THE HIPPOCAMPUS IN NOCICEPTION

by

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ABSTRACT

Limbic structures, including the hippocampus, are thought to be involved in pain though not much is known of their neuronal responses to noxious stimuli. Experiments were therefore performed in lightly anaesthetized rats to determine the effect of noxious heat stimuli on the excitability of dorsal hippocampal field CA1 pyramidal neurones. A prolonged and substantial depression of the CA1 population spike was produced by a brief but intense noxious stimulus applied to the tail. This depression was temperature-dependent and habituated to subsequent noxious stimuli applied more than 1 hr later. In other animals, a similar depression and habituation was also obtained with noxious heat stimuli applied to the left hind paw. However, following this habituation of the hind paw, a persistent depression of the CA1 population spike was seen if the tail was exposed to a noxious heat stimulus.

The persistent depression was absent when noxious heat was applied in the presence of hippocampal theta rhythm. If, however, the hippocampal electroencephalographic (EEG) activity was in an irregular pattern at the time noxious heat was applied, a 4-6 Hz theta rhythm was produced along with the depression of the population spike. The latency and intensity of the reflex response was combined into a reflex-reaction score. There appeared to be a relationship between the reflex-reaction score and the duration of theta rhythm induced by different intensities of noxious heat stimuli but there was no habituation to these responses.

The CA1 population spike evoked either by ipsilateral or contralateral CA3 stimulation was similarly depressed following a noxious stimulus. Concomitantly, the persistent depression and habituation of the commissural CA1 population spike was also accompanied by similar changes in the corresponding dendritic field excitatory postsynaptic

potential (EPSP). However, the amplitude of the CA1 antidromic spike was increased in the majority of cases. These findings suggest that a presynaptically mediated decrease in synaptic transmission may account for the depression of the population spike and dendritic field EPSP.

There is evidence to suggest that the noxious stimulus-induced persistent depression of CA1 pyramidal cell synaptic excitability is due to a cholinergic projection from the medial septal-vertical limb of the diagonal band of Broca complex (MS-VLDBB). Thus, atropine sulphate (40 mg/kg, i.p.) prevented the persistent depression of the CA1 population spike to a noxious stimulus. It also antagonized the septal tetanus-evoked, cholinergic mediated facilitation of the CA1 commissural population spike but had no effect on the corresponding paired-pulse facilitation. Atropine, applied iontophoretically to the cell body region antagonized the iontophoretic acetylcholine-induced facilitation of the CA1 population spike but not its depression to a noxious stimulus. On the other hand, apical dendritic application of atropine antagonized iontophoretic acetylcholine and noxious stimulus-induced depression of the CA1 dendritic field EPSP. However, such iontophoretic application of atropine had no effect on dendritically applied gamma aminobutyric acid (GABA)-induced depression of the CA1 dendritic field EPSP. These results support the notion that acetylcholine release in the dendritic region of CA1 neurones is involved in the depression of synaptic excitability of these neurones evoked by a noxious stimulus.

Abstract

The persistent depression and habituation of CA1 pyramidal cell synaptic excitability may relate to the noxious stimulus-induced stress and the resultant adaptive changes in animal behaviour to pain.

J.G. Sinclair, Ph.D.

Supervisor

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LIST OF ABBREVIATIONS

Ach	acetylcholine
AchE	acetylcholinesterase
AHP	afterhyperpolarization
α	alpha
Atr	atropine
β	beta
C	centigrade
Ca^{2+}	calcium ion
CER	conditioned emotional response
ChAT	cholineacetyltransferase
ChAT-LI	cholineacetyltransferase-like immunoreactivity
cm	centimeter
CNS	central nervous system
Co^{2+}	cobalt ion
CS	conditioned stimulus
DAP	depolarizing afterpotential
δ	delta
DG	dentate gyrus
DH	dorsal hippocampus
DMPP	dimethylphenyl piperazine
dTC	d-tubocurarine
EEG	electroencephalogram
EPSP	excitatory postsynaptic potential

Fig	figure
FIM	fimbria
fiss	fissure
g	gram(s)
GABA	gamma aminobutyric acid
HRP	horseradish peroxidase
HSV	higher stimulation voltage
HTM	high threshold mechanoreceptor
Hz	hertz
IPSP	inhibitory postsynaptic potential
K+	potassium ion
kg	kilogram
KHz	kilohertz
L	lateral
LHP	left hind paw
LSV	lower stimulation voltage
M	molar
mA	milliamperes
mf	mossy fibres
mg	milligram
min	minute
μm	micrometer
mm	millimeter
mM	millimolar

Mn^{2+}	manganese ion
MS-VLDBB	medial septal-vertical limb of the diagonal band of Broca
msec	millisecond
N_2	nitrogen
nA	nanoamperes
Na^+	sodium ion
NaCl	sodium chloride
NGC	nucleus reticularis gigantocellularis
NS	nociceptor-specific
P	posterior
pp	perforant path
PHA-L	Phaseolus vulgaris leucoagglutinin
PMN	polymodal nociceptor
psi	pounds per square inch
[3H] QNB	tritiated 3-quinuclidinyl benzilate
RSA	rhythmic slow activity
S	septal
Sc	Schaffer-collaterals
sec	second
SEM	standard error of mean
str	stratum
str. gran	stratum granulosum
str. lac	stratum lacunosum
str. mol	stratum moleculare

str. or	stratum oriens
str. pyr	stratum pyramidale
str. rad	stratum radiatum
SUB	subiculum
T	temporal
TFR	tail-flick reflex
TTX	tetrodotoxin
US	unconditioned stimulus
V	ventral
WDR	wide dynamic range

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INTRODUCTION

Pain has been defined as " an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage " (Merskey et al., 1979). Pain in humans is a subjective experience which includes three components, namely (a) sensory-discriminative, (b) affective-motivational and (c) cognitive-evaluative (Mayer and Price, 1982; Melzack and Casey, 1968; Melzack and Wall, 1982). Animals cannot verbally express the subjective quality of pain but it is assumed they experience an unpleasant sensation when exposed to tissue damaging stimuli (or noxious stimuli). The equivalent of pain in animals is termed nociception. In response to a noxious stimulus animals exhibit motor responses that are similar to those seen in humans. Such responses, termed nocifensive behaviour, are taken as indices of nociception in animals.

The sensory-discriminative aspect of pain functions to analyze nociceptive information in terms of location, rate of onset and intensity. The great majority of work has been done on this component. It has been suggested that the spinothalamic system, the ventrobasal thalamic complex and the somatosensory cortex are associated with the sensory-discrimination of nociceptive information (Melzack and Casey, 1968).

The affective-motivational component of pain in humans is characterized by depression, anger, anguish, misery, and frustation (Mayer and Price, 1982; Melzack and Casey, 1968; Melzack and Wall, 1982). In animals it is manifested as escape, attack or avoidance behaviour (Melzack and Casey, 1968). The affective-motivational responses are thought to be subserved by the ascending fibre system from the spinal cord, the medial reticular formation, the medial thalamic structures and the limbic system including the hippocampus (Melzack and Casey, 1968; Melzack and Wall, 1982).

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The cognitive-evaluative component allows a comparison of the present input with past experience (Mayer and Price, 1982; Melzack and Casey, 1968). Such cognitive-evaluation of the situation in which the subject is exposed to a noxious stimulus influences the affective-motivational aspect of pain. Other cognitive factors which may influence pain include anticipation of pain, anxiety, suggestion, the placebo effect and the meaning of the pain producing situation. Melzack and Casey (1968) proposed that the cerebral cortex is involved in the cognitive-evaluative component of pain.

Thus, pain or nociception is not subserved by a single hardwired system, but involves multiple anatomical substrates. Some of these neural structures are more important for one aspect of pain in comparison to the other aspects. The various substrates subserving different aspects of nociception or pain interact. For example, at the spinal cord level, Wall (1988) reported that spinal cord nociceptive transmission is modulated by inhibitory and facilitatory influences impinging on spinal neurones from a variety of subcortical and cortical structures implicated in nociception. Such an interaction will influence the final response to a noxious stimulus. The author also emphasised the plasticity of responses to a noxious stimulus.

The hippocampus has been implicated in number of functions that may be associated with pain. For example, stimulation of the anterior hippocampus in cats and monkeys produces responses such as vocalization, offensive and defensive escape responses and autonomic manifestations, which mimick behaviour in response to pain (Delgado, 1955; Maclean and Delgado, 1953). Lesioning of the dorsal region or larger areas of the rat hippocampus has also been shown to increase threshold to vocalization (Blanchard and Fial, 1968) and decrease aggressive behaviour (Eichelman, 1971). In humans, surgical section of the cingulum bundle, which connects the posterior frontal cortex to the hippocampus, results

in a loss of " negative affect " associated with intractable pain (Foltz and White, 1962). Based on various lines of evidence, including some mentioned above, Melzack and Casey (1968) proposed that the limbic forebrain structures, including the hippocampus, are involved in " aversive drive and affect that comprise the motivational dimension of pain ". According to O'Keefe and Nadel (1978), the hippocampus is involved in making a cognitive map of the animal's environment which may indirectly affect the animals " motivational process ". For example, the hippocampus may be involved in associating pain or fear with a particular place where the animal received a painful stimulus. This may explain deficits in avoidance behaviour to escape electric footshock in hippocampal lesioned animals (Teitelbaum and Milner, 1963; Miller et al., 1975). The hippocampal formation is also suggested to be involved in coping behaviour to stress including noxious stimuli-induced stress (Henke, 1990). Such a coping behaviour to noxious stimulus-induced stress may be part of affective-motivational component of nociception or pain. Henke (1990) reported that in stressed animals showing gastric pathology the hippocampal formation dentate gyrus population spike was depressed. Conversely, animals who did not show gastric pathology to stress had an enhanced dentate gyrus population spike.

Evidence from hippocampal single cell and electroencephalogram (EEG) recordings also showed that nociceptive stimuli can produce changes in hippocampal neuronal activity. For example, noxious stimulation of the tooth pulp (Brankack and Buzsaki, 1986) or heating of the tail (Sinclair and Lo, 1986) alters the activity of hippocampal neurones. Soulairac et al. (1967) reported that strong electrical stimulation of the tail in conscious animals synchronizes the hippocampal EEG which lasts several seconds and is correlated with the animal vocalizing and biting the stimulating electrode. Similarly, hippocampal EEG synchronization, or theta rhythm of 5-6 Hz frequency, was reported in unanaesthetized

rabbits after a strong noxious stimulus (Jung and Kornmuller, 1938). The hippocampal CA1 synaptic transmission is depressed during spontaneous and sensory-induced theta EEG activity (Herreras et al., 1987, 1988a). Further, during hippocampal EEG theta activity the hippocampal pyramidal neurones alter their responsiveness to sensory stimuli (Brankack and Buzsaki, 1986). The hippocampal theta rhythm is due to medial septal-vertical limb of the diagonal band of Broca (MS-VLDBB) input (Bland, 1986). Consistent with this, septo-hippocampal neurones are activated by peripheral noxious heat stimuli (Dutar et al., 1985).

Although it is clear that hippocampal activity results from a noxious stimulus, little is known of the neuronal processing which takes place within the hippocampus to such a stimulus. We elected to examine the effect of noxious stimuli on the hippocampal CA1 pyramidal excitability since the pyramidal neurones are the principle cell type in the hippocampus (Buzsaki, 1984) and their axons comprise the major efferent output from this structure. Afferent or efferent stimulation produces a synchronous discharge of a number of pyramidal neurones recorded extracellularly as an orthodromic population spike or antidromic spike, respectively (Andersen, 1975; Andersen et al., 1971a). The orthodromic population spike appears to be causally related to the corresponding dendritic field excitatory field potential (EPSP; Andersen, 1975). In the current study the effect of noxious heat was studied on the CA1 orthodromic and antidromic field potentials.

There are two major input pathways to the hippocampas: (a) entorhinal cortical afferents and (b) MS-VLDBB input. We predicted that at least the latter input would be activated by a noxious stimulus based on the following reports: septo-hippocampal neurones are activated following peripheral noxious heat exposure, many septo-hippocampal are cholinergic (Amaral and Kurz, 1985; Fonnum and Walaas, 1978; Matthews et al., 1987) and acetylcholine is released in the hippocampus following a non-noxious sensory stimulus or

stress (Dudar et al., 1979; Imperato et al., 1989). Therefore, an attempt was made to determine whether the changes in CA1 pyramidal neuronal excitability were cholinergically mediated, and if so, to identify the site of action.

A noxious stimulus was found to induce a prolonged depression of the CA1 pyramidal cell synaptic excitability. However, habituation to this response occurred when the noxious stimulus was repeated more than an hour later. The depression and habituation of CA1 synaptic excitability was topographically specific. A depression of CA1 population spike evoked upon Schaffer-collateral or commissural afferent stimulation was observed following a noxious heat stimulus. However, the CA1 antidromic spike amplitude tended to be enhanced following a noxious heat exposure suggesting that the noxious stimulus-induced depression was not postsynaptically mediated. The depression evoked by a noxious stimulus appeared to be cholinergically mediated since the effect was blocked by systemic atropine administration. The site of action would seem to be in the apical dendritic region of CA1 pyramidal neurones since the depression was blocked by atropine applied iontophoretically to dendrites but not on the cell body region of the CA1 pyramidal neurones.

REVIEW OF LITERATURE

INTRINSIC PHYSIOLOGY OF THE HIPPOCAMPUS

General morphology

The hippocampal formation, part of the limbic system, consists of the hippocampus (or Ammon's Horn), the dentate gyrus and the subicular complex. The subicular complex is further subdivided into the subiculum, the parasubiculum and the presubiculum. The rat hippocampal formation, arching above the thalamus, appears bent in a C - shaped manner along its septotemporal axis (Fig. 1; Swanson et al., 1978). The dentate gyrus caps the free edge of the hippocampal formation. It has an outer blade adjacent to the diencephalon, an inner blade lying along the hippocampal fissure and a crest between the two blades. The part of the hippocampus above the thalamus is often called the dorsal hippocampus and the temporal part reaching into the the lateral ventricles, the ventral hippocampus. In the rat, the dorsal hippocampus is predominant. The rostral or septal end of the hippocampus merges with the subiculum.

The hippocampus and the dentate gyrus are allocortex. In a dorsal to ventral order in the transverse section, the layers of the hippocampus - dentate gyrus system are the: alveus (which is a fibre bundle marking the outer boundary of the hippocampus fields), stratum (str.) oriens, str. pyramidale, str. radiatum, str. lacunosum, str. moleculare, str. granulosum and str. polymorphe (Fig. 2; Ramon y Cajal- from translation of his work by L.M. Kraft, 1968). The fimbria is a fibre tract for afferents and efferents of the hippocampal formation. Ramon y Cajal described in detail the cellular structure of the hippocampus and
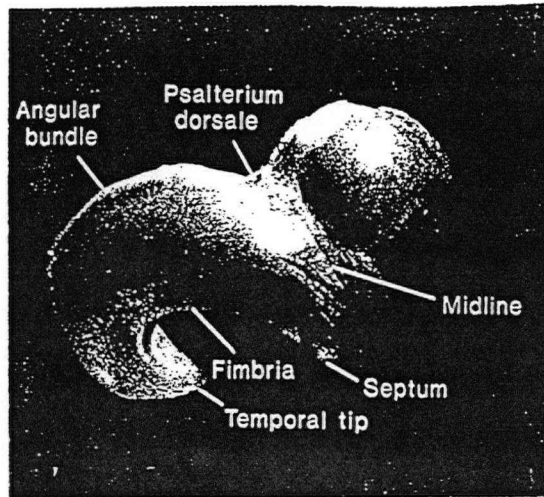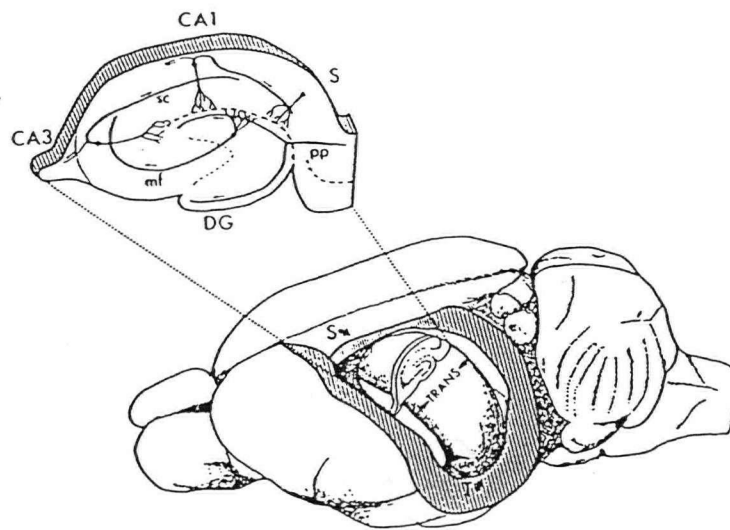
Figure 1: A view of the hippocampal formation of the rat brain. A: Antero-dorso-lateral view of the C-shaped hippocampal formation removed by dissection from a rat brain (Gaarskjaer, 1982a). B: Diagrammatic representation of the hippocampal formation in the outline of the rat brain (Amaral and Witter, 1989). S and T are the septal and temporal ends of the long axis of the hippocampal formation. " Trans " is the short transverse axis of the hippocampal formation perpendicular to the septo-temporal axis. A transverse lamellae of the hippocampus and dentate gyrus with neuronal elements and intrinsic connections is represented in B at top. Abbreviations: DG, dentate gyrus; mf, mossy fibres; pp, perforant path; sc, Schaffer-collaterals.

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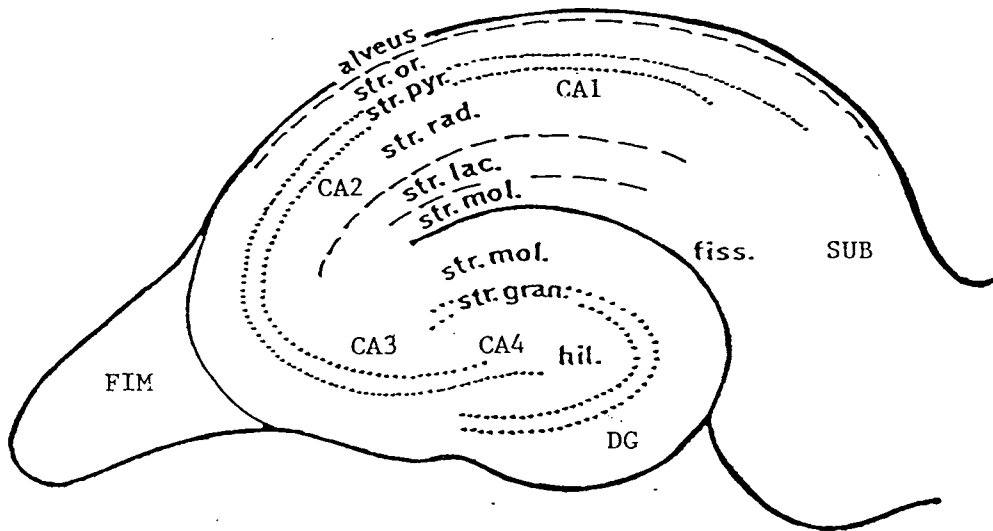
Figure 2: Diagrammatic representation of the transverse lamellae of the hippocampus and dentate gyrus. The major hippocampal fields and the dorso-ventral layers of the hippocampus and dentate gyrus are represented. Abbreviations: str. or., stratum oriens; str. pyr., stratum pyramidale; str. rad., stratum radiatum; str. lac., stratum lacunosum; str. mol., stratum moleculare; str. gran., stratum granulosum; FIM, fimbria; DG, dentate gyrus; fiss., fissure; SUB, subiculum.

the dentate gyrus in a number of species using Weigert-Pal, Golgi or Cox preparations. In the hippocampus, the str. oriens has a zone of polymorphic cells (fusiform or irregular shaped) and a plexiform zone. The polymorphic interneurones have axons which also terminate among pyramidal neurones of the hippocampus. The plexiform zone has dendritic processes from pyramidal cells, nerve plexus and few interneurones. Ramon y Cajal suggested that an interneurone in the str. oriens region, by virtue of its wide axonal arborization in str. pyramidale, may influence the activity of a large number of pyramidal cells.

The str. pyramidale has 3-4 rows of pyramidal cells. The cell bodies of these neurones are fusiform or ovoid and elongated. All pyramidal cells have basal and apical dendrites directed towards str. oriens and str. radiatum, respectively. The pyramids of regio inferior (the hippocampal area adjacent to the dentate gyrus and coinciding with field CA2 and CA3, see below) are larger in size compared to similar neurones of regio superior (the hippocampal area adjacent to the subiculum and coinciding with field CA1, see below). The pyramidal neurones in the hilus (field CA4, see below) are of more irregular body shape with shorter, stronger and coarser dendrites than others. Ramon y Cajal also described dendritic branches of pyramidal cells covered with spines. The pyramidal cells have myelinated axons which travel to the white matter (alveus and the fimbria). These axons yield collaterals which terminate among polymorphic cells in str. oriens and str. radiatum. The axons may also bifurcate, especially the axons from the pyramidal cells of regio inferior.

The str. radiatum is a layer of nerve plexus and also contains apical dendritic processes from pyramidal cells. A few apparently displaced pyramidal cells and interneurones are also present in this layer. The dendritic processes of these interneurones

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may extend to str. oriens and the molecular zone of the hippocampus. Their axons ramify in str. radiatum, str. lacunosum or in str. pyramidale.

The str. lacunosum contains numerous interneurones with dendrites extending up to str. oriens and axons terminating in str. lacunosum or molecular zone and occasionally in the pyramidal cell layer. A number of fibres are also found in this layer. The str. moleculare has interneurones and as well as multiple fibres.

An important cell type in the dentate gyrus is the granule cell located in str. granulosum (Ramon y Cajal). These neurones are ovoid with dendrites emerging from the apical poles. In a section through the hippocampal formation the dentate granule cell layer is seen curved around the pyramidal cell layer of the hippocampus (Fig. 2). The dentate granular layer is thus divided into a suprapyramidal and an infrapyramidal layer. The axons of dentate granule cells are called mossy fibre collaterals and project towards regio inferior of the hippocampus. Buzsaki (1984) indicated that the hippocampal pyramidal cells and the dentate granule cells comprise 96-98 % of the neuropil in these regions.

Lorente de No (1934) divided the hippocampus into various fields, namely, CA1, CA2, CA3 and CA4. The following description of these fields in the rat is consistent with that described by Blackstad (1956) in silver sections of the hippocampus. Field CA4 is a transition zone starting within the hilus of the dentate gyrus (i.e. the dentate region between the two granule cell layer blades) to the field CA3 of the hippocampus. The border between fields CA4 and CA3 is marked by the appearance of a dense fibre plexus in the str. radiatum when using reduced silver impregnations. At the distal end of this fibre plexus (i.e. away from the dentate) is the field CA1 with a small transition zone called " upper end " (Blackstad, 1956) or field CA2 (Lorente de No, 1934). Swanson et al. (1978) described the " unfolded " hippocampal formation with its various subdivisions arranged as

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adjoining longitudinal strips of cortex, starting at the rhinal fissure with the lateral entorhinal cortex, followed in order by the medial entorhinal cortex, para and presubiculum, subiculum, CA1-CA4 fields and the dentate gyrus.

Intrinsic circuitry

The intrinsic connections of the hippocampus described below include those between the dentate granule cells and the hippocampal field CA3 as well as the projection from CA3 neurones to field CA1. Also described are the association and commissural afferents of the hippocampus and dentate gyrus. As indicated above, the granule cells and pyramidal neurones are the principal cells of the dentate gyrus and the hippocampus, respectively. The connection between these neurones has influenced views on the information processing in the hippocampal formation.

The axonal projection from the dentate granule cells to the regio inferior of the hippocampus constitutes the *mossy fibre bundle*. The dentate granule cell axons are called mossy fibres because of their varicose appearance that is similar to the mossy fibres of the cerebellum (Ramon y Cajal). The mossy fibre system is described in the rat as a result of using a variety of techniques including terminal and axonal degeneration following lesions in the dentate granule cell layer (Blackstad et al., 1970; Gaarskjaer, 1978a, b), anterograde transport of tritiated amino acids (Swanson et al., 1978) or horseradish peroxidase (HRP; Claiborne et al., 1986).

The mossy fibres are unmyelinated (Blackstad and Kjaerheim, 1961; Claiborne et al., 1986) and oriented in a transverse direction to the septotemporal axis of the hippocampal formation (eg: Blackstad et al., 1970; Swanson et al., 1978). The mossy fibre bundle traverses the length of the field CA3. Whereas, Blackstad et al. (1970) indicated

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that the mossy fibre system may extend along field CA2, Swanson et al. (1978) did not find such a projection. In either case the mossy fibres do not extend into field CA1 of the hippocampus.

The mossy fibres can travel above the pyramidal cell layer along the length of field CA3 (Blackstad et al., 1970; Claiborne et al., 1986; Swanson et al., 1978). This bundle of mossy fibres is sometimes called the suprapyramidal bundle. Mossy fibres are also observed in the superficial part, the middle and deep portions of the pyramidal cell layer (Claiborne et al., 1986). Consistent with this, mossy fibre terminals are described within the pyramidal cell layer of the hippocampus (Gaarskjaer, 1978a, b). Further, using Timm's preparation (Swanson et al., 1978) or the electron microscope (Blackstad and Kjaerheim, 1961), the mossy fibre varicosities are described making contact with thorn-like spines or dendritic shafts of the apical dendrites of pyramidal cells in field CA3.

Granule cells in all parts of the dentate gyrus, including the two blades and the crest, at all septotemporal levels contribute to the suprapyramidal mossy fibre system (Claiborne et al., 1986; Swanson et al., 1978). Further, Claiborne et al. (1986) found granule cells from different regions of the two dentate granule cell blades contribute differentially to the intrapyramidal mossy fibre bundle. These authors also described the trajectories of mossy fibres from 18 granule cells stained intracellularly with HRP.

The mossy fibre bundle is organized in a lamellar fashion. That is, the mossy fibres arising from granule cells at a septotemporal level innervate a restricted septotemporal level of field CA3 (Amaral and Witter, 1989; Blackstad et al., 1970; Swanson et al., 1978). However, Swanson et al. (1978) found that, at the septal level of the hippocampal formation, the mossy fibres near the field CA1 run caudally and parallel to the long axis of the hippocampal formation for as much as 2 mm.

The mossy axons of dentate granule cells also release collaterals in the hilar region of the hippocampal formation (Claiborne et al., 1986). The hilar projection from the dentate granule cells has also been suggested from degeneration studies (Blackstad et al., 1970; Gaarskjaer, 1978 b) and tracer experiments (Amaral and Witter, 1989; Swanson et al., 1978). The granule cell axonal collaterals in the hilus have a number of varicosities which make synaptic contacts upon dendrites of hilar neurones (Claiborne et al., 1986).

There is an *ipsilateral associational fibre system* from the hilus to the dentate gyrus. Zimmer (1971) first described such a system in degeneration studies when lesions were placed in field CA4 (or hilus) and proximal CA3 (field CA3c of Lorente de No) but not after lesioning other areas of the hippocampal formation of decommissurated rats. The author indicated that such a fibre system had a considerable septotemporal extent and that it terminated in the inner third of the molecular layer of the dentate gyrus. Degeneration studies (Laurberg, 1979) as well as autoradiographic and HRP transport studies (Laurberg, 1979; Swanson et al., 1978) have localized the origin of this fibre system to the hilus. The axons constituting this fibre system are from medium to large fusiform and spindle shaped cells, as well as stellate neurones of the hilus (Swanson et al., 1981). Swanson et al. (1978) have described a hilar associational projection to str. oriens and str. radiatum of hippocampal field CA3 and CA1. However, later studies failed to show such a hilar projection to the hippocampus (Laurberg, 1979; Sorensen and Laurberg, 1981; Swanson et al., 1981).

The associational fibres from the dentate hilus, both at septal and middle levels, project to a considerable septotemporal extent of the hippocampal formation (Fricke and Cowan, 1978; Swanson et al., 1978). However, the temporal hilus has a relatively restricted projection to the dentate gyrus corresponding more closely to the lamellar organization

characteristic of the mossy fibre system. A differential labelling of the two blades of the dentate gyrus by the ipsilateral associational fibre system has also been described (Fricke and Cowan, 1978).

A projection from *field CA3 to ipsilateral fields CA3 and CA1* has been described. For example, in the Golgi preparation of the hippocampal formation from young rabbits and pigs, Schaffer (1892) noticed that the pyramidal cells in regio inferior have axons which bifurcate in str. oriens with one branch going to the alveus and the other continuing in the str. oriens. From the latter, or from the undivided initial part of the axon, a branch projects to the str. radiatum and str. lacunosum. This branch is designated as a *"Schaffer-collateral "*. The Schaffer-collateral has several side branches to str. oriens and str. radiatum and these fibres also enter regio superior. Comparable results were obtained by Ramon y Cajal in man, rabbit, guinea pig and mouse. Ramon y Cajal was able to follow the collaterals close to the subiculum. Terminal ramifications were described as being present in both the str. radiatum and lacunosum. Lorente de No (1934) indicated that the Schaffer-collaterals arise mainly from the regio inferior close to the hilus (subfield CA3c of Lorente de No) and only to a minor extent from pyramidal cells in other parts of regio inferior.

In an agreement with the above studies terminal degeneration is obtained in both fields CA3 and CA1 of the hippocampus in adult rats following lesions in field CA3 (Hjorth-Simonsen, 1973; Laurberg, 1979). However, terminal degeneration is observed in str. radiatum and str. oriens and not in other layers of the hippocampus.

The projection from the septal and middle field CA3 to the str. radiatum has an extensive septotemporal length (Laurberg, 1979), whereas, the projection from the temporal field CA3 is more restricted in its septotemporal extent (Swanson et al., 1978). In a lesion study (Laurberg, 1979), the projection from CA3 along the transverse axis of the

hippocampal field CA3 and CA1 was most dense at the level of the lesion and progressively

decreased with increasing distance. However, based on anterograde transport of Phaseolus

vulgaris leucoagglutinin (PHA-L), Amaral and Witter (1989) stated that the greatest

density of fibre and terminal labelling is located at a distance of 1 mm from the injection site.

In tracer transport experiments, various authors have reported that Schaffer-collaterals

from pyramidal cells in all regions of field CA3 project to the field CA1 (Swanson et al.,

1981; Swanson et al., 1978).

Swanson et al. (1978) also found that the temporal half of field CA3, which is closer

to regio superior (subfield CA3a of Lorente de No), gave rise to an ascending fibre system,

that courses the str. radiatum and oriens of this part of field CA3. The authors suggested

that this fibre bundle may be similar to the *longitudinal association bundle from field CA3*

as described by Lorente de No (1934). This fibre bundle is suggested to interrelate widely

separated zones of regio inferior along its septotemporal axis.

More recently, based on anterograde transport of PHA-L, Amaral and Witter (1989)

described the following organizational features of the CA3 projections to ipsilateral field CA1

at all septotemporal levels. They described a subiculodentate gradient in the projection of

CA3 cells in which those located close to the dentate gyrus distribute preferentially to the

field CA1 near the subicular border where they terminate in the superficial portion of the str.

radiatum. CA3 neurones that are located progressively closer to the field CA1 border project

preferentially to parts of CA1 that are progressively closer to CA3 and to deeper portions of

str. radiatum and into str. oriens. Further, CA3 cells located close to the dentate gyrus tend

to project further and more heavily in the septal direction. Cells located near the CA1 border

tend to project further and more heavily in the temporal direction. The terminal labelling in

CA1 tended to be organized along a septotemporal gradient such that at the septal level the

terminal labelling is closer to the CA3/CA1 border and in deeper parts of str. radiatum and str. oriens. However, as progressively more temporal levels are approached the terminals are located closer to the subicular border and more superficially in str. radiatum.

