NORADRENERGIC FUNCTION IN THE DENTATE GYRUS: AN ELECTROPHYSIOLOGICAL INVESTIGATION IN VITRO

JEAN-CLAUDE LACAILLE

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NORADRENERGIC FUNCTION IN THE DENTATE GYRUS: AN ELECTROPHYSIOLOGICAL INVESTIGATION IN VITRO

BY

© Jean-Claude Lacaille, B.Sc.

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Department of Psychology
Memorial University of Newfoundland

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Abstract

Although the hippocampal formation is known to receive an extensive noradrenergic input, there is some controversy about the effects of norepinephrine (NE) on hippocampal neurons and it is not known whether the effects of NE are identical in all the major subdivisions of the hippocampal formation. The objectives of the present study were to evaluate in detail the physiological effects of NE in the rat dentate gyrus in vitro and to compare these effects with those previously reported for hippocampal pyramidal cells and for dentate gyrus granule cells.

Granule cell responses were measured electrophysiologically by recording perforant path evoked field potentials. Responses were digitized and analyzed by a microprocessor based system for EPSP slope or amplitude, population spike onset latency and population spike amplitude.

NE produced dose-dependent changes in evoked responses which were maximal after superfusion of 10 uM NE for 10 minutes. Typical effects of 10 uM NE were to increase population spike amplitude and decrease population spike onset latency. Effects of NE on extracellular EPSP parameters were less consistent: EPSP slope was generally increased and EPSP amplitude was most often unchanged although sometimes increased. The effects of NE on

population spike amplitude were of the greatest magnitude and duration. Long-lasting increases in population spike amplitude were observed in 24% of the cases.

The effects of NE were mediated by beta-adrenergic receptors. Timolol but not phentolamine blocked them and isoproterenol but not phenylephrine mimicked them. The population spike-EPSP relationship suggested that 50% of the increase in population spike amplitude was due to synaptic effects and 50% to extrasynaptic effects of NE. NE did not increase the antidromic population spike, thus the enhanced responsiveness was not due to granule cell soma membrane excitability changes. NE superfusion without concurrent perforant path stimulation produced enhancement of subsequently evoked responses. Hence, these effects of NE were activity-independent. In contrast to NE, 5-HT produced decreases in perforant path evoked responses.

These results suggest that the noradrenergic input to the dentate gyrus facilitates the granule cell responses to afferent inputs. These results are discussed in terms of noradrenergic function in the dentate gyrus, are related to hippocampal function in physiological and behavioral perspectives, and are compared to the effects of NE in other central nervous system (CNS) regions.

Key words: norepinephrine - dentate gyrus - hippocampal slice - EPSP - population spike - long-lasting potentiation - beta receptors

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List of Abbreviations

5-HT	- Serotonin
5-OHDA	- 5-hydroxydopamine
6-OHDA	- 6-hydroxydopamine
ACh	- Acetylcholine
ACSF	- Artificial cerebrospinal fluid
ACTH	- Adrenocorticotropin
A-D	- Analog-to-digital
CA1	- Ammon's horn regio superior
CA3	- Ammon's horn regio inferior
cAMP	- Cyclic adenosine 3',5'-monophosphate
CNS	- Central nervous system
CRF	- Corticotropin-releasing factor
D-A	- Digital-to-analog
DBH	- Dopamine beta hydroxylase
DC	- Direct current
DG	- Dentate gyrus
ECl	- Lateral entorhinal cortex
ECm	- Medial entorhinal cortex
EPSP	- Excitatory postsynaptic potential
GABA	- Gamma-aminobutyric acid
GLU	- Glutamate
HRP	- Horseradish peroxidase
IPSP	- Inhibitory postsynaptic potential
ISO	- Isoproterenol
MAO	- Monoamine oxidase

NE	- Norepinephrine, noradrenaline
PA	- Phentolamine
PARASUB	- Parasubiculum
PE	- Phenylephrine
PRESUB	- Presubiculum
RF	- Rhinal fissure
SUB	- Subiculum
TIM	- Timolol
VOR	- Vestibulo-ocular reflex

Chapter 1: Introduction

Early investigations of the function of the central noradrenergic system reported mostly suppressant effects of norepinephrine (NE) on neuronal responses. However, more recent studies have observed facilitatory effects of NE on spontaneous and evoked responses in many CNS regions. These facilitatory effects have been interpreted by many as increases in signal-to-noise ratio produced by NE. Such enhancement of evoked neuronal transmission may have implications for attentional processes. Similarly initial reports suggested suppressant effects of NE on granule cell responses in the dentate gyrus. Yet, more recent evidence indicates that NE also produces facilitation of granule cells' evoked responses. The present study was undertaken to characterize physiologically and pharmacologically the effects of NE on dentate granule cell responses in vitro.

However, before describing the present study, the literature on the central noradrenergic system will be reviewed. The anatomical localization of central noradrenergic fibers, the physiological effects of NE and the functional aspects of the central noradrenergic system are covered. Then the neurobiology of the hippocampus, including its anatomy, physiology, pharmacology and function, is described. A review of noradrenergic function

in Ammon's horn and the dentate gyrus follows. Then, the localization of hippocampal noradrenergic fibers, enzymes and receptors will be considered as well as the previously reported physiological effects of NE. Finally, the objectives of the present study are described.

Norepinephrine in the central nervous system

Identification

The presence of norepinephrine in the central nervous system was suggested by Euler (1946) on the basis of his observations that spinal cord and brain extracts contained "sympathin", a substance possessing physiological effects similar to those of norepinephrine. Later, Holtz (1950) demonstrated that sympathin was composed of 90% norepinephrine and 10% epinephrine. By performing a number of biological and chemical tests on discrete brain extracts, Vogt (1954) showed that norepinephrine was present in varying amounts in the spinal cord, medulla, mesencephalon, diencephalon and telencephalon. Based on these studies, the idea developed that norepinephrine could possibly serve as a neurotransmitter for discrete populations of neurons in the CNS.

Anatomical localization

With the development of the formaldehyde fluorescence method, it became possible to visualize the noradrenergic fiber terminals in the brain (Carlsson, Falck & Hillarp, 1962; Fuxe, 1965) and spinal cord (Dahlstrom & Fuxe, 1965) as well as the noradrenergic neurons themselves (Dahlstrom & Fuxe, 1964) and their axonal pathways (Ungerstedt, 1971).

Norepinephrine-containing cell bodies in the rat were only found in certain nuclei of the brain stem (Dahlstrom & Fuxe, 1964). Proceeding roughly from caudal to rostral, these cell groups are referred to as A1, A2, A3, A4, A5, A6 and A7 (Dahlstrom & Fuxe, 1964). Cell group A1 is located in the region of the inferior olivary complex and of the lateral reticular nucleus. Cells of the A2 group are found in the area of nucleus of the solitary tract, dorsal motor nucleus of the vagus nerve and commissural nucleus. The few neurons of the group A3 are situated in the dorsal accessory olivary nucleus. Cells of group A4 are seen in the lateral part of the roof of the fourth ventricle under the ependyma at the level of the nucleus of the facial nerve. Neurons forming group A5 are located among the fibers of the rubrospinal tract at the level of the superior olivary nucleus. Cells of group A6 are identical with the nucleus locus coeruleus and represent the largest noradrenergic cell

group. Finally, a small group of cells forming A7 are found in the reticular formation, at the caudal third of the central grey, ventral to the ventral portion of the superior cerebellar peduncle.

With regard to the noradrenergic terminals, following the initial localization of fluorescing fibers and varicosities in the hypothalamus (Carlsson et al., 1962), they were found to be widely distributed in the CNS from the cortex to the brain stem (Fuxe, 1965) as well as in the spinal cord (Dahlstrom & Fuxe, 1965). Fuxe (1965) reported that, in the telencephalon, noradrenergic terminals were found in the neocortex, olfactory lobe, entorhinal and piriform cortex, cingulate gyrus, hippocampal formation, amygdaloid complex, septal area and interstitial nucleus of the stria terminalis.

In the diencephalon noradrenergic terminals were seen in many hypothalamic nuclei, specifically the dorsomedial, paraventricular, supraoptic, periventricular, lateral, ventromedialand anterior nuclei, as well as in the retrochiasmatic and preoptic areas, and in the nuclei of the mammillary body. The majority of thalamic nuclei were also shown to contain noradrenergic terminals. In the cerebellum, noradrenergic terminals were observed scattered in all layers of the cortex. In the brain stem, Fuxe (1965) showed that noradrenergic terminals were present in the

majority of somatic afferent and efferent nuclei, as well as in many visceral afferent and efferent nuclei and in certain parts of the reticular formation.

With respect to the innervation of the spinal cord, Dahlstrom and Fuxe (1965) reported the presence of noradrenergic nerve terminals in the spinal grey matter throughout the extent of the spinal cord. A higher density of terminals was observed in the sympathetic lateral column, in the more ventral portion of the ventral horn and in the most dorsal portion of the dorsal horn (substantia gelatinosa). Detailed descriptions of the distribution of noradrenergic terminals have appeared elsewhere: see Levitt and Moore (1979) and Jones and Friedman (1983) for brain stem; Olson and Fuxe (1971) for cerebellum; Blackstad, Fuxe and Hokfelt (1967) for hippocampus; Fuxe, Hamberger and Hokfelt (1968) for cerebral cortex; and Lindvall, Bjorklund, Nobin and Stenevi (1975) for thalamus.

By combining formaldehyde fluorescence with 6-hydroxydopamine (6-OHDA) lesions, Ungerstedt (1971) visualized the axonal pathways by which the projections of the brain stem noradrenergic neurons ascend to reach their terminal areas. Using the glyoxylic acid method, which resulted in improved visualization of fluorescing noradrenergic fibers, Lindvall and and Bjorklund (1974) confirmed and expanded on these observations in the intact

rat. A description of the descending pathway from the brain stem to the terminal fields in the spinal cord has been given by Dahlstrom and Fuxe (1965). Ungerstedt (1971) described two major ascending pathways, a ventral pathway originating mostly from cell groups A1, A2, A5 and A7, and a dorsal ascending pathway originating mostly from locus coeruleus (A6). He additionally described a descending pathway originating from locus coeruleus that projects to lower brain stem nuclei, and a lateral pathway also originating from locus coeruleus which enters the cerebellum medial to the pedunculus cerebellaris medius. Lindvall and Bjorklund (1974) additionally identified another major fiber system, the periventricular system containing axons originating from cell groups A2, A4 and locus coeruleus.

Proceeding from caudal to rostral, axons from cell groups A1, A2 and A5 ascend together in the mid-reticular formation where they overlap with the descending pathway from locus coeruleus to form the medullary bundle. Both of these pathways contribute to the terminal innervation in the pons and medulla (Ungerstedt, 1971). At the level of the locus coeruleus this ascending pathway receives contributing fibers from locus coeruleus (A6) and A7 cell group neurons and courses ventromedially to form loosely the ventral noradrenergic bundle (Ungerstedt, 1971; Lindvall & Bjorklund, 1974). However, the majority of fibers from the

locus coeruleus remain dorsal and project rostro-medially to form the dorsal noradrenergic bundle (Ungerstedt, 1971). More medially, fibers originating in locus coeruleus, A2 and A4 groups project rostrally to form the periventricular system (Lindvall & Bjorklund, 1974). These noradrenergic fibers in the periventricular system in turn project to and provide noradrenergic innervation to the tectal, pretectal, thalamic, epithalamic and hypothalamic regions.

Further rostrally at the hypothalamic level, the dorsal and ventral noradrenergic bundles join ventrally to form part of the medial forebrain bundle (Ungerstedt, 1971; Lindvall & Bjorklund, 1974). Along the medial forebrain bundle, fibers from the ventral bundle exit to provide noradrenergic innervation to hypothalamic, thalamic, preoptic, septal, amygdaloid and pyriform regions (Ungerstedt, 1971; Lindvall & Bjorklund, 1974). Finally, the fibers in the medial forebrain bundle, originating from the dorsal bundle and locus coeruleus, continue rostrally and terminate mostly in the thalamus, hippocampus and neocortex but also in the tectum, pretectum, metathalamus, amygdaloid-piriform region, septum and olfactory bulb (Ungerstedt, 1971; Lindvall & Bjorklund, 1974).

It can be seen from this brief description of the projections of the noradrenergic system that it is highly divergent in nature. Noradrenergic neurons, especially

those of the locus coeruleus, give off collateral branches which innervate different brain areas. These divergent projections have been directly demonstrated for single locus coeruleus neurons using retrograde double-labeling techniques in rats (Nagai, Satoh, Imamoto & Maeda, 1981; Room, Postema & Korf, 1981) and mice (Steindler, 1981). Nagai et al. (1981) observed double labeled locus coeruleus neurons projecting to frontal cortex and occipital cortex, frontal cortex and cerebellar cortex, frontal cortex and olfactory bulb, cerebellar cortex and spinal cord, and hippocampus and olfactory bulb.

Room et al. (1981) reported divergent projections of locus coeruleus neurons between any pairing of cerebral cortex, thalamus, hippocampus and spinal cord. Finally, Steindler (1981) demonstrated divergent projections of the same locus coeruleus neurons to cerebellar cortex and visual cerebral cortex as well as to cerebellar cortex and somatosensory cerebral cortex. These double-labeling studies complement the observations from histofluorescence and suggest that the noradrenergic system in the CNS is divergent in nature with collaterals branching from each axon and projecting to wide-spread areas. Although the projections of the noradrenergic system are divergent, they also display neuronal specificity in that they project only to certain specific areas of the CNS and not diffusely to

all areas.

Ultrastructural localization

Descarries and Lapierre (1973) demonstrated that the noradrenergic axon terminals in the cerebral cortex could be radioautographically visualized following topical application of [^{3}H]-norepinephrine in the presence of monoamine oxidase inhibitors. The distribution and the morphology of the radioautographically labeled noradrenergic terminals were found to be similar at the light microscope level to that described previously with histofluorescence (Descarries & Lapierre, 1973). Additionally, their radioautographic presence was shown to be sensitive to destruction of noradrenergic terminals by 6-OHDA (Descarries & Lapierre, 1973).

This method also permitted the serial visualization of the labeled axon terminals at the electron microscope level (Descarries & Lapierre, 1973; Lapierre, Beaudet, Demianczuk & Descarries, 1973; Descarries, Watkins & Lapierre, 1977). Ultrastructurally, [^{3}H]-norepinephrine was found predominantly in axonal enlargements (varicosities) in association with small agranular vesicles and often large dense-core vesicles but was also found in axonal segments devoid of synaptic vesicles (Descarries & Lapierre, 1973).

Quantitatively, labeled axons were observed to be unmyelinated and fine (0.35 μm) whereas the varicosities were spaced at varying intervals (1-3 μm) and were approximately 1 μm in diameter (Lapierre et al., 1973; Descarries et al., 1977).

An important ultrastructural difference observed between noradrenergic synaptic boutons and others in the adjacent neuropil was that only a low proportion of noradrenergic varicosities (less than 5% for NE vs 50% for unlabeled) were found to form typical junctional complexes with asymmetric or symmetric membrane differentiations (Descarries et al., 1977). Therefore most noradrenergic varicosities were found to lack typical synaptic structures. These results seemed to confirm earlier ultrastructural observations obtained from the periventricular nucleus of the hypothalamus with permanganate perfusion (Hokfelt, 1968). Swanson, Connely and Hartman (1978) using intraventricular injection of 5-hydroxydopamine (5-OHDA) for labeling and aldehyde fixation also found a low incidence (20%) of noradrenergic varicosities forming conventional asymmetric specialized contacts in the paraventricular nucleus of the hypothalamus.

In contrast, using either permanganate fixation or 5-OHDA labeling and aldehyde fixation, Landis and Bloom (1975) observed a greater incidence of specialized synapses in the cerebellar cortex. More recently, using immunocytochemical

localization of dopamine-beta-hydroxylase (DBH), the enzyme which synthesizes norepinephrine from dopamine, a higher incidence of typical synaptic contacts at noradrenergic varicosities was observed in a number of brain areas (Olschowka, Molliver, Grzanna, Rice & Coyle, 1981). The percentage of varicosities with synaptic contacts varied between 50-62% and such synaptic contacts, mostly of the asymmetric type, were found in the anteroventral and paraventricular nuclei of the thalamus, paraventricular nucleus of the hypothalamus, hippocampal dentate gyrus, retrosplenial granular cortex and cerebellar cortex (Olschowka et al., 1981).

Thus it appears that although their respective proportions remain controversial, two types of noradrenergic varicosities may be present in the CNS, those with specialized synaptic contacts and those with none. Functionally, it suggests two types of specificity for the neuronal actions of the noradrenergic system. First noradrenergic neurons will specifically influence those neurons with which they form conventional synapses and second they may diffusely influence other noradrenaline-receptive neurons which are located in the vicinity of the varicosities and which lack specialized synaptic contacts.

Physiological effects of norepinephrine

The initial attempts at evaluating the central effects of NE used the technique of iontophoretic application and reported no physiological effects of NE on the evoked activity of spinal cord motor neurons or interneurons (Curtis, Phillis & Watkins, 1961) or on the spontaneous activity of mesencephalic reticular formation neurons (Curtis & Koizumi, 1961) of barbiturate-anesthetized cats. Soon after however, it was reported that iontophoretically applied NE depressed the evoked unit activity of neurons of the lateral geniculate nucleus of the thalamus in barbiturate anesthetized cats (Curtis & Davis, 1962) and mostly facilitated but sometimes depressed the spontaneous activity of neurons of the brain stem in decerebrate cats (Bradley & Wolstencroft, 1962).

Following these initial reports, the effects of iontophoretically applied NE were investigated in a number of brain areas of decerebrate or non-barbiturate anesthetized animals and were found to be mostly depressant in nature. NE was observed to depress the spontaneous and evoked activity of neurons in cat neocortex (Krnjevic & Phillis, 1963a; 1963b). It primarily depressed, although sometimes facilitated, the spontaneous activity of neurons in cat hypothalamus mostly in paraventricular and ventral median nuclei (Bloom, Oliver & Salmoiraghi, 1963), of tufted

mitral or granule cells in rabbit olfactory bulb (Baumgarten, Bloom, Oliver & Salmoiraghi, 1963; Bloom, Costa & Salmoiraghi, 1964) and of neurons in the caudate nucleus of the cat (Bloom, Costa & Salmoiraghi, 1965). NE was found to suppress evoked or spontaneous neuronal activity in cerebellar cortex (Krnjevic & Phillis, 1963b; Yamamoto, 1967), as well as evoked activity in medial (Tebecis, 1967) and lateral (Phillis, Tebecis & York, 1967) geniculate nuclei of the thalamus. Re-examination of the effects of NE in the spinal cord showed that the spontaneous and evoked activity of interneurons, motor neurons and Renshaw cells was mostly depressed by NE (Biscoe, Curtis & Ryall, 1966; Engberg & Ryall, 1966), although the activity of some interneurons and Renshaw cells was facilitated (Weight & Salmoiraghi, 1966a; 1966b). In contrast, NE was observed to mostly facilitate the spontaneous and evoked activity of neurons in the lateral vestibular nucleus and cerebellar flocculus (Yamamoto, 1967).

Initial attempts at characterizing the NE effects in term of receptor types showed the relationship to be intricate. Bloom et al. (1964) reported antagonism of the depressant effects of NE in the olfactory bulb by alpha-receptor antagonists. Weight and Salmoiraghi (1966b) reported antagonism of the facilitatory effects of NE on Renshaw cells of the spinal cord also by alpha-receptor

antagonists, while Yamamoto (1967) observed antagonism of the facilitatory effects of NE on neurons of the lateral vestibular nucleus by a beta receptor antagonist.

Effects of norepinephrine on cerebellar Purkinje cells

A closer examination of the effects of NE on Purkinje cells of the cerebellar cortex revealed however that the action of NE was not simply depressant or inhibitory.

Early studies on Purkinje cells in rat cerebellum established that NE either released by iontophoresis (Hoffer, Siggins & Bloom, 1969) or synaptically by stimulation of the nucleus locus coeruleus (Siggins, Hoffer, Oliver & Bloom, 1971) produced a depression of the spontaneous activity of Purkinje cells. It was suggested that this depression was mediated via a postsynaptic beta receptor (Hoffer, Siggins & Bloom, 1971) and to involve the formation of cyclic adenosine 3',5'-monophosphate (cAMP) (Siggins, Hoffer & Bloom, 1971). Intracellular recordings from Purkinje cells showed that synaptic release of NE by locus coeruleus stimulation produced a membrane hyperpolarization associated with either no change or a decrease in membrane conductance (Siggins, Hoffer, Oliver & Bloom, 1971; Hoffer, Siggins, Oliver & Bloom, 1973). Similar membrane effects were produced by iontophoresis of

NE and cAMP (Hoffer et al., 1973).

However, when the action of NE on the evoked activity of Purkinje cells was investigated a different effect was found. Freedman, Hoffer, Woodward and Puro (1977) reported that during iontophoresis of NE, the spontaneous activity of the Purkinje cells is more depressed than the evoked activity produced by climbing fiber activation or by mossy fiber activation. Also, the depression evoked by stimulation of basket-stellate cells was augmented more than the depression of spontaneous activity during NE iontophoresis (Freedman et al., 1977). The augmentation of evoked inhibition was sometimes observed with current ejections of NE which produced only minimal changes in spontaneous activity (Freedman et al., 1977). Thus, the net effect of NE was to enhance the evoked activity (excitatory or inhibitory) relative to the spontaneous activity, or in other words, to increase the signal-to-noise ratio of the neuronal activity.

Similarly, Moises, Woodward, Hoffer and Freedman (1979) reported that, relative to spontaneous activity, iontophoretically applied NE preferentially augmented the responses of Purkinje cells to iontophoresis of the putative neurotransmitters for the pathways activated by Freedman et al. (1977). That is, NE preferentially augmented the excitation produced by iontophoresis of glutamate (GLU) as

well as the inhibition produced by gamma-aminobutyric acid (GABA) relative to spontaneous activity (Moises et al., 1979). Furthermore, the augmentation of glutamate and GABA responses was sometimes observed with NE levels causing minimal or no change in spontaneous activity (Moises et al., 1979). These facilitatory effects were specific to NE since dopamine did not produce such changes (Moises et al., 1979). Also importantly, these effects of NE were specific to the putative cerebellar neurotransmitters glutamate and GABA since the depressions produced by glycine (Moises et al., 1979), taurine or beta-alanine (Yeh, Moises, Waterhouse & Woodward, 1981) were not augmented.

Synaptic release of NE induced by locus coeruleus stimulation also augmented excitatory evoked activity (Moises, Waterhouse & Woodward, 1981) and inhibitory evoked activity (Moises & Woodward, 1980; Moises, Waterhouse & Woodward, 1983) of Purkinje cells. With respect to the pharmacological characterization of the facilitatory effects of NE, a beta-1-adrenergic antagonist effectively antagonized the augmentation of GABA inhibitory responses produced by iontophoresis of NE (Waterhouse, Moises, Yeh & Woodward, 1982; Yeh & Woodward, 1983). Although facilitation of GABA inhibitory responses seems to involve a beta-1 receptor, identification of the receptor type mediating facilitation of the excitatory responses has not

been achieved.

Based on these studies in the cerebellum the suggestion was made that the central function of NE was that of a neuromodulator which modifies neuronal responsiveness to conventional afferent synaptic inputs (Woodward, Moises, Waterhouse, Hoffer & Freedman, 1979).

Modulatory action of norepinephrine in other central nervous system areas

Since the initial description of the modulatory action of NE in the cerebellum, similar actions of NE have also been reported in other brain areas. In rat somatosensory cortex iontophoretically applied NE enhanced both excitatory and inhibitory synaptically-evoked responses relative to spontaneous activity (Waterhouse & Woodward, 1980). In some cells NE produced an increase only in the evoked excitatory response.

Similarly, Waterhouse, Moises and Woodward (1980) have shown that iontophoretically applied NE augmented, relative to background activity, the excitatory response of neurons of the somatosensory cortex to the putative neurotransmitter acetylcholine (ACh) applied iontophoretically (however see Reader, Ferron, Descarries & Jasper, 1979, for suppressant effects of NE on ACh responses and spontaneous activity in

frontoparietal cortex). The inhibitory responses to iontophoresis of GABA were also augmented relative to background activity following iontophoresis of NE (Waterhouse et al., 1980). The facilitation of both responses (ACh and GABA) was sometimes observed with minimal or no change in spontaneous activity and was specific to NE since application of dopamine did not facilitate the responses (Waterhouse et al., 1980). Waterhouse, Moises and Woodward (1981) have suggested that the facilitation by NE of excitatory responses in somatosensory cortex is mediated by an alpha adrenergic receptor since alpha but not beta agonists produced similar facilitation of iontophoretic and synaptic excitatory responses and alpha antagonists blocked the facilitation of ACh responses by NE. The nature of the facilitation of the inhibitory responses in terms of adrenoceptors has not been characterized in this brain region.

In cat visual cortex iontophoresis of NE enhanced visually-evoked excitatory responses in almost all simple cells but had less effect on complex cells, again relative to background activity (Kasamatsu & Heggelund, 1982). In many cells the evoked excitatory response itself was augmented (Kasamatsu & Heggelund, 1982). In area 17 of rat visual cortex, iontophoretically applied NE facilitated the excitatory and inhibitory responses evoked by visual

stimulation with either no change or suppression of spontaneous activity (Waterhouse, Azizi, Burne & Woodward, 1983). In this instance, two types of adrenoceptors were involved with alpha agonists producing facilitation of excitatory evoked responses and beta agonists, facilitation of inhibitory evoked responses (Waterhouse et al., 1983).

In monkey auditory cortex iontophoresis of NE also reduced spontaneous activity to a greater extent than excitatory responses evoked by species-specific vocalization, thereby producing a greater net evoked response (Foote, Freedman & Oliver, 1975).

In the lateral geniculate nucleus of the thalamus of cats and rats, the excitatory neuronal activity evoked by stimulation of the optic tract or by light flashes is augmented by NE released synaptically by locus coeruleus stimulation (Nakai & Takaori, 1974; Rogawski & Aghajanian, 1980a) or applied by iontophoresis (Rogawski & Aghajanian, 1980a; 1980b). Although the spontaneous activity of lateral geniculate neurons is also facilitated by iontophoretically (Rogawski & Aghajanian, 1980b; 1980c) or synaptically (Rogawski & Aghajanian, 1982) released NE, the net effect on the evoked activity is enhancement of the signal-to-noise ratio (Rogawski & Aghajanian, 1980b).

Pharmacological characterization suggested that the facilitatory effect of NE released iontophoretically or

synaptically on the evoked and spontaneous activity of lateral geniculate neurons is mediated via a postsynaptic alpha-1 receptor (Rogawski & Aghajanian, 1980a; 1980c; 1982). Whether NE facilitates lateral geniculate relay neurons directly or indirectly by removing an inhibitory effect of interneurons remains controversial. Nakai and Takaori (1974) reported that the neuronal activity of two types of interneurons in the lateral geniculate nucleus is depressed by locus coeruleus stimulation whereas Kayama, Negi, Sugitani and Iwama (1982) observed one type of interneuron to be depressed and another to be facilitated by locus coeruleus stimulation.

NE enhancement of evoked activity via a disinhibitory process has been reported in the olfactory bulb (Jahr & Nicoll, 1982). With intracellular recordings, Jahr and Nicoll (1982) observed that norepinephrine, acting on granule cells, attenuated the GABA mediated inhibitory feedback from granule to mitral cells, thereby facilitating mitral cell firing. The mechanism of action of NE on granule cells and the receptor type mediating the effect have not been determined.

In the hippocampus, as will be explained in greater detail below, the synaptically evoked activity of pyramidal cells is enhanced by norepinephrine (Mueller, Hoffer & Dunwiddie, 1981). In the dentate gyrus, the synaptically

evoked activity of the granule cells is also enhanced by iontophoretically applied NE (Neuman & Harley, 1983). In both pyramidal and granule cells, the facilitation of evoked activity appears to be mediated via a beta receptor (Mueller et al., 1981; Neuman & Harley, 1983).

In the superior colliculus, electrical stimulation of the locus coeruleus (Kayama & Sato, 1982) and iontophoresis of NE (Sato & Kayama, 1983) produced either inhibition or facilitation of spontaneous or visually- and electrically-evoked activity. Since the facilitation was more preponderant in the deeper layers, Kayama and Sato (1982) suggested that the differential effects were probably observed on separate populations of neurons. In many instances of the inhibitory action of NE, the depression of spontaneous activity was greater than that of evoked activity, resulting in improved signal-to-noise ratio (Kayama & Sato, 1982; Sato & Kayama, 1983).

In the facial motor nucleus of the rat brain stem, iontophoretically applied NE produced facilitation of the response of motor neurons evoked iontophoretically by glutamate or synaptically by stimulation of the cortex (McCall & Aghajanian, 1979). Alpha antagonists blocked the facilitatory effect of NE on facial motor neurons, thereby indicating that the NE effect is probably mediated via an alpha receptor (McCall & Aghajanian, 1979). With

intracellular recordings from facial motor neurons, VanderMaelen and Aghajanian (1980) reported that iontophoresis of NE produced a subthreshold slow membrane depolarization accompanied by a decrease in membrane conductance and an increase in neuronal excitability as estimated by evoked spiking in response to long depolarizing pulses.

In the spinal cord, as mentioned above, both facilitatory and depressant effects of NE have been observed. More recently, White and Neuman (1980) have reported that iontophoresis of NE enhanced the responses of lumbar motor neurons evoked by iontophoresis of glutamate or by stimulation of ventral or dorsal roots. The enhancement of the evoked response was often long-lasting, persisting six minutes after termination of NE iontophoresis (White & Neuman, 1980; 1983). White and Neuman (1983) suggested that the facilitatory effects of NE were mediated via an alpha receptor since alpha antagonists blocked the enhancement of the glutamate-evoked response by NE. Similarly, recording intracellularly from lumbar motor neurons, Fung and Barnes (1981) observed that NE released synaptically by locus coeruleus stimulation facilitated the motor neuron response evoked by dorsal root stimulation, also probably via an alpha adrenergic receptor. Synaptically released NE produced a slow near threshold membrane depolarization in

some but not all motor neurons (Fung & Barnes, 1981).

Similar intracellularly recorded results have been obtained by Marshall, Pun, Hendelman and Nelson (1981) with cultured spinal neurons. Marshall et al. (1981) co-cultured neuronal explants from locus coeruleus and from spinal cord and reported that either electrical stimulation of the locus coeruleus explant or iontophoresis of NE onto spinal neurons produced a slow membrane depolarization accompanied by no change or by small decreases in membrane conductance in the cultured spinal neurons.

Also, in the spinal cord, Hodge, Apkarian, Stevens, Vogelsang and Wisnicki (1981) have observed that NE synaptically released by locus coeruleus stimulation enhanced the responses of neurons of dorsal horn lamina 6 evoked by cutaneous stimulation.

Thus the recent experimental evidence suggests that facilitation of central neuronal activity is a genuine effect of NE. This is in contrast with most earlier and some recent results suggesting a suppressant effect of NE on neuronal activity. More detailed reviews of central effects of NE have appeared elsewhere (Moore & Bloom, 1979; Szabadi, 1979; Van Dongen, 1981; Foote, Bloom & Aston-Jones, 1983; Reader, 1983). Several reasons for the discrepancy between facilitatory and suppressant effects have been suggested.

In the thalamus, it has been observed that facilitatory

effects of NE are seen predominantly with low iontophoretic ejection currents whereas suppressant effects appear with high ejection currents (Rogawski & Aghajanian, 1980c). Similar dose-dependent effects of NE were observed in the cerebellum where a large injection sometimes produced reduction of spontaneous and evoked activity, whereas a lower ejection current sometimes enhanced evoked activity without changing spontaneous activity (Moises et al., 1979). Indeed, in some instances when the concentration of applied NE could be measured precisely, dose-dependent effects with suppression at high doses and facilitation at low doses have been clearly documented. Such observations were made with bath application of NE in the in vitro hippocampal slice (Mueller et al., 1981) and cerebellar slice (Basile & Dunwiddie, 1984), as well as with concurrent monitoring of NE concentration by electrochemical analysis during iontophoresis of NE in somatosensory cortex in vivo (Armstrong-James & Fox, 1983).

It is also possible that the dose-dependent effects are related to specific receptor types. In the in vitro hippocampus and cerebellum, the suppressant effect of NE at high doses is apparently mediated by an alpha receptor, whereas the low dose facilitatory effect is mediated by a beta receptor (Mueller et al., 1981; Basile & Dunwiddie, 1984). However, in the in vivo cerebellum the enhancement

of inhibitory evoked responses appears to be mediated via a beta receptor (Waterhouse et al., 1982) as is the suppressant effect on spontaneous activity (Hoffer et al., 1971). Thus the underlying principles for facilitatory vs suppressant effects may involve interactions of different factors such as concentration of NE, receptor types present, method of application as well as type of neuronal activity recorded.

Mechanism of action of norepinephrine

A ubiquitous characteristic of the action of NE is that its duration of action seems to outlast the period of application. This long duration of action was noted in the early studies and was contrasted with the duration of action of "classical" neurotransmitters such as glutamate and GABA (Bradley & Wolstencroft, 1962; Bloom et al., 1963; Bloom et al., 1965; Biscoe et al., 1966; Weight & Salmoiraghi, 1966a). Whereas GABA and glutamate actions ended almost immediately upon termination of application, the effects of NE often did not dissipate until 2 to 5 minutes had elapsed.

Indeed some of the facilitatory effects of NE have been observed to be relatively long-lasting. In the spinal cord, White and Neuman (1980; 1983) observed the facilitation of motor neuron excitability to be still present 6 minutes

after the application of NE. In the cerebellum, NE released by iontophoretically-applied amphetamine produced facilitation of GABA inhibitory responses, and the facilitation was observed to last for 60 minutes (Michael, Waterhouse & Woodward, 1983). In somatosensory cortex, the excitation of some neurons of the deep layers by iontophoretically applied NE was observed to last up to one hour (Armstrong-James & Fox, 1983). Finally in the hippocampus, as will be seen in greater detail later, a short iontophoretic application of NE produced enhancement of synaptically evoked activity of the granule cells which was sometimes observed to last more than 11 hours (Neuman & Harley, 1983).

As for the cellular mechanism of action of NE, excluding that in the hippocampus which will be covered further below, actions which are consistent with both depression and facilitation of neuronal activity have been reported.

In accounting for the direct suppressant effects of NE, initial intracellular studies of NE action on spinal cord motor neurons of the cat reported a membrane hyperpolarization resulting in a decrease of excitatory (EPSP) and inhibitory (IPSP) postsynaptic potential amplitude during iontophoresis of NE (Phillis, Tebecis & York, 1968; Engberg & Thaller, 1970). The membrane hyperpolarization was accompanied by a decrease in membrane

conductance (Engberg & Marshall, 1971). The membrane hyperpolarization produced by NE was found to increase at hyperpolarized membrane levels and to decrease at depolarized membrane levels (Engberg & Thaller, 1970; Engberg & Marshall, 1971). Impalement of a motor neuron with two independent electrodes permitted an estimate of the reversal potential of the NE-induced hyperpolarization of -20 mV (Marshall & Engberg, 1979). Since the NE-induced hyperpolarization was not altered by intracellular injection of chloride ions, it was suggested that the hyperpolarization was more likely due to a decrease in sodium and potassium membrane conductance, although other mechanisms such as a decrease in release of tonic excitatory neurotransmitter, or a combination of an activation of an ionic pump (e.g. sodium-potassium) and a membrane conductance decrease were also possible (Marshall & Engberg, 1979).

Similar hyperpolarizing membrane effects have been reported in cerebellar Purkinje cells during iontophoresis of NE or stimulation of locus coeruleus (Siggins, Hoffer, Oliver & Bloom, 1971; Hoffer et al., 1973). Since iontophoresis of cAMP also produced similar membrane changes it was suggested that noradrenergic action is mediated via the formation of cAMP (Hoffer et al., 1973). In Purkinje cells, the locus coeruleus-induced hyperpolarizations were

also observed to increase at more hyperpolarized membrane levels (Hoffer et al., 1973) suggesting that the ions involved might be similar to those in spinal motor neurons. Membrane hyperpolarizations accompanied by a membrane conductance decrease were also reported with iontophoresis of NE on dissociated neonatal mouse brain neurons in culture (Bonkowski & Dryden, 1977).

With intracellular recording from cat cortical neurons, Phillis (1977, fig 1.) reported that iontophoresis of NE produced a membrane hyperpolarization but one which was generally not accompanied by any change in membrane conductance.

More recently, North and Yoshimura (1984) have recorded intracellularly from substantia gelatinosa neurons in slices of adult rat spinal cord maintained in vitro. They report that NE applied in the superfusion medium or by pressure micro-ejection produced a membrane hyperpolarization accompanied by an increase in conductance (North & Yoshimura, 1984). The membrane hyperpolarization reversed at -88 mV and varied with the extracellular potassium concentration as predicted by the Nernst equation, thus suggesting that the NE membrane hyperpolarization was mediated by an increase in potassium conductance (North & Yoshimura, 1984). The hyperpolarization produced by NE was maintained in low calcium medium and was blocked by alpha-2

antagonists, thereby suggesting that it was mediated via postsynaptic alpha-2 adrenergic receptors (North & Yoshimura, 1984).

A different cellular mechanism of NE action on central neurons has been suggested by Phillis (1974a). He hypothesized that the NE-induced membrane hyperpolarization was produced by NE stimulating cAMP formation which in turn activated a protein kinase which phosphorylated Na^+-K^+ ATPase, thereby activating an electrogenic sodium-potassium pump. Following the initial report that NE stimulated Na^+-K^+ ATPase in rat brain synaptosomes (Schaefer, Unyi & Pfeifer, 1972) a number of reports have further substantiated the activation of a ouabain-sensitive Na^+-K^+ ATPase by NE in brain (see Phillis & Wu, 1981, for review). The observations that ouabain, an inhibitor of the sodium-potassium pump, effectively antagonized the depression of cerebral cortical (Phillis, 1974b) and cerebellar Purkinje (Yarbrough, 1976) neurons produced by iontophoresis of NE provided support for such a mechanism. The antagonism by ouabain was selective for NE suppressant effects, since it did not antagonize the inhibition of Purkinje cells by GABA (Yarbrough, 1976). Additionally, Wu and Phillis (1981) showed that NE stimulated ^{22}Na efflux and ^{42}K influx in rat cerebral cortical slices which could be antagonized by ouabain as well as by alpha and beta noradrenergic

antagonists.

Since activation of the sodium pump in skeletal muscle of frogs produced a decrease in membrane conductance (Geduldig, 1968), Phillis and Wu (1981) suggested that similar changes could occur in central neurons and that NE, by activating an electrogenic sodium pump, could produce the observed membrane hyperpolarization associated with a decrease in conductance.

In contrast more recent studies on motor neurons and dissociated spinal neurons in culture report different effects of NE which could acccount for the observed facilitatory effects of NE on spontaneous and evoked excitatory responses. VanderMaelen and Aghajanian (1980) observed a slow sub-threshold depolarization of the membrane of motor neurons of the rat facial nucleus during iontophoresis of NE. The membrane depolarization was accompanied by an increase in neuronal excitability as estimated by long depolarizing pulses and also by a decrease in membrane conductance. Fung and Barnes (1981) also reported a slow membrane depolarization in spinal motor neurons with stimulation of locus coeruleus in cats resulting in increased afferent synaptic efficacy.

Marshall et al. (1981) observed similar changes in co-cultured explants of neonatal locus coeruleus and spinal neurons. Electrical stimulation of the locus coeruleus

explant or iontophoresis of NE caused a slow depolarization of the membrane, lasting up to two minutes, which was associated with a decrease or no change in conductance (Marshall et al., 1981). Injection of depolarizing current resulted in a greater membrane depolarization by NE and injection of hyperpolarizing current resulted in a smaller depolarization or sometimes abolished the depolarizing response to NE (Marshall et al., 1981). Thus, since the depolarization was accompanied by a conductance decrease and was greater at more depolarized levels, it was suggested that it resulted from inactivation of resting conductance to potassium ions (Marshall et al., 1981).

With regard to the cellular mechanism of action of NE in cerebellum and neocortex resulting in enhanced evoked activity (signal-to-noise ratio) for both excitatory and inhibitory responses, no intracellular recordings have been made during such effects and the cellular mechanism remains to be determined. But the observation that NE produces facilitation of inhibitory responses to GABA and not to glycine, taurine or beta-alanine (Moises et al., 1979; Yeh et al., 1981) suggests that the NE effects are not mediated by a general membrane change (such as activation of a sodium-potassium ionic pump) since the post-synaptic response to all active neurotransmitters would be facilitated by such a mechanism. Although receptor-specific

actions of glycine, taurine and beta-alanine have not been demonstrated intracellularly in the cerebellum, it has been suggested that they alter Purkinje neuron responses via a chloride conductance change similar to GABA but via different receptors (Yeh et al., 1981). Additionally, the observation of enhanced evoked responses with no changes in spontaneous activity suggests that the membrane hyperpolarization reported during depression of spontaneous activity (Siggins, Hoffer, Oliver & Bloom, 1971) might not be necessary for the facilitation.

Thus, four mechanisms of action have been proposed to account for the direct actions of NE on CNS neurons (excluding hippocampus). First, a decrease in sodium and potassium membrane conductance leading to hyperpolarization of the membrane (Marshall & Engberg, 1979). Second, an increase in potassium conductance resulting in hyperpolarization (North & Yoshimura, 1984). Third, an activation of a sodium-potassium electrogenic pump also leading to hyperpolarization of the membrane (Phillis & Wu, 1981). These three mechanisms have been suggested to mediate the suppressant effects of NE on neuronal responses. Finally, a decrease in the potassium resting conductance producing a slow depolarization of the membrane has been observed and was suggested as a mechanism for the facilitation of evoked excitatory responses by NE

(VanderMaelen & Aghajanian, 1980).

However, in the olfactory bulb facilitatory effects of NE on evoked excitatory responses have been shown to involve indirect effects of NE. NE was observed to produce disinhibition of the mitral cells of the olfactory bulb by its inhibitory action on the granule cells (Jahr & Nicoll, 1982), thus indicating possible indirect effects of NE in facilitating evoked excitatory responses of some central neurons.

Finally, effects of NE on astrocytes in culture have recently been reported. With intracellular recordings from astrocytes, NE added to the medium directly or by iontophoresis produced a depolarization of the glial membrane which could be antagonized by an alpha receptor antagonist. These results were obtained in rat neocortical astrocytes in primary cultures (Hirata, Slater & Kimelberg, 1983) and in astrocytes in explant cultures from rat cerebellum and brainstem (Hosli, Hosli, Zehnter, Lehman & Lutz, 1982). Such results, if they are shown to occur in situ, would suggest that some of the facilitatory actions of NE on excitatory neuronal responses may involve glial cells and result in or from altered extracellular potassium levels.

Functional aspects of the central noradrenergic system

Based on the divergent and widespread nature of its terminal innervation, early hypotheses suggested that the central noradrenergic system, and more specifically the locus coeruleus, functions as a central arousal system which, when active in temporal contiguity with other neurons of specific pathways, acts as reinforcement and permits the development of persistent changes in these pathways (Crow, 1968; Kety, 1970; Crow & Arbuthnott, 1972). Initial evidence from studies with intracranial self-stimulation as well as from pharmacological and biochemical analyses of NE turn-over rates supported this hypothesis (see Kety, 1970, for review). Subsequent work with central depletion of NE using 6-OHDA indicated that no impairments in intracranial self-stimulation or in learning abilities were present following severe depletion of central NE (see Mason, 1981 for review).

However, Mason and Iversen (1975) reported a specific behavioral deficit for NE-depleted rats. In 6-OHDA treated rats, they observed that following acquisition of an alleyway running task with food as the reward, NE-depleted animals showed an increased resistance to extinction when the task was no longer reinforced. This deficit, termed the dorsal bundle extinction effect, formed the basis of the selective attentional filter hypothesis for noradrenergic

function which has been recently reformulated (Mason, 1981). In this hypothesis, the function of the NE system is to filter out incoming sensory stimuli by labeling these stimuli as irrelevant to the present task and suppressing them (Mason, 1981). However, recent reports have failed to replicate the dorsal bundle extinction effect (Tombaugh, Pappas, Roberts, Vickers, & Szostak, 1983) and others have reported intact selective attention in NE-depleted rats (Pisa & Fibiger, 1983).

Recent experiments on the physiology of locus coeruleus neurons have provided other insights into the function of the central noradrenergic system. The firing rate of locus coeruleus neurons appears related to the behavioral state of the animal in that the discharge rate in rats is highest during the waking states, lower during slow wave sleep and virtually absent during paradoxical sleep (Aston-Jones & Bloom, 1981a). Despite earlier reports of increased activity during paradoxical sleep in cat (Chu & Bloom, 1974), a similar relationship between locus coeruleus firing rate and awake-sleep states has been found in cats (Hobson, McCarley & Wyzinski, 1975) and monkeys (Foote, Aston-Jones & Bloom, 1980).

Locus coeruleus neurons have also been reported to be activated by noxious stimuli, or "stressors". Foot-shock and tail-pinch increased NE turnover, presumably as a result

of activity in NE neurons, in various brain regions (see Amaral & Sinnamon, 1977 for review). With extracellular unit recordings in anesthetized rats, the neurons of the locus coeruleus have been shown to be activated by noxious stimuli such as toe pressure (Korf, Bunney & Aghajanian, 1974), deep pressure and pin pricks to the body surface (Cedarbaum & Aghajanian, 1978) and by stimulation of peripheral cutaneous nerves (Takigawa & Mogenson, 1977; Cedarbaum & Aghajanian, 1978). The firing rate of locus coeruleus neurons is also activated by iontophoresis of adrenocorticotropin (ACTH) (Olpe & Jones, 1982) and corticotropin-releasing factor (CRF) (Valentino, Foote, & Aston-Jones, 1983), which are secreted in response to stressful stimuli. These results with noxious stimuli support the hypothesis that the NE system is a central analogue of a sympathetic ganglion being activated during noxious, stressful situations (Amaral & Sinnamon, 1977).

