulse delivered to the LOT followed by a test pulse to the ADTN resulted in

a reduction of EPSP amplitude of 19% ($p<0.05$) at an ISI of 35 ms and 23% at an ISI of 50 ms, but little change (+5%) at an ISI of 100 ms (Table 3).

Effects of Lidocaine Injection or PP Lesion

When Lidocaine was injected into the PP, PP-evoked potentials were reduced in amplitude to a maximum of 15% of baseline, or were abolished (n=3). The ADTN EPSP remained intact, although with a reduced EPSP amplitude (79% of pre-injection baseline) and slope (80% of baseline). An electrolytic lesion to the PP (n=2) that abolished the PP-evoked potential also resulted in a decrease in ADTN-evoked potential amplitude to 47% of baseline and a reduction in EPSP slope of 27% (see Figure 9). The latency to peak of the ADTN potential was not changed by either Lidocaine or lesion of the PP. Reductions in EPSP amplitude were not significant for either lesion or Lidocaine injection alone, but did reach significance when results from all 5 animals were pooled (see Table 4).

Effects of PGi Stimulation

PGi stimulation led to significant and reliable potentiation of the PP population spike, with an average increase of 33% over baseline (range 14 to 42%), while not consistently or significantly altering the amplitude or slope of the PP elicited event upon which the population spike was superimposed. Potentiating effects on the population spike were replicated within animals with PGi stimulations spaced 10 min apart. The effects of PGi stimulation paired with ADTN stimulation on ADTN EPSP amplitude and slope was inconsistent between animals. Each animal showed either a clear increase or

reduction of ADTN EPSP amplitude that was consistent at most ISIs; the effects on slope were not as consistent within animals (Table 5). Within the animals that showed an increase in ADTN EPSP amplitude, that increase reached significance ($p<0.05$) only at a PGi-ADTN ISI of 70 ms; changes in EPSP slope did not reach significance at any ISI. Within the subjects that showed ADTN EPSP amplitude decreases, the decrease was significant at ISIs of 40, 45, 55 and 65 ms, while the decrease in slope reached significance at ISIs between 40 to 60 ms inclusive, and at 70 and 75 ms. The effect of PGi stimulation on ADTN EPSP amplitude did not correlate with the anterior-posterior position of the PGi stimulating electrode as it was determined from the histology; those animals that showed decreases tended to have PGi stimulating loci that were medial and ventral to those in subjects that showed increases. However, Figure 13 shows that PGi stimulating loci from animals showing either effect were, in some cases, very close to the same site.

Effects of Idazoxan Injection

Figures 11 summarizes the effect of idazoxan on the amplitude and slope of the ADTN and LOT EPSPs. While 350 μg/kg idazoxan had no significant effect on ADTN-evoked potential amplitude or slope, a significant change in amplitude (a decrease of 18%) was seen at a dose (500 μg/kg) that also caused a significant change in LOT EPSP amplitude (an increase of 19%; n=4, Table 6). The changes in ADTN and LOT slope (-9% and +45%, respectively) were in directions consistent with the changes in amplitude, but did not reach statistical significance. Injection of vehicle (0.9% saline) alone had no effect on ATDN or LOT EPSP amplitude or slope (see Table 6). Idazoxan effects were apparent within 4 min post-injection and lasted for at least 15 min in all animals tested; in

two animals in which the experiment continued beyond 15 min post-injection, recovery of ADTN amplitude to baseline was observed between 22 and 25 min post-injection.

FIGURES

All measures are indicated in units of one increment,

unless otherwise noted.

Anatomical plates are taken from

Paxinos (1999) Atlas of the Rat Brain.

Figure 1: Typical evoked potentials recorded in the granular cell layer of the dentate gyrus by monopolar stimulation of the ADTN.

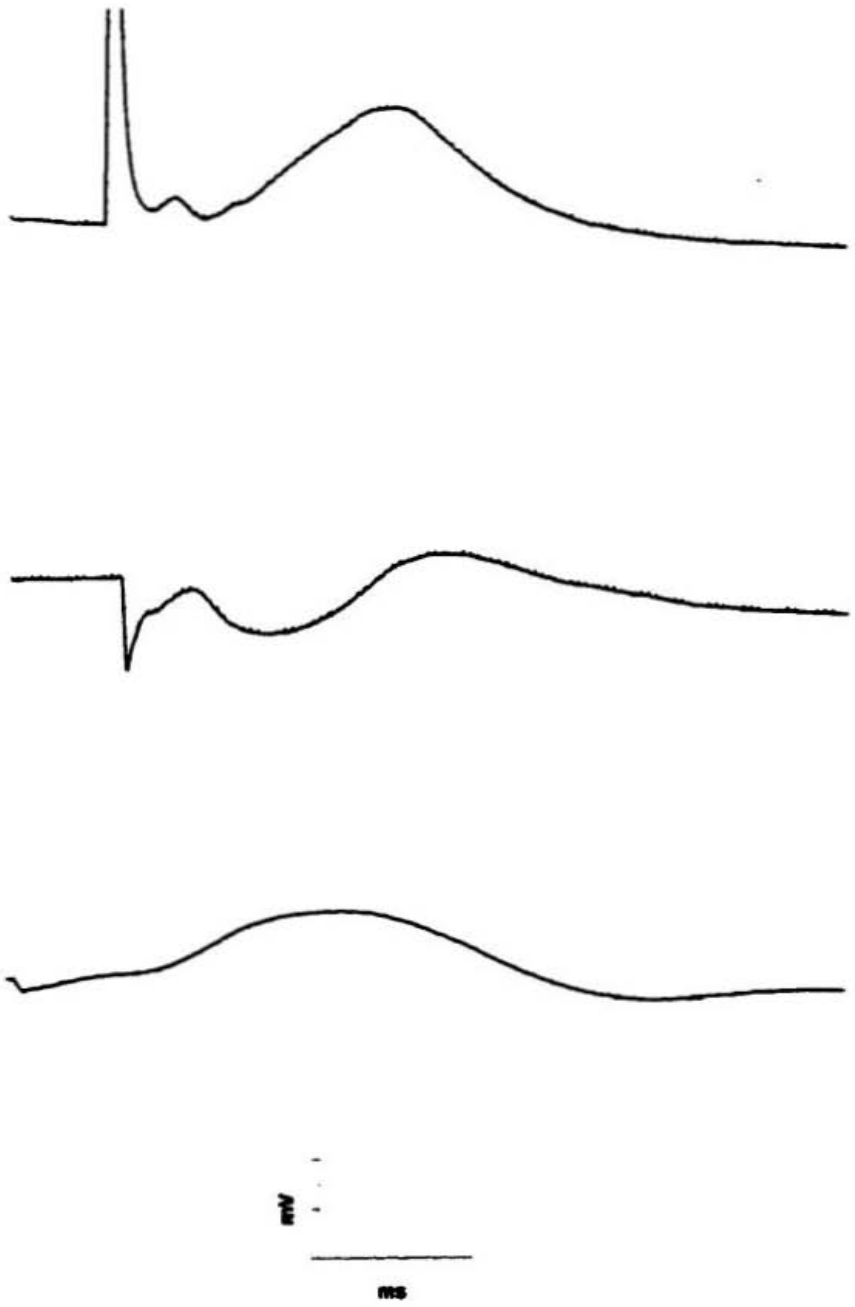


Figure 2: Sample waveforms recorded at various depths in the dentate gyrus after stimulation of the perforant path or the anterodorsal thalamic nucleus.
Recording depth is relative to the granular layer of the DG as indicated by the maximal size of the PP EPSP; all waveforms are from one animal.

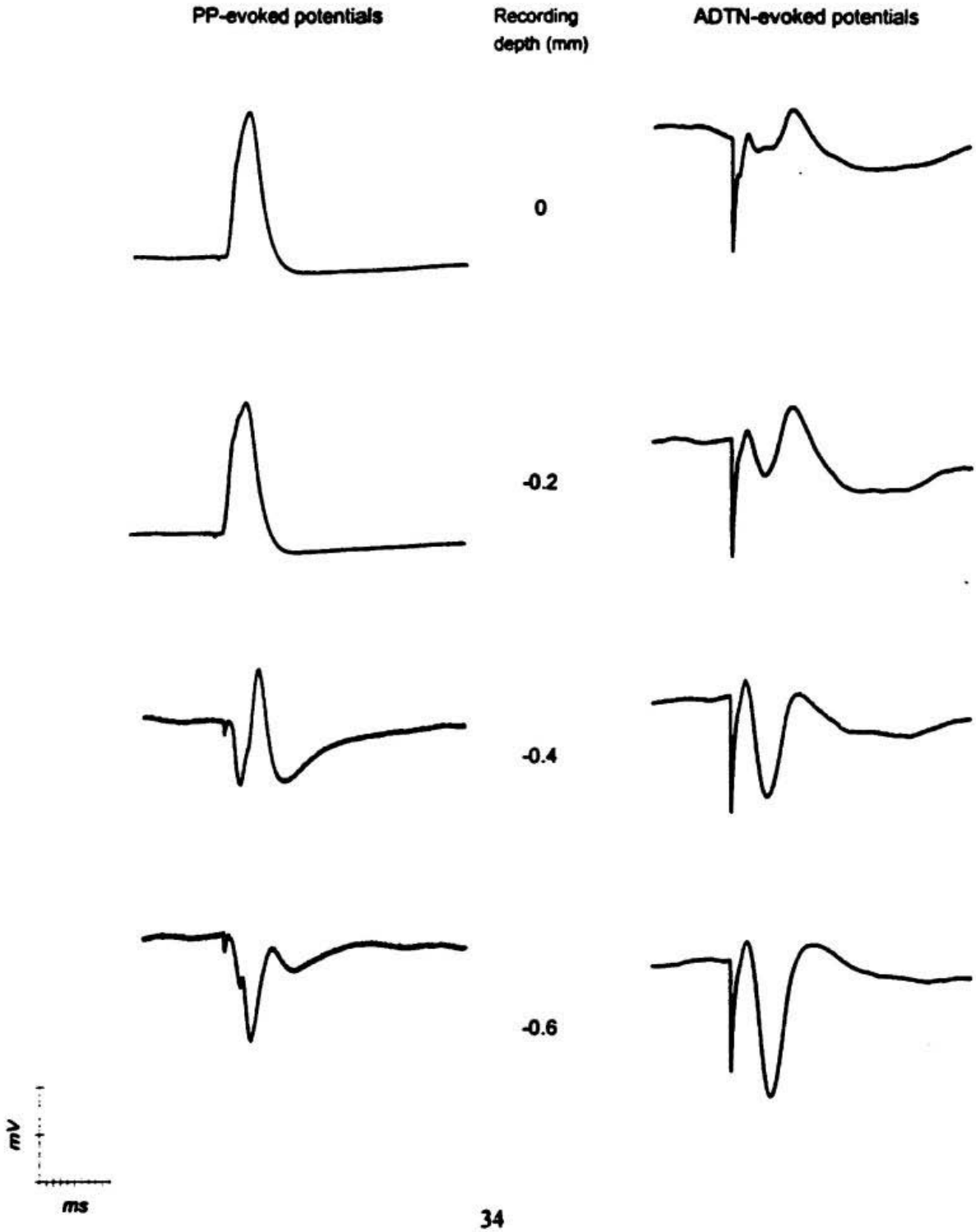


Figure 3: Evoked potentials recorded from the perforant path and dentate gyrus following stimulation of the anterodorsal thalamic nucleus (potentials are from two animals; ADTN stimulation 170 uA and 250 uA, respectively).