The *CA1 pyramidal cells* axons project outside the hippocampus and do not have an extensive hippocampal projection.

Commissural afferents to the hippocampus and the dentate gyrus have also been identified. For example, in rats, an ipsilateral lesion of the hippocampus and the dentate gyrus or in the ventral hippocampal commissure revealed contralateral terminal degeneration chiefly in str. oriens and radiatum of the hippocampus and in the inner third of str. moleculare of the dentate gyrus (Blackstad, 1956; Hjorth-Simonsen and Laurberg, 1977; Laurberg, 1979). The commissural afferents course through the ventral hippocampal commissure and the fimbria entering the hippocampus.

The commissural afferents to the dentate area and the hilus are from the contralateral dentate hilus, while the commissural projection to the hippocampal fields CA1 and CA3 is from the contralateral field CA3 (Hjorth-Simonsen and Laurberg, 1977; Laurberg, 1979). Laurberg (1979) found that a lesion in field CA3 produced commissural degeneration along considerable septotemporal extent of the fields CA1 and CA3. The most dense projection to field CA3 contralateral to the lesion was at the same level as the site of the lesion. Commissural projection from hippocampal field CA3 and the dentate hilus have also been described with anterograde transport of tritiated amino acids (Fricke and Cowan, 1978; Swanson et al., 1978). The description of the commissural projections using this method agrees with that described following lesions of field CA3 or the dentate hilus. Consistent with a widespread septotemporal commissural projection from the dentate hilus, injections of HRP or of fluoroscent dyes in the septal part of the dentate gyrus retrogradely

labelled hilar neurones along a considerable septotemporal extent (Laurberg, 1979;

Sorensen and Laurberg, 1981). In a multiple retrograde labelling study, Swanson et al.

(1981) reported that all parts of field CA3 contribute to the commissural projections and

that more than two-thirds of CA3 pyramidal neurones contribute axonal collaterals both as

Schaffer-collaterals and to the commissural projection. Similarly, more than 80 % of hilar

neurones contributed collateral projection to both the ipsilateral and contralateral dentate

gyrus.

Based on electrophysiological studies in rabbits, Andersen et al. (1971 b) suggested

that the hippocampus and the dentate gyrus are organized in a series of " parallel lamellae "

in a plane approximately normal to the septotemporal axis of the hippocampal formation

(Fig. 1). Each lamellae has a 4-component excitatory chain consisting of (1) perforant path

fibres from the entorhinal cortex (see later in the text), (2) mossy fibres of the dentate

gyrus granule cells, (3) Schaffer-collaterals of CA3 pyramidal cells and (4) alvear fibres of

CA1 pyramidal cells. Stimulation of perforant path fibres led to a sequential activation of

other members of the 4-component chain in the same lamellae. A similar lamellar

organization has also been suggested for the hippocampus of the rat (Rawlins and

Green, 1977). However, Amaral and Witter (1989), based on the anatomical evidence

available, suggested that the hippocampus and dentate gyrus are organized for processing of

information both in the transverse lamellae and in the septotemporal axis. Thus, the

processing of information for a particular task will take place simultaneously over much of

the septotemporal axis of the hippocampus and dentate gyrus.

Finally, the hippocampus has a number of interneurones localized to various layers

(see text above). These interneurones exercise a strong influence on the activity of

hippocampal pyramidal neurones. For example, Schwartzkroin and Kunkel (1985) have

described the morphology and synaptic contacts of electrophysiologically identified, HRP filled interneurones in hippocampal slices. These neurones have electrophysiological characteristics similar to interneurones involved in feed-back or feed-forward inhibition of pyramidal cell activity (see text below). The cell body of HRP filled interneurones, as well as neurones with comparative morphology in golgi preparations, were located at the border of str. pyramidale and str. oriens or within str. pyramidale itself. The interneurones had large somas with dendrites projecting basally to the alveus and apically through str. pyramidale and reaching str. radiatum and str. lacunosum. Some of the interneurones were ovoid and described as basket cells. The axons of interneurones make synaptic contact (both bouton terminal and en passant type) mostly onto pyramidal cell somata and proximal apical and basal dendritic shafts. These interneurones make synaptic contact on a large number of pyramidal cells as well as other interneurones and also receive synaptic contacts onto their soma and dendrites. Similarly, Lacaille and Schwartzkroin (1988) described str. lacunosum-moleculare interneurones which, from electrophysiological evidence, are involved in the modulation of CA1 pyramidal neuronal activity. They exhibit dendritic spread into str. lacunosum-moleculare and str. radiatum while their axons branch mostly in str. radiatum and str. pyramidale.

Physiological characteristics of hippocampal neurones

Characteristics of hippocampal neurones in a variety of species have been documented *in vivo*. For example, presumed hippocampal CA3/CA2 pyramidal cells in cats were antidromically invaded by stimulation in the fornix (Kandel et al., 1961; Kandel and Spencer, 1961). The antidromic spike was usually followed by an inhibitory postsynaptic potential (IPSP) and a decrease in the spontaneous activity of the cell. An IPSP was also

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produced in these neurones by fornix stimulation at thresholds lower than that required for antidromic activation. These neurones fired with a mix of single and complex-spikes. In rats, hippocampal CA1 and CA3 neurones with similar physiological characteristics have been morphologically identified as pyramidal cells following intracellular HRP injections (Finch et al., 1983). The CA3 pyramidal cells were observed to send their axons in the alveus and also project as Schaffer-collaterals to field CA1. The CA1 pyramidal cells all had axons in the alveus which could be traced to the subiculum. A few of these also had rostrally directed collaterals projecting towards the fimbria.

In addition, Kandel et al. (1961) reported that single spike or burst firing of pyramidal neurones was associated with a depolarizing post-spike potential. It appears that these after-potentials were additive and the magnitude corresponded to the number of spikes in a burst. In some neurones there appeared to be what was termed a fast prepotential which is a small potential that preceeds the somatic spike (Spencer and Kandel, 1961a). Since the potential was so small, it was concluded that it was generated at some site distant from the soma. Antidromic activation of the cell failed to evoke any fast prepotentials so it is unlikely that they arose from axons. It was suggested that the prepotentials represented dendritic spikes. These electrophysiological characterisitics are mimicked by hippocampal pyramidal neurones when recorded *in vitro* in the slice preparation (eg: Schwartzkroin, 1975).

Based on evidence from cell recordings in the slice preparation, Wong et al. (1986) suggested that the voltage-dependent inward currents supporting action potentials in hippocampal pyramidal neurones are (a) a tetrodotoxin (TTX)-sensitive component that is carried by Na^+ and (b) a calcium component that is insensitive to TTX but is suppressed by Co^{2+} or Mn^{2+}. There are also outward currents of which one component is elicited during a

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burst and is activated by increases in intracellular calcium and is mediated by K+. These authors suggested the following scheme for burst generation in pyramidal neurones: membrane depolarization above threshold elicits the Na+-dependent upstroke of an action potential; this activates the slowly deactivating calcium conductance which sustains the secondary depolarization following the action potential (the depolarizing afterpotential or DAP). The amplitude of DAP reaches threshold in the bursting cells and triggers a sequence of action potentials. The increased intracellular calcium during bursting produces an activation of K+ conductance. When the strength of K+ conductance is sufficient it terminates the burst and sustains a slowly decaying afterhyperpolarization. Tetrodotoxin - sensitive presumed Na+ channels are also implicated in population discharge of pyramidal cells to orthodromic and antidromic stimulation (Turner et al., 1989).

The complex-spike pyramidal cells of the CA1/CA3 region have also been characterized with respect to various specific behaviours and spatial locations (Ranck, 1973). The recording sites were histologically localized in the str. pyramidale of the hippocampus (Fox and Ranck, 1975). Following single shock stimulation of septal, commissural or entorhinal cortex, the complex-spike pyramidal cells generally responded with a single spike (Andersen et al. 1964a, b; Fox and Ranck, 1981; Rose and Pang, 1985). Afferent stimulation can also evoke large extracellular potentials in the hippocampus and the dentate gyrus. Andersen (1975) presented evidence indicating the nature of these field potentials. For example, while stimulating in the perforant path/entorhinal cortex and recording from the dentate gyrus, a large negative potential is recorded whose amplitude is maximal in the middle part of the dentate molecular layer. This potential reverses in the granule cell layer with a large negative population spike superimposed. The negative wave in the molecular layer is thought to be causally related to the population spike, since the

spike occurs only when the negative wave in the molecular layer reaches a certain magnitude. Intracellularly recorded EPSP from the dentate granule cells coincide in time with the negative wave in the molecular layer. Therefore, the molecular layer negative wave is interpreted as the extracellular sign of EPSP produced upon perforant path stimulation. The population spike correlates in time with unit discharges and the amplitude of the spike is a function of the number of units discharging (Andersen et al., 1971a; Andersen, 1975). Such unit discharges were recorded from the dentate granule cell layer. Consequently, the population spike recorded upon perforant path stimulation may reflect the synchronous discharge of a number of granule cells. Further, after a population spike, a large positive wave is recorded. This corresponds to an intracellularly recorded hyperpolarization with an inhibition of unitary discharge or a decrease in the population spike due to a subsequent test volley (Andersen, 1975). This granule cell layer positivity is, therefore, accepted as the extracellular equivalent of the intracellular IPSP.

Similarly, stimulation of mossy fibres or Schaffer-collaterals produces an extracellular field EPSP and population spike in the apical dendrites and pyramidal cell body layer of field CA3 and CA1, respectively (Andersen, 1975). Stimulation of the commissural input to field CA1 also produces a large negative field EPSP in the apical dendritic region and a discharge of pyramidal neurones. Further, electrical stimulation of CA1 pyramidal cell axons within the alveus evokes a sharp, short-latency, negative going antidromic spike which also represents the summed synchronous discharge of a population of pyramidal neurones (Andersen et al., 1971a). Indeed, in intracellularly recorded CA1 pyramidal neurones, the latency of the orthodromic spike coincides with the negative going extracellular population spike (eg: Richardson et al., 1984). Similarly, the intracellular

antidromic action potential corresponds in time to the extracellular antidromic spike (Richardson et al., 1984).

In a laminar profile analysis of evoked field potentials and current source density in the hippocampal field CA1 in the slice preparation, Richardson et al. (1987) suggested that the cell body region is the point for spike initiation in pyramidal cells for both antidromic and orthodromic afferent stimulation. Thus, the antidromic spike or the orthodromic population spike and associated current sink of the shortest peak latency was found in the region of the pyramidal cell body layer. The authors indicated that the spike field potential from the cell body layer retrogradely invaded the basal and apical dendritic arborizations and is distinguished on the dendritic synaptic field potential as a positive or positive/negative deflection occurring at a latency greater than at the cell body layer. However, in an *in vivo* laminar profile analysis of the orthodromic field potential upon Schaffer-collateral stimulation,the negativity latency of the evoked population spike was shortest in the proximal dendrites in str. radiatum (Andersen, 1960). This indicated that the spike was initiated at proximal dendrites and propagated towards the soma. Consistent with this conclusion is very recent evidence which suggests that voltage-dependent conductances in proximal apical dendrites are contributing to spike generation at the cell body layer (Turner et al., 1989). They found that stimulation of afferent Schaffer-collateral/commissural input to distal apical dendrites evoked a negative going field potential in the proximal apical dendrites and an orthodromic population spike in the CA1 cell body layer. Local administration of TTX in the proximal apical dendrites decreased the proximal apical dendritic field potential and the Schaffer-collateral stimulation-evoked population spike, but did not influence str. oriens afferent stimulation-evoked population spike or antridromic spike. In contrast, TTX applied in str. pyramidale decreased the Schaffer-collateral

stimulation-evoked population spike without affecting the proximal apical dendritic field potential. These manipulations with TTX do not affect the synaptic field EPSP at the distal apical dendrites. The authors conclude that active conductance through TTX-sensitive presumed Na+ channels in the proximal apical dendrites is instrumental in the generation of the Schaffer-collateral stimulation-evoked population spike at the cell body layer.

Further, based on sensitivity to TTX, Turner et al. (1989) identified a slow field potential and a fast Na+ spike at the level of proximal apical dendrites which they referred to as a dendritic spike. The dendritic spike occurred before the population discharge and in some cases had a lower stimulus threshold than the cell body population spike. The duration of both the dendritic spike and the population spike was identical.

Turner et al. (1989) also identified a positive going deflection in the synaptic field EPSP which decreased in size when the population spike or the dendritic spike was depressed following TTX application in the cell body layer or proximal apical dendrites. Therefore, and consistent with Richardson et al. (1987), they suggested that the positive deflection is due to retrograde invasion of the dendritic or population spike.

In addition to synaptic activation, ephaptic interactions may also contribute to the excitation of pyramidal neurones following afferent stimulation. For example, in the hippocampal slice preparation, recording intracellularly from CA1 pyramidal neurones or the adjacent extracellular space revealed a transmembrane depolarization in pyramidal neurones that occurred synchronously with the negative going phase of the antidromic or orthodromic population spike (Richardson et al., 1984; Taylor and Dudek, 1984). These authors suggested that such changes in pyramidal cell excitability due to the extracellular electrical field would aid in the synchronous activation of the neuronal population following afferent stimulation since the ephaptic depolarization of each neurone is restricted in time to

the peak of the population spike. The electrical field effect could also lead to the recruitment of otherwise subthreshold neurones. This could substantially increase the amplitude of the population spike beyond that resulting from synaptic activation alone.

Another class of neurone has been physiologically recognized in the hippocampus which generally responds with multiple discharges to afferent septal, commissural or entorhinal cortex single shock stimulation (Andersen et al., 1964b; Fox and Ranck, 1981; Buzsaki and Eidelberg, 1982; Ashwood et al., 1984; Rose and Pang, 1985). Fox and Ranck (1975 and 1981) called these neurones theta cells since they were phase-locked to theta rhythm. These cells usually had high spontaneous activity and were recorded chiefly from regions of str. oriens of CA1 and str. radiatum of CA3 (Fox and Ranck, 1975; Buzsaki and Eidelberg, 1982). Stimulation in the alveus, fimbria or contralateral hippocampal commissure failed to antidromically activate the cells of this class, whereas complex-spike pyramidal cells were antidromically driven (Bland et al., 1980; Fox and Ranck, 1981). Indeed, in the CA1 region, alveus stimulation produced an activation (multiple discharge) of theta neurones which followed the antidromic field potential (Ashwood et al., 1984). Further, in the *in vitro* slice preparation, neurones with the above physiological characteristics were identified using intracellular HRP injection as having locally ramifying axons (Schwartzkroin and Mathers, 1978; Schwartzkroin and Kunkel, 1985). The above evidence, taken together suggests that most neurones of this class are local interneurones.

Electrophysiological evidence suggests that at least some hippocampal interneurones mediate recurrent inhibition of pyramidal neurones following their activation. Thus, Spencer and Kandel (1961b) in the chronic hippocampal deafferentated cat reported that stimulation in the fornix resulted in antidromic invasion followed by an IPSP in intracellularly recorded CA2, CA3 and CA4 pyramidal cells. Thus the inhibition might be

mediated via collaterals of the efferent axons. Afferent stimulation also produced similar changes in cat CA3 pyramidal cells (Andersen et al. 1964a). The IPSP is associated with an extracellular wave which is maximal at the cell body layer suggesting that the inhibition took place at the pyramidal cell body itself. The authors suggest that an interneurone is involved in the inhibition because (a) the latency to onset of the IPSP lengthened as stimulation of the afferent source weakened, and (b) a high frequency ripple or wave-like increments (up to 1000 Hz) was observed on the rising phase of a weak IPSP which may correspond to interneuronal activation. Further, the latency to onset of the IPSP was typically longer than that of either orthodromic or antidromic activation. Andersen et al. (1964b) described presumed interneurones in str. oriens of the hippocampus which fired at high frequency following afferent stimulation. Stimulation of deafferented fimbria evoked activity in str. oriens presumed interneurones after antidromic invasion of pyramidal cells. These authors therefore concluded that following afferent or antidromic stimulation of pyramidal neurones, the recurrent axonal collaterals of these neurones excite the interneurones which then inhibit the pyramidal cells. Consistent with this idea, other authors have also reported that hippocampal CA1/CA3 pyramidal cells fired on the population spike or after the population spike threshold had been reached to afferent stimulation (Fox and Ranck, 1981; Rose and Pang, 1985) while putative interneurones fire on the post-population-spike positive field potential (Fox and Ranck, 1981). Similarly, the putative interneurones fired after an antidromic spike of pyramidal neurones (Ashwood et al., 1984). Further, it has been demonstrated in *in vitro* slice preparations that intracellular stimulation of non-pyramidal cells produced IPSP's in CA1 pyramidal cells, while stimulation of pyramidal cells produced EPSPs or spikes in non-pyramidal cells (Knowles and Schwartzkroin, 1981). This gives credence to the concept of feed-back inhibition.

In addition to the classical recurrent inhibition described above, there appears to be feed-forward inhibition in the hippocampus. This has been suggested on the basis of the following: (a) afferent stimulus threshold to activate some interneurones is lower than that required to evoke a population spike (Buzsaki and Eidelberg, 1982) and (b) interneurones can respond to afferent stimulation at a shorter latency than that of the population spike (Fox and Ranck, 1981; Buzsaki and Eidelberg, 1982; Ashwood et al., 1984; Rose and Pang, 1985) and in some instances fire on the positive field potential preceding the population spike (Ashwood et al., 1984). Indeed, Lacaille and Schwartzkroin (1988) reported a number of str. lacunosum-moleculare interneurone-pyramidal cell pairs in which stimulation of the interneurone resulted in an IPSP in the pyramidal cell. However, pyramidal cell stimulation did not elicit any correlated changes in membrane potential of these paired interneurones.

Feed-forward excitation of CA1 pyramidal neurones by interneurones might also occur. For example, in interneurone-pyramidal cell pairs, subthreshold stimulation of afferents evoked a larger and a faster rising depolarization in the interneurone than in the pyramidal cell (Lacaille and Schwartzkroin, 1988). And increasing the intensity of afferent stimulation resulted in an EPSP of sufficient amplitude in the interneurone to elicit an action potential, whereas the depolarization in the pyramidal cell was still of subthreshold amplitude. At still higher intensity, afferent stimulation-evoked an action potential in both cells, but the action potential in the interneurone was evoked at shorter latency.

AFFERENTS TO THE HIPPOCAMPUS

Afferents to the hippocampus have been described from the entorhinal cortex, MS-VLDBB complex , diencephalic and brainstem structures. The entorhinal and MS-VLDBB afferents to the hippocampus have been implicated in sensory stimuli-induced changes in the hippocampal formation.

Entorhinal cortical afferents

The entorhinal cortex is a major source of afferent input to the hippocampus and the dentate gyrus. A number of investigators have described the projections from the entorhinal cortex to the hippocampal formation. For example, in the rat, Steward (1976) has indicated, based on degeneration studies, that the entorhinal cortex-hippocampal formation afferents project to (a) ipsilateral dentate gyrus with a sparse projection to contralateral dentate gyrus, (b) ipsilateral field CA3 of the hippocampus, and (c) bilaterally to field CA1. Evidence from similar studies (Hjorth-Simonsen, 1972; Hjorth-Simonsen and Jeune, 1972; Ruth et al., 1988) and from anterograde transport of tritiated amino acids (Steward, 1976; Wyss, 1981) indicates that medial entorhinal cortex afferents terminate in the middle third of the molecular layer of the dentate gyrus and in the depth of str. lacunosum-moleculare of subfield CA3. The lateral entorhinal cortex afferent terminals are localized in the outer third of the molecular layer of dentate gyrus and the outer two-thirds of the str. lacunosum-moleculare of subfield CA3.

The bilateral projection from the entorhinal cortex to subfield CA1 also terminates in str. lacunosum-moleculare (Steward, 1976; Wyss, 1981). The ipsilateral entorhino-CA1 projection is organized in a medio-lateral gradient fashion such that the medially located neurones within the entorhinal area project to the CA1-CA2 zone and progressively more

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laterally located cells in the entorhinal cortex project towards the subicular zone of field CA1. A similar organization of entorhino-CA1 afferents is seen in the contralateral rostral subfield CA1 (septal pole). However, in the contralateral temporal regions of CA1 there is no entorhinal projection to the CA1-subicular transition zone (Steward, 1976). Using anterograde transport of PHA-L, Witter et al. (1988) have indicated that the most rostrolateral position of the entorhinal cortex projects only to subfield CA1 and the subiculum of the hippocampal formation.

The entorhinal cortical afferents to the dentate gyrus and subfield CA3/CA1 run in the angular bundle and penetrate the subiculum on a course along the hippocampal fissure or the CA1 to reach their terminal fields (Hjorth-Simonsen, 1972; Hjorth-Simonsen and Jeune, 1972; Steward, 1976). This fibre bundle is usually described as the perforant tract. Steward (1976) has also described entorhino-CA1 afferents travelling through the alveus.

There is conflicting evidence on the septotemporal extent of entorhinal cortical projection to the dentate gyrus. Based on degeneration studies, Hjorth-Simonsen and Jeune (1972) suggested that there is a lamellar organization of medial entorhino-dentate gyrus afferents in the hippocampal formation. Thus, the dorsal part of the medial entorhinal cortex projects to the septal pole of the dentate gyrus and the more ventral aspect of the medial entorhinal cortex projects to increasingly temporal segments of the dentate gyrus. A similar organization of lateral entorhinal-dentate gyrus afferents is also reported based on degeneration as well as retrograde HRP transport studies (Ruth et al., 1988). However, in a retrograde HRP transport study, the cells of the medial entorhinal afferents to the hippocampal formation lamellae were organized along the anterior-posterior axis of the entorhinal cortex (Ruth et al. 1982). Cells in the posteriolateral and posteriomedial walls of the entorhinal cortex along the dorsoventral axis projected to the septal pole of the dentate

gyrus. Cells in increasingly anteromedial aspect of the medial entorhinal cortex projected to more temporal regions of the dentate gyrus.

Whereas, contrary to the above proposed lamellar organization of the entorhinal afferents, anterograde tracer transport studies have provided evidence that the entorhinal afferents may project to a considerable septotemporal extent of the hippocampal formation regions. For example, Wyss (1981) showed that localized injections of tritiated amino acids in the dorsal aspect of the medial entorhinal cortex and in the lateral entorhinal cortex labelled a substantial septotemporal extent of the hippocampal formation. However, the author described a lamellar organization in the hippocampal formation of the afferents from the ventral aspect of the medial entorhinal cortex. A non-lamellar organization of entorhinal afferents to the hippocampal along its septotemporal axis is also indicated by Amaral and Witter (1989) based on anterograde transport of PHA-L following its discrete injection into several regions of the rat entorhinal cortex. The authors found that a PHA-L injection which reached approximately 10 % of the length of the entorhinal cortex heavily labelled 25 % of the length of the dentate gyrus. A sparse projection could be traced to approximately 40 % of the total length of dentate gyrus. Indeed, in monkey, employing retrograde transport of two tracers, entorhinal cortical cells were described which gave rise to collaterals innervating distant septotemporal levels of the dentate gyrus (Witter et al., 1989).

The stellate cells and pyramidal cells found in layer II and III, respectively, of the entorhinal cortex are thought to be cells of origin projecting to the dentate gyrus and field CA1, respectively (Steward and Scoville, 1976; Ruth et al., 1982 and 1988).

Physiological studies also parallel the findings of anatomical studies. Stimulation in the entorhinal cortex/angular bundle in rabbits (Andersen et al., 1966; Lomo, 1971) and rats (Fox and Ranck, 1979) produced an extracellular recorded field EPSP in the dentate

gyrus which is maximal in the str. moleculare. This is consistent with the anatomical localization of perforant path synapses in the hippocampal lamellae. Short latency field potential changes have also been described in field CA1 to entorhinal cortex/angular bundle stimulation (Fox and Ranck, 1979). The authors suggest that this short latency response is due to activation of the projection fibre system from the entorhinal cortex to the field CA1.

The effect of entorhinal cortex and/or angular bundle stimulation on hippocampal pyramidal cells has also been reported. For example, Ben-Ari et al. (1979), found that intracellularly recorded CA3 pyramidal cells were hyperpolarized upon entorhinal cortex stimulation. Such stimulation could also activate both CA1 and CA3 pyramidal cells followed by inhibition (Fox and Ranck, 1981). Segal (1972), *in vivo*, in rats and Doller and Weight (1982), in guniea pig slice preparations, have described both a short and a longer latency activation of CA1 pyramidal cells to entorhinal cortex/angular bundle stimulation. These authors suggested that the short latency activation of CA1 pyramidal cells is due to stimulation of entorhinal cortex fibres projecting to CA1. The reason being that the short latency response could not be abolished by blocking the activation of CA3 pyramidal and dentate granule cells which, however, blocked the longer latency activation of CA1 pyramidal neurones. The authors therefore suggested that the longer latency response in CA1 pyramidal cells might have been due to sequential activation of the 4-component excitatory chain of the hippocampal lamellae following entorhinal cortex stimulation. The sequential activation of dentate granule cells and the hippocampal pyramidal cells which evoke field potential changes and the population spike have been described earlier in the text.

The putative interneurones in the hippocampal fields CA1/CA3 are also activated following entorhinal stimulation (Fox and Ranck, 1981; Buzsaki and Eidelberg, 1982; Rose

and Pang, 1985). These neurones generally respond with a multiple discharge to entorhinal afferent stimulation.

MS-VLDBB afferents

The MS-VLDBB afferents to the hippocampus in rats have been described using various methods including retrograde and anterograde transport of HRP, anterograde transport of tritiated amino acids and terminal degeneration following septal lesions (Mosko et al., 1973; Segal and Landis, 1974; Meibach and Siegel, 1977; Swanson and Cowan, 1979; Crutcher et al., 1981). These reports have described an input from the MS-VLDBB complex to the hippocampus and the dentate gyrus. A dense projection to str. oriens and str. radiatum of CA3 and the hilus of the dentate gyrus has been consistently reported. Meibach and Siegel (1977) and Monmaur and Thomson (1983) have also reported MS-VLDBB projection to the field CA1 of the hippocampus. Using tracer techniques Meibach and Siegel (1977) described a medio-lateral organization of the MS-VLDBB neurones projecting to the hippocampus. Thus, MS-VLDBB neurones located close to the midline project through the dorsal fornix to terminate in the dorsal hippocampus fields CA1 to CA4 and the dentate gyrus, while neurones located progressively more lateral in the MS-VLDBB project through progressively more lateral parts of the fornix and the fimbria to terminate in progressively more postero-ventral hippocampus and the dentate gyrus. More recently, Nyakas et al. (1987) have studied the MS-VLDBB neuronal axonal projection and termination in the hippocampus and the dentate gyrus using anterograde transport of locally applied PHA-L in the MS-VLDBB coupled with light microscopy. In general, they confirmed a strong projection from the MS-VLDBB to the hippocampus and the dentate gyrus. However, they found differences in the hippocampal projection from the medial septal nucleus and the

VLDBB. They described a medio-lateral organization of the hippocampal projection from the medial septal nucleus which was somewhat similar to that described by Meibach and Siegel (1977). The projection from the midline region of the medial septal nucleus was substantial on field CA3 and dentate gyrus but moderate to low in field CA1 of the dorsal hippocampus. The projection from the lateral region of the medial septal nucleus was exclusively to ventral hippocampal field CA1, CA2 and dentate gyrus. The rostral and the caudal VLDBB project only to the dorsal hippocampus field CA1 to CA3 and dentate gyrus, while the intermediate VLDBB had afferents throughout the extent of the hippocampus and the dentate gyrus. The projection to field CA1 from the VLDBB was substantial as compared to the projection from the medial septal nucleus. The axons from the MS-VLDBB to field CA1 were of variable thickness. The thinner axons make en passant contact on pyramidal cell bodies and dendrites while the thicker axons innervate mostly in str. oriens and radiatum/moleculare.

Andersen et al. (1961), in rabbits, have described field potential changes in field CA1/CA3 of the hippocampus to MS-VLDBB stimulation. However, Fox and Ranck (1979) in conscious rats and Krnjevic and Ropert (1982) in urethane anaesthetized rats have reported either small or no field potential changes in the hippocampus field CA1/CA3 to such stimulation. Septal stimulation can increase the excitability of hippocampal pyramidal cells to afferent stimulation. Thus, in anaesthetized rats, a brief tetanus (50-100 Hz) to the medial septal nucleus preceding single shock stimulation of the ventral hippocampal commissure evoked a population spike on the positive field potential (Krnjevic and Ropert, 1982). This tetanic stimulation of the MS-VLDBB region also suppressed the spontaneously occurring IPSPs in intracellularly recorded field CA1-CA3 pyramidal neurones (Krnjevic et al., 1988). There is also an increase in membrane resistance in the majority of these neurones. Similarly, in the majority of hippocampal pyramidal cells, MS-VLDBB

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stimulation also reduced the amplitude of IPSPs (and the accompanying conductance increase) following fimbria or alveus stimulation. These effects of septal stimulation may promote excitation of pyramidal neurones to afferent stimulation and facilitation of the population spike observed extracellularly (Krnjevic et al., 1988; Krnjevic and Ropert, 1982). Septal stimulation also produces depolarization in hippocampal pyramidal cells recorded intracellularly with non-chloride containing electrodes (Krnjevic et al., 1988). When recording with the chloride containing electrodes (IPSPs reversed in depolarizing direction), septal stimulation usually had a hyperpolarizing effect on the membrane potential. The authors suggested that this effect of septal stimulation was consistent with suppression of ongoing, predominantly chloride mediated IPSPs. This may result from septal stimulation-induced inhibition of GABAergic interneurones which exercise inhibitory control on hippocampal pyramidal cell somatic excitability via chloride currents. Consistent with this notion, septal stimulation in anaesthetized rats inhibited the spontaneous activity of the majority of CA1 theta neurones (or putative interneurones; Mizumori et al., 1989). But such stimulation did not alter the spontaneous activity in a majority of hippocampal pyramidal cells. This latter effect is consistent with the modulatory influence of septal stimulation. Conditioning septal stimulation, which enhanced the CA1 population spike, also decreased the CA1 dendritic field EPSP (Rovira et al., 1983a).

In anaesthetized rats, a conditioning septal stimulation can also enhance the population spike in the dentate gyrus evoked upon perforant path stimulation (Bilkey and Goddard, 1985 and 1987; Mizumori et al., 1989). Septal stimulation *per se* did not evoke a significant field potential change in the dentate gyrus. As outlined above, a disinhibitory mechanism for septal facilitation is favoured because (a) septal facilitation is blocked when the inhibitory effect of GABAergic interneurones is abolished by local infusion of picrotoxin

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in the dentate hilus (Bilkey and Goddard, 1985), (b) medial septal stimulation, if temporally close to the first perforant path stimulus, prevented the depression of the population spike to paired perforant stimuli. The paired-pulse depression may be due to feedback activation of interneurones by the first perforant path test pulse. Septal stimulation when temporally close to the first test pulse may prevent or reduce the activation of inhibitory interneurones. Similarly, septal stimulation temporally close to commissural stimulation reduced the depressive effect of the latter on the perforant path stimulation-evoked population spike (Bilkey and Goddard, 1987), and (c) the probability of activation of putative interneurones to perforant path stimulation is decreased by preceding septal conditioning stimulation (Mizumori et al., 1989). The conditioning septal stimulation does not effect the somatic field EPSP in the dentate population spike evoked by perforant path stimulation (Bilkey and Goddard, 1985 and 1987; Mizumori et al., 1989).

MS-VLDBB stimulation may also sometimes evoke IPSPs in intracellularly recorded hippocampal pyramidal neurones (Krnjevic et al., 1988). These IPSPs occurred after relatively long latencies (of 10-12 ms). The authors stated that such IPSPs were more dominant when the stimulating electrode abutted structures such as the dorsal fornix. Therefore, these IPSPs could be due to activation of afferents/efferents in the dorsal fornix due to current spread. Alternately, MS-VLDBB afferents may, by a direct effect or by activation of inhibitory interneurones, inhibit the pyramidal cells.