However, unit recordings in unanesthetized rats and monkeys have now shown that locus coeruleus neurons are also activated phasically by non-noxious sensory stimuli (Foote et al., 1980; Aston-Jones & Bloom, 1981b). Indeed in monkeys the most efficient stimulus for eliciting activation of locus coeruleus neurons was the presentation of complex arousing stimuli such as preferred food (Foote et al., 1980). In rats the magnitude of the evoked sensory response

varied as a function of the animal's vigilance level, being depressed during consumatory behaviors such as grooming and drinking (Aston-Jones & Bloom, 1981b). Thus it appears that the locus coeruleus is involved in a more general function than the mediation of a central response to pain or noxious stress. As pointed out by Aston-Jones and Bloom (1981b) the fact that locus coeruleus neurons are excited phasically also suggests that its function is not one of tonic modulation.

Therefore, it appears that the locus coeruleus acts as an integrator of two types of influences, excitatory influences from arousing sensory stimuli and inhibitory influences reflecting the vigilance state of the animal (wake vs sleep, and/or consumatory behaviors). The net interaction of these influences will determine the phasic activation of the locus coeruleus. The end result of the activation of locus coeruleus neurons would then, according to the reviewed postsynaptic effects of NE, result in selective enhancement of synaptic transmission in certain CNS areas and suppression of neuronal activity in others. Indeed in some instances this enhancement could be long-lasting.

Therefore the physiological evidence suggests a similar type of function to that proposed earlier in terms of enhancement of synaptic transmission as a function of the

arousing properties of sensory stimuli (Kety, 1970).

Norepinephrine and neuronal plasticity

A related functional aspect of the central noradrenergic system which has recently come into focus is its involvement in neuronal plasticity.

In the cat visual cortex, Wiesel and Hubel (1963) have shown that monocular lid suture during a critical period results in an ocular dominance shift. A greater number of cortical cells are found that respond to visual stimulation of the non-deprived eye, and fewer are found that respond to the deprived eye than in normal kittens. Kasamatsu and Pettigrew (1976; 1979), however, reported that central depletion of NE with daily intraventricular injections of 6-OHDA over 10 days resulted in failure to observe an ocular dominance shift in cortical cells following monocular lid suture, and consequently more cells were found that could be driven binocularly.

It was further observed that microperfusion of 6-OHDA over the visual cortex with osmotic minipumps during one week of monocular lid suture also blocked the ocular dominance shift (Pettigrew & Kasamatsu, 1978; Kasamatsu, Pettigrew & Ary, 1979; Bear, Paradiso, Schwartz, Nelson, Carnes & Daniels, 1983). However, if NE was applied by

microperfusion for one week after treatment with 6-OHDA it was found that the ocular dominance shift did occur and cortical cells were now mostly responsive to the non-deprived eye (Pettigrew & Kasamatsu, 1978; Kasamatsu et al., 1979).

Since in NE-depleted kittens the ocular dominance shift did not occur, and since with replacement of NE in NE-depleted kittens the ocular dominance shift did occur, it appears that cortical NE is necessary for this neuronal plasticity. Kasamatsu, Pettigrew and Ary (1981) have now shown that the plasticity taking place during the recovery from monocular deprivation (recovery from monocular dominance to binocularity) also involves NE since it is suppressed by 6-OHDA microperfusion and accelerated by NE microperfusion following re-opening of the closed eye.

The cellular mechanism by which NE permits the functional alterations in synaptic connectivity in the visual cortex remains undetermined. Preliminary evidence indicates a receptor mediated action possibly involving the formation of cAMP. Kasamatsu (1983) has reported that the beta receptor antagonist propranolol is also effective in blocking the ocular dominance shift and that superfusion of dibutyryl cAMP results in an ocular dominance shift in 6-OHDA treated, monocularly deprived kittens.

A similar role for NE in neuronal plasticity has been

reported for adaptation of the vestibulo-ocular reflex (VOR). In the VOR, compensatory eye movements opposite to the direction of movement of the head result in stabilization of an image on the retina.

If the image is rotated with the head for 5 hours, the VOR is suppressed and there is adaptation in the VOR gain (a decrease in amplitude of compensatory eye movements) (Keller & Smith, 1983). However the same cats depleted of central NE following intracisternal injection of 6-OHDA show a suppression of VOR adaptation with very little decrease in the VOR gain (Keller & Smith, 1983). Hence it appears that NE is necessary for plasticity to occur in the VOR.

Similarly, central NE depletion with subcutaneous 6-OHDA injections in newborn rats resulted in the suppression of the consequences of rearing in an enriched environment (O'Shea, Saari, Pappas, Ings & Stange, 1983; Brenner, Mirmiran, Uylings & Van der Gugten, 1983). The increased forebrain weight, decreased hypothalamic weight and enhanced maze acquisition in a Lashley Type III maze which are found in rats reared in an enriched environment were not present in NE depleted rats reared in the same enriched environment (O'Shea et al., 1983). Similarly, Brenner et al. (1983) found that in NE-depleted rats the increased cerebral cortical weight usually associated with rearing in an enriched environment was not present. Thus NE appears also

to be necessary for plasticity to occur during rearing in an enriched environment.

Other recent reports indicate that central NE depletion in rats results in impaired acquisition of locomotor tasks involving precise paw placement. Injections of 6-OHDA intracisternally (Watson & McElligott, 1983) or into the coerulo-cerebellar pathway (Watson & McElligott, 1984) resulted in significant impairment in acquisition of the new locomotor task.

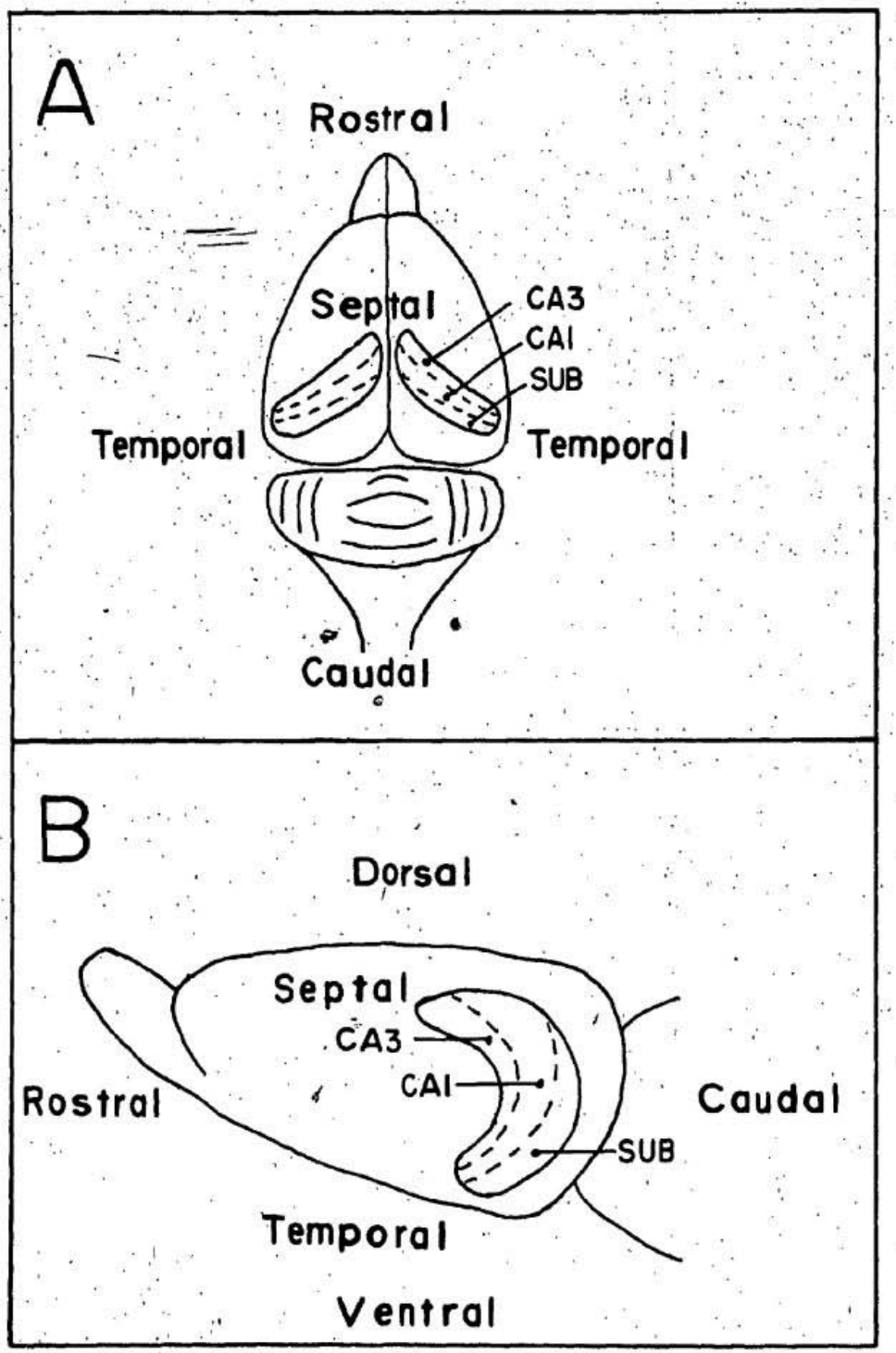
Together these experiments indicate an additional function of NE somewhat different from its physiological action on neuronal activity. Thus not only does NE suppress or facilitate neuronal activity in specific CNS areas but it also permits the establishment of more permanent changes in neuronal function under the appropriate circumstances.

Neurobiology of the hippocampus

Anatomy of the hippocampal formation

The location of the hippocampal formation in the CNS is expressed diagrammatically for the rat from a dorsal view in Figure 1A and a lateral view in Figure 1B. It is a roughly bean-shaped structure lying between the neocortex and the diencephalon which is oriented longitudinally along a septo-

Figure 1. Location of the hippocampal formation in the rat CNS. Diagrammatic representation as viewed through the overlying neocortex. Subfields CA3, CA1 and subiculum are represented as well as the septo-temporal longitudinal axis of the hippocampal formation. A: Dorsal view (redrawn from Blackstad et al., 1970). B: Lateral view (redrawn from Swanson & Cowan, 1977).

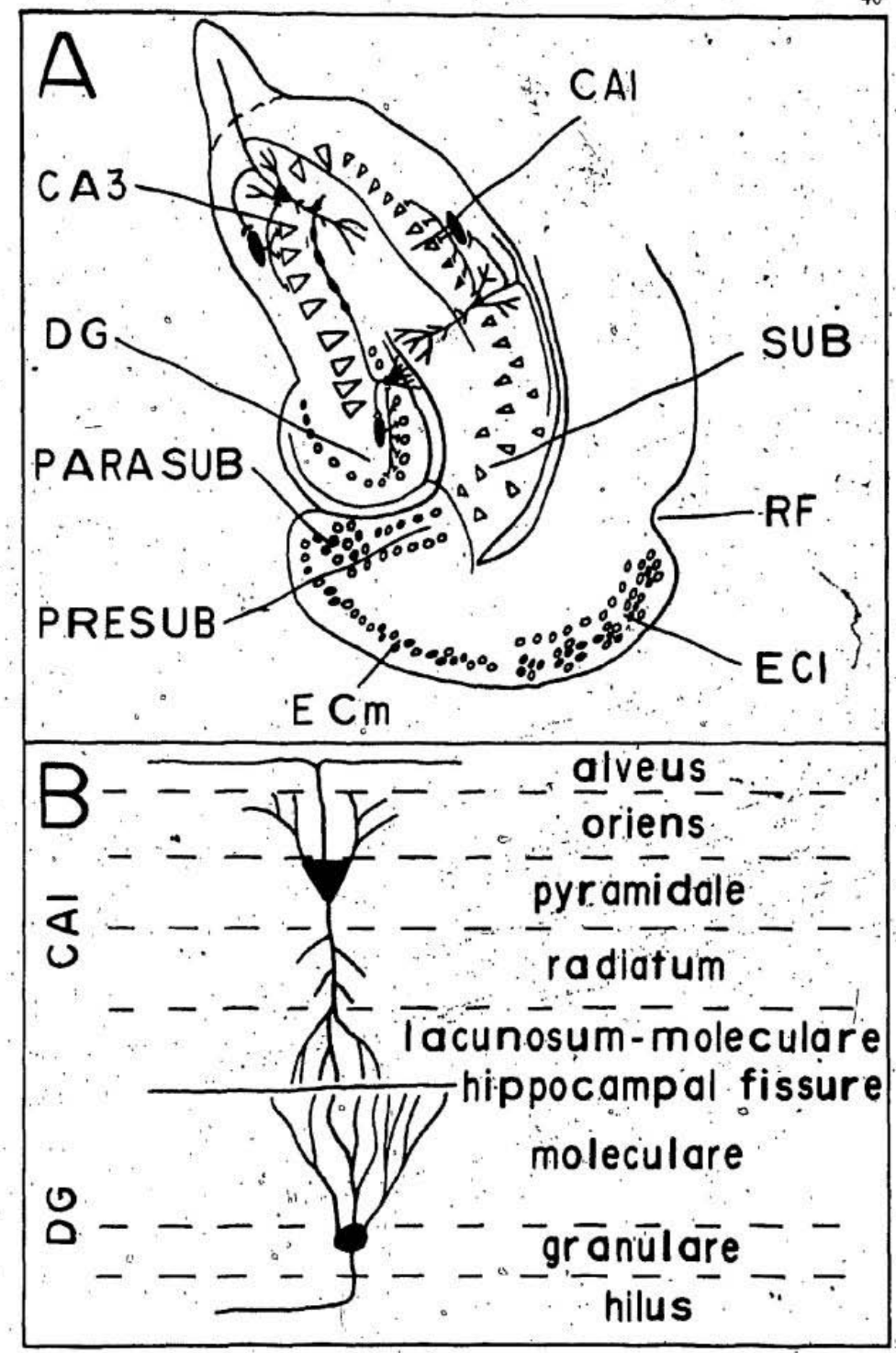

A
Rostral
Septal
CA3
CAI
SUB
Temporal
Temporal
Caudal
B
Dorsal
Septal
CA3
CAI
Rostral
Caudal
SUB
Temporal
Ventral

temporal axis (Blackstad, Brink, Hem & Jeune, 1970). The cytoarchitecture of its various sub-fields is expressed diagrammatically in Figure 2 in horizontal section, transverse to the longitudinal axis near the temporal pole. The hippocampal formation refers to four major fields described in Figure 2A: the entorhinal cortex (EC1, ECm), dentate gyrus (DG), Ammon's horn (CA3, CA1) and the subicular complex (SUB, PRESUB, PARASUB) (Swanson & Cowan, 1977; Amaral, 1978; Wyss, Swanson & Cowan, 1979a; Amaral & Cowan, 1980).

The entorhinal cortex is the major periallocortical component of the hippocampal formation and it extends from the border with the parasubiculum to the rhinal fissure or more dorsally to the neocortex (Blackstad, 1956; Steward, 1976). It is composed of two major zones and an intermediate one. Adjacent to the parasubiculum is the medial entorhinal cortex which is characterized by a prominent layer II consisting of tightly packed stellate cells (Steward, 1976). Moving laterally there is a small transitional zone, the intermediate entorhinal cortex, and more laterally the lateral entorhinal cortex (Steward, 1976). The lateral entorhinal cortex is characterized by cell islands in layer II and a greater density of cells in layer III (Steward, 1976).

The dentate gyrus is a curved structure consisting of a

Figure 2. Cytoarchitectonics of the hippocampal formation. A: Diagrammatic representation of the subfields of the hippocampal formation from an horizontal section, transverse to the septo-temporal axis, near the temporal pole of the right hemisphere. Abbreviations: CA1, Ammon's horn regio superior; CA3, Ammon's horn regio inferior; DG, dentate gyrus; EC1, lateral entorhinal cortex; ECm, medial entorhinal cortex; PARASUB, parasubiculum; PRESUB, presubiculum; RF, rhinal fissure; SUB, subiculum. B: Diagram of the strata of area CA1 and the dentate gyrus.


A
CA1
CA3
DG
SUB
PARASUB
RF
PRESUB
ECl
ECm
B
alveus
oriens
pyramidale
radiatum
lacunosum-moleculare
hippocampal fissure
moleculare
granulare
hilus
CA1
DG

supra- and an infrapyramidal limb (Amaral, 1978). It lies adjacent to the subiculum and CA1 region from which it is separated by the hippocampal fissure. The principal cells of the dentate gyrus are the granule cells which are aligned in dense rows to form the granule cell layer (Cajal, 1968). The dendrites of the granule cells extend radially through the extent of the molecular layer (Cajal, 1968). The region under the rows of granule cells is the hilus through which the axons of the granule cells (the mossy fibers) course toward Ammon's horn (Cajal, 1968). Scattered among the hilus are numerous polymorphic cells (Cajal, 1968) which recently have been characterized on the basis of their morphology into 21 different cell types (Amaral, 1978).

Ammon's horn is also a curvilinear structure which contains two major subdivisions: regio inferior or CA3 which is partly adjacent to the hilus and regio superior or CA1 which is partly adjacent to the subiculum (Swanson & Cowan, 1977). The layers of CA3 and CA1 have a similar nomenclature and thus only those of CA1 and the dentate gyrus are illustrated in Figure 2B. The principal cells of areas CA1 and CA3 are pyramidal cells which are aligned to form the pyramidal layer and consist of three or four rows of tightly packed cells (Cajal, 1968). The CA3 pyramidal cells are larger than those of CA1 and both types possess basal processes branching into stratum oriens and apical

processes extending into stratum radiatum and radiating into stratum lacunosum-moleculare (Cajal, 1968). Additionally in CA1 region there is another layer, the alveus, through which course the axons of the pyramidal cells (Cajal, 1968).

The last major division of the hippocampal formation is the subicular complex consisting of subiculum, presubiculum and parasubiculum (Cajal, 1968; Blackstad, 1956; Swanson & Cowan, 1977). The junction of CA1 area and subiculum is characterized by a less dense layer of pyramidal cells scattered over a larger area (Cajal, 1968). The transition between subiculum and presubiculum takes place where the cell poor superficial layer of the subiculum contacts layers II-III of the presubiculum (Sorensen & Shipley, 1979). And finally, the parasubiculum is characterized by scattered cells in the superficial layers (Shipley, 1975).

Afferent and efferent connections of the entorhinal cortex

The cortical afferents to the entorhinal area arise from piriform cortex (Powell, Cowan & Raisman, 1965), lateral olfactory tract (Kerr & Dennis, 1972), cortical, basolateral and lateral nuclei of the amygdala (Krettek & Price, 1974) and from multimodal associational areas in orbital-frontal and temporal neocortical areas (Van Hoesen, Pandya &

Butters, 1972; 1975; Van Hoesen & Pandya, 1975). Subcortical afferents to the entorhinal area originate from locus coeruleus, dorsal and median raphe, nucleus reuniens, medial septum and diagonal band of Broca (Segal, 1977). The entorhinal area also receives local projections from the hippocampal formation, notably from pre- and parasubiculum bilaterally (Shipley, 1975; Segal, 1977; Swanson & Cowan, 1977) and from CA3 (Hjorth-Simonsen, 1971; Swanson & Cowan, 1977).

The efferent connections of the entorhinal area are mostly unilateral but to some extent bilateral and project to the dentate gyrus and Ammon's horn (Steward, 1976; Swanson & Cowan, 1977; Hjorth-Simonsen & Jeune, 1972; Hjorth-Simonsen, 1972). Ipsilateral fibers originating from neurons of layer II of the entorhinal cortex (Schwartz & Coleman, 1981; Ruth, Collier & Routtenberg, 1982) form two distinct fiber systems, the lateral and medial perforant paths (Hjorth-Simonsen, 1972; Hjorth-Simonsen & Jeune, 1972). The medial perforant path originates from the medial entorhinal cortex and projects to the middle third of the molecular layer of the dentate gyrus as well as to the deeper half of stratum lacunosum-moleculare of CA3 (Hjorth-Simonsen, 1972; Hjorth-Simonsen & Jeune, 1972; Steward, 1976). Projections from the lateral entorhinal cortex form the lateral perforant path and project to the superficial

layers of the molecular layer of the dentate gyrus and of the stratum lacunosum-moleculare of CA3 (Hjorth-Simonsen, 1972; Hjorth-Simonsen & Jeune, 1972; Steward, 1976). A similar pattern of termination is present in the crossed entorhinal-dentate gyrus and CA3 pathways (Steward, 1976).

Projections to stratum lacunosum-moleculare of the CA1 region however are organized differently with medial entorhinal afferents projecting to the CA1 area adjacent to the CA1-CA2 transition and the lateral entorhinal afferents to the CA1-subiculum transition (Steward, 1976). A similar arrangement was found in the crossed temporo-ammonic tract (Steward, 1976).

The projections from medial entorhinal cortex to dentate gyrus are also organized topographically along the hippocampal septo-temporal axis. Neurons of the medial entorhinal cortex project in a parallel fashion to the dentate gyrus with those from the posterodorsolateral area of medial entorhinal cortex terminating in the septal pole of the dentate gyrus and those from the anteroventromedial area terminating in the temporal pole (Ruth et al., 1982). A similar parallel projection system from dorso-ventral entorhinal cortex to septo-temporal dentate gyrus was previously suggested by Hjorth-Simonsen and Jeune (1972). Although this type of lamellar organization apparently characterizes the projections of the medial and intermediate

entorhinal cortex (Wyss, 1981; Ruth et al., 1982), the lateral entorhinal cortex appears to project much more broadly along the septo-temporal axis (Wyss, 1981).

The entorhinal cortex, mostly the lateral part, also projects to non-hippocampal areas. These projections appear to be reciprocal to the major extrinsic inputs to the entorhinal cortex and terminate in piriform and periamygdaloid cortex, endopiriform nucleus, the lateral, basolateral and cortical nuclei of the amygdala, the nucleus of the olfactory tract, the olfactory tubercule, the anterior olfactory nucleus, the taenia tecta and the indusium griseum (Wyss,1981).

Afferent and efferent connections of the dentate gyrus

As mentioned above, the dentate granule cells receive projections from the entorhinal cortex on their dendritic processes in the molecular layer. Fibers from the lateral perforant path terminate in the outer third of the molecular layer, those from the medial perforant path in the middle third and those originating from intermediate entorhinal cortex at intermediate locations between middle and outer third (Hjorth-Simonsen & Jeune, 1972; Hjorth-Simonsen, 1972; Steward, 1976). The dendrites of the granule cells also receive commissural projections from the contralateral

homotopic dentate gyrus which terminate in the inner third of the molecular layer (Blackstad, 1956). Associational fibers from the ipsilateral dentate gyrus also terminate in the inner third of the molecular layer (Zimmer, 1971). Recently it was shown that the commissural and associational fibers are collaterals originating from the same hilar neurons (Laurberg & Sorensen, 1981). In the rabbit, these hilar neurons were identified as polymorph neurons and not hilar pyramidal neurons (Berger, Semple-Rowland & Basset, 1980).

Subcortical afferents to the dentate gyrus have been demonstrated using the method of retrograde axonal transport of horseradish peroxidase (HRP) with large injections incorporating various subfields of the hippocampal formation in monkey (Amaral & Cowan, 1980) and rat (Segal & Landis, 1974; Pasquier & Reinoso-Suarez, 1976; 1978; Wyss et al., 1979a). More recently these afferents have been more selectively demonstrated in the rat with iontophoretic applications of HRP restricted to the dentate gyrus (Riley & Moore, 1981). It was found that the afferents to the dentate gyrus were primarily ipsilateral and originated from the septum (medial septum and diagonal band of Broca), the supramamillary area of the hypothalamus, the dorsal and median nuclei of raphe and from the locus coeruleus. Riley and Moore (1981) additionally documented afferents from the

interpeduncular nucleus, ventromedial dorsal tegmental nucleus, as well as scattered neurons in ventral central gray and lateral dorsal tegmental nucleus.

With respect to the zone of termination of these subcortical afferents, those from the supramamillary area terminate from the superficial one half of the granular layer to the inner one fifth of the molecular layer (Segal, 1979; Wyss et al., 1979b; Dent, Galvin, Stanfield & Cowan, 1983). The septal projections terminate in two narrow bands in the infra- and supragranular regions (Raisman, Cowan & Powell, 1965; Mosko, Lynch & Cotman, 1973). The terminal projections of the raphe form a narrow infragranular band as well as a moderately sparse innervation of the molecular layer and hilus (Moore & Halaris, 1975). Azmitia and Segal (1978) suggested that the dorsal raphe projections terminate in the molecular layer while those from the median raphe terminate in the hilus. Locus coeruleus projections, which will be covered in greater detail below, form a dense terminal plexus in the hilar region and innervate to a lesser degree the granular and molecular layers (Loy, Koziell, Lindsey & Moore, 1980).

With reference to the efferent projections of the dentate gyrus, there are only two major systems. As mentioned above, axons of hilar neurons project ipsilaterally to form the associational fibers and

contralaterally to form the commissural fibers (Blackstad, 1956; Zimmer, 1971; Berger et al., 1980; Laurberg & Sorenson, 1981). Secondly, the axons of the granule cells (mossy fibers) leave the hilus and course suprapyramidally in the stratum radiatum of the CA3 region in a distinct layer referred to as stratum lucidum (Blackstad et al., 1970; Swanson & Cowan, 1977). This mossy fiber system projects to CA3 in a lamellar fashion, transverse (perpendicular) to the septo-temporal axis of the hippocampus and ends at the CA3-CA1 transition (Blackstad et al., 1970).

Anatomical connections of other regions of the hippocampal formation.

Since this thesis concentrates on the dentate gyrus, the anatomical connections of the other regions of the hippocampal formation will not be covered in detail. Detailed accounts of their afferent (Segal & Landis, 1974; Pasquier & Reinoso-Suarez, 1978; Wyss et al., 1979a; Riley & Moore, 1981; Irle & Markowitsch, 1982) and efferent (Swanson & Cowan, 1977) connections have appeared elsewhere.

Briefly, extrahippocampal projections to the CA3 and CA1 regions are similar to those of the dentate gyrus except that these regions receive additional fibers from thalamic

nuclei (Wyss et al., 1979a). The major efferents of the CA3 region are to the septum, CA1 pyramidal cells and subiculum (Swanson & Cowan, 1977). CA1 cells also project mainly to septum and subiculum (Swanson & Cowan, 1977). The subicular region however projects to hypothalamic and thalamic nuclei as well as to the entorhinal cortex (Swanson & Cowan, 1977). The Schaffer-collateral projections from CA3 to CA1 are also oriented in a lamellar fashion, transverse to the septo-temporal hippocampal axis (Hjorth-Simonsen, 1973). Thus there is a major sequential projection system within the hippocampal formation which is lamellar in nature and involves perforant path -> granule cells -> CA3 pyramidal cells -> CA1 pyramidal cells.

Physiology of the hippocampus

Initial extracellular electrophysiological studies reported the activation of hippocampal pyramidal cells by stimulation of the entorhinal cortex (Renshaw, Forbes & Morison, 1940), Schaffer-collaterals or mossy fibers (Cragg & Hamlyn, 1955) or commissural afferent fibers (Cragg & Hamlyn, 1957). Cragg and Hamlyn (1957) also reported the activation of the dentate granule cells by stimulation of the perforant path.

Later intracellular and extracellular studies confirmed

the excitatory nature of the hippocampal tri-synaptic circuit: perforant path -> granule cells (Andersen, Holmqvist & Voorhoeve, 1966), mossy fibers -> CA3 pyramidal cells (Andersen & Lomo, 1966), Schaffer-collaterals -> CA1 pyramidal cells (Andersen & Lomo, 1966). Detailed topographical mapping of stimulation and recording sites confirmed electrophysiologically the lamellar organization of the intra-hippocampal circuit. Lomo (1971a) demonstrated that restricted stimulation of the perforant path monosynaptically activated a narrow band of granule cells oriented transversely to the septo-hippocampal axis. Andersen, Bliss and Skrede (1971b) showed that the monosynaptic activation of CA3 pyramidal cells by the granule cells was also oriented similarly. Monosynaptic activation of CA1 pyramidal cells via the Schaffer-collaterals as well as the trajectory of the CA1 axons in the alveus were also found to be oriented transversely to the septo-temporal axis (Andersen et al., 1971b; Andersen, Bland & Dudar, 1973).

A more detailed physiological account of the hippocampal formation will be restricted to the dentate gyrus, except for the action of norepinephrine in Ammon's horn which will be covered below. A comprehensive review of the neurobiology of the hippocampal formation has recently been published (Seifert, 1983) to which the reader is referred

for further information.

Physiology and pharmacology of the dentate gyrus

Stimulation of the perforant path elicits in the granule cells an EPSP followed 2-4 milliseconds later by an IPSP lasting some 100 milliseconds (Andersen et al., 1966; Lomo, 1971a).

Experimental studies have indicated that the excitatory amino acids glutamate and aspartate are taken up (Storm-Mathisen & Iversen, 1979) and released (Nadler, White, Vaca, Redburn & Cotman, 1977; White, Nadler, Hamberger & Cotman, 1977) in the dentate gyrus. Furthermore, pharmacological antagonism of the perforant path evoked synaptic response of the granule cells by antagonists of these excitatory amino acids has been reported (White et al., 1977; Wheal & Miller, 1980; Crunelli, Forda & Kelly, 1983). These results indicate that glutamate or aspartate may be the neurotransmitter released at the perforant path synapse. Thus far the evidence favors glutamate as the endogenous excitatory neurotransmitter in the perforant path (White et al., 1977; Wheal & Miller, 1980; Crunelli et al., 1983).

The delayed, long duration IPSPs evoked by stimulation of the perforant path synapse are thought to reflect two processes. As suggested earlier by Andersen et al. (1966) a

component of the IPSP is a hyperpolarization produced by recurrent inhibition via basket cells, involving GABA and an increase in chloride conductance (Fricke & Prince, 1984). However, a second component is due to a delayed, late calcium-activated increase in potassium conductance (Thalmann & Ayala, 1982; Fricke & Prince, 1984).

Other afferents to the dentate molecular layer are also excitatory in nature. Stimulation of the commissural and associational afferents produce monosynaptic excitation of the granule cells (Deadwyler, West, Cotman & Lynch, 1975; Steward, White & Cotman, 1977; Buzsaki & Czeh, 1981). Buzsaki and Czeh (1981) and Buzsaki and Eidelberg (1982) have additionally suggested that commissural activation also produces feed-forward inhibition of the granule cells.

Another afferent system to the dentate gyrus, the septo-hippocampal system, is also thought to be excitatory in nature. Stimulation of the septal area produced activation of the granule cells (Andersen, Bruland & Kaada, 1961; Wheal & Miller, 1980; Krug, Ott & Matthies, 1980) and enhancement of the perforant path evoked response (Alvarez-Leefmans & Gardner-Medwin, 1975; Fantie & Goddard, 1982). It has been suggested that the excitation of the granule cells by septal afferents is mediated by acetylcholine via muscarinic receptors (Wheal & Miller, 1980) although the modulation of the perforant path evoked response by septal stimulation was

found to be non-muscarinic and non-nicotinic (Fantie & Goddard, 1982).

The influence of the raphe system on the granule cells, however, is thought to involve serotonin and to be inhibitory in nature. Stimulation of the median raphe produced inhibition of unit activity of the granule cells (Assaf & Miller, 1978). Iontophoretic application of serotonin inhibits granule cells post-synaptically by depolarizing the membrane, probably via an increase in chloride conductance (Assaf, Crunelli & Kelly, 1981). In contrast to these inhibitory effects on granule cells, stimulation of the median raphe produced an increase in the perforant path evoked response of the granule cells (Assaf & Miller, 1978; Winson, 1980; Srebro, Azmitia & Winson, 1982). Assaf & Miller (1978) have suggested that the increased evoked response resulted from serotonergic inhibition. But since Srebro et al. (1982) observed facilitation of the perforant path evoked response by raphe stimulation despite depletion of hippocampal serotonin, they suggested that the facilitation observed was due to non-serotonergic factors.

The actions of the hypothalamic afferents in the dentate gyrus remain unclear. Segal (1979) reported that stimulation of the supra-mammillary region produced monosynaptic inhibition of the granule cell spontaneous unit activity. However, Dent et al. (1983) observed at the

electron microscope level that the hypothalamic terminals in the dentate gyrus formed synaptic contacts in the supragranular region which were typical of excitatory synapses. Thus this latter report suggested an excitatory action of the hypothalamic afferents on the granule cells in contrast to the observations of Segal (1979).

Various neuropeptides are also present in the dentate gyrus. Substance P (Vincent & McGeer, 1981; Vincent, Kimura & McGeer, 1981), cholecystokinin (Greenwood, Godar, Reaves & Hayward, 1981; Handelmann, Meyer, Weinfeld & Oertel, 1981), somatostatin (Finley, Maderdrut, Roger & Petrusz, 1981), angiotensin II (Haas, Felix, Celio & Inagami, 1980) and vasoactive intestinal polypeptide (Kohler, 1983; Loren, Emson, Fahrenkrug, Bjorklund, Alumets, Akansson & Sundler, 1979) have been identified in the dentate gyrus. However, their physiological actions on granule cells have not been demonstrated.

On the other hand, opiates have been shown to produce excitatory effects on the granule cells (Haas & Ryall, 1980; Linseman & Corrigall, 1982) and are present in the dentate gyrus (Gall, Brecha, Karten & Chang, 1981) but their physiological role remains unclear. It has also been shown that the granule cells concentrate subcutaneously injected tritiated corticosterone (Gerlach & McEwen, 1972) and that systemically injected corticosterone produced a decrease in

spontaneous unit activity in the dentate gyrus (Pfaff, Silva & Weiss, 1971). However, the physiological role of the corticosterone action also remains unclear.

The physiological action of the other major afferent system to the dentate gyrus, the noradrenergic innervation from locus coeruleus, will be covered in detail below.

As mentioned above, there are also numerous intrinsic neurons in the dentate gyrus, mostly in the hilar region (Amaral, 1978). Although it has been suggested that some mediate feedback and feedforward inhibition of the granule cells (Andersen et al., 1966; Buzsaki & Czeh, 1981; Buzsaki & Eidelberg, 1982), there has not been any direct intracellular investigation from interneurons in the dentate gyrus of this matter. Nor is it known whether any of the interneurons have excitatory actions on the granule cells.

Function of the hippocampal formation

Although the role of the hippocampal formation in brain function remains to be defined specifically, the evidence from humans who have undergone surgical removal of the hippocampal formation initially suggested its involvement in the establishment of long-term memory (Scoville & Milner, 1957). Patients that underwent medial-temporal lobe resections which included the hippocampal region showed

severe post-operative anterograde amnesia with only partial retrograde amnesia (Scoville & Milner, 1957). Furthermore, the anterograde amnesia was still present 14 years following the lobectomy (Milner, Corkin & Teuber, 1968). The patients' perceptual and other intellectual capacities, including short-term memory, remained intact (Scoville & Milner, 1957; Milner et al., 1968).

With respect to animal studies on hippocampal function, they have recently been reviewed and integrated by O'Keefe and Nadel (1978; 1979) in their proposal of the hippocampus as a cognitive map. The cognitive map theory is based on the observation that hippocampal neurons are found to be specifically responsive to the presence ("place" units) or absence ("misplace" units) of cues, or spatial factors, in their environment or, alternatively, to be responsive to the displacement of the animal in its environment ("displace" units) (O'Keefe & Nadel, 1979).

Thus, it was suggested that the hippocampus (Ammon's horn and dentate gyrus) constructs and may store cognitive maps which represent the experienced spatial environment of the animal (O'Keefe & Nadel, 1979). It was also suggested that the spatial map/memory system exists in humans but because of lateralization of function the hippocampus of the language hemisphere is involved in semantic rather than spatial mapping (O'Keefe & Nadel, 1979).

Although the process by which such maps are formed remains undetermined, synapses in the hippocampus have been shown to undergo changes in synaptic efficacy with activation. First, decrements in cellular responses following low-frequency stimulation similar to behavioral habituation (Thompson & Spencer, 1966) have been observed in the granule cells (Teyler & Alger, 1976; Harris, Lasher & Steward, 1978). And secondly, long-lasting increases in cellular responses (long-term potentiation) have been shown to occur following brief high-frequency stimulation of the afferent fibers to the granule cells (Bliss & Lomo, 1973; Bliss & Gardner-Medwin, 1973) and to CA3 and CA1 pyramidal cells (Schwartzkroin & Wester, 1975; Alger & Teyler, 1976). The long-term potentiation of the granule cell responses was observed to last sometimes over two months (Douglas & Goddard, 1975). Although the cellular changes underlying long-term potentiation have not been completely identified (see Swanson, Teyler & Thompson, 1982 for recent review) such changes have been suggested as taking place during the formation of long-term memory in the hippocampus (Douglas & Goddard, 1975; Swanson et al., 1982).

The firing of hippocampal pyramidal cells has also been shown to be correlated with, and predictive of, the conditioned response in classical conditioning of the rabbit nictitating membrane (see Swanson et al., 1982 for review).

This increase in hippocampal cellular responses developing with learning appears closely related to long-term potentiation and could possibly arise from such changes taking place in the hippocampal circuit during learning (Swanson et al., 1982).

Noradrenergic function in Ammon's horn and the dentate gyrus

Localization of noradrenergic terminals, enzymes and receptors

Noradrenergic terminals have been described in all regions of the hippocampal formation including Ammon's horn and the dentate gyrus (Blackstad et al., 1967). The initial observations of Blackstad et al. (1967) have been extended by Loy et al. (1980) using a more sensitive fluorescence histochemical technique. Hence, fluorescent fibers with varicosities are observed in stratum oriens, pyramidale, radiatum and lacunosum-moleculare of CA1 region (Loy et al., 1980). In the CA3 region, the fluorescent fibers are also present in stratum oriens, pyramidale and radiatum but a distinct plexus of fibers is observed in stratum lucidum (Loy et al., 1980; Blackstad et al., 1967).

In the dentate gyrus some fluorescent fibers and terminals are seen in the molecular and granular layers but

a much denser plexus of fibers and varicosities is present in the hilus (Blackstad et al., 1967; Loy et al., 1980). Within the hilus, the innervation appears denser in the infragranular zone (Blackstad et al., 1967; Loy et al., 1980). Loy et al. (1980) observed that varicosities were often clustered around somas or closely apposed to dendrites of granule and pyramidal cells.

Loy et al. (1980) also reported that the noradrenergic fibers originating from the locus coeruleus and projecting into the dorsal noradrenergic bundle enter Ammon's horn and the dentate gyrus via three pathways: fasciculus cinguli, fornix and ventral amygdaloid bundle-ansa peduncularis. Specifically, the dentate gyrus receives its innervation via the ipsilateral and contralateral fasciculus cinguli as well as the fornix and the ventral amygdaloid bundle-ansa peduncularis (Loy et al., 1980). A similar distribution of noradrenergic fibers in Ammon's horn and the dentate gyrus was observed with immunocytochemical localization of dopamine-beta-hydroxylase (DBH), the noradrenergic synthesizing enzyme which converts dopamine to norepinephrine (Swanson & Hartman, 1975).

With respect to the topography of the locus coeruleus neurons projecting to the dentate gyrus, a recent report indicates that the noradrenergic fibers originate mostly from neurons of the dorsal part of the locus coeruleus

(Haring & Davis, 1983). Furthermore, following double-label injections into septal and temporal dentate gyrus, some locus coeruleus neurons were doubly stained (Haring & Davis, 1983). Thus it appears that the locus coeruleus projections to the dentate gyrus are divergent in nature with collaterals of the same neuron innervating both septal and temporal poles of the hippocampus.

Ontogenically in the rat, noradrenergic axons appear in the CA3 region at embryonic day 18 and in the dentate gyrus at postnatal day 4 (Loy & Moore, 1979). The maturation of these afferents is relatively rapid and the innervation pattern is fairly complete throughout those regions by postnatal day 10 (Loy & Moore, 1979).

At the electron microscope level, the noradrenergic terminals have been examined in the dentate gyrus with the permanganate-glyoxylic acid fixation method (Koda & Bloom, 1977; Koda, Wise & Bloom, 1978; Koda, Schulman & Bloom, 1978). The small granular vesicles that appear with this method are thought to be the ultrastructural correlates of aldehyde-induced fluorescent noradrenergic varicosities (Koda & Bloom, 1977). The distribution of boutons containing small granular vesicles in the dentate is similar to that previously described for fluorescent fibers. A greater number of boutons are observed in the hilus, particularly in the infragranular zone, and fewer boutons

are found in the granular and molecular layers (Koda & Bloom, 1977). Proportionally, the molecular layer contains approximately a sixth and the granular layer a third of the small granular bouton density of the hilus (Koda & Bloom, 1977).

Although the permanganate method does not stain synaptic specializations prominently, synaptic profiles were assessed by the presence of a thickened or parallel contact with a dendrite in association with an aggregation of vesicles near that contact (Koda, Schulman & Bloom, 1978). It was found that about 20% of the noradrenergic boutons in the molecular layer and hilus formed such synaptic contacts (Koda, Schulman & Bloom, 1978).

This proportion was similar to that of other non-adrenergic boutons in the dentate gyrus (Koda, Schulman & Bloom, 1978). Although this estimate was not achieved with serial sectioning and provides only an indirect estimate of synaptic contacts, it suggests that some noradrenergic boutons make specialized contacts with postsynaptic structures in the dentate gyrus. Olschowka et al. (1981) reached a similar conclusion using non-serial dopamine-beta-hydroxylase immunocytochemistry, although no details were given about the distribution and the density of the noradrenergic varicosities they observed.

Biochemical measurements have confirmed the presence of

norepinephrine and dopamine-beta-hydroxylase as well as norepinephrine uptake mechanisms in Ammon's horn and the dentate gyrus as a whole (Heller & Moore, 1968; Zigmond, Chalmers, Simpson & Wurtman, 1971; Thierry, Stinus, Blanc & Glowinski, 1973; Ross & Reis, 1974; Storm-Mathisen & Guldberg, 1974). These parameters are reduced by 50-80% following lesions to the medial forebrain bundle (Heller & Moore, 1968), lateral hypothalamus (Zigmond et al., 1971), locus coeruleus (Thierry et al., 1973; Ross & Reis, 1974) or fimbria/fornix superior and cingulum (Storm-Mathisen & Guldberg, 1974). Following transection of the three noradrenergic afferent routes, fimbria/fornix superior, cingulum and amygdaloid, hippocampal noradrenergic uptake was completely suppressed (Storm-Mathisen & Guldberg, 1974). Such studies suggest that the endogenous NE and dopamine-beta-hydroxylase levels as well as NE uptake mechanisms in the hippocampal formation are derived from noradrenergic terminals of extrinsic origin.

More recently, however, biochemical assays of more discrete areas of the hippocampal formation have been carried out (Loy et al., 1980). At the septal end, NE content was found to be greater in the dentate gyrus (364 ng/g) than in Ammon's horn (201 ng/g). Similarly, at the temporal end the dentate gyrus contained more NE (642 ng/g) than Ammon's horn (346 ng/g) (Loy et al., 1980). Thus,

throughout the hippocampus, the dentate gyrus contained more NE than Ammon's horn and the temporal end contained more than the septal end (Loy et al., 1980). Bilateral lesions of the locus coeruleus produced a 88-90% decrease in the endogenous NE levels in Ammon's horn and in dentate gyrus (Loy et al., 1980). Selective deafferentation indicated that the dentate gyrus receives most of its noradrenergic innervation via the ipsilateral cingulum and approximately equal contributions via the fornix, amygdaloid bundle-ansa peduncularis and contralateral cingulum (Loy et al., 1980).

Histochemical visualization of monoamine oxidase (MAO), an enzyme involved in the metabolism of norepinephrine and other monoamines, indicated a distribution of the enzyme different from the noradrenergic innervation (Mellgren & Geneser-Jensen, 1972; Geneser-Jensen, 1973). In the rat, the MAO distribution is densest in the hilus, almost absent in the granule cell layer and homogeneously moderate throughout the molecular layer with a denser supragranular zone. (Mellgren & Geneser-Jensen, 1972). The MAO distribution in the guinea pig dentate gyrus is similar but much more stratified, especially in the molecular layer with a dense supragranular zone, weaker superficial layer and almost unstained intermediate zone (Geneser-Jensen, 1973).

The relative distribution of noradrenegic receptors in Ammon's horn and the dentate gyrus remains controversial.

Using a fluorescent beta noradrenergic blocker, Atlas and Segal (1977) reported that beta receptors were located around cell bodies and proximal basal and apical dendrites in stratum oriens and pyramidale of both CA3 and CA1 regions. In the dentate gyrus they reported less beta receptor fluorescence in the hilus than in Ammon's horn and still less fluorescence in the granular and molecular regions (Atlas & Segal, 1977).