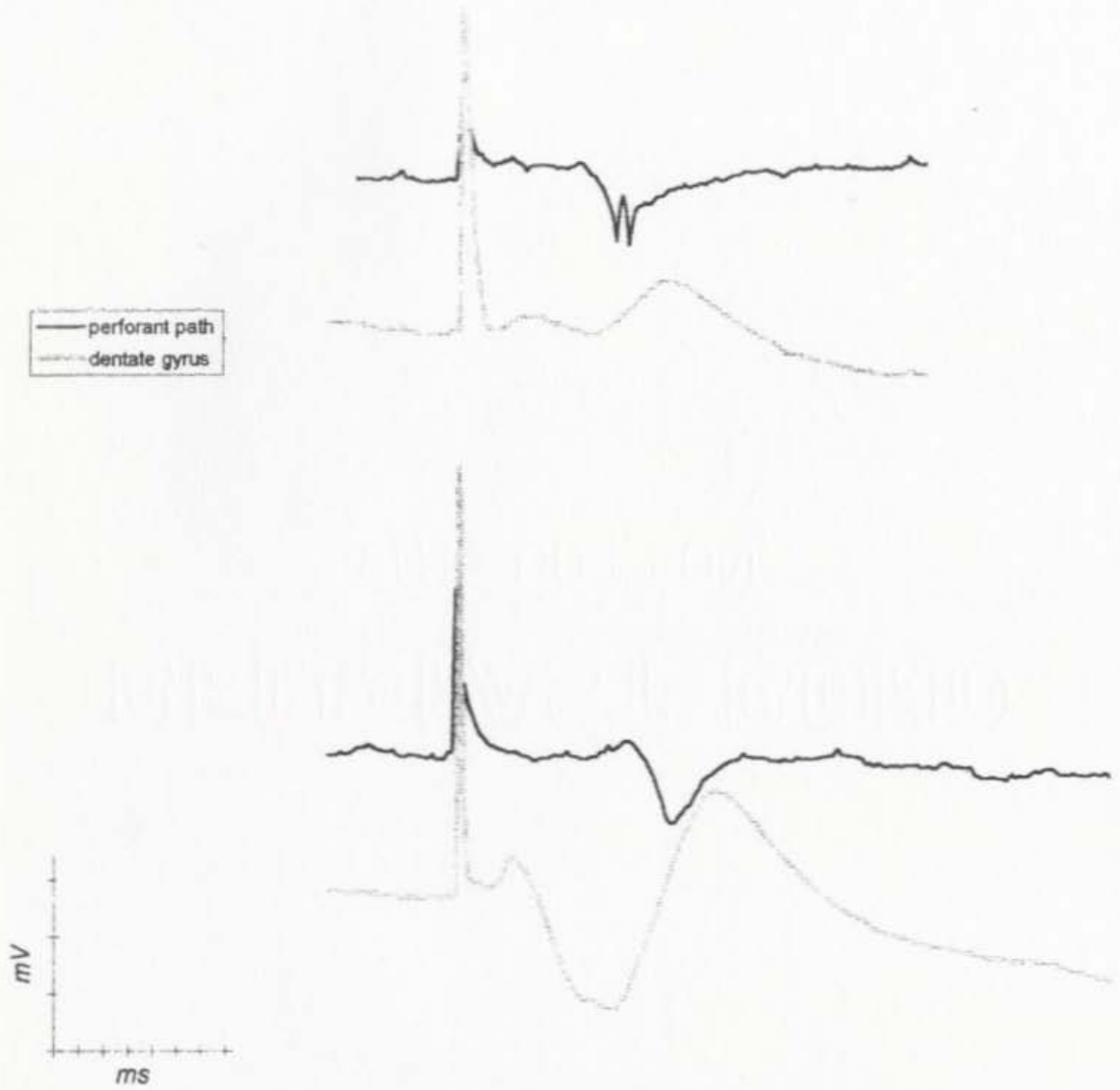


Figure 4: A. Paired pulse effects on anterodorsal thalamic nucleus EPSP amplitude and slope.
B. Sample anterodorsal thalamic nucleus -evoked potential waveforms elicited by paired pulse stimulation (ISI 20 - 50 ms).

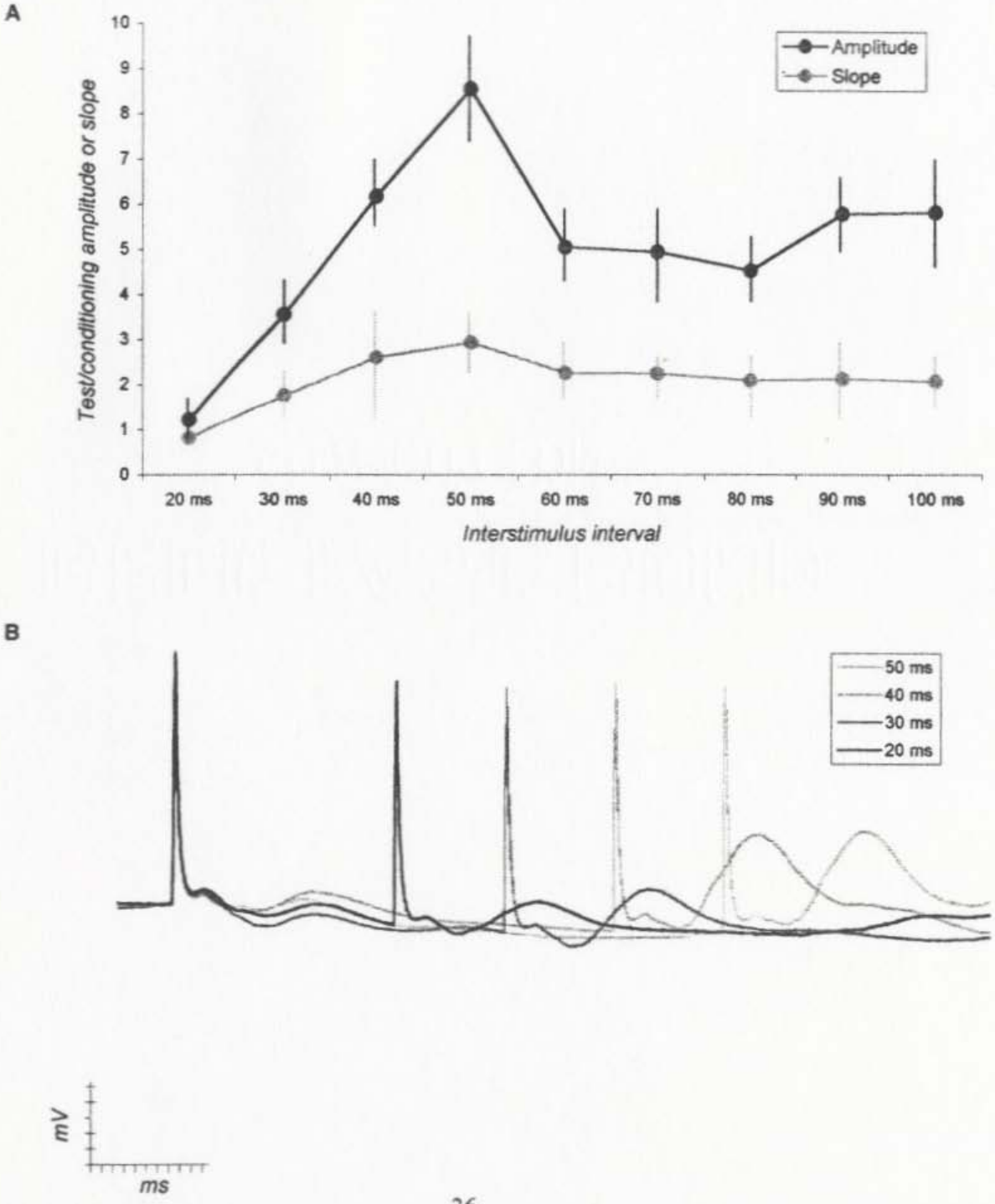
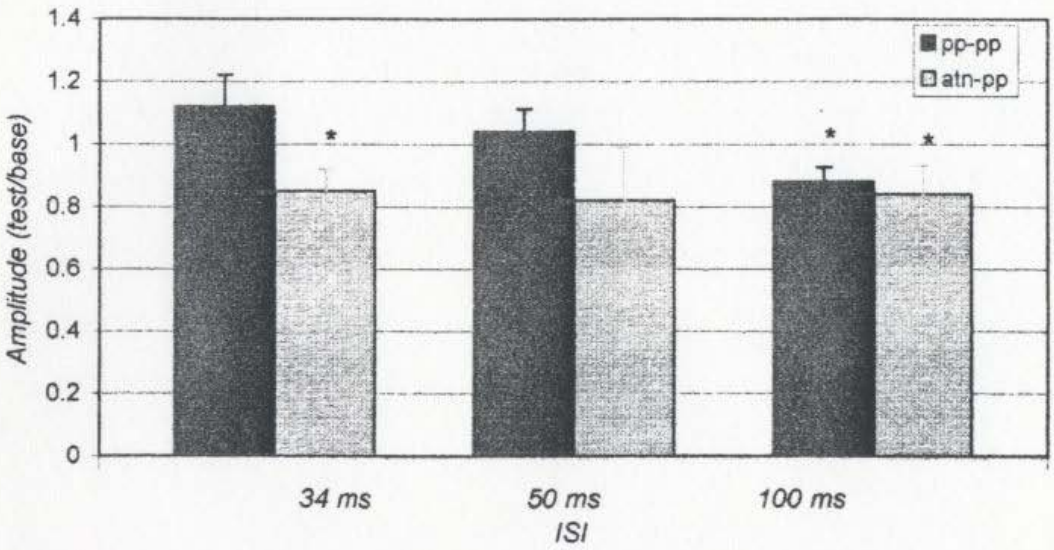


Figure 5: A. Perforant path EPSP test/base amplitude (conditioning pulse to the perforant path or the anterodorsal thalamic nucleus).
B. Typical base and test perforant path -evoked potentials (conditioning pulse to perforant path or anterodorsal thalamic nucleus; ISI 34 ms).

A



* indicates the test amplitude was significantly different from base in a paired samples t-test ($p<0.05$).