The MS-VLDBB complex is implicated in the modulation of *theta rhythm* in the hippocampal (Bland, 1986). Theta rhythm (or rhythmic slow activity; RSA) is sinusoidal wave-like activity recorded in the hippocampus EEG. Bland (1986), in a comprehensive review, has enumerated the following reasons for associating the MS-VLDBB in the regulation of hippocampal theta rhythm: (a) lesions of the MS-VLDBB complex completely

abolished theta activity, (b) electrical stimulation of the MS-VLDBB complex, at a frequency that is consistent with the frequency of naturally occurring theta, induces theta rhythm in the hippocampus, (c) a group of neurones in the MS-VLDBB complex fire in a rhythmic fashion and the total frequency distribution of the discharge of these neurones produced a curve similar to the theta wave in shape, (d) hippocampal units fire in phase to the theta rhythm. The rhythmic firing of these neurones is abolished by localized lesions in the medial septal nucleus, and (e) disconnection of the hippocampal afferent input to the septal nuclei does not decrease the proportion of rhythmically firing neurones in the medial septal nucleus.

Theta rhythm has been subdivided into 2 types (Bland, 1986): (1) type-1 which occurs associated with a certain class of movement, has a frequency of 7-12 Hz in rats and is abolished by anaesthestics, (2) type-2, or immobility-related theta, has a lower overall frequency range of 4-9 Hz in rats, occurs during immobility and is resistant to anaesthetics such as urethane. This type of theta rhythm may also be produced upon sensory stimulation of the animal.

In an analysis of hippocampal neuronal firing in relation to the theta wave, Fox et al. (1986) found that, in urethane anaesthetized rats, the majority of hippocampal CA1 complex-spike pyramidal cells and interneurones were phase locked to the theta rhythm. The pyramidal cells and interneurones showed a greater probability of firing on the negative and the positive phase of the CA1 theta wave, respectively. Similar findings were reported by Buzsaki and Eidelberg (1983). Consistent with these reports, the amplitude of evoked population spike is maximal in the CA1 region if the commissural afferent stimulus is presented just after the positive peak of the dentate theta wave which corresponds with the negative phase of the CA1 theta wave (Rudell and Fox, 1984).

Intracellularly recorded CA1 pyramidal cells show a transmembrane oscillation of membrane potential in the theta frequency range (Leung and Yim, 1986) which is approximately 180° phase-reversed from the extracellular theta recorded in str. oriens of the hippocampus. Further, a reversal of evoked IPSPs in these neurones by current injection or ion leakage also reverses intracellular theta resulting in an in phase relationship with extracellular theta. From these observations the authors suggest that the theta rhythm in CA1 pyramidal cells is caused by a rhythmic modulation of somatic IPSPs. This may occur by regulating the activity of interneurones having inhibitory input onto pyramidal cells.

PHARMACOLOGY OF THE CHOLINERGIC SEPTO-HIPPOCAMPAL AFFERENTS

The hippocampus and the dentate gyrus receive their cholinergic input mainly from the MS-VLDBB complex. Thus, lesions placed in this complex or of fornix/fimbria result in a massive loss of cholineacetyltransferase (ChAT) and acetylcholinesterase (AchE) activity in the hippocampal formation (Fonnum and Walaas, 1978; Lewis et al., 1967; Matthews et al., 1987). Consistent with this finding, destruction of both the hippocampal pyramidal neurones and dentate granule cells with an intrahippocampal kainic acid injection did not alter the distribution and intensity of AchE staining or ChAT activity (Fonnum and Walaas, 1978). Further, electrical stimulation of the MS-VLDBB region evoked the release of acetylcholine (Ach) in the hippocampal formation (Dudar, 1975). Indeed, MS-VLDBB neurones, identified by retrograde transport of tracer as projecting to the hippocampus can also be stained for ChAT-like immunoreactivity (ChAT-LI; Amaral and Kurz, 1985).

Matthews et al. (1987) have provided a detailed description of the distribution of ChAT-LI fibres and terminals in the hippocampus and the dentate gyrus. The ChAT-LI fibres have a varicose appearance and traverse the fimbria, the alveus and are also observed

in the dorsal fornix. They are distributed in all regions of the hippocampus and the dentate gyrus but are more abundant in regio inferior. The ChAT-LI terminals are distributed throughout the hippocampus and the dentate gyrus. They are also observed in all laminae including the str. oriens and the str. radiatum. A limited number of ChAT-LI neurones are also observed in the hippocampus and the dentate gyrus. In the hippocampus these neurones are located primarily in the str. lacunosum-moleculare of field CA1. MS-VLDBB lesions virtually abolished all ChAT-LI terminals and fibres in the hippocampus and the dentate gyrus indicating their septal origin. Frotscher and Leranth (1985), in an electron microscopic analysis, have indicated that the ChAT-LI axons in the hippocampal formation are unmyelinated. The axonal terminals make synaptic contact with dendritic spines in the dendritic layers of the hippocampus and in the molecular layer of the dentate gyrus. The authors suggested that, since most neurones in the hippocampus and the dentate gyrus having dendritic spines are pyramidal cells or granule cells, the ChAT-LI fibres make terminal contact with the dendrites of these neurones. However, synaptic contact of these fibres with aspinous dendrites of non-pyramidal neurones has also been described (Frotscher and Leranth, 1985). The ChAT-LI fibres also make synaptic contact with the cell bodies of pyramidal cells and dentate granule cells.

Radioligand binding studies using cholinergic antagonists have identified both muscarinic and nicotinic binding sites in the hippocampal formation. Thus, specific binding of tritiated 3-quinuclidinyl benzilate ([3H] QNB), a potent cholinergic muscarinic antagonist, is observed in different laminae of the hippocampus and the dentate gyrus including in the str. oriens, str. radiatum and str. moleculare (Kuhar and Yamamura, 1976). Kuhar et al. (1981), in an electron microscopic analysis, have indicated that a significant fraction of muscarinic ligand binding sites are localized over synapses on

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dendrites. Electrophysiological studies have also suggested that Ach has a strong effect on the hippocampal pyramidal neuronal activity mediated by muscarinic receptors. For example, iontophoretic Ach increased the activity of hippocampal pyramidal cells in anaesthetized rats and was antagonized by iontophoretically applied muscarinic antagonists, scopolamine and quinuclidinyl benzilate (Bird and Aghajanian, 1976). The antagonism was specific since the increase in activity of pyramidal cells to iontophoretic glutamate was not altered. The effect of Ach was mimicked by muscarinic agonists such as muscarine, methacholine and bethanechol applied iontophoretically. In the hippocampal slice preparation, Ach, applied either by superfusion (Cole and Nicoll, 1983) or in form of droplets near the recording site (Segal, 1982), induced a variety of changes in intracellularly recorded CA1/CA3 pyramidal neurones. These changes included (a) depolarization which may be accompanied by an increase in cell membrane resistance, (b) block of afterhyperpolarization (AHP) evoked in response to a brief depolarizing current pulse, and (c) a reduction in accomodation of cell activity to depolarizing pulses. These effects of Ach were also mimicked by muscarinic agonists (muscarine, pilocarpine and carbachol) and antagonized by atropine (Cole and Nicoll, 1983). Further, Ach and muscarinic agonists like carbachol applied to hippocampal slices reduced the intracellular EPSP evoked in CA1 pyramidal cells by stimulation of Schaffer-collaterals/ commissural afferents in str. radiatum (Dutar and Nicoll, 1988; Segal, 1982). This effect is also antagonized by atropine. Studies involving field potential analyses have further indicated a strong muscarinic receptor mediated effect of Ach on hippocampal neurones. Thus, iontophoretically applied Ach or muscarinic agonists in the cell body region enhanced the CA1 population spike evoked by Schaffer-collateral/commissural stimulation (Rovira et al., 1983b). These responses could be antagonized by muscarinic antagonists. When applied in

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the apical dendritic region, Ach or muscarinic agonists decreased the apical dendritic synaptic field EPSP and the concomitantly recorded population spike in the CA1 cell body region. Iontophoretically applied muscarinic antagonists, atropine and scopolamine, antagonized the effect of Ach on the synaptic field EPSP.

A sub-classification of muscarinic receptors into M_1 and M_2 receptor types is also proposed based on high and low affinity binding of the muscarinic antagonist, pirenzipine, to these sites (Hammer et al., 1980; Watson et al., 1986). Muscarinic antagonists, such as AF-DX 116 and gallamine, bind preferentially to the M_2 site (Watson et al., 1986; Cortes and Palacios, 1986). Atropine, however, does not differentiate between these two sites. Both M_1 and M_2 binding sites have been identified in the hippocampus with the former predominating (Cortes and Palacios, 1986; Hammer et al., 1980; Watson et al., 1986). In functional studies, both atropine and the M_2 ligand, AF- DX 116, antagonized the decrease in K^+-evoked Ca^{2+}-dependent release of glutamate produced by Ach from rat hippocampal synaptosomes (Marchi and Raiteri, 1989). The M_1 ligand, pirenzipine, and the nicotinic antagonist, mecamylamine, had no effect on this Ach response. The AF-DX 116 antagonism of the Ach effect is dose-dependent. AF-DX 116 also antagonized, in a dose-dependent fashion, the inhibition by Ach of K^+evoked release of acetylcholine from hippocampal synaptosomes (Marchi and Raiteri, 1989) or slices (Gulya et al., 1989). However, AF-DX 116 was 80-100 times less potent in antagonizing this cholinergic response (Marchi and Raiteri, 1989). Consistent with the above effects, the putative M_2 antagonist, gallamine, antagonized the depression of the synaptic EPSP by the muscarinic agonist, carbachol, recorded intracellularly from CA1 pyramidal neurones in the slice preparation (Dutar and Nicoll, 1988). This putative M_2 antagonist also prevented the carbachol blockade of a voltage- and time-dependent K^+ current, called the M-current. But gallamine had a very

weak antagonistic effect on carbachol-induced depolarization, increase in cell membrane resistance and block of AHP in pyramidal neurones.

The M_1 ligand, pirenzipine, antagonized in a dose-dependent fashion the various effects of carbachol on CA1 pyramidal neuronal excitability (Dutar and Nicoll, 1988). Atropine behaved similarly when tested at a single dose.

Nicotinic binding sites are also implicated in the hippocampus (Schwartz, 1986). However, the involvement of the nicotinic receptor in the Ach effect on hippocampal pyramidal cells is less clear. For example, *in vivo* iontophoretic application of nicotinc agonists near the cell body recording site did not produce a consistent or specific effect on pyramidal cell activity (Bird and Aghajanian, 1976). Similarly, many nicotinic antagonists iontophoresed at cell body recording sites also produced nonspecific changes in pyramidal cell excitability. However, nicotinic antagonists, dihydro-β-erythroidine and gallamine, produced a specific antagonism of the excitatory effects of Ach as well as the muscarinic agonists, muscarine and methacholine. The Ach evoked changes recorded intracellularly from pyramidal neurones in hippocampal slices are not antagonized by the nicotinic antagonist, d-tubocurarine (dTC; Cole and Nicoll, 1983; Segal, 1982). However, cholinergic pharmacological manipulation of hippocampal field potentials recorded *in vivo* in anaesthetized rats indicate a nicotinic involvement (Rovira et al., 1983b). Thus, both muscarinic and nicotinic agonists, when applied iontophoretically near the cell body recording site, enhanced the CA1 population spike evoked upon Schaffer-collateral/commissural afferent stimulation. This effect was antagonized by respective antagonists. When applied in apical dendrites, muscarinic agents and Ach reduced the synaptic field EPSP, and concomitantly, the population spike. This effect was antagonized by iontophoretic atropine and scopolamine. In contrast, the nicotinic agonist,

dimethylphenyl piperazine (DMPP), enhanced the field EPSP with an accompanying increase in the population spike. This effect was antagonized by the nicotinic antagonist, dTC, but not by atropine or scopolamine.

Stimulation of cholinergic afferents to the hippocampus mimicks the response to exogenously applied Ach. For example, in the hippocampal slice, tetanic stimulation of str. oriens, through which cholinergic hippocampal afferents course, produces a slow depolarization in intracellularly recorded CA1 pyramidal neurones accompanied by an increase in membrane resistance (Cole and Nicoll, 1983). These effects were blocked by superfusion of the slice with atropine. Superfusing physostigmine, a reversible cholinesterase inhibitor, increased the size and duration of the stimulation-induced depolarization resulting in a spike discharge. Thus, the authors concluded that the str. oriens stimulation-induced depolarization is a slow muscarinic EPSP. Such stimulation also reduced AHP and accomodation and these effects were also enhanced by physostigmine (Cole and Nicoll, 1983).

In experiments in anaesthetized rats, tetanic stimulation of the MS-VLDBB facilitated the CA1 population spike, an effect which was also observed with iontophoretic Ach applied in the pyramidal cell body region (Krnjevic and Ropert, 1982). Both the septal and Ach facilitation of the CA1 population spike was antagonized by a sytemically administered muscarinic antagonist (atropine 7-26 mg/kg, i.v. or 39 mg/kg, i.p., scopolamine 4-40 mg/kg, i.v.; Krnjevic and Ropert, 1982). These effects were also antagonized by iontophoretic atropine and scopolamine (Rovira et al., 1983 a) and enhanced by iontophoretic physostigmine (Krnjevic and Ropert, 1982) when these drugs were applied near the cell body recording site. Intraventricular administration of hemicholinium-3, which strongly reduces the hippocampal acetylcholine content, diminished the facilitation of the

CA1 population spike by septal tetanic stimulation (Glavinovic et al., 1983). The above observations strongly suggest that the MS-VLDBB facilitation of the CA1 population spike is mediated via Ach released in the hippocampus. The septal-cholinergic facilitatory effect on the CA1 population spike may be due to disinhibition (Ben-Ari et al., 1981; Krnjevic et al., 1981; Krnjevic et al., 1988). Indeed, in anaesthetized rats, MS-VLDBB tetanic stimulation suppressed the spontaneously ongoing IPSPs in pyramidal cells and decreased the amplitude of IPSPs evoked in these neurones following fimbria or alveus stimulation (Krnjevic et al., 1988). Similarly, iontophoretic Ach, applied near the pyramidal cell body recording site, reduced the IPSP due to fimbrial or entorhinal cortical stimulation (Ben-Ari et al., 1981). However, such application of Ach did not reduce the hyperpolarization due to GABA iontophoretically applied near the cell body recording site. Since GABA is a putative inhibitory neurotransmitter in the hippocampus, the authors concluded that Ach produced disinhibition by reducing the release of GABA from inhibitory interneurones impinging on pyramidal cells. Indeed, iontophoretic bicuculline, a GABAa antagonist, when applied near the cell body recording site, increased the hippocampal population spike amplitude, whereas, similarly applied GABA depressed the spike amplitude (Krnjevic et al., 1981). Further, hippocampal paired-pulse depression, which has a GABAergic component, was reduced by cholinomimetics iontophoretically applied near the pyramidal cell body recording site (Rovira et al., 1983b).

Tetanic stimulation of the MS-VLDBB complex reduced the apical dendritic synaptic field EPSP of hippocampal pyramidal cells, an effect which is mimicked by the apical dendritic iontophoresis of Ach (Rovira et al., 1983a). Muscarinic antagonists, when administered either systemically or iontophoretically near the pyramidal cell dendritic recording site, antagonized both the MS-VLDBB tetanus- and Ach-induced depression of the

synaptic field EPSP. The septal-cholinergic mediated reduction in CA1 pyramidal cell apical dendritic synaptic field EPSP may be due to presynaptic inhibition of the excitatory drive. Indeed, iontophoretic application of Ach in the apical dendrites depressed the Schaffer-collateral stimulation-evoked synaptic EPSP in pyramidal neurones recorded intracellularly, but it did not attenuate depolarizations evoked in these neurones by brief apical dendritic applications of glutamate (Valentino and Dingledine, 1981). These authors ruled out an effect of Ach on the postsynaptic dendritic membrane because the time course of the EPSP was not altered.

From the above it follows that the septal-cholinergic input to the hippocampus can, by decreasing either the inhibitory input impinging on the pyramidal cell bodies or the excitatory drive at the apical dendrites of the pyramidal neurones, alter the synaptic excitability of CA1 pyramidal neurones. The decrease of the excitatory drive in the hippocampus by MS-VLDBB-cholinergic input may be particularly sensitive to antagonism by putative M_2 antagonists.

Bland (1986) also suggested that the hippocampal type-2 theta rhythm is mediated by the septo-hippocampal cholinergic input based on the following evidence: (a) type-2 theta is abolished by injections of atropine sulphate (5 mg/kg, i.v., or 25-50 mg/kg, i.p.) but not by atropine methylnitrate. Type-1 theta was relatively unaffected by these doses of atropine sulphate, (b) physostigmine (0.8-1 mg/kg, i.p.) elicited type-2 theta and (c) intraventricular administration of hemicholinium-3, which depletes the endogenous stores of Ach, severely attenuated type-2 theta. The same treatment had no effect on type-1 theta. Indeed, there is a parallelism in the effect of the above drugs at the doses used on the type-2 theta rhythm and the inhibition of septal facilitation on CA1 pyramidal cell excitability.

HIPPOCAMPUS IN NOCICEPTION

Pain and nociception

The International Association for the Study of Pain subcommittee on taxonomy defined pain as " an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage " (Merskey et al., 1979). Stimuli which produce or are potentially capable of producing tissue damage are called noxious (Merskey et al., 1979). Pain is a subjective experience. However, the word pain is not used with reference to animals since they cannot verbally communicate the subjective quality to a noxious stimulus. Nevertheless, it is assumed that they experience unpleasant sensations when exposed to noxious stimuli. The equivalent of pain in animals is termed nociception. Animals exhibit motor responses to noxious stimuli that are similar to those seen in humans. Such responses in animals comprise nocifensive behaviour which includes reflex responses (flexion reflex) and voluntary reactions (eg. escape and avoidance reactions). Noxious stimuli is also stressful to animals as evidenced by increased plasma glucocorticoid levels (Endroczi and Lissak, 1962; Endroczi et al., 1959; Guillemin et al., 1977).

Melzack and Casey (1968) identified three components of pain, namely (a) sensory-discriminative, (b) affective-motivational and (c) cognitive-evaluative. The sensory-discriminative component functions to analyze the location, rate of onset and intensity of noxious stimuli. The affective-motivational response may manifest as frustation, depression or anger (Mayer and Price, 1982). Some pain experiences are also characterized by misery, anguish, desperation and urgency (Melzack and Wall, 1982). Further, the affective-motivational aspect of nociception is intimately influenced by cognitive evaluation of the

situation in which the subject is exposed to a noxious stimulus (Mayer and Price, 1982; Melzack and Casey, 1968). This cognitive-evaluative component allows a comparison of the present input with past experience. Other cognitive factors which may influence nociception or pain include anticipation of pain, anxiety, attention, suggestion, placebos, and evaluation of the meaning of the pain producing situation.

The complexity of nociception or pain suggests that a wide variety of central nervous system (CNS) structures are involved following a noxious stimulus producing pain. The involvement of some of the CNS structures in various aspects of nociception is briefly described below.

CNS substrate for nociception

Melzack and Casey (1968) proposed that the following neural substrates subserve the different aspect of nociception: (a) the sensory-discriminative aspect of nociception is associated with the spinothalamic system, the ventrobasal complex of the thalamus and somatosensory cortex, (b) the affective-motivational component is related to a paramedial ascending system from the spinal cord to the reticular formation which in turn connects to the limbic system including the hippocampus, and (c) the cognitive-evaluative dimension which is associated, at least in part, with the cerebral cortex. The bulk of experimental work has been done to study the sensory-discriminative component of nociception/pain while relatively little is known about nociceptive transmission affecting the affective-motivational and cognitive-evaluative component. In the following pages a brief description is provided of evidence involving some of the CNS structures in nociception/pain.

Primary afferents

Painful sensory information from the skin can access the CNS via cutaneous nerves. The cutaneous nerves consist of several fibre types, including large diameter myelinated Aαβ-fibres, the small diameter Aδ-fibres and the unmyelinated C-fibres. Of these various types of cutaneous nerve fibres, it is the Aδ- and C-fibres which convey noxious information from the skin nociceptors to the spinal cord sites in the CNS (Perl, 1984). In humans, selective block of transmission in Aδ- and C-fibres in the radial nerve by application of the local anaesthetic, lidocaine, prevented pain sensation to electrical stimulation of the dorsum of the hand (Hallin and Torebjork, 1976). However, sensation to touch was retained. Indeed, repetitive microstimulation of a single nociceptive C-fibre evoked a sensation of pain which was, however, was not evoked by such stimulation of large diameter fibre types including Pacinian corpuscle, hair follicle and Merkel afferents (Willis 1985).

The characteristics of cutaneous nociceptive Aδ- and C-fibres have been investigated in detail in animals using extracellular recording techniques. These units have been classified into high threshold mechanoreceptor units (HTM) with Aδ- and C-axons (Burgess and Perl, 1967; Bessou and Perl, 1969) and polymodal nociceptor units (PMN) with C-axons (Bessou and Perl, 1969). HTM units responded to strong noxious pressure applied to their receptive field with a linear relationship between discharge rate and stimulus intensity. These units were resistant to activation from noxious radiant heat of their receptive fields. However, application of noxious radiant heat to the receptive field of HTM units with Aδ-axons frequently resulted in sensitization so that they became responsive to subsequent heat stimulation (Campbell et al., 1979; Fitzgerald and Lynn, 1977). They, however, did not respond to irritant chemicals (Burgess and Perl, 1967).

The unmyelinated PMN units responded to noxious pressure, noxious heat and application of irritant chemicals to their receptive fields (Bessou and Perl, 1969). The authors observed a graded increase in response of these units to increasing skin temperature. Heat sensitization of these units also occurred after a single strong heating of the receptive field.

Anatomical studies using tracer techniques have found that the small diameter myelinated and unmyelinated primary afferents, some of which respond to noxious stimuli terminate principally in the superficial spinal cord dorsal horn (Laminae I and II; Willis 1985).

spinal cord

There are at least two types of spinal cord dorsal horn neurones which are excited by the noxious information entering the spinal cord. These are the wide dynamic range (WDR) or multireceptive neurones and nociceptor-specific (NS) neurones. The former class of neurones is excited by both noxious and innocuous stimuli whereas NS neurones are excited only by noxious stimuli. The heaviest concentration of nociceptive neurones occurs in the region of laminae I-II and V-VII of the spinal cord.

The WDR neurones are mostly concentrated laminae V-VI of the spinal cord deep dorsal horn. The electrophysiological characterisitcs of these neurones have been described by a number of authors in anaesthetized (Handwerker et al., 1975; Price and Browe, 1973; Willis, 1985) and conscious animals (Hoffman et al., 1981). These neurones, as mentioned earlier, are excited by both innocuous mechanical stimulation and noxious pinch of their peripheral receptive fields. A high proportion of such neurones are also activated to be stimulated by noxious heating of their receptive fields with a graded increase in the response

to increasing skin temperature. There is a prominent after-discharge following the removal of the thermal stimulus. Some of these neurones also discharge to innocuous cooling or warming of their receptive fields. The deep dorsal horn WDR neurones have been reported to receive inputs from both large diameter (Aαβ) and small diameter (Aδ, C-fibre) afferents (Handwerker et al., 1975; Light and Durkovic, 1984).

Some of the deep dorsal horn WDR neurones have axons which ascend the spinal cord to supraspinal structures. For example, some of the spinocervical tract (Cervero et al., 1977), spinothalamic tract (Rucker et al., 1984), spinomesencephalic (Yezierski and Schwartz, 1984), spinoreticular (Willis, 1985) and spinohypothalamic tract neurones (Burstein et al., 1987) have WDR characteristics.

Spinal dorsal horn neurones which are driven solely by noxious stimulation of their receptive fields have been described by various authors (see review by Willis, 1985). Although NS neurones have also been reported in the deep dorsal horn of the spinal cord (Light and Durkovic, 1984), most of these neurones are concentrated in laminae I and II of the superficial dorsal horn. These neurones respond to both Aδ- and C-fibre input. At least some NS lamina I neurones project to supraspinal structures. For example, Craig and Kniffki (1985) reported that a number of NS neurones from lamina I could be antidromically activated from the contralateral thalamus.

Ventrobasal thalamus and somatosensory cortex

The ventroposterior lateral nucleus (VPL) of the ventrobasal thalamus may subserve the sensory-discriminative aspect of nociception. Nociceptive neurones have been identified in the thalamic VPL in rats, cats and monkeys (Willis, 1985). These neurones have WDR or NS characteristics (Willis, 1985) and can often be shown to encode the

intensity of noxious heat stimuli. Further, in monkeys, the nociceptive neurones have restricted receptive fields which are somatotopically organized (Willis, 1985).

Nociceptive neurones are also reported in the cerebral cortical region SI (Willis, 1985). However, not much is known about the characteristics of these neurones.

Reticular formation and the medial system

The nucleus reticularis gigantocellularis (NGC) and central gray of the medullary and mesencephalic reticular formation are considered important for the affective-motivational response to pain/nociception (Mayer and Price, 1982; Melzack and Casey, 1968; Melzack and Wall, 1982). In animals, stimulation of the NGC or the central gray results in escape while lesions of these areas markedly reduced the animal's responsiveness to noxious stimuli (Mayer and Price, 1982; Melzack and Wall, 1982). Indeed, in conscious cats, neurones in the NGC have WDR characteristics and fire maximally when the animal escapes to avoid electric shock to a cutaneous nerve (Casey, 1971; Casey et al., 1974). These structures are part of the medial pain system which also includes the intralaminar and medial thalamic nuclei and the limbic forebrain strucutres (Melzack and Wall, 1982; Vogt, 1985; see discussion).

Involvement of hippocampus in nociception

Melzack and Casey (1968) proposed, based on experimental evidence involving stimulation and lesioning limbic structures as well as limited clinical evidence, that the limbic forebrain structures, including the hippocampus, are involved in " aversive drive and affect that comprise the motivational dimension of pain " . Based on lesion studies, O'Keefe and Nadel (1978) suggested that the hippocampus is involved in making of a cognitive map

of the animals environment. This can indirectly affect the animals " motivational process ".

For example, the hippocampus may be involved in associating pain or fear with a particular

place where the animal received a painful stimulus. This may explain deficits in avoidance

behaviour to escape electric footshock in hippocampal lesioned animals (Miller et al., 1975;

Teitelbaum and Milner, 1963; see later in the text).

The following is a description of the evidence involving the hippocampus in

nociception organized under four sections: (a) clinical evidence, (b) electrical and chemical

stimulation of the hippocampus, (c) lesioning of the hippocampus, and (d)

electrophysiological evidence.

Clinical evidence

There is limited evidence for hippocampal involvement in pain in human subjects

and the evidence available is indirect. For example, surgical section of the cingulum bundle,

which connects the posterior frontal cortex to the hippocampus, results in a loss of " negative

effect " associated with intractable pain (Foltz and White, 1962). More recently, Hebben et

al. (1987) reported that a patient who underwent bilateral removal of medial temporal

structures, including the anterior hippocampus and parahippocampal gyrus, was poorly

responsive to even the most intense noxious stimulus applied to his forearm or chest.

Electrical and chemical stimulation of the hippocampus

MacLean and Delgado (1953) and Delgado (1955) reported that electrical

stimulation in the anterior hippocampus and the crus fornix in cat and monkey produced

responses which mimicked painful behaviour such as vocalization, offensive and defensive

escape responses and autonomic manifestations.

Others have also stimulated in the hippocampus and studied changes in animal behaviour and hippocampal electrical activity (Andy and Akert, 1955; Delgado and Sevillano, 1961; MacLean, 1957; Siegel and Flynn, 1968). Stimulation in the hippocampus can produce an epileptic-like afterdischarge which can be propagated to other structures. Electrical stimulation of the posterior, dorsal and ventral hippocampus at intensities at or below threshold for eliciting an afterdischarge in cats (MacLean, 1957; Siegel and Flynn, 1968) or rats (MacLean, 1957) failed to elicit any dramatic or consistent behavioural response. Similarly, no overt changes in animal behaviour were observed when the hippocampal afterdischarge was confined to this structure (Andy and Akert, 1955; Delgado and Sevillano, 1961). However, behavioural changes occurred when the hippocampal afterdischarges propagated to other structures. The most frequently described behavioural change in cats and rats was a lack of spontaneous movement (Andy and Akert, 1955; MacLean, 1957; Delgado and Sevillano, 1961). MacLean (1957) described the animals to be in a " catatonia-like state ". Occasional vocalization and autonomic manifestations such as dilatation of the pupils, changes in blood pressure or respiration have also been reported (Delgado and Sevillano, 1961; Maclean, 1957). Convulsive movements involving facial twitching have been described. Delgado and Sevillano (1961) emphasized that motor, autonomic and postural changes become pronounced with repeated hippocampal stimulation producing an afterdischarge in this structure. Following hippocampal afterdischarge the animal may be hyperreactive, walking and running (Delgado and Sevillano, 1961) and show enhanced pleasure and grooming reactions (MacLean, 1957).

Animal responses to sensory stimulation can also be altered during the period of propagated hippocampal afterdischarges. For example, Delgado and Sevillano (1961) reported deficits in animal avoidance behaviour to painful electric footshock. Similarly,

MacLean (1957) found that animals were underreactive to noxious stimuli of moderate intensity. Abbott and Melzack (1978) have also reported a prolonged antinociception in the rat formalin test following afterdischarges evoked by stimulating in the dorsal hippocampus. This antinociception coincided with increased exploratory behaviour in the animals. Antinociception in the rat tail-flick test has also been reported following electrical stimulation in the dorsal hippocampus (Prado and Roberts, 1985). However, these authors did not study the effect of such electrical stimulation on the hippocampal EEG.

Finally, microinjection of acetylcholine into the anterior hippocampus can produce changes in animal behaviour mimicking painful responses (MacLean and Delgado, 1953). However, in a later study, MacLean (1957) failed to observe any overt behavioural changes to acetylcholine microinjection in the posterior hippocampus. Angry, attacking behaviour of the animal was observed when the drug was injected into the ventricles. MacLean (1957) noticed that carbachol injected into the hippocampus produced epileptic afterdischarges and behavioural changes as described above for electrical stimulation in this structure.

In summary, in some experimental studies electrical or chemical stimulation of the hippocampus evoked responses which mimicked nociceptive behaviour or produced antinociception. However, stimulation studies involving the hippocampus are handicapped due to stimulation-produced epileptic-like afterdischarges in this structure.

Lesioning of the hippocampus

Earlier investigations have concentrated on qualitative changes in animal behaviour following lesions involving the hippocampal formation. For example, Bard and Mountcastle (1947) and Rothfield and Harmon (1954) reported that bilateral removal of the cat hippocampus or fornix along with the overlying neocortex failed to alter animal reactions to

rough handling and noxious stimuli. Similarly, Brady and Hunt (1955) reported that bilateral ablation of the hippocampus in rats did not alter the animal " affective " responses to pinching of the tail. However, the authors noticed that these animals were deficient in their ability to localize and attack the source of the noxious stimulus.

Hippocampal lesions produced deficits in animal behaviour to noxious stimuli under more contrived experimental conditions. For example, Brady and Hunt (1955) reported that rats trained to press a lever for a water reward, when conditioned with noxious electric footshock, showed deficit in lever pressing, accompanied by crouching, immobility and defecation (conditioned emotional response, CER). Bilateral damage of the hippocampus prevented such an CER in animals. In another paradigm, rats trained to enter a compartment for food reward were footshocked during one such entry (Issacson and Wickelgren, 1962). Following escape, neocortical ablated animals were reluctant to re-enter the compartment (i.e. showed passive avoidance) but the hippocampal lesioned animals did not alter their reward directed behaviour (i.e. showed deficit in passive avoidance). In a simpler version of these experiments, rats were exposed to a compartment which had an electrified floor and a safe platform (Teitelbaum and Milner, 1963). Both normal and hippocampus lesioned animals receiving an electric footshock escaped to the safe platform, but only normal animals showed a reluctance to step down from the safe area. Similar observations have also been made in slightly different versions of this test procedure (Blanchard and Fial, 1968; Best and Orr, 1973; Miller et al., 1975).