However, using radioligand binding methods, Crutcher and Davis (1980) reported an approximately equal density of beta adrenergic receptors in Ammon's horn and the dentate gyrus. In Ammon's horn the beta receptors were found to be uniformly distributed in stratum pyramidale and radiatum (Crutcher & Davis, 1980). In contrast there were 30% more alpha binding sites in the dentate gyrus than in Ammon's horn (Crutcher & Davis, 1980). In the dentate gyrus, the concentration of alpha receptors was found to be twice that of beta adrenergic receptors (Crutcher & Davis, 1980). Thus it appears that the distribution of beta adrenergic receptors differs from the pattern of noradrenergic innervation in the hippocampal formation. The distribution of alpha receptors, however, appears more similar to the pattern of innervation. More recently, Rainbow, Parsons and Wolfe (1984), using quantitative autoradiography, have evaluated the distribution of beta-1 and beta-2

noradrenergic receptors in the hippocampal formation. They reported a greater percentage of beta-1 receptors (60-90%) in all hippocampal areas (Rainbow et al., 1984). In the septal pole of the dentate gyrus the proportions were 75% beta-1, 25% beta-2 whereas in the temporal pole it was 85% beta-1 and 15% beta-2 (Rainbow et al., 1984).

With respect to the release of norepinephrine in the hippocampal formation, it has been studied in synaptosomal preparations from Ammon's horn and the dentate gyrus (West & Fillenz, 1981; Fung & Fillenz, 1983). Norepinephrine is released from hippocampal synaptosomes in a calcium-dependent fashion spontaneously (Fung & Fillenz, 1983) and in response to increased extracellular potassium (25-60 mM) (West & Fillenz, 1981; Fung & Fillenz, 1983). Furthermore, GABA was found to increase the spontaneous release of NE, whereas it decreased the potassium-evoked release (Fung & Fillenz, 1983). Fung and Fillenz (1983) have suggested that the increase in spontaneous release is the result of an increase in resting calcium conductance because of the depolarizing presynaptic inhibition of noradrenergic terminals via GABA-A receptors and an increase in chloride conductance. The decrease in potassium-evoked release, however, was proposed to arise from non-depolarizing presynaptic inhibition of noradrenergic terminals mediated by GABA-B receptors and by a decrease in voltage-dependent

calcium conductance (Fung & Fillenz, 1983).

Reports of NE release from hippocampal slices indicated that kainic acid (0.3 mM) also evokes a calcium-dependent release of NE although 20-fold less effective than potassium (40mM) (Nelson, Zaczek & Coyle, 1980). Electrical field stimulation also produces a calcium-dependent release of NE which is inhibited by alpha-2 adrenergic agonists and by an adenosine analogue (Fredholm, Jonzon & Lindgren, 1983). Thus it appears from release studies that evoked norepinephrine release from noradrenergic terminals can be inhibited by mechanisms involving $GABA_B$, adenosine or alpha-2 adrenergic receptors.

Physiological effects of norepinephrine on hippocampal pyramidal cells

Initial reports of iontophoretic application of NE near hippocampal pyramidal cells described a suppression of the spontaneous and evoked activity recorded extracellularly in the anesthetized cat (Stefanis, 1964; Biscoe & Straughan, 1966).

Later reports confirmed the suppressant effects on the spontaneous activity of pyramidal cells with iontophoretically applied NE (Segal & Bloom, 1974a) and locus coeruleus stimulation in anesthetized (Segal & Bloom,

1974b) and awake rats (Segal & Bloom, 1976a; 1976b). The suppressant effects of NE were relatively long-lasting and outlasted the period of iontophoresis or locus coeruleus stimulation by 5 seconds to 6 minutes (Segal & Bloom, 1974a; 1974b). In the awake, restrained cat, locus coeruleus stimulation also produced suppression of spontaneous activity (Finch, Feld & Babb, 1978).

Intracisternal 6-OHDA injections abolished the effects of locus coeruleus stimulation (Segal & Bloom, 1974b; 1976a; 1976b) but not of iontophoresis of NE (Segal & Bloom, 1974a). The suppressant effect of iontophoretically applied NE and locus coeruleus stimulation in anesthetized rats could be blocked by a beta adrenergic antagonist (sotalol) and augmented by the NE uptake blocker desmethylimipramine (Segal & Bloom, 1974a; 1974b). The suppressant effects of NE iontophoresis could be mimicked by iontophoresis of cAMP or dibutyryl cAMP (Segal & Bloom, 1974a). From these studies it appeared that the effect of NE on hippocampal pyramidal cells was inhibition mediated via a beta receptor and involving cAMP.

Experiments with intracellular recordings from pyramidal cells have yielded conflicting data concerning the cellular mechanism of action for the suppressant effect of NE. Herrling (1981) observed a hyperpolarization of the membrane accompanied by a conductance decrease following

iontophoresis of NE in the in vivo cat. Synaptically-evoked EPSPs were also observed to increase in amplitude during iontophoresis of NE (Herrling, 1981).

In contrast, in the in vitro hippocampal slice, Segal (1981) and Langmoen, Segal and Andersen (1981) reported a hyperpolarization of pyramidal cells accompanied by an increase in membrane conductance following a "microdrop" (topical) application of NE. These membrane effects were present in low calcium medium and thus the effects of NE were suggested to be post-synaptic in nature (Segal, 1981; Langmoen et al., 1981). In normal medium, synaptically-evoked EPSPs were either increased (7 cells) or decreased (8 cells) (Langmoen et al., 1981) or were mostly decreased (Segal et al., 1981). Since the hyperpolarizing effects of NE were partly sensitive to extracellular chloride concentration and partly sensitive to ouabain, Segal (1981) suggested that NE activates two mechanisms, an increase in chloride conductance and an increase in sodium-potassium pump activity.

On the other hand, Langmoen et al. (1981) have suggested that NE decreases an inward Ca^{2+}-Na^{+} current, thereby decreasing anomalous rectification (Hotson, Prince & Schwartzkroin, 1979) and resulting in an apparent increase in membrane conductance. Anomalous rectification in pyramidal cells is thought to be mediated by an inward Ca^{2+}-

Na^+ current which increases with membrane depolarization and which produces an apparent increase in membrane resistance by adding to the voltage change caused by an intracellular current pulse (Hotson et al., 1979).

More recently, facilitatory effects of NE on the evoked activity of pyramidal cells have been described (Mueller et al., 1981). Mueller et al. (1981) reported dose-dependent, receptor specific, differential effects of NE on the synaptically-evoked firing of CA1 pyramidal cells as measured by the population spike amplitude of field potentials in the in vitro slice. When added to the medium, low doses of NE (5-10 μM) produced an increase in the evoked potential which was mediated by beta receptors, while high doses of NE (50 μM) produced decreases in the evoked activity mediated via alpha receptors (Mueller et al., 1981). It was suggested that the beta-mediated increase in excitability involved cAMP since cAMP derivatives also produced increases in evoked responses (Mueller et al., 1981).

Similar facilitatory effects of bath applied NE have now been reported for spontaneous and synaptically-evoked unit activity in the CA3 region (Otmakhov & Bragin, 1982), and for synaptically evoked field potentials in the CA1 region (Anwyl & Rowan, 1984). Iontophoretically applied NE has also been reported to facilitate synaptically evoked field

potentials in the CA1 region (Marciani, Calabresi, Stanzione & Bernardi, 1984).

The facilitatory effects of NE on the evoked activity of pyramidal cells have also been observed intracellularly in vitro (Segal, 1982; Madison & Nicoll, 1982; Haas & Konnerth, 1983). Segal (1982) reported that glutamate-induced membrane depolarizations are augmented by iontophoresis of NE, or of beta agonists, on the pyramidal cell soma but not on the dendrites. The NE effects were not accompanied by any systematic or major change in membrane polarization or resistance (Segal, 1982).

Madison and Nicoll (1982) and Haas and Konnerth (1983) reported that in the in vitro slice the pyramidal cell firing evoked by intracellular depolarizing pulses or by extracellular iontophoresis of glutamate was augmented during bath application of NE. It was observed that NE produced a decrease in the calcium-dependent potassium conductance mediating the late hyperpolarization (Madison & Nicoll, 1982; Haas & Konnerth, 1983). Thus by decreasing the late hyperpolarization which normally inactivates the cell firing, NE produced an increase in evoked firing. This effect of NE is thought to be mediated following the entry of calcium into the pyramidal cells since calcium spikes recorded intracellularly are not affected by NE (Madison & Nicoll, 1982; Haas & Konnerth, 1983). Additionally, the

facilitatory effect of NE was found to be mediated via beta receptors (Madison & Nicoll, 1982; Haas & Konnerth, 1983) and could be mimicked by cAMP derivatives (Madison & Nicoll, 1982).

Thus, to summarize, in Ammon's horn, NE appears to produce dose- and receptor-dependent effects. Low doses of NE produce facilitatory effects on pyramidal cells via beta receptors and possibly cAMP. At the cellular level this facilitation of firing appears to be caused by a reduction of the calcium-dependent potassium conductance mediating the late hyperpolarization. High doses of NE produce a suppressant effect on pyramidal cells via alpha receptors. The cellular mechanism of the suppressant effect remains unclear, both conductance increases and decreases have been observed and at least three mechanisms have been suggested: an activation of the sodium-potassium pump, an increase in chloride conductance and a decrease in anomalous rectification.

Physiological effects of norepinephrine on dentate granule cells

In comparison to some other brain areas, little is known about the physiological effects of NE on the dentate granule cells. An initial abstract reported suppressant effects of

NE on the spontaneous and evoked activity of neurons of the dentate gyrus (Stefanis, 1964). Later, Segal and Bloom (1976a) in the course of their investigation of the effects of NE on hippocampal pyramidal cells, reported that locus coeruleus stimulation inhibited the spontaneous activity of most extracellularly recorded hippocampal cells, including 8 cells from the dentate gyrus.

However, more recently, the effect of NE on identified dentate granule cells has also been evaluated. In a conference report, Assaf, Mason & Miller (1979) observed that stimulation of the locus coeruleus augmented the synaptically evoked firing of the granule cells as measured by the amplitude of the population spike of the evoked field potential. Dendritic potentials representing the summation of EPSPs were not altered. The increase in evoked cell discharge following stimulation of locus coeruleus was abolished by 6-OHDA injections into the dorsal noradrenergic bundle which depleted 91% of hippocampal noradrenaline (Assaf et al., 1979).

In a more detailed account, Neuman and Harley (1983) reported that iontophoresis of NE also augmented the amplitude of the evoked population spike representing the synaptically evoked firing of the granule cells. They observed a 20-400% increase in the evoked response which in 39% of the cases had not recovered to control levels 30

minutes after the iontophoretic application of NE. In some instances the effect was followed over a long period of time and the enhancement of the evoked response was shown to last as long as eleven hours (Neuman & Harley, 1983).

Characteristically, the long-lasting effects developed as a continous, sustained increase in the minutes following the iontophoretic application (1-2 minutes) reaching a plateau after 15-30 minutes (Neuman & Harley, 1983). Sometimes however, the increase was slower and more gradual achieving a plateau only 3 hours after the iontophoretic application (Neuman & Harley, 1983). Typically, iontophoresis of NE did not affect the synaptic potentials (EPSP), although this was not systematically evaluated (Neuman & Harley, 1983). Preliminary evidence indicated that enhancement produced by NE was probably mediated via beta adrenergic receptors (Harley & Neuman, 1980).

Preliminary reports (Harley, Lacaille & Milway, 1982) indicate that locus coeruleus stimulation can produce an enhancement of the perforant path evoked population spike indicative of an enhanced evoked firing of the granule cells. Furthermore, with sufficient repetition (50) of the paired locus coeruleus and perforant path stimulation, the subsequent enhancement of the evoked responses to perforant path stimulation alone was observed to last for more than 30 minutes in 60% of the animals tested (Harley et al., 1982).

Thus, it would appear that the effect of NE on dentate granule cells in vivo is to increase their firing in response to synaptic activation. Furthermore, this facilitation of the granule cell response is often long-lasting. Preliminary evidence suggests the involvement of beta receptors.

Objectives

The main objective of this study is to characterize the effect of norepinephrine on the evoked responses of the dentate granule cells using the in vitro hippocampal slice preparation (Bliss & Richards, 1971; Skrede & Westgaard, 1971; Yamamoto, 1972; Schwartzkroin, 1975; Dingledine, Dodd & Kelly, 1980).

The in vitro slice preparation is used because it offers the following advantages. The extracellular space is directly accessible to the experimenter and thus drugs such as NE and various adrenergic agonists and antagonists can be applied directly in the superfusion medium at a precise concentration. The condition of the slice is under the control of the experimenter, hence secondary drug effects related to changes in blood pressure, heart rate, temperature or anesthesia are eliminated. Finally, stimulating and recording electrodes can be positioned in

the slice under visual control thus permitting the precise placement of electrodes within specific areas.

However, the slice preparation also has its limitations. The cells are removed from their normal CNS environment and there is a progressive metabolic run-down of the slice.

The evoked responses of granule cells will be recorded electrophysiologically using extracellular field potentials. This method is used because it offers the advantage of simultaneously measuring evoked EPSPs and evoked action potentials in populations of granule cells. Thus the effects of NE will be assessed in terms of its action on synaptic activation as well as on the firing of granule cells. With reference to the physiological effects of NE on the evoked responses of dentate granule cells, another objective of this study will be to examine the incidence of long-lasting NE effects in vitro. Thus, the effects of NE will be systematically recorded for 30 minutes following the application of NE.

Other objectives of this study aim to characterize the mechanism of action of NE:

1. To identify pharmacologically which type of adrenergic receptor (alpha and/or beta) mediates the effects of NE on the granule cell responses. Therefore, experiments will be conducted with adrenergic agonists (phenylephrine and isoproterenol) and antagonists (phentolamine and

timolol).

2. To evaluate whether NE produces suppressant and/or facilitatory effects which are dose-dependent. Thus NE will be applied at different concentrations (1-200 μM) in the superfusion medium.

3. To determine the relationship between synaptically evoked EPSPs and action potentials in granule cells and to examine how NE affects this relationship. Therefore, the normal relationship will be measured and the effects of NE on these parameters will be evaluated.

4. To examine whether NE alters the excitability of granule cell somas. Thus the effects of NE on the antidromically evoked responses of the granule cells will be examined.

5. To determine whether concurrent evoked activity of the granule cells is necessary for the effects of NE to take place. Thus NE will be applied in the superfusion medium with and without concurrent perforant path stimulation.

6. To evaluate the effects of serotonin on the evoked response of the granule cells and to compare serotonin effects with those of NE. Thus serotonin will be applied in the superfusion medium and the effects on perforant path evoked responses will be measured.

Chapter 2: Method

Subjects

The experiments were performed on 81 Sprague-Dawley albino rats, male (n=19) or female (n=62), weighing 250-300 grams. The rats were obtained either from the Memorial University breeding colony or from Charles River Inc., Quebec, and were housed in the Animal Care Unit, Faculty of Medicine, Memorial University of Newfoundland.

Apparatus

Perfusion chamber

Hippocampal slices were maintained in a perfusion chamber based on the design of Haas, Schaerer and Vomansky (1979). The design was modified to accomodate submerged slices in a similar manner to Corrigall and Linseman (1980). The slice perfusion chamber was built of Plexiglass by Technical Services, Memorial University and is illustrated in Figures A-1 to A-4 in Appendix A.

The perfusion chamber consisted of an enclosed water chamber with a lid into which three recording chambers were machined. The water chamber was filled to three quarters

with distilled water which was maintained at 37.5 ± 0.5 degrees Celsius by a direct current (DC) proportional temperature controller.

The temperature controller was built by the Electronics Technical Services, Department of Psychology, Memorial University. The electronic circuit diagrams for the temperature controller are included in Figure B-1 and B-2 of Appendix B. The temperature probe was a two-terminal integrated circuit temperature transducer, model AD 590, from Analog Devices.

A mixture of 95% oxygen/5% carbon dioxide was bubbled through the water chamber. The humidified O_2/CO_2 was then led via air holes to the recording chamber to provide a warm, humidified source of oxygen to prevent drying of the slices. Polyethylene tubing (inside diameter 1.02 mm) running in the water chamber carried the artificial cerebrospinal fluid (ACSF) by gravity from a storage container to the recording chamber while warming it to a final temperature of 35.5 ± 0.5 degrees Celsius. Solution flow rates were monitored with a Gilmont (size no.12) precision flowmeter. Different solutions could be selected for superfusion with a two-way tap. Prior to superfusion, solutions were continously bubbled with O_2/CO_2 while warmed to 40 degrees Celsius to prevent bubble formation in the recording chamber.

Only one of the three recording chambers was used at a time. Polyethylene tubing carrying the superfusion medium was led into and out of the water chamber via the air holes of an adjacent recording chamber. Air holes from the unused recording chambers were blocked to avoid O_2/CO_2 leakage.

The recording chamber consisted of a Plexiglass inner resting stage with fine nylon net (Nytex) on its surface and a Plexiglass outer square frame with nylon net on its topmost surface. The slice was laid on the inner resting stage which was then inserted into the outer square frame thereby creating a 500 micron space between the two nets. This space containing the slice was then filled with ACSF (see Figure A-3).

The superfusion medium was supplied to the recording chamber by positioning the end of the polyethylene tubing with micromanipulators onto the topmost nylon net and continously superfusing at a rate of 4-6 milliliters per minute (gravity-fed). The superfusion medium exited the recording chamber by capillary action along the nylon net which extended over the edge of the lid (see Figure A-3). Usually filter paper was also used on and/or under the nylon net to increase the capillary draw over the edge of the recording chamber. A sliding cover was maintained as much as possible over the slice to prevent drying of the slices.

Electrophysiological recording and stimulating apparatus

Monopolar field potentials were recorded with respect to a ground electrode in the recording chamber. Monopolar recordings were made with micropipettes pulled from Omega Dot capillaries (1.2 mm O.D. x 4 in.) filled with 3M NaCl. Size of the tips ranged from 5-15 microns and impedances were 0.4-1.8 megohms.

The electrophysiological signal was differentially amplified (WPI, model DAM-6A) at a bandwidth of 1 Hz to 3 KHz and then displayed on a storage cathode-ray oscilloscope (Tektronix, model R5031). The amplified electrophysiological signal was also sent to a microprocessor-based system for analog to digital (A-D) conversion, signal analysis, digital to analog (D-A) conversion and subsequent write-out of the amplified signal on an ink-pen recorder (Beckman, model R-411).

Slices were electrically stimulated with bipolar Teflon-coated tungsten microelectrodes (F. Haer Co.) with shank diameter of 0.010 inch, blunt tip profiles, an exposed tip length of 25 microns and tip separation of 100 microns. Biphasic constant-voltage stimulating pulses were generated by an Ortec stimulator (model 4710) and isolated from ground by Grass stimulus isolation units (model SIU5). Stimulation pulses were also led to a level slicer (BRS, model LS201),

the output of which was then led to the microprocessor system to act as a trigger.

Microprocessor systems

Two microprocessor systems were used in the course of the experiments. Initially, a Z80 microprocessor (Exidy, model Sorcerer) with 48K of memory was used in conjunction with either a tape recorder or disk drives for data storage. The A-D and D-A conversion was done with an 8-bit converter with 2 A-D and one D-A channels (Model Ratzapper) built by the Center for Engineering Design, Department of Engineering, Memorial University.

The second microprocessor used was an 8086 microprocessor (Seattle, model Gazelle 1) with 8-inch disk drives (Qume, model Data Trak 8) and 128K of memory. The A-D converter was a 12-bit, 16 channel converter (Tecmar, model TM-AD212) with timer and counters and the D-A converter was a 12-bit, 4 channel converter (Tecmar, model TM-DA100). Additionally with this system, a dot-matrix printer (Epson, model MX-80F/T) was used for on-line print-out of data analysis.

Procedure

Solutions

All solutions were mixed on the day of the experiment. The artificial cerebrospinal fluid (ACSF) was the same as that used by Yamamoto (1972) for submerged slices and consisted of (in mM, final concentrations) NaCl, 124; KCl, 5; $CaCl_2$, 2.4; KH_2PO_4, 1.24; $MgSO_4$, 1.3; $NaHCO_3$, 26; glucose, 10.

For the dose-response experiments, stock solutions of NE were mixed fresh before each experiment. NE was dissolved at a final concentration (1, 5, 25, 50, 100 & 200 μM) in pre-oxygenated ACSF and stored in a refrigerator. Fifteen minutes prior to bath application, the solution was warmed to room temperature in a water bath and continuously bubbled with O_2/CO_2.

For all other experiments concentrated drug solutions were mixed and stored in a refrigerator. Before application they were diluted to their final concentration with warm, oxygenated ACSF. Norepinephrine (NE) (Sigma, l-arterenol) was generally mixed at 300 μM in 0.01% (w/v) ascorbic acid (Sigma) in ACSF. Timolol (TIM) (C.E. Frosst & Co.) was mixed to 150 μM in ACSF. Phentolamine (PAQ (Ciba Pharmaceuticals) was made up to 1.5 mM in ACSF. Isoproterenol (ISO) (Sigma, l-isoproterenol) was dissolved to 150 μM in 0.01% ascorbic acid ACSF. Phenylephrine (PE)

(Winthrop Laboratories) was mixed to 900 μM or 1.5 mM in ACSF. Finally, serotonin (5-HT) (Sigma, 5-hydroxytryptamine) was mixed to 300 μM in ACSF.

Slicing procedure

Hippocampal slices were obtained by a method similar to the Vibratome method of Dingledine et al. (1980). The animals were decapitated and the skull was exposed. Using rongeurs the dorsal aspect of the skull was removed thus exposing the brain. A metal spatula was inserted under the brain and the cranial nerves were severed. The brain was then removed from the skull and placed on a filter paper moistened with AGSF at room temperature.

With the brain lying on its ventral side, a 7.5 mm thick block of tissue including the left hippocampus was obtained by making two oblique near- and far-sagittal cuts with a razor blade. Such cuts were nearly perpendicular to the septo-temporal axis of the hippocampus. With the block of tissue lying on its medial side, additional cuts were made rostral and caudal to the hippocampus to remove unnecessary tissue. The lateral aspect of the tissue block was then fixed to the Vibratome stage with cyanoacrylate glue (Krazy Glue). The stage and block of tissue were then immersed in the Vibratome (Oxford, model G) bath which was filled with

ACSF at room temperature and bubbled with 95% O_2/5% CO_2. At this point the procedure was repeated to obtain a block of tissue containing the right hippocampus.

The stage and block of tissue were then clamped in the bath with the alvear side of the hippocampus facing the Vibratome blade. Hippocampal slices, transverse to the septo-temporal axis and 300-400 microns thick, were cut with the Vibratome blade advancing at slow speed. After each slice was cut it was transferred with a wide-bored pipette to a storage beaker which was filled with ACSF at room temperature and continuously bubbled with O_2/CO_2. After the left hippocampus had been sliced the procedure was repeated for the right one. Normally, 4-5 slices were obtained from each hippocampus. From the time of decapitation to immersion of both tissue blocks in the Vibratome bath the procedure usually took less than 5 minutes. The slicing procedure of the left hippocampus was generally finished by 15 minutes and that of the right one by 25 minutes post-decapitation. The slices were then allowed to rest for an hour in the storage beaker to overcome the trauma of the slicing procedure.

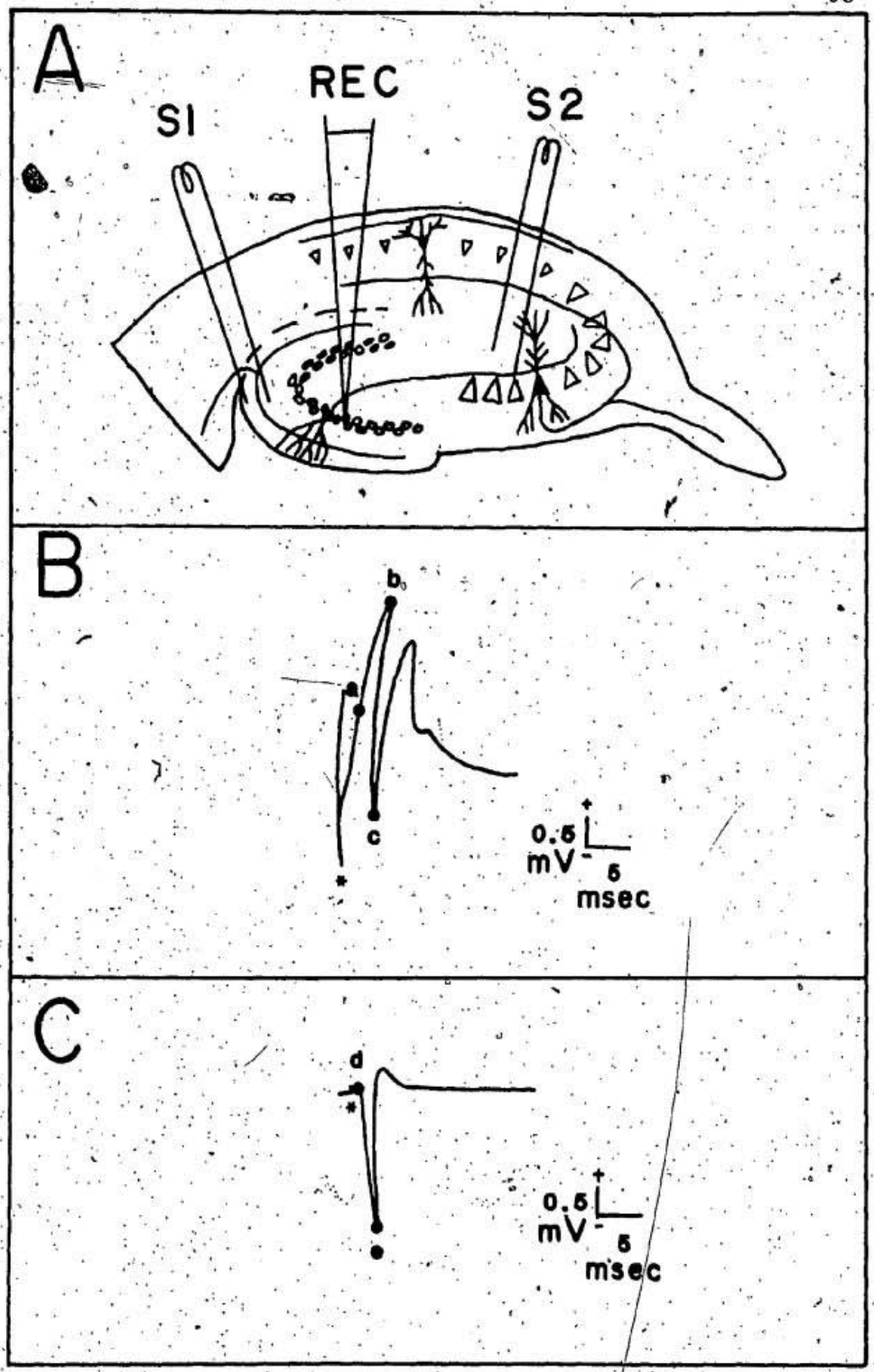
Recording procedure

A slice was transferred from the storage beaker to the

nylon net on the inner resting stage of the recording chamber with a wide-bored pipette. The slice was positioned with a fine paint brush and sometimes held down onto the stage with nylon strings. The inner stage was inserted into the outer square frame and positioned into the recording chamber where superfusion with warm ACSF was started. The slice was then allowed to rest an additional 15-20 minutes to permit recovery from the transition from cold to warm medium.

The microelectrodes were lowered into the recording chamber through openings in the nylon net and then positioned on the slice under visual guidance with a dissecting microscope (Wild, model M7, magnification 60-310). Figure 3A illustrates diagrammatically the placements of the electrodes in a slice. The recording micropipette (REC) was positioned in the granule cell layer. For orthodromic stimulation, bipolar stimulating electrodes (S1) were positioned in the outer two-thirds of the molecular layer and away from the recording electrode so as not to stimulate directly the dendrites of the recorded cells. For antidromic stimulation, bipolar stimulating electrodes (S2) were positioned in stratum radiatum of CA3 to stimulate the mossy fibers. In all experiments field potentials were evoked at a rate of 0.1 Hz and only slices which displayed a stable baseline of evoked responses were kept for

Figure 3. Electrode placement and evoked responses. A: Diagram of the slice with orthodromic (S1) and antidromic (S2) stimulating electrodes and recording micropipette (REC). B: Example of an orthodromically evoked field potential. C: Example of an antidromically evoked field potential. Asterisk (*) in B and C indicates stimulus artefact. See text for explanation of a to e.


A
SI
REC
S2
B
b
a
c
0.5
mV
5
msec
C
d
0.5
mV
5
msec

experiments. Slices with increasing or decreasing baseline responses which did not stabilize were discarded. Usually a 10% change over 10 minutes was easily noted and slices were discarded on that basis. In a few cases the slice showed a change in evoked responses with superfusion of the vehicle solution alone. These slices were also discarded.

Figure 3B is an example of an orthodromic field potential recorded in the granule cell layer after perforant path stimulation. Figure 3C is an example of an antidromic field potential recorded in the granule cell layer after mossy fiber stimulation. Simultaneous recordings of extracellular field potentials and extracellular unit activity or intracellular recordings have demonstrated the cellular events underlying these field potentials (Andersen, Bliss & Skrede, 1971a; Lomo, 1971a). For the orthodromic field potential, the first positivity ("*" to b in Figure 3B) is termed the extracellular EPSP and reflects the synaptic current generated by intracellular EPSPs in a number of granule cells. The first negativity (b to c in Figure 3B) is called the population spike and reflects the number of granule cells discharging action potentials nearly synchronously. For the antidromic field potential, the negativity (d to e in Figure 3C) is the antidromic population spike also reflecting the number of granule cells firing action potentials nearly synchronously. More

detailed discussions of the membrane currents generating the field potentials and underlying cellular events have appeared elsewhere (Andersen et al., 1966; Andersen et al., 1971a; 1971b; Lomo, 1971a; 1971b; Winson & Abzug, 1978; Jefferys, 1979).

Microprocessor-based data acquisition

Data acquisition was basically the same with the two microprocessor systems used. Thus, the procedure will be explained only for the Seattle-based system.

The computer programs for the data acquisition and analysis are included in Appendix C. The language used was 8086 FORTH (Laboratory Microsystems) running under CP/M-86 (E.D.S.). The instructions for the analog-to-digital and digital-to-analog conversions consisted of machine language subroutines.

For the data acquisition two analog-to-digital channels were used. One carried the electrophysiological signal and the other a trigger signal from the level slicer. The analog-to-digital conversion was done at a rate of one point every 75 microseconds.

First, the trigger channel was continuously sampled for the presence of a trigger signal. When a trigger signal was found the analog-to-digital converter was multiplexed to the

other channel carrying the electrophysiological signal and the A-D conversion was started. Two hundred and fifty six points representing 19.2 milliseconds of signal were thus digitized and stored in memory.

Following on-line data analysis (covered in next section) the digitized waveform was then converted back to a voltage signal at a rate of 1 point every 29.4 milliseconds (34 Hz). The output of the D-A converter was continuously plotted on an ink-pen recorder.

On-line data analysis

The data analysis consisted of both on- and off-line analysis. The on-line analysis consisted of the measurements of parameters of the digitized waveform and the off-line analysis consisted of statistical analyses on these parameters across experimental situations.

For the on-line analysis the parameters measured were the following: extracellular EPSP, population spike onset latency, population spike peak latency and population spike amplitude. For both orthodromically and antidromically evoked responses all parameters were measured although the EPSP and latency measurements were not considered for the antidromic response.

For the orthodromic response the parameters were

measured in the following way. For the extracellular EPSP, two methods of measurement were used. First, the amplitude of the first positivity was measured at a fixed latency after the stimulus artefact and the baseline measurement was subtracted from it (in Figure 3B, amplitude of point a minus baseline taken prior to stimulation). This parameter is referred to as the EPSP amplitude. However, in the course of the experiments it was thought that the EPSP slope measurement might provide a more powerful measurement of the extracellular EPSP component. Thus, in the transition to the second microprocessor system the algorithm was changed to measure the extracellular EPSP slope. The EPSP slope was measured as the change in amplitude over the change in time between a point during the rise time of the first positivity and the peak of the first positivity. With reference to Figure 3B the EPSP slope was measured as the change in amplitude between points b and a divided by the change in time between points b and a. Before an experiment, the time at which point a was measured was set by the experimenter so that the time interval between the two points (a and b in Figure 3B) was approximately 1-1.25 milliseconds.

In the present experiments, the "pure" extracellular EPSP uncontaminated by the population spike was not measured. It is acknowledged that the present EPSP measurement from the compound signal could be altered by

changes in the population spike (such as decreases in onset latency). However, since the effects of NE on the population spike were of primary interest, it was decided to measure the extracellular EPSP from the compound signal as an initial estimation of the effects of NE on synaptic potentials.

For the population spike onset latency, the latency of the peak of the first positivity after the stimulus artefact was measured. With reference to Figure 3B the population spike onset latency equals the latency of point b after the stimulus artefact "*".

For the population spike peak latency, the latency of the peak of the first negativity after the stimulus artefact was measured. In Figure 3B the peak latency of the population spike equals the latency of point c after the stimulus artefact "*".

For the population spike amplitude, the difference between the peak of the first positivity and the peak of the first negativity was measured. With reference to Figure 3B the population spike amplitude equals the amplitude of point b minus the amplitude of point c.

For the antidromically evoked field potentials the antidromic population spike amplitude was measured as the amplitude difference between the peak of the first negativity and baseline. With reference to Figure 3C the

antidromic population spike amplitude equals amplitude of point d minus amplitude of point e.

This on-line analysis was performed after each A-D conversion of the signal. Then the results were displayed, printed and stored for batch dumping to disk. Finally a D-A conversion took place and a record made on the polygraph.

Off-line data analysis

In evaluating effects on perforant path evoked responses the extracellular EPSP measures, the population spike onset latency and the population spike amplitude were considered. When antidromically evoked responses were evaluated only the effects on population spike amplitude were examined.

For statistical analysis, the data from a recording session was transferred from disk to memory.

For a given experimental manipulation a control period was specified. The parameters (EPSP, population spike onset latency and amplitude) were averaged in blocks of 3 measures (i.e. blocks of 30 seconds) for that period. The mean for each parameter was then measured for the control period. Then, the 95% confidence interval of the control period (two-tailed, n-1 degrees of freedom) was measured for each parameter. Afterward the parameters during the given period of experimental manipulation were averaged in blocks of 3.

Finally the parameters during both control and experimental periods were expressed as percentages of their respective mean for the control period. The results of the statistical analysis were printed-out on a line printer.

Therefore, for each experimental manipulation the statistical analysis would yield a print-out giving the mean of each parameter (EPSP, population spike onset latency and amplitude) for the control period, the respective 95% confidence interval of the control period for each parameter and the means of each block of 3 data points for each parameter for the whole experiment expressed as the percentage of the mean of the control period.

Effective NE concentration: Dose-response experiments

For the dose-response experiments, the vehicle solution and different concentrations of NE (1, 5, 10, 25, 50 µM) were applied consecutively to the same slice in four slices. In another slice all solutions but 1 µM NE were applied. In two of the slices 100 and 200 µM NE were also individually applied. The order of presentation of the drug solutions was random.

Since repeated applications of NE to the same slice were used, short control periods were taken and the effects of NE were not systematically followed for a long period. Thus,

following a control period of 2 minutes of evoked responses the solution containing NE or the vehicle solution alone was applied to the slice for 10 minutes. After this period, the slices were again superfused continuously with ACSF. After the return to ACSF superfusion the slices were allowed to stabilize. Following stabilization of the evoked response (less than 10% variability) the procedure was repeated with a different concentration of NE. For data analysis the magnitude of the evoked response (as % of the immediately preceding control period) during the seventh and tenth minute of superfusion with NE was compared with the responses during the same interval of superfusion with the vehicle solution. Since these were a priori comparisons the statistical significance of the changes in response for each drug concentration compared to vehicle was assessed with a t-test for correlated means (Runyon & Haber, 1977).

Effective NE concentration: Physiological characterization of NE effects

On the basis of the dose-response experiments, the most effective dose of NE was chosen for the physiological characterization of NE effects. In 38 slices, the effects of superfusion of 10 μM NE on granule cell responses evoked by stimulation of the perforant path were assessed. First,

a control period of 10 minutes was taken. Then ACSF containing 10 μM NE was superfused for 10 minutes. After this period the slices were again superfused with normal ACSF. The recovery from the effects of NE was followed for 30 minutes after the return to superfusion with normal ACSF (wash-out) or until the evoked responses had recovered to control levels, whichever came first.

The effect of NE on the EPSP amplitude, EPSP slope, population spike onset latency and population spike amplitude of the evoked response was monitored. The following five measures were computed for each NE effect on these parameters: onset of effect, peak effect, time of peak effect, time of recovery and presence of long-lasting effects. The onset of effect was defined as the time after the beginning of the NE application of the first of two consecutive means of blocks of 3 data points which fell outside the 95% confidence interval of the control period. The peak effect was defined as the maximum effect (% of control) which occurred after the onset of effect. It had to be one of two consecutive significant means. The time of peak effect was defined as the time after the beginning of the NE application at which the peak effect took place. Finally, the time of recovery was defined as the time after the beginning of NE wash-out (return to control ACSF) of the last significantly different mean of blocks of 3 data

points. The last significantly different mean was defined as the mean of a block of 3 data points after the peak effect which fell outside the 95% confidence interval and which preceded the first two consecutive means of blocks of 3 data points which were within the 95% confidence interval.

Long-lasting effects were defined as effects which had not recovered to within the 95% confidence interval of the control period 30 minutes after the removal of NE and the beginning of superfusion with control ACSF. In some instances the duration of effect was measured. It was defined as the difference between the time of recovery and the time of onset for a given effect.

Effective NE concentration: Pharmacological characterization of NE effects

In the pharmacological experiments the effects of 10 uM NE were characterized in terms of their mediation by alpha or beta adrenergic receptors. To evaluate mediation by an alpha receptor, NE was applied in conjunction with phentolamine (PA), a reversible, competitive, non-selective alpha receptor antagonist (Goodman Gilman, Goodman & Gilman, 1980). Also, phenylephrine (PE) a non-selective alpha receptor agonist with little effect on beta receptors (Goodman Gilman et al., 1980) was superfused on the slices.

To evaluate the mediation of NE effects via a beta receptor, timolol (TIM), a non-selective beta adrenergic antagonist without demonstrable local anesthetic properties (Goodman Gilman et al., 1980) was superfused in conjunction with NE. Also, isoproterenol (ISO) a non-selective beta receptor agonist with almost no action on alpha receptors (Goodman Gilman et al., 1980) was superfused on the slices.

The effectiveness of the adrenergic antagonists was evaluated by their action on the peak effect produced by 10 μM NE. The effectiveness of the adrenergic agonists was evaluated with the peak effect they produced compared to that of 10 μM NE. The effectiveness of the antagonists (TIM & PA) was evaluated in different slices whereas the effectiveness of the agonists (ISO & PE) was evaluated in the same slices.

In these experiments the general procedure was, first, a 10 minute control period, followed by 10 minutes of superfusion with the drug solution and finally wash-out with control ACSF. In the experiments with antagonists, 10 μM NE was first applied for 10 minutes. After recovery from the effects of NE, the antagonist (TIM or PA) was applied concurrently with 10 μM NE. After recovery from these effects, the antagonist (TIM or PA) was applied alone.

In the experiments with agonists, 10 μM NE was first applied. After recovery from the effects of NE, the first

agonist was applied. After recovery from these effects, the second agonist was then applied. In two slices isoproterenol was applied before phenylephrine and in five it was the reverse order.

Effective NE concentration: EPSP slope and population spike amplitude relationship

Since NE was found to increase both the EPSP slope and the population spike amplitude (see Results) these results indicated that NE could increase both the postsynaptic depolarization (extracellular EPSP) and the firing of the granule cells (population spike). Thus the possibility arose that the increase in granule cell firing (population spike amplitude) was the result of the greater postsynaptic depolarization (EPSP slope). To assess this possibility, experiments were performed to examine the relationship between EPSP slope and population spike amplitude under normal conditions. After establishment of the EPSP slope and population spike amplitude function in a given slice, it was asked whether, for that slice, the effects of NE on the population spike amplitude could be accounted for by the effects of NE on the EPSP slope alone.

A procedure similar to that used by others was employed (Bliss and Lomo, 1973; Wilson, 1981; Bliss, Goddard and

Riives, 1983; Low, BeMent & Whitehorn, 1983). First, for subsequent measurement of the functional relationship, the EPSP slope and population spike amplitude were recorded over a range of perforant path stimulus intensities. Care was taken to measure the parameters mostly over the linear portion of the stimulus-response function.

After the measurement of the range of evoked responses, the stimulus intensity was adjusted so that the magnitude of the evoked response was in the low end of the range. This was done to ensure that the increase in responses produced by NE would fall within the range of responses previously recorded. A control period of 10 minutes was then taken, followed by 10 minutes of superfusion with 10 μM NE and then return to control ACSF. During this time the peak effect of NE on EPSP slope and population spike amplitude was measured.

To measure the EPSP slope and population spike amplitude function, a linear regression analysis was performed on the range of evoked responses. Thus the regression of the population spike amplitude on the EPSP slope was computed (Kerlinger & Pedhazur, 1973). This analysis resembled that used by Low et al. (1983) for similar purposes.

With the regression analysis an equation was obtained that predicted the population spike amplitude from the EPSP slope. By using the peak value of the EPSP slope produced

by 10 μM NE in the regression equation it was possible to compute the predicted value of the population spike that could be accounted for by the increment in EPSP slope during NE perfusion. Then by comparing this predicted population spike amplitude value with the observed one, it could be seen whether the increase in population spike amplitude was accounted for by the increase in EPSP slope. The value of the EPSP slope necessary to predict the observed peak value of the population spike amplitude produced by 10 μM NE was also computed. Furthermore, to find the percentage of the increase in population spike amplitude accounted for by the increase in EPSP slope, equation (1) was computed:

$$\frac{POPpre - POPctrl}{POPobs - POPctrl} \times 100 \qquad (1)$$

In the equation (1) POPpre was the population spike amplitude predicted from the observed EPSP slope during 10 μM NE superfusion, POPctrl was the population spike amplitude observed during the control period and POPobs was the population spike amplitude observed during 10 μM NE superfusion. Thus the equation yielded the percentage of the observed increase in population spike amplitude accounted for by the increase in EPSP slope.

The functional relationship of EPSP slope and population

spike amplitude was examined in 6 slices.

Effective NE concentration: Effect of NE on antidromically evoked responses

To evaluate whether the effect of NE on the orthodromically evoked population spike amplitude was due to changes at the soma membrane of the granule cells, the effect of NE on the antidromically evoked population spike amplitude was assessed. Since the extracellularly recorded antidromic population spike amplitude reflects the spatio-temporal summation of the current flow across the soma membrane resulting from antidromic propagation of action potentials, changes in the soma membrane characteristics produced by NE would be reflected by changes in the antidromically evoked population spike amplitude. Thus if the increase in orthodromic population spike amplitude was due to a change in soma membrane characteristics, the antidromic population spike amplitude would also be increased. This possibility was assessed in 8 slices.

The general procedure for superfusion of NE in these experiments was the same as before with a 10 minute control period, 10 minutes of superfusion with NE and a recovery period with superfusion of ACSF.

In these experiments, the responses of the granule cells

were first evoked orthodromically by stimulation of the perforant path and the effect of NE on the evoked responses was measured. Subsequently, granule cell responses were evoked antidromically by stimulation of the mossy fibers. Care was taken to adjust the stimulus intensity to evoke an antidromic response which was of approximately the same magnitude range as the orthodromic response. NE was then superfused and the effect of NE on the antidromically evoked responses was measured.

Additionally, in 6 of the 8 slices, granule cell responses were later evoked orthodromically again by stimulating the perforant path. NE was superfused again and the effects on the orthodromic responses were reassessed. This additional procedure was done to ensure that the granule cell responses were still sensitive to the effect of NE and to show that any lack of effect of NE on the antidromic response was not due to a lack of responsiveness on the part of the granule cells or the slices.

Effective NE concentration: Activity-independence of NE effects

Since the preceding experiments had shown that the effect of NE was to increase the perforant path evoked response and since this increase was observed to last longer

than the period of NE superfusion, the question of whether NE had to be present in association with the evoked response to produce the increase was of interest. In terms of neuronal events the question asked was the following. Given that there are two synapses (A and B) on the dendrite of a granule cell and that synapse A is active but B is inactive during superfusion of NE, will both synapses be equally affected by NE? Following superfusion and then removal of NE, based on the results of the previous experiments, one would predict that the response of the granule cell to activation of synapse A would be increased. But what would happen to the response of the granule cell to activation of synapse B? Would it be increased or unchanged? Are the effect of NE activity-dependent (only the response to synapse A would be increased) or are they activity-independent (the responses to both synapse A and B would be increased)?

Since the experimental preparation did not allow direct measurement of the response of a single granule cell to two different afferent synapses, the following experiments were designed to answer the question. First, a 10 minute control period was taken, followed by superfusion of 10 μM NE for 10 minutes and then return to control ACSF. The effects of NE on the evoked responses were measured. By analogy to the example, this experiment represented the test for synapse A

active during NE superfusion.

After recovery from the effect of NE, a second 10 minute control period was taken. After this period, 10 μM NE was superfused for 10 minutes but during this period the perforant path was not stimulated and, therefore, no responses were evoked. Upon return to superfusion with control ACSF, the perforant path was stimulated again and the effect of NE on these evoked responses was measured. By analogy to the example this experiment represented the test for synapse B inactive during NE superfusion but subsequently active.

In these experiments the effect of NE on the evoked responses was assessed by measuring the peak effect (% of the respective control period) produced by NE but obtained after the superfusion period. However in the course of the experiments it was noted that following the 10 minute period without perforant path stimulation, the subsequently evoked responses were transiently enhanced compared to the control period. Thus to permit a direct comparison of the evoked responses obtained following the inactive period with those obtained following the active period, this transient increase was subtracted from the measures. The value to be subtracted was obtained by measuring the increase in evoked responses (as % of control) produced following the inactive period during which the vehicle solution was superfused.