B

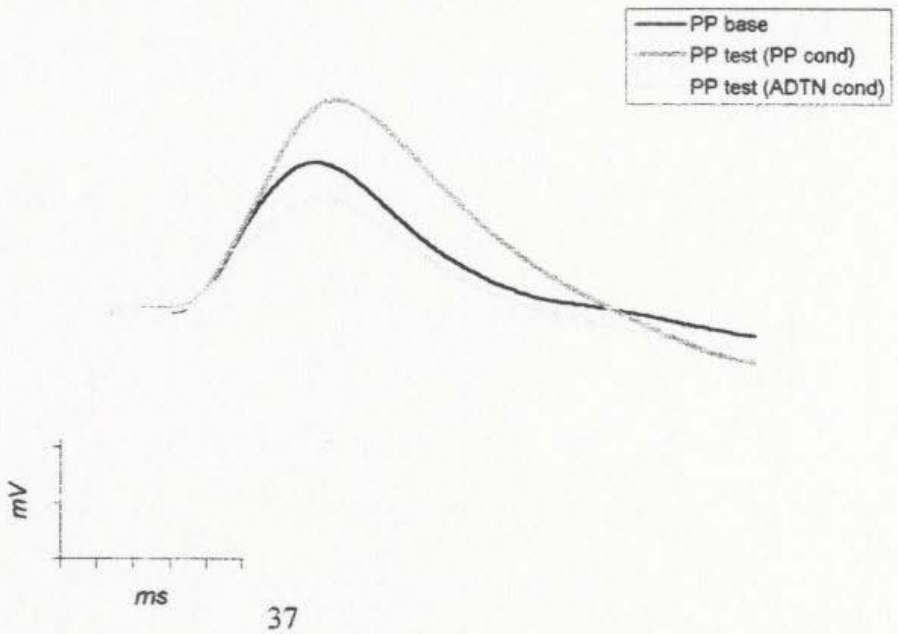
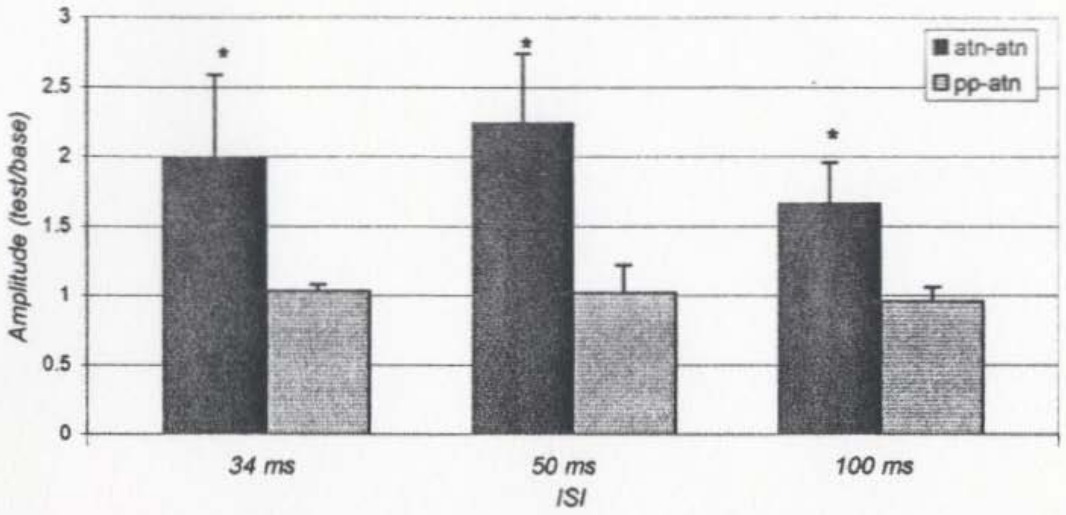


Figure 6: A. Anterodorsal thalamic nucleus EPSP test/base amplitude (conditioning pulse to perforant path or anterodorsal thalamic nucleus).
B. Typical base and test anterodorsal thalamic nucleus -evoked potentials (conditioning pulse to perforant path or anterodorsal thalamic nucleus;

A



* indicates the test amplitude was significantly different from base in a paired samples t-test($p<0.05$).

B

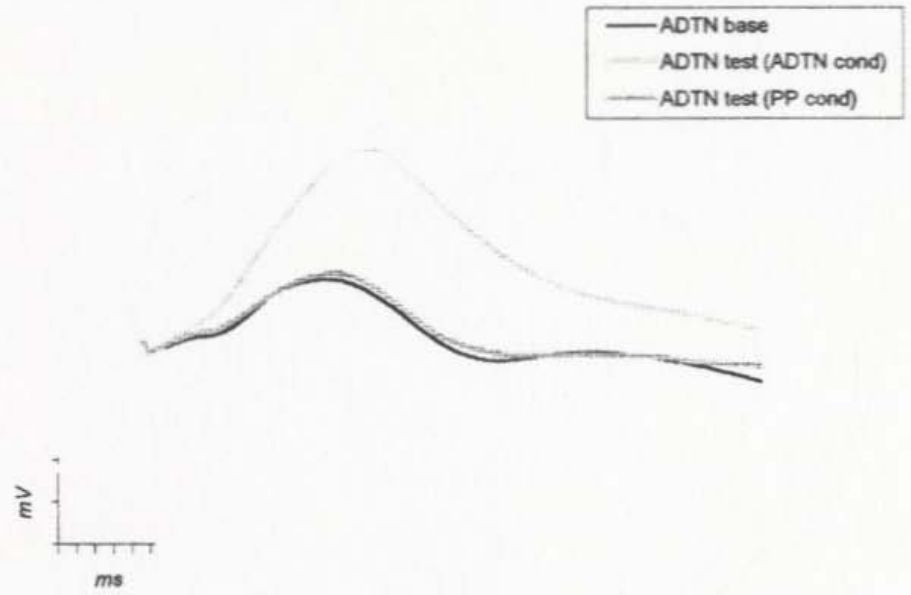
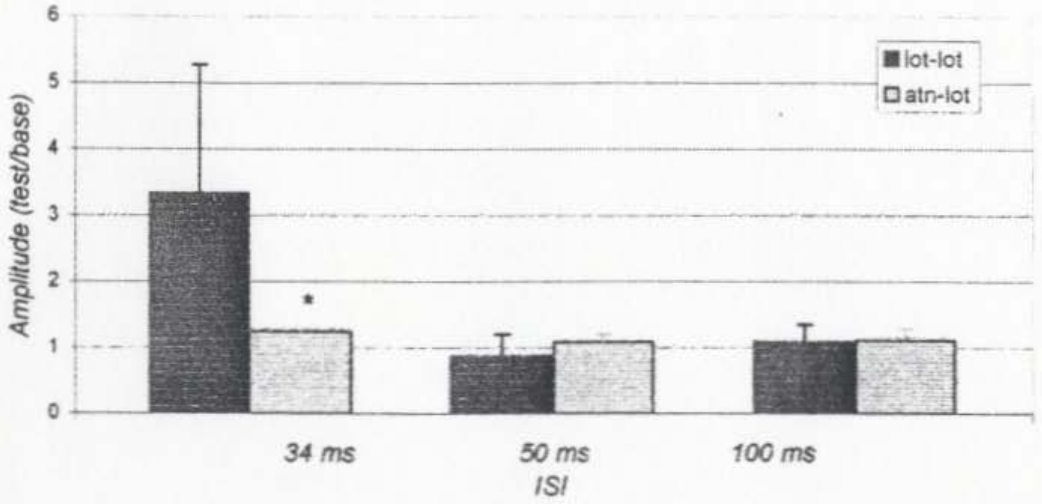


Figure 7: A. Lateral olfactory tract EPSP test/base amplitude (conditioning pulse to the lateral olfactory tract or the anterodorsal thalamic nucleus).
B. Typical base and test lateral olfactory tract -evoked potentials (conditic pulse to lateral olfactory tract or the anterodorsal thalamic nucleus; ISI

A



* indicates the test amplitude was significantly different from base in a paired samples t-test ($p<0.05$).

B

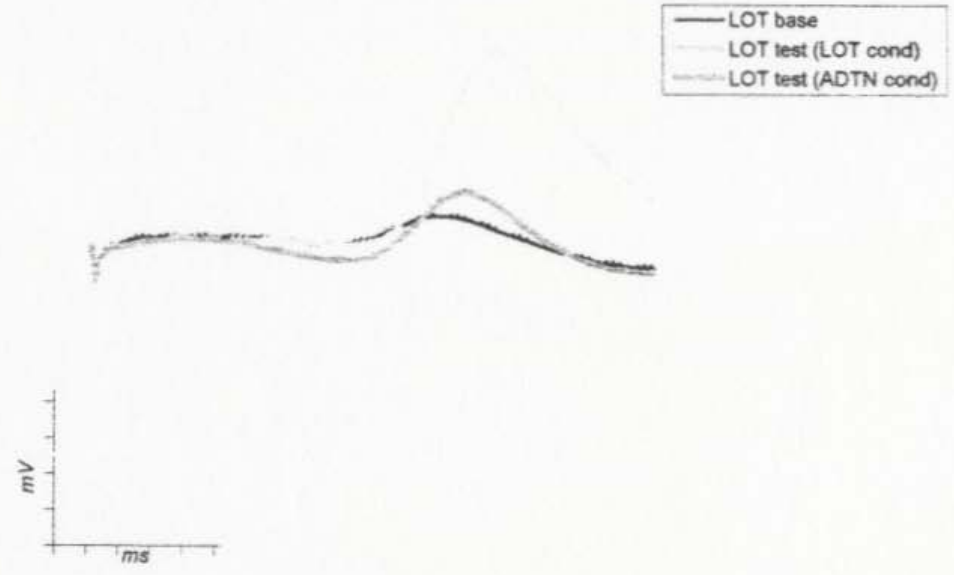
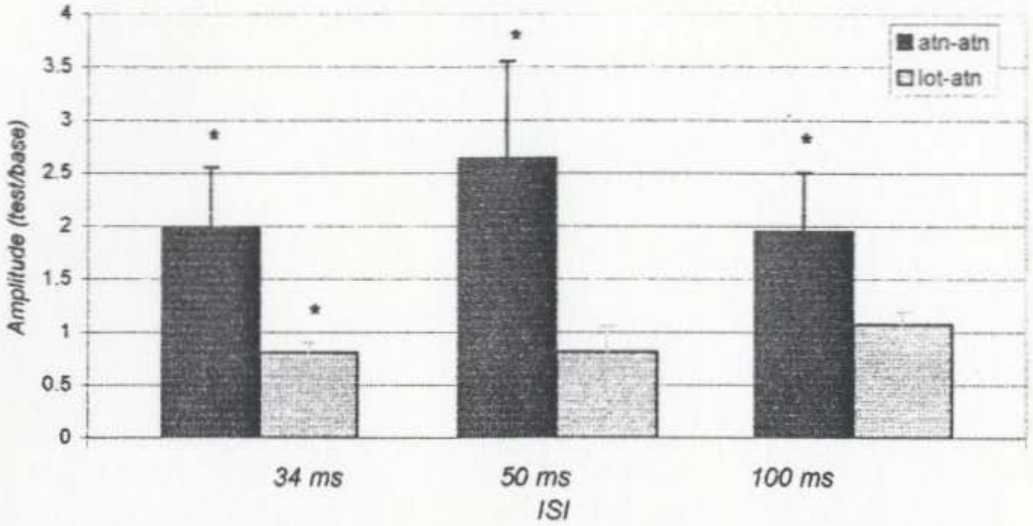


Figure 8: A. Anterodorsal thalamic nucleus EPSP test/base amplitude (conditioning pulse to ADTN or LOT).
B. Typical base and test anterdorsal thalamic nucleus -evoked potentials (conditioning pulse to ADTN or LOT; ISI 50 ms).