Hippocampal lesions can improve avoidance behaviour in a two-way avoidance task. In this task, the animals, in order to avoid being footshocked in either of two connected compartments, cross over to the unoccupied compartment (Olton and Issacson, 1968). Blanchard et al. (1970) suggested that deficits in passive avoidance and improved two-way

avoidance following hippocampal lesions may be related to increased locomotor activity in the animals. Because the hippocampus lesioned animals are more active than controls they would be more inclined to step down from the safe area in the passive avoidance test and move more often from one compartment to another in a two-way avoidance task. However, improved avoidance is not seen in hippocampus lesioned rats when the animals have to escape footshock from one compartment to a connecting, safe compartment (i.e., one-way task; Olton and Issacson, 1968; Black et al., 1977).

Other authors (Black et al., 1977; O'Keefe and Nadel, 1978) suggested that hippocampal lesions disrupt " place strategy " or the " cognitive spatial map ". That is, the animals are unable to associate nociception or fear with a particular place where they received a noxious stimulus. This results in deficits in avoidance behaviour in passive avoidance and one-way avoidance. In the two-way avoidance task, hippocampectomized animals would not exhibit a tendency to avoid previously shocked locations and, therefore, move more readily between the two compartments.

The footshock threshold to induce a jump response is decreased in hippocampus lesioned rats (Blanchard and Fial, 1968; Eichelman, 1971). An increase in footshock threshold to induce vocalization is also observed in hippocampus lesioned rats (Blanchard and Fial, 1968).

Electrophysiological evidence

Electrophysiological recordings of evoked potentials and single cell activity in the hippocampus have provided information on changes in neuronal excitability to sensory stimuli including a few instances of noxious stimuli. For example, Brankack and Buzsaki (1986) found that acoustic or toothpulp stimulation in conscious rats elicited potentials in

the CA1/CA3 fields of the hippocampus and in the dentate gyrus. In each case the evoked potential consisted of a negative deflection preceded by a smaller positive potential. Based on laminar-depth profile analysis, the authors suggested that the negative potential was due to: (a) an excitatory entorhinal cortical input to the dendritic layers of granule and CA3 pyramidal neurones, and (b) an excitatory Schaffer-collateral input to the apical dendrites of CA1 pyramidal neurones. The short latency positive field, having the largest amplitude above the CA1 pyramidal cell layer and the hilus, was suggested to be due to postsynaptic inhibition of the principal neurones by inhibitory neurones activated by subcortical inputs. Similarly, Deadwyler et al. (1981) suggested that a subcortical input to the hippocampal formation from the medial septal nucleus may exercise a suppressive influence on neuronal excitation. They reported that in medial septal nucleus lesioned rats, but not in normal rats, an unconditioned sensory tone stimulus produced a late negative going potential (N_2) which had its maximum amplitude in the molecular layers of the dentate gyrus. Indeed, unidentified neurones of the medial septal nucleus are activated by an auditory tone stimulus as well as air puffs applied to the cornea of conscious rabbits (Berger and Thompson, 1977). Furthermore, septo-hippocampal neurones are activated by intense peripheral noxious heat stimuli applied in anaesthetized rats (Dutar et al., 1985).

The spontaneous activity of unidentified hippocampal CA1/CA3 and dentate gyrus neurones may be decreased and/or increased in response to noxious (footshock) or non-noxious (visual, auditory and touch) stimuli to conscious, paralyzed rats (Miller and Groves, 1977). Brankack and Buzsaki (1986) reported that, in conscious rats, the spontaneous activity of CA1 pyramidal neurones is depressed to tooth pulp or auditory stimuli. They illustrated one CA1 interneurone whose spontaneous activity increased following auditory stimulation. Granule cell and multiple unit activity recorded from the

dentate granule cell layer was also increased following tooth pulp or auditory stimulation. Tooth pulp stimulation also produced an increased discharge in the hilar pyramidal neurones. Similarly, Vinogradova (1975) and Vinogradova and Brazhnik (1977) reported that the spontaneous activity of the majority of presumed hippocampal pyramidal cells was decreased following the presentation of auditory stimuli in conscious rabbits. There was a habituation to repeated auditory stimuli which was accompanied by adaptive changes in the animal's orienting response. In conscious rats, fur stroking depressed the hippocampal CA1 population spike produced upon Schaffer-collateral or perforant path stimulation and, at the same time, enhanced the amplitude of perforant path stimulation-induced dentate gyrus population spike (Herreras et al., 1988a). These authors also reported that fur stroking decreased the probability of orthodromic activation of CA1 pyramidal neurones but increased the spontaneous activity and the probability of orthodromic activation of CA1 theta neurones. In anaesthetized rats, noxious heating of the tail increased the discharge rate of unidentified hippocampal CA1 neurones (Sinclair and Lo, 1986).

Consistent with the suggestion that MS-VLDBB input exerts an inhibitory influence, lesioning the medial septal nucleus prevented the depression of spontaneous activity of the hippocampal formation neurones to sensory stimuli (Miller and Groves, 1977). Such lesions also markedly reduced the number of hippocampal neurones showing depression of spontaneous activity to the presentation of sensory stimuli (Miller and Groves, 1977; Vinogradova and Brazhnik, 1977).

Further, strong electrical stimulation or pinching of the tail in conscious rats synchronizes the hippocampal EEG which lasts several seconds (Soulairac et al., 1967; Stewart and Vanderwolf, 1987). Similarly, hippocampal EEG synchronization, or theta

rhythm of 5-6 Hz frequency, was reported in unanaesthetized rabbits after a strong noxious stimulus (Jung and Kornmuller, 1938).

RATIONALE

There is some evidence described above that implicates hippocampus in nociception or pain. The clinical evidence is suggestive of hippocampal and the temporal lobe involvement in pain. In some animal experiments, stimulation of the hippocampus produced responses which mimicked nociceptive behaviour. Lesioning of the hippocampus increased the threshold to vocalization and decreased aggressive behaviour. Such lesions also disrupted the avoidance response, but not escape, of animals to a noxious stimulus. Such a subtle effect of the hippocampal lesions on avoidance affective-motivational behaviour may be due to disruption of the spatial cognitive map (Black et al., 1977; O'Keefe and Nadel, 1978). There is more recent evidence involving hippocampal formation in affective-motivational behaviour of the animal. Henke (1990) has reported that animals stressed using various procedures, including exposure to noxious stimuli, showed gastric pathology if the dentate population spike was depressed. Conversely, animals who did not show gastric pathology to stress had an enhanced dentate gyrus population spike.

Electrophysiological studies provide evidence that the hippocampal field CA1 neurones are involved in nociceptive transmission (Brankack and Buzsaki, 1986; Sinclair and Lo, 1986). Further, noxious stimuli can activate MS-VLDBB afferents to the hippocampus. The MS-VLDBB is the major source of cholinergic innervation of the hippocampal formation and stimulation of this complex produced cholinergically mediated changes in the excitability of CA1 pyramidal neurones (see above). Therefore, this study was aimed at investigating the effect of noxious stimuli on the excitability of hippocampal

CA1 pyramidal output neurones. Further, to investigate whether the MS-VLDBB cholinergic projection was involved in such excitabiltiy changes and if so to investigate the mechanism involved.

SPECIFIC OBJECTIVES

(a) To examine the effect of noxious heat stimuli on the CA1 population spike, tail-flick reflex and the hippocampal EEG.

(b) To examine the effects of hippocampal theta rhythm on the noxious heat-induced changes in the CA1 population spike.

(c) To investigate whether noxious heat induced changes in the CA1 population spike were topographically specific.

(d) To determine if noxious stimulus-induced change in CA1 pyramidal cell synaptic excitability was afferent specific.

(e) To determine whether pre- and/or postsynaptic components were involved in changes in CA1 excitability to a noxious heat stimulus.

(f) To determine whether the noxious heat-induced changes in CA1 pyramidal cell excitability were cholinergically-mediated.

METHODS AND MATERIALS

Anaesthesia and surgical procedure

The experiments were carried out in a Faraday radio frequency shielded room on male Sprague-Dwaley rats (250-300 g). The animals were lightly anaesthetized with urethane 1.0 g/kg, i.p. Deep anaesthesia during surgery was produced by supplementation with halothane (Fluothane[R]). Most animals underwent very limited surgery. The animals were held in a flat-skull position in a stereotaxic headholder (Narashigi) using the method of Paxinos and Watson (1982). The skin over the skull was cut along the midline and retracted. To permit the positioning of stimulating and recording electrodes, burrholes (about 3.0 mm in diameter) were drilled in the cranium with a Moto-tool (Dremel Manufacturing Company). Rectal temperature was monitored by an electronic thermometer and maintained within physiological limits by a feedback controlled heating blanket (Harvard). The entire preparation was isolated from the floor by inflating the supporting table (N_2 40 psi, Zero-G isolation Table). This " floating " table minimized inherent floor vibrations from being transmitted through the animal frame and microcarrier to the recording electrode.

In addition, the blood pressure in four animals was monitored via a polyethylene cannula (No. 60, Clay Adams) prefilled with dilute sodium heparin (Upjohn Company) and placed in a carotid artery.

Set up for stimulation and recording of CA1 field potentials

Under a stereoscope (Olympus), the duramater of the exposed brain areas was cut and reflected. In all experiments a concentric bipolar stimulating electrode (David Kopf

Instruments, Model NE-100) was directed towards field CA3 of the hippocampus either ipsilateral or contralateral to the CA1 recording site using the following landmarks : P 2.3 mm from bregma, L 1.4 mm from midline and V 3.5 mm from the cortical surface (Paxinos . and Watson, 1982). In a few experiments stimulating electrodes were positioned both in the ipsilateral and the contralateral CA3. The following stimulating electrode (David Kopf, Model NE-100) placements were also made in different experiments to stimulate (a) alveus, ipsilateral to CA1 recording site at P 4.8 mm from bregma, L 1.4 mm from midline, (b) medial septal-vertical limb of the diagonal band of Broca (MS-VLDBB) A 0.2 mm from bregma, L 0.0 and V 7.0 mm from the cortical surface. In this case the final vertical depth of the stimulating electrode was adjusted so that tetanic stimulation produced a facilitation of the CA1 population spike.

In most experiments electrical stimulation parameters were controlled using a Digitimer D4030 stimulator. This stimulator had multiple outputs which were connected to Digitimer isolation units which, in turn, were connected to the stimulating electrodes. A Grass S88 stimulator was used in some experiments. The D4030 or S88 stimulator was also used to trigger the oscilloscope sweep and the Apple IIe or Nora systems computer. This enabled the display of evoked field potentials on an oscilloscope and computer screen. Such field potentials were stored on a floppy or hard disk in the computer for later analysis.

The following field potential recordings were made from dorsal hippocampal field CA1 in different experiments: (a) orthodromic population spike evoked upon ipsilateral CA3 stimulation, (b) orthodromic population spike or antidromic spike evoked upon ipsilateral CA3 and alveus stimulation, respectively, (c) orthodromic population spikes evoked upon ipsilateral or contralateral CA3 stimulation, (d) orthodromic population spike evoked upon contralateral CA3 stimulation, (e) orthodromic population spike or corresponding apical

dendritic field excitatory postsynaptic potential (EPSP) evoked upon contralateral CA3 stimulation, and (f) apical dendritic field EPSP evoked upon contralateral CA3 stimulation. The recording site in field CA1 was P 3.8 mm from bregma and L 1.4 mm from midline (Paxinos and Watson, 1982). The piamater over this site was carefully removed under the stereoscope using a pair of fine watchmaker's forceps. The recording microelectrode (carbon fibre) was secured to a hydraulic microdrive holder (David Kopf Instruments) which, in turn, was mounted to a fine adjustable electrode carrier (Narashigi). In most experiments the electrode was placed perpendicular to the cortical surface over the pial opening and lowered by remote control to penetrate the hippocampus. In experiments where both the CA1 population spike and apical dendritic field EPSP were recorded, a second recording microelectrode, inclined at an angle of 20 from vertical, was directed into the apical dendritic region of field CA1 to record at the same mediolateral plane as the vertically directed electrode positioned in pyramidal cell region of CA1.

The signal picked up by the recording microelectrode was fed into a high impedance preamplifier (M707, W.P. Instruments), bandpass filtered at 3 KHz, amplified and subsequently displayed on a storage oscilloscope (Tektronix, Model 5111). The output of the oscilloscope was usually fed to an Apple IIe computer via an A/D interface (Stoelting Company, Cat. No. 47105) and stored using the Waveman program (Teyler et al., 1982; Stoelting Cat. No. 47101). In experiments where both orthodromic and antidromic field potentials were recorded, the evoked potentials were fed into an Nora systems computer and stored using a program written by Dr. David Harris.

In some experiments multibarrelled microiontophoretic electrodes were used to record either the CA1 population spike or the apical dendritic field EPSP. The use of this electrode assembly is described later in the text.

Except where noted, in all other experiments the contralateral dorsal hippocampal (DH) EEG was continuously monitored on a polygraph. For this purpose a concentric bipolar electrode (David Kopf Instruments, Model NE-100) was positioned in region CA1 of the contralateral hippocampus corresponding to the ipsilateral CA1 recording site. The output from the electrode was fed into a Grass A.C. preamplifier with 1/2 amplitude low and high filters set at 1 Hz and 35 Hz, respectively.

At the end of each experiment the recording and stimulating sites were lesioned and later histologically verified.

Application of noxious heat

Water in a porcelain dish was heated to and maintained at the desired noxious temperature by pumping hot water through a copper coil placed in the dish. The hot water in the coil was heated, maintained at constant temperature and pumped through the coils using a Haake circulating pump. The noxious stimulus consisted of placing and maintaining 3.0 cm of the distal end of the rat's tail in water at 50, 55 or 60° C for 15 sec. In some experiments 2.0 cm of the distal end of the left hind paw was similarly exposed to hot water at 55° C.

Preparation of carbon fibre and microiontophoretic microelectrodes

Carbon fibre electrodes were prepared according to the method of Armstrong-James and Millar (1979). A carbon fibre of suitable length was inserted into a glass capillary (KIMAX-51 capillaries of 0.8-1.10 x 100 mm size; Kimble Products) and pulled on a vertical microelectrode puller (Narashigi). The electrode formed had several centimeters of carbon fibre protruding from the tip. Under a light microscope, the fibre was trimmed and etched in

chromic acid at 0.12 mA current so that only 15 mm of carbon fibre protruded from the micropipette. The microelectrode was then filled with 4M NaCl solution and used for extracellular recording.

The four or five barrelled microelectrodes were constructed from glass capillaries with 1.5 mm outside diameter (Glass Company of America, Omega Dot Brand). These capillaries contained a single glass fibre strand to facilitate their filling by capillary action. Individual capillaries were bent to an obtuse angle at about 0.5 cm from one end. The vertical length of these bent capillaries from the bend to the opposite end was about 4.0 cm. Three or four of these capillaries were glued together at the bend with a straight capillary protruding about 3.0 cm. The straight capillary was 10 cm long. The lower end of this assembly was also held together by glue. After curing, the capillary assembly was pulled into a multibarrelled microelectrode using the Narashigi vertical puller. Here, the unit was heated via a coil midway between the glued ends and gently twisting by 180 and allowed to fall 0.5-1 cm by gravity as the glass melted. The heat source was turned off and the glass allowed to cool. It was then pulled in the normal fashion. Under a light microscope the electrode was broken so that the tip diameter was 4-5 mm and the various barrels were filled with appropriate solutions. The recording barrel of the microiontophoretic electrode containing the 4M NaCl solution and had a DC resistance of 10-20 megohms.

Physiological experiments

Experiments were designed to investigate the effect of peripheral noxious heat on field CA1 pyramidal cell excitability. Before describing the individual experiments, a few generalities should be mentioned. The recording electrode, upon penetrating the hippocampus (approximately 2500 μm deep from the cortical surface), picks up cellular

activity including the characteristic complex-spike bursting of hippocampal pyramidal cells. The position was then adjusted to produce the maximal field potentials upon CA3 stimulation. The CA1 population spike and apical dendritic field EPSP was evoked by stimulating CA3 with a square wave pulse at 0.1 or 0.2 Hz, 0.2 msec pulse duration. A given stimulating frequency was used throughout a series of experiments constituting an experimental group. In such cases, an output from the D4030 was obtained from the pulse mode on the matrix board. Except where noted, the CA3 stimulus intensity was adjusted to produce a population spike which was about 80 % of maximal. The apical dendritic field EPSP, when recorded, was also evoked at the same stimulus intensity. Alveus stimulation intensity was adjusted to produce an antidromic spike similar in amplitude to the population spike.

A given animal was exposed to noxious heat at one temperature only. In initial experiments, in order to ensure recording stability, the evoked CA1 population spike was monitored for about 1 hr before a heat stimulus was applied. Since little fluctuation occurred over this time period, control recordings were reduced to 20 min in later experiments. The dendritic field EPSP or the antidromic spike was also monitored for 20 min before the first noxious heat application. The following experiments were performed:

Temperature-dependent effect of noxious heat applied to the rat tail on the CA1 population spike

In these experiments ipsilateral CA3 was stimulated to evoke a CA1 population spike. Different groups of animals were exposed to noxious heat at 50, 55 or 60°C applied for 15 sec to the tail. A second application of noxious heat to the tail was given 72 min after the first application. Subsequent noxious exposures were made at shorter time intervals. In

these experiments, especially to compare changes accompanying repeated noxious heat applications at 50 and 55°C, the animal's reflex-reaction score and the duration of theta rhythm following noxious heat exposure were also determined. The animal tail-flick reflex reaction to a noxious heat stimulus was scored as explained later in the text. The relationship between spontaneously occurring hippocampal theta rhythm and CA1 pyramidal cell excitability to noxious stimulus was also identified. For this purpose, in different experiments, a noxious heat stimulus (55°C, 15 sec) was applied to the tail when the EEG was either desynchronized or in theta rhythm, and changes in the CA1 population spike were recorded.

In 4 experiments the tail was exposed to 55°C water only until a tail-flick occurred. A series of at least 5 tests, each at 4 min intervals, were performed while evaluating the effect on the CA1 population spike amplitude.

Effect of noxious heat stimuli applied to the left hind paw and later to the tail on the CA1 population spike

As described by Khanna and Sinclair (1989), and also later in the text, a noxious heat application to the tail at either 55 or 60°C for 15 sec produced a prolonged depression of the CA1 population spike. A habituation of this response was observed to subsequent noxious heat applications to the same receptive field. These experiments were designed to investigate whether the habituation of the persistent depression of the CA1 population spike showed topographical specificity.

The CA1 population spike was evoked upon ipsilateral CA3 stimulation. The left hind paw (LHP) of the animal was first exposed to a noxious heat stimulus (55°C, 15 sec). The change in the CA1 population spike in response to the first noxious heating of the left

hind paw was followed for 1 hr. Thereafter, another noxious stimulus was applied to the left hind paw. However, the effect of the second or a subsequent noxious exposure was followed for shorter time periods. The experiment was then repeated following the same protocol except that noxious stimuli were applied to the tail rather than the LHP.

Effect of a noxious heat stimulus on the antidromic spike and the population spike in field CA1

These experiments were performed to determine if postsynaptic excitability changes in CA1 pyramidal neurones are involved in the change in synaptic excitability of these neurones to a noxious stimulus. The alveus was stimulated alternately every 5 sec with the ipsilateral CA3 stimulation to produce a short latency antidromic spike and a CA1 population spike, respectively. Once control records were obtained a noxious heat stimulus (55°C, 15 sec) was applied to the tail and its effect on these potentials observed. At the end of the experiment a 20 or 50 Hz train for 100 msec was applied to the alveus to confirm the antidromic nature of the field potential.

Effect of a noxious heat stimulus on the CA1 commissural population spike

These experiments were done to determine whether noxious stimulus-induced persistent depression of CA1 pyramidal cell synaptic excitability was afferent input specific. Thus, CA1 population spikes were evoked upon Schaffer-collateral and commissural afferent-input activation following ipsilateral and contralateral CA3 stimulation, respectively. In these experiments, the stimulation source was manually alternated every minute via means of a switch box. Once control records were obtained, the noxious heat stimulus (55°C for 15

sec) was applied to the left hind paw. The change in the population spikes were recorded until recovery occurred.

Effect of noxious heat stimuli on the commissural CA1 population spike and apical dendritic field EPSP

These experiments were performed to determine whether the noxious stimulus-induced persistent depression of the CA1 population spike and the habituation of this response to subsequent noxious heat exposure involved similar changes in apical dendritic field EPSP. Thus, both the the CA1 population spike and the apical dendritic field EPSP, produced upon contralateral CA3 stimulation, were recorded in the same experiment. The depth of maximal dendritic field EPSP ranged 300-500 μm ventral to the CA1 population spike recording site. The population spike and the apical dendritic field EPSP were recorded alternately with a one minute run of the population spike followed by a minute run of the field EPSP. The noxious stimulus consisted of exposing the tail to hot water at 55°C for 15 sec. A second application of noxious heat to the same receptive area was given 1 hr after the first application.

Pharmacological experiments

Experiments were designed to investigate whether the cholinergic system of the hippocampus is involved in the noxious stimulus-induced persistent depression in synaptic excitability of the CA1 pyramidal cells. The cholinergic involvement was tested by (a) intraperitoneal administration of atropine sulphate, and (b) iontophoretic application of atropine either at the cell body or the apical dendritic recording site of the CA1 pyramidal neurones. The protocols for these experiments are described below:

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Effect of systemic administration of atropine sulphate on the noxious stimulus-induced

change in the amplitude of CA1 population spike

In these experiments the following stimuli were applied: (a) contralateral CA3 to evoke a commissural CA1 population spike, (b) the CA1 population spike was facilitated by a conditioning MS-VLDBB tetanic stimulus (0.4 msec pulse duration at 100 Hz for 70 msec) applied 30 msec preceding CA3 stimulation, and (c) paired-pulse stimulation, at inter-pulse interval of 100 msec, of the contralateral CA3.

Initially during the experiment, the CA3 stimulation intensity was adjusted to evoke a threshold CA1 population spike. Then two different intensities of MS-VLDBB and paired-pulse stimuli were selected to evoke facilitation of the CA1 population spike. Thereafter, the CA3 stimulation intensity was adjusted to evoke a population spike 80 % of the maximal (submaximal population spike) and recorded for 20 min. Then a noxious stimulus (55°C, for 15 sec) was applied to the LHP. Following recovery from the persistent depression of the submaximal CA1 population spike, the CA3 stimulation intensity was again adjusted to evoke a threshold CA1 population spike and evoked facilitation was re-examined. Atropine sulphate (40 mg/kg) was then infused intraperitoneally over 4 min via a cannula inserted during surgical preparation of the animal. The effect of atropine was followed on the submaximal population spike for 20 min. Then, between 20-30 min following atropine administration, the evoked-facilitation was tested on the threshold population spike. Finally, 50 min after atropine administration, the effect of a noxious heat stimulus applied to the tail was tested on the submaximal population spike.

Effect of iontophoretic atropine on noxious stimulus-induced persistent depression of CA1 synaptic excitability

Four or five barrelled microiontophoretic electrodes were used in these experiments. The recording barrel, filled with 4M NaCl, was used for recording CA1 field potentials evoked from contralateral CA3 stimulation. The remaining barrels contained 0.5 M Ach (pH 4.0; Sigma), 1.0 mM Atr in 165 mM NaCl or 0.2 M Atr sulphate (pH 5.0; Sigma) and normal saline. GABA (1.0 M, pH 4.5; Sigma) was also included in five barrel electrodes.

In one series of experiments, atropine and other drugs were iontophoretically applied at the cell body recording site in field CA1. Initially in each experiment, the effect of iontophoretic Ach and its antagonism by iontophoretic atropine was determined on the CA1 threshold population spike. Following recovery of the Ach effect from atropine antagonism, a submaximal commissural CA1 population spike was evoked and recorded for 20 min. Noxious heat (55°C, for 15 sec) was then applied to the LHP. Later, following recovery of the population spike amplitude, Atr was iontophoretically applied for up to 5 min at the recording site prior to the application of noxious heat to the tail.

In a second series of experiments, the drugs were iontophoretically applied at the apical dendritic recording site in field CA1. Here, throughout the course of an experiment, the apical dendritic field EPSP was evoked by a constant intensity of CA3 stimulation. The CA3 stimulation intensity was adjusted such that it evoked a CA1 population spike 80 % of maximal if recorded from the CA1 pyramidal cell layer. The same protocol was used to test the drugs and noxious heat effect on this field EPSP as described above for the population spike.

In both series of experiments a common protocol was followed for testing of drugs (Ach and Atr) on either the CA1 population spike or dendritic EPSP. However, GABA was

tested only on the dendritic EPSP. The protocol included testing the effect of iontophoretic Ach on evoked potentials using, in most cases, 3 iontophoretic currents of 40, 80 and 160 nA. Each iontophoretic administration of Ach was initiated at the onset of a CA3 stimulus and maintained for 40 sec. Thus, iontophoretic Ach was applied 2 or 3 times at each current level and the field potentials for the iontophoretic period were averaged.

The effect on the CA1 field potentials of the highest iontophoretic dose of Ach was also tested against a single iontophoretic dose of Atr. Here, 2 or 3 control responses of the evoked field potentials to Ach were taken. Then iontophoretic Atr was either applied preceding a test Ach iontophoresis or simultaneously with the onset of Ach iontophoresis and maintained for 1 or 1.5 min. Ach iontophoresis was repeated with intervening intervals of one minute until recovery was observed.

To determine whether iontophoretic Atr was selective in preventing the depression of field EPSP, it was also tested on iontophoretic GABA-induced changes in the dendritic field EPSP. The iontophoretic dose of GABA was adjusted to produce a change in the dendritic field EPSP comparable to that seen with the highest iontophoretic dose of Ach. GABA was iontophoresed for 10 sec initiated at the onset of a CA3 stimulus. The iontophoresis was repeated with intervening intervals of one minute. Thereafter, the selected iontophoretic current of Atr was applied in the manner described above before a noxious heat stimulus to the tail. The effect of iontophoretic GABA on the dendritic field EPSP was monitored for 5 min from the start of Atr iontophoresis.

Computer programs for recording and analysis of data

The Waveman and Dataman programs (Teyler et al., 1982; Stoelting Cat. No. 47101 for Waveman and Cat. No. 47102 for Dataman) were used for recording and analysis of field

potentials on an Apple IIe computer. Field potentials from the oscilloscope were fed into the computer via an A/D converter and stored in digitized form at 8 KHz frequency using the Waveman program. The amplitude of the population spike was calculated with Dataman by measuring the vertical from the peak of the spike to the tangent across the mouth of the spike. The Waveman program was also used to calculate the slope of the somatic field EPSP at a fixed latency near the middle of the first positive wave of the population spike. Similarly, the amplitude of the dendritic field EPSP was measured at a fixed latency near the peak of this negative going waveform.

In experiments where both the orthodromic population spike and the antidromic spike were recorded, a computer program written for the laboratory by Dr. David Harris was used for storing and determining the amplitude of these field potentials.

Statistical analysis

The electrophysiological data were analyzed, except where indicated, using one-way analysis of variance and the computed F ratio was used to determine significant difference among group means. The Significant difference between pairs of means was then determined using the Duncan multiple range test. In a few experiments, as indicated later in the text, the electrophysiological data were analyzed using the paired t-test. The reflex-reaction score was compared within the same group of animals using the paired t-test and between different groups of animals with the unpaired t-test. Statistically significant difference between compared values was accepted at $p < 0.05$.

Except where noted, the absolute values were analyzed for statistical significance. In some cases electrophysiological data were also analyzed using regression and correlation analysis. The use of this method of analysis is described in the text where they were applied.

RESULTS

Physiological experiments

Temperature-dependent effect of noxious heat applied to the tail on the CA1 population spike amplitude

The animals generally reacted to noxious heat with reflex movements but did not vocalize. Further, when the tail was exposed to hot water at 50°C for 15 sec, a tail-flick generally occurred with a latency of 5-10 sec, whereas, the tail-flick latency in 55°C water was always less than 5 sec. In the latter case the tail-flick was also more vigorous and the tail would have been withdrawn from the hot water if released by the experimenter. These reflexes were scored using the following scale:

Reaction	Score
Mild tail-flick response (TFR), latency > 5.0 sec	1
Mild TFR, latency < 5.0 sec	2
Vigorous TFR, latency < 5.0 sec	3

Using this scale the reflex reaction score was evaluated for comparison when the tail was exposed to hot water at 50 or 55°C for 15 sec. In these experiments the duration of EEG synchronization (theta rhythm) was also determined following noxious heat exposure. Table I shows the duration of the evoked theta rhythm and the reflex-reaction score in

TABLE 1

NOXIOUS HEAT INDUCED THETA AND REFLEX-REACTION SCORE

TEST GROUP	50° C (n=6)		55^oC (n=7)		55° C (n=5) with prior theta
	1 st Exposure	2 nd Exposure	1 st Exposure	2nd Exposure	1 st Exposure
REFLEX-REACTION SCORE (+ S.E.M)	1.3 ± 0.2	1.5 ± 0.2	2.7 ± 0.2	2.6 ± 0.2	2.8 ± 0.2
DURATION OF EVOKED THETA (SEC ± S.E.M)	6 ± 1	7 ± 1	11 ± 2	12 ± 2	–

response to noxious stimuli for different test groups. The duration of the evoked theta rhythm was dependent on the intensity of the noxious stimulus and was not significantly changed with a second exposure of the same stimulus. Similarly, the reflex-reaction score was dependent on the stimulus intensity and did not habituate to a second exposure of noxious heat.

The CA1 population spike, which was stable over the control time period, occurred at a latency of 5-8 msec following the stimulation (Figs. 3, 4). Exposure of the tail to noxious heat depressed the CA1 population spike amplitude in a temperature-dependent manner (Fig. 4). No consistent effect was seen at 50°C. However, 2-5 min following a 15 sec application of noxious heat at 55 and 60°C, a mean peak depression of 68 ± 13 % (range 27-100 %) and 83 ± 6 % (range 63-100 %) occurred, respectively. The depression was long lasting in that the population spike amplitude remained significantly inhibited 8 min following heat exposure at 55°C and 18 min at 60°C (Fig. 4B,C; F ratio of 3.23 and 7.29, respectively, $p < 0.01$). The depression of the population spike amplitude was not accompanied by a significant change in the slope of the its field EPSP (e.g., 55°C, 15 sec). An intense noxious stimulus was required to depress the CA1 population spike. If the tail remained in the water only long enough to evoke a tail-flick reflex no depression of the population spike occurred.

The heat-evoked depression of the population spike was greatly attenuated or eliminated (i.e., habituated) on subsequent exposure(s) of noxious heat to the tail (Fig. 5B). This occurred whether the noxious stimulus was hot water at 55°C or 60°C.