Hence the resulting corrected value represented the evoked response (in % of control) produced following superfusion of NE during an inactive period, with the spontaneously enhanced responsiveness removed. This value could then be compared with the evoked response obtained following superfusion of NE with concurrent perforant path stimulation. These experiments were done in 6 slices.

Effects of serotonin on evoked responses

The effects of serotonin on the perforant path evoked responses were measured and compared to the effects of NE in 5 slices.

In these experiments 10 μM NE was applied for 10 minutes following a 10 minute control period. After the return to superfusion with control ACSF, the evoked responses were followed until the effects of NE had disappeared. Then, after another 10 minute control period, serotonin (10-25 μM 5-HT) was superfused for 10 minutes. After the return to superfusion with ACSF the effects of serotonin were followed until recovery. The effects of serotonin on the perforant path evoked responses were assessed in terms of peak effect, onset of effect, time of peak effect and time of recovery on the EPSP slope, population spike onset latency and population spike amplitude.

Chapter 3: Results

Overall, the results indicated that superfusion of NE on hippocampal slices produced a facilitation of the perforant path evoked responses in the dentate gyrus. The facilitation of the synaptically evoked responses was expressed in the field potential as an increase in the EPSP amplitude and/or slope, a decrease in the population spike onset latency and an increase in the population spike amplitude. In many slices the facilitation of the evoked responses was found to be long-lasting. The facilitation of the evoked responses was also found to be mediated via a beta adrenergic receptor. The facilitation of the population spike amplitude by NE could not be accounted for solely by the increase in the EPSP slope also produced by NE. Superfusion of NE did not produce facilitation of the antidromically evoked field potentials. NE did facilitate the subsequently evoked perforant path responses even when the perforant path was not concurrently stimulated during superfusion with NE. Finally, superfusion of 5-HT produced a decrease in the perforant path evoked field potentials expressed as a decrease in EPSP slope, population spike onset latency and population spike amplitude. The effects of 5-HT were not found to be long-lasting.

Dose-response experiments

Superfusion of NE produced significant dose-dependent increases in some components of the perforant path evoked responses. Tables 1, 2 and 3 summarize the means for all slices of each parameter (EPSP amplitude, population spike onset latency and population spike amplitude, respectively) as percent of the control period during the seventh and tenth minute of superfusion for each concentration of NE or the vehicle application. Table 4 summarizes the t-values for correlated means for the difference between vehicle and NE applications for EPSP amplitude, population spike onset latency and population spike amplitude during the seventh and tenth minute of superfusion. Figures 4, 5 and 6 illustrate semi-logarithmically the effects of NE during the seventh and tenth minute of superfusion on the EPSP amplitude, population spike onset latency and population spike amplitude, respectively, as a function of the concentration of NE applied.

The statistical analysis indicated that the changes in EPSP amplitude and population spike onset latency observed during superfusion of NE (1-50 µM) were not significantly different from those observed during vehicle application ($p > .05$, two-tailed). The population spike amplitude changes produced by 1 µM NE were also not statistically significant.

Table 1

Dose-response summary table, effects of NE on EPSP amplitude. Means (% of control) of EPSP amplitude during 7th and 10th minute of superfusion with NE (1-200 μM) or vehicle in n slices.

Solution	n	7th	10th
Vehicle	5	105.6	100.4
1 μM	4	97.0	102.5
5 μM	5	110.4	99.7
10 μM	5	99.7	109.5
25 μM	5	108.3	102.4
50 μM	5	99.1	106.2
100 μM	1	103.4	93.8
200 μM	1	96.8	93.8

Note. Means during NE superfusion not significantly different from vehicle application $p > .05$ two-tailed.

Table 2

Dose-response summary table, effects of NE on population spike onset latency. Means (% of control) of population spike onset latency during 7th and 10th minute of superfusion with NE (1-200 µM) or vehicle in n slices.

Solution	n	7th	10th
Vehicle	5	98.7	98.3
1 µM	4	99.5	98.9
5 µM	5	100.2	98.8
10 µM	5	99.9	98.7
25 µM	5	98.3	97.6
50 µM	5	98.2	97.0
100 µM	1	97.8	99.6
200 µM	1	99.6	100.4

Note. [c] Means during NE superfusion not significantly different from vehicle application $p > .05$ two-tailed.

Table 3

Dose-response summary table, effects of NE on population spike amplitude. Means (% of control) of population spike amplitude during 7th and 10th minute of superfusion with NE (1-200 µM) or vehicle in n slices.

Solution	n	7th	10th
Vehicle	5	98.3	98.5
1 µM	4	98.3	98.7
5 µM	5	104.0*	97.8
10 µM	5	114.0*	118.0*
25 µM	5	113.8*	113.6*
50 µM	5	111.7*	109.6*
100 µM	1	109.1	114.8
200 µM	1	109.3	105.7

Note. * mean significantly different from vehicle application $p<.05$ two-tailed.

Table 4

Dose-response summary table, t-values for correlated means. Statistic t for differences between NE and vehicle applications during 7th and 10th minute for EPSP amplitude (EPSP), population spike onset latency (ONSET) and population spike amplitude (POP. SPIKE).

		EPSP		ONSET		POP. SPIKE	
NE	d.f.	7th	10th	7th	10th	7th	10th
1 µM	7	1.33	1.08	0.40	0.73	0.50	0.17
5 µM	9	0.89	0.11	1.53	0.50	3.71*	0.38
10 µM	9	1.25	0.78	0.75	0.23	5.01*	6.15*
25 µM	9	0.57	0.25	0.31	0.61	4.43*	4.36*
50 µM	9	1.10	1.04	0.47	2.04	3.48*	2.95*

Note. * Statistically significant t, $p<.05$, two-tailed.

Figure 4. EPSP amplitude dose-response curve. Semi-logarithmic graph of mean EPSP amplitude ± s.e.m. during superfusion with NE or vehicle. Number of slices in parentheses.

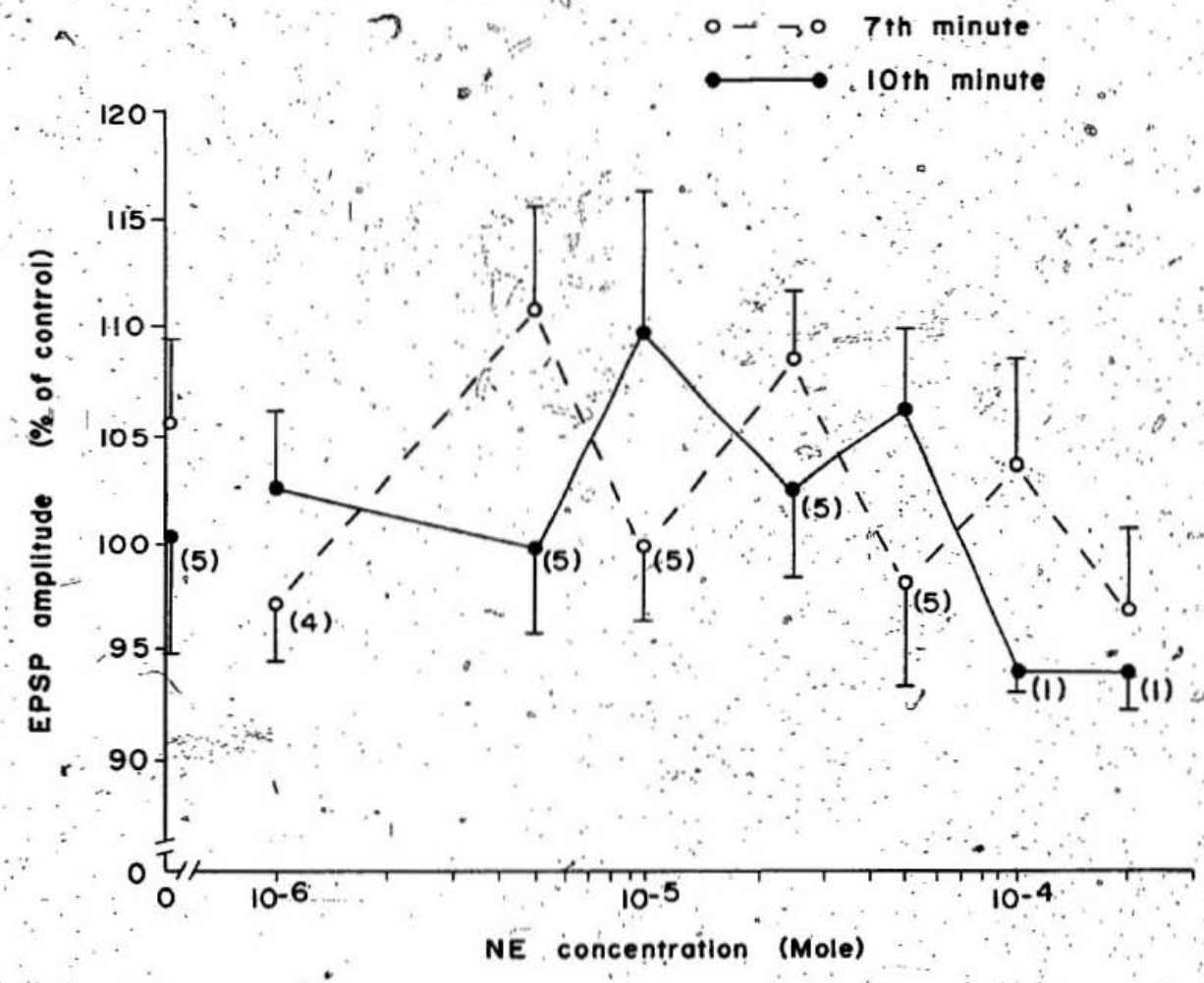

7th minute
10th minute
EPSP amplitude (% of control)
120
115
110
105
100
95
90
0
(5)
(4)
(5)
(5)
(5)
(5)
(1)
(1)
0
10-6
10-5
10-4
NE concentration (Mole)

Figure 5. Population spike onset latency dose-response curve. Semi-logarithmic graph of mean population spike onset latency ± s.e.m. during superfusion with NE or vehicle. Number of slices in parentheses.

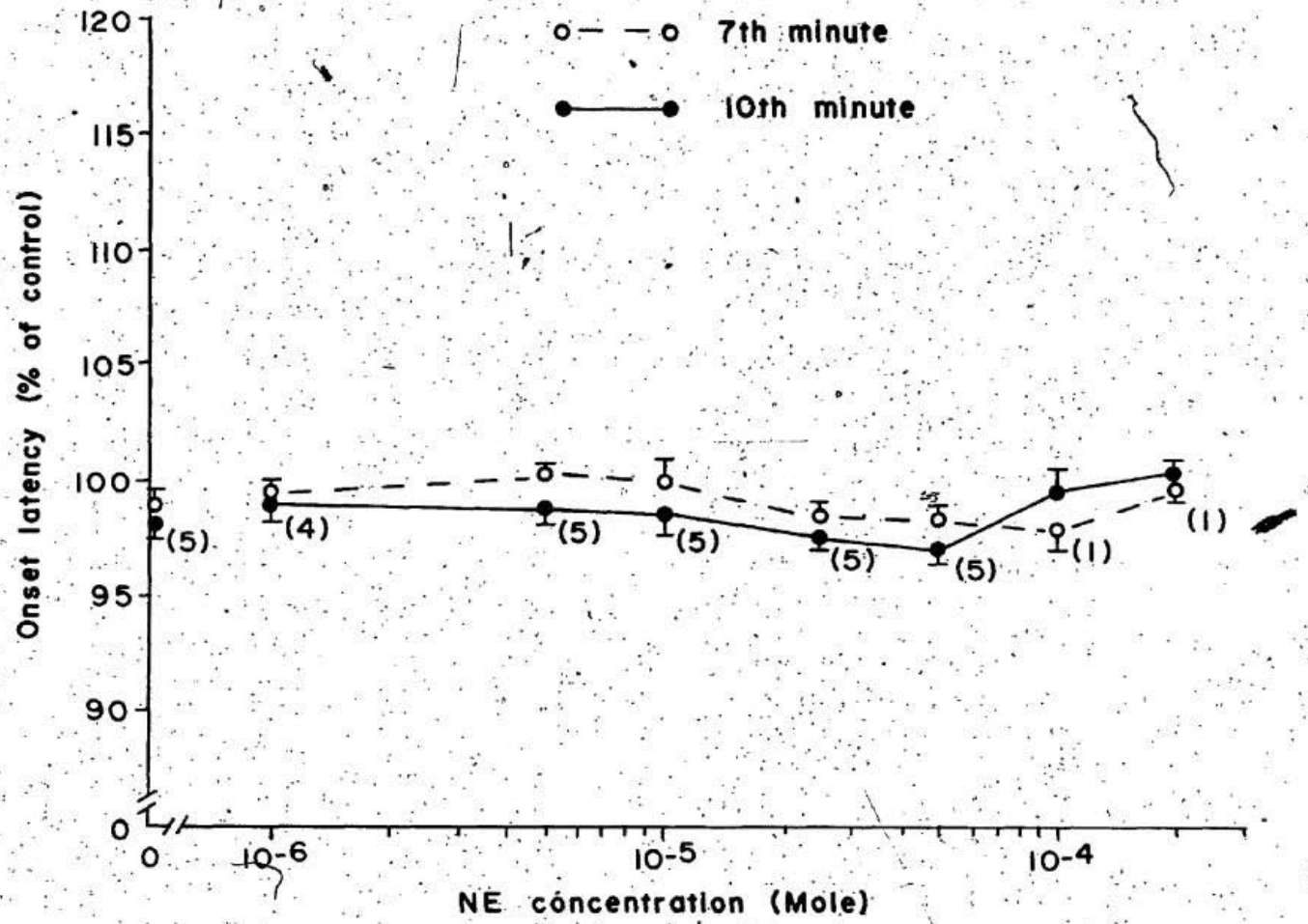
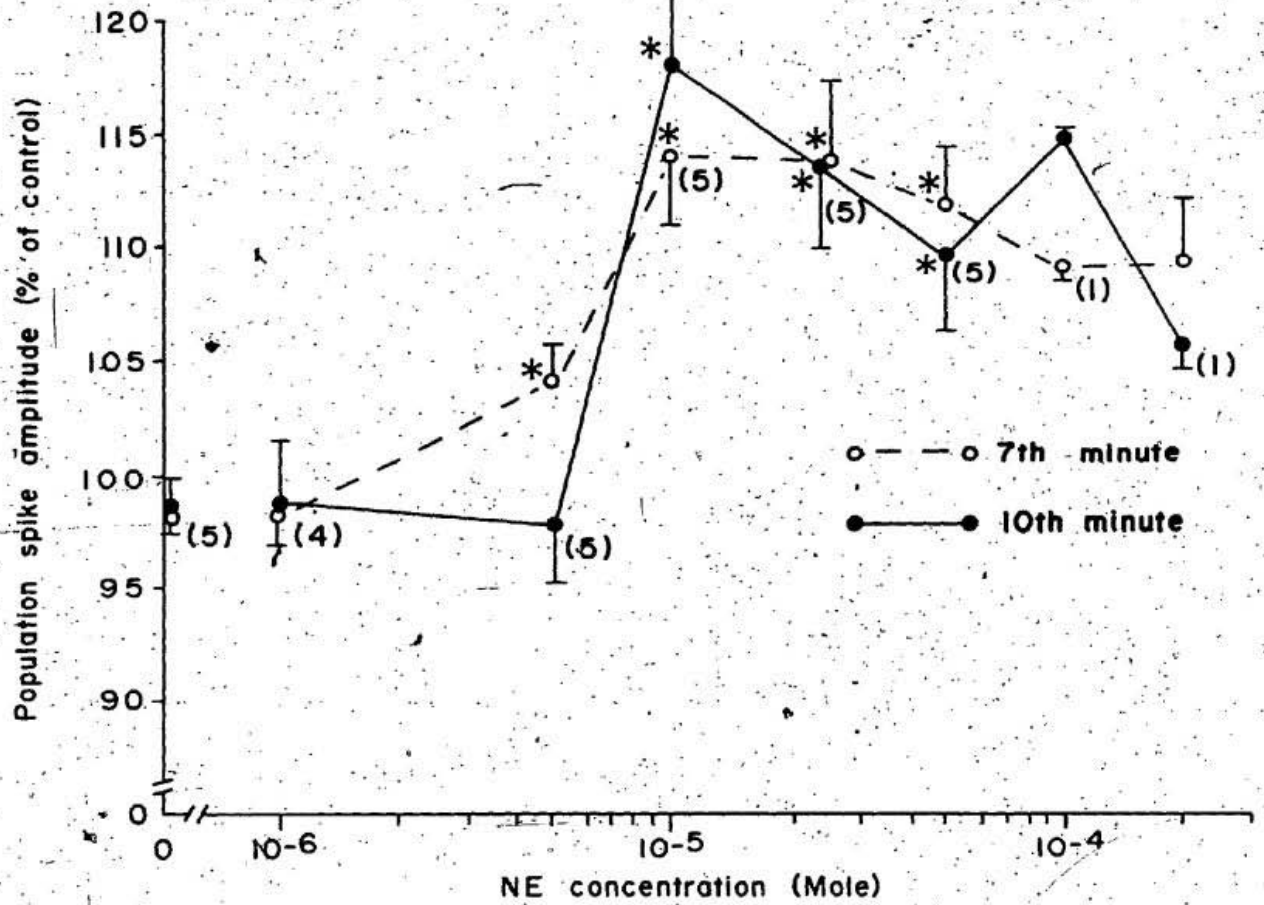

7th minute
10th minute
Onset latency (% of control)
120
115
110
105
100
95
90
0
(5)
(4)
(5)
(5)
(5)
(5)
(1)
(1)
0
10-6
10-5
10-4
NE concentration (Mole)

Figure 6. Population spike amplitude dose-response curve. Semi-logarithmic graph of mean population spike amplitude ± s.e.m. during superfusion with NE or vehicle. Number of slices in parentheses. Asterisk (*) indicates mean significantly different from vehicle application, $p<.05$, two-tailed.


120
115
110
105
100
95
90
0
Population spike amplitude (% of control)
0
10-6
10-5
10-4
NE concentration (Mole)
(5)
(4)
(5)
(5)
(5)
(1)
(1)
7th minute
10th minute

However, the population spike amplitude showed significant increases with superfusion of 5-50 μM NE ($p<.05$, two-tailed). With 5 μM NE the increase was significant during the seventh minute but not during the tenth. At other concentrations (10, 25, 50 μM) the increase in population spike amplitude was significant during both the seventh and tenth minute. As illustrated in Figure 6 the mean increase in population spike amplitude was the greatest (118% of control) during the tenth minute of superfusion with 10 μM NE. A smaller but significant increase was also observed at 25 and 50 μM NE. NE was also applied at 100 and 200 μM, in one slice each, and both concentrations produced a clear increase in the population spike amplitude (see Table 3 and Figure 6). Since 100 and 200 μM NE were only applied in one slice each, they were not included in the statistical analysis.

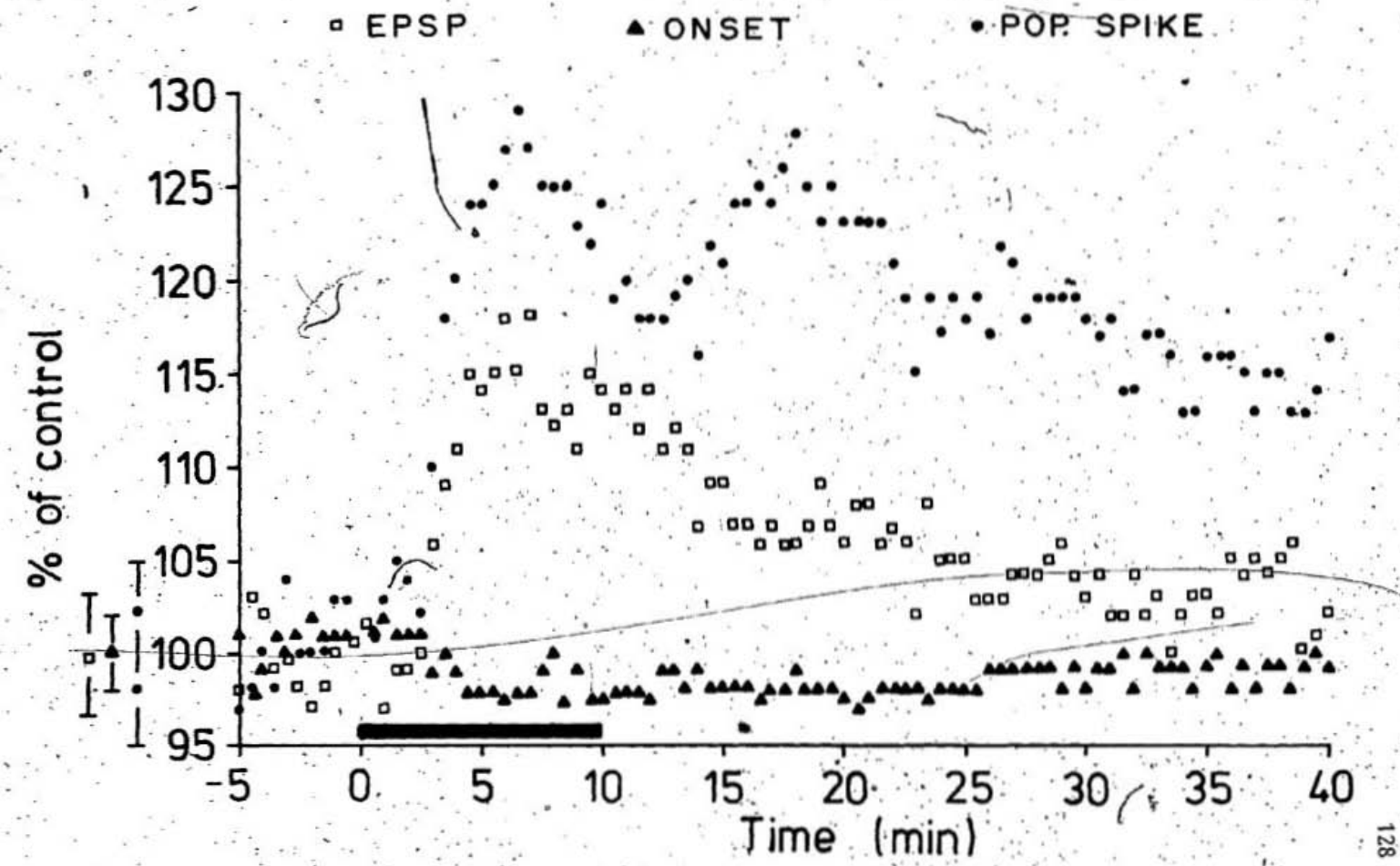
Physiological characterization of NE effects

In the present and following experiments, the effects of NE were not compared with vehicle application but rather with the respective control period preceding NE superfusion. In these experiments slices showing changes in evoked responses during superfusion of the vehicle solution were discarded.

Superfusion of 10 μM NE for 10 minutes typically produced no change (or sometimes an increase) in EPSP amplitude, an increase in EPSP slope, a decrease in population spike onset latency and an increase in the population spike amplitude. Long-lasting effects were mostly seen on the population spike amplitude.

Figure 7 illustrates a representative example of the effects of 10 μM NE on EPSP slope, population spike onset latency and population spike amplitude of the perforant path evoked responses in an individual slice displaying a long-lasting increase in population spike amplitude. Three minutes following the beginning of superfusion with NE there was a significant increase in the EPSP slope. This increase reached a peak of 118% of control at 7 minutes following the onset of superfusion. Following the return to superfusion with control ACSF the increase slowly decayed and reached control levels at 15 minutes following wash-out. In this slice, the effect of NE on the population spike onset latency was smaller, more delayed and short-lived than usual. There was a significant decrease in the onset latency 9.5 minutes following the beginning of NE superfusion. It reached a peak effect of 98% of control at this time and had recovered to control level a minute later. The population spike amplitude showed a significant increase 3 minutes after the onset of NE superfusion. The amplitude

Figure 7. Effects of NE on perforant path evoked responses. Typical effects of 10 minutes of superfusion of 10 μM NE (bar above abscissa) on EPSP slope (EPSP), population spike onset latency (ONSET) and population spike amplitude (POP. SPIKE) for slice #34. Intervals at the left of the ordinate indicate the respective 95% confidence intervals of the control period. Only the last 5 minutes of the control period are illustrated.


EPSP
ONSET
POP. SPIKE
% of control
130
125
120
115
110
105
100
95
-5
0
5
10
15
20
25
30
35
40
Time (min)

128

increased further until it reached a peak effect of 129% of control at 6.5 minutes following the onset of superfusion. After the peak effect and the return to superfusion with control ACSF there was a small decay in the amplitude increase, but 30 minutes after removal of NE the population spike amplitude was still significantly greater than during the control period.

Table 5 summarizes the effects of 10 μM NE on EPSP amplitude and Table 6 on EPSP slope for all slices tested. Note that EPSP amplitude and slope were not measured in the same slices (see Method).

Usually superfusion with 10 μM NE did not produce a significant change in EPSP amplitude. However in a third of the slices a significant increase was observed (mean 114% of control, range 106-122%). In contrast superfusion with NE produced an increase in the EPSP slope (mean 118% of control, range 107-152%) in a greater proportion of slices (15/20). In only two slices were no significant changes in EPSP slope observed. The onset of effect, time of peak effect and time of recovery were generally earlier for the EPSP slope than for the EPSP amplitude. Long-lasting NE effects were observed only on the EPSP slope (2 slices).

Table 7 summarizes the effects of 10 μM NE on population spike onset latency. The change most often observed was a significant decrease (mean 94% of control, range 88-98%) in

Table 5

Summary table of NE effects on EPSP amplitude. Means (± s.e.m.) of effects produced by a 10 minute superfusion of 10 μM NE on EPSP amplitude.

	EPSP amplitude		
	Decrease	No change	Increase
No. of slices	1	10	6
Peak effect (% of control)	94	-	114 ± 2.4
Onset of effect (min.)	11	-	7.8 ± 1.2
Time of peak effect (min.)	11	-	13.3 ± 0.8
Time of recovery (min.)	2.55	-	11.3 ± 4.5
Long-lasting effects (no. of slices)	0	-	0

Note. One slice showed biphasic (decrease, increase) effects.

Table 6

Summary table of NE effects on EPSP slope. Means (± s.e.m.) of effects produced by a 10 minute superfusion of 10 μM NE on EPSP slope.

	EPSP slope		
	Decrease	No change	Increase
No. of slices	1	2	15
Peak effect (% of control)	91	-	118 ± 3.1
Onset of effect (min.)	8.5	-	3.9 ± 0.3
Time of peak effect (min.)	14	-	10.1 ± 2.0
Time of recovery (min.)	9	-	8.5 ± 2.9
Long-lasting effects (no. of slices)	0	-	2

Note. One slice showed biphasic (increase, decrease) and another triphasic (increase, decrease, increase) effects.

Table 7

Summary table of NE effects on population spike onset latency. Means (± s.e.m.) of effects produced by a 10 minute superfusion of 10 μM NE on onset latency.

	Population spike onset latency		
	Decrease	No change	Increase
No. of slices	28	8	1
Peak effect (% of control)	94 ± 0.4	-	106
Onset of effect (min.)	5.2 ± 0.6	-	17
Time of peak effect (min.)	10.3 ± 0.7	-	17
Time of recovery (min.)	12.4 ± 1.7	-	11.5
Long-lasting effects (no. of slices)	3	-	0

Note. One slice showed biphasic (decrease, increase) effects.

onset latency. The observed times of onset, peak and recovery of onset latency decrease were generally similar to those of the increase in EPSP measures. Long-lasting decreases in onset latency were observed in three slices.

Table 8 summarizes the effects of 10 μM NE on the population spike amplitude. The change most often observed was a significant increase in amplitude (mean 131% of control, range 106-200%). The percentage increase in population spike amplitude (mean 131%) was generally greater than the increase observed in EPSP amplitude (mean 114%) or slope (mean 118%). The onset and time of peak increases in population spike amplitude were generally similar to those of increases in extracellular EPSP and decreases in onset latency. However, a greater proportion of slices showed long-lasting effects for population spike amplitude increases (24%) than for extracellular EPSP increases (10%) and population spike onset latency decreases (11%). Generally, long-lasting changes in EPSP slope or onset latency were observed in slices showing long-lasting changes in population spike amplitude. However, the long-lasting changes in EPSP slope and onset latency occurred in different slices.

The time to recovery from population spike amplitude increases was generally longer (mean approx. 17 minutes) than that of extracellular EPSP increases (mean approx. 9

Table 8

Summary table of NE effects on population spike amplitude. Means (± s.e.m.) of effects produced by a 10 minute superfusion of 10 μM NE on population spike amplitude.

	Population spike amplitude		
	Decrease	No change	Increase
No. of slices	2	3	29
Peak effect (% of control)	91 ± 4	-	131 ± 4
Onset of effect (min.)	7.2 ± 0.2	-	4.3 ± 0.4
Time of peak effect (min.)	9 ± 1.5	-	10.1 ± 0.8
Time of recovery (min.)	7.5 ± 2.5	-	17.2 ± 1.9
Long-lasting effects (no. of slices)	0	-	7

Note. One slice showed triphasic (increase, decrease, increase), two slices biphasic (increase, decrease) and another slice biphasic (decrease, increase) effects.

minutes for slope and amplitude data pooled) and population spike onset latency decreases (mean approx. 12 minutes).

Figure 8 illustrates graphically the difference in time of recovery for population spike amplitude increases and extracellular EPSP increases (slope and amplitude data pooled) produced by 10 μM NE.

Pharmacological characterization of NE effects

The results indicated that the effects of 10 μM NE were mediated by a beta adrenergic receptor. The effects on perforant path evoked responses were antagonized by a beta, but not an alpha, receptor antagonist and were mimicked by a beta, but not an alpha, receptor agonist.

Figures 9 and 10 give representative examples, in the same slice, of the antagonistic effects of the beta antagonist timolol (5 μM) on the increase in EPSP slope and population spike amplitude, respectively, produced by 10 μM NE. As illustrated in Figure 9, approximately 5 minutes after the onset of superfusion with NE the EPSP slope increases significantly. With continued superfusion, the EPSP slope increased further. Finally it reached a peak effect of approximately 115% of control about 4 minutes after the removal of NE. However, in the same slice when NE was superfused with the beta receptor antagonist TIM, no

Figure 8. Histogram of time of recovery from NE effects. Distribution of time of recovery, in 10 minute intervals, from increases in EPSP slope and amplitude (EPSP) and from increases in population spike amplitude (POP. SPIKE) produced by 10 μM NE applied for 10 minutes.

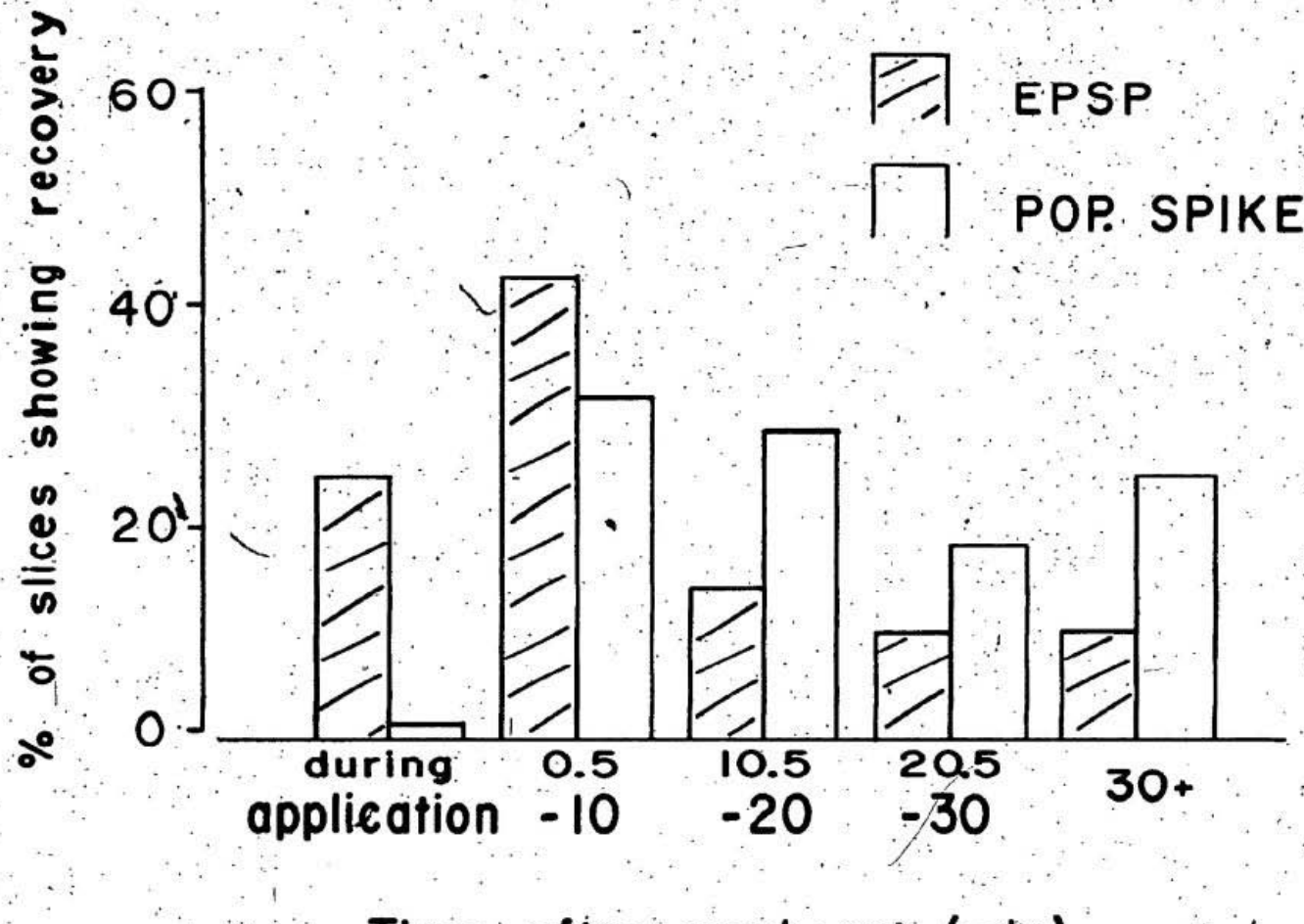

% of slices showing recovery
60
40
20
0
EPSP
POP. SPIKE
during application
0.5 -10
10.5 -20
20.5 -30
30+
Time after wash-out (min)

Figure 9. Effects of timolol and NE on EPSP slope. Example of pharmacological antagonism of NE effects by timolol (TIM) for slice #15. Bar above the abscissa indicates drug superfusion. Intervals to the left of the ordinate indicate the respective 95% confidence interval of the control periods.

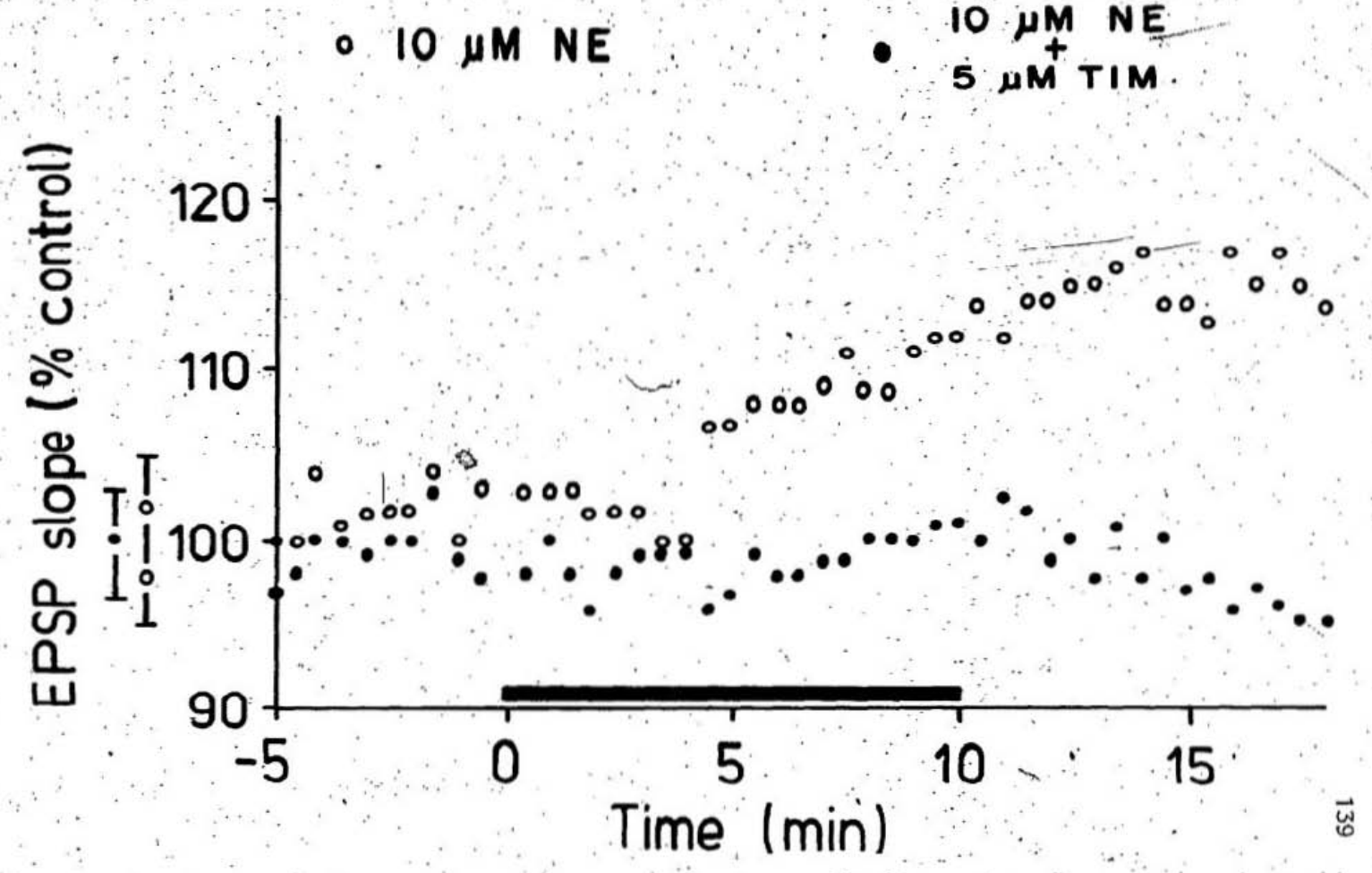
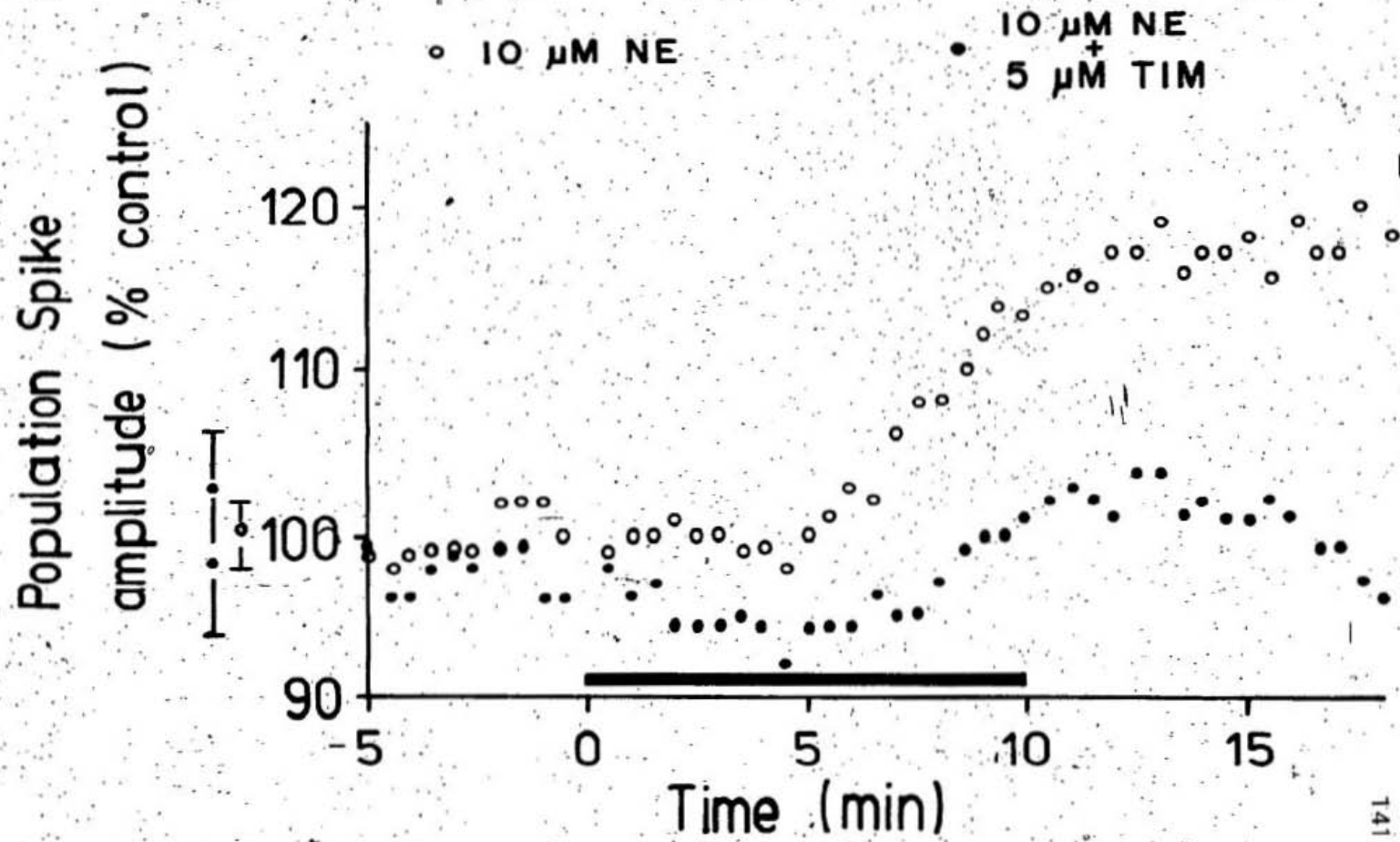

o 10 µM NE
● 10 µM NE + 5 µM TIM
EPSP slope (% control)
120
110
100
90
-5
0
5
10
15
Time (min)

Figure 10. Effects of timolol and NE on population spike amplitude. Example of pharmacological antagonism of NE effects by timolol (TIM) for slice #15. Bar above abscissa indicates drug superfusion. Intervals to the left of the ordinate indicate the respective 95% confidence interval of the control periods.


10 µM NE
10 µM NE
+
5 µM TIM
Population Spike
amplitude (% control)
120
110
100
90
-5
0
5
10
15
Time (min)

significant change was observed in the EPSP slope.

Similarly as illustrated in Figure 10, after approximately 6 minutes of superfusion with NE the population spike amplitude showed significant increases. With continued superfusion, the population spike amplitude augmented to reach a peak effect of 118-120% of control 3 minutes following cessation of NE superfusion. Yet in the same slice, when NE was superfused with TIM, no significant changes were observed in the population spike amplitude.

Tables 9, 10 and 11 summarize the effect of the beta adrenergic antagonist TIM on the action of 10 μM NE on the EPSP slope, population spike onset latency and population spike amplitude, respectively, in 8 slices.

When applied alone, TIM usually had no effects on the perforant path evoked responses. The effective concentration of TIM required to reliably antagonize the effects of 10 μM NE on the perforant path evoked responses was found to be 5 μM.

In the experiments with 1 μM TIM, NE produced an increase in EPSP slope in 2 slices which 1 μM TIM blocked in 1 slice. NE brought about a decrease in population spike onset latency in 4 slices which 1 μM TIM blocked in 2 slices. Finally, NE caused an increase in population spike amplitude in 4 slices which 1 μM TIM blocked in 1 slice.

In one slice, 2.5 μM TIM blocked the population spike

Table 9

Summary table of action of timolol on EPSP slope. Peak effects (% of control) on EPSP slope produced by 10 μM NE alone (NE), NE with timolol (NE+TIM) and timolol alone (TIM).

Slice	TIM Dose (μM)	EPSP slope		
		NE	NE+TIM	TIM
#8	1	91, 123	81	100
#12	1	112	100	100
#13	1	100	83	100
#15	1	117	107	-
#15	2.5	117	107	-
#15	5	117	100	100
#16	5	100	76	100
#17	5	90	100	100
#18	5	87, 120	100	111
#21	5	118	100	100

Note. Double entries indicate biphasic effects.

Table 10

Summary table of action of timolol on population spike onset latency. Peak effects (% of control) on population spike onset latency produced by 10 μM NE alone (NE), NE with timolol (NE+TIM) and timolol alone (TIM).

Slice	TIM Dose (μM)	Population spike onset latency		
		NE	NE+TIM	TIM
#8	1	93	96	100
#12	1	95	100	100
#13	1	93	94	100
#15	1	94	100	-
#15	2.5	94	100	-
#15	5	94	100	105
#16	5	94	96	100
#17	5	100	100	100
#18	5	100	91	100
#21	5	94	100	100

Table 11

Summary table of the action of timolol on population spike amplitude. Peak effects (% of control) on population spike amplitude produced by 10 μM NE alone (NE), NE with timolol (NE+TIM) and timolol alone (TIM).