A



* indicates the test amplitude was significantly different from base in a paired samples t-t

B

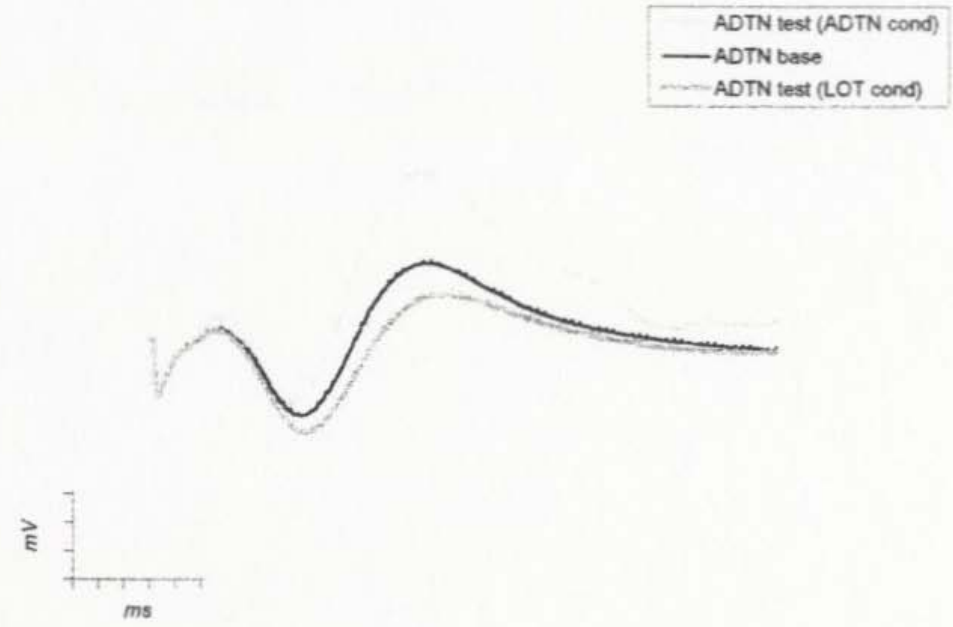
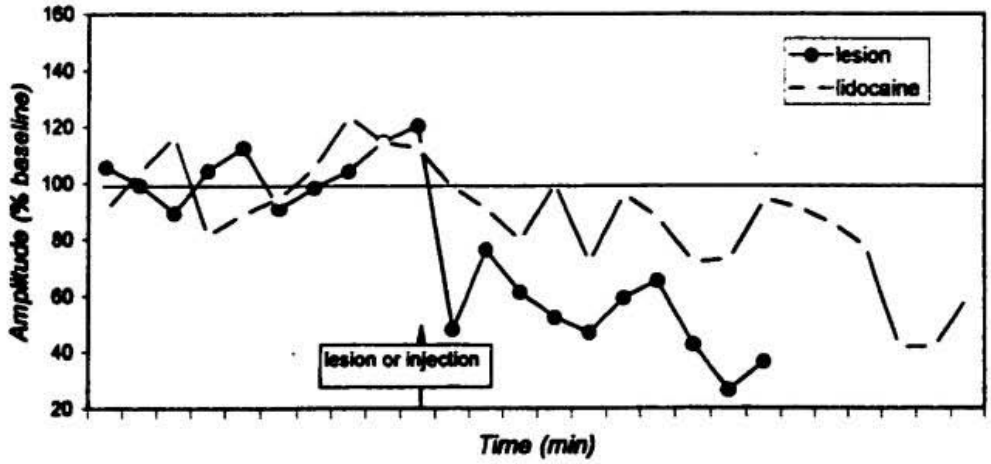


Figure 9: A. Effect of electrolytic lesion or Lidocaine application to perforant path on anterodorsal thalamic nucleus EPSP amplitude.
B. Typical ADTN-evoked potentials before and after Lidocaine application to the perforant path.

A



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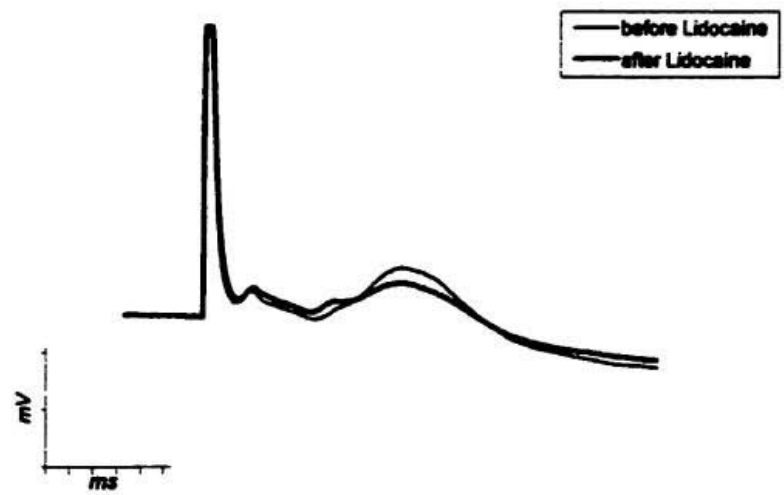
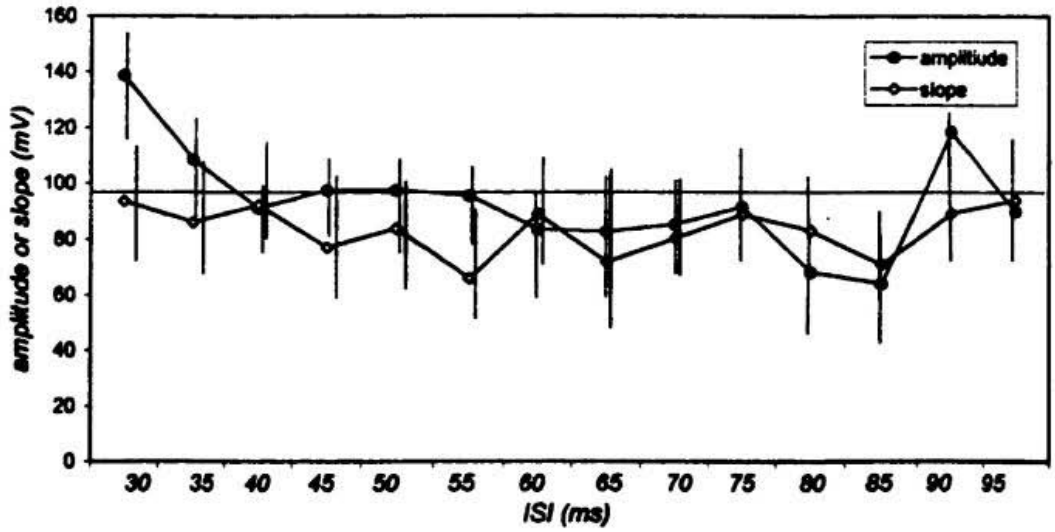


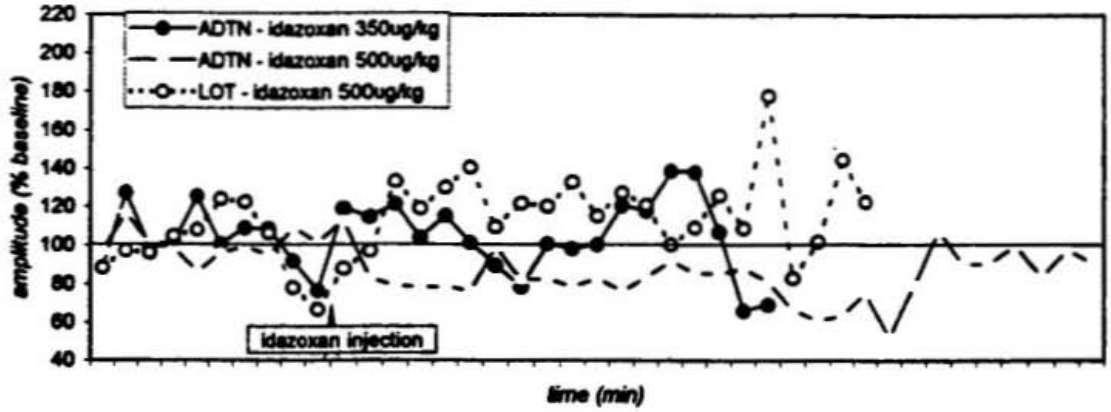
Figure 10: Effect of PGi stimulation on ADTN-evoked potential amplitude and slope.



* indicates the epsp parameter significantly differed from baseline in a paired samples t-test ($p<0.05$).

Figure 11: A. Effect of i.v. idazoxan injection on ADTN and LOT EPSP amplitude.
B. Effect of i.v. idazoxan injection on ADTN and LOT EPSP slope.
C. Typical ADTN and LOT -evoked potentials before and after injection of 500 ug/kg idazoxan.

A



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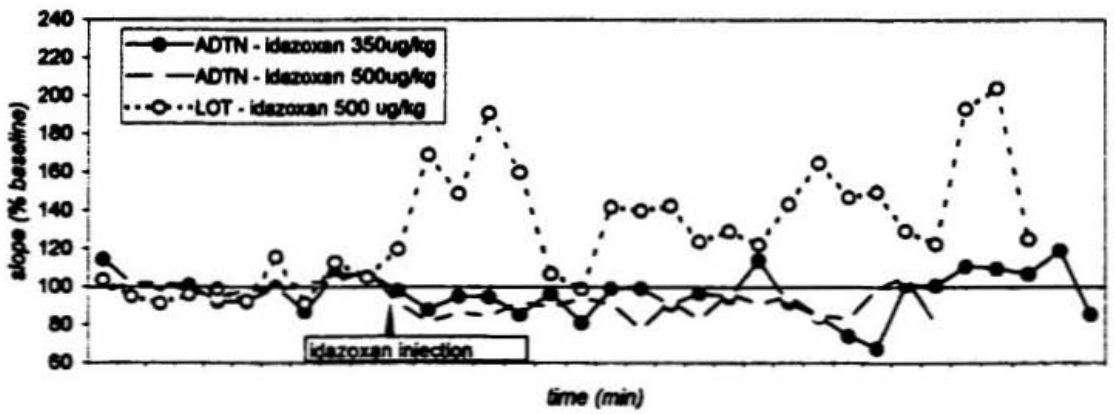


C

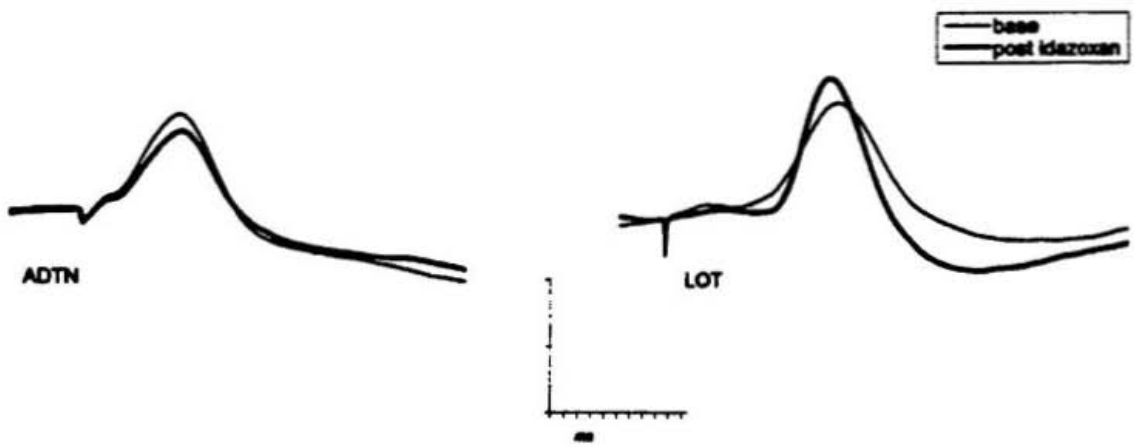


Figure 12: Placements of ADTN stimulating electrodes.