The dorsal hippocampal EEG spontaneously alternated between irregular activity
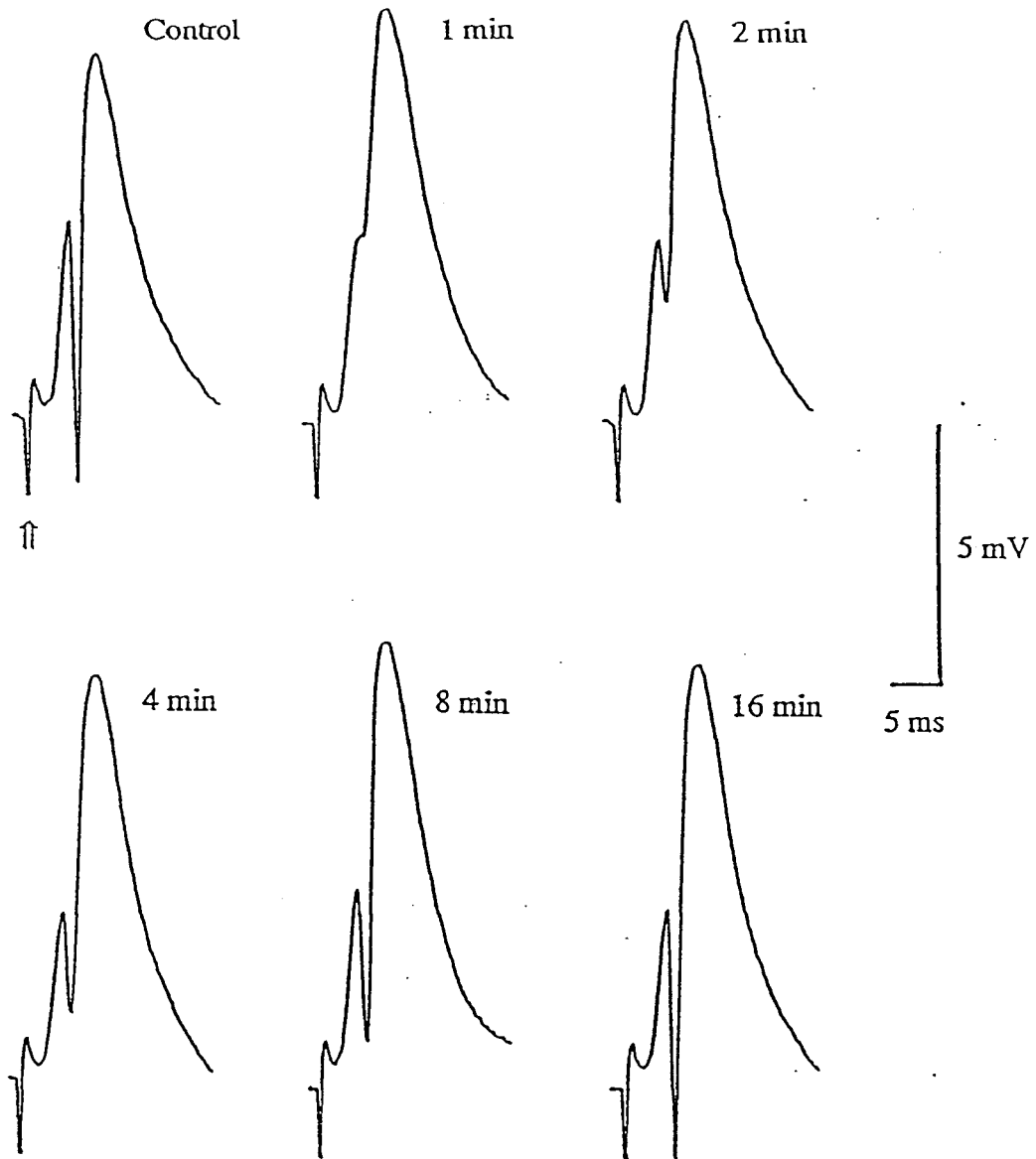
Figure 3: Trace representation of the effect of noxious heat (55°C, 15 sec) applied to the tail on the dorsal hippocampal CA_1 population spike amplitude. Each trace in the figure is an average of 4 consecutive sweeps. Positive is up in these traces. The control trace is an average of population spikes within the minute prior to noxious heat application. The arrow under the control trace indicates the stimulus artifact. The other traces were collected after the noxious heat application around the times indicated above the trace. Note the marked and prolonged depression of spike amplitude following noxious heat exposure.
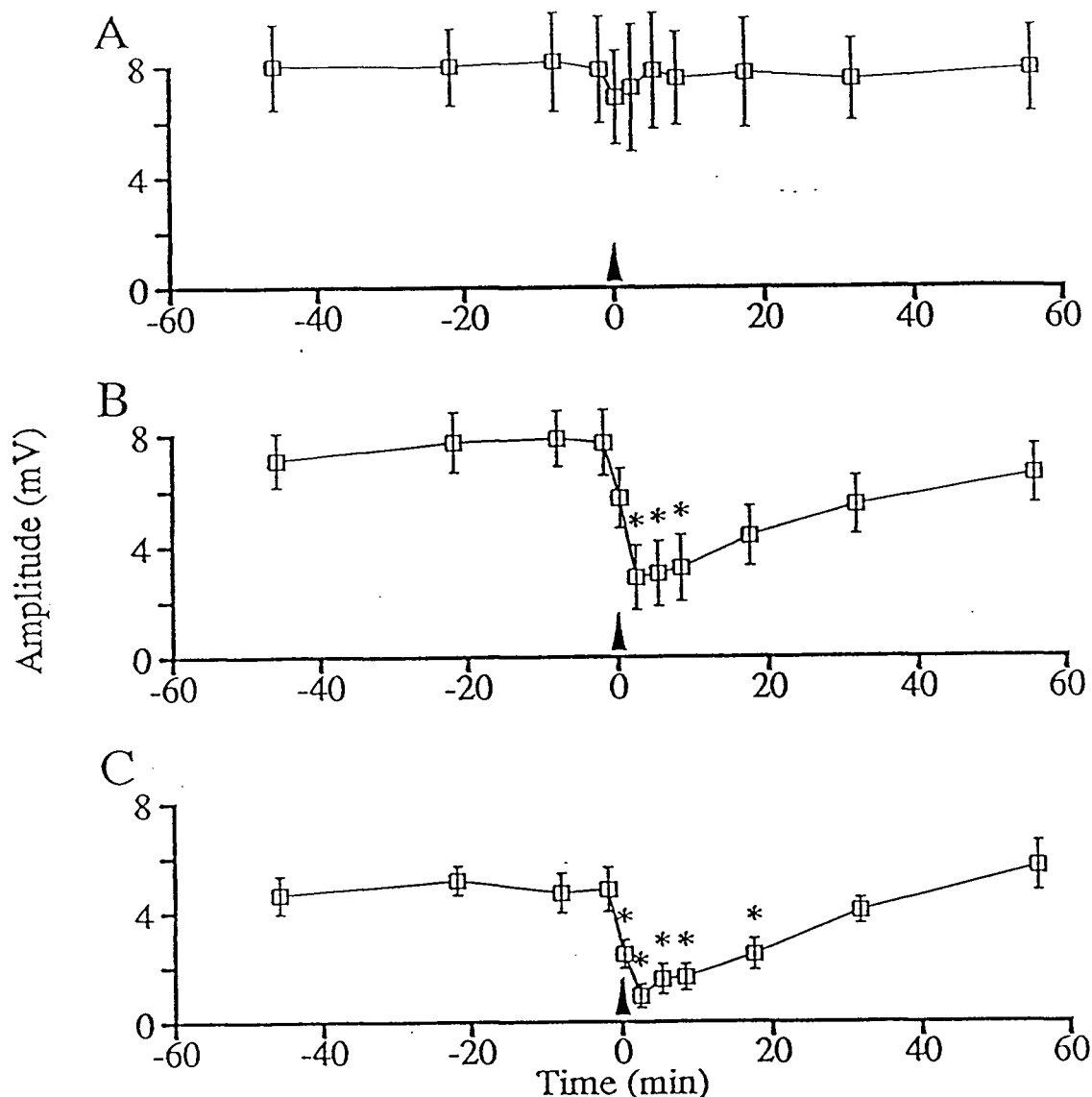
Figure 4: Temperature-dependent effect of noxious heat applied to the tail on the CA_1 population spike amplitude. Files of population spikes were collected and their amplitude averaged periodically over the course of an experiment. These files were collected more frequently around the noxious heat stimuli. Noxious heat was applied for 15 sec starting at the time indicated by the arrowhead. Each point on the plot represents the mean ± SEM of population spike amplitude. No change was observed in spike amplitude at 50°C (A; n=6), but prominent and long-lasting depression of amplitude is seen at 55°C (B; n=7) and a greater effect at 60°C (C; n=5). Significant change in value from preheat controls are indicated by an asterisk (* p<0.05).

Figure 5: The influence of repeated noxious heat exposures and the hippocampal EEG state on the CA1 population spike. The upper traces in each section are contralatral hippocampal EEG records and each point in the graphs represent the mean ± SEM population spike amplitude. In these experiments noxious heat to the tail was applied at 55°C for 15 sec as indicated by solid bar below the trace and the arrowhead in each graph. A: The first application of noxious heat produced a long-lasting depression of the population spike when the EEG was in an irregular state at the time of application (n=7). An example is shown in the upper trace. Also note that noxious stimulus induces theta rhythm. B: The time course in B is a continuation of A. Note that there is an habituation of the heat-induced depression of the population spike on repeated applications of the stimulus. However, theta rhythm is reproducibly evoked with each stimulus. The example used for illustration in the upper trace was obtained during the second application of noxious heat (72 min after the first application) in the same experiment shown in A. In this experiment there was a marked inhibition of the population spike with the first application and none with the second application. C: In another set of animals the population spike failed to be inhibited by the first application of noxious heat to the tail if the hippocampal EEG was in theta rhythm at the time of heat application (upper trace; n=5). The large regular spikes in the EEG traces resulted from contralatral CA3 stimulation to evoke the CA1 population spike. Significant differences from preheat controls are indicated by asterisks (* p<0.05).
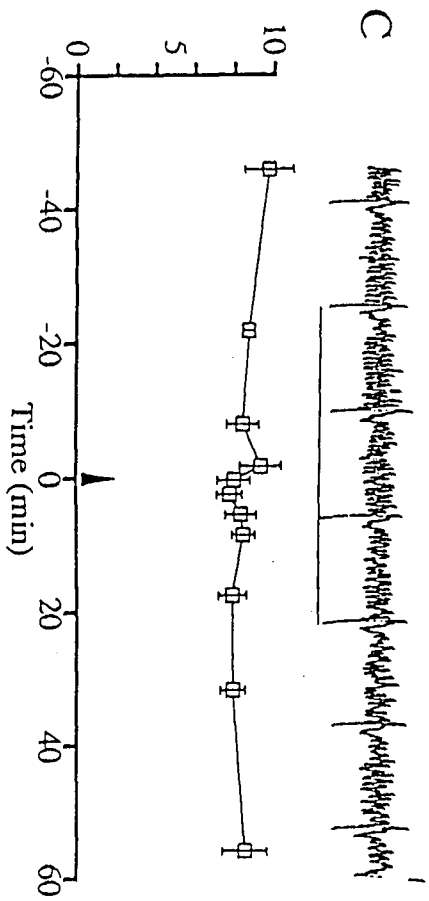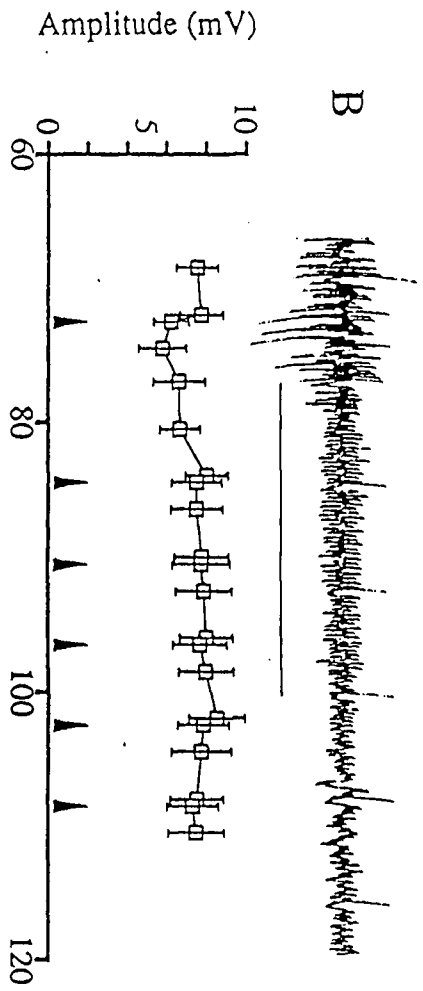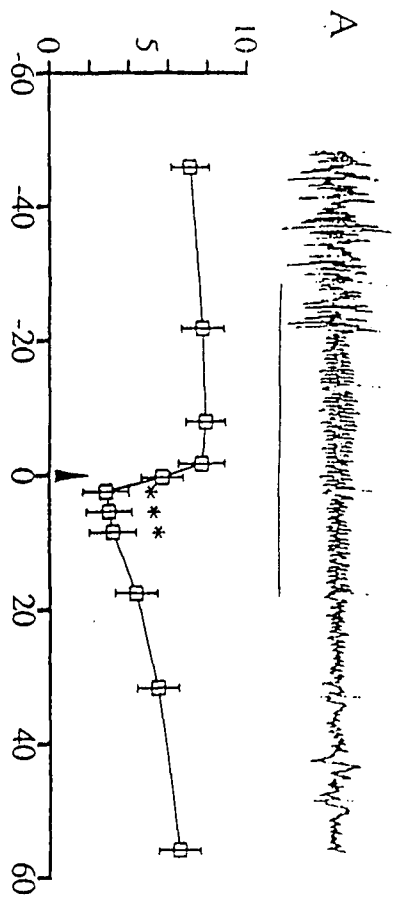
and a 4-6 Hz rhythmic theta rhythm. Interestingly when noxious heat (55°C, 15 sec, tail) was applied with the hippocampal EEG in theta rhythm, no depression of the population spike was observed (Fig. 5C). If, however, the hippocampal EEG was in an irregular pattern at the time noxious heat was applied, a 4-6 Hz theta rhythm was produced (Fig. 5A, B) along with the depression of the population spike. However, it appears that there is a dissociation between the persistent depression of the population spike and the induction of theta rhythm in response to noxious stimulus. For example, at 50°C noxious heat exposure, a theta rhythm lasting an average of 6 ± 1.0 sec was produced with no significant change in population spike amplitude. Furthermore, two successive exposures of 55°C evoked theta rhythm lasting 11 ± 2.0 sec and 12 ± 2.0 sec while only the first application resulted in a consistent depression of CA1 population spike (Fig. 5A, B; Table 1).

It is perhaps noteworthy that the inhibition of the population spike was markedly attenuated in the 4 animals prepared for blood pressure recording. The mean inhibition upon placing the tail in 55°C water was 31 ± 10 % and was observed only during the application of the noxious stimulus. These experiments were not included in the data shown. Thus it appears that the additional surgery required for the cannulation of the carotid artery is responsible for the attenuation. Similarly, Clarke and Matthews (1985) showed that trauma due to surgery is involved in increasing the jaw opening reflex threshold in cats. In our experiments two applications of noxious heat separated by more than 1 hr produced similar increases in blood pressure lasting approximately 30 sec. Thus, the noxious stimulus-induced depression of the CA1 population spike is not due to blood pressure changes.

Effect of noxious heat stimuli applied to the left hind paw and later to the tail on the CA1

population spike

Noxious heat exposure of the left hind paw produced a persistent depression of the population spike amplitude and it remained significantly depressed at 18 min following the noxious stimulus (Fig. 6A, n=6). The mean peak depression was 84 ± 7 % (range 52-100 %). The noxious heat-evoked depression of the population spike habituated on subsequent exposure(s) of noxious heat to the left hind paw. Following this, noxious heat exposure of the tail produced a depression of the population spike (Fig. 6B, n=6). The mean peak depression was 73 ± 10 % and the population spike was significantly inhibited 8 min following noxious heat exposure. This depression is similar to that seen when the tail alone is exposed to noxious heat. In 5 of 6 experiments, the tail was again exposed to the same noxious heat stimulus. In the remaining animal, tail was not exposed a second time for it appeared to be recovering from anaesthesia. In the five animals tested with second exposure, the mean peak depression was 66 ± 13 % (Fig. 6B, n=5) and the population spike was significantly depressed 8 min after heat exposure. A strong depression was produced in 4 of 5 cases. This was unlike experiments in which the tail alone was exposed to noxious heat at 55°C in which a habituation of the persistent depression of population spike was observed with the second noxious heat exposure.

Effect of a noxious heat stimulus on the antidromic spike and the population spike in field

CA1

Noxious heat applied to the tail depressed the CA1 population spike for at least 4 min in this group of animals (Fig. 7A, n=7). It should be noted that the mean
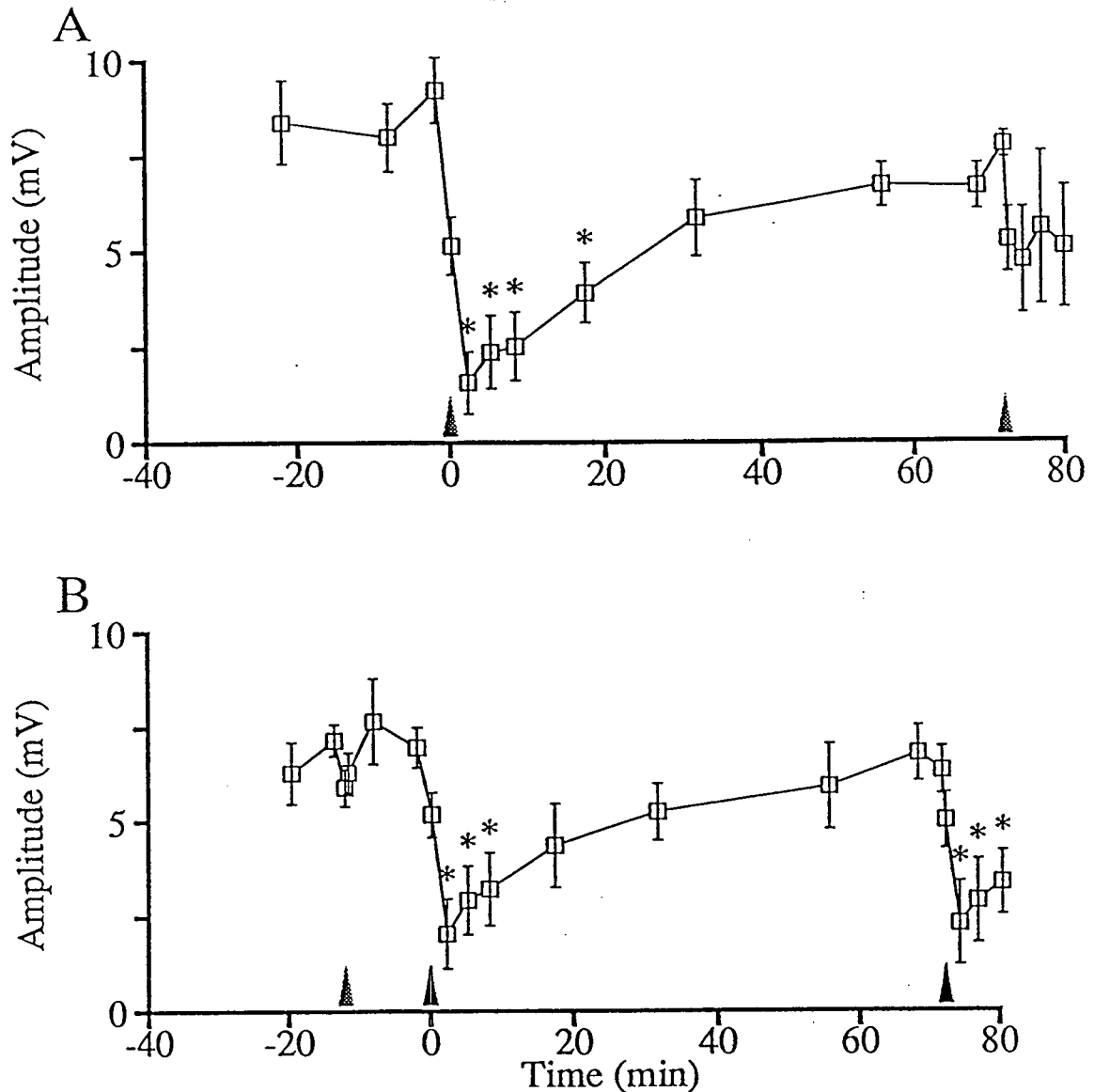
Figure 6: The effect of noxious heat applied to the left hind paw and then to the tail on the CA1 population spike amplitude. A: Noxious heat (55°C, 15 sec) was first applied to the left hind paw (light arrowheads). The population spike was significantly depressed 18 min following the first noxious heat application with mean peak depression of 84 ± 7 % (n=6). Habituation was seen to the second noxious heat application. B: In all cases, total adaptation occurred after the second or third noxious heat application (light arrowhead). Noxious heat applied to the tail following left hind paw habituation resulted in a marked depression of the population spike (dark arrowhead). The spike amplitude was significantly depressed 8 min following exposure with mean peak depression of 73 ± 10 % (n=6). A second heat application to the tail significantly depressed the population spike 8 min following exposure with mean peak depression of 66 ± 13 % (n=5).
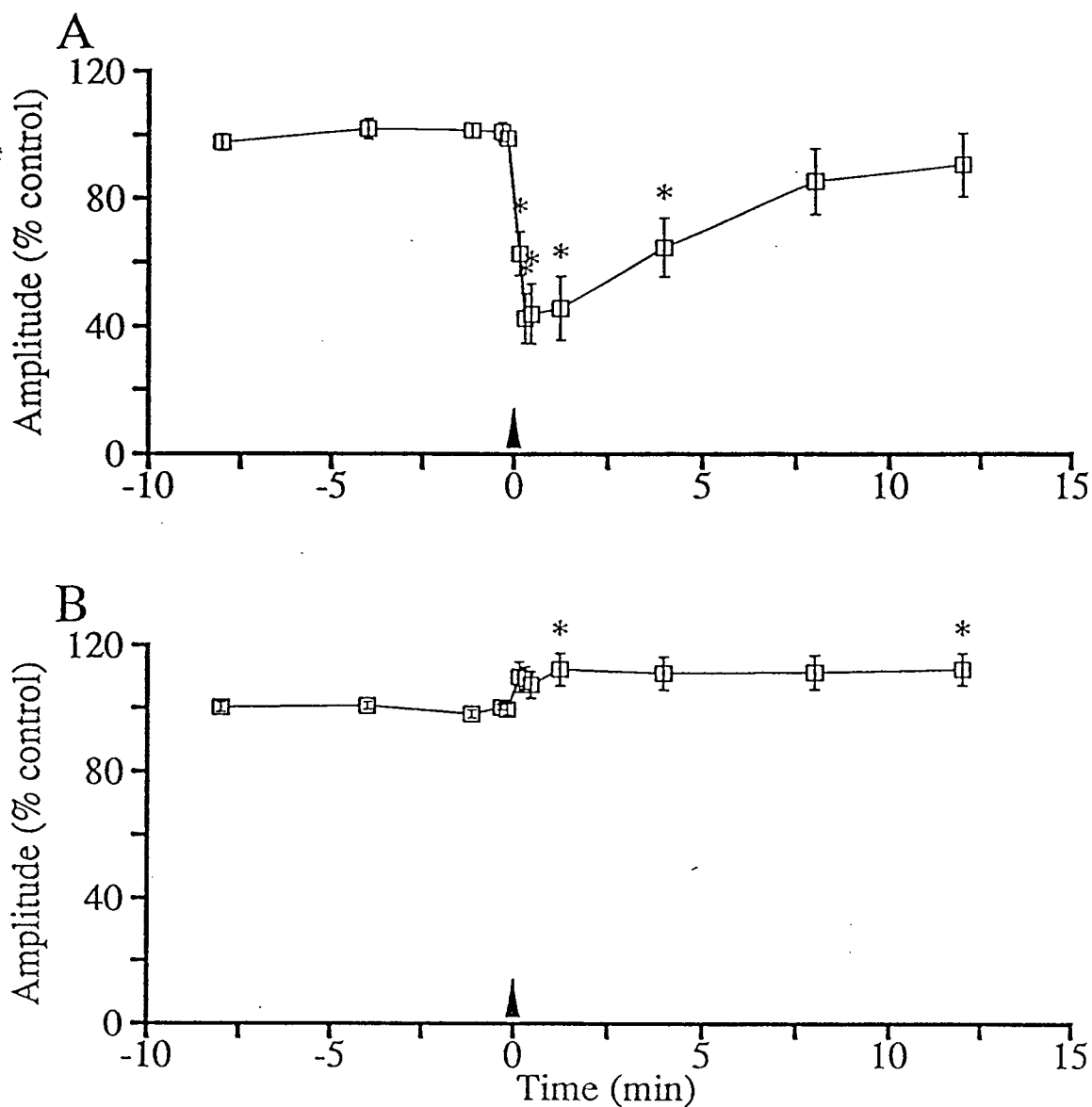
Figure 7: The effect of noxious heat (55°C, 15sec) applied to the tail on the dorsal hippocampal CA1 population spike (A) and antidromic spike amplitude (B) evoked upon alternate stimulation of the ipsilateral CA3 and alveus, respectively. Noxious heat was applied at time 0 min (arrowhead). The mean of all preheat spike amplitudes was taken as 100 % control. Each point represents mean ± SEM of spike amplitude expressed as % of control. n value for A and B is 7. Significant differences from preheat control are indicated by asterisks (* $p < 0.05$).

duration of inhibition was less than described above in another group of animals. In this series of experiments, the hippocampal EEG was not recorded and therefore we suspect that some of the animals may have been in theta rhythm when the noxious stimulus was applied. Previous experiments, as described above, clearly showed that the depression of the CA1 population spike was larger in magnitude and duration during irregular EEG, whereas, minimal inhibition was seen when the hippocampal EEG exhibited theta rhythm. Here, 2 of 7 animals showed weak inhibition.

One-way analysis of variance of the absolute values revealed no significant change in the antidromic spike amplitude at various time points following noxious heat application when compared with preheat control values. However, if the absolute values of the amplitude of the antidromic spike were normalized to take into account the variability due to different baselines, a statistically significant difference was calculated. In this method, for each experiment the absolute value of the antidromic population spike of all preheat control responses at various time points was averaged. This value was equated to 100 % and individual values were expressed as a percentage of this control. The antidromic spike amplitude was increased in 5 of 7 experiments following a noxious stimulus while no change in amplitude was observed in the other 2 experiments. It should be noted that in the 5 animals showing increased antidromic spike amplitude, the CA1 population spike was markedly depressed in 3 of these and showed a weak depression in the remaining 2 experiments. In 2 experiments, no change in antidromic spike was observed, although the CA1 population spike was markedly depressed. In this group of 7 animals the F ratio for one-way analysis of variance of normalized antidromic spike amplitude was 2.25 with probability (p) of 0.021. The following analysis with Duncan test indicated that the

antidromic spike amplitude at 1.28 min and 12 min after noxious heat application was different from the control values (Fig. 7B).

Similar analysis of 5 experiments (excluding the 2 which showed no change) gave a F ratio of 2.95, p value of 0.005 and the antidromic spike was significantly enhanced in amplitude in the 12 min recorded following heat exposure (Fig. 8B). The maximum enhancement ranged from 120-140 % of control. In these 5 animals the CA1 population spike was significantly depressed for at least 4 min following noxious heat exposure (Fig. 8A).

Effect of noxious heat stimulus on CA1 commissural population spike

In 4 experiments ipsilateral and contralateral CA3 were stimulated alternately to produce population spikes in CA1. A noxious stimulus applied to the left hind paw produced identical changes in these spikes (Fig. 9). The mean peak depression was 70 ± 17 % and 71 ± 17 %, respectively, and the population spikes were significantly depressed for 20 min following noxious heat exposure.