Slice	TIM Dose (μM)	Population spike amplitude		
		NE	NE+TIM	TIM
#8	1	124	86	100
#12	1	122	109	90
#13	1	111	107	88
#15	1	120	116	-
#15	2.5	120	112	-
#15	5	120	100	100
#16	5	111	88	100
#17	5	115	100	100
#18	5	114	90	92
#21	5	128	100	100

onset latency decrease but not the increase in EPSP slope and in population spike amplitude produced by NE.

In the experiments with 5 μM TIM, NE caused an increase in EPSP slope in 2 slices which TIM blocked. NE produced a decrease in population spike onset latency in 3 slices and 5 μM TIM antagonized the decrease in 2 slices. Finally, NE brought about an increase in population spike amplitude in 5 slices which was blocked by 5 μM TIM in all 5 instances.

Figures 11 and 12 give representative examples, in the same slice, of the lack of antagonistic effects of the alpha antagonist phentolamine (50 μM) on the increase in EPSP slope and population spike amplitude, respectively, produced by 10 μM NE.

As illustrated in Figure 11, superfusion of NE produced significant increases in the EPSP slope 3 minutes following the onset of superfusion. The effect increased and reached a peak of approximately 118% of control at 5 minutes of superfusion. Afterwards the effect decayed. When NE was applied with PA similar effects were found. A significant increase in EPSP slope was observed after 4 minutes of superfusion. The effect increased until reaching about 115% of control after 5 minutes of superfusion. Thereafter the effect decayed.

Similarly, as shown in Figure 12, with superfusion of NE, a significant increase was observed in the population

Figure 11. Effects of phentolamine and NE on EPSP slope. Example of lack of pharmacological antagonism of NE effects by phentolamine (PA) for slice #26. Bar above abscissa indicates drug superfusion. Intervals at the left of the ordinate indicate the respective 95% confidence interval of the control periods.

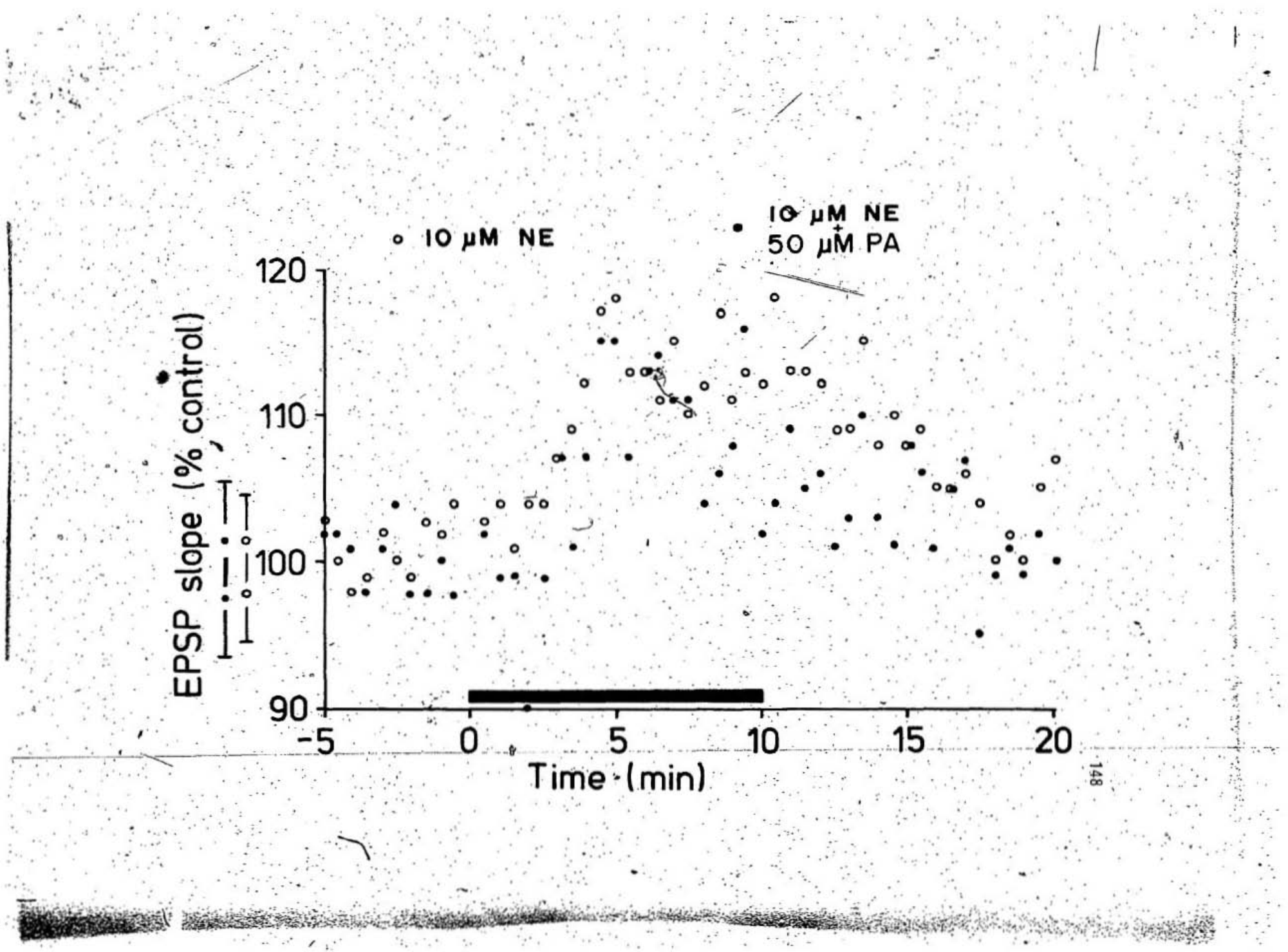
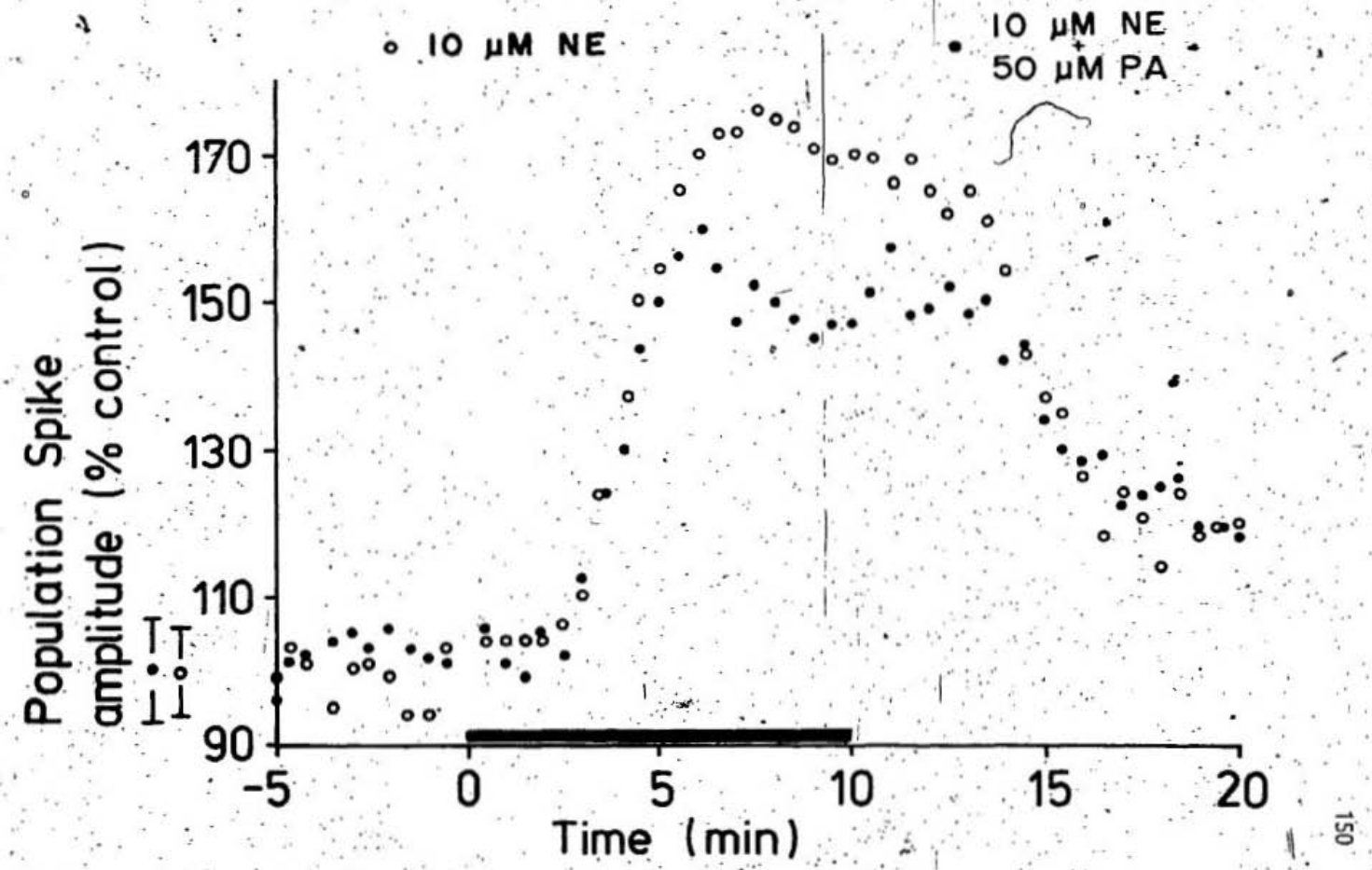

10 µM NE
10 µM NE
+
50 µM PA
EPSP slope (% control)
120
110
100
90
-5
0
5
10
15
20
Time (min)

Figure 12. Effects of phentolamine and NE on population spike amplitude. Example of lack of pharmacological antagonism of NE effects by phentolamine (PA) for slice #26. Bar above abscissa indicates drug superfusion. Intervals to the left of the ordinate indicate the respective 95% confidence interval of the control periods.


o 10 µM NE
• 10 µM NE
+
50 µM PA
Population Spike
amplitude (% control)
170
150
130
110
90
-5
0
5
10
15
20
Time (min)

spike amplitude after 3 minutes of superfusion. The effect continued to increase to a peak of over 170% of control after about 7 minutes of superfusion. After return to superfusion with ACSF the effect decayed. When NE was superfused with PA, similar results were obtained. After 3 minutes of superfusion, significant increases in population spike amplitude were observed. The effect peaked after 6 minutes of superfusion at over 150% of control. After return to superfusion with ACSF the effect decayed similarly.

Tables 12, 13 and 14 summarize the effect of PA on the action of 10 μM NE on the EPSP slope, population spike onset latency and population spike amplitude, respectively, in 6 slices.

In all these experiments a concentration of 50 μM PA was used. Bath applications of 50 μM PA have been shown to be effective in antagonizing the alpha-mediated effects of NE on CA1 pyramidal cells (Mueller et al., 1981; Marciani et al., 1984). NE caused an increase in EPSP slope in 4 slices which was blocked by PA in one instance only. NE produced a decrease in population spike onset latency in 5 slices and PA blocked the decrease in one slice only. NE brought about an increase in population spike amplitude in 5 slices and PA never blocked the increases.

In one slice NE produced a biphasic effect on EPSP slope

Table 12

Summary table of action of phentolamine on EPSP slope. Peak effects (% of control) on EPSP slope of 10 μM NE alone (NE), NE with 50 μM phentolamine (NE+PA) and phentolamine alone (PA).

Slice	EPSP slope		
	NE	NE+PA	PA
#23	107,93	109,91	92
#24	113	100	100
#26	118	116	100
#27	100	100	100
#40	106	113	100
#42	111	110	100

Note. Double entries indicate biphasic effects.

Table 13

Summary table of the action of phentolamine on population spike onset latency. Peak effects (% of control) on population spike onset latency of 10 μM NE alone (NE), NE with 50 μM phentolamine (NE+PA) and phentolamine alone (PA).

Slice	Population spike onset latency		
	NE	NE+PA	PA
#23	95	97	100
#24	91	92	100
#26	95	95	100
#27	105	93	100
#40	97	100	100
#42	94	96	100

Table 14

Summary table of the action of phentolamine on population spike amplitude. Peak effects (% of control) on population spike amplitude of 10 μM NE alone (NE), NE with 50 μM phentolamine (NE+PA) and phentolamine alone (PA).

Slice	Population spike amplitude		
	NE	NE+PA	PA
#23	115,86	118,79	112,85
#24	133	118	100
#26	176	160	118
#27	144	167	132
#40	136	158	118
#42	129	127	116

Note. Double entries indicate biphasic effects.

and population spike amplitude (increase followed by decrease) and PA did not block either increase or decrease.

PA when applied alone showed partial adrenergic agonistic properties, producing an increase in population spike amplitude in 4 out of 5 slices. PA alone had no effect on EPSP slope or population spike onset latency.

Figures 13 and 14 give individual examples, in the same slice, of the agonistic effects of the beta agonist isoproterenol (1 μM) on the EPSP slope and population spike amplitude, respectively.

For the slice shown in Figure 13 superfusion with NE produced a significant increase in EPSP slope after approximately 5 minutes of superfusion. The effect peaked rapidly at about 5 minutes to approximately 108% of control. Afterwards there was only a very small decay. When ISO was superfused a significant increase was observed after 3 minutes of superfusion. The increase reached a peak of over 110% of control after 4-5 minutes of superfusion. However the increase decayed rapidly and had recovered to control levels before the end of superfusion.

Similarly, as illustrated in Figure 14, superfusion with NE resulted in a significant increase in the population spike amplitude occurring after 3 minutes of superfusion. A peak effect of about 125% of control was observed after approximately 5 minutes of superfusion then the effect

Figure 13. Effects of adrenergic agonists on EPSP slope. Example of agonistic properties of isoproterenol (ISO) but not of phenylephrine (PE), compared to the effect of NE for slice #36. Bar above abscissa indicates drug superfusion. Intervals to the left of the ordinate indicate the respective 95% confidence interval of the control periods.

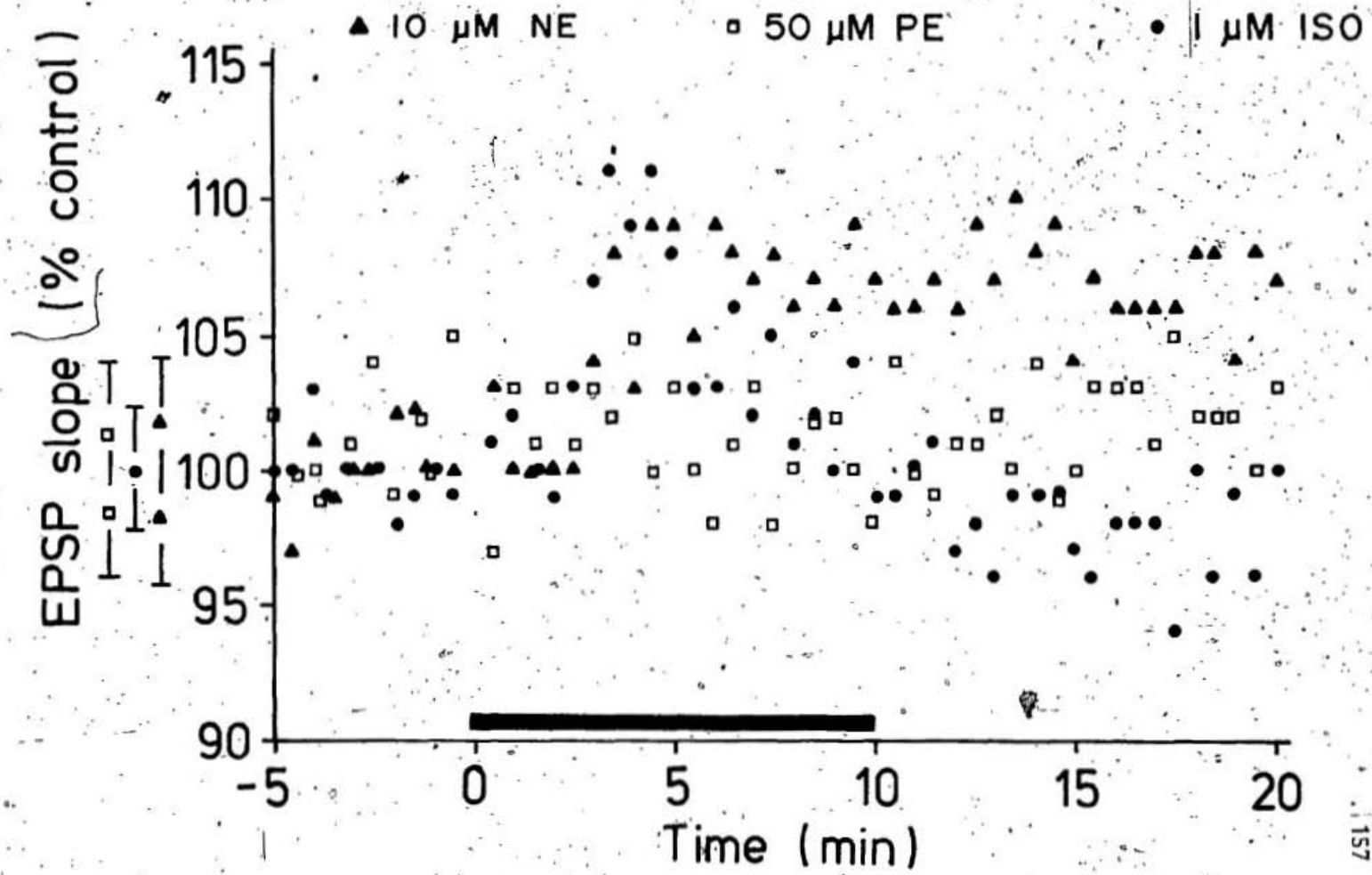
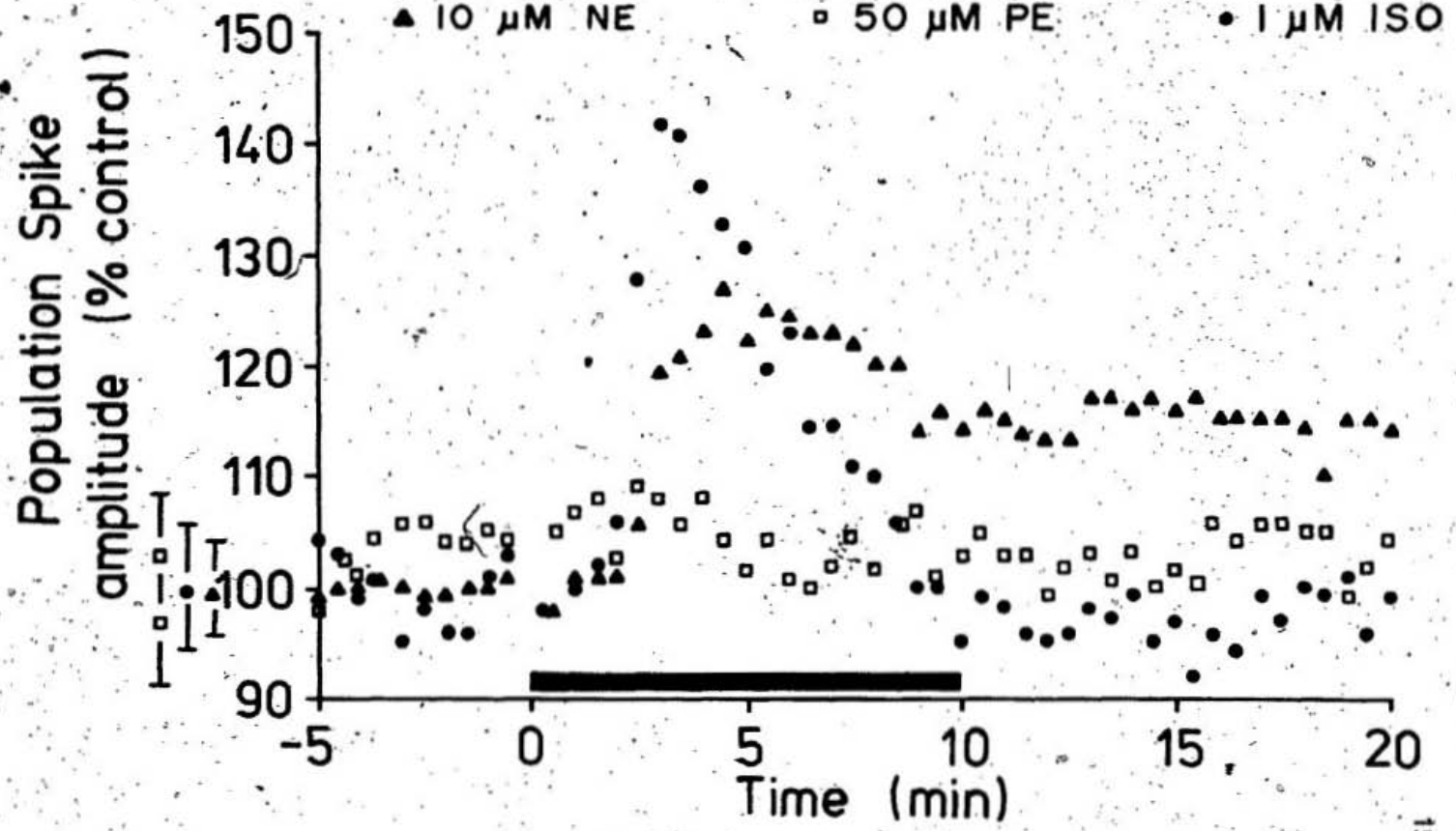

▲ 10 µM NE
▫ 50 µM PE
• 1 µM ISO
EPSP slope (% control)
115
110
105
100
95
90
-5
0
5
10
15
20
Time (min)
157

Figure 14. Effects of adrenergic agonists on population spike amplitude. Example of agonistic properties of isoproterenol (ISO) but not of phenylephrine (PE), compared to the effect of NE for slice #36. Bar above abscissa indicates drug superfusion. Intervals to the left of the ordinate indicate the respective 95% confidence interval of the control periods.


▲ 10 μM NE
▫ 50 μM PE
• 1 μM ISO
Population Spike amplitude (% control)
150
140
130
120
110
100
90
-5
0
5
10
15
20
Time (min)

159

slowly decayed. When ISO was superfused similar effects were observed. During the third minute of superfusion significant increases in the population spike amplitude occurred. After 3 minutes of superfusion the effect had reached a peak of over 140% of control. Afterwards the effect decayed rapidly and recovered to control levels before the superfusion period was finished.

Table 15 compares the effects of ISO to those of NE on EPSP slope, population spike onset latency and population spike amplitude for the 7 slices tested.

A concentration of 1 μM ISO was found to be necessary to produce reliable noradrenergic agonistic effects on the perforant path evoked responses.

In the experiments with 500 nM ISO, NE produced an increase in EPSP slope in one slice and ISO did not. NE produced a decrease in population spike onset latency in one slice and ISO did not. NE caused an increase in population spike amplitude in 2 slices and ISO in one slice only.

In the experiments with 1 μM ISO, NE brought about an increase in EPSP slope in 2 slices, and ISO in 6 slices. NE caused a decrease in population spike onset latency in 5 slices and ISO in 6 slices. NE produced an increase in population spike amplitude in 6 slices and ISO also in 6 slices. Thus 1 μM ISO uncovered typical noradrenergic effects that 10 μM NE did not produce in 4 slices for the

Table 15

Summary table of action of isoproterenol. Peak effects (% of control) on EPSP slope (EPSP), population spike onset latency (ONSET) and population spike amplitude (POP. SPIKE) of 10 μM NE (NE) or isoproterenol (ISO).

Slice	ISO Dose (μM)	EPSP		ONSET		POP. SPIKE	
		NE	ISO	NE	ISO	NE	ISO
#36	0.5	110	93	100	100	127	89
#47	0.5	100	100	94	100	139	127
#36	1	110	111	100	96	127	142
#39	1	107	116	93	93	152	193
#46	1	100	115	95	96	122	117
#47	1	100	114	94	96	139	166
#48	1	100	111	93	94	114	150
#49	1	70	115	92	95	118	130
#35	5	107	107	100	97	130	131

EPSP slope and in one slice for the population spike onset latency. Also in 5 out of 6 slices the effect of 1 μM ISO on the population spike amplitude was greater than the effect of 10 μM NE.

In the experiment with 5 μM ISO, NE caused an increase in EPSP slope, no change in population spike onset latency and an increase in population spike amplitude. ISO produced similar effects with a decrease in population spike onset latency.

With respect to the duration of ISO effects compared to those of NE, the differences observed in Figures 13 and 14 was uncommon. For the increase in population spike amplitude produced by 1 and 5 μM ISO the mean duration of effect for 7 slices was 14.4 minutes. For the increase produced by 10 μM NE, the mean duration of effect was 16.8 minutes.

In terms of individual slices, the effects of ISO lasted longer than those of NE in 3 slices whereas those of NE lasted longer than the effects of ISO in the remaining 4 slices. Thus, although the effects of ISO were of shorter duration in a greater number of slices, the difference observed was not great.

It should be noted that in one slice, ISO produced a long-lasting increase in population spike amplitude which was still present 30 minutes after removal of ISO. In that

slice the effect of NE recovered 18.5 minutes after removal of NE.

Figures 13 and 14 give representative examples, in the same slice, of the lack of noradrenergic agonistic properties of the alpha agonist phenylephrine (50 μM) on EPSP slope and population spike amplitude, respectively.

As mentioned earlier, in the slice illustrated in Figure 13, superfusion with NE resulted in an increase in EPSP slope to approximately 108% of control after 5 minutes of superfusion. However in the same slice superfusion with PE did not produce significant changes in EPSP slope.

Similarly as shown in Figure 14, superfusion with NE caused a significant increase in population spike amplitude to approximately 125% of control after 5 minutes of superfusion. Yet, in the same slice, superfusion with PE failed to produce any significant changes in population spike amplitude.

Table 16 summarizes the effect of PE on EPSP slope, population spike onset latency and population spike amplitude in comparison to those of 10 μM NE in 7 slices.

The effects of PE (10-50 μM) on the perforant path evoked responses proved to be complex. PE rarely mimicked the effects of 10 μM NE, but sometimes produced opposite effects which were generally smaller and more transient.

In these experiments, NE produced an increase in EPSP

Table 16

Summary table of action of phenylephrine. Peak effects (% of control) on EPSP slope (EPSP), population spike onset latency (ONSET) and population spike amplitude (POP. SPIKE) of 10 μM NE (NE) or phenylephrine (PE).

Slice	PE Dose (μM)	EPSP		ONSET		POP. SPIKE	
		NE	PE	NE	PE	NE	PE
#36	10	110	100	100	100	127	100
#36	25	110	88	100	104	127	72
#36	25	110	100	100	100	127	92
#35	30	107	100	100	100	130	100
#36	50	110	100	100	100	127	100
#39	50	107	90	93	104	152	100
#46	50	100	100	95	100	122	91
#47	50	100	90	94	103	139	93
#48	50	100	93	93	95	114	89,109
#49	50	70	94	92	100	118	95,106

Note. Double entries indicate biphasic effects and 25 μM PE was applied twice to slice #36.

slope in 3 slices and PE (10-50 μM) never produced such an effect. NE caused a decrease in population spike onset latency in 5 slices and PE (50 μM) did so in only one slice. NE brought about an increase in population spike amplitude in 8 slices and PE (50 μM) also produced an increase, although smaller and delayed, in only 2 slices. The two slices in which PE produced an increase in population spike amplitude were obtained from the same animal.

However, more consistent effects of PE were a decrease in EPSP slope, increase or no change in population spike onset latency and a decrease in population spike amplitude. PE (25 μM in one slice; 50 μM in 4 slices) produced a mean decrease in EPSP slope to 91% of control for a mean duration of 2.2 minutes. PE (25 μM in one slice; 50 μM in 2 slices) caused a mean increase in population spike onset latency to 104% of control for a mean duration of 2.8 minutes. PE (same slice twice at 25 μM; 4 slices at 50 μM) brought about a mean decrease in population spike amplitude to 89% of control for a mean duration of 5.5 minutes.

EPSP slope and population spike amplitude relationship

The results of these experiments indicated that in general the increase in population spike amplitude produced by NE could not be accounted for entirely by the increase in

EPSP slope also produced by NE.

The functional relationship between population spike amplitude and EPSP slope for dentate granule cell evoked responses is indicated by the linear regression curve shown in Figure 15 for slice #38. As described in the Method section, this curve was produced by measuring evoked responses to different intensities of perforant path stimulation.

Figure 15 also illustrates for slice #38 the parameters of the evoked responses obtained during the control period preceding NE superfusion and the peak effects produced by 10 uM NE superfusion (observed EPSP slope and observed population spike amplitude). The predicted value of the population spike amplitude that would be expected from the regression curve to be associated with the EPSP slope observed during NE application is also indicated. Also illustrated is the necessary value of the EPSP slope which would be expected from the regression curve to predict the population spike amplitude observed during superfusion of NE.

As can be seen in Figure 15, the population spike amplitude observed during NE application was too large to be accounted for solely by the magnitude of the EPSP slope during NE superfusion. Indeed, as calculated from equation (1) in the Method section and as shown in Table 17 the

Figure 15. Functional relationship between population spike amplitude and EPSP slope. Linear regression analysis of population spike amplitude (POP. SPIKE) on EPSP slope (EPSP) for slice #38. Intervals on the control period projections represent the respective 95% confidence interval. Refer to text in Results for the explanation of control, observed, predicted and necessary values. Note that the term "Direction of prediction" is used figuratively and not statistically. The results indicate that the population spike amplitude predicted from the increase in EPSP slope produced by NE, was smaller than the population spike amplitude value observed during NE superfusion.

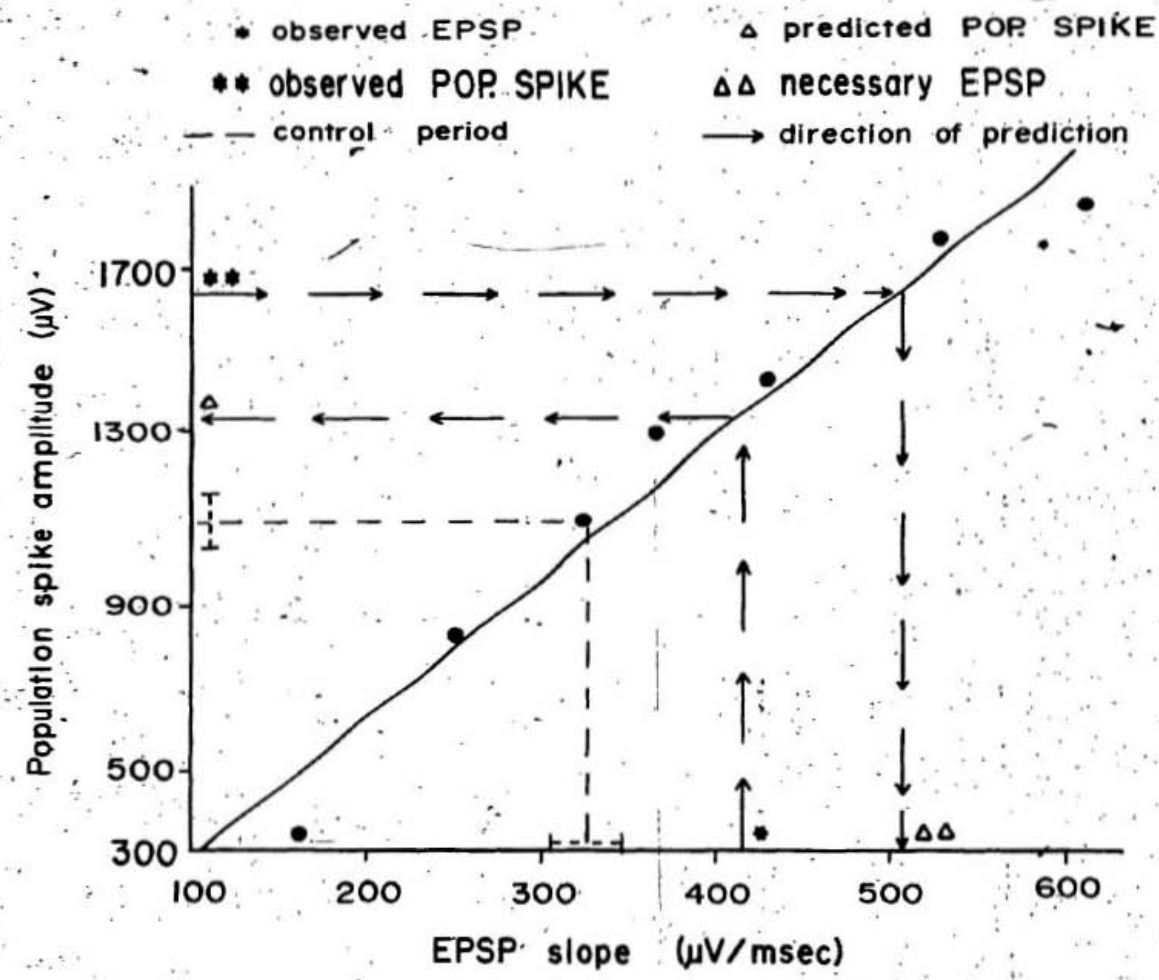

* observed EPSP
** observed POP. SPIKE
— — control period
△ predicted POP. SPIKE
△△ necessary EPSP
→ direction of prediction
Population spike amplitude (μV)
1700
1300
900
500
300
100
200
300
400
500
600
EPSP slope (μV/msec)

Table 17

Summary table of EPSP slope and population spike amplitude relationship. Control (CON), observed (OBS), necessary (NEC) and predicted (PRED) values (see text of Results for explanation) of EPSP slope (EPSP, in μV/msec) and of population spike amplitude (POP. SPIKE, in μV) with the percentage of the POP. SPIKE increase produced by NE which can be accounted for by the EPSP increase, for all slices.

Slice	EPSP			POP. SPIKE			POP. SPIKE Percent
	CON	OBS	NEC	CON	OBS	PRED	Predicted
#34	832	981	974	2043	2628	2658	105%
#33	1355	1445	1670	1421	1924	1657	47%
#35	709	761	997	1521	1973	1503	-4%
#36	832	918	1063	1885	2401	2036	29%
#38	326	412	507	1094	1649	1329	42%
#39	352	378	408	638	971	847	63%

percentage of the population spike amplitude increase that was produced by NE in slice #38 which could be accounted for solely by the EPSP slope increase was only 42%. Similarly, as indicated in Figure 15, an EPSP slope value greater than the one observed during NE was necessary to predict the population spike amplitude value observed during NE.

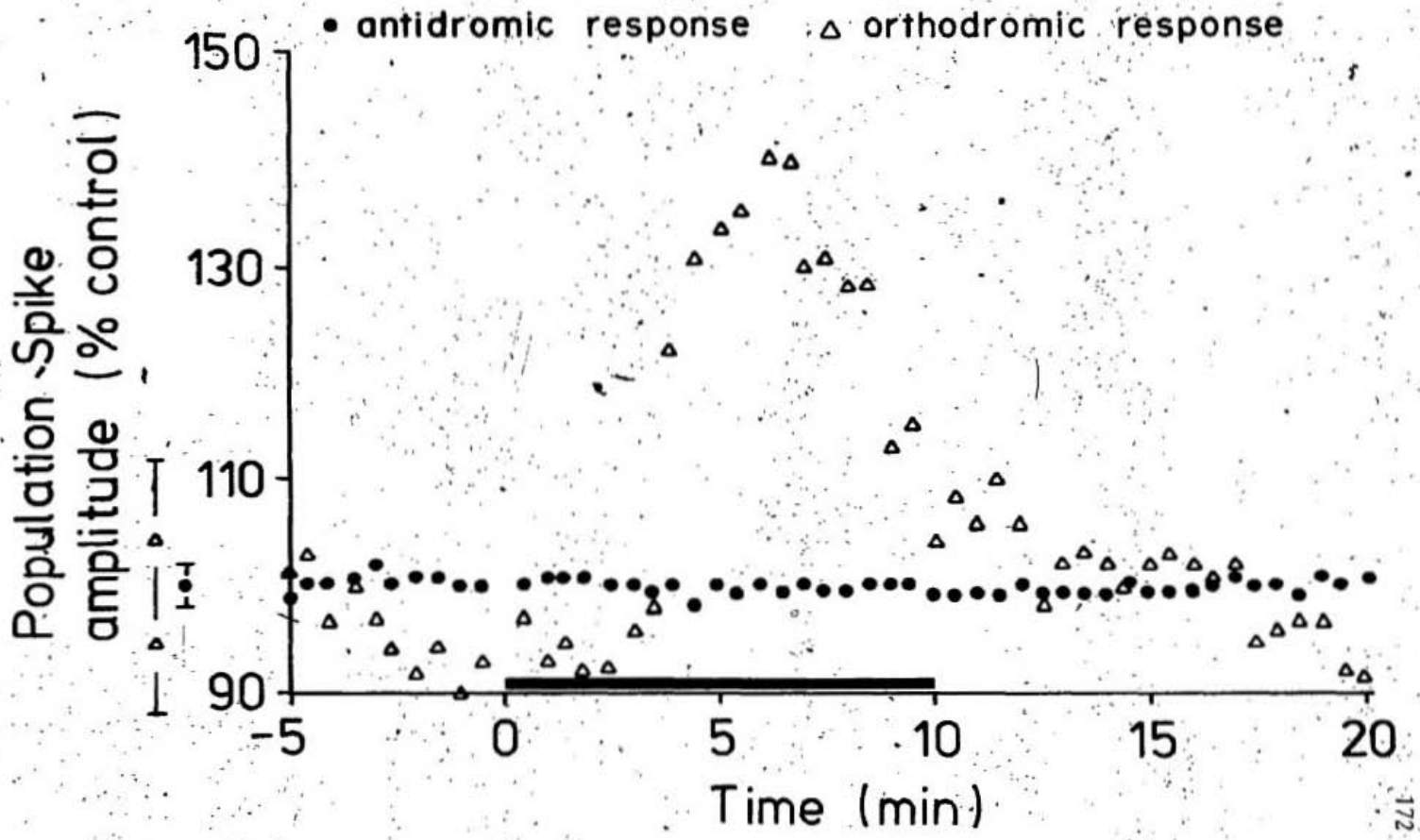
Table 17 indicates that out of 6 slices, similar results were found in 5 slices. Hence, in one slice only, the EPSP slope increase was sufficient to account for the population spike increase. In the other five slices the EPSP slope increase accounted for a mean value of 35% of the increase in population spike amplitude. For all slices, a mean of 47% of the population spike amplitude increase produced by NE could be accounted for by the increase in EPSP slope also produced by NE.

Effect of NE on antidromically evoked responses

The results indicated that in contrast to the orthodromic responses, NE rarely produced an increase in the antidromically evoked population spike amplitude. Generally, the antidromic population spike measure showed no change or a small decrease of short duration.

Figure 16 illustrates an example of the lack of effect of NE on antidromic responses compared to the increase

Figure 16. Lack of effect of NE on antidromic responses. Example of the lack of effect of 10 μM NE on the antidromic population spike amplitude in contrast to the increase produced in the orthodromic population spike amplitude for slice #28. Bar above abscissa indicates NE superfusion. Intervals to the left of the ordinate represent the 95% confidence interval of the respective control period.


• antidromic response
△ orthodromic response
Population Spike amplitude (% control)
150
130
110
90
-5
0
5
10
15
20
Time (min)

produced in orthodromic responses. Table 18 summarizes the effect of NE on the antidromic and orthodromic responses for the 8 slices on which these experiments were performed.

As illustrated in Figure 16 for slice #28 superfusion of 10 μM NE did not produce any increase or significant change in the antidromic population spike amplitude. In contrast, in the same slice, 10 μM NE produced a significant increase in the orthodromic population spike amplitude. Note that the effect of NE on the orthodromic responses illustrated in Figure 16 was obtained subsequent to the experiment on the antidromic responses of the same population of granule cells. Thus the lack of effect of NE on the antidromic responses was not due to deterioration of the slice preparation since an increase to 140% of control could subsequently be produced by NE on the orthodromic responses.

As indicated in Table 18, NE produced an increase in antidromic responses in one of eight slices. Even in this case the magnitude of the effect on the antidromic responses (112% of control) was much less than the effect on the orthodromic responses (200%, and subsequently 141%). In three of the eight slices no changes in the antidromic responses were observed following superfusion with NE.

In contrast in the remaining four slices a decrease in the antidromic population spike amplitude was noted (mean 96% of control, s.e.m. 0.6). The mean time of onset was 4

Table 18

Summary table of the effects of NE on antidromic population spikes. Values of the peak effect (% of control) produced by 10 μM NE on the orthodromic population spike amplitude (Orthodromic), on the antidromic population spike amplitude (Antidromic) or on the orthodromic population spike amplitude evoked subsequently to the antidromic experiment (Subsequent orthodromic).

Slice	Orthodromic	Antidromic	Subsequent Orthodromic
#30	200	112	141
#28	147	100	140
#44	132	100	116
#45	139	100	151
#31	128	95	-
#34	129	98	135
#40	136	96	153
#43	117	96	-

minutes (s.e.m. 0.4), the time of peak effect was 6.4 minutes (s.e.m. 0.7) and the mean time of recovery was 2 minutes (s.e.m. 2.7), thus yielding a mean duration of effect of 8.5 minutes. Hence the effects of NE on the antidromic response were generally no change or a small decrease in magnitude of relatively short-duration.

In six of the eight slices in which orthodromic responses could be reliably evoked subsequent to the antidromic experiments, NE was always observed to increase significantly the orthodromic population spike amplitude. Therefore the absence of an increase in the antidromic response during superfusion with NE was not due to a lack of responsiveness on the part of the granule cells or the slices.

Activity-independence of NE effects

The results of these experiments indicated that the effects of NE on the perforant path evoked responses were activity-independent. Thus even though no concurrent perforant path stimulation was applied during superfusion of NE, upon subsequent return to superfusion with control ACSF and perforant path stimulation, the evoked responses were enhanced compared to the control period.

Figures 17 and 18 illustrate an example of the effect of

Figure 17. Activity-independence of NE effects on EPSP slope. Effects of 10 μM NE (bar above abscissa) applied with and without concurrent perforant path stimulation on EPSP slope for slice #41. Intervals to the left of the ordinate represent the 95% confidence interval of the respective control period.