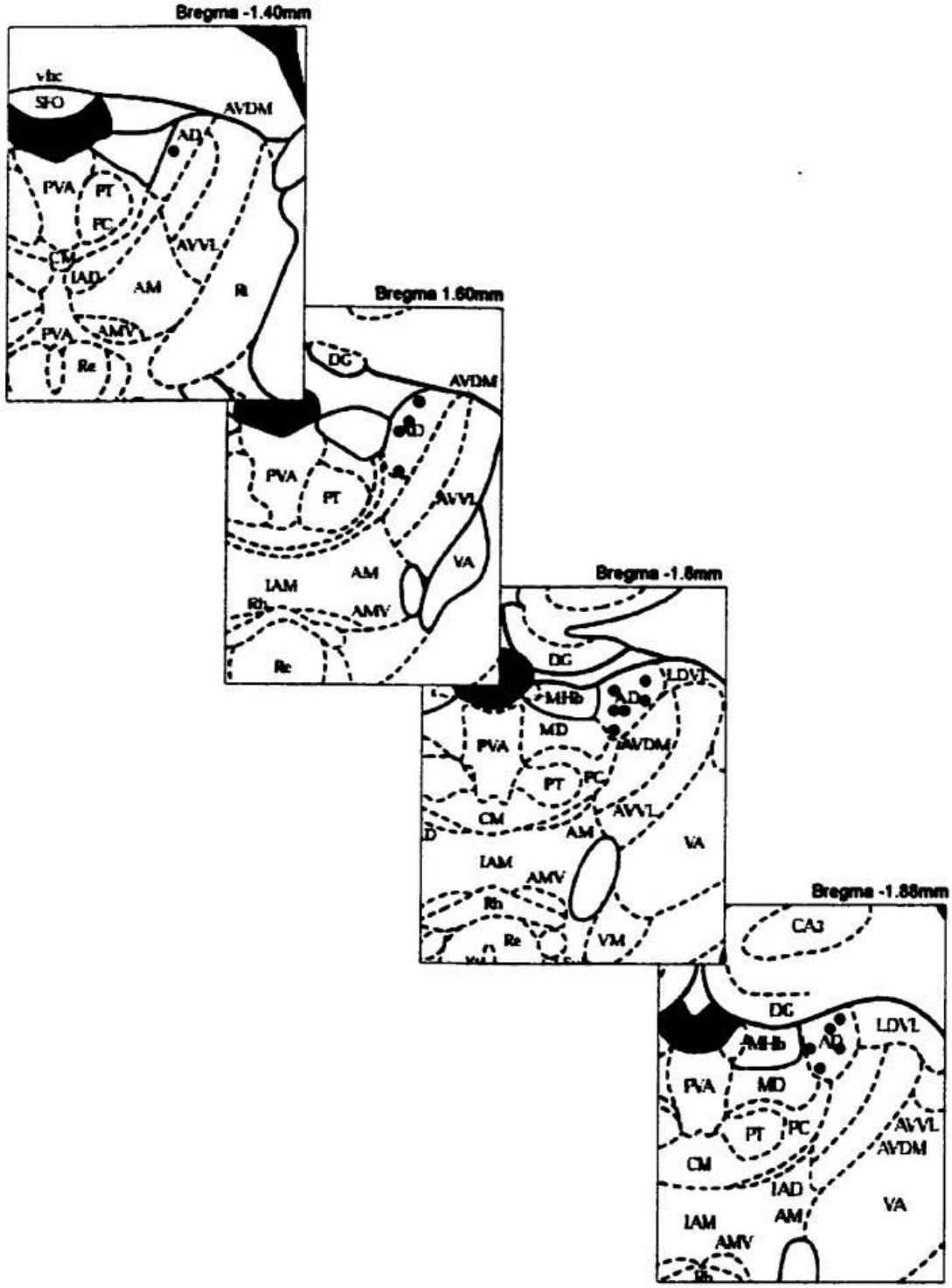
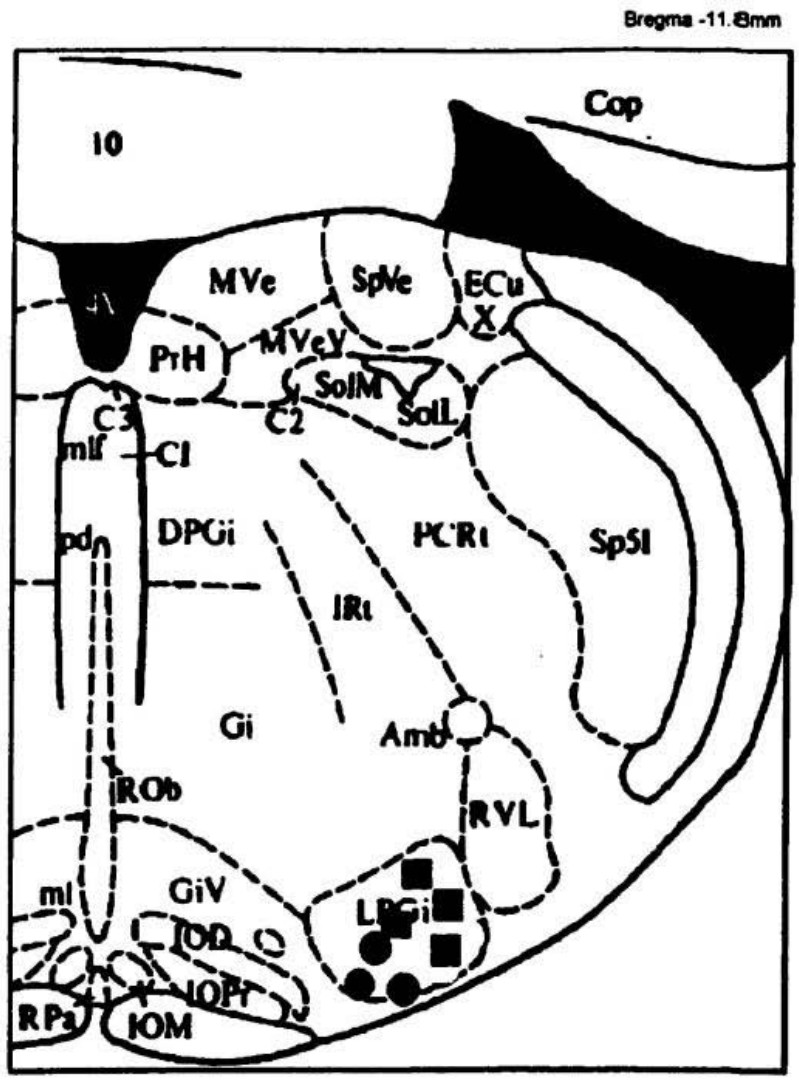


Figure 13: Placements of PGi stimulating electrodes.



stimulation followed by ADTN EPSP amplitude reduction
stimulation followed by ADTN EPSP amplitude increase

Table 1: Effect of paired pulse stimulation on ATDN-evoked potential amplitude and slope.

		Amplitude				Slope			
ISI (ms)	n	Conditioning avg (mV)	Test avg (mV)	t-value *p<0.05	Test/ base %	Conditioning avg (mV)	Test avg (mV)	t-value *p<0.05	Test/ cond %
20	6	0.958	1.17	0.595	122	0.438	0.400	0.393	91
30	6	0.873	2.39	3.124 *	274	0.416	0.628	3.055 *	151
40	6	0.817	4.21	4.506 *	516	0.387	0.846	5.107 *	219
50	6	0.801	4.58	5.664 *	572	0.397	0.958	4.288 *	241
60	5	1.1625	4.51	7.781 *	388	0.416	0.957	5.212 *	230
70	5	1.142	4.49	7.207 *	393	0.477	0.985	4.455 *	206
80	5	1.250	4.36	5.945 *	349	0.486	0.957	3.958 *	197
90	5	1.093	4.38	5.138 *	401	0.467	1.017	3.647 *	217
100	5	1.215	4.43	5.039 *	365	0.507	1.057	3.441 *	208

Table 2: Effect of paired pulse stimulation at various interstimulus intervals on ADTN and PP -evoked potential amplitude.

Site of conditioning stimulus		Site of test stimulus									
		PP					ADTN				
	ISI (ms)	n	Base avg (mV)	Test avg (mV)	t-value p<0.05*	Test/ base %	n	Base avg (mV)	Test avg (mV)	t-value p<0.05*	Test/ base %
PP	34	6	1.999	2.231	1.751	112	6	1.735	1.787	0.727	103
	50	6	2.067	2.147	1.322	104	6	1.681	1.721	0.478	102
	100	4	1.564	1.377	1.809*	88	4	2.613	2.504	0.923	96
ADTN	34	6	1.755	1.486	2.128*	85	6	1.939	3.851	3.855*	199
	50	6	1.798	1.476	1.908	82	6	1.686	3.779	5.646*	224
	100	4	1.575	1.321	4.924*	84	4	2.706	4.504	2.321*	166

Table 3: Effect of paired pulse stimulation at various interstimulus intervals on ADTN and LOT -evoked potential amplitude.

Site of conditioning stimulus		Site of test stimulus									
		LOT					ADTN				
	ISI (ms)	n	Base avg (mV)	Test avg (mV)	t-value p<0.05*	Test / bas	n	Base avg (mV)	Test avg (mV)	t-value p<0.05*	Test/ base %
LOT	34	5	0.855	2.845	1.974	333	5	2.614	2.112	3.516*	81
	50	4	1.063	1.222	0.449	87	4	2.127	1.632	1.456	77
	100	4	1.057	1.139	0.404	108	4	2.356	2.478	1.716	105
ADTN	34	5	1.192	1.480	2.856*	124	5	2.581	5.140	12.404*	199
	50	4	1.303	1.402	0.566	108	3	2.010	5.307	38.064*	264
	100	4	1.024	1.141	0.823	111	3	2.419	4.725	6.159*	195

Table 4: Effect of electrolytic lesion or application of 2-4 µl Lidocaine to the perforant path on ADTN-evoked potential parameters.