In another 5 experiments only contralateral CA3 was stimulated. The combined mean peak depression of the resultant commissural CA1 population spike (i.e evoked upon contralateral CA3 stimulation) in these and above 4 experiments was 74 ± 9 % (range 40-100%). The population spike was significantly depressed for at least 20 min following noxious heat exposure.
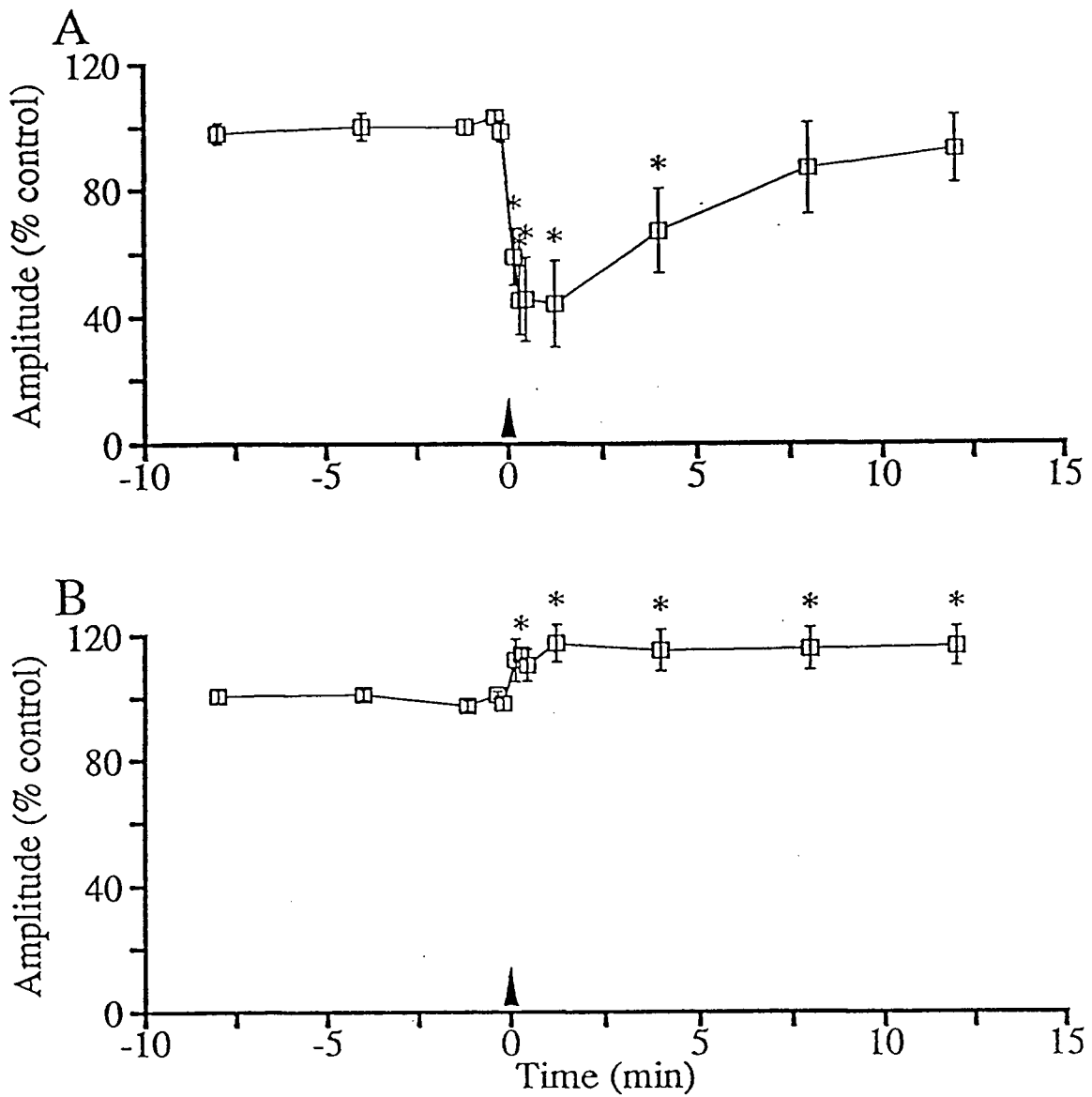
Figure 8: The effect of noxious heat (55°C, 15sec) applied to the tail on the dorsal hippocampal CA1 population spike (A) and antidromic spike amplitude (B) evoked upon alternate stimulation of ipsilateral CA3 and alveus, respectively. The data used here is from 5 experiments which were from larger group of 7 experiments reported in Fig. 7. This figure is made similar to Fig. 7. Significant differences from preheat control are indicated by asterisks (* p<0.05).
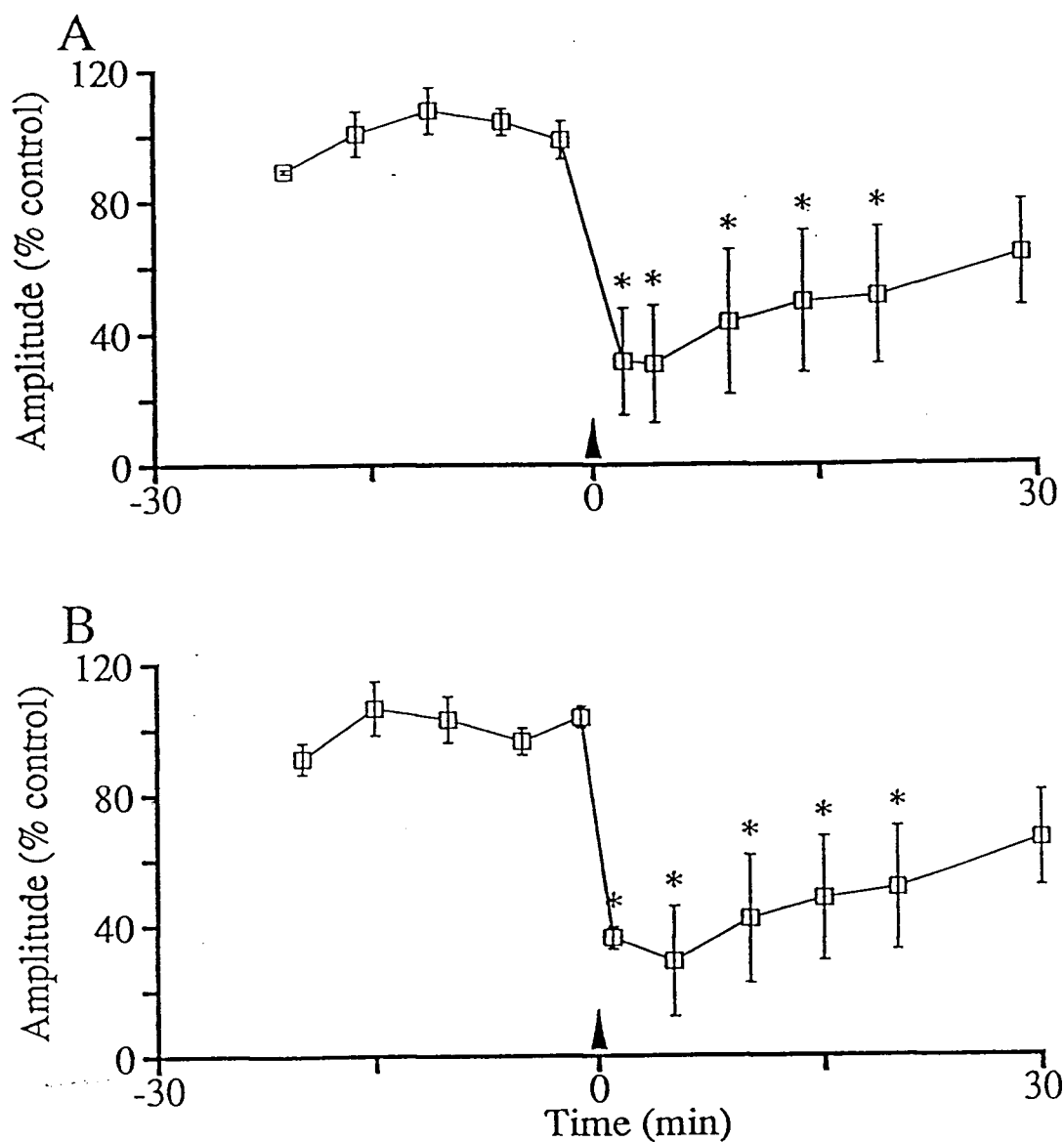
Figure 9: The effect of noxious heat (55°C, 15sec) applied to the left hind paw on the ipsilateral (A) and contralateral (B) CA3 stimulation-evoked CA1 population spike amplitudes. The ipsilateral and contralateral CA3 were stimulated alternately every 10 sec. Noxious heat was applied at time 0 min (arrowhead). The mean of all preheat spike amplitudes was taken as 100 % control. Each point represents mean ± SEM of spike amplitude expressed as % of control. n value for A and B is 4. Significant differences from preheat control are indicated by asterisks (* p<0.05).

Effect of noxious heat stimuli on the commissural CA1 population spike and apical dendritic field EPSP

Following exposure of the tail to hot water the CA1 population spike and dendritic field EPSP were significantly depressed for 6 min and 20 min, respectively (Fig. 10, 11). Regression and correlation analysis indicated that in 2 of the experiments there was a linear relationship between changes in population spike and dendritic field EPSP amplitude (r=0.95 and 0.98; r^2=0.90 and 0.96). This was not the case in the remaining experiments (r=0.71, 0.78 and 0.81; r^2=0.50, 0.60 and 0.65). Regression analysis was done by comparing the amplitude of the population spike at various time points 20 min before and 40 min after the first noxious heat exposure as a function of the dendritic field EPSP amplitude corresponding to the same time points. Correlation analysis was done between the dendritic field EPSP and the population spike amplitude measured at the same time points. Both the dendritic field EPSP and the population spike amplitude were considered as independent variables for the correlation analysis.

The mean peak depression of the population spike and dendritic field EPSP to the first noxious heat exposure was 72 ± 14 % (range 30-100 %) and 57 ± 8 % (range 30-78 %), respectively. The heat-evoked depression of these potentials was greatly attenuated or eliminated (i.e., habituated) on a subsequent exposure of noxious heat to the tail one hour later (Fig. 11).

Figure 10: Trace representation of the effect of noxious heat (55°C, 15 sec) applied to the tail on the dorsal hippocampal CA1 population spike (A) and apical dendritic field excitatory postsynaptic potential (EPSP; B). The population spike and EPSP were recorded from the same mediolateral CA1 field. Each trace in the figure is an average of 4 consecutive sweeps. Positive in A is up and down in B. The control traces in A and B are the average population spikes and EPSP's, respectively, within two and one minute prior to noxious heat application, . The arrow under the control trace indicates the stimulus artifact. The other traces in A and B were collected after the noxious heat application around the times indicated above each trace. Note the marked and prolonged depression of the population spike and EPSP amplitude following noxious heat exposure.
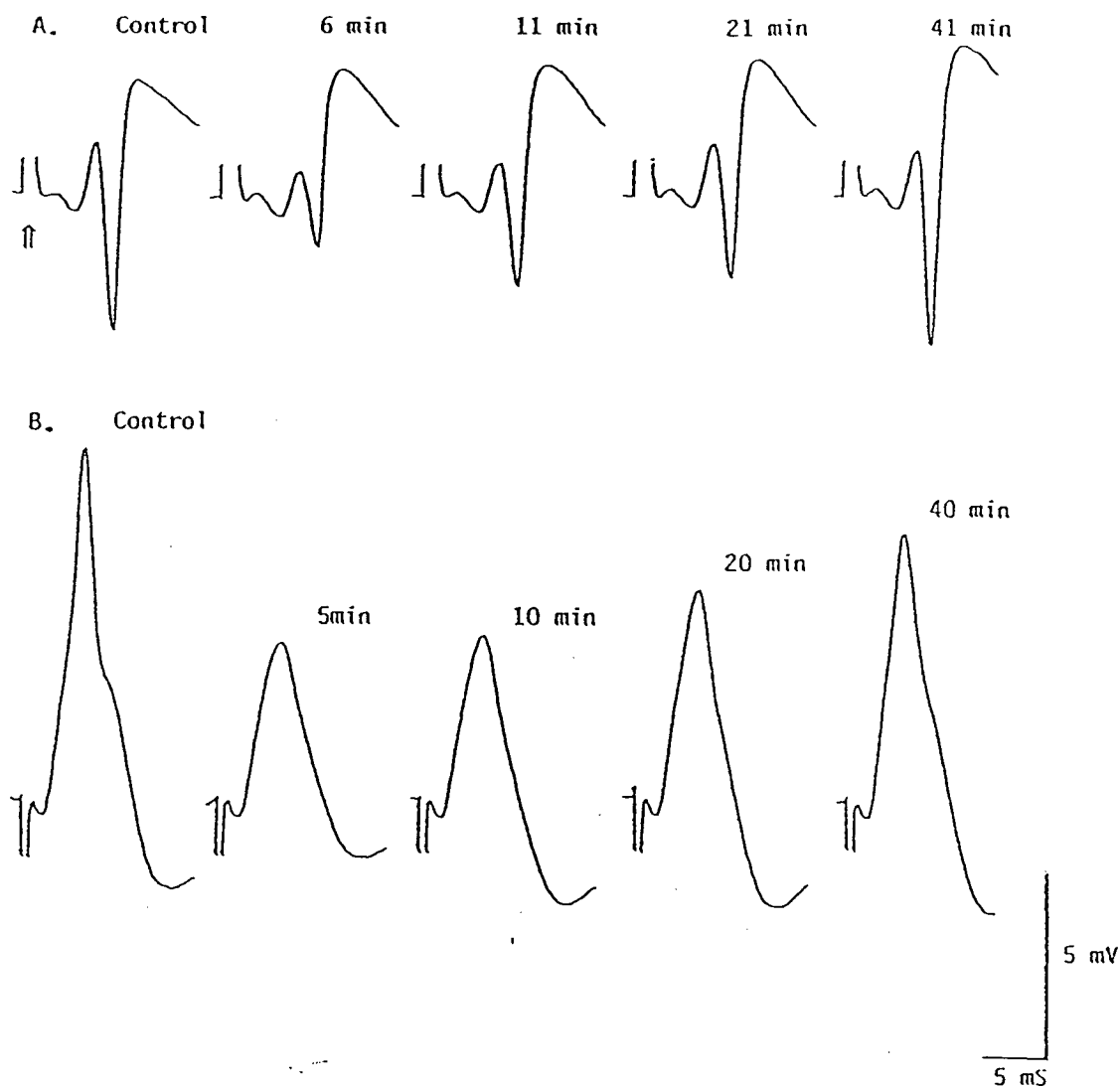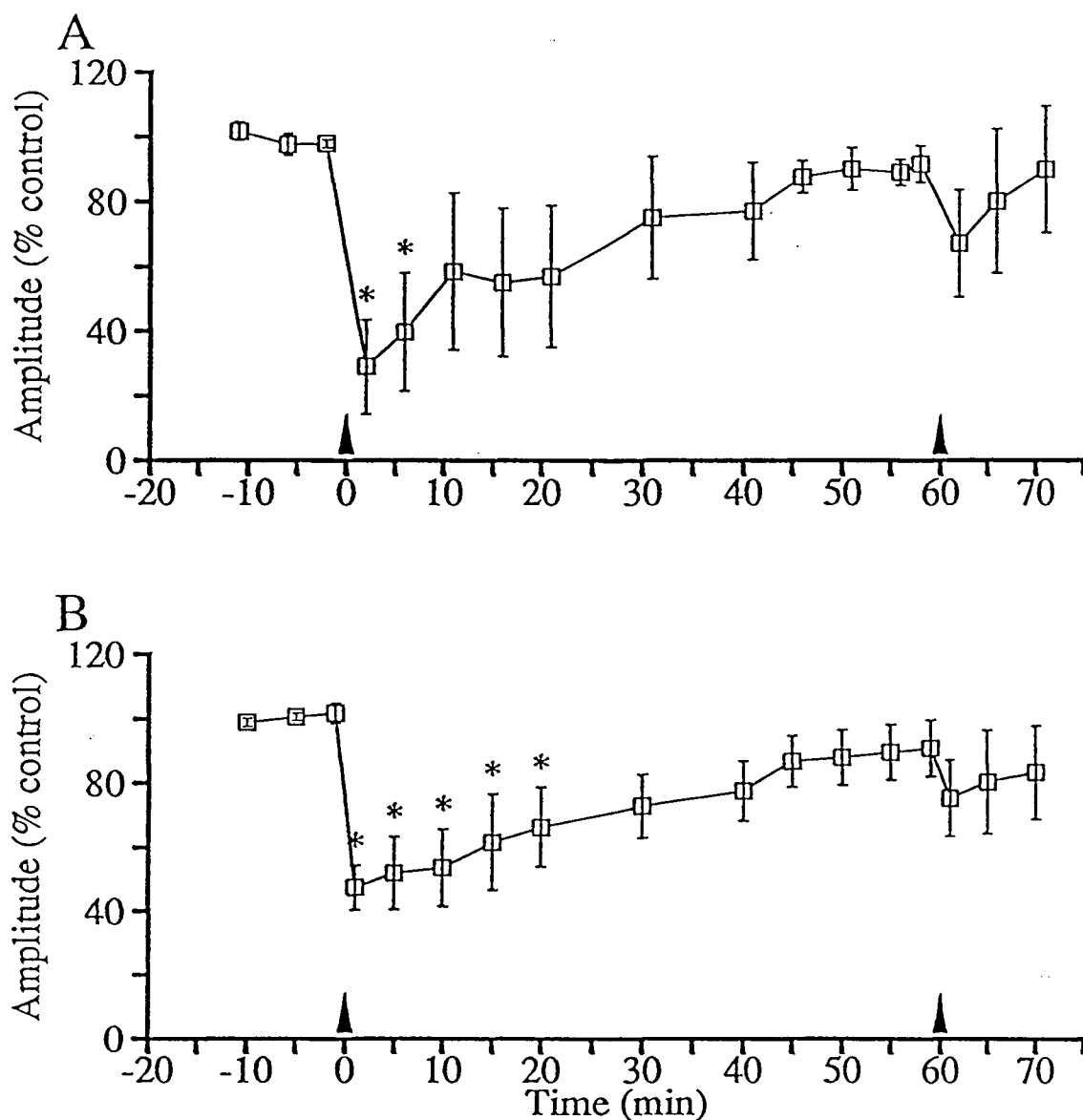
Figure 11: Graphical representation of the effect of noxious heat (55°C, 15 sec) applied to the tail on the dorsal hippocampal CA1 population spike amplitude (A) and the apical dendritic field excitatory postsynaptic potential (EPSP; B). Noxious heat was applied at time 0 and 60 min (arrowheads). The mean amplitude of the population spike or the EPSP recorded over a period of 20 min before the first noxious exposure was taken as 100 % control. The amplitude of population spike and EPSP at various time points are expressed as % of respective control. Each point in A and B represents mean ± SEM % control amplitude, n=5. Significant differences from preheat control are indicated by asterisks (* p<0.05).

Pharmacological experiments

Effect of systemic administration of atropine sulphate on the noxious stimulus-induced

persistent depression of the CA1 population spike

Exposure of the left hind paw to noxious heat produced a persistent depression of the CA1 commissural population spike (Fig. 12A). The mean peak depression was 77 ± 10 % (range 41-100 %) and the population spike was significantly depressed for 15 min following heat exposure. However, pretreatment with atropine sulphate (40 mg/kg, i.p) 50 min prior to a noxious stimulus applied to the tail prevented the depression of the CA1 population spike (Fig. 12B). Atropine alone had no effect on the CA1 commissural population spike amplitude.

Fig. 13 illustrates the effect of atropine sulphate on the septal stimulation-induced facilitation of the CA1 population spike. The MS-VLDBB tetanus (100 Hz for 70 msec) preceded contralateral CA3 stimulation by 30 msec (Krnjevic and Ropert, 1982). The higher intensity of septal stimulation facilitated the amplitude of the CA1 commissural population spike to approximately double that produced by lower intensity MS-VLDBB stimulation. The facilitation of population spike by both intensities of septal stimulation was greatly attenuated following administration of atropine. Further, the difference in facilitation between the two intensities of MS-VLDBB stimulation was abolished. However, the effect of atropine was selective in that it did not affect the paired-pulse facilitation (at inter-pulse interval of 100 msec) of CA1 commissural population spike (Fig. 14). Spontaneous and noxious stimulus-induced theta rhythm was also blocked following atropine administration (type-2 theta).
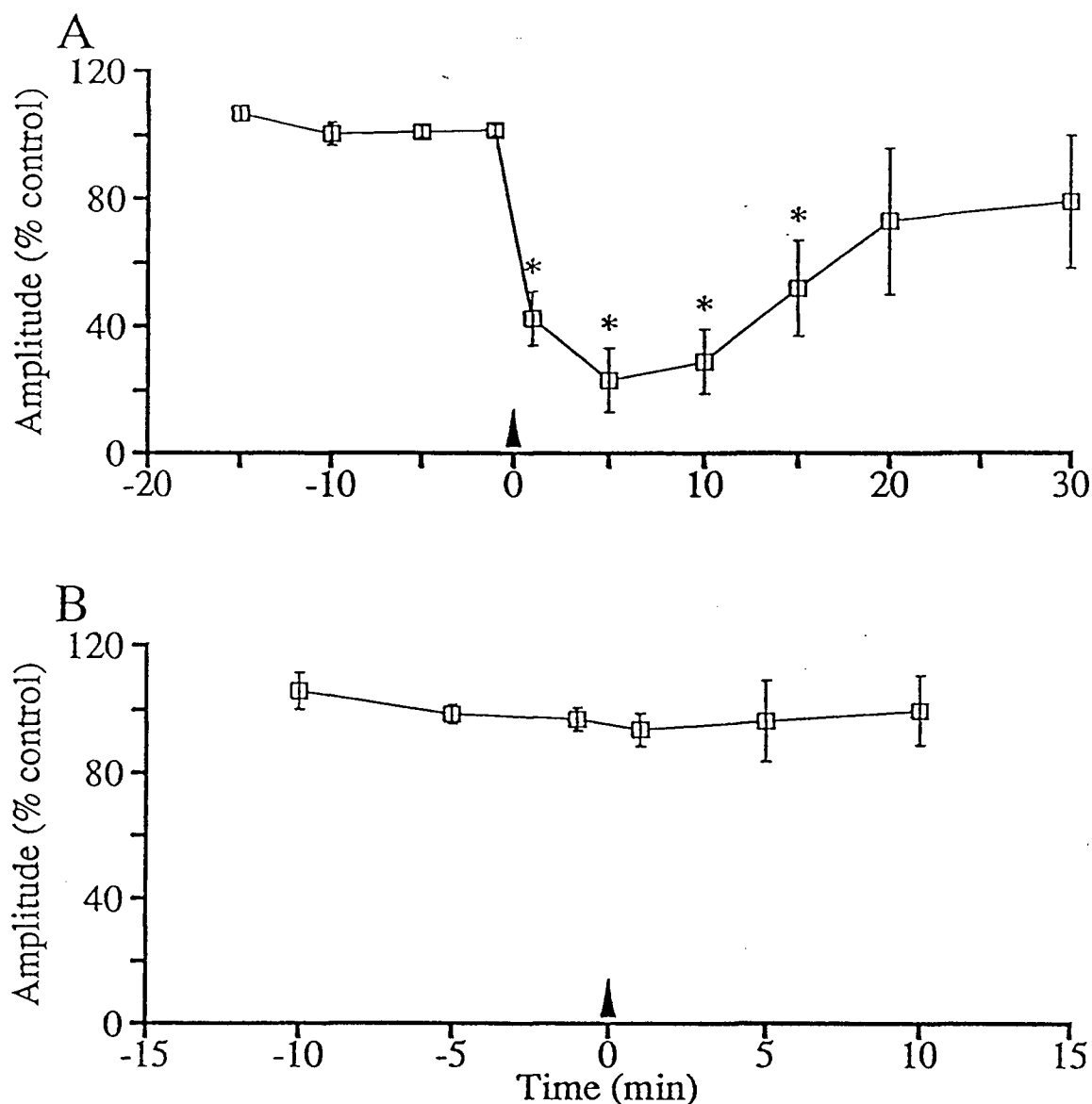
Figure 12: Atropine sulphate (40 mg/kg, i.p.) blocks the noxious heat-induced persistent depression of CA1 population spike. Noxious heat (55°C, 15 sec) was applied at time indicated by arrowheads. The mean of all preheat spike amplitudes was taken as 100 % control. Each point represents mean ± SEM of spike amplitude expressed as % of control. In these experiments, first the left hind paw of the animal was exposed to noxious heat (A). The population spike was significantly depressed 15 min following the noxious heat application with mean peak depression of 77 ± 10 % (n=5). An hour or more later, when the population spike amplitude had recovered, atropine sulphate was administered. Fifty minutes after administration of atropine sulphate, noxious heat applied to the tail failed to depress the CA1 population spike amplitude (B; n=5).
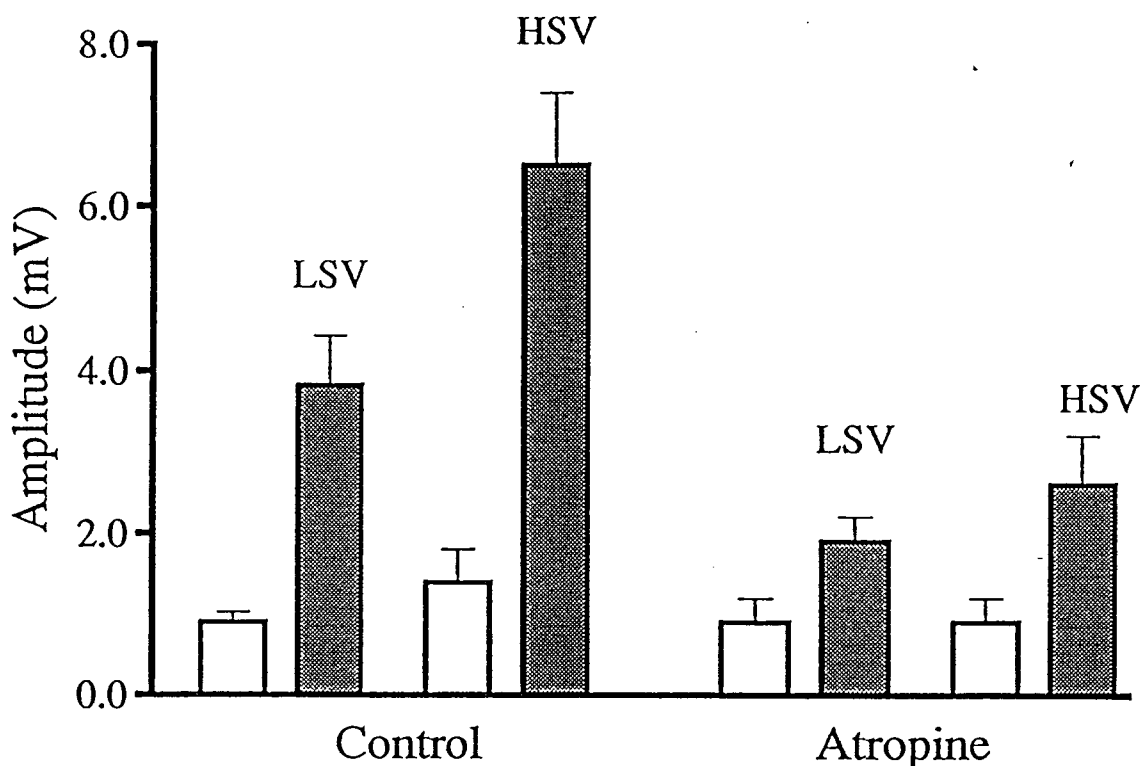
Figure 13: Septal stimulation-induced facilitation of the CA1 population spike amplitude and the antagonism of this effect by systemic atropine sulphate (40 mg/kg, i.p.). The open bars symbolize the control population spike amplitude before septal stimulation. The shaded bars represent the facilitated population spike amplitude following septal stimulation. Each bar indicates the mean ± SEM of spike amplitude (n=6). LSV and HSV are the lower and higher septal stimulation voltage, respectively. Paired t-test was used to analyse the data and significant difference between various bar means was accepted with $p < 0.05$. The open bars were not different from each other. Septal stimulation significantly enhanced the population spike compared to corresponding controls. Average facilitation at HSV was approximately double that of LSV before atropine administration. Atropine drastically attenuated septal facilitation and abolished the difference between HSV and LSV facilitation.
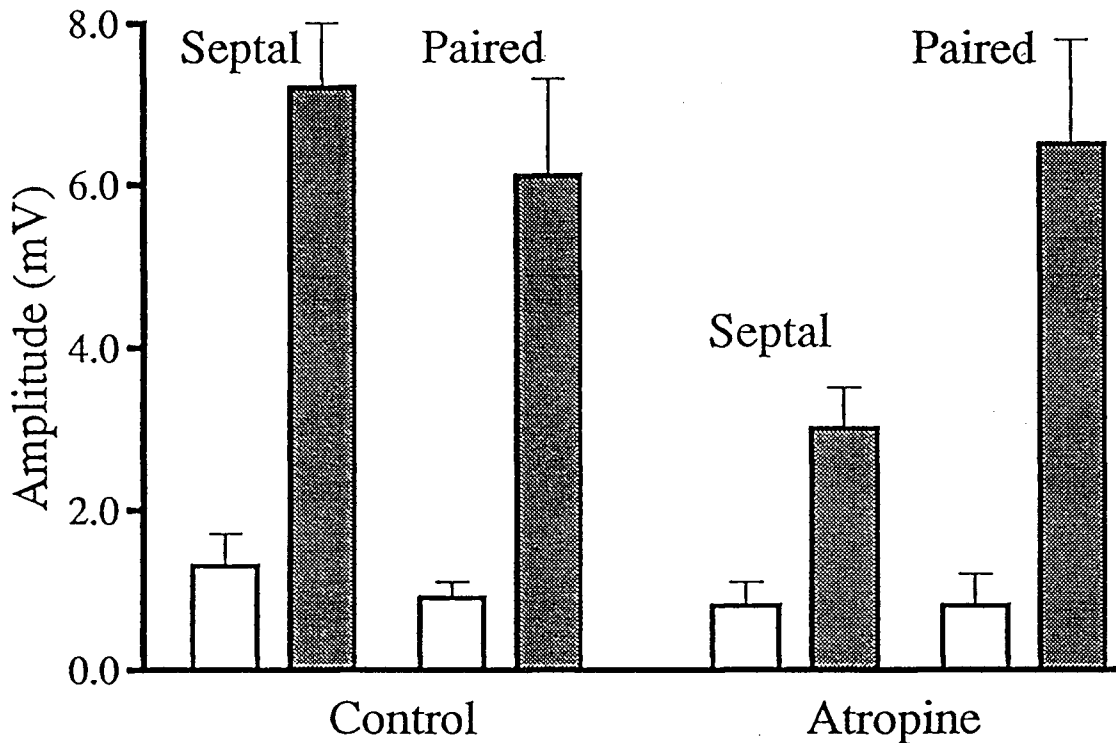
Figure 14: Differential effect of atropine (40 mg/kg, i.p.) on septal and paired-pulse facilitation. The open bar symbolize the control CA1 population spike amplitude before septal or paired-pulse stimulation of region CA3. The shaded bars represent the facilitated population spike amplitude following septal or paired-pulse stimulation. Each bar indicates the mean ± SEM of spike amplitude (n=5). Septal stimulation was using higher stimulation voltage as in Fig. 13. Paired-pulse facilitation of CA3 was produced by stimuli with an interpulse interval of 100 msec. Paired t-test was used to analyse the data and significant difference between various bar means was accepted with p<0.05. The open bars were not different from each other. Before atropine administration, septal and paired-pulse stimulation significantly enhanced population spike from corresponding controls. However, these facilitated spike amplitudes were not different from each other. Following atropine administration, septal facilitation was drastically attenuated with no significant change in paired-pulse facilitation.

Effect of iontophoretic atropine on noxious stimulus-induced persistent depression of CA1

synaptic excitability

Iontophoretic application of Ach at the cell body recording site produced a facilitation of the CA1 commissural population spike. These results are in line with reports in the literature (Krnjevic and Ropert, 1982). The facilitation was dose-dependent (e.g., Fig. 15). This effect of iontophoretic Ach was blocked by iontophoretic Atr (Fig. 16).

Exposure of the left hind paw to noxious heat (55° C, 15 sec) produced a persistent depression of the CA1 population spike amplitude. Iontophoresis of Atr (40, 100 and 180 nA applied for up to 5 min in 3 experiments) before application of noxious heat to the tail failed to block the persistent depression of CA1 population spike amplitude in any of the experiments. An individual example is illustrated in Fig. 17.

Iontophoretic Ach, when applied at the dendritic recording site, produced a dose-dependent reduction in the amplitude of the dendritic field EPSP (Fig. 18). The depression of the dendritic field EPSP by the iontophoretic dose of Ach tested was blocked by iontophoretic Atr (Fig. 19).