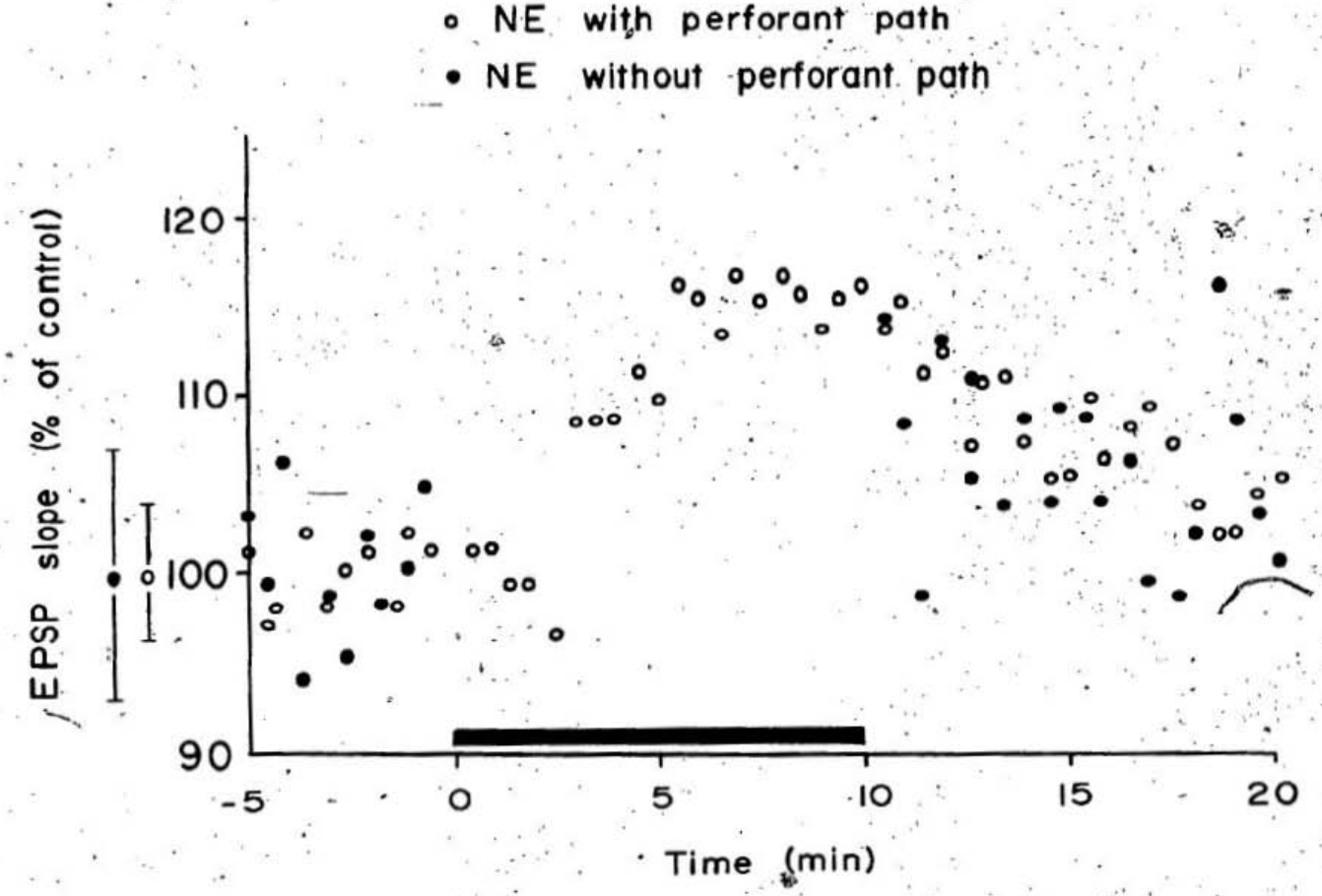
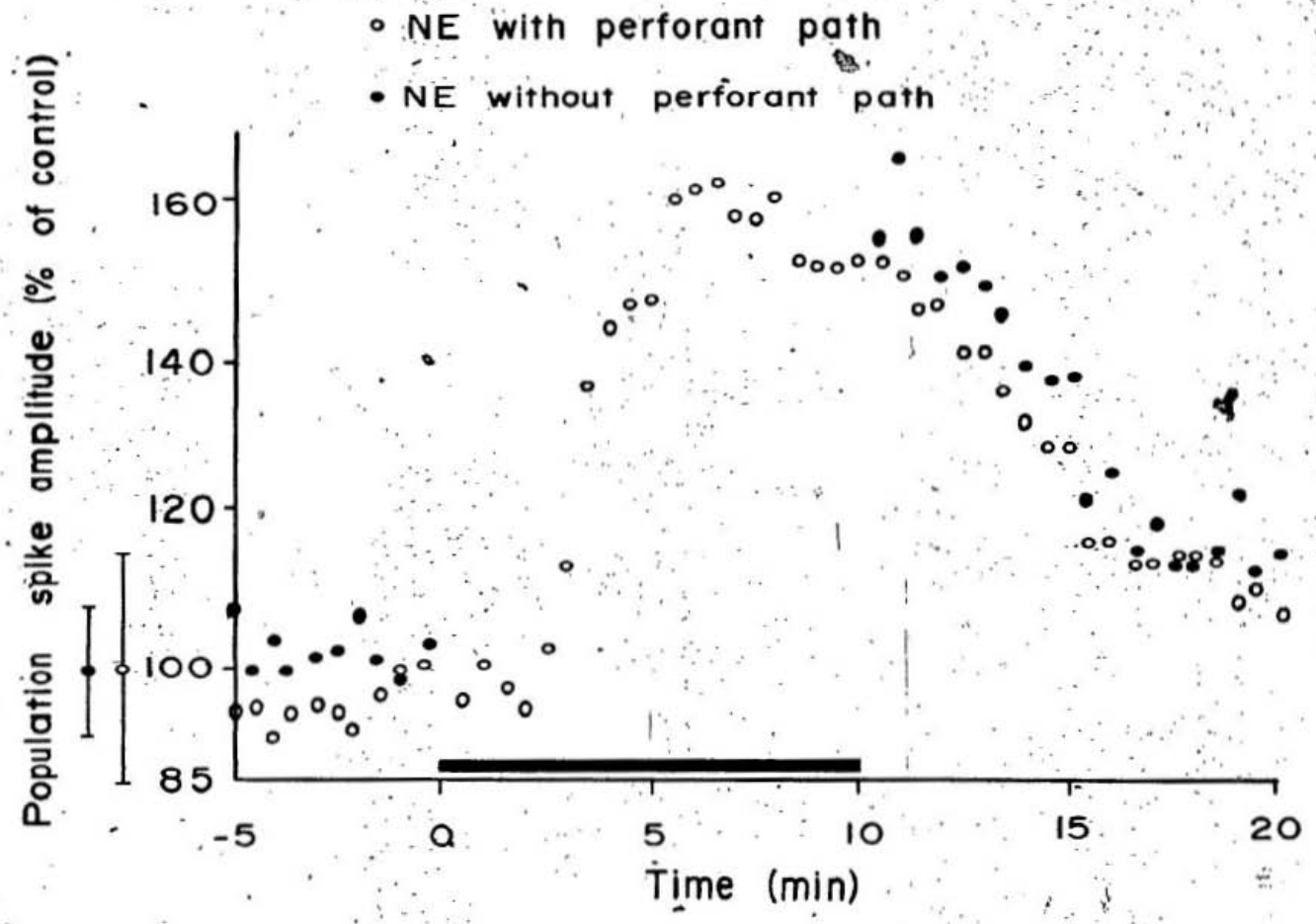

o NE with perforant path
• NE without perforant path
EPSP slope (% of control)
120
110
100
90
-5
0
5
10
15
20
Time (min)

Figure 18. Activity-independence of NE effects on population spike amplitude. Effects of 10 μM NE (bar above abscissa) applied with and without concurrent perforant path stimulation on population spike amplitude for slice #41. Intervals to the left of the ordinate represent the 95% confidence interval of the respective control period.


o NE with perforant path
• NE without perforant path
Population spike amplitude (% of control)
160
140
120
100
85
-5
0
5
10
15
20
Time (min)

NE on the EPSP slope and population spike amplitude, respectively, of responses evoked subsequently to superfusion with NE with and without concurrent perforant path stimulation. Table 19 represents a summary table of the effect of NE on evoked responses obtained subsequent to superfusion with and without concurrent perforant path stimulation in the 6 slices tested.

The results shown in Figures 17 and 18 indicate that superfusion of NE produced an increase in the EPSP slope and population spike amplitude of the responses evoked concurrently during the superfusion period. Following the peak effect and the return to ACSF the effect decayed slowly. The maximum effect produced after superfusion with concurrent perforant path stimulation was 115% for the EPSP slope and 152% for the population spike amplitude. The responses evoked after superfusion of NE but without concurrent perforant path stimulation were similarly increased with respect to the control period. Peak effects of 117% for EPSP slope and 165% for population spike amplitude were observed. Figures 17 and 18 also show that the effects produced after superfusion without concurrent perforant path stimulation decayed at approximately the same rate as those produced after superfusion with concurrent perforant path stimulation.

The results of Table 19 indicate that in all slices

Table 19

Summary table of the effects of NE applied with and without concurrent perforant path stimulation. Peak effects (% of control) obtained after superfusion of 10 μM NE with (With PP) and without perforant path stimulation (Without PP) on EPSP slope (EPSP), population spike onset latency (ONSET) and population spike amplitude (POP. SPIKE).

Slice	EPSP		ONSET		POP. SPIKE	
	With PP	Without PP	With PP	Without PP	With PP	Without PP
#18	100	66	94	100	120	100
#28	119	111	93	92	142	122
#29	100	76	94	94	124	113
#41	115	114	96	92	152	165
#42	115	100	95	100	143	118
#43	100	111	94	93	117	119

tested superfusion of NE without concurrent perforant path stimulation generally produced similar effects to those of superfusion of NE with concurrent perforant path stimulation. In these experiments NE applied without concurrent perforant path stimulation produced an increase in EPSP slope (mean 112%) in three slices. In comparison NE applied with concurrent perforant path stimulation produced a mean increase of 116% in 3 slices. NE applied without perforant path stimulation caused no significant change in EPSP slope in one slice whereas when applied with perforant path stimulation NE caused no significant change in 3 slices. NE applied without concurrent perforant path stimulation also brought about a decrease (mean 71%) in EPSP slope in 2 slices.

NE applied without concurrent perforant path stimulation produced a mean decrease of 93% in population spike onset latency in 4 slices and no significant changes in 2 slices. Similarly NE applied with concurrent perforant path stimulation caused a mean decrease of 94% in population spike onset latency in 6 slices.

Finally, NE without concurrent perforant path stimulation brought about a mean increase of 127% in population spike amplitude in 5 slices and no significant changes in one slice. Similarly NE applied with concurrent perforant path stimulation produced a mean increase of 133%

in population spike amplitude in 6 slices.

In these experiments long-lasting effects of NE were not observed, thus the effects of NE superfusion with and without concurrent perforant path stimulation were never assessed on long-lasting NE effects.

Effects of serotonin on evoked responses

Serotonin produced different effects than NE on the perforant path evoked responses. Generally serotonin produced a decrease in EPSP slope, a small, transient decrease in population spike onset latency and a decrease in population spike amplitude.

Figure 19 illustrates a representative example of the effects of 5-HT on EPSP slope, population spike onset latency and population spike amplitude in one slice.

Table 20 summarizes for the 5 slices tested the peak effects of 5-HT on the EPSP slope, population spike onset latency and population spike amplitude, compared with the peak effects of 10 μM NE in the same slice.

As illustrated in Figure 19 superfusion with 25 μM 5-HT produced a significant decrease in EPSP slope after 7 minutes of superfusion. The decrease reached a peak effect (85% of control) 13 minutes following the onset of superfusion. Afterwards the effect decayed and recovered to

Figure 19. Effects of 5-HT on perforant path evoked responses. Representative example of the effect of 10 minutes of superfusion of 25 μM 5-HT (bar above abscissa) on EPSP slope (EPSP), population spike onset latency (ONSET) and population spike amplitude (POP. SPIKE) for slice #46. Intervals to the left of the ordinate represent the respective 95% confidence intervals of the control period.

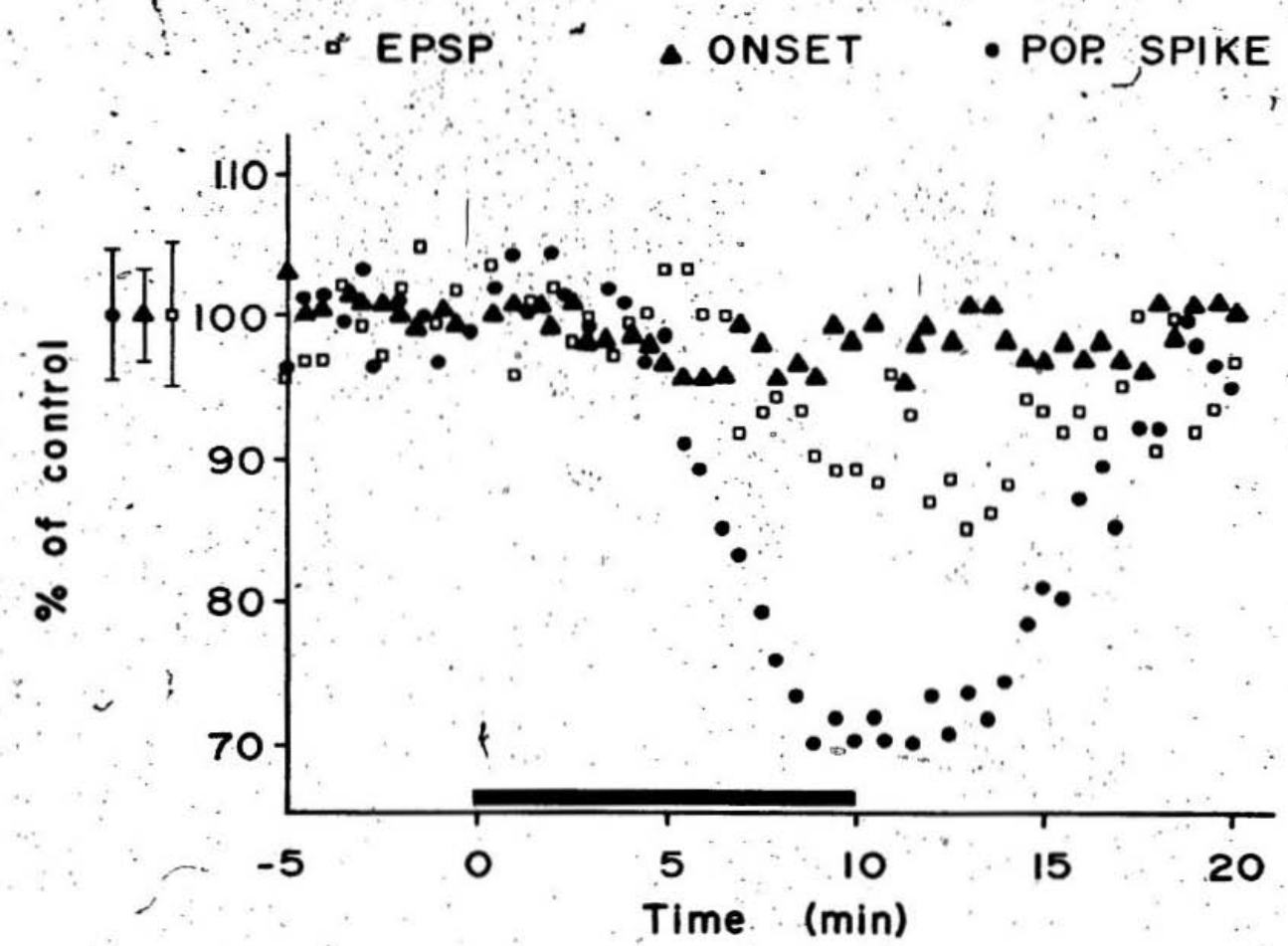

EPSP
ONSET
POP. SPIKE
110
100
90
80
70
% of control
-5
0
5
10
15
20
Time (min)

Table 20

Summary table of the effects of 5-HT and NE in the same slices. Peak effects (% of control) produced by superfusion with 10 μM NE (NE) or with 25 μM 5-HT (5-HT) on EPSP slope (EPSP), population spike onset latency (ONSET) and population spike amplitude (POP. SPIKE).

Slice	EPSP		ONSET		POP. SPIKE	
	NE	5-HT	NE	5-HT	NE	5-HT
#35	107	100	100	97	130	81
#39	107	84	93	95	152	117,63,124
#46	100	85	95	96	122	70
#48	100	84	93	95,109	114	108,65
#49	70	85	92	96	118	72,131

Note. Double entries indicate biphasic effects and triple entry indicates triphasic effect.

control levels at 17 minutes.

The effects of 5-HT on the population spike onset latency were smaller and more transient. In this slice, a significant decrease (96% of control) was observed at 5.5 minutes after the onset of superfusion and it had recovered to control levels at 7 minutes. However the effect of 5-HT on the population spike amplitude was of a greater magnitude. As illustrated in Figure 19, 5.5 minutes after the onset of superfusion a significant decrease in population spike amplitude was observed. The effect increased with continued superfusion reaching a peak at 70% of control 10 minutes after the onset of superfusion. Gradually thereafter the effect decayed and the population spike amplitude had recovered to control levels 8 minutes after the removal of 5-HT.

As indicated in Table 20 similar effects were observed in most slices. A concentration of 25 μM of 5-HT was found to be necessary to observe these effects. In slice #35, 10 μM 5-HT was applied for 10 minutes and no significant changes were observed. Thus, 25 μM 5-HT produced a decrease in EPSP slope in four slices and no change in one. A mean decrease to 84% of control was observed with a mean time of onset of 4.5 minutes, a mean time of peak effect of 10.6 minutes and a mean time of recovery of 2.2 minute following removal of 5-HT.

Twenty five μM 5-HT caused a decrease in population spike onset latency in all 5 slices. In one slice, the decrease was followed by an increase to 109% of control. A mean decrease to 96% of control was observed with a mean time of onset of 4.1 minutes, a mean time of peak effect of 4.8 minutes and a mean duration of effect of 2 minutes.

Serotonin brought about a decrease in population spike amplitude in all 5 slices. In two slices the decrease was preceded by a transient (1 minute) increase and also in two slices the decrease was followed by an increase which lasted 10.5-19 minutes. The mean decrease observed in population spike amplitude was to 70% of control, with a mean time of onset of 3.8 minutes, a mean time of peak effect of 10.8 minutes and a mean time of recovery of 9.6 minutes after the removal of 5-HT.

As indicated in Table 20, these effects of 5-HT differed from the effects of NE on the same slices. NE was observed to increase the EPSP slope in two slices (mean of 107%), to decrease it (70%) in one slice and not to affect it in two slices. NE caused a mean decrease to 93% in population spike onset latency in 4 slices and produced no change in one slice. NE produced a mean increase to 127% of control in population spike amplitude in the 5 slices. In these slices NE effects on the population spike amplitude recovered faster than in general (mean recovery 8.5 minutes

after wash-out).

Chapter 4: Discussion

The results indicate that superfusion of NE produces an enhancement of the perforant path evoked responses of the granule cells in the dentate gyrus in vitro. This increased responsiveness to synaptic activation in vitro is consistent with the effects of NE observed in the dentate gyrus in vivo. The enhancement of the population spike amplitude observed with superfusion of NE was similar to the increase reported following synaptic release of NE by locus coeruleus stimulation (Assaf et al., 1979; Harley et al., 1982) or following iontophoretic application of NE (Neuman & Harley, 1983). Additionally, in agreement with the in vivo studies of iontophoretic application of NE (Neuman & Harley, 1983) and of locus coeruleus stimulation (Harley et al., 1982), the increase in population spike amplitude in the in vitro slice preparation was often observed to be long-lasting.

However, the results also indicated other aspects of the effect of NE on the perforant path evoked responses in the dentate gyrus hitherto undemonstrated. Notably, superfusion with NE produced dose-dependent effects which were found to be maximal with superfusion of 10 μM NE. In the physiological characterization experiments, in which no changes were observed with vehicle superfusion, NE also generally caused either increases or no change in EPSP

amplitude and/or slope as well as decreases in population spike onset latency. However the most marked and long-lasting effects were found on the population spike amplitude.

The effects of NE on the evoked responses were mediated via beta adrenergic receptors. A beta receptor antagonist, but not an alpha receptor antagonist, blocked the effects, whereas a beta receptor agonist, but not an alpha receptor agonist, produced similar changes in responses. NE was also found to increase the granule cells' responsiveness by affecting both the EPSP slope and the population spike amplitude. The increased responses brought about by NE were not caused by changes taking place at the granule cell soma since the amplitude of the antidromically evoked population spike was not enhanced by NE. The effects of NE were activity-independent since superfusion of NE without concurrent perforant path stimulation produced enhancement of the subsequently evoked responses. Finally, the effects of NE were found to differ from those of 5-HT. Superfusion with 5-HT produced decreases in perforant path evoked responses.

Dose-response experiments

The results indicated that a concentration of 10 μM NE

applied for 10 minutes was most effective in producing an increase in population spike amplitude. Lesser concentrations produced small or no effects. Greater concentrations also produced significant increases but generally smaller in magnitude.

However, in the dose-response experiments, no significant changes were observed on population spike onset latency and on EPSP amplitude at the same concentrations of NE producing changes in population spike amplitude. Although small decreases were observed in onset latency, these changes were not significantly different from the vehicle application. In the case of the EPSP amplitude the large variability of the measures tended to obscure any small effect which might have been present. The physiological characterization experiments confirmed this by showing that although the most common effect observed on EPSP amplitude was no change, in some instances significant increases were observed. Moreover, as discussed in the next section, when the EPSP slope, which was slightly less variable than the amplitude, was measured, the effect most often observed following 10 μM NE was a significant increase.

These results, although similar in general, differ in some aspects from those obtained by Mueller et al. (1981) with bath application of NE on CA1 pyramidal cells. They

reported increases in population spike amplitude at approximately the same effective concentration of NE (5-10 μM). Similarly to the results obtained here, they also reported no significant changes in EPSP amplitude with superfusion of NE at various doses.

However, high doses of NE (25-50 μM) were reported to produce decreases in the population spike amplitude of CA1 cells (Mueller et al. 1981). Also in CA1, Marciani et al. (1984) observed similar dose-dependent inhibitory effects on the population spike with iontophoretic application of NE, but Segal (1982) reported only facilitatory effects of iontophoretically applied NE on excitatory responses produced by iontophoresis of glutamate. The dose-response experiments reported here indicate that for the granule cells of the dentate gyrus higher concentrations of NE (25-50 μM) do not produce inhibition of evoked responses but rather increases of smaller magnitude. Similarly, Neuman and Harley (1983) failed to observe suppressant effects of NE on granule cell responses with higher or longer iontophoretic ejection currents. Assaf et al. (1979) and Harley et al. (1982) also reported only facilitatory effects of NE on granule cell evoked responses with stimulation of the locus coeruleus. Although their results cannot be directly compared with results obtained from CA1, they indicate that in the dentate gyrus following locus coeruleus

stimulation no suppressant effects of NE are observed, supporting the results obtained here in the slice. Thus in the CA1 region dose-dependent effects with facilitation at low doses and suppression at high doses of NE are observed, whereas in the dentate gyrus only dose-dependent facilitatory effects are produced. These results suggest differences in noradrenergic function in different parts of the hippocampal formation.

Although the lack of a suppressant effect in the dentate gyrus at high doses may represent differences in the mechanism of action of NE on the granule cells, it may also reflect a different weighting of the magnitude of effects mediated by the different receptor sub-types in the two regions. Hence, whereas the alpha receptor action mediating the suppressant effect of NE in the CA1 region (Mueller et al., 1981) is sufficient to overcome the facilitatory effect mediated by the beta receptor action in that region, it may not be so in the dentate gyrus. This point will be further discussed in relation to the pharmacological experiments.

Physiological characterization of NE effects

The results indicated that the effects most often observed following 10 minutes of superfusion with 10 μM NE were no change or sometimes an increase in EPSP amplitude,

an increase in EPSP slope, a decrease in population spike onset latency and an increase in population spike amplitude.

The different effects on the EPSP measures are noteworthy. The generally unchanged EPSP amplitude measure following superfusion with NE is consistent with other reports of a general lack of effect of locus coeruleus stimulation (Assaf et al., 1979; Harley et al., 1982) or iontophoresis of NE (Neuman & Harley, 1983) on the EPSP amplitude of the granule cells (but see Neuman & Harley, 1983, for some effects). Similarly in CA1, Mueller et al. (1981) reported an unchanged EPSP amplitude with superfusion of NE. Intracellular studies in CA1 have given mixed results. In vivo, Herrling (1981) reported increases in intracellular EPSP amplitude. In vitro, Langmoen et al. (1981) reported mostly equal incidences of increases and decreases, whereas Segal (1981) observed mostly decreases in intracellular EPSP amplitude.

However, the more consistently observed changes in EPSP slope in this study indicated that one effect of NE was to increase the excitatory synaptic drive on the granule cells. Thus, these results suggest that the EPSP slope may represent a more sensitive extracellular measure of the excitatory synaptic activation of the granule cells than the EPSP amplitude measure. At least in conditions similar to the present ones where the EPSP parameters were measured on

a suprathreshold evoked response which included a population spike. The reason for the discrepancy in effects between the EPSP measures remains unclear. Although a greater mean effect was observed on the slope (118%) than on the amplitude (114%) and the variability of the slope measure was less (mean 95% confidence interval ± 5.6% of control) than that of the amplitude measure (mean 95% confidence interval ± 7.1% of control) these differences are small. The fact remains, however, that an increase in excitatory synaptic drive produced by NE was more often observed on the EPSP slope than EPSP amplitude and therefore suggests that an effect of NE on the granule cells is to increase such excitatory synaptic activation.

It should be noted that with the present method of measuring the EPSP slope, a decrease in population spike onset latency associated with no change in the extracellular EPSP could result in an artefactual increase in the EPSP slope measure. Thus the effect of NE on the EPSP slope may arise from the observed decrease in population spike onset latency. However there are three arguments suggesting that NE produced a true effect on the extracellular EPSP.

First, during all experiments the evoked responses were displayed at high magnification as a stored trace on the cathode-ray oscilloscope. With superfusion of NE, a shift in population spike onset latency which was accompanied by a

prior increase in EPSP slope was commonly observed on the stored traces.

Second, the computerized measurements of the EPSP amplitude in the first part of the physiological characterization experiments represented a measure of the extracellular EPSP which would not be affected by a decrease in population spike onset latency. In these experiments superfusion of NE produced a significant increase in EPSP amplitude in 6 slices, whereas no changes were observed in 10 slices. Thus, in more than a third of the slices true increases in the extracellular EPSP measure were produced by NE.

Finally, to assess directly whether the increase in EPSP slope was artefactual, the effect of NE was also measured on the true extracellular EPSP in two slices. Thus, following measurement of the effects of NE on the evoked responses recorded at the soma, the recording electrode was positioned in the outer half of the molecular layer and the effects of NE on the amplitude of the pure extracellular EPSP was measured. In the 2 slices, whereas NE produced an increase in EPSP slope of 120% and 112% of control, following superfusion with NE the amplitude of the pure extracellular EPSP was increased to 116% and 112% of control respectively. Thus in these two slices the increase in EPSP slope reflected an increase in the extracellular EPSP truly

produced by NE. These three observations therefore suggest that the effects of NE observed on the EPSP slope were not artefactual. However the question can only be definitely resolved by measuring the effects of NE on the pure EPSP response recorded either intra- or extracellularly.

Regarding the decrease in population spike onset latency observed with superfusion of 10 μM NE, these changes differed from the results obtained during the dose-response experiments. In the dose-response experiments, although a small decrease in onset latency was observed with superfusion of NE, a decrease was also observed with superfusion of the vehicle solution. However, in the physiological characterization experiments showing NE-induced decreases in onset latency, in only one out of twenty eight slices did superfusion with vehicle solution produce a decrease in onset latency. In terms of neuronal events, the decrease in population spike onset latency indicated that with superfusion of NE the evoked action potential firing of the granule cells occurred earlier, or more synchronously, following perforant path stimulation.

The increased population spike amplitude, in turn, suggested that with superfusion of NE a greater number of action potentials were evoked in the granule cells following perforant path stimulation. Alternatively, a greater current could have been generated by the firing of the same

number of granule cell action potentials either because of synchronous firing or other factors such as increases in membrane conductance.

The effects of NE on the different parameters of the evoked responses occurred with similar time of onset (means approximately 4-5 minutes) and time of peak effect (means approximately 10 minutes). However differences were noted. First, the effect of NE was greater in magnitude on the population spike amplitude (mean 131% of control) than on the extracellular EPSP measure (means 114-118% of control). Second, the effect of NE on the population spike amplitude generally recovered later (mean approximately 17 minutes after removal of NE) than its effect on extracellular EPSP or onset latency (means 8.5-12.4 minutes). Secondary to this difference in duration of effect, a greater incidence of long-lasting effects (still present 30 minutes after removal of NE) was observed for the population spike amplitude (24%) than for extracellular EPSP and onset latency measures (10-11%).

Thus, the effect of NE on extracellular EPSP, population spike onset latency and population spike amplitude developed with a similar time-course. However, the effect of NE on the population spike amplitude could be differentiated from its effect on extracellular EPSP measures and population spike onset latency on the basis of the respective

magnitudes and durations of effect. These observations suggest that NE produced distinct effects on extracellular EPSP and onset latency and on population spike amplitude. This point will be further discussed below with respect to the experiments on the EPSP slope and population spike amplitude relationship.

The effects on extracellular EPSP measures and population spike onset latency are the first demonstration of the effect of NE on these parameters of the granule cell evoked responses. As mentioned above, Assaf et al. (1979) and Harley et al. (1982) typically did not observe an increase in EPSP amplitude following locus coeruleus stimulation and neither did Neuman and Harley (1983) following iontophoresis of NE. The present results are, however, consistent with more recent observations of increases in EPSP slope following stimulation of the locus coeruleus (Harley, Lacaille & Milway, unpublished observations). Similarly, an enhancement by NE of the perforant path evoked extracellular EPSP of the granule cells has recently been suggested by Dahl, Bailey and Winson (1983). These authors reported that the increase in extracellular EPSP observed in freely moving rats during the still-alert state relative to slow-wave sleep was not present following depletion of hippocampal NE with 6-OHDA. Therefore, these authors suggested that the increase

observed in normal rats was mediated by NE.

As mentioned above, the effects on the population spike amplitude are consistent with previous reports of increases in population spike amplitude following locus coeruleus stimulation (Assaf et al., 1979; Harley et al., 1982) and iontophoresis of NE (Neuman & Harley, 1983). However they are inconsistent with the initial reports of suppressant effects of NE on spontaneous and evoked activity of dentate gyrus cells (Stefanis, 1964; Segal & Bloom, 1976a).

The effect of NE on the population spike amplitude in the slice (131%) was in the same direction and of similar magnitude to that observed following locus coeruleus stimulation (140-150% of control) or iontophoresis of NE (130-140%) in the anesthetized rat (Assaf et al., 1979; Harley et al., 1982; Neuman & Harley, 1983). However, the development and subsequent decay of the increase in population spike amplitude was not uniform with iontophoresis of NE, stimulation of locus coeruleus or superfusion of NE in the slice.

In the iontophoretic studies, following an ejection period of 1-2 minutes, the population spike amplitude increased slowly to a plateau over a period of 15-30 minutes. Sometimes the population spike amplitude continued to increase after this period and was followed by a slow decay over hours (Neuman & Harley, 1983). With locus

coeruleus stimulation, the first pairing of locus coeruleus and perforant path stimulation produced a near peak increase in population spike amplitude (Harley et al., 1982). Following 50 repetitions of such pairing (i.e. over a 10 minute period), when the perforant path was stimulated alone the population spike amplitude was still elevated relative to control although less than during paired locus coeruleus-perforant path stimulation. Afterwards, either the effect decayed back to baseline or remained elevated for 30 minutes (Harley et al., 1982). In the slice, a similar pattern to that produced by locus coeruleus stimulation was observed. The population spike amplitude increased relatively rapidly after the onset of superfusion, and then peaked near the end of the superfusion period. Afterwards either the effect decayed back to baseline or decayed much more slowly and remained significantly elevated for 30 minutes. The similarity of the effect with superfusion in the slice and locus coeruleus stimulation in vivo suggests that the superfusion method in vitro provides a good approximation of the effect of physiologically released NE in vivo.

With respect to the long-lasting potentiation of the population spike amplitude produced by NE, its incidence was found to be lower in the slice than in vivo. Whereas long-lasting effects were observed in 60% of the animals following locus coeruleus stimulation (Harley et al., 1982)

and at 39% of the potentiated sites following iontophoresis of NE (Neuman & Harley, 1983), following superfusion of NE they were observed in 24% of the slices showing an increase in population spike amplitude. The lower incidence of long-lasting effects in the slice was not unexpected given that there is always a progressive metabolic run-down of the slice. However the fact that long-lasting increases in evoked responses were observed in vitro suggests that the neuronal substrate necessary for such long-lasting changes in neuronal responsiveness is present in the simplified slice preparation. The lower incidence observed in vitro may reflect the absence in the slice of factors facilitating the effects of NE in vivo. For example, co-localization of vasopressin and NE in locus coeruleus cells has been suggested on the basis of immunocytochemistry (Caffe & Van Leeuwen, 1983). Additionally, vasopressin has been reported to potentiate the stimulation of cyclic AMP accumulation produced by NE in mouse hippocampus (Church, 1983). Thus perhaps the presence in vivo of endogenous factors such as vasopressin which are co-released with NE or which are under other extrinsic influences facilitates the effects of NE and results in a greater incidence of long-lasting effects than in vitro.

Pharmacological characterization of NE effects

The pharmacological results indicate that the effects of NE on the EPSP slope, population spike onset latency and population spike amplitude were mediated via beta adrenergic receptors. The effects of NE on the evoked responses were antagonized by the beta receptor antagonist timolol but not by the alpha receptor antagonist phentolamine. Additionally, similar effects to those produced by NE were caused by the beta receptor agonist isoproterenol but not by the alpha receptor agonist phenylephrine.

The effective concentration of timolol necessary to reliably antagonize the effect of 10 μM NE was found to be 5 μM. The effective concentration of isoproterenol necessary to reliably mimick the effect of 10 μM NE was noted to be 1 μM. The greater potency of isoproterenol relative to norepinephrine in producing changes in the granule cell evoked responses further suggests that these effects were mediated via beta receptors (Goodman Gilman et al., 1980).

The greater potency of isoproterenol resulted in effects of greater magnitude being produced on EPSP slope and population spike amplitude than were produced by NE. While superfusion with isoproterenol did not appear to result in a greater incidence of long-lasting effects, this question was not thoroughly investigated in the present study. Since isoproterenol is more potent than NE, it may be that shorter

periods of superfusion would produce long-lasting effects. In this respect, Anwyl and Rowan (1984) mentioned that superfusion of isoproterenol on CA1 cells produced increases in population spike amplitude sometimes lasting up to 2 hours. Alternatively, a more selective beta-1 or beta-2 adrenergic agonist could be necessary to cause the long-lasting effects in the dentate gyrus.

Although the effects of NE on EPSP slope and population spike amplitude could be differentiated in terms of magnitude and duration of effect, the effects on both parameters appeared to be mediated via beta receptors. Timolol blocked and isoproterenol mimicked the increase in both EPSP slope and population spike amplitude produced by NE.

The results of the experiments with superfusion of the alpha receptor agonist phenylephrine indicated that some alpha-mediated actions of norepinephrine were also present in the dentate gyrus. These alpha-mediated actions resulted in decreases in EPSP slope and population spike amplitude but no changes in population spike onset latency. However, compared to the beta-mediated effects, the alpha actions were of shorter duration and smaller magnitude. Thus, with superfusion of NE, the beta-mediated effects predominated and increases in EPSP slope and population spike amplitude as well as in population spike onset latency were mostly

observed.

The present demonstration of beta-mediated increases in population spike amplitude produced by NE confirmed the preliminary evidence of Harley and Neuman (1980) that the increases in population spike amplitude produced by iontophoresis of NE were mediated by beta receptors. The present data are also consistent with the beta receptor mediated increase in population spike amplitude (Mueller et al., 1981; Marciani et al., 1984) and the beta-mediated increase in evoked firing (Madison & Nicoll, 1982; Haas & Konnerth, 1983) observed in hippocampal CA1 cells. However the present data provide the first demonstration that the increase in evoked excitatory synaptic activation also produced by NE in granule cells (increased EPSP slope) is mediated via beta adrenergic receptors.

Hence the results indicate that the physiological effects of NE on the evoked responses of the granule cells of the dentate gyrus are predominantly mediated by beta receptors. In contrast, the physiological effects of NE on the evoked responses of the pyramidal cells of the CA1 region appear to be mediated via both alpha and beta receptors (Mueller et al., 1981). Thus between the two regions there appears to be a difference in terms of weighting, or relative strength, of alpha vs beta mediated effects. In contrast to the physiological results,

radioligand binding studies indicated that, whereas a relatively equal density of beta receptors are found in Ammon's horn and the dentate gyrus, 30% more alpha binding sites are present in the dentate gyrus than in Ammon's horn (Crutcher & Davis, 1980). The present results suggest that the higher density of alpha binding sites in the dentate gyrus represents a relatively inactive population of receptors if they are situated on granule cells. Alternatively these receptors could be situated on neurons other than the granule cells, or on glial cells, which may not affect evoked potential parameters in the slice preparation.

EPSP slope and population spike amplitude relationship

The results of the experiments examining the population spike amplitude measure as a function of the EPSP slope indicated that the increase in the EPSP slope produced by NE was not of a sufficient magnitude to account for the observed increase in population spike amplitude. Thus it was concluded that NE produced distinct effects on EPSP slope and population spike amplitude.

Regression analyses permitted a quantitative assessment of the contribution of each factor to the observed increase in population spike amplitude. It was observed that the

increase in the EPSP slope contributed to a mean of 47% of the increase in population spike amplitude. Consequently a mean of 53% of the increase in population spike amplitude was due to other extrasynaptic factors.

Therefore it is concluded that roughly 50% of the effect of NE on the perforant path evoked firing of the granule cells is due to an enhanced excitatory synaptic activation of the granule cells (effect on extracellular EPSP measure). The remaining 50% is due to other effects of NE on the granule cells which result either in a given extracellular EPSP producing a greater firing of action potentials by the granule cells, or in a greater current being generated by the same number of action potentials fired by the granule cells. This point will be further discussed in the next section with regards to the results on antidromic responses.

These results further emphasize the need for careful monitoring of extracellular EPSP measures in assessing changes in granule cell evoked responses. The present results suggest that a previously undemonstrated increase in the extracellular EPSP component of the perforant path evoked responses produced by NE, accounts for roughly half of the effect of NE observed on the population spike component.

It should be noted that if the decrease in the population spike onset latency was causing an artefactual

increase in EPSP slope, the regression based estimate of the percentage of population spike increase induced by NE would be affected. The results, in that case, would overestimate the percentage of the population spike increase that could be accounted for by the increase in EPSP slope. Given the magnitude of the onset latency change usually observed with superfusion of NE, the overestimate would approximately be of 5-10%.

Effects of NE on antidromically evoked responses

The results of the experiments on antidromic stimulation of the granule cells showed that, in contrast to the effect on orthodromically evoked responses, superfusion of NE did not cause an increase in the antidromic population spike amplitude. These results suggested that the increase in orthodromic population spike amplitude produced by NE was not due to voltage-dependent membrane changes occurring at the soma since such changes would also have resulted in an increase in the antidromic population spike amplitude.

In terms of neuronal changes produced by NE these experiments help clarify the changes resulting in a distinct increase in population spike amplitude. As mentioned in the preceding section the increase in orthodromic population spike amplitude could arise either from a greater current

flowing across the soma membrane as a result of a greater number of action potentials being generated orthodromically in the granule cells or from a greater current being generated across the soma membrane by the same number of action potentials generated in granule cells.

However the antidromic population spike amplitude represents the current flowing across the soma membrane as a result of the somatic invasion of action potentials evoked antidromically in granule cell axons.

Thus, assuming that the antidromic stimulation evoked a relatively constant number of action potentials, if the effects of NE on the orthodromic population spike had arisen from a greater current being generated by the same number of action potentials in granule cells, the antidromic population spike should have been altered in the same manner as the orthodromic one. The results indicated that this was not the case, and it is suggested that the distinct increase produced by NE on the orthodromic population spike amplitude arises from a greater number of action potentials being generated by the granule cells to the synaptic input.

The results of antidromic stimulation also show that a commonly observed effect of NE on the antidromic population spike amplitude was a small decrease of short duration. Thus it would appear that part of the action of NE on the granule cell evoked responses involves a decrease in

population spike amplitude arising from changes taking place at the granule cell soma. Since the alpha agonist phenylephrine was also observed to produce similar changes on the orthodromic population spike it is suggested that these effects on orthodromic and antidromic responses represent alpha-mediated effects of NE on the granule cells. This suggestion could be tested experimentally by examining the effect of NE on antidromic responses in the presence of an alpha receptor antagonist or using an alpha agonist. Alternatively, the decrease in antidromic population spike amplitude could reflect an increase in passive membrane resistance produced by NE. Such a decrease in leakage membrane conductance may result in a decrease in antidromic responses, but result in an increase in orthodromic responses by facilitating electrotonic conduction of synaptic current to the axon hillock. Clearly, intracellular studies are needed to clarify this point.

Activity-independence of NE effects

The results of these experiments indicated that following superfusion with NE, subsequently evoked granule cell responses were increased whether or not the perforant path was concurrently stimulated during the superfusion period.

These experiments indirectly showed that all perforant path inputs to the granule cells, regardless of whether they were active or not during the period of application of NE, were probably enhanced following superfusion with NE. Thus, contiguity in time between synaptic activation and the presence of NE may not be necessary for the development of the effects of NE on the granule cell evoked response.

The present results confirmed similar conclusions of Harley et al. (1982) who showed that 10 Hz stimulation of locus coeruleus neurons produced an increase in the amplitude of the population spike evoked at 10 second intervals during and subsequent to locus coeruleus stimulation. In this instance, contiguity in time between the perforant path evoked response and locus coeruleus stimulation was not necessary for the development of the effects of NE.

The present experiments additionally demonstrated that the effects of NE on the EPSP slope measure were activity-independent.

In the present activity-independence experiments, long-lasting NE effects were not observed and therefore, the activity-independence of long-lasting NE effects was not assessed. Further experiments should address this question since the demonstration of activity-dependent long-lasting effects would have great functional implications. Such a

demonstration would provide a model of neurotransmitter-induced long-lasting associative changes in neuronal responsiveness in mammalian CNS.

Effects of serotonin on evoked responses

Whereas application of NE produced increases in the perforant path evoked responses, superfusion with 5-HT caused decreases in the evoked responses. It was observed that 25 μM 5-HT generally produced decreases in EPSP slope, population spike amplitude and population spike onset latency which were of shorter duration than the changes usually observed with NE. Sometimes the decrease in population spike amplitude was preceded by a transient increase and on some occasions the decrease was followed by an increase.

These results provide the first demonstration of the effect of direct application of 5-HT on the perforant path evoked field responses of the granule cells. These results are consistent with the intracellular observations of Assaf et al. (1981) who reported inhibition of granule cells with iontophoresis of 5-HT and who suggested that 5-HT produced an increase in conductance to chloride ions. Such actions of 5-HT would reduce the synaptic depolarization produced by perforant path stimulation by shunting the granule cell

membrane. Such a mechanism of action would predict that in the presence of 5-HT the EPSP slope and population spike amplitude of the perforant path evoked responses would be reduced, as was observed in the present experiments with superfusion of 5-HT.

The present observations of decreased evoked responses with superfusion of 5-HT appear to contradict the observation of Assaf and Miller (1978) of increased population spike amplitude following raphe stimulation. They suggested that this increase resulted from the inhibitory action of 5-HT on the granule cells. However the observations of decreased evoked responses with 5-HT superfusion presented here lend further support to the proposal of Srebro et al. (1982) that the increase in evoked responses following raphe stimulation is non-serotonergic, since in their hands this increase was still observed following depletion of hippocampal serotonin.

An increase in the granule cell's population spike amplitude was sometimes observed to follow the initial decrease produced by 5-HT. This response is similar to the relatively long duration (30-40 minutes) facilitation which has been reported to follow an initial period of suppression of unit activity in CA3 cells after superfusion of 5-HT (Otmakhov & Bragin, 1982). Thus it would appear that some of the action of serotonin on the granule cells as on CA3

pyramidal cells may be more complex than simple inhibition.

Noradrenergic actions in the dentate gyrus

In summary, the results presented here indicate that norepinephrine produces distinct effects on the responses of granule cells to perforant path stimulation. The results suggest that NE produces an increase in evoked synaptic activation as well as an increase in evoked firing of the granule cells. Furthermore, these effects are mediated via beta adrenergic receptors and are activity-independent. Additionally, it was shown that the observed increase in evoked firing is not due to membrane changes at the soma and that in 24% of the cases the increase in evoked firing produced by NE is long-lasting. Finally, norepinephrine was also observed to sometimes produce a small decrease in evoked response which was mediated via alpha receptors. It was suggested that these relatively weak alpha mediated effects are taking place at the soma membrane.

The present experiments did not attempt to characterize further the mechanism of action of NE on the granule cells. Clearly, intracellular studies are needed to resolve this question. In this regard the present experiments provide a much needed physiological and pharmacological characterization of the effects of NE in the slice

preparation. Given that intracellular studies of granule cells can be more easily achieved in the slice preparation than in vivo, the characterization of the effects of NE obtained here should prove useful in designing the appropriate intracellular studies on the cellular mechanism of action of NE.

However, mechanisms of action of NE which have been demonstrated elsewhere in the CNS could account for the observed increase in the granule cell evoked response if they took place in the dentate gyrus.

First, Dolphin (1982) reported that norepinephrine, or the beta-2 agonist salbutamol, potentiated the potassium-evoked release of glutamate from rat cerebellum. If NE produced such a potentiation of the synaptic release of glutamate from the perforant path fibers in the dentate gyrus, then with superfusion of NE one would observe an increase in the synaptic activation of the granule cells following perforant path stimulation. Indeed this was an effect of NE observed here in the dentate gyrus.

Second, Madison and Nicoll (1982) and Haas and Konnerth (1983) reported that norepinephrine applied to hippocampal pyramidal cells produced an increase in evoked firing via a decrease in the calcium-dependent potassium conductance mediating the late hyperpolarization. This beta-mediated effect has been referred to by Madison and Nicoll (1982) as

a decrement in spike accommodation. If NE produced such a decrement in spike accomodation in granule cells, then with superfusion of NE one would observe an increase in evoked firing following perforant path stimulation. In this report such an effect was observed in the dentate gyrus following superfusion of NE. Similarly, since the late hyperpolarization is not observed following antidromic stimulation (Thalmann & Ayala, 1982), the lack of facilitatory effects of NE on the antidromic responses observed in this report further supports this hypothesis.

Therefore, it is suggested that these two beta-mediated effects of NE shown to occur elsewhere in the CNS could account for the observed beta-mediated effects of NE on the granule cell evoked responses. Although there is no direct evidence that these cellular actions of NE are occurring in the dentate gyrus, they provide a parsimonious explanation of the effects observed. However, yet undemonstrated actions of NE may instead take place in the dentate gyrus. Hypothetically, the effect of NE on the EPSP measure could be caused by an increase in the sensitivity, or in the number, of receptors for the excitatory amino acid neurotransmitter in the perforant path. Alternatively, the effect could arise from membrane changes taking place in the dendrites of the granule cells. In turn the effect of NE on the population spike amplitude could be mediated by changes

taking place at the spike initiation segment resulting in a lower firing threshold for the granule cells. The population spike increase could also be caused by factors increasing the coupling between synaptic drive and action potential firing. Such an increase in coupling has been suggested by Mueller et al. (1981) to account for the facilitatory effects of NE on hippocampal pyramidal cells.

With respect to the weak alpha-mediated effects of NE observed on the granule cells, any of the numerous mechanisms of action of the suppressant effects of NE demonstrated elsewhere in the CNS could also take place in the dentate gyrus. Such actions as a decrease in sodium and potassium membrane conductance (Marshall & Engberg, 1979), an increase in potassium conductance (North & Yoshimura, 1984), an activation of a sodium-potassium electrogenic pump (Phillis & Wu, 1981; Segal, 1981), an increase in chloride conductance (Segal, 1981) or a decrease in anomalous rectification (Langmoen et al., 1981) could result in the weak suppressant effects of NE observed in the dentate gyrus. Among these mechanisms of action, only the increased potassium conductance observed by North and Yoshimura (1984) in spinal neurons has been shown to be mediated via alpha receptors.

As mentioned above, although these proposed mechanisms could account for the effects of NE in the dentate gyrus,

intracellular studies are needed to directly resolve these questions.