		Amplitude				Slope			
Action at PP	n	Base avg (mV)	Post-lesion avg (mV)	t-value ($p<0.05^*$)	Post/base %	Base avg (mV)	Post-lesion avg (mV)	t-value ($p<0.05^*$)	Post/base %
lesion	2	1.22	0.571	3.733	47	0.470	0.395	2.049	73
lidocaine	3	1.33	1.061	1.585	79	0.534	0.435	2.697	80
either	5	1.29	0.865	2.962*	67	0.614	0.470	3.478*	77

Table 5: Effect of PGi stimulation on ADTN EPSP amplitude and slope.

		Amplitude Increase					Amplitude Decrease				
Parameter	ISI (ms)	n	Baseline avg (mV)	PGi-paired avg (mV)	t-value *p<0.05	PGi-paired/ base %	n	Baseline avg (mV)	PGi-paired avg (mV)	t-value *p<0.05	PGi-paired/ base %
Amplitude	15	2	1.481	2.050	6.825	138	0	n/a	n/a	n/a	n/a
	20	2	1.204	1.831	3.221	152	3	0.901	0.626	2.912	69
	25	2	0.816	1.361	2.301	167	3	0.724	0.247	2.971*	34
	30	4	1.361	1.535	2.096	131	3	0.936	0.216	3.094*	23
	35	4	0.841	1.167	2.739	138	3	0.878	0.391	2.093	45
	40	4	1.258	1.561	0.956	124	3	0.656	0.143	5.825*	22
	45	4	1.734	1.781	0.176	102	3	1.226	0.573	1.899	47
	50	4	1.489	1.568	0.333	105	3	1.136	0.487	1.431	43
	55	3	0.984	0.948	0.315	96	3	0.972	0.719	1.083	74
	60	1	1.367	2.023	n/a	147	3	0.920	0.584	3.015*	63
	65	1	0.545	0.760	n/a	139	3	0.921	0.496	1.761	54
	70	1	0.852	1.837	n/a	215	3	1.183	0.324	1.577	27
	75	1	0.551	1.038	n/a	188	2	0.796	0.752	0.387	94
	80	1	0.599	1.136	n/a	190	2	0.799	0.418	2.055	52
Slope	15	2	0.311	0.291	2.224	93	0	n/a	n/a	n/a	n/a
	20	2	0.347	0.383	0.248	110	3	0.459	0.336	2.694	73
	25	2	0.287	0.339	4.752	118	3	0.406	0.326	2.958*	79
	30	4	0.406	0.366	2.383*	90	3	0.431	0.276	5.874*	64
	35	4	0.381	0.369	0.266	96	3	0.418	0.298	20.845**	71
	40	4	0.449	0.412	0.395	91	3	0.428	0.203	10.280*	48
	45	4	0.676	0.706	0.349	105	3	0.537	0.407	4.125*	76
	50	4	0.480	0.456	0.865	95	3	0.554	0.321	2.748	58
	55	3	0.329	0.301	2.889	91	3	0.496	0.372	3.158*	75
	60	1	0.414	0.565	n/a	136	3	0.532	0.405	3.561*	76
	65	1	0.469	0.400	n/a	85	3	0.529	0.435	1.504	82
	70	1	0.455	0.450	n/a	98	3	0.553	0.348	2.004	63
	75	1	0.437	0.379	n/a	87	2	0.384	0.347	0.613	90
	80	1	0.374	0.43	n/a	107	2	0.396	0.350	2.824	88

Table 6: The effect of 0.9% saline and idazoxan (300 & 500 µg/kg) on ADTN and LOT-evoked potential parameters.

			Amplitude				Slope			
Site	Drug & Dose	n	Base avg (mV)	Post-inject avg (mV)	t-value p<0.05*	Post/ base %	Base avg (mV/ms)	Post-inject avg (mV/ms)	t-value (p<0.05*)	Post/ base %
ADTN	saline	2	2.625	2.680	2.303	102	0.454	0.449	0.975	99
	idaz 300	4	0.739	0.785	0.485	106	0.403	0.386	1.046	96
	idaz 500	5	1.905	1.558	2.499*	82	0.469	0.428	1.235	91
LOT	saline	2	1.252	1.273	0.293	104	0.302	0.268	1.346	91
	idaz 500	4	0.246	0.292	2.777*	119	0.320	0.464	1.461	145

DISCUSSION

ADTN Stimulation Evokes an EPSP in the DG

Electrical stimulation in the region of the ADTN does elicit an evoked potential in the DG. Although the potential shows variability in latency to peak between animals, a signal is reliably evoked from stimulation that histology shows to be within the borders of the ADTN.

The ADTN is located directly beneath the posterior portion of the hippocampus, so the evoked potential could have resulted from stimulation of DG fibres directly. If this were so, however, one would expect the signal to have a shorter latency to peak than that which was observed. Monopolar stimulation was used to minimize the spread of activation; the bipolar Rhodes SNE 100 electrodes have a barrel to tip length, and therefore area of activation, of 1 mm. Due to the small size of the ADTN, it is likely that the tip and the barrel end of the bipolar electrodes would not both be in the ADTN in most subjects. Monopolar stimulation results in a spherical field of stimulation of which the strongest activation is at the electrode tip. Monopolar stimulation also minimizes lesions and metal deposition near the stimulation site (Yeomans, 1990). Indeed, the lesions made with the monopolar electrodes were much smaller than with the bipolar electrodes. Monopolar stimulation should better insure ADTN stimulation without direct activation of hippocampal fibres.

Localizing the Point of Stimulation to the ADTN

In some early experiments (N=3), lesions were made when the signal suspected to result from ADTN stimulation appeared (as a stimulating electrode moved ventrally through the hippocampus and ADTN) and disappeared. The lesions bordered the ADTN

in two of these animals; in one of those animals and several others not included in this analysis, the monopolar electrode tip lesion was found to be slightly ventral to the ADTN. In several, but not all, of these animals, a response in the DG was recorded from stimulation of the anteroventral nucleus that exhibited morphology similar to the response recorded from ADTN stimulation. It is reasonable to expect this, given the similarity in connection matrix of the ADTN and the anteroventral thalamic nucleus; both nuclei send projections to the retrospenial cortex, the thalamic reticular nucleus, and to the subicular complex, in particular the presubiculum. Another similarity between the two nuclei is that they have both been found to contain HD cells (Taube, 1995).

In all animals in which a DG signal was recorded with the stimulating electrode wholly or partially in the ADTN, the stimulation site that elicited the maximal response was at least 2 mm ventral to the hippocampus, making stimulation of hippocampal fibres unlikely. Based on these observations, one can conclude that stimulation within the ADTN elicited an evoked potential in the DG. There is, therefore, electrophysiological evidence for an anatomical link between neurons of the ADTN and those of the DG.

Route of travel from ADTN to DG is polysynaptic

The variability of latency to peak, the long latency, and the inability of the response to follow stimulation of 50 Hz suggest that the EPSP was evoked polysynaptically. This is consistent with anatomical data which show no direct connection between the ADTN and the DG.

The ADTN-evoked signal could be recorded in the hilus and molecular layer of the DG and was apparent only when the recording electrode could also record PP-evoked potentials. When the recording electrode was raised into CA1, the ADTN -evoked signal was reduced to a slight, negative-going wave; similar effects of moving from the DG to

CA1 with PP stimulation have been observed by other investigators (Liu & Bilkey, 1997), and would be consistent with the ADTN input traveling via the LPP, which innervates the stratum-moleculare region of CA1 (Witter & Amaral, 1991). The greater strength of the ADTN signal in the DG proper suggests that the signal is entering the DG itself, and is not a result of spread of depolarization originating in CA1. Further studies using ADTN stimulation and recording in the infrapyramidal blade of the DG would confirm that the pattern of activation is localized to the DG, specifically the molecular layer.

Often, a small depolarization was recorded in the DG with ADTN stimulation which preceded the characteristic ADTN-evoked pEPSP. The latency to peak of this depolarization was extremely stable (approximately 3 msec from the onset of the stimulation artifact as seen in the top waveform of Figure 1). This small potential resembles the shorter latency presynaptic fibre volleys observed in the DG with PP stimulation by Lomo (1971) and follows stimulation frequencies that the ADTN EPSP does not. The wave was not affected by pharmacological manipulation of the EPSP, but was slightly reduced in amplitude by lesion of Lidocaine application to the PP. Taken together, these observations suggest the wave is a presynaptic fibre volley mediated by a relatively rapidly conducting circuit. The presence of a presynaptic fibre volley preceding the ADTN potential supports the hypothesis of a locally generated ADTN EPSP within the DG. A systematic study of this potential and its interaction with PP fibre volleys might make a useful adjunct in the functional analysis of the ADTN input pathway.

The ADTN EPSP Reverses in the DG Molecular Layer

The latency to peak of the reversing ADTN EPSP changed as the recording electrode was moved through the molecular layer of the DG in a way that was similar to the change recorded in the latency to peak of the reversing PP EPSP. This reversal of the

ADTN EPSP suggests that local depolarization of DG granule cells did occur following ADTN stimulation. The place in the molecular layer at which ADTN EPSP reversal occurred was closer to the granule cell layer than that of the PP-evoked potential. This suggests that the depolarization of DG granule cells that followed ADTN stimulation originated proximal to the medial molecular layer, closer to the granule cells than the site of termination of the stimulated PP inputs.

ADTN EPSP Transmission may be via the PP

The population spike resulting from PP stimulation in the depth-profile animals had a latency to onset of 4.2 ms. This latency to onset is consistent with other research (i.e. McNaughton & Barnes, 1977) in which the population spike onset latencies and the MPP and LPP EPSPs were compared; the 4.2 ms latency seen here suggests that the PP-evoked potentials in these two depth profiles were elicited mainly by stimulation of the MPP. However, it has been noted that population spike onset latency decreases with increasing intensity of PP stimulation (Lomo, 1971), so it is possible that the PP-evoked potential seen in these depth profiles reflected a mixture of LPP and MPP stimulation. Future comparisons of ADTN to PP responses should use EPSPs alone, without population spikes; the rise-time of the PP-evoked EPSPs would give a more accurate indication as to the portion of the PP being stimulated, as would a current source density analysis (Nicholson & Freedman, 1975).

PP division aside, the reversal of the ADTN EPSP in the molecular layer of the DG is consistent with transmission of the EPSP via the PP. The lesser latency to peak of the corresponding negative-going waves recorded in the outer molecular layer were consistent with PP EPSPs evoked by other investigators (i.e. Lomo, 1971) and may reflect the closer proximity of the recording electrode tip to the site of origin of the

depolarization.

PP Depolarization Does Not Predict the ADTN EPSP

Following ADTN stimulation, the event recorded in the PP did not predict that of the EPSP recorded in the DG in a way that would be consistent with the hypothesis of a common route of entry into the DG.