Exposure of the left hind paw to a noxious heat stimulus produced a persistent depression of the dendritic field EPSP (Fig. 20A, 21A). However, iontophoretic Atr (35-70 nA) prevented the persistent depression of CA1 dendritic field EPSP when the tail was exposed to a noxious heat stimulus (Fig. 20B and 21B). This was seen in all experiments whether the atropine iontophoretic time was one minute (n=1) or five minute (n=3). In the former case, a iontophoretic time of 1 min was used to test whether this short period of iontophoresis would block the noxious heat-induced EPSP depression. Similar doses of iontophoretic Atr, 1 min or 5 min, did not prevent the depression of the dendritic field EPSP to iontophoretic GABA (Fig. 22).
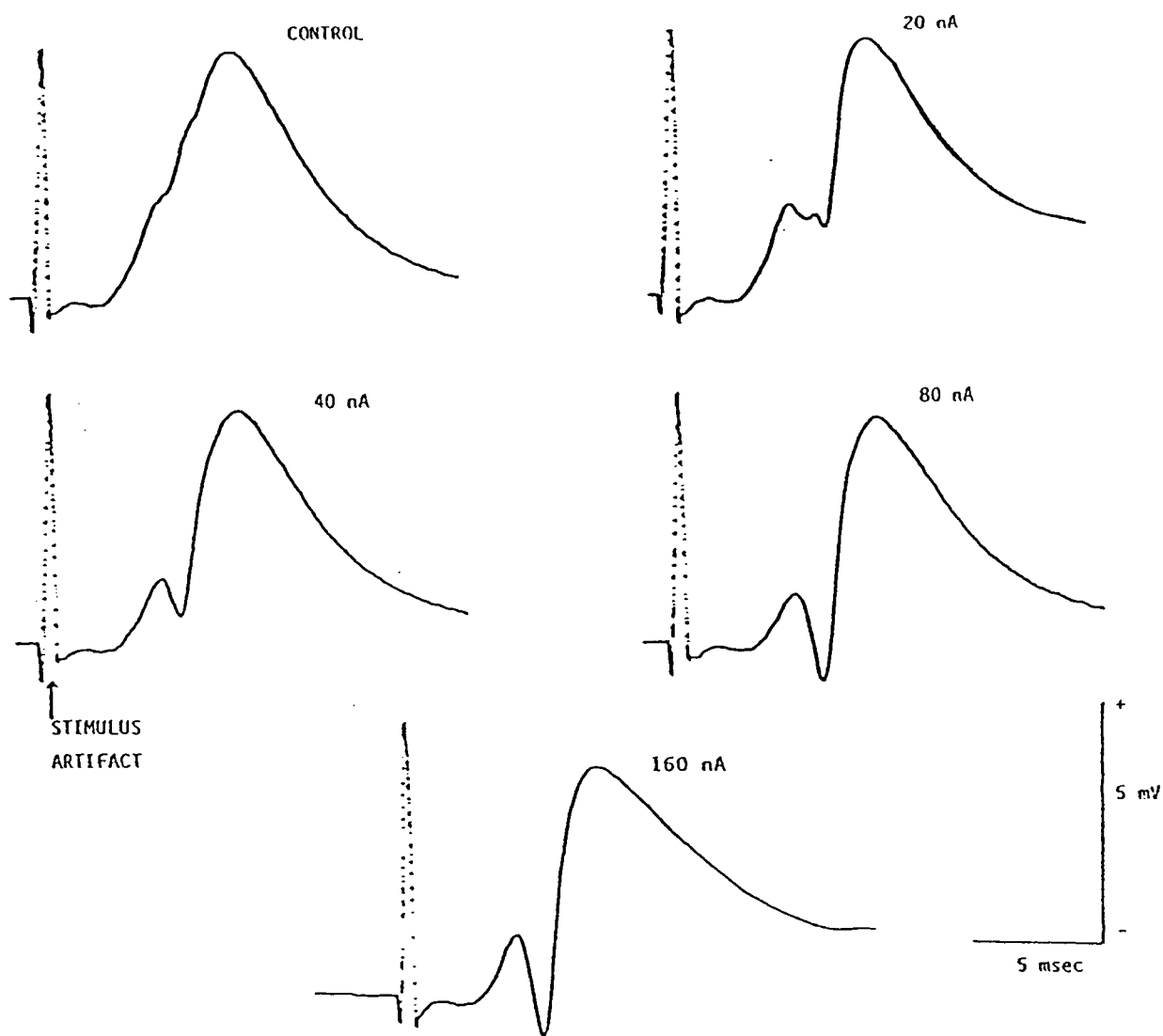
Figure 15: Facilitation of CA1 commissural population spike by iontophoretic acetylcholine (Ach). Ach was applied iontophoretically at the recording site in str. pyramidale. The various Ach iontophoretic currents (applied for 40 sec with the onset initiated with a CA3 stimulation) are indicated above the trace. Each trace in the figure is an average of 4 consecutive sweeps. The control trace is a positive going field potential. Iontophoretic Ach produced a negative going population spike. This effect of Ach was dose-dependent.
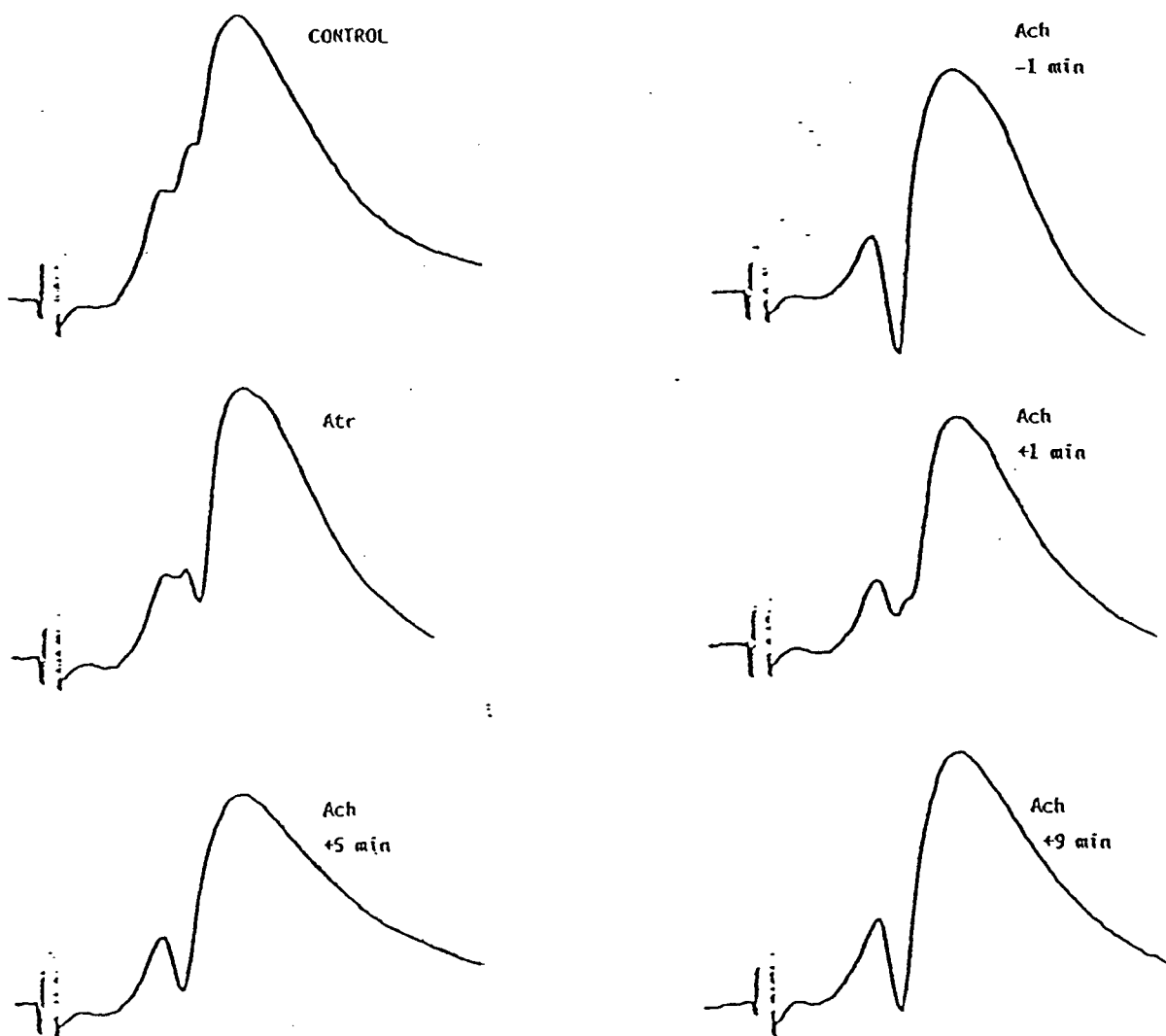
Figure 16: Iontophoretic atropine (Atr) blocks the facilitatory effect of iontophoretic acetylcholine (Ach) on the CA1 population spike amplitude evoked upon contralateral CA3 stimulation. Each trace in the figure is an average of 4 consecutive sweeps except the trace marked Atr. Atropine (80 nA) was iontophoresed for 1.5 min and traces were averaged for this time period. The control trace is a positive going field potential recorded within 2 min prior to start of atropine iontophoresis. The other traces were collected before and after the middle of atropine iontophoresis around the time indicated above the trace. Note the marked facilitation to iontophoretic Ach (160 nA, 40 sec) at time -1 min, but a block of this effect following atropine iontophoresis. The facilitatory effect of Ach had recovered by 9 min. Atropine per se had a minor facilitatory effect. The scale and directions (i.e. positive/negative) are similar to Fig. 15.
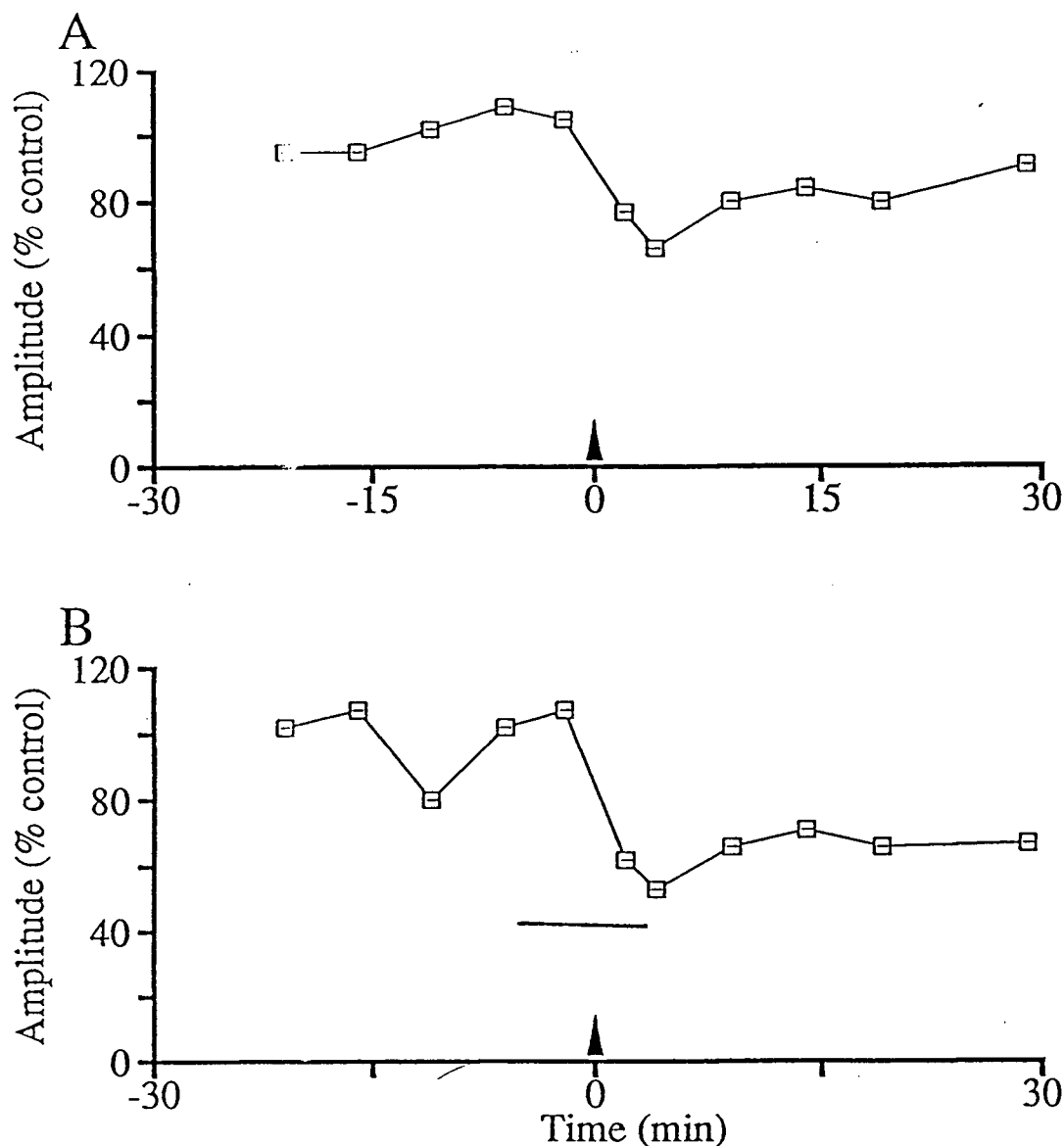
Figure 17: Lack of effect of iontophoretic atropine on noxious stimulus-induced persistent depression of CA1 population spike amplitude. Noxious heat (55°C, 15 sec) was applied at time indicated by arrowheads. The mean of all preheat spike amplitudes was taken as 100 % control. Each point represents mean ± SEM of spike amplitude expressed as % of control from an individual experiment. A: Noxious heat was first applied to the left hind paw. The population spike was depressed for 20 min following the noxious heat application with peak depression of 66 % of control. B: Atropine (180 nA) was applied iontophoretically near the cell body recording site for the period indicated by the bar (i.e. 5 min before and 2 min after application of noxious heat). This did not prevent the persistent depression of CA1 population spike to noxious heat applied to the tail.
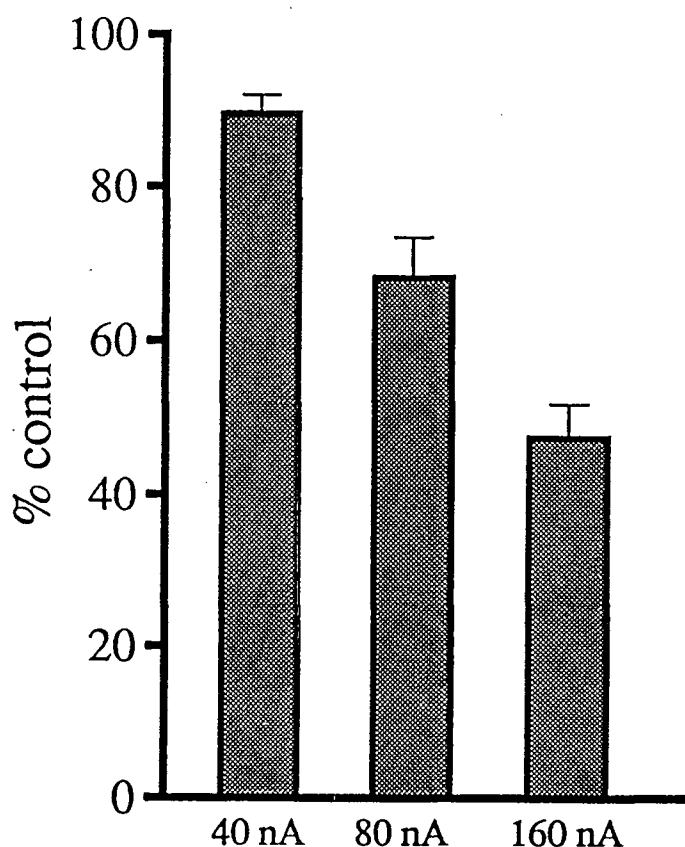
Figure 18: Iontophoretic acetylcholine (Ach) produces a dose-dependent depression of the CA1 field excitatory postsynaptic potential (EPSP). The EPSP was recorded from the apical dendrites where the drug was also iontophoresised. The Ach was iontophoretically applied for 40 sec. The various Ach iontophoretic currents are indicated below the corresponding EPSP amplitude bars. The mean amplitude of the dendritic EPSP recorded for 40 sec before Ach iontophoresis was taken as 100 % control. The amplitudes of EPSP recorded during the period of Ach iontophoresis were averaged and expressed as % of preceding control (Mean ± SEM, n=4).
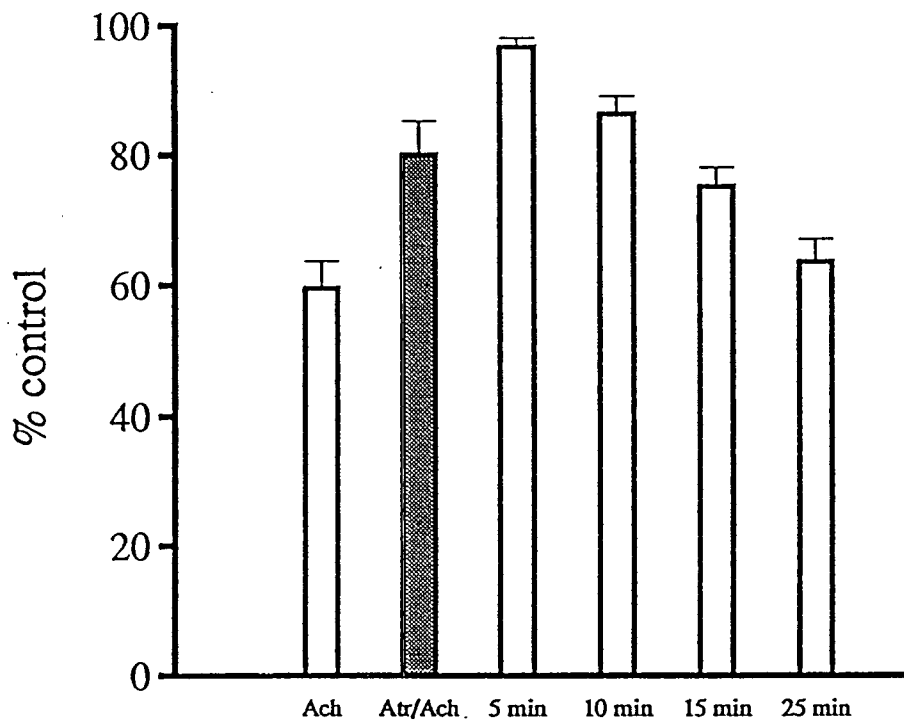
Figure 19: Iontophoretic atropine (Atr) blocks the iontophoretic acetylcholine (Ach)-induced depression of the CA1 dendritic field excitatory postsynaptic potential (EPSP). The recording and iontophoretic site was in the apical dendrites. Each histogram is developed as described for Fig.18. Ach iontophoretic current was 160 nA for 40 sec. Atr iontophoretic currents were 35-70 nA for 1 - 1.5 min. The first histogram, labelled Ach, reflects the depression of EPSP amplitude by iontophoretic Ach. The filled histogram, marked Atr/Ach, indicates the developing antagonism of Ach effect by iontophoretic Atr applied simultaneously during Ach iontophoresis. The other histograms indicate the time course of recovery of Ach effect following the end of Atr iontophoresis. The various time points are indicated underneath the corresponding histograms. n=4.
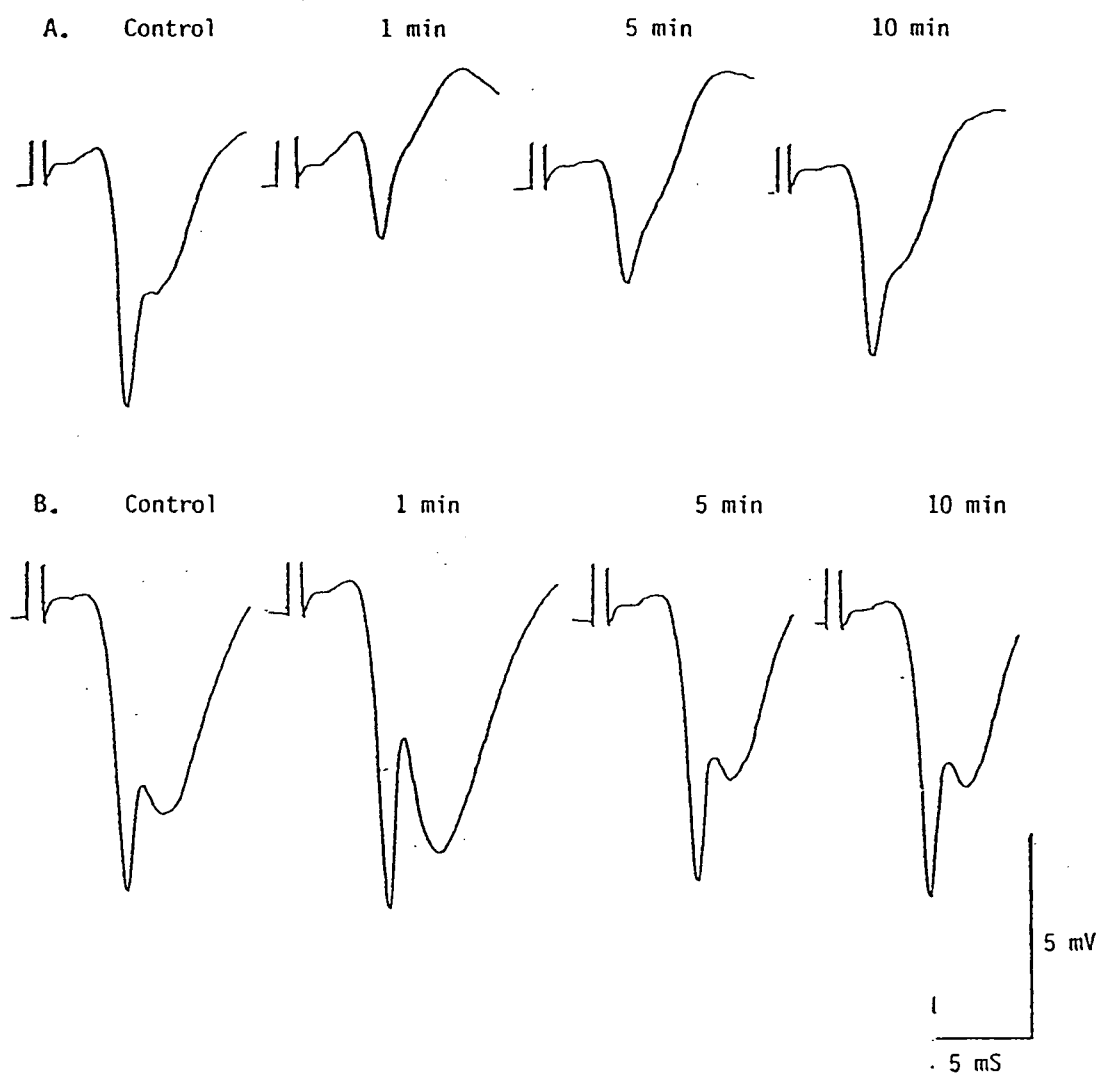
Figure 20: Trace representation where iontophoretic atropine applied near the apical dendritic recording site in field CA1 prevents the noxious heat-induced depression of the field excitatory postsynaptic potential (EPSP). In each trace the first negative peak represents dendritic field EPSP and the second negative peak (apparent in B) may be due to retrograde invasion of the population spike. Noxious heat (55°C, 15 sec) was applied either to the left hind paw (A) or to the tail (B). Each trace in the figure is an average of 4 consecutive sweeps. Positive is up in these traces. In A the control trace is an average of the EPSP within a minute prior to noxious heat application. The other traces in A and B were collected after the noxious heat application around the times indicated above each trace. Note the marked and prolonged depression of EPSP amplitude following noxious heat exposure in A. In B the control trace is an average of EPSP collected during the last 40 sec of a 1 min period of atropine iontophoresis (35 nA) applied immediately before noxious heat was applied to the tail. The noxious stimulus failed to depress the dendritic EPSP.
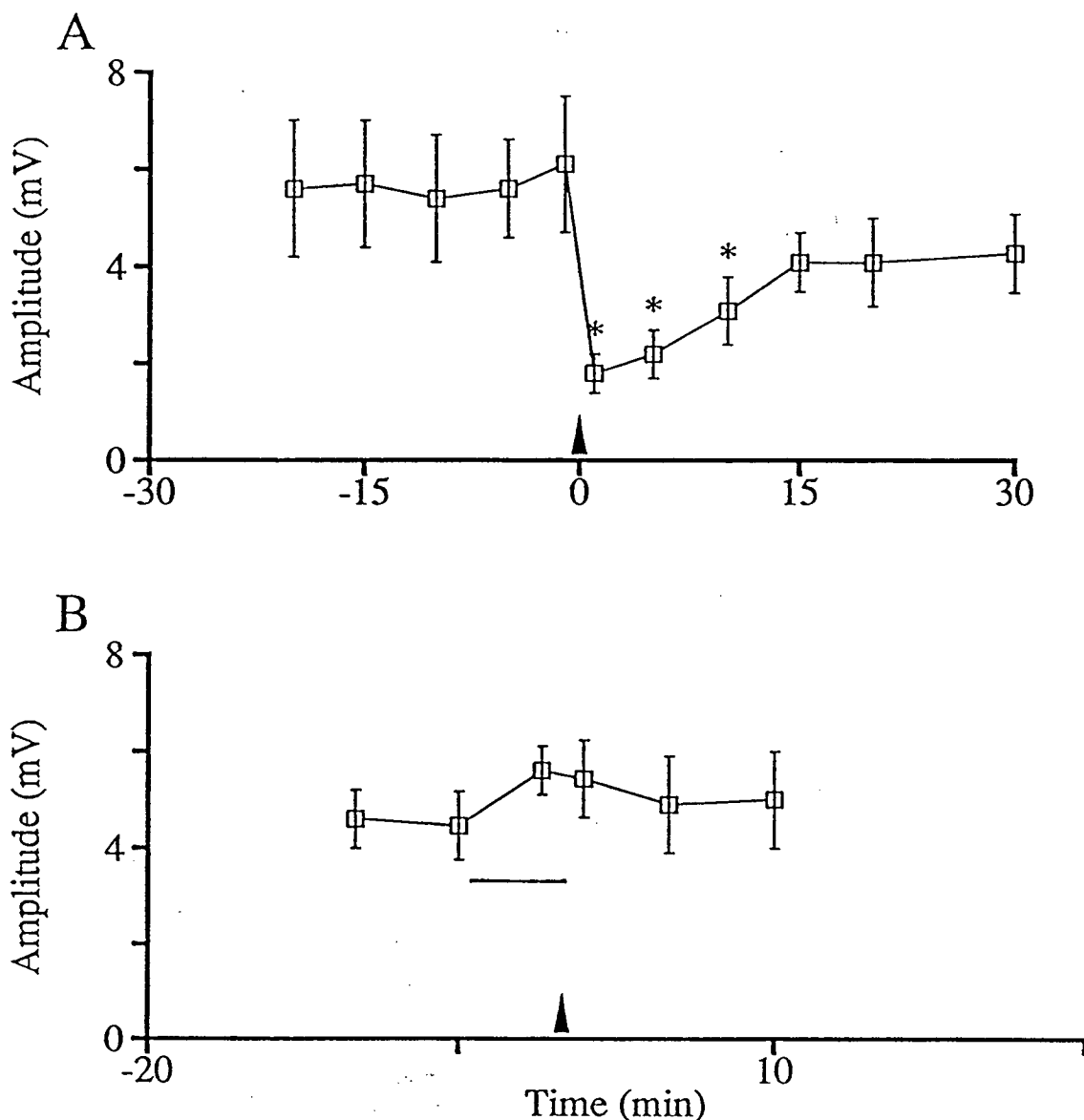
Figure 21: Graphical representation where iontophoretically applied atropine (Atr) prevents the persistent depression of dendritic field excitatory postsynaptic potential (EPSP) to a noxious stimulus. The recording and the drug iontophoretic site was in the apical dendrites of hippocampal field CA1. Noxious heat (55°C, 15 sec) was applied at the time indicated by the arrowhead. Each point is the mean ± SEM of EPSP amplitude (n=4). In the experiment noxious heat was first applied to the left hind paw. The EPSP was significantly depressed 10 min following noxious heat application (A; n=4). An hour or more later, following recovery of the EPSP amplitude, tail was exposed to hot water (B). However, in this case, Atr (35-70 nA) was applied iontophoretically prior to noxious heat exposure. The time of application of Atr in most experiments is indicated by the bar (B). The depression of EPSP to noxious heat applied to the tail was blocked (B; n=4). Significant differences from preheat control are indicated by asterisks (* p<0.05).

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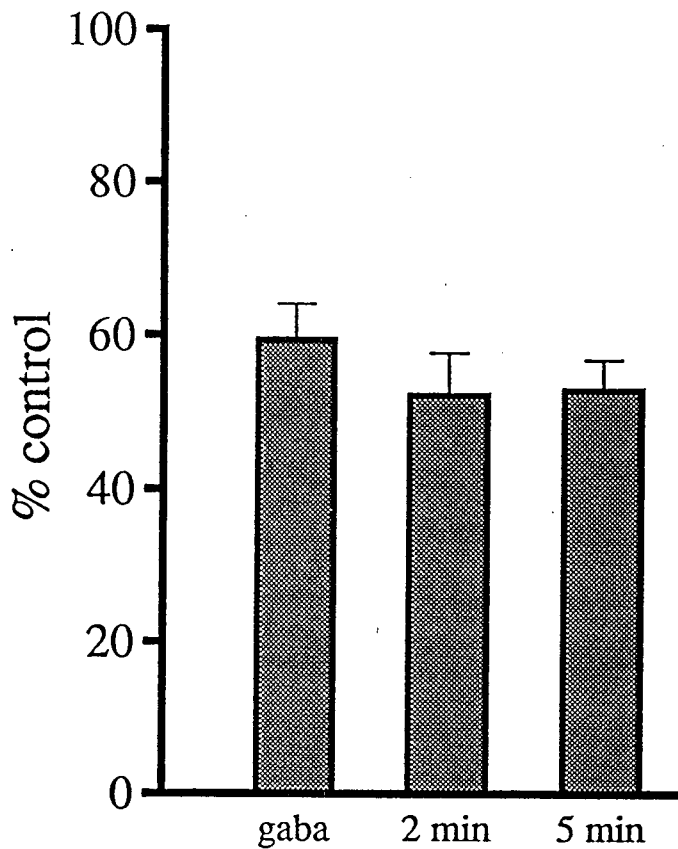
Figure 22: Iontophoretic atropine (Atr) fails to block the depression of CA1 dendritic field excitatory postsynaptic potential (EPSP) produced by iontophoretic gamma aminobutyric acid (GABA). The recording and iontophoretic site was in the apical dendrites. Each bar is developed as described for Fig.18. GABA iontophoretic currents were 30-35 nA for 10 sec. Atr iontophoretic currents were 35-70 nA for 1 or 5 min. The iontophoretic application of GABA and Atr was initiated with a CA3 stimulus. The bar labeled gaba reflects the depression of EPSP amplitude by iontophoretic GABA. The remaining bars reflect the effect of iontophoretic GABA on the EPSP amplitude 2 and 5 min after the start of Atr iontophoresis (n=4).

DISCUSSION

Experimental evidence obtained chiefly from lesioning the hippocampus suggests that this structure is involved in aversive cognitive-motivational aspect of pain that leads to an avoidance behaviour. However, there is a paucity of electrophysiological studies describing the influence of noxious stimuli on the activity of hippocampal neurones. In the present study, using lightly anaesthetized rats, a noxious stimulus to a receptive area induced a persistent depression of CA1 pyramidal cell excitability. This response was greatly attenuated or eliminated (i.e., habituated) on repeated applications of the noxious stimuli to the same receptive area. These experiments do not provide evidence linking directly the aforementioned electrophysiological changes to animal pain behaviour. However, as discussed in the following pages, the current findings are consistent with some of the available experimental evidence providing clues for an electrophysiological basis of the involvement of the hippocampus in an adaptive behaviour of the animal.

Discussion of the current experimental findings is provided under the following headings: (a) physiological findings, (b) pharmacological findings, and (c) functional implication of the current findings.

PHYSIOLOGICAL FINDINGS

Persistent depression

Intensity of stimulation, effect of anaesthesia and the noxious stimuli used

We demonstrated that an intense noxious stimulus was required to evoke a depression of CA1 synaptic excitability. Such changes were not observed with non-noxious

sensory stimulation such as stroking the fur although others have shown this to be the case in conscious rats albeit of shorter duration (Herreras et al., 1988a). The difference may have been due to the presence of the anaesthetic in our case or to the strength in afferent drive in evoking the CA1 field potentials (population spike and dendritic field EPSP). Herreras et al. (1988a) reported the greatest effect of fur stroking when the CA1 population spike was near threshold. In the current experiments, the stimulation intensity was adjusted to produce a population spike which was approximately 80 % of maximal.

Immersion of either the tail or left hind paw in hot water at 55 or 60°C produced a reflex-withdrawl but the animal did not vocalize. However, in conscious animals, a noxious stimulus of similar intensity affects the animal behaviour. For example, following exposure of a rat's hind paw to water at 55° C for 15 to 20 sec, the animal responds by elevating and periodically licking the exposed paw (Coderre and Melzack, 1987). However, within an hour these responses subside and the animal returns to normal eating, grooming and locomotor behaviour. Furthermore, such an noxious exposure produces hyperalgesia marked by decreased foot withdrawl latency of the exposed as well as the contralateral paw (Coderre and Melzack, 1987).

Characteristics

We found that an exposure of the tail to noxious hot water produces a prolonged and substantial depression of the CA1 population spike evoked upon ipsilateral CA3 stimulation. This depression is temperature-dependent. Further, exposure of the left hind paw to water at 55° C also similarly produced a persistent depression of the CA1 population. Both the ipsilateral and contralateral CA3 stimulation-evoked CA1 population spikes were similarly depressed. Thus, the excitability of CA1 pyramidal neurones to both Schaffer-collateral and

commissural afferent stimulation is depressed following an intense noxious stimulus. Since the population spike reflects the synchronous discharge of pyramidal neurones following synaptic depolarization of the cell population (Andersen et al., 1971a), a depression of the population spike amplitude following a noxious stimulus indicates a decrease in synaptic excitability of CA1 pyramidal neurones. Indeed, an intense noxious stimulus reduced the amplitude of both the CA1 commissural population spike and the corresponding apical dendritic field EPSP. This response habituated on repeated nociceptive exposures.