Long-lasting effects of NE in the dentate gyrus

A remarkable aspect of the effects of NE in the dentate gyrus is the ability to produce long-lasting physiological changes in the granule cells. With the demonstration in humans that the hippocampal formation may be involved in the establishment of long-term memories (Scoville & Milner, 1957), an active area of research has been to try to identify the neuronal substrate in the hippocampal formation for this memory process. The primary requirement for such a neuronal substrate is that the neuronal changes be long-lasting in nature. With the demonstration by Bliss and Lomo (1973) and Bliss and Gardner-Medwin (1973) that long-lasting changes in synaptic efficacy are produced in the dentate granule cells following high-frequency stimulation of the perforant path, the phenomenon of long-term potentiation has been suggested as a neuronal substrate for memory formation (Swanson et al., 1982). In fact, since its demonstration, long-term potentiation has been regarded as the only type of long-lasting change occurring in the hippocampal formation. Thus, it was readily attractive as a potential model for a memory substrate.

However, the recent demonstration by Neuman and Harley (1983) that iontophoresis of NE produces long-lasting increases in perforant path evoked responses indicated that long-lasting changes in synaptic efficacy in the hippocampal formation could also be produced by other physiological means. Harley et al. (1982) have confirmed these results and shown that NE released physiologically by locus coeruleus stimulation also produces long-lasting changes in granule cell responses. The present report further confirms the genuine long-lasting effects of NE and extends them by showing that superfusion of NE also produces long-lasting changes in synaptic efficacy in the in vitro slice preparation. Furthermore, the present results indicate that the long-lasting effects of NE are mostly seen on the evoked firing of the granule cells. However, occasionally they are also observed on the evoked synaptic activation.

Therefore, because of its ability to produce long-lasting changes in synaptic efficacy, NE-induced long-lasting potentiation (Neuman & Harley, 1983) satisfies the primary requirement of a neuronal model of memory formation. Thus, NE-induced long-lasting potentiation provides an alternate neuronal model of memory formation to long-term potentiation.

Indeed, NE-induced long-lasting potentiation closely resembles conceptually the heterosynaptic facilitation

produced by serotonin in Aplysia which is thought to be the neuronal correlate of classical conditioning in that preparation (Carew, Hawkins & Kandel, 1983; Hawkins, Abrams, Carew & Kandel, 1983; Walters & Byrne, 1983). Thus, NE-induced long-lasting potentiation may represent a mammalian CNS analogue which has evolved from an evolutionarily early monoamine action.

However at the present time, further experiments are needed at the physiological and behavioral level to test these hypotheses. It should be noted that the long-lasting changes taking place during NE-induced long-lasting potentiation and those occurring during frequency-induced long-term potentiation may be related. Bliss et al. (1983) reported that long-term potentiation of the extracellular EPSP of the dentate granule cells is reduced by 50% in rats depleted of NE with reserpine or 6-OHDA. Such results suggest a relationship between NE and long-term potentiation.

Although NE-induced long-lasting effects have been reported mostly in the dentate gyrus, others have recently observed similar changes in other regions. Armstrong-James and Fox (1983) reported that some neurons of the deep layers of rat primary somatosensory cortex responded to low levels of iontophoresis of NE with an increase in spontaneous firing rate which lasted for up to one hour.

More recently, Anwyl & Rowan (1984) noted that bath application of isoproterenol produced increases in the population spike amplitude of CA1 pyramidal cells in vitro which lasted up to 2 hours. In contrast, other investigators who have applied NE to CA1 cells and have observed facilitatory effects do not report long-lasting effects (Mueller et al., 1981; Madison & Nicoll, 1982; Haas & Konnerth, 1983). Since Anwyl & Rowan (1984) observed long-lasting changes with a beta receptor agonist, the long-lasting effects not observed by others may have been masked or prevented by the alpha-mediated effects which are also produced by NE on CA1 cells.

Finally in the cerebellum, Michael et al. (1983) observed that the facilitation of GABA inhibitory responses produced by NE released by iontophoretically applied amphetamine can last for 60 minutes. This last report indicates that NE can facilitate inhibitory responses as well as excitatory ones for long periods.

Noradrenergic function in the dentate gyrus

As mentioned above in the Introduction it is generally thought that there are four major excitatory inputs to the dentate gyrus: perforant path, associational fibers, commissural fibers and septal projections. Whether the

hypothalamic afferents are excitatory or inhibitory remains controversial.

Based on electrophysiological experiments in awake freely-moving rats, Winson (1980) had suggested that the two brain stem afferents to the dentate gyrus exert modulatory influences on the granule cells excitability. Thus, by their action on the granule cells it was suggested that raphe and locus coeruleus nuclei modulate the transmission through the dentate gyrus which arises from activation of other inputs to the granule cells. Such a mechanism was suggested to account for the variations in neuronal transmission observed in the dentate gyrus during different behavioral states (Winson & Abzug, 1978; Winson, 1980).

However in the light of recent evidence this suggestion needs reformulating. First, it was assumed that locus coeruleus and raphe influences on the granule cells were tonic in nature (Winson, 1980). But recent evidence indicates that locus coeruleus neurons are also activated phasically (Foote et al., 1980; Aston-Jones & Bloom, 1981b). Thus the influences of locus coeruleus on the dentate gyrus have to be considered not only in terms of tonic influences according to behavioral state, but also in terms of phasic influences arising from arousing sensory stimuli in the environment.

Second, it was assumed that both the serotonergic input

from raphe and the noradrenergic input from locus coeruleus were inhibitory in nature (Winson, 1980). However the present results and those of others (Assaf et al., 1979; Harley et al., 1982; Neuman & Harley, 1983) indicate that the noradrenergic input from locus coeruleus is facilitatory in nature. Thus the modulatory action of the locus coeruleus on the granule cells results in increased neuronal transmission in the dentate gyrus. In contrast the action of the serotonergic input from raphe nuclei causes a decrease in neuronal transmission in the dentate area.

In reformulating Winson's hypothesis (1980) of a modulatory function of the brain stem afferents on neuronal transmission through the dentate gyrus, these factors must be taken into account. Thus, the influence of the raphe nuclei results in a decrement in neuronal transmission while that of the locus coeruleus produces an increment in transmission. This modulatory influence not only occurs during the tonic activation of these nuclei but also as a function of their phasic activity.

The experimental evidence of Winson & Abzug (1978) of an increased population spike amplitude during slow-wave sleep does not appear consistent with the proposal since the tonic activity of the locus coeruleus is low in this behavioral state (Aston-Jones & Bloom, 1981a). Yet the extracellular EPSP increase observed during the still-alert state (Winson

& Abzug, 1978) is consistent with the hypothesis, since the locus coeruleus is more active in this behavioral state (Aston-Jones & Bloom, 1981a).

As noted above, these changes in evoked responses during different behavioral states represent changes arising from changes in tonic activity. Recent evidence suggests that a more functionally important modulatory role for the locus coeruleus may arise from its phasic activity (Aston-Jones & Bloom, 1981b). Therefore, a more appropriate test of the hypothesis would involve monitoring the perforant path evoked responses in awake animals during the presentation of arousing sensory stimuli which produce phasic activation of the locus coeruleus.

The observation that NE produces long-lasting effects on the granule cells further suggests that a transient, phasic activation of the locus coeruleus could produce long-lasting changes in neuronal transmission through the dentate gyrus. Thus, the phasic modulatory influence of the locus coeruleus on neuronal transmission might be considered to be long-lasting in nature in some circumstances.

It should be emphasized that these functional inferences are based on experimental work on perforant path evoked transmission in the dentate gyrus. Before generalizing to other inputs, further experiments are needed to test whether NE and 5-HT also affect the responses evoked from activation

of commissural, associational, septal and hypothalamic afferents. It should also be assessed whether NE and 5-HT modulate the action of other intrinsic neurotransmitters (GABA, substance P, etc...) in the dentate gyrus.

Another aspect of the action of NE which requires further characterization in order to assess its functional implications are the long-lasting effects of NE. A characterization of the factors necessary for the reliable establishment of long-lasting effects is particularly needed. More specifically it would be very informative to determine if the long-lasting changes produced by NE are associative, or activity-dependent, in nature.

Noradrenergic contribution to hippocampal function

Winson and Abzug (1978) have suggested that the physiological function of the dentate gyrus is to provide a gating process regulating the flow of information through the hippocampal formation. Thus depending on its "state of activation" the gating of the dentate gyrus would vary from being open and allowing unrestricted transmission of information through to CA3 neurons, to being closed and severely restricting the flow of information to the CA3 area. In this respect, the proposed action of the noradrenergic input to the dentate gyrus would be to

modulate neuronal transmission through it and thus in conjunction with other modulatory influences to adjust such a gating process. Since NE was found to enhance evoked responses, it is suggested that it would enhance the flow of information through the dentate to CA3 and thus adjust the gating process to a more open setting.

In terms of hippocampal behavioral function, as mentioned above, O'Keefe and Nadel (1978; 1979) have suggested that the hippocampal formation is involved in spatial/cognitive mapping. Thus if one transposes from physiological function to behavioral function, it suggests that the noradrenergic input to the dentate gyrus modulates the flow or processing of spatial information through the dentate area. In this manner activation of the noradrenergic input would allow enhanced transmission or processing of spatial information. Thus if the noradrenergic input to the dentate gyrus is to have significant behavioral implications, one would predict that behavioral tasks requiring spatial/cognitive mapping would be facilitated with activation of the noradrenergic input or impaired with removal of it.

If the long-lasting effects of NE in the dentate gyrus contribute to long-lasting changes in hippocampal function, one would predict that some behavioral tasks involving long-lasting changes in spatial/cognitive mapping would be

enhanced by activation of the noradrenergic input or impaired by its removal.

Although these behavioral inferences are speculative they are also experimentally testable. Behavioral tasks involving spatial/cognitive mapping strategies are well documented (Seifert, 1983) as are noradrenergic stimulation and depletion techniques (Amaral & Sinnamon, 1977; Foote et al., 1983).

The proposed function of the noradrenergic input in the dentate gyrus is similar to the function of the central noradrenergic system proposed earlier by Kety (1970). Additionally, the suggested involvement of NE in long-lasting changes in hippocampal function is also similarly related to recent reports of the involvement of NE in neuronal plasticity. In these reports NE appears to play a permissive role in the establishment of relatively permanent changes in neuronal function (Kasamatsu, 1983; Keller & Smith, 1983; O'Shea et al., 1983; Watson & McElligott, 1983). With respect to Kety's proposal, it was suggested that synaptic release of NE in temporal contiguity with activation of specific pathways would result in enhancement of synaptic transmission in those specific pathways (Kety, 1970). Although initially this was suggested as a physiological mechanism to account for reinforcement in learning paradigms, subsequent work indicated instances of

intact learning in NE-depleted animals (Mason, 1981).

However it is now apparent that there may be more than one cellular mechanism underlying learning. So far at least three different cellular mechanisms have been demonstrated as taking place during learning in classical conditioning paradigms (Hawkins et al., 1983; Woody, Swartz & Gruen, 1978; Alkon, 1983). Thus different learning situations may involve different cellular mechanisms, some of which may not involve NE at all. On the other hand it appears that some but not all of the effects of NE are related to long-lasting changes in synaptic transmission. As mentioned above, long-lasting effects have been observed in roughly 25-60% of the cases in the dentate gyrus. Thus some of the effects of NE may be related to learning and/or long-lasting functional changes.

In trying to establish the behavioral and physiological relevance of the action of the central noradrenergic system, the hippocampal formation provides a model system from which general functional principles may be extracted. At the cellular level, its physiology and pharmacology have been intensively investigated and provide the background knowledge necssary for further investigations into the cellular and molecular mechanism of action of NE. At the neuronal systems level, its major inputs and outputs have been described and can be selectively manipulated.

Therefore the effects of NE could also be evaluated on these processes. Finally at the behavioral level, tasks involving spatial/cognitive mapping which require an intact hippocampal formation have been described. Hence, the effects of NE could also be evaluated at the behavioral level. Furthermore, behaviorally, the hippocampal formation appears to be involved in memory processes, thus it offers a unique opportunity to evaluate the contributions of NE to neuronal plasticity in a manner that could be directly relevant behaviorally. Therefore the hippocampus offers the opportunity to investigate and characterize the effects of NE in terms of molecular, cellular and behavioral actions. The opportunity to relate molecular to behavioral actions and vice versa will certainly prove to be beneficial in establishing the function of the central noradrenergic system.

Comparison of noradrenergic function in the dentate gyrus and in other CNS regions

Conceptually, the observed facilitation by NE of excitatory evoked responses in the dentate gyrus is similar to the concept of increases in signal-to-noise ratio of conventional afferent synaptic inputs which was proposed for the central action of NE by Woodward et al. (1979). However

in the dentate gyrus, NE has only been shown to enhance evoked excitatory responses. As mentioned above; in the increase in signal-to-noise ratio paradigm, NE usually produces a decrement in spontaneous activity (Woodward et al., 1979), and increases in inhibitory evoked responses have been shown in the cerebellum (Freedman et al., 1977) and somatosensory cortex (Waterhouse & Woodward, 1980). But in the dentate gyrus, neither the effects of NE on the spontaneous activity of granule cells nor its influence on inhibitory evoked responses have been systematically evaluated.

However, enhancement by NE of excitatory evoked responses similar to that produced in the dentate gyrus has been described in many other CNS areas. These include the cerebellum (Moises et al., 1979), somatosensory cortex (Waterhouse & Woodward, 1980), visual cortex (Kasamatsu & Heggelund, 1982), lateral geniculate nucleus of the thalamus (Nakai & Takaori, 1974; Rogawski & Aghajanian, 1980a), superior colliculus (Kayama & Sato, 1982), facial motor nucleus (McCall & Aghajanian, 1979), dorsal horn of the spinal cord (Hodge et al., 1981) and lumbar motor neurons (White & Neuman, 1980; 1983).

Thus the results obtained so far in the dentate gyrus are consistent with the concept of Woodward et al. (1979). However, the effects of NE on spontaneous and inhibitory

evoked activity in the dentate gyrus remain to be determined.

Another difference between the enhancement of excitatory evoked responses produced by NE in the dentate gyrus and elsewhere concerns the adrenergic receptor type mediating these effects. In the visual cortex (Waterhouse et al., 1983), somatosensory cortex (Waterhouse et al., 1981), thalamus (Rogawski & Aghajanian, 1982), facial motor nucleus (McCall & Aghajanian, 1979) and on lumbar motor neurons (Fung & Barnes, 1981; White & Neuman, 1983) the facilitation of excitatory evoked responses by NE is mediated via an alpha adrenergic receptor. However in the dentate gyrus the enhancement of excitatory responses was noted in the present report to be mediated via a beta receptor. In this respect the beta-mediated effects of NE in the dentate gyrus are not unique since similar beta-mediated effects have also been reported on hippocampal pyramidal cells (Mueller et al., 1981; Madison & Nicoll, 1982; Haas & Konnerth, 1983). Thus it would appear that, pharmacologically, the enhancement of excitatory evoked responses produced by NE in the CNS can be mediated via two adrenergic receptor types, alpha and beta. However, so far, the beta-mediated effects on excitatory responses have been observed only in the hippocampal formation.

Lastly, another functional difference in the action of

NE in the dentate gyrus is the relatively weak suppressant effect of NE. Although it was initially reported that the effects of NE on unit activity in the dentate gyrus were suppressant in nature (Stefanis, 1964; Segal & Bloom, 1976a), more detailed investigation indicated that mostly facilitatory effects are produced by NE on population field responses (Neuman & Harley, 1983; Harley et al., 1982; present report). Since the difference in results could be explained by the difference in neuronal response recorded, a confirmation of the facilitatory effects of NE on single granule cell responses is needed.

However, in the meantime, the present results and those of Neuman and Harley (1983) and Harley et al. (1982) strongly suggest that the function of the noradrenergic input to the dentate gyrus is to facilitate the neuronal responsivity of the granule cells to excitatory inputs. As mentioned above these facilitatory effects are not unique to the dentate gyrus, but they are in contrast to the inhibitory effects of NE produced elsewhere (see Van Dongen, 1981; Foote et al., 1983 for recent reviews). In fact the relatively weak alpha-mediated suppressant effects reported here for the dentate gyrus are in contrast to the relatively strong alpha-mediated suppressant effects in the CA1 region of the hippocampal formation (Mueller et al., 1981). These results suggest that noradrenergic function may vary from

region to region in the CNS and that it may also vary within subdivisions of a given CNS region.

Recommendations for further research

Further research on noradrenergic function in the dentate gyrus and the hippocampal formation is required at three levels: the cellular level, the neuronal systems level and the behavioral level.

At the cellular level, an investigation of the effect of NE on single granule cell responses recorded intra- or extracellularly would be welcomed. Since intracellular responses from the relatively small granule cells are more easily recorded in the slice preparation, the present report provides information which will be helpful for such investigations.

A question which should be addressed in intracellular experiments relates to the mechanism of action of NE. In such preparations the hypothesis of a decrement in spike accomodation could be tested with intracellular depolarizing currents or extracellular iontophoresis of glutamate during superfusion of NE. The question of the mechanism of action of NE effects on synaptic activation, however, requires a different experimental preparation. An investigation of the release of pre-loaded tritiated-glutamate from perforant

path fibers, before and after application of NE, would answer that question.

Other questions about the cellular mechanism of action of NE also require attention. A further pharmacological characterization of the beta-mediated effects of NE in terms of beta-1 and beta-2 adrenergic receptor subtypes using more selective agonists and antagonists would be helpful. Such characterization would further specify the mechanism of action of NE and would allow more specific and selective control of the conditions producing the effects of NE. Additionally in the in vitro slice, the effect of synaptically released NE could be investigated using amphetamine or tyramine, agents which produce pre-synaptic release of NE (Goodman Gilman et al., 1980). In this manner more physiological conditions would be approximated than with bath superfusion of NE.

The biochemical mediation of the cellular mechanism of action of NE also needs to be determined. Since other beta receptor mediated effects of NE elsewhere in the CNS have been suggested to be mediated via the synthesis of cAMP (Siggins, Hoffer & Bloom, 1971; Mueller et al., 1981), this possibility should also be evaluated in the granule cells. In the slice preparation, intracellular injection of cAMP or its derivatives as well as factors influencing its synthesis and metabolism could be achieved. In this manner the effect

of cAMP on granule cell evoked responses could be evaluated and compared with those of NE.

Finally it would be informative to determine the mechanism for biochemical mediation of the long-lasting effects of NE. Notably, the difference at the cellular level between effects which recover within 20 minutes and those which are long-lasting should be determined.

At the neuronal systems level, the effects of NE on the other afferent inputs to the granule cells should be evaluated. The effects should be assessed on inhibitory as well as excitatory inputs. This could be tested on unit responses with iontophoresis of the putative neurotransmitters in vitro or in vivo. Alternatively, the effects of NE could be assessed on the action of the electrically stimulated afferents on granule cells in vivo.

A systematic evaluation of the contributing factors to the establishment of long-lasting effects is also needed. It should be assessed whether the long-lasting effects are produced directly by NE or represent effects of NE of shorter duration which are facilitated by some other factor. As mentioned above, the lower incidence of long-lasting effects in vitro may be due to the absence of such facilitating factors in the slice preparation. In this respect, it was suggested that the involvement of vasopressin be assessed.

Also with respect to the long-lasting effects of NE, it should be evaluated whether these effects are activity-dependent, or associative, in nature. As mentioned above this question has important functional implications in terms of memory or learning models. Although the shorter duration effects of NE have been reported here to be activity-independent, the possibility remains that the long-lasting ones are activity-dependent.

Finally, at the neuronal systems level, the effects of NE should be evaluated in the other regions of the hippocampal formation. In this respect the effects of NE in the subicular complex and entorhinal area need to be determined. Additionally, in Ammon's horn, the effects of locus coeruleus stimulation should be evaluated on the pyramidal cell evoked responses. Although in vitro bath superfusion of NE was shown to produce alpha- and beta-mediated suppressant and facilitatory effects respectively, it should be assessed whether these effects are also produced in vivo with synaptic release of NE and what triggers one effect versus the other.

At the behavioral level, the most pressing questions concern the contribution of the observed physiological effects of NE to hippocampal function. It was suggested above, that on physiological grounds the function of the noradrenergic input to the dentate gyrus was to modulate the

flow of information through the dentate gyrus and consequently through the hippocampal formation. Thus it would appear that in this fashion the noradrenergic input could modulate hippocampal function. It is possible to test this suggestion by evaluating the effects of electrical stimulation of the locus coeruleus on the performance of rats on behavioral tasks known to require spatial/cognitive mapping stategies. Also, hippocampal depletion of NE with 6-OHDA or DSP-4 injections, or pharmacological antagonism should interfere with any effects of NE on such performance.

Finally, the contribution of the long-lasting effects of NE to hippocampal function needs to be determined. It was suggested above that if these long-lasting effects are behaviorally relevant then activation of the noradrenergic input during the performance of tasks requiring spatial/cognitive mapping should result, in the proper circumstances, in long-lasting changes in behavioral performance. Again in this context, locus coeruleus stimulation during the performance of hippocampal-related tasks could test this hypothesis. Furthermore, as with other instances in which NE has been shown to be necessary for neuronal plasticity, it should be assessed whether hippocampal NE-depletion prevents the learning of tasks involving spatial/cognitive mapping stategies.

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Appendix A

Brain slice perfusion chamber

FIGURE A-1. Perfusion chamber top view. Drawing of the chamber showing the chamber lid and the three recording chambers. Note that the inner resting stage and the outer square frame are represented separately. Refer to Figure A-3 for a view of the assembled recording chamber.

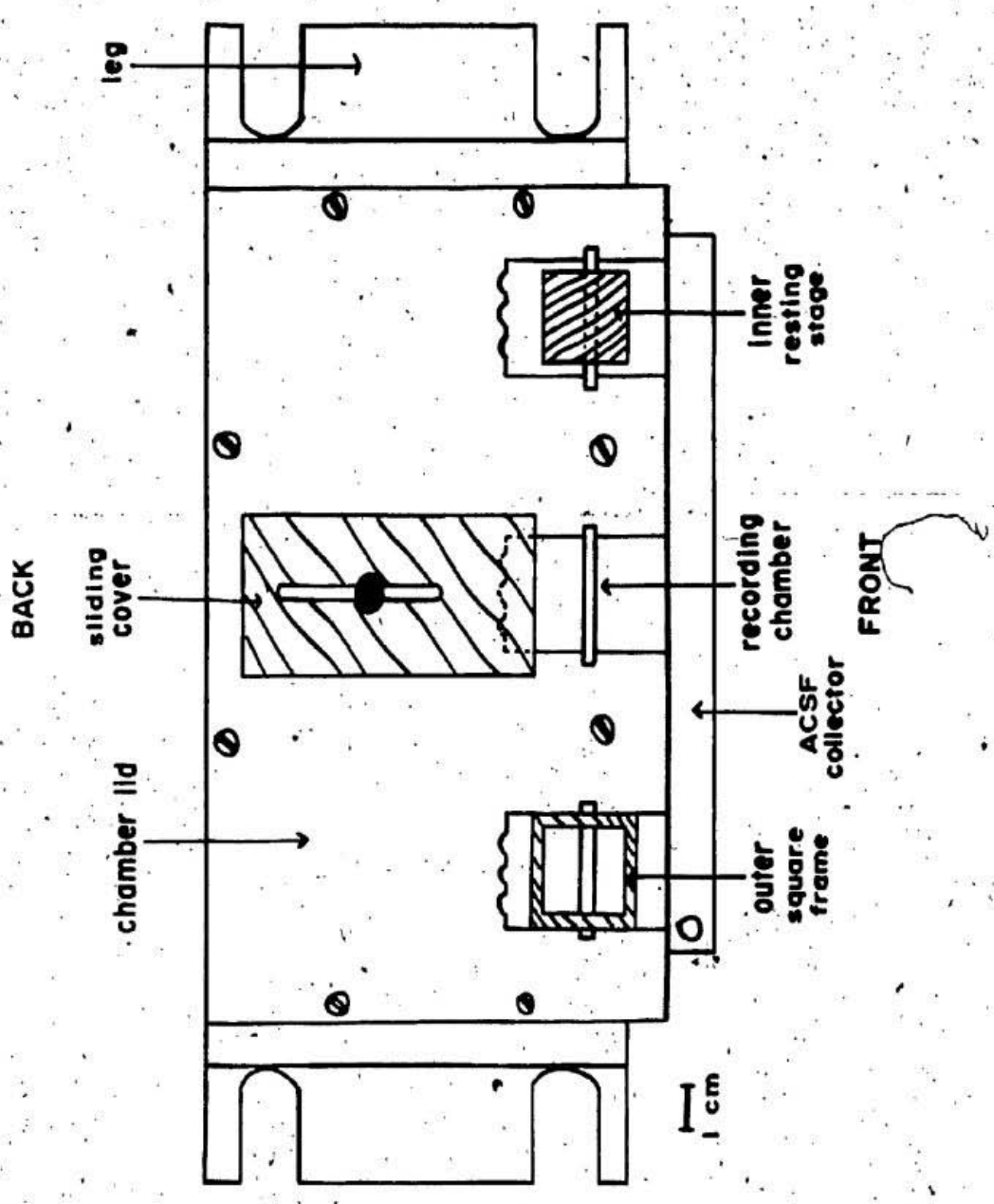

leg
inner resting stage
BACK
sliding cover
recording chamber
FRONT
ACSF collector
chamber lid
outer square frame
1 cm

FIGURE A-2. Perfusion chamber side view. Drawing of the chamber without the assembled recording chamber.

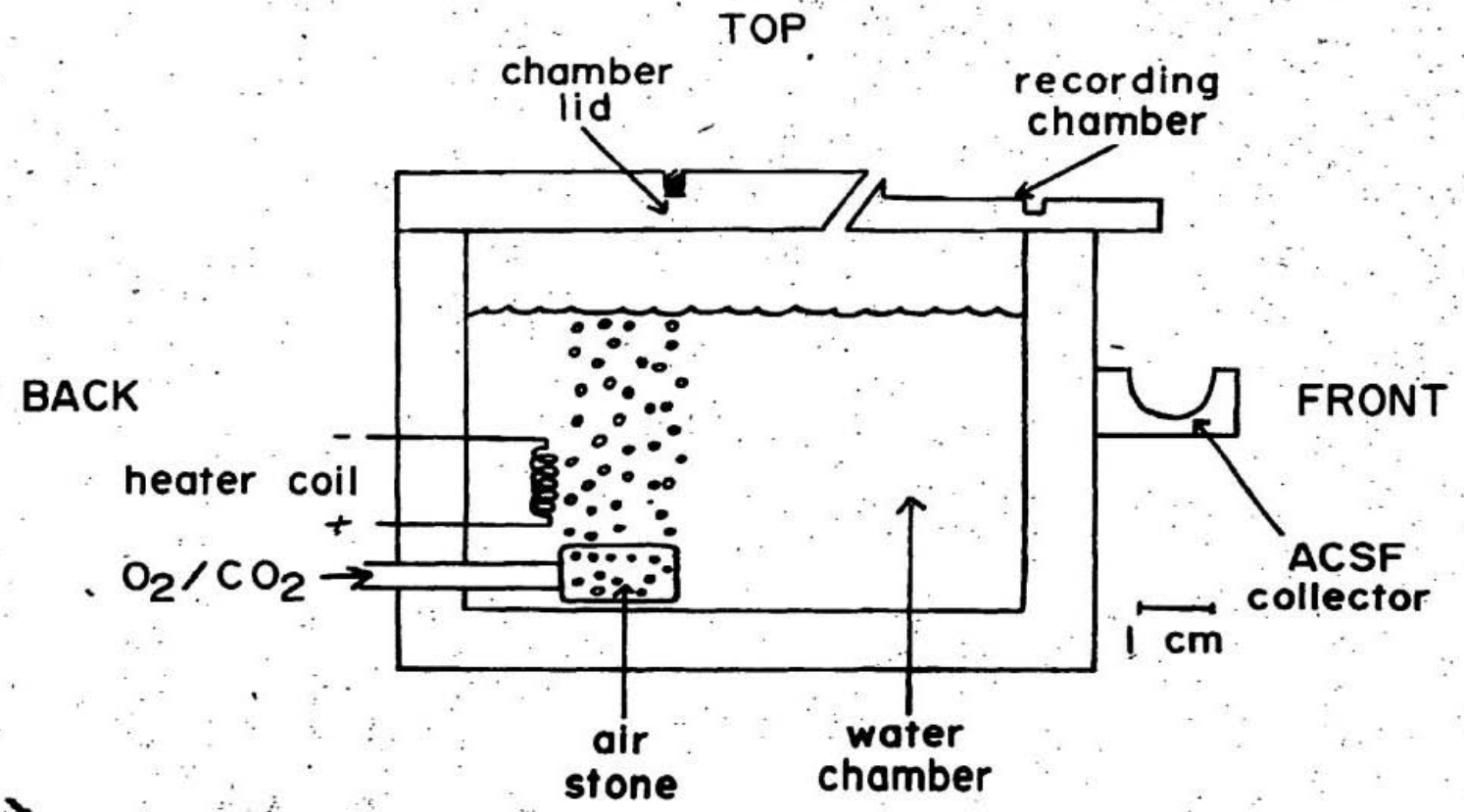

TOP
chamber lid
recording chamber
BACK
FRONT
heater coil
-
+
O_2/CO_2
ACSF collector
1 cm
air stone
water chamber
BOTTOM

FIGURE A-3. Recording chamber side view. Drawing through the assembled recording chamber in the chamber lid. Also a top view of the inner resting stage and the outer square frame showing the nylon netting between which the brain slice lies.

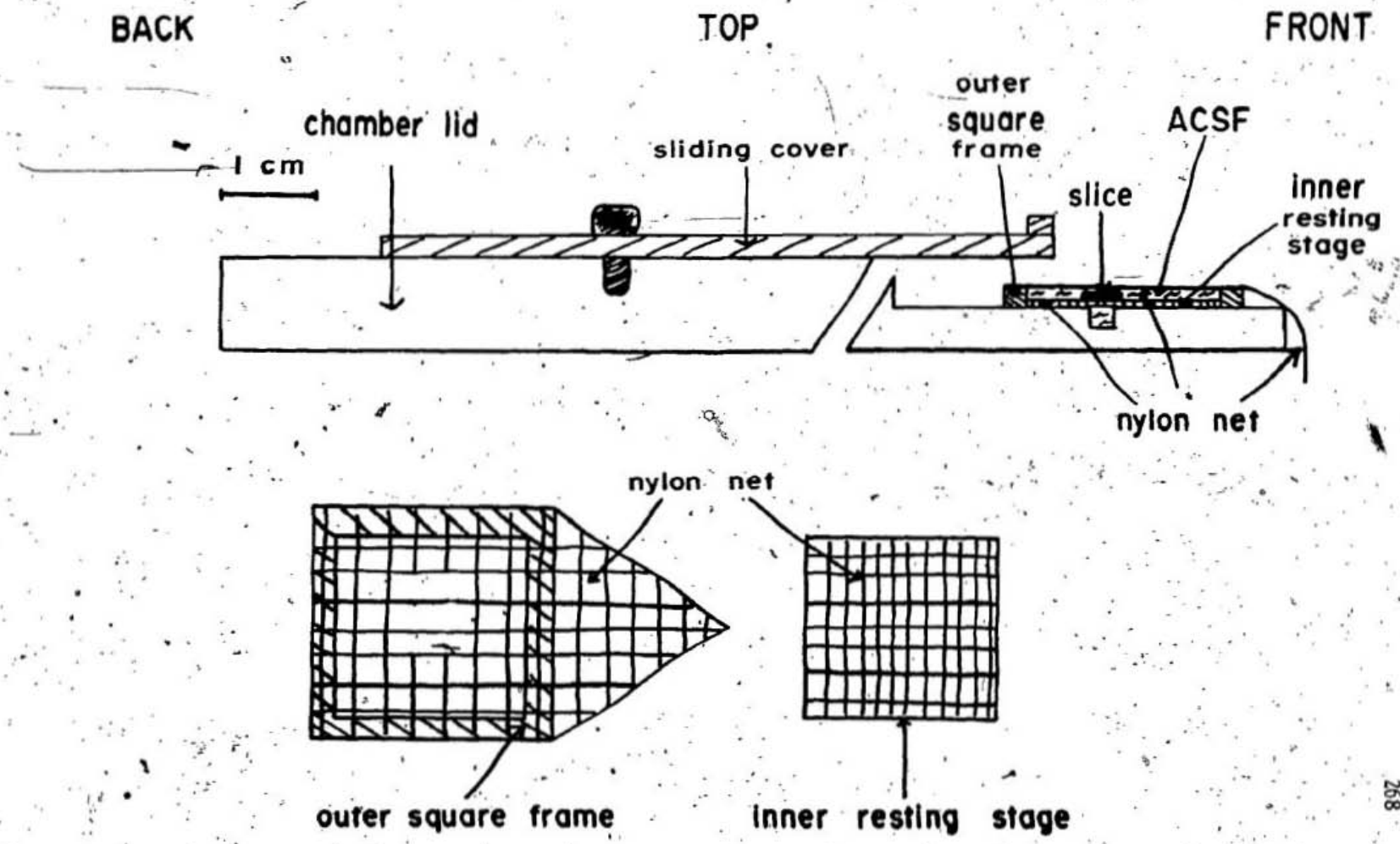
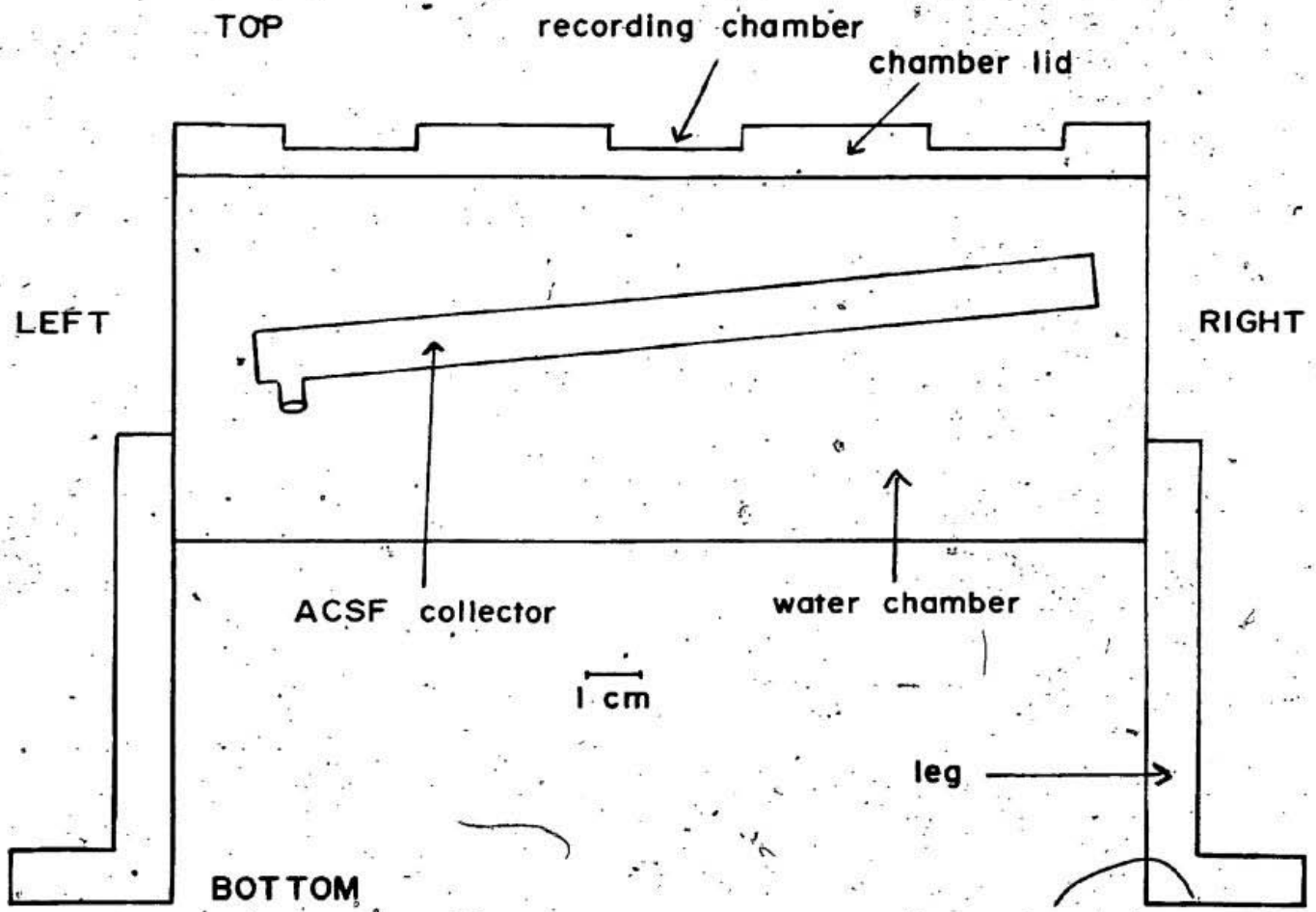

BACK
TOP
FRONT
chamber lid
1 cm
sliding cover
outer
square
frame
ACSF
slice
inner
resting
stage
nylon net
nylon net
outer square frame
inner resting stage

FIGURE A-4. Perfusion chamber front view.


TOP
recording chamber
chamber lid
LEFT
RIGHT
ACSF collector
water chamber
1 cm
leg
BOTTOM

Appendix B

DC proportional temperature controller

FIGURE B-1. Temperature controller circuit part one. First part of the circuit diagram of the DC proportional temperature controller used for the water chamber.

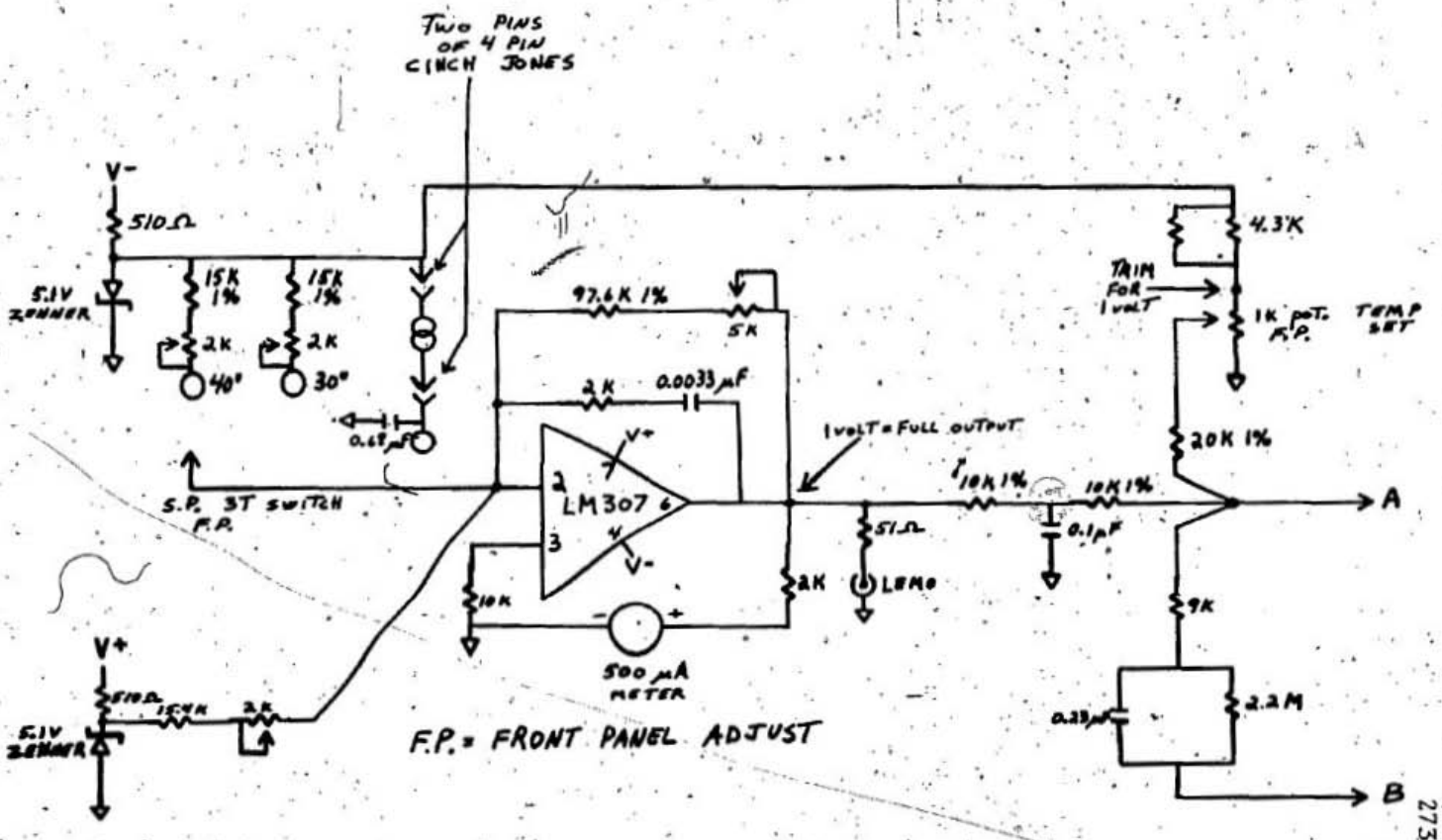
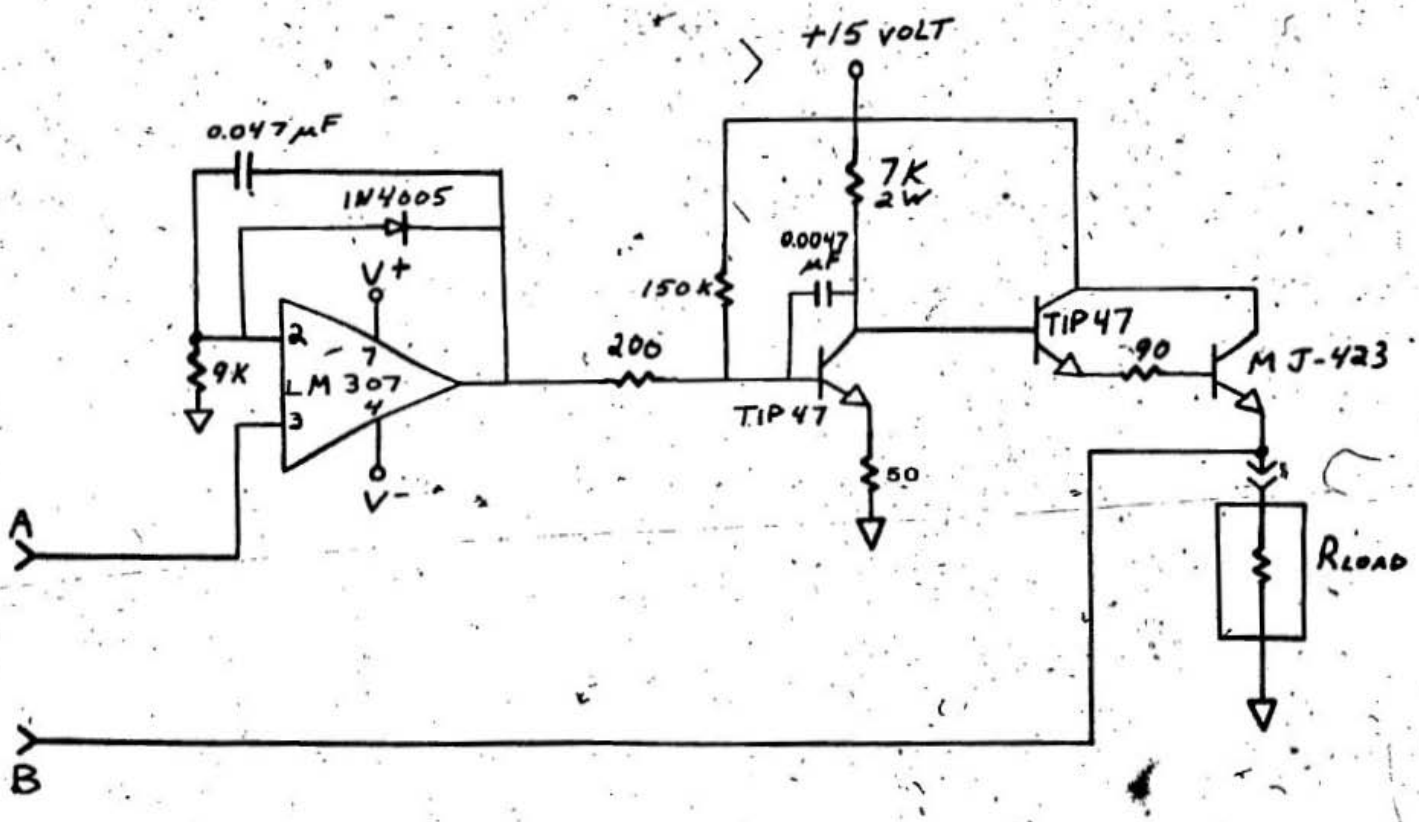

TWO PINS OF 4 PIN CINCH JONES
V-
510Ω
5.1V ZENNER
15K 1%
15K 1%
2K
2K
40°
30°
0.47μF
97.6K 1%
5K
2K
0.0033μF
S.P. 3T SWITCH F.P.
LM307
V+
V-
1VOLT = FULL OUTPUT
10K 1%
10K 1%
A
51Ω
LEMO
0.1μF
2K
10K
500μA METER
4.3K
TRIM FOR 1 VOLT
1K POT. F.P.
TEMP SET
20K 1%
9K
2.2M
0.33μF
B
V+
510Ω
1.5K
2K
5.1V ZENNER
F.P. = FRONT PANEL ADJUST

FIGURE B-2. Temperature controller circuit part two.
Second part of the circuit diagram of the DC proportional temperature controller.