Between-event latency is insufficient

If the ADTN signal enters the DG through the PP, the peak of the PP-recorded event should precede the maximal amplitude of the DG-recorded event by the amount of fibre volley travel time from the PP to the DG, plus the latency of granular cell response to the fibre volley. The travel time of such a volley was recorded as 1 to 2 ms by Lomo (1971), who reported the speed of the volley along PP axons to be approximately 3.3 m/s; this value is consistent with the range of estimates of the speed at which depolarization travels along PP axons found in the literature (1.5 - 3.3 m/s; Gloor et al., 1963; Lomo, 1971; Tielen et al., 1981). Here, where the travel distance is estimated at 4.28 mm, the fibre volley travel time can be estimated at 1.4 ms, assuming a conduction velocity of 3.3 m/s. The latency to maximal granular cell response (nonspiking EPSP peak) is estimated at 3 to 5 ms; the two latencies combined yield the 5-6 ms latency to peak exhibited by EPSPs normally elicited from electrical stimulation of the PP. In these experiments, the total latency between PP and DG -recorded events should be a minimum of 1.4 ms plus 3 ms, for a total of 4.4 ms. In contrast, the latency between the peaks of the PP-recorded event, which would presumably reflect the passing fibre volley, and the DG-recorded event in response to ADTN stimulation was only 2-3 ms. The appearance of the ADTN associated presynaptic fibre volley in the DG prior to the presumed PP fibre event also

suggests the PP event is not mediating the ADTN input. These results would suggest that only a late component of the ADTN EPSP, if any of it, is due to depolarization of PP axons.

PP EVENT DETECTION MAY NOT HAVE BEEN ADEQUATE

The low impedance stimulating electrode that was used to record the PP response may not have been a sufficiently sensitive instrument with which to record the activation of PP axons. Employing a proven method of measuring activation of passing fibres could shed some light on the possible ADTN-DG connection.

ADTN EPSP POTENTIATES WITH PAIRED-PULSE STIMULATION

While it is interesting for characterization purposes that the ADTN-evoked potential is facilitated by paired-pulse stimulation, it is to be expected with a polysynaptic route. Interestingly, the pattern of paired pulse facilitation is different from the LOT-evoked potential; LOT paired-pulse facilitation was evident only at an ISI of 34 ms, while that of the ADTN was significant at 34, 50 and 100 ms ISIs. LOT stimulation gave rise to a DG response consistent with previous studies (Liu & Bilkey, 1997) and histology showed electrolytic lesions in or near the LOT. If both the LOT and ADTN signals entered the DG via the LPP and the locus of potentiating activity were in the LPP-DG synapse alone, one would expect the LOT and ADTN paired-pulse profile to look very similar. This confirms that potentiating effects seen here occur at other synapses in these pathways, and/or may reflect different routes of entry to the DG. Because facilitatory mechanisms could be occurring at any of the synapses which comprise the ADTN-PP and LOT-DG pathways, paired-pulse facilitation data alone cannot yield information as to the route by which the signal enters the DG.

CROSS-STIMULATION USED TO INFER ROUTE OF ADTN EPSP

Cross-stimulation studies can indicate if polysynaptic routes converge on the same pathway; the cross-stimulation between the ADTN and the LOT and PP could indicate if the ADTN signal was conveyed by a route similar to either of these two pathways.

ADTN-PP CROSS-STIMULATION - INHIBITORY OR EXCITATORY?

The PP-PP stimulation led to small but significant potentiation at ISIs of 34 and 50 ms, which changed to depression at an ISI of 100 ms. This is consistent with stimulation of the MPP, paired pulses to which result in test pulse potentiation of ~10% at 20 ms, which gives way to depression before an ISI of 100 ms (McNaughton, 1980). The rise-times of the PP-evoked EPSP were also consistent with MPP activation. A conditioning pulse to the ADTN resulted in depression of PP-evoked potentials at all ISIs, suggesting that stimulation from the ADTN was not traveling via the MPP and may have shunted currents in the DG molecular layer.

The ADTN stimulation could be simultaneously activating another pathway that depressed the PP EPSP. There is some evidence that activation of the LPP via stimulation of the LOT leads to some brief granule cell inhibition originating in the inner molecular layer or in the hilus that becomes evident only after the LPP-elicited current sink in the outer molecular layer has passed (Liu & Bilkey, 1997). Such multiple EPSP components could also result from ADTN stimulation. In the case of the LOT, both the outer ML and inner ML/hilus sinks seem to be dependent on PP transmission; both components were lost only when the PP, but not the LEC, was lesioned. The same would not necessarily be true in the case of ADTN stimulation, because there are a variety of ways that ADTN input could reach the hippocampus.

The depression in LOT-PP cross-stimulation is not observed in paired-pulse

effects of homosynaptic LOT or ADTN stimulation, possibly because the facilitatory actions occurring at the multiple synapses in these pathways mask any LOT-PP or ADTN-PP inhibition present. Alternatively, if there are two-time displaced current flows, the first could lead to facilitation that is unaffected by the later inhibition.

The depth-profile data from this experiment are somewhat consistent with a hypothesis of inhibition, in that the ADTN input appears to arrive in the DG at the level of the inner molecular layer, the same layer as inhibitory LOT input, as well as inhibitory input from associational and commissural fibres. Such inhibition may be reflected by the long (>30 ms) negative-going wave (recorded at the cell body later) observed to follow the ADTN EPSP in some animals, and could be of greater duration than inhibition resulting from LOT stimulation. Such inhibitory input could serve to shunt PP activation of granule cell dendrites, reducing the amplitude of the PP evoked potential, as was observed. As PP stimulation would be not be expected to reciprocate this shunting effect, this theory is also consistent with the lack of effect of PP stimulation on subsequent ADTN-evoked potentials seen in this experiment, in contrast with the high amplitude increase that resulted from ADTN-ADTN stimulation.

ADTN PAIRED-PULSE FACILITATION MAY CONFOUND HYPOTHESIS OF INHIBITION

If ADTN stimulation did exert an inhibitory effect, ADTN-ADTN stimulation should not have yielded such a large EPSP amplitude increase. If, in contrast, the ADTN stimulation has an excitatory effect, and enters the DG at a point in the ML that is between the PP input and the granule cell threshold zone for inhibition, then it is possible that the input from ADTN shunts the excitatory input from PP postsynaptically but sums with its own input presynaptically. An excitatory ADTN input would be consistent with the ADTN-LOT results, in that ADTN-LOT stimulation led to slight potentiation of the LOT

EPSP.

ADTN-LOT CROSS-STIMULATION

LOT-LOT stimulation led to a test EPSP amplitude increase at an ISI of 34 ms, while ADTN- LOT stimulation also led to potentiation, slight but significant, at that ISI and at no other, somewhat consistent with the hypothesis that the ADTN EPSP travels via the LPP. However, the LOT-ADTN data would seem to refute this notion also. A conditioning pulse to the LOT significantly decreased the amplitude of the ADTN evoked potential at an ISI of 34 ms; a similar reduction was observed at an ISI of 50 ms, without reaching significance. LOT stimulation has not been shown to have this inhibitory effect on PP EPSPs at an ISI of 34 ms (Wilson & Steward, 1978), possibly because the inhibitory sink observed in response to LOT stimulation was no longer present by approximately 35 ms post stimulation. This is difficult to evaluate from the Wilson & Steward study because they do not report the ISIs used in testing for cross-potentiation between the LPP and the LEC. If the inhibitory sink resulting from LOT stimulation observed by Liu and Bilkey is of sufficient strength to inhibit subsequent granule cell firing, one would predict a reduction in LEC EPSP amplitude if the LOT-LEC ISI were reduced to 35 ms or less. Wilson & Steward did report an unexplained increase in onset latency of the LEC test response after an LOT conditioning stimulus, but did not report the ISI(s) at which this was observed.

Alternatively, the ADTN response could be the result of conduction block caused by the LOT EPSP, supporting the hypothesis that the LOT and ADTN enter the DG via the same portion of the PP. The present data, therefore, neither confirm nor refute the theory that the LOT and ADTN activate similar pathways.

PP Lesion or Lidocaine Injection Decreases ADTN EPSP Amplitude and Slope

The application of Lidocaine or an electrolytic lesion to the PP resulted in abolishment of the PP-evoked potential and a decrease in ADTN EPSP amplitude and slope. If the ADTN EPSP entered the DG via the PP, one would expect both evoked potentials to disappear simultaneously. It is possible that the manipulations to the PP were incomplete and that ADTN stimulation was activating alternative PP fibres. However, the PP lesions were quite large, making alternate fibre transmission unlikely, and the amount of Lidocaine used was sufficient to quiet the entire PP in other experiments (Liu & Bilkey, 1997), making transmission by unaffected PP fibres unlikely.

Parallel Input into the DG?

An alternate explanation would be that the ADTN evoked potential does not travel solely by way of the PP, but may in fact be the result of parallel input into the DG. Another study which showed reduction, but not abolishment of a DG-recorded signal was done by Liu & Bilkey (1997), who showed that electrolytic lesion to the perirhinal cortex resulted in a significant decrease in the amplitude of the LOT evoked potential. Similarly, a portion of ADTN input to the DG could be mediated by the PP, while another, parallel pathway to the DG mediates either the remainder or the majority of the ADTN signal.

Another possible reason for the results observed is that the reduction in ADTN EPSP amplitude is due to incidental damage done to the presubiculum, if the ADTN signal in the DG travels through this area. Further study into the effects of presubiculum lesions alone, combined with studies using Lidocaine carrying a dye to evaluate its spread, may shed some light as to the validity of this alternative explanation.

The above results taken together do not provide clear evidence as to the route by which the ADTN-evoked potential reaches the hippocampus, but suggest that at least a

portion of it is carried by either the presubiculum or the PP.

ALTERNATE ROUTES OF PASSAGE TO THE DG

While the data are somewhat consistent with travel of the ADTN EPSP via the LPP, there are several alternate, or perhaps parallel, routes of entry of the response into the DG that are equally consistent with the present data.

A DEEP ENTORHINAL PROJECTION

Deller et al. (1996) report the existence of an entorhinal projection to the DG that projects horizontally into the DG, not via the PP, that they discovered using anterograde tracing techniques. The EC axons they describe enter the crest of the DG, send collaterals to the outer two thirds of the molecular layer, and branch into the inner molecular layer, the granule cell layer, and the hilus. In the inner molecular layer, these fibres synapsed on dendrites of neurons that stained for GABA; none of the EC fibres themselves stained for GABA. Within the granule cell layer, EC fibres synapsed on granule cell dendrites and somata. In the hilus, EC fibres synapsed on dendrites both proximal to and distant from the granular cell layer. Some fibres traversed deeply into the hilus, terminating near the granule cells of the infrapyramidal blade. Anterograde label injection sites giving rise to these EC fibres were typically in the deep layers of the medial entorhinal area. EC axons traveling via the PP were labeled in response to label injections in the superficial layers of the EC and were not found to branch out of the outer two- thirds of the molecular layer.