There are reports in the literature supporting the notion that sensory stimulation depresses CA1 neuronal excitability. For example, analysis of evoked field potentials suggests that a sensory auditory click or tooth pulp stimulation in conscious rats produces an inhibition of CA1 pyramidal cells (Brankack and Buzsaki, 1986). Such sensory stimuli decreased the spontaneous activity of CA1 pyramidal cells and the probability of orthodromic activation of these neurones (Brankack and Buzsaki, 1986; Herreras et al., 1988a). Further, inhibitory influences generated by an auditory tone presentation in conscious rats regulate the development of excitatory field potentials in the dentate gyrus (Deadwyler et al., 1981).

In comparing the work of Herreras et al. (1988a) to our findings, they reported that stroking the body fur decreased the amplitude of both the CA1 population spike and the corresponding apical dendritic field EPSP evoked upon ipsilateral CA3 (Schaffer-collateral) stimulation. Our findings were similar except that a noxious heat stimulus to rats produced a prolonged depression of the CA1 pyramidal cell population spike and corresponding dendritic field EPSP. In addition, Herreras et al. (1988a) found that non-noxious sensory stimulus-induced depression of CA1 pyramidal cell synaptic excitability occurred concurrently with the generation in the hippocampus of a 5-6 Hz theta rhythm. Further,

during spontaneously occurring theta rhythm, the CA1 population spike was also depressed (Herreras et al., 1987). Therefore, Herreras et al. (1988a) suggested that sensory stimuli-induced changes in CA1 synaptic excitability were due to generation of hippocampal theta rhythm.

The 5-7 Hz hippocampal theta rhythm has also been correlated with arousal or an alert state in conscious animals (including rabbits, cats, monkeys) induced by sensory stimulation (such as visual, auditory or tactile; Green and Arduini, 1954). However, in the present study, in lightly anaesthetized rats, there is a dissociation between the " arousal " theta rhythm and the persistent depression of the CA1 population spike. Thus, while theta rhythm is invariably elicited in rats in response to repeated noxious stimuli (50 or 55° C), depression of the CA1 population spike is only seen at 55° C, and then, only following the first noxious exposure. In addition, persistent depression is not produced by a simple tail-flick reflex although theta rhythm is produced by the stimulus.

Such prolonged depression of CA1 synaptic excitability may also be related to the hippocampal involvement in stress. Indeed, in conscious stressed animals there occurs a prolonged depression of dentate population spike produced upon perforant path stimulation (Henke, 1990).

The depression of the dendritic field EPSP following a noxious heat exposure may be due to either (a) a presynaptic inhibition with decreased release of excitatory neurotransmitter from the Schaffer/commissural afferents terminals in the apical dendritic region of field CA1, and/or (b) postsynaptic changes in dendritic and somatic membrane excitability producing a decreased activation of pyramidal neurones. Postsynaptic changes in pyramidal cell membrane excitability affecting CA1 synaptic field potentials (i.e., population spike and dendritic field EPSP) can include decreased resistance and increased

conductance across the soma-dendritic membrane. As a result, a decreased potential change across the membrane will be reflected by a decreased amplitude of the synaptic field potentials. However, the following findings argue against such a possibility:

(a) a noxious stimulus, while it depressed the amplitude of CA1 orthodromic population spike, enhanced the antidromic spike amplitude in the majority of experiments. This enhanced antidromic spike amplitude persisted after the population spike amplitude has recovered suggesting that the change in cell body membrane excitability cannot account for the depression of the CA1 population spike. Further, the increase in antidromic spike amplitude may reflect an enhanced ephaptic interactions due to increased cell body membrane excitability.

(b) while the dendritic field EPSP is still depressed, the corresponding orthodromic population spike tends to recover towards the control value. This may be partly accounted by enhanced ephaptic interactions. Since, the CA3 stimulation intensity was suprathreshold for evoking the dendritic field EPSP and the population spike, a recovery in synaptic transmission would be amplified by increased ephaptic interactions leading to greater recovery in population spike amplitude.

(c) the slope of the somatic field EPSP is not altered following a noxious heat stimulus (Khanna and Sinclair, 1989).

The other alternative is that the depressed synaptic transmission in field CA1 is due to presynaptic mediated decreased neurotransmitter release. Evidence from the pharmacological studies favour this notion (see below).

As noted above, the time of depression of the orthodromic population spike is less than that of the field EPSP or the antidromic spike enhancement. It is not clear whether there is a correlation in the time course between the antidromic spike enhancement and the EPSP depression. If both effects coincide in time, it may indicate that longer lasting changes occur both in the presynaptic input and postsynaptic CA1 neuronal excitability than is apparent when the population spike alone is recorded.

Modulation of persistent depression during hippocampal theta rhythm

The hippocampal response to a noxious stimulus is markedly influenced by the state of the hippocampal EEG. A noxious stimulus failed to produce an inhibition of the population spike if the EEG was in theta rhythm at the time of noxious stimulus application. Similarly, hippocampal tooth pulp stimulation-evoked potentials were absent during theta activity in conscious animals (Brankack and Buzsaki, 1986). It is known that during theta rhythm the hippocampal CA1 pyramidal cells undergo fluctuation in excitability related to different phases of the theta wave (Buzsaki and Eidelberg, 1983; Fox et al., 1986). Possibly this limits the extent or duration of influence of external input onto these cells.

The hippocampal theta rhythm requires a medial septal input (Bland, 1986). It is noteworthy that hippocampal theta rhythm is invariably elicited in response to noxious stimuli. A similar finding has been reported by others in response to an intense electrical stimulation (Soulairac et al., 1967) or pinching the tail (Stewart and Vanderwolf, 1987).

Consistent with this notion is the finding that the septal-hippocampal neurones respond to intense peripheral noxious heat (Dutar et al., 1985).

Afferent input

As indicated above, evidence suggests that noxious input can reach the hippocampus via MS-VLDBB afferents. Nociceptive information can reach the MS-VLDBB complex via afferents from the spinal cord. In a retrograde tracer transport study, Burstein and Giesler (1989) reported that neurones throughout the length of the spinal cord project to the medial septal nucleus. A similar projection was identified using anterograde transport of PHA-L injected in the cervical spinal cord (Burstein et al., 1987). However, the spinal neurones projecting to this region were few compared to spinohypothalamic or spinothalamic projecting neurones identified with similar retrograde tracer transport technique (see discussion Burstein and Giesler, 1989). Alternately or conjointly, nociceptive information may reach the MS-VLDBB complex via a spinal-lateral hypothalamic-MS-VLDBB fibre projection system. Spinal cord-lateral hypothalamic afferents are described by antidromic activation of spinal neurones and by anterograde and retrograde transport of tracers (Burstein et al., 1987). Lateral hypothalamic projecting neurones were found throughout the length of the spinal cord. The majority of these spinohypothalamic neurones were localized in the lateral reticulated area of the spinal dorsal horn. Other spinohypothalamic neurones were localized in the lateral spinal nucleus, in the gray matter around the central canal and in the marginal zone. Burstein and Giesler (1989) indicated, using identical retrograde tracing techniques, that comparable large number of spinal neurones were labelled following tracer injection in the thalamus or the hypothalamus.

Spinal neurones projecting to the lateral hypothalamus respond to noxious heat. For example, Burstein et al. (1987) reported that 6 of 7 antidromically identified spinohypothalamic neurones were multireceptive and the remaining neurone was nociceptive specific. These neurones responded incrementally to increasing noxious thermal stimuli applied to their receptive field.

A number of lateral hypothalamic neurones increased or decreased their activity in response to noxious stimuli (Hamba, 1988; Sikdar and Oomura, 1985). Indeed, rats with lesions of the lateral hypothalamus showed a lack of responsiveness to sensory stimuli (Marshall et al., 1971). Further, lateral hypothalamic afferents to the MS-VLDBB have been identified using anterograde transport of tritiated amino acids (Saper et al., 1979; Swanson and Cowan, 1979).

The MS-VLDBB also receives a dense afferent input from various brainstem nuclei, including the locus coeruleus (Vertes, 1988). Neurones of the locus coeruleus respond to noxious stimuli (Elam et al., 1986; Jacobs, 1986) which, in turn, might influence MS-VLDBB neuronal activity.

Nociceptive related information can also access the hippocampus via a projection system including the spinal cord-medial reticular formation-medial and intralaminar thalamus-cingulate cortex-and entorhinal cortex. The entorhinal cortex projection to the hippocampus and the dentate gyrus has been detailed earlier in the text. The entorhinal cortical area receives a substantial projection from the cingulate cortex via the cingulum fibre bundle (Raisman et al., 1965). Cingulate cortical neurones are responsive to sensory stimulation, especially in relation to acquired behavioural significance of the stimuli. For example, in a dual tone discrimination-avoidance behaviour, the cingulate cortical neurones responded with enhanced activity to tone stimuli (CS) paired to nociceptive footshock (US),

but not to the unpaired tone stimulus (Gabriel et al., 1980). The differential response developed early with the first CS-US exposure. The authors suggested that this differential response of the cingulate cortical neurones coded for the associative significance of the conditional stimulus. Such associative significance of a stimulus to pain or nociception may be transferred to the hippocampus and the dentate gyrus via the entorhinal cortical afferents to these structures. Indeed, entorhinal cortical neurones respond to noxious stimuli (cf Witter et al., 1989). Further, both the hippocampal pyramidal neurones and dentate granule cells show a differential response to conditional stimuli in a discrimination task (Foster et al., 1987; Wiener et al., 1989). Additionally, lesioning of the entorhinal cortical-perforant path abolished the conditional stimuli discharge in the dentate granule cells (Foster et al., 1988).

The intralaminar thalamic nuclei, which include the centrolateral and paracentral nucleus and the centromedian-parafascicular complex, might be involved in transmission of nociceptive information to the cingulate cortex. Indeed, there are afferents projecting from the intralaminar thalamic nuclei to the cingulate cortex (Jones and Leavitt, 1974; Vogt, 1985) and many neurones in the intralaminar thalamic region respond to noxious stimuli. For example, in anaesthetized rats, neurones in the centrolateral and centromedian-parafascicular region showed an increase or decrease in their spontaneous activity to noxious mechanical stimuli applied anywhere on the animal body surface (Peschanski et al., 1981). Noxious heat stimuli also altered the activity of most neurones so tested. In most cases, an intense noxious heat stimulus (50-55°C for 15 sec applied to the tail) was required to alter the activity of these neurones. Further, these neurones did not respond in a graded fashion to increasing noxious heat temperatures.

The intralaminar thalamic nuclei may receive nociceptive information from the medial reticular nuclei. For example, Peschanski and Besson (1984) using anatomical tracing techniques in the rat, have identified a projection from the nucleus reticularis gigantocellularis (NGC) to the above mentioned intralaminar thalamic nuclei. They have suggested that, since many NGC neurones respond to noxious stimuli, the afferents from this nucleus might relay nociceptive information to the intralaminar thalamic region. The NGC, in turn, receives afferents from the spinal cord. These originate chiefly from laminae VII and VIII in the ventral horn, around the central canal, and to a lesser extent, from the neck of the dorsal horn (Peschanski and Besson, 1984). Further, in anaesthetized rats, many lumbar spinal cord neurones antidromically driven from the medial reticular areas, including NGC, were responsive to noxious stimuli (Menetrey et al., 1980). Generally, these neurones had large receptive fields that included at least half the paw.

Peschanski and Besson (1984) argued that the nociceptive information transmitted along the spino-reticulo-thalamic pathway might be involved in the motor and/or behavioural reaction of the animal to pain. Indeed, the electrophysiological response of intralaminar thalamic neurones suggests that the thalamic output along this pathway does not code for discrete spatial or temporal information. Based on lesion and stimulation studies, Melzack and Casey (1968) proposed that the intralaminar thalamic nuclei were involved in aversive drive and affect in response to pain. The spino-reticular-thalamic-cingulate cortical pathway relevant to affective-motivational aspect of pain has often been described as the medial pain system (Melzack and Casey, 1968; Vogt, 1985).

Taking into account the above described evidence, it appears that the noxious stimulus-induced persistent depression of the CA1 pyramidal cell synaptic excitability is due to a septal afferent input to the field CA1. The major evidence highlighting MS-VLDBB

involvement are: (a) MS-VLDBB input to the hippocampus is suggested to be activated during noxious and non-noxious stimulation (see above) as well as during stress (Gilad, 1987), and (b) MS-VLDBB input is implicated in sensory stimulation-induced decrease in hippocampal pyramidal cell excitability. Indeed, pharmacological evidence discussed later in the text is consistent with the notion that MS-VLDBB cholinergic input is involved in depression of CA1 synaptic transmission to a noxious stimulus.

Habituation

The noxious stimulus-induced depression of CA1 pyramidal cell synaptic excitability habituates in response to the same noxious stimulus applied repeatedly . This is in marked contrast to the reflex-reaction and the duration of theta rhythm which did not habituate with repeated noxious stimuli. Similarly, ventromedial medullary neurones, including from the nucleus reticularis paragigantocellularis, showed a habituation of evoked activity to repeated noxious heat stimuli in conscious rats (Oliveras et al., 1990). The habituation persisted for the 15 min tested. There was, however, no habituation of the accompanying skin twitch reflex. The application of repetitive innocuous stimulus did not induce a habituation of the neuronal response. The authors suggested that habituation to repeated noxious stimuli may be related to stress. Indeed, spinal and thalamic neurones which subserve sensory-discriminative aspects of nociception or " pain " do not show habituation to repeated noxious stimuli (Oliveras et al., 1990).

The mechanism(s) involved in the observed habituation of CA1 synaptic excitability is not clear. Various possibilities exist for producing habituation. For example, such a process may be localized within the hippocampus. Not consistent with this idea is the finding that, after habituation of the depression following repeated noxious stimuli applied to

the left hind paw, exposure of the tail to a noxious stimulus produced synaptic depression when recording from the same site within the field CA1. If local effects were important, habituation to a noxious stimulus applied to the left hind paw might be expected to produce a failure of response to the tail exposure. More so, because a near maximal depression was observed with LHP or tail exposure (84 ± 7 % and 73 ± 10 %, respectively) which decreases the possibilty that different subset of neurones comprising the population response might be inhibited each time.

Habituation in the hippocampus may occur secondary to changes elsewhere in the central nervous system (CNS). For example, in a multiple unit recording from the MS-VLDBB region, Berger and Thompson (1977) found that the evoked activity produced by an auditory tone or air puff to the cornea decreased with repeated presentations of the stimulus. Similar findings were reported by Vinogradova (1975) in a minority (31 %) of medial septal neurones. It is not clear whether MS-VLDBB neurones would show decrement of response to repeated noxious stimuli. However, this region receives a substantial projection from the locus coeruleus nucleus (Vertes, 1988) which has been implicated in habituation to the repeated noise stress in conscious cats (Abercrombie and Jacobs, 1987). Locus coeruleus neurones respond to a variety of stimuli including noxious stimuli (Elam et al., 1986; Jacobs, 1986). However, it is not clear whether locus coeruleus neuronal response habituates to repeated intense noxious stimuli. This nucleus in turn receives a very prominent afferent input from the nucleus paragigantocelluaris (Aston-Jones et al., 1986). As indicated above neurones in the nucleus paragigantocelluaris can show habituation of response to repeated noxious stimuli (Oliveras et al., 1990). Thus, neurones in CNS, which may subserve input to the hippocampus, can show habituation or a decrement in response to repeated

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presentation of a stimulus. Similarly, a habituation in the current study may be a continum of this response which may reflect processing to stress or novelty.

In the current study, where the tail was exposed to noxious heat after an identical exposure to the left hind paw, there was no habituation of response to the second noxious heat exposure of the tail. But, the time course of persistent depression to additional noxious stimuli was not tested and thus it is not clear whether habituation would have occurred to subsequent noxious heat exposures.

PHARMACOLOGICAL FINDINGS

We found that intraperitoneal administration of the cholinergic muscarinic antagonist, atropine, blocked the effects produced by a noxious stimulus on the CA1 population spike amplitude. The dose of atropine sulphate (40 mg/kg) used was high. However, evidence in the literature, as well as from our experiments, suggests that this dose of atropine is selective in its antagonism of cholinergic-mediated synaptic events in the hippocampus. For example, a high dose of atropine (39 mg/kg, i.p), which blocked septal stimulation-induced facilitation of the CA1 population spike, also antagonized the effect of muscarinic agents, acetylcholine and methacholine, but not that of the nicotinic agent, dimethylphenyl piperazine (DMPP), when these agents were applied iontophoretically on CA1 neurones (Krnjevic and Ropert, 1982). Further, this dose of atropine blocked the cholinergic septal stimulation-induced facilitation of the CA1 population spike (Krnjevic and Ropert, 1982) but spared the presumably non-cholinergic facilitation of the dentate population spike following septal stimulation (Fantie and Goddard, 1982). Similarly, in the current study the dose of atropine used to markedly attenuate the septal facilitation of the CA1 population spikes spared the non-cholinergic paired-pulse facilitation of the population

spike. The facilitation of population spike by both the lower and higher intensities of septal stimulation was greatly attenuated following administration of atropine. Further, the difference in facilitation between the two intensities of MS-VLDBB stimulation was abolished. This may indicate that at the dose given, atropine sulphate strongly antagonized endogenous Ach effects in the hippocampus. This is further supported by the absence of hippocampal EEG theta rhythm in these animals following atropine administration. This latter effect is consistent with reports in the literature, where comparable doses of atropine blocked the cholinergic-mediated type-2 theta rhythm in the hippocampus but have little effect on non-cholinergic type-1 theta rhythm (Bland, 1986). Further, a high dose of atropine (50 mg/kg, i.p or i.m) produced a substantial displacement of saturated 3H QNB binding to presumed muscarinic receptors in the hippocampus (Yamamura et al., 1974). Such doses of the drug also disrupted animal performance in a spatial task in which the hippocampus is involved (Whishaw, 1989).

It is likely that atropine prevented the noxious stimulus-induced persistent depression of the CA1 population spike by blocking the cholinergic septo-hippocampal input. Septo-hippocampal neurones are known to be excited by noxious stimuli (Dutar et al., 1985). Some of these neurones may be cholinergic and release Ach in the hippocampus to depress the CA1 population spike. Indeed, Ach is released in the hippocampus of conscious or anaesthetized rats following sensory stimulation such as stroking the body fur (Dudar et al., 1979). Such stimulation also produced a short lasting depression of the CA1 population spike (Herreras et al., 1988a). This depression was antagonized by atropine locally infused in the hippocampus (Herreras et al., 1988b). In the current experiments, iontophoretic atropine applied at the cell body recording site failed to block the persistent depression of the CA1 population spike to a noxious stimulus. However, this lack of effect may be due to (a)

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the failure to block the action of released Ach near the recording site, or more likely (b) synaptically released Ach near the cell body site of CA1 pyramidal neurones is involved in enhancement but not depression of the population spike amplitude (Krnjevic and Ropert, 1982; Rovira et al., 1983a; Valentino and Dingledine, 1981).

Acetylcholine, if released in the hippocampus following a noxious stimulus, may depress the CA1 population spike by its action in the dendritic region. Septal afferents are known to terminate in the apical and basal dendritic region of CA1 pyramidal cells (see review of literature) and septal stimulation reduced the apical dendritic field EPSP to commissural afferent stimulation (Rovira et al., 1983a). This effect of septal stimulation was also antagonized by muscarinic antagonists, scopolamine administered systemically, or atropine applied iontophoretically at the dendritic recording site. Consistent with these findings, iontophoretically applied Ach in the apical dendritic region of CA1 pyramidal neurones reduced the dendritic field EPSP and abolished the population spike (Rovira et al., 1983b; Valentino and Dingledine, 1981). We were able to reproduce these findings and show that iontophoretic atropine applied in the apical dendritic region prevented the depression of the dendritic field EPSP to iontophoretic Ach and to a noxious stimulus. The effect of atropine is selective in that it blocked the depression of the dendritic field EPSP to iontophoretic Ach but not to iontophoretic GABA.

Valentino and Dingledine (1981) suggested that Ach depressed synaptic transmission in CA1 by presynaptic mediated decrease of the synaptic afferent drive. Their conclusion was partly based on the observation that, while dendritic Ach depressed the EPSP in pyramidal neurones recorded intracellularly, it did not attenuate depolarizations evoked in these neurones by brief dendritic applications of glutamate. Indeed, in the rat hippocampal synaptosomal preparation, Ach reduced the Ca^{2+}-dependent K^+-evoked release

of glutamate (Marchi and Raiteri, 1989). Glutamate is a putative excitatory neurotransmitter in the Schaffer-collaterals and the commissural afferents to field CA1.

The effect of iontophoretic atropine applied at the dendritic recording site is consistent with the effect of systemic atropine in preventing the depression of the CA1 population spike. Part of the effect of systemic atropine may be at the apical dendrites, preventing the depression of the dendritic field EPSP and therefore the population spike to a noxious stimulus. Further, the major source of cholinergic input to the hippocampus is from the MS-VLDBB (see review of literature). These pharmacological findings favour the notion that the septal-cholinergic input is involved in the persistent depression of the CA1 synaptic transmission to a noxious stimulus.

Our findings that in the majority of experiments a noxious stimulus increased the amplitude of the CA1 antidromic spike is also consistent with activation of septo-hippocampal cholinergic afferents. Dalkara et al. (1986) found that Ach iontophoretically applied in the pyramidal cell body region enhanced the antidromic spike amplitude in field CA3. The effect was most evident if the control antidromic spike amplitude was between 20 and 90 % of maximum. The submaximal antidromic spike was enhanced 8-38 % by various iontophoretic Ach doses, or by different intensities of medial septal stimulation (Dalkara et al., 1986). This is reminiscent of our findings where the submaximal antidromic spike was maximally enhanced 20-40 % following a noxious stimulus. Dalkara et al. (1986) suggested that the enhancement of the antidromic spike was due to enhanced ephaptic interactions. This results from Ach mediated disinhibition making the pyramidal neurones more excitable (Dalkara et al., 1986; see review of literature). The failure in some experiments to produce an enhanced antidromic spike following noxious stimulus maybe because they were more

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closer to maximal Consistent with this notion is the finding that while the dendritic field EPSP is still depressed the population spike amplitude tends to recover.

FUNCTIONAL IMPLICATIONS

Noxious stimuli-induced stress and adaptive behaviour

It appears that the noxious stimuli-induced persistent depression and habituation of the CA1 pyramidal cell excitability may reflect neural changes in the hippocampus which accompany stress. The evidence is as follows : (a) persistent depression of hippocampal formation dentate population spike was observed following restraint or footshock stress (Henke, 1990), (b) stress induced an activation of cholinergic MS-VLDBB input to the hippocampus (Gilad, 1987) and increased acetylcholine release in the hippocampal formation (Imperato et al., 1989). Consistent with this notion, in our experiments the persistent depression of CA1 population spike and apical dendritic field EPSP is antagonized by systemic atropine or iontophoretic application in the apical dendritic recording sites, respectively, and (c) habituation of response to repeated stress is observed in certain CNS neurones such as those in the locus coeruleus (Abercrombie and Jacobs, 1987). Habituation of response to repeated noxious heat stimuli is also observed in reticular formation neurones in conscious rats which may relate to stress (Oliveras et al., 1990). Interestingly, restraint stress in conscious rabbits depressed the hippocampal stimulation-evoked potentials in the hypothalamic medial basal tuberal region (Kawakami et al., 1971). This may be in line with decreased synaptic excitability of hippocampal formation neurones following stressful exposure. Indeed, in rabbits repeated immobilization stress produced a gradual habituation

of the depression of hippocampal stimulation-evoked potentials in the medial tuberal region of the hypothalamus (Kawakami et al., 1971).

Henke (1990) reported that the depressed dentate population spike in stressed rats was associated with increased gastric pathology and escape latency to noxious footshock stimulation. The author speculated that this resulted from an impaired ability to cope with stress. In contrast, gastric pathology was not observed in animals exhibiting an enhanced dentate population spike to stress exposure. Similarly, in hippocampal slices from rats, the ability to induce long-term potentiation in field CA1 upon Schaffer-collateral stimulation was severly impaired if the animals had been exposed to inescapable shock (Shors et al., 1989). We suggest that a noxious stimulus-induced persistent depression of CA1 synaptic transmission, subsequent recovery of amplitude and habituation to repeated exposure may relate to the animal's adaptive changes to pain. The adaptive changes may be manifest in the hippocampal formation mediated passive avoidance behaviour after an initial nociceptive exposure. For example, both normal and hippocampal lesioned animals receiving an electric footshock escaped to a safe platform, but only normal animals showed a reluctance to step down from the safe area (Teitelbaum and Milner, 1963). Consistent with this that notion the hippocampal neuronal activity relate to adaptive behaviour are the findings that habituation of the hippocampal field CA1 and CA3 pyramidal cell depression accompanied adaptive changes of the animal's orienting response (Vinogradova, 1975). Of particular interest are the findings reported in a recent paper by Coderre and Wall (1988). They found that ankle joint urate arthritis in the rat was associated with a decreased response to noxious stimuli of the distal foot. Interestingly, they also noted that the forebrain was essential for the induction but not maintenance of this reduced responsiveness.

In conclusion, the hippocampus has been suggested to be involved in a variety of activities including learning and memory, cognitive mapping and adaptive behaviour of animals in stress. There is also an association between pain and these activities. Both non-noxious and noxious sensory stimuli clearly produce electrophysiological changes within the hippocampus although it is difficult to relate these directly to functional significance. Nevertheless, the characteristics of the changes produced by noxious stimuli noted in this study-prolonged depression of the CA1 synaptic transmission which habituates to repeated stimuli- are different from those observed by non-noxious stimuli. For example, stroking of fur in rats produced a short lasting depression of CA1 synaptic excitability and there was no habituation to repeated tests. It can further be said that the prolonged depression of the CA1 population spike and consequent habituation is not likely related to the sensory-discriminative aspect of pain for it is dissociated from the sensory stimuli-induced theta rhythm. More likely, although not conclusively, it is related to noxious stimuli induced-stress and the resultant adaptive behaviour.

SUMMARY AND CONCLUSION

The effects of noxious peripheral heat on the excitability of dorsal hippocampal field CA1 pyramidal neurones were examined. Experiments were also done to investigate whether the septo-hippocampal cholinergic system was involved in the observed changes. The following observations were made and conclusions reached:

(1). An intense noxious stimulus (55 or 60°C, 15 sec) applied to the tail or the left hind paw produce a prolonged and substantial depression of the CA1 population spike. The noxious stimulus also induced a 4-6 Hz theta rhythm in the hippocampal electroencephalogram (EEG) as well as a reflex response. The latency and the intensity of the tail-flick reflex was combined into a reflex-reaction score.

(2). The depression of CA1 population spike induced by noxious heat exposure of the tail was temperature-dependent. Thus, no consistent effect was seen at 50°C, while significant depression was observed 8 min and 18 min following exposure at 55 and 60°C, respectively. However, placing the tail in hot water at 55°C only until a tail-flick reflex occurred (about 5 sec) failed to produce a depression of the CA1 population spike.

(3). The depression of the population spike in response to a noxious stimulus habituated to subsequent noxious stimuli, whereas, the reflex-reaction score and the duration of theta rhythm did not.

(4). The persistent depression of the CA1 population spike was not seen when noxious heat (55°C, 15 sec) was applied in the presence of hippocampal theta rhythm. It is known that

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the hippocampal CA1 pyramidal cells during theta rhythm undergo fluctuation in excitability related to different phases of the theta wave (Fox et al., 1986). Possibly, this limits the extent or duration of influence of the external input onto these cells.

(5). Exposure of the tail to hot water (55°C, 15 sec) after habituation of the depression to left hind paw exposure to the same noxious stimulus, produced a depression of the CA1 population spike. A second noxious heat stimulus to the tail also depressed the CA1 population spike. Since a maximal depression of > 70 % occurred when either the left hind paw or tail was exposed to noxious heat, it is clear that many of the same neurones are depressed by the noxious stimulus. This perhaps suggests that the mechanism leading to habituation is not localized within field CA1 of the hippocampus. However, on theoretical grounds, other mechanisms can be envisioned which would account for habituation being generated within the hippocampus.

(6). The CA1 population spike evoked by either ipsilateral or contralateral CA3 stimulation was depressed by the noxious heat stimulus (55°C, 15 sec). The ipsilateral or contralateral CA3 stimulation presumably excited the Schaffer-collateral or commissural afferent input to the field CA1, respectively. It follows that there is a persistent depression of synaptic transmission in field CA1 to different afferent inputs.

(7). The dendritic field EPSP was also depressed following a noxious heat stimulus to the tail. This response also habituates to subsequent applications of the noxious stimulus to the same receptive area. On the other hand, the antidromic spike amplitude was enhanced to a noxious heat exposure in the majority of cases. These results indicate that presynaptic

changes are involved in the depression of synaptic transmission through field CA1 in response to a noxious stimulus. This inference is also consistent with the results obtained from pharmacological experiments.

(8). Systemic atropine sulphate (40 mg/kg, i.p) prevented the persistent depression of the CA1 population spike to a noxious stimulus. This dose of atropine also greatly attenuated the facilitation of CA1 population spike by both the lower and higher intensity septal tetanic stimulation. The higher intensity of septal stimulation induced a population spike approximately twice the amplitude induced by lower septal stimulation. However, following atropine administration, the facilitation in both cases was antagonized and the difference in facilitation between the two intensities of MS-VLDBB stimulation was abolished. This may indicate that at the dose given, atropine sulphate strongly antagonized endogenous Ach effects in the hippocampus. This is further supported by the absence of hippocampal EEG theta rhythm in these animals following atropine administration. Atropine pretreatment did not effect the non-cholinergic paired-pulse facilitation of the CA1 population spike.

(9). Iontophoretic atropine, when applied in the apical dendrites, prevented depression of dendritic field EPSP to a noxious stimulus, whereas, it had no effect on the depression of the CA1 population spike when applied in the cell body region. Thus, a site of action for systemic atropine in preventing the depression of synaptic transmission may be at the apical dendrites of pyramidal cells in field CA1.

Since the MS-VLDBB is the major source of the hippocampal cholinergic system (Fonnum and Walaas, 1978; Matthews et al., 1987), the above findings (8 and 9) suggest

summary and conclusion

that this afferent input is involved in noxious stimulus-induced persistent depression of CA1 synaptic transmission. In support of this idea, the septo-hippocampal neurones are activated by peripheral noxious heat stimuli (Dutar et al., 1985) and acetylcholine is released in the hippocampal formation by sensory stimulation (Dudar et al., 1979) and stress (Imperato et al., 1989). Further, the effective antagonism of noxious heat-induced CA1 depression by the iontophoretically applied dendritic muscarinic antagonist, atropine, may be due to antagonism of a dendritically released Ach-mediated presynaptic effect.   Indeed, iontophoretic application of Ach in the apical dendrites depressed the Schaffer-collateral stimulation-evoked synaptic EPSP in pyramidal neurones recorded intracellularly, but it did not attenuate depolarizations evoked in these neurones by brief apical dendritic application of glutamate (Valentino and Dingledine, 1981). These authors ruled out an effect of Ach on the postsynaptic dendritic membrane because the time course of the EPSP was not altered. Furthermore, in the rat hippocampal synaptosomal preparation, Ach reduced the Ca^{2+}-dependent K^{+}-evoked release of glutamate (Marchi and Raiteri, 1989). Glutamate is a putative excitatory neurotransmitter in the CA3 Schaffer-/commissural afferents.

In conclusion, the hippocampus has been suggested to be involved in a variety of activities including learning and memory, cognitive mapping and adaptive behaviour of animals in stress. There is also an association between pain and these activities. Both non-noxious and noxious sensory stimuli clearly produce electrophysiological changes within the hippocampus although it is difficult to relate these directly to functional significance. Nevertheless, the characteristics of the changes produced by noxious stimuli noted in this study -prolonged depression of the CA1 synaptic transmission which habituates to repeated stimuli- are different from those observed by non-noxious stimuli. For example, stroking of fur in rats produced a short lasting depression of CA1 synaptic excitability and there was no

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