The deep-layer EC projection described by Deller et al. could lead to feedforward inhibition (inhibition through direct activation of interneurons) of granule cell firing such as was observed in the crossover paired-pulse measures in this experiment. If the ADTN EPSP were transmitted along this pathway, the reversal point of the EPSP in the

molecular layer would be close to the granular layer, as was observed. The relatively large lesions used in the PP in this experiment could also have damaged this second EC projection, decreasing the amplitude of the ADTN EPSP. There is anatomical evidence for ADTN contribution to such an EC pathway; the ADTN sends some efferents directly to layers VI and V of the rostrolateral EC, bypassing the cingulate gyrus (Shibata, 1993).

However, if the ADTN input were entering the DG via the Deller et al. deep-layer entorhinal projection, ADTN stimulation would be predicted to elicit stronger EPSPs in the temporal portion of the DG, as the projection seen by Deller et al. was focused there, with no such fibres being found in the septal portion. Such a difference in signal strength was not found in these experiments; some of the present recordings were in septal areas, and the ADTN EPSP in these records were not distinguishable from those recorded closer to the temporal portion of the DG.

Presubiculum-alveus route

Another route by which the ADTN signal could enter the DG is via the alveus from the presubiculum. The ADTN sends projections to layers I and III - VI of the presubiculum and to layers I and IV-VI of the parasubiculum, both of which innervate the DG via the alveus and not the PP (Patton & McNaughton, 1995). These projections terminate in the middle molecular layer. A series of lesion studies in addition to a thorough current density analysis would provide information about the true path(s) of the ADTN signal.

The Role of ADTN Output in Place Cell Firing

The existence of the ADTN signal is consistent with current theory regarding the role of the hippocampus in spatial navigation, specifically in path integration and generation of the place cell code. Since the discovery of HPCs and HD cells in the dorsal

presubiculum, and subsequently, in the ADTN, it has been assumed that ADTN cells were indirectly connected to those in the hippocampus. In studies that show that HPCs are influenced by vestibular input, the ADTN has been included as one of the loci mediating such information.

Several suggested representations of the anatomical connections important for the generation and maintenance of HPC representation of the environment have suggested that input from the ADTN reaches the hippocampus via the postsubiculum to entorhinal cortex connection. The models that include the EC as part of the connections in the HD/HPC circuit are strengthened by the recent finding of cells in the superficial layers of the MEC that exhibit positional firing variations that are location-specific (Quirk et al., 1991). In light of the present experiment's findings, it is unlikely that these cells are strongly informed by input from the ADTN, as such information would be expected to travel to the DG via the MPP from the MEC, a transfer for which this experiment found no evidence.

MEC LOCATION-SPECIFIC CELL RESPONSE

Ideothetic and HD cell information must reach the MEC in order for the positional firing of MEC cells to occur. Such information could reach the MEC through a variety of routes; for example, through the presubiculum, the efferents of which terminate primarily in MEC layers I and III. Alternatively, HD information could reach the deep layers of the MEC via the retrosplenial cortex, to which the ADTN has an input, with subsequent transfer of information from the deep layers of the MEC to the superficial layers.

Unfortunately, the study on the MEC location specific cells did not provide information as to the effect of vestibular input on these cells' firing. The experimenters did suggest that MEC cells are bound more tightly to sensory information than HPCs; they

based this on differences they saw in MEC cell and HPC firing when the shape of the animals' enclosure was changed from a circle to a square. The HPCs altered their place fields entirely, while the MEC cells fields only stretched to match the new topography. The authors suggest this is a result of similar features of portions of the circle and the square, presumably visual cues. The MEC cells are likely not wholly driven by visual cues, however, because their location specificity persisted upon removal of the only obvious visual cue in the experimental environment. It would be informative to see what effects navigating in the dark would have on MEC cell firing specificity.

HPCs : MORE ATTUNED TO IDEOTHETIC INFORMATION THAN ARE MEC CELLS?

Should MEC cells be more attuned to visual, rather than ideothetic cues, than their hippocampal counterparts, it would be reasonable to suggest that HPCs are informed by a source of ideothetic information not available to MEC cells. Parallel entry of ideothetic and/or HD information into the hippocampus is also possible, with some information transferred via the MEC "place cells", and some transferred via a different route, such as the aforementioned deep-layer EC connection, via a direct pathway from the postsubiculum via the alveus, or through the postsubiculum and subiculum projections to the LEC, and, subsequently, the LPP. The data in the present experiment are insufficient to discriminate between these possibilities, but the depth profile is least consistent with the last.

PGi EFFECTS

Stimulation of the PGi was confirmed through histological analysis and by its effects on the PP- evoked potential. The ISI used to test PGi effects on the PP-evoked potential was optimal, according to Babstock & Harley (1992), for producing significant

effects on the population spike. Consistent with their results, PGi stimulation resulted in an increase in population response amplitude by an average of 33% over baseline, without causing significant changes in synaptic drive as recorded at the granule cell layer. No recordings were made from the dendritic layer; synaptic drive changes observed there could have served as another confirmation of PGi activation, as investigators have reported NE-induced EPSP slope changes when recording at the dendritic, but not the cell body, layer of the DG (Dahl & Winson, 1985). The histological criteria and effects on the PP-evoked potential strongly suggest that the PGi was activated in these experiments.

PGi STIMULATION YIELDS MIXED RESULTS

The PGi effects on the ADTN EPSP are difficult to interpret, in part because of the division in response between animals, and because previous studies regarding PGi, or even NE, effects on DG EPSPs alone are so few. Other researchers have observed some decreases in population spike amplitude with electrical LC stimulation (Dahl & Winson, 1985) in addition to increases; this may be attributed to mixed stimulation of the LPP and MPP. Results regarding population spike effects may not be relevant to effects on synaptic drive in this case, as researchers have noted that NE appears to uncouple the relationship between cell population response and synaptic drive. Other studies have found that LC stimulation causes both PP EPSP slope increases (Stanton & Sarvey, 1987) and decreases (Dahl & Winson, 1985) as recorded within the middle ML; no slope effects have been reported while recording at the granule cell layer. The present results are significant in that significant EPSP slope changes were observed while recording at the granule cell layer or in the hilus. These changes may be indicative of complex alterations in source-sink relationships in the DG caused by NE release, as has been postulated in other studies (Dahl & Winson, 1985), or may reflect the characteristics of the ADTN input

to the DG.

PGi EFFECTS DEPEND ON PATHWAY CONCERNED

That NE can act differently on projections to the DG from the EC has been demonstrated; NE potentiates the population response resulting from MPP stimulation, while reducing that presumably conveyed by the LPP (Dahl & Winson, 1989). In one experiment on PGi effects on the LOT EPSP, and presumably the LPP, PGi stimulation resulted in an EPSP amplitude and slope decrease in 8 of 9 animals. The failure of experimenters to observe a slope increase at the cell layer in previous experiments may be due to mixed LPP and MPP stimulation. The mixed results in the present experiment are consistent with the ADTN evoked potential either entering the DG via a non-PP route that has a complex relationship with NE effects, or a parallel input that combines to produce one visible EPSP, the elements of which are affected differently by NE. They are also consistent with their being more than one effect of PGi stimulation depending on location, as there are direct projections from the PGi to the DG as well as to the LC (Zagon et al., 1994). Further study regarding the mode of entry of the ADTN EPSP into dentate gyrus is required, as is further characterization of NE and PGi stimulation effects in the dentate gyrus.

EFFECTS OF IDAZOXAN

The effect of α_2-receptor blockade on LPP EPSPs has not previously been studied, but several researchers have reported that NE ejection in the DG is followed by a reduction in size of the LPP-evoked potential (Dahl & Sarvey, 1989). One study reported that idazoxan caused an increase in population spike amplitude in the DG, while simultaneously decreasing the slope of the EPSP (upon which a population spike was always superimposed) , but it was not clear which portion of the PP was being stimulated

(Richter - Levin et. al., 1990). In the present study, i.v. injection of the α_2-antagonist idazoxan resulted in a significant and reliable increase in LOT EPSP amplitude. The latency to onset and duration of the increase were reliable and contingent on idazoxan injection, as the effect was not observed following injection of vehicle alone. Although local-injection α_2-antagonist effects have been investigated in the DG and were reported to increase NE efflux in the DG (Abercrombie et al., 1988), the intradentate effects of systemic injection of an α_2-antagonist have not been assessed.

Systemic and local injection of α_2-antagonists increase LC firing, presumably as a result of the loss of negative feedback, but it has not been determined that this is reflected in a significant increase in NE concentration in the DG, or that such an effect would be the only consequences of systemic injection that would have effects on DG function. If presynaptic α_2-receptors are related to the decrease that NE has been reported to cause in LPP EPSPs, then their blockade may in fact decrease tonic NE inhibition of LPP-evoked responses, leading to the facilitation of response observed in the present experiment. Further characterization of the effects of systemic and local application of α-receptor agonists and antagonists on NE levels in the DG and on MPP and LPP -evoked responses would help clarify the complex actions of NE in the DG and might aid in understanding the effect of idazoxan injection on the ADTN EPSP also.

Based on previous findings regarding LPP evoked responses and NE, the reduction in ADTN EPSP amplitude following idazoxan injection seen in this experiment would be consistent with the theory that the ADTN signal arrives in the DG via the LPP. However, given that the effects on the ADTN EPSP were opposite those on the LOT EPSP, presumed to arrive at the DG via the LPP, this conclusion cannot be made without further investigation into the nature of α_2-receptor-mediated effects in the DG.

SUMMARY

In these experiments an EPSP was reliably elicited in the DG by monopolar ADTN stimulation, suggesting there exists an anatomical connection between the two structures. This is consistent with the Taube's ideas that HD cells in the ADTN inform hippocampal place cells by way of a multisynaptic route. The route of entry of this ADTN-elicited signal is not clear. While a DG-recorded depth profile suggests that axons carrying the ADTN signal terminate in the ML, experiments to determine if those axons comprised part of the PP had mixed results. Cross-potentiation experiments between the PP and ADTN, and between the LOT and ADTN, yielded changes in EPSP amplitude and slope that were difficult to interpret. Lesioning or applying Lidocaine to the PP only diminished the size of the ADTN EPSP, even when the PP EPSP was abolished. 500 μg/kg idazoxan did not have the same effect on the LOT EPSP as on the ADTN EPSP, suggesting they may enter the DG via different routes, but the effect of idazoxan on the LOT EPSP was not consistent with previously reported norepinephrine effects on the LPP-DG connection, so these results must be subject to confirmation and further investigation before assertions about ADTN EPSP entry into the DG can be made. PGi stimulation yielded mixed results with respect to its effects on the ADTN EPSP, even when effects on the PP elicited signal were consistent. These results taken together do not allow conclusions to be made about the route by which ADTN information may reach the DG, but may illustrate that the ADTN signal is not a strong signal into the DG via strictly the LPP or the MPP; if it were, results in these experiments should have been more consistent, as they were for the PP and LOT EPSPs. The ADTN EPSP may have multiple components that enter the DG by a single or multiple pathways, or may enter the DG via a pathway not investigated here, such as the alveus. Further investigation into the connectivity between the ADTN and the DG could yield further clues as to the generation of HD firing and place codes, as well as

further information as to the functions of both structures.

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