+15 VOLT
0.047 μF
IN4005
V+
9K
2
3
7
4
LM 307
V-
200
150K
0.0047 μF
7K 2W
TIP47
90
MJ-423
TIP47
50
A
B
RLOAD

Appendix C

Computer programs for data acquisition and analysis

```
Screen #0
 0 ( Program called TI-PET to collect & analyze field )
 1 ( potentials. It is a hybrid of 8086 FORTH configured )
 2 ( for 64K memory with 32 BUFFERS; it contains a full )
 3 ( SCREEN EDITOR as well as an 8086 ASSEMBLER. TI-PET )
 4 ( uses machine language subroutines to digitize the )
 5 ( field potentials and the baseline measurements. It )
 6 ( performs on-line analysis of user set parameters on )
 7 ( the digitized WAVEFORM and it stores the RESULTS in )
 8 ( SCREEN format as well as sending them to the parallel )
 9 ( printer.  It does a D/A conversion of WAVEFORM at a )
10 ( frequency compatible for a pen recorder.  This version)
11 ( can only READ&PLACE in memory 3 SCREENS from disk at )
12 ( one time and then PRINT-OUT the RESULTS. Technical )
13 ( notes: A/D range -10 to +10 Volts; D/A range -2.5 to )
14 ( +2.5 Volts. )
15

Screen #1
 0 ( SET COUNTER 5 FOR A/D FREQUENCY ) HEX       : J-C ;
 1 0 VARIABLE WAVEFORM 202 ALLOT WAVEFORM 2+ WAVEFORM !
 2 (5653) CODE SET*COUNTER*5
 3 AL, # 17    MOV    ( SET DATA POINTER TO MASTER )
 4 1E , AL     OUT    ( MODE REGISTER )
 5 AL, # B0    MOV    ( SET MASTER MODE: BCD,F1/1,8-BIT, )
```

```
 6 1C , AL     OUT    ( REST 0 )
 7 AL, # 81    MOV
 8 1C , AL     OUT
 9 AL, # 05    MOV    ( SET DATA POINTER TO COUNTER )
10 1E , AL     OUT    ( MODE REGISTER 5 )
11 AL, # 31    MOV    ( SET COUNTER 5: F1 FALLING EDGE, )
12 1C , AL     OUT    ( RELOAD FROM LOAD, COUNT BCD DOWN )
13 AL, # 1B    MOV    ( REPETITIVELY, ACTIVE HIGH TC, )
14 1C , AL     OUT    ( NO GATING )
15 -->
```

Screen #2

```
 0 ( SET COUNTER 5 CONT'D )
 1 AL, # 75    MOV    ( SET COUNTER 5 LOAD TO 75 )
 2 1C , AL     OUT
 3 AL, # 00    MOV
 4 1C , AL     OUT
 5 AL, # 70    MOV    ( LOAD & ARM COUNTER 5 )
 6 1E , AL     OUT
 7 NEXT
 8 -->
 9
10
11
12
```

```
13
14
15

Screen #3
 0 ( A/D ROUTINE LOOK FOR TRIGGER )
 1 CODE A/D*ROUTINE
 2 AL, # 00     MOV    ( SET MUX TO CHANNEL 0 )
 3 12 , AL      OUT
 4 AL, # 0C     MOV    ( ENABLE EXTERNAL TRIGGER )
 5 10 , AL      OUT
 6 AL, 10       IN     ( READ STATUS )
 7 AL, # 80     AND    ( MASK OUT DONE )
 8 AL, # 00     CMP    ( LOOP TO READ STATUS UNLESS DONE )
 9 58A6         JZ
10 DX, # 0400   MOV    ( TRESHOLD TRIGGER= 1/2 OF - RANGE )
11 AL, 12       IN     ( READ LO BYTE )
12 CL, AL       MOV    ( & STORE IT )
13 -->
14
15

Screen #4
 0 ( A/D ROUTINE DIGITIZE AND TEST TRIGGER )
 1 AL, 14       IN     ( READ HI BYTE )
```

```
 2 AL, # 08     ADD    ( ADJUST HI BYTE BOTTOM=00 )
 3 CH, AL       MOV    ( & STORE IT )
 4 DX, CX       CMP    ( IF BELOW THRESHOLD LOOP BACK )
 5 58A6         JNB    ( TO READ STATUS )
 6 AL, # 04     MOV    ( DISABLE EXTERNAL TRIGGER )
 7 10 , AL      OUT
 8 CX, # 0100   MOV    ( SET A/D LENGTH )
 9 BX, # 5653   MOV    ( SET ADDRESS 1 OF WAVEFORM )
10 AL, # 01     MOV    ( SET MUX TO CHANNEL 1 )
11 12 , AL      OUT
12 AL, # 0C     MOV    ( ENABLE EXTERNAL TRIGGER )
13 10 , AL      OUT
14 AL, 10       IN     ( READ STATUS )
15 AL, # 80     AND    ( MASK OUT DONE. ) -->

Screen # 5
 0 ( A/D ROUTINE DIGITIZE & STORE IN WAVEFORM )
 1 AL, # 00     CMP    ( LOOP BACK TO READ STATUS )
 2 58D4         JZ     ( UNLESS DONE )
 3 AL, 12       IN     ( READ LO BYTE )
 4 [BX], AL     MOV    ( & STORE IT IN WAVEFORM )
 5 BX           INC    ( INCREMENT WAVEFORM POINTER )
 6 AL, 14       IN     ( READ HI BYTE )
 7 AL, # 08     ADD    ( ADJUST HI BYTE BOTTOM=0 )
 8 [BX], AL     MOV    ( & STORE IT IN WAVEFORM )
```

```
 9 BX          INC    ( INCREMENT WAVEFORM POINTER )
10 CX          DEC    ( DECREMENT & IF NOT END OF )
11 58D4        JNZ    ( CONVERSION,  LOOP TO READ STATUS )
12 AL, # 04    MOV    ( DISABLE EXTERNAL TRIGGER )
13 10 , AL     OUT
14 NEXT
15 -->
```

Screen #6

```
 0 ( SET COUNTER 4 FOR D/A FREQUENCY )
 1 : SET*COUNTER*4 ( SET COUNTER 4 FOR 34 Hz OUTPUT )
 2   17 1E PCI ( SET POINTER TO MASTER MODE )
 3   E0 1C PCI ( SET MASTER: BIN, 8-BITS, 1MHz/4096 )
 4   01 1C PCI
 5   04 1E PCI ( SET POINTER TO COUNTER 4 MODE )
 6   31 1C PCI ( SET COUNTER 4: NO GATING, RELOAD FROM LOAD )
 7   1E 1C PCI ( COUNT BCD, DOWN, REPETITIVELY, ACTIVE HIGH )
 8   07 1C PCI ( SET COUNTER 4 LOAD TO 7 )
 9   00 1C PCI
10   68 1E PCI ( LOAD & ARM COUNTER 4 ) ;
11
12
13
14
15 -->
```

```
Screen # 7
 0 ( D/A ROUTINE CONVERT WAVEFORM )
 1 : STATUS  ( test STATUS REGISTERS for OUT4=1 )
 2       1E P@ 10 AND   ;
 3 : OUT*PORT*1 ( addr  ---      : D/A of WORD at addr )
 4       ( 2's complement HI byte, out HI, out LO on #1 )
 5       DUP 1+ C@ 08 - F0 PC! C@ F1 PC! ;
 6 : ARM*COUNTER*4 ( LOAD & ARM COUNTER 4 ) 68 1E PC! ;
 7 : -D/A  ( out most - value ) F8 F0 PC! 00 F1 PC! ;
 8 : +D/A  ( out most + value ) 07 F0 PC! FF F1 PC! ;
 9 : D/A   ( D/A conversion of WAVEFORM at 34 Hz )
10         ( on D/A port 1 )
11       SET*COUNTER*4 WAVEFORM @ DUP 202 + -D/A SWAP
12       DO BEGIN STATUS UNTIL ARM*COUNTER*4 I OUT*PORT*1
13       2 +LOOP -D/A 80 0 DO NOOP LOOP +D/A ;
14
15 -->


Screen # 8
 0 ( VARIABLES & CONVERT )
 1 : V VARIABLE ;
 2 0 V SCRBUFFER 0 V FIELDPOT 0 V 1EPSP 0 V SAVED
 3 0 V LATADD 0 V ONSETSPIKE 0 V PEAKSPIKE 0 V POINT
 4 0 V POPSPIKE 0 V AMPMAX 0 V PPOINT 0 V GAIN 0 V LATADDR
```

```
 5 0 V AMPMINI 0 V VOLTBASE 2 ALLOT 0 V 1POINT
 6 0 V 2POINT 0 V 3POINT DECIMAL
 7 : CONVERT ( conversion of amplitude value to uVolts )
 8    20000 M* 4095 M/ SWAP DROP 1000 M* GAIN @ M/MOD ROT
 9    DROP 10000000. GAIN @ M/MOD ROT DROP DMINUS D+ ;
10 HEX
11 -->
12
13
14
15
```

Screen # 9

```
 0 ( USER SET PARAMETERS )
 1 : GETNUMBER ( EXPECTs a string from keyboard & converts )
 2             ( it to a signed double number & leaves )
 3             ( it on the stack )
 4     S0 @ 50 EXPECT 0 IN ! HERE 20 WORD NUMBER ;
 5 : .?USER ( instructions to user to set parameters )
 6      ." Enter latencies at which to measure the field "
 7      ." potential's parameters " CR ." in microseconds "
 8   ." and it will be rounded to multiples of 75 usec. " CR
 9      ." Terminate each entry with <CR>. " CR ;
10 : .?EPSP ( ask for EPSP time window )
11 ." 1. Choose EPSP SLOPE interval: " CR ." [ Population "
```

```
12    ." spike onset latency ] - xxxxx usec [post-stim.]-? "
13       CR ;
14 : ROUND75USEC 26 + 4B / 4B * DUP . ." usec. " ;
15 -->

Screen # 10
 0 ( USER SET PARAMETERS CONT'D )
 1 : .?ONSET ( ask for ONSET LATENCY time window )
 2    ." 2. Choose POPULATIN SPIKE ONSET LATENCY interval: "
 3    CR ." Between 975 usec & xxxxx usec ? " CR ;
 4 : .?PEAK ( ask for PEAK LATENCY time window )
 5    ." 3. Choose POPULATION SPIKE PEAK LATENCY interval: "
 6    CR ." Between  ONSET LATENCY  and xxxxx usec ? " CR ;
 7 : .?GAIN ( ask for amplifier GAIN )
 8       ." 4. What is the amplifier gain ? " CR ;
 9 : +D->S ( convert signed double length to signed single )
10          1 M/ SWAP DROP ;
11 : SETEPSP ( set EPSP SLOPE parameter )
12       .?EPSP GETNUMBER +D->S ROUND75USEC 1POINT ! CR ;
13 : SETONSET ( set ONSET LATENCY parameter )
14       .?ONSET GETNUMBER +D->S ROUND75USEC 2POINT ! CR ;
15 -->

Screen # 11
 0 ( USER SET PARAMETERS CONT'D )
```

```
1 : SETPEAK ( set PEAK LATENCY parameter )
2       .?PEAK GETNUMBER +D->S ROUND75USEC 3POINT ! CR ;
3 : SETGAIN ( set amplifier GAIN )
4       .?GAIN GETNUMBER +D->S GAIN ! CR ;
5 : ?PARAMETERS ( ask user to set PARAMETERS )
6       CR .?USER SETEPSP SETONSET SETPEAK SETGAIN ;
7
8
9
10
11
12
13
14
15 -->
```

Screen # 12

```
0 ( SET BUFFERS )
1 : SETBUFFERS ( store POTENTIAL # & initialize BUFFERS )
2     EMPTY-BUFFERS EB80 USE ! FB7C PREV ! EFA0 EB80 DO
3     SCRBUFFER @ I !  1 SCRBUFFER +! 84 +LOOP ;
4
5
6 -->
7
```

```
 8
 9
10
11
12
13
14
15
```

Screen # 13

```
 0 ( CREATE DATA & FILL BUFFER )
 1 0 V DATA 400 ALLOT ( create RESULTS BUFFER )
 2 DATA '2+ DATA !
 3 : FILLBUFFER ( transfer from DATA to BUFFERS )
 4                ( preserve STATUS CELL & trailing blanks )
 5       8 0 DO I 80 * DATA @ + I 84 * USE @ 2+
 6       + 80 CMOVE LOOP ;
 7 : SET ( set DATA pointers to TOP, fill excess )
 8   ( memory with 0's ) ?PARAMETERS 1 FIELDPOT ! DATA @ 400
 9  + DUP 6 - 1EPSP ! DUP 8 - ONSETSPIKE ! DUP A - PEAKSPIKE
10   ! 0E - POPSPIKE ! 8000 SCRBUFFER ! 0 DATA @ C! 0 DATA @
11       1+ C! DATA @ DUP 3FE + 0 SWAP C! 3FF + 0 SWAP C! ;
12
13
14
```

```
15 -->

Screen # 14
 0 ( CONVERT USER SET LATENCIES )
 1 : LAT->ADDR  ( convert latencies to WAVEFORM addresses )
 2     4B / 1- 2 * WAVEFORM @ + ;
 3 : CONVERT.LATENCIES  ( convert EPSP, ONSET & PEAK )
 4      ( latencies ) 1POINT @ LAT->ADDR 1POINT !
 5      2POINT @ LAT->ADDR 2POINT !
 6      3POINT @ LAT->ADDR 3POINT !   ;
 7 : ADDR->LAT (convert WAVEFORM addresses to latencies )
 8      WAVEFORM @ - 2 / 1+ 4B * ;
 9 : POPMAX ( find & store max amplitude & its latency )
10       ( between 975 usec and user set 2POINT )
11    3CF LAT->ADDR DUP LATADD ! DUP POINT ! DUP @ AMPMAX !
12   2POINT @ 2+ SWAP DO LATADD @ @ POINT @ 2+ DUP POINT ! @
13      < IF POINT @ DUP LATADD ! @ AMPMAX ! ENDIF 2 +LOOP
14      LATADD @ ADDR->LAT ONSETSPIKE @ ! ;
15 -->

Screen # 15
 0 ( DOUBLE-LENGTH OPERATIONS )
 1 : 2SWAP >R ROT ROT R> ROT ROT ;
 2 : 2ROT >R >R 2SWAP R> R> 2SWAP ;
 3 : 2DROP DROP DROP ;
```

```
 4 : D*10 ( Multiply a double-length by 10, double-length )
 5  ( product ) 2DUP 09 0 DO 2DUP 2ROT D+ 2SWAP LOOP 2DROP ;
 6 : MD* ( d1  u1  ---  d2 : d1*u1=d2; d2max = +2147483648 )
 7     >R 2DUP R> DUP CASE
 8      0 OF DROP 2DROP 2DROP 0. ENDOF
 9      1 OF DROP 2DROP          ENDOF
10   >R  1 DO 2DUP 2ROT D+ 2SWAP LOOP 2DROP R> ENDCASE ;
11 : D* ( d1 ud2  ---  d3 : d1*ud2=d3; d3max = +2147483648 )
12      >R >R 2DUP R> MD* 4 PICK 4 PICK R> MD* D*10
13      D+ ROT DROP ROT DROP ;
14
15 -->

Screen # 16
 0 ( MEASURE EPSP SLOPE )
 1 : EPSPSLOPE ( measure EPSP SLOPE between POPMAX & user )
 2  ( set 1POINT & store it; SLOPE is in microvolts/second )
 3        ( ???? keep d-length: use M/MOD ???? ) ( YES )
 4        ( ???? keep signed: use M/ ???? ) ( NO )
 5       AMPMAX @ CONVERT 1POINT @ @ CONVERT D- 3E8 MD*
 6      ONSETSPIKE @ @ 1POINT @ ADDR->LAT -
 7       M/MOD ROT DROP 1EPSP @ 2! ;
 8 -->
 9
10
```

```
11
12
13
14
15

Screen # 17
 0 ( MEASURE PEAK & SPIKE AMPLITUDE )
 1 : POPMINI ( find the value & latency of the minimum )
 2           ( between POPMAX & 3POINT & store them.)
 3   LATADD @ DUP LATADDR ! DUP PPOINT ! AMPMAX @ AMPMINI !
 4     3POINT @ 2+ SWAP DO
 5     LATADDR @ @ PPOINT @ 2+ DUP PPOINT ! @ > IF
 6     PPOINT @ DUP LATADDR ! @ AMPMINI ! ENDIF 2 +LOOP
 7     LATADDR @ ADDR->LAT PEAKSPIKE @ ! ;
 8 : SPIKEAMP ( Find population spike amplitude in uvolts )
 9            ( store it )
10   AMPMAX @ CONVERT AMPMINI @ CONVERT DMINUS D+ POPSPIKE
11     @ 21 ;
12 -->
13
14
15

Screen # 18
```

```
 0 ( RESET POINTERS, PRINT RESULTS )
 1 : RESET ( Advance DATA pointers )
 2     1 FIELDPOT +! -0C 1EPSP +! -0C ONSETSPIKE +!
 3     -0C PEAKSPIKE +! -0C POPSPIKE +! 0 SAVED ! ;
 4 : HEADING ( heading for PRINT-OUT of results )
 5     CR 4 SPACES ." FIELD" 7 SPACES ." EPSP" F SPACES
 6     ." POPULATION SPIKE" CR 2 SPACES ." POTENTIAL" 5
 7     SPACES ." SLOPE" 4 SPACES ." ONSET LATENCY" SPACE
 8     ." PEAK LATENCY" 2 SPACES ." AMPLITUDE" CR ;
 9 : RESULTS ( print RESULTS )
10     3 SPACES FIELDPOT @ 5 .R 7 SPACES 1EPSP @ 2@ 6 D.R 6
11   SPACES ONSETSPIKE @ @ 6 .R 8 SPACES PEAKSPIKE @ @ 6 .R
12     7 SPACES POPSPIKE @ 2@ 6 D.R CR ;
13 -->
14
15


Screen # 19
 0 ( A/D FOR BASELINE )
 1 0 VARIABLE BASELINE*A/D 66 ALLOT ( Create array to )
 2 BASELINE*A/D 2+ BASELINE*A/D !  ( store BASELINE values )
 3 CODE A/D*CONVERSION   ( addr  n  ---          )
 4  ( Expects destination address & length of A/D on stack )
 5     ( will perform A/D at rate set by external trigger )
 6 CX       POP   ( set A/D length )
```

```
 7 BX        POP   ( set destination address )
 8 AL, # 01  MOV   ( set MUX to 1 )
 9 12 , AL   OUT
10 AL, # 0C  MOV   ( enable external trigger )
11 10 , AL   OUT
12 AL, 10    IN    ( read STATUS )
13 AL, # 80  AND   ( mask out DONE bit )
14 -->
15
```

```
Screen # 20
 0 ( BASELINE A/D ROUTINE CONT'D )
 1 AL, # 00  CMP   ( loop to read STATUS )
 2 685D      JZ    ( unless DONE )
 3 AL, 12    IN    ( read LO byte )
 4 [BX], AL  MOV   ( store it in ARRAY )
 5 BX        INC   ( increment ARRAY pointer )
 6 AL, 14    IN    ( read HI byte )
 7 AL, # 08  ADD   ( adjust HI byte, bottom=0 )
 8 [BX], AL  MOV   ( store it in ARRAY )
 9 BX        INC   ( increment ARRAY pointer )
10 CX        DEC   ( decrement A/D counter )
11 685D      JNZ   ( jump to read STATUS unless counter=0 )
12 AL, # 04  MOV   ( disable EXTERNAL TRIGGER )
13 10 , AL   OUT
```

```
14 NEXT
15

Screen # 21
 0 ( BASELINE & DOIT command )
 1 : BASELINE ( average 50 consecutive points & store )
 2            ( converted voltage )
 3    0. VOLTBASE 2! SET*COUNTER*5 BASELINE*A/D @ 32
 4    A/D*CONVERSION BASELINE*A/D @ DUP 68 + SWAP 2+ DO
 5    I @ CONVERT VOLTBASE 2@ D+ VOLTBASE 2! 2 +LOOP
 6    VOLTBASE 2@ 32 M/ S->D VOLTBASE 2! DROP ;
 7 : DOIT ( enable BASELINE, A/D, analysis of field )
 8          ( potential PRINT-OUT of RESULTS and D/A )
 9    BASELINE SET*COUNTER*5 A/D*ROUTINE POPMAX EPSPSLOPE
10    POPMINI SPIKEAMP PRINTER RESULTS CONSOLE RESULTS
11    RESET D/A ;
12 -->
13
14
15

Screen # 22
 0 ( RESET pointers following disk storage & )
 1 ( PRINT PARAMETERS )
 2 : ?ENDBUFFER ( test for end of data buffer )
```

```
 3       DATA @ A - POPSPIKE @ = ;
 4 : RESETBLOCKS ( RESET pointers to start of DATA )
 5       DATA @ 400 + DUP 6 - 1EPSP ! DUP 8 - ONSETSPIKE !
 6       DUP A - PEAKSPIKE ! 0E - POPSPIKE ! 0 DATA @ C!
 7       0 DATA @ 1+ C! ;
 8 : .GAIN ( print user set GAIN )
 9       ." AMPLIFIER GAIN IS " GAIN @ . CR CR ;
10 : .EPSP ( print user set EPSP latency )
11       ." EPSP SLOPE IS MEASURED BETWEEN " 1POINT @ U.
12       ." uSEC AND POPULATION " CR ." SPIKE ONSET LATENCY "
13       CR CR ;
14 -->
15
```

```
Screen # 23
 0 ( PRINT PARAMETERS CONT'D )
 1 : .ONSET ( print user set ONSET latency )
 2       ." POPULATION SPIKE ONSET IS MEASURED WITHIN THE "
 3       ." INTERVAL " CR ." 975 TO " 2POINT @ U. ." uSEC"
 4       CR CR ;
 5 : .PEAK ( print user set PEAK latency )
 6       ." POPULATION SPIKE PEAK IS MEASURED WITHIN THE "
 7       ." INTERVAL " CR ." OF ONSET LATENCY TO " 3POINT @
 8       U. ." uSEC " CR CR ;
 9 : .PARAMETERS ( print user set PARAMETERS )
```

```
10      CR ." USER DEFINED PARAMETERS " CR
11      ." ********************************************" CR
12      .GAIN .EPSP .ONSET .PEAK
13 ." ********************************************" CR ; -->
14
15
```

Screen # 24

```
 0 ( LE CREMAGE )
 1 : LETSGO ( run PROGRAM until a key is depressed )
 2    DECIMAL SET SETBUFFERS PRINTER .PARAMETERS HEADING
 3  CONSOLE HEADING CONVERT.LATENCIES BEGIN DOIT ?ENDBUFFER
 4      IF FILLBUFFER FLUSH RESETBLOCKS SETBUFFERS 1 SAVED !
 5      ENDIF ?TERMINAL UNTIL
 6     SAVED @ IF PRINTER ." LAST SCREEN WAS #" SCRBUFFER
 7     @ 8009 - 8 / . ." AND IT WAS FULL " CR CONSOLE
 8     ELSE 1EPSP @ 4 + DATA @ 2+ DO B1 I C! LOOP
 9     FILLBUFFER FLUSH PRINTER ." LAST SCREEN WAS #"
10     SCRBUFFER @ 8001 - 8 / . CR CONSOLE DECIMAL ENDIF ;
11 -->
12
13
14
15
```

```
Screen # 25
 0 ( VARIABLES FOR RESULTS ANALYSIS )
 1 0 VARIABLE MEMPOINTER 0 VARIABLE FPOT
 2 0 VARIABLE SCR1 0 VARIABLE SCR#
 3 0 VARIABLE 1FIELDPOT
 4 0 VARIABLE BUFPOINTER
 5 : .1ST ( print 1st SCREEN & 1st FIELD POTENTIAL # in )
 6  ( memory ) CR ." 1st SCREEN IN MEMORY IS #" SCR1 @ . CR
 7     ." 1st FIELD POTENTIAL IN MEMORY IS #"
 8       1FIELDPOT @ . CR ;
 9 : BLOCK0 ( move 1st BUFFER of SCREEN into memory )
10        SCR# @ 8 * BLOCK 2+ MEMPOINTER @ 2+
11        3FE - 7E CMOVE ;
12 --->
13
14
15


Screen # 26
 0 ( READ & MOVE DATA IN RAM )
 1 : BLOCK1-6-( move BUFFERS 1-6 of SCREEN into memory )
 2       7 1 DO SCR# @ 8 * I + BLOCK MEMPOINTER @
 3       2+ 8 I - 80 * - 80 CMOVE LOOP ;
 4 : BLOCK7  ( move BUFFER 7 of SCREEN into memory )
 5       SCR# @ 8 * 7 + BLOCK MEMPOINTER @
```

```
 6      7E - 7E CMOVE ;
 7 : SETUSE ( set USE & PREV to point to lower BUFFER )
 8     EB80 USE ! FB7C PREV ! ;
 9 : READ&PLACE ( n1  n2  ---   : READ from disk, SCREEN n1 )
10       ( to SCREEN n2 & PLACE in memory from FC00 down )
11      FC00 MEMPOINTER ! 1+ SWAP DUP DUP SCR1 ! 55 * 1+
12      1FIELDPOT ! .1ST DO I SCR# ! SETUSE BLOCK0 .
13      BLOCK1-6 BLOCK7 -3FC MEMPOINTER +! LOOP ;
14 -->
```

```
Screen # 27
 0 ( PRINT-OUT DATA FROM MEMORY )
 1 : .RESULTS ( PRINT DATA placed in memory by READ&PLACE )
 2      3 SPACES FIELDPOT @ 5 .R
 3      7 SPACES FC0C FPOT @ 0C * - 4 - 2@ 6 D.R
 4      6 SPACES FC0C FPOT @ 0C * - 6 - @ 6 .R
 5      8 SPACES FC0C FPOT @ 0C * - 8 - @ 6 .R
 6      7 SPACES FC0C FPOT @ 0C * - 0C - 2@ 6 D.R CR ;
 7 : PRINT-OUT ( n1  n2  ---       : print DATA from FIELD )
 8          ( POTENTIAL # n1 to FIELD POTENTIAL # n2 )
 9      1+ SWAP DUP FPOT ! PRINTER HEADING DO I FIELDPOT !
10      .RESULTS 1 FPOT +! LOOP ." THE END " CR CONSOLE ;
11 DECIMAL ;S
12
13
```

```
14
15

Screen # 28
 0 ( Utility to reset bootup literals and rewrite )
 1 ( TI-PET.CMD. May be used to add new compiled functions )
 2 ( to the load file.  Requires a dummy FORTH.CMD on the )
 3 ( disk, because SAVE rewrites FORTH.CMD. After the disk )
 4 ( storage REN TI-PET.CMD=FORTH.CMD. )
 5 ( To execute type: 28 LOAD <RETURN> )
 6 FORTH DECIMAL
 7 LATEST 12 +ORIGIN ! ( top NFA )
 8 HERE 28 +ORIGIN ! ( FENCE )
 9 HERE 30 +ORIGIN ! ( DP )
10 VOC-LINK @ 32 +ORIGIN ! ( VOC-LINK )
11 SAVE
12 ;S
13
14
15

Screen # 30
 0 ( ANALYZE is a program which moves data from DISK to )
 1 ( RAM.  DATA is stored from 1FC00 down [seg 0000, off )
 2 ( FC00] starting with SCREEN #0 & possibly up to )
```

```
 3 ( SCREEN #63. CONTROL averages for a control period )
 4 ( and measures the 95% confidence interval.  %CONTROL )
 5 ( measures the means of blocks of 3 data points and )
 6 ( expresses them as % of control.  ANALYZE is a )
 7 ( modified version of 8086 FORTH configured for 128K )
 8 ( of memory. )  -->
 9
10
11
12
13
14
15
```

Screen # 31

```
 0 ( VARIABLES & CONSTANTS ) HEX
 1 0 VARIABLE MEMPOINTER 0 VARIABLE FPOT
 2 0 VARIABLE SCR1 0 VARIABLE SCR#
 3 0 VARIABLE 1FIELDPOT 0 VARIABLE BUFPOINTER
 4 0 VARIABLE FIELDPOT
 5 0 VARIABLE LENGTH 0 VARIABLE CTRLONSET 2 ALLOT
 6 0 VARIABLE CTRLPOP 2 ALLOT 0 VARIABLE CTRLEPSP 2 ALLOT
 7 0 VARIABLE 1CTRL 0 VARIABLE 2CTRL 0 VARIABLE POPCOUNT
 8 0 VARIABLE POPPOINT 0 VARIABLE MEANONSET
 9 0 VARIABLE MEANEPSP 2 ALLOT 0 VARIABLE MEANPOP 2 ALLOT
```

```
10 0 VARIABLE T
11 -->
12
13
14
15

Screen # 32
 0 ( READ & MOVE DATA in RAM )
 1 : .1ST ( print 1st SCREEN & 1st FIELD POTENTIAL # in )
 2   ( memory ) CR ." 1st SCREEN IN MEMORY IS #" SCR1 @ . CR
 3      ." 1st FIELD POTENTIAL IN MEMORY IS #"
 4      1FIELDPOT @ . CR ;
 5 : READ&PLACE ( n1  n2  ---  : READ from disk, SCREEN n1 )
 6      ( to SCREEN n2 & PLACE in memory from 1FC00 down )
 7      F804 MEMPOINTER ! 1+ SWAP DUP DUP SCR1 ! 55 * 1+
 8    1FIELDPOT ! .1ST DO 1000 I BLOCK 2+ 0000 MEMPOINTER @
 9      3FC CMOVEL -3FC MEMPOINTER +! LOOP ;
10
11 -->
12
13
14
15
```

```
Screen # 33
 0 ( print RESULTS & HEADING )
 1 : .RESULTS ( PRINT DATA placed in memory by READ&PLACE )
 2      3 SPACES FIELDPOT @ 5 .R
 3      7 SPACES 0000 FCOC FPOT @ 0C * - 2 - @L
 4               0000 FCOC FPOT @ 0C * - 4 - @L 6 D.R
 5      6 SPACES 0000 FCOC FPOT @ 0C * - 6 - @L 6 .R
 6      8 SPACES 0000 FCOC FPOT @ 0C * - 8 - @L 6 .R
 7      7 SPACES 0000 FCOC FPOT @ 0C * - 0A - @L
 8               0000 FCOC FPOT @ 0C * - 0C - @L 6 D.R CR ;
 9 : HEADING ( heading for PRINT-OUT of results )
10      CR 4 SPACES ." FIELD" 7 SPACES ." EPSP" F SPACES
11      ." POPULATION SPIKE" CR 2 SPACES ." POTENTIAL" 5
12      SPACES ." SLOPE" 4 SPACES ." ONSET LATENCY" SPACE
13      ." PEAK LATENCY" 2 SPACES ." AMPLITUDE" CR ;
14 -->
15

Screen # 34
 0 ( PRINT-OUT of DATA from memory & printer control )
 1 ( commands )
 2 : PRINT-OUT (  n1  n2  ---      : print DATA from FIELD )
 3              ( POTENTIAL #n1 to FIELD POTENTIAL #n2 )
 4      1+ SWAP DUP FPOT ! PRINTER HEADING DO I FIELDPOT !
 5      .RESULTS 1 FPOT +! LOOP ." THE END " CR CONSOLE ;
```

```
 6 : D->S ( 32-bit number into 16-bit ) DROP ;
 7 : HOR132 ( 132 characters per line ) OF EMIT ;
 8 : HOR80 ( 80 characters per line ) 12 EMIT ;
 9 : LFSHORT ( line-feed = 1/10 inch ) 1B EMIT ." 1" ;
10 : LFRET ( line-feed = 1/6 inch ) 1B EMIT ." 2" ;
11
12 -->
13
14
15
```

```
Screen # 35
 0 ( double-length global & print CONTROL DATA )
 1 : 2@L     (  seg   off   ---   d   )
 2           ( fetch-long double-length number [32-bits] )
 3       2DUP 2+ @L ROT ROT @L ;
 4 : 2       (  d   seg   off   ---       )
 5           ( store-long double length number [32-bits] )
 6       2DUP 5 ROLL ROT ROT  2+  ;
 7
 8 : PRINTCONTROL ( print means for CONTROL period )
 9       PRINTER HOR132 CR ." POTENTIAL #" 1CTRL @ . ." TO "
10     2CTRL @ . 3 SPACES ." MEAN EPSP =" CTRLEPSP 2@ D. 3
11       SPACES ." MEAN ONSET = " CTRLONSET 2@ D. 3 SPACES
12    ." MEAN POP. SPIKE = " CTRLPOP 2@ D. CR HOR80 CONSOLE ;
```

```
13
14 -->
15

Screen # 36
 0 ( SET & SUM CONTROL means )
 1 : SETCONTROL ( set VARIABLES for SUMCONTROL )
 2      0. CTRLEPSP 2! 0. CTRLONSET 2! 0. CTRLPOP 2!
 3      DUP 2CTRL ! 1FIELDPOT @ - 1+ 0C * MINUS FC00 + SWAP
 4    DUP 1CTRL ! 1FIELDPOT @ - 1+ 0C * MINUS FC00 + 2CTRL @
 5      1CTRL @ - 1+ 3 / LENGTH ! ;
 6
 7 : SUMCONTROL ( sum control period means )   DO
 8      0 I 8 + 2@L 0 I 4 - 2@L D+ 0 I 10 - 2@L D+ 3 M/MOD
 9      ROT DROP CTRLEPSP 2@ D+ CTRLEPSP 2!
10      0 I 6 + @L 0 I 6 - @L + 0 I 12 - @L + 3 / S->D
11      CTRLONSET 2@ D+ CTRLONSET 2!
12      0 I 2@L 0 I 0C - 2@L D+ 0 I 18 - 2@L D+ 3 M/MOD ROT
13      DROP CTRLPOP 2@ D+ CTRLPOP 2! -24 +LOOP ;
14
15 -->

Screen # 37
 0 ( AVERAGE control means ; HEADING & SET for %CONTROL )
 1 : AVERAGECONTROL ( averages means of control period )
```

```
 2       CTRLEPSP 2@ LENGTH @ M/MOD CTRLEPSP 2! DROP
 3       CTRLONSET 2@ LENGTH @ M/MOD CTRLONSET 2! DROP
 4       CTRLPOP 2@ LENGTH @ M/MOD CTRLPOP 2! DROP ;
 5
 6 : %HEADING ( print heading )
 7       3 0 DO 3 SPACES ." FIELD" 3 SPACES 3 0 DO
 8       ." % CONTROL" SPACE LOOP ." *" LOOP CR 3 0 DO SPACE
 9       ." POTENTIAL" 4 SPACES ." EPSP" 6 SPACES ." ONSET"
10       5 SPACES ." SPIKE " ." *" LOOP CR ;
11 : SET%CONTROL ( set variables for summing for %CONTROL )
12   0 POPCOUNT ! 0 MEANONSET ! 0. MEANEPSP 2! 0. MEANPOP 2!
13   1FIELDPOT @ - 1+ OC * MINUS FC00 + SWAP DUP POPPOINT !
14       1FIELDPOT @ - 1+ OC * MINUS FC00 + ;
15 -->
```

Screen # 38

```
 0 ( Means as % of control )
 1 : MEANBLOCK3 ( calculate means for blocks of 3 )
 2  0 T @ 8 + 2@L 0 T @ 4 - 2@L D+ 0 T @ 10 - 2@L D+ 3 M/MOD
 3  MEANEPSP 2! DROP 0 T @ 6 + @L 0 T @ 6 - @L + 0 T @ 12 -
 4  @L + 3 / MEANONSET ! 0 T @ 2@L 0 T @ OC - 2@L D+ 0 T @
 5       18 - 2@L D+ 3 M/MOD MEANPOP 2! DROP 1 POPCOUNT +! ;
 6
 7: MEAN%CONTROL ( calculate & print means as % of control )
 8       SPACE POPPOINT @ 4 .R ." -" POPPOINT @ 2+ 4 .R
```

```
 9      4 SPACES MEANEPSP 2@ D->S 2710 M* CTRLEPSP 2@ D->S
10      M/ 5 .R DROP 5 SPACES MEANONSET @ 2710 M* CTRLONSET
11      2@ D->S M/ 5 .R DROP 5 SPACES MEANPOP 2@ D->S 2710 M*
12    CTRLPOP 2@ D->S M/ 5 .R DROP 2 SPACES ." *" 3 POPPOINT
13      +! POPCOUNT @ 3 MOD IF ELSE CR ENDIF ;
14 -->
15

Screen # 39
 0 ( %CONTROL & t-table p<0.05 two-tailed )
 1 : %CONTROL ( n1 n2 %CONTROL  : from FIELD POTENTIAL )
 2     ( n1 to n2 calculate means of blocks of 3 as % of )
 3     ( control period ) DECIMAL PRINTER HOR132
 4    SET%CONTROL %HEADING DO I T. ! MEANBLOCK3 MEAN%CONTROL
 5      -24 +LOOP CR HOR80 CONSOLE ;
 6 DECIMAL 0 VARIABLE T.975 404 ALLOT
 7 1270 T.975 2+ ! 430 T.975 4 + ! 318 T.975 6 + !
 8 278 T.975 8 + ! 257 T.975 10 + ! 245 T.975 12 + !
 9 236 T.975 14 + ! 231 T.975 16 + ! 226 T.975 18 + !
10 223 T.975 20 + ! 220 T.975 22 + ! 218 T.975 24 + !
11 216 T.975 26 + ! 214 T.975 28 + ! 213 T.975 30 + !
12 212 T.975 32 + ! 211 T.975 34 + ! 210 T.975 36 + !
13 209 T.975 38 + ! 209 T.975 40 + ! 208 T.975 42 + !
14 207 T.975 44 + ! 207 T.975 46 + ! 196 T.975 402 + !
15 -->
```

```
Screen # 40
 0 ( t-table cont'd )
 1 : T1 54 48 DO 206 T.975 I + ! 2 +LOOP ;
 2 : T2 60 54 DO 205 T.975 I + ! 2 +LOOP ;
 3 : T3 70 60 DO 204 T.975 I + ! 2 +LOOP ;
 4 : T4 80 70 DO 203 T.975 I + ! 2 +LOOP ;
 5 : T5 90 80 DO 202 T.975 I + ! 2 +LOOP ;
 6 : T6 120 90 DO 201 T.975 I + ! 2 +LOOP ;
 7 : T7 140 120 DO 200 T.975 I + ! 2 +LOOP ;
 8 : T8 200 140 DO 199 T.975 I + ! 2 +LOOP ;
 9 : T9 320 200 DO 198 T.975 I + ! 2 +LOOP ;
10 : T10 402 320 DO 197 T.975 I + ! 2 +LOOP ;
11 T1 T2 T3 T4 T5 T6 T7 T8 T9 T10
12 ( t-table, two-tailed, p<0.05 )
13 ( For n d.f.: t= T.975 n 201 MIN 2 * + @ )
14 (          Leaves: t value x 100 )
15 -->

Screen # 41
 0 ( SQUARE ROOT for large numbers ) DECIMAL
 1 0 VARIABLE R1 0 VARIABLE R2 0 VARIABLE RCOUNT
 2 : /TEST ( X/Y=Z, DOES Y=Z )
 3     2DUP R1 @ U/ R2 ! DROP R1 @ R2 @ = RCOUNT @ 10 =
 4     SWAP IF DROP 0 ELSE IF 0 ELSE 1 ENDIF ENDIF ;
```

```
 5 : YSET ( IF NOT, SET Y= [Y+Z]/2 )
 6      R1 @ 1 U* R2 @ 1 U* D+ 2 U/ R1 ! DROP 1 RCOUNT +! ;
 7: >SQUAREROOT ( d >SQUAREROOT: leaves the square root of )
 8   ( the double number d on the stack, last digit )
 9   ( unrounded ) 30000 R1 ! 0 RCOUNT ! BEGIN /TEST WHILE
10                YSET REPEAT DROP DROP R1 @ ;
11 : <SQUAREROOT ( d <SQUAREROOT: finds the square root )
12   ( of d to 2 decimals and rounds to integer format, )
13   ( 64000>d ) D->S 10000 U* 3000 R1 ! 0 RCOUNT ! BEGIN
14     /TEST WHILE YSET REPEAT DROP DROP R1 @ 100 /MOD
15     SWAP 50 > IF 1+ ENDIF ; -->
```

Screen # 42

```
 0 ( 95% confidence interval ) HEX
 1 0 VARIABLE SUMEPSP 2 ALLOT 0 VARIABLE SUMONSET 2 ALLOT
 2 0 VARIABLE SUMPOP 2 ALLOT 0 VARIABLE SDEPSP
 3 0 VARIABLE SDONSET 0 VARIABLE SDPOP
 4 0 VARIABLE EPSP-95% 2 ALLOT 0 VARIABLE EPSP+95% 2 ALLOT
 5 0 VARIABLE ONSET-95% 2 ALLOT 0 VARIABLE ONSET+95% 2 ALLOT
 6 0 VARIABLE POP-95% 2 ALLOT 0 VARIABLE POP+95% 2 ALLOT
 7
 8
 9 : SETSUMSQUARES ( set variables for sum of squares )
10      0. SUMEPSP 2! 0. SUMONSET 2! 0. SUMPOP 2!
11      2CTRL @ 1FIELDPOT @ - 1+ 0C * MINUS FC00 +
```

```
12      1CTRL @ 1FIELDPOT @ - 1+ 0C * MINUS FCOO + ;
13 -->
14
15

Screen # 43
 0 ( Calculate sums of squares & standard deviations )
 1 : SUMSQUARES ( sum of squares of means of blocks of 3 )
 2 DO 0 I 8 + 2@L 0 I 4 - 2@L D+ 0 I 10 - 2@L D+ 3 M/MOD ROT
 3  DROP CTRLEPSP 2@ D- D->S DUP M* SUMEPSP 2@ D+ SUMEPSP 2!
 4  0 I 6 + @L 0 I 6 - @L + 0 I 12 - @L + 3 / S->D CTRLONSET
 5     2@ D- D->S DUP M* SUMONSET 2@ D+ SUMONSET 2! 0 I 2@L
 6  0 I 0C - 2@L D+ 0 I 18 - 2@L D+ 3 M/MOD ROT DROP CTRLPOP
 7     2@ D- D->S DUP M* SUMPOP 2@ D+ SUMPOP 2! -24 +LOOP ;
 8 : SQUAREROOT ( <SQUAREROOT < 64000 < >SQUAREROOT )
 9       2DUP FAOO. D> IF >SQUAREROOT ELSE <SQUAREROOT
10       ENDIF ;.
11 : STANDARDDEVIATION ( Trivia: What does this do ? )
12     SUMEPSP 2@ LENGTH @ M/MOD ROT DROP SQUAREROOT SDEPSP !
13  SUMONSET 2@ LENGTH @ M/MOD ROT DROP SQUAREROOT SDONSET !
14     SUMPOP 2@ LENGTH @ M/MOD ROT DROP SQUAREROOT SDPOP ! ;
15 -->

Screen # 44
 0 ( Calculate 95% confidence interval ) DECIMAL
```

```
 1 : 95%INTERVAL ( Mean +/- T.975[s.d.] )
 2      CTRLEPSP 2@ T.975 LENGTH @ 1- 201 MIN 2 * + @
 3      SDEPSP @ M* 100 M/ S->D ROT DROP 2DUP CTRLEPSP
 4      2@ D+ EPSP+95% 2! D- EPSP-95% 2!
 5      CTRLONSET 2@ T.975 LENGTH @ 1- 201 MIN 2 * + @
 6      SDONSET @ M* 100 M/ S->D ROT DROP 2DUP CTRLONSET
 7      2@ D+ ONSET+95% 2! D- ONSET-95% 2!
 8      CTRLPOP 2@ T.975 LENGTH @ 1- 201 MIN 2 * + @
 9      SDPOP @ M* 100 M/ S->D ROT DROP 2DUP CTRLPOP
10      2@ D+ POP+95% 2! D- POP-95% 2! ;
11 HEX -->
12
13
14
15
```

Screen # 45

```
 0 ( Print 95% confidence interval )
 1 : .95%EPSP ( Print confidence interval for EPSP )
 2      ." 95% CONFIDENCE INTERVAL FOR EPSP: " EPSP-95%
 3     2@ D. ." - " EPSP+95% 2@ D. ." uVOLTS; AS %CONTROL: "
 4      EPSP-95% 2@ D->S 2710 M* CTRLEPSP 2@ D->S M/ . DROP
 5      ." - " EPSP+95% 2@ D->S 2710 M* CTRLEPSP 2@ D->S
 6      M/ . DROP ." %." CR ;
 7
```

```
 8 : .95%ONSET ( Print confidence interval for ONSET )
 9       ." 95% CONFIDENCE INTERVAL FOR ONSET: " ONSET-95%
10     2@ D. ." - " ONSET+95% 2@ D. ." uSEC; AS %CONTROL: "
11     ONSET-95% 2@ D->S 2710 M* CTRLONSET 2@ D->S M/ . DROP
12       ." - " ONSET+95% 2@ D->S 2710 M* CTRLONSET 2@ D->S
13       M/ . DROP ." %." CR ;
14
15 -->
```

Screen # 46

```
 0 ( Command for 95% confidence interval )
 1 : .95%POP (Print confidence interval for pop. spike )
 2       ." 95% CONFIDENCE INTERVAL FOR POPULATION SPIKE: "
 3       POP-95% 2@ D. ." - " POP+95% 2@ D. ." uVOLTS; AS "
 4       ." %CONTROL: " POP-95% 2@ D->S 2710 M* CTRLPOP
 5       2@ D->S M/ . DROP ." - " POP+95% 2@ D->S 2710
 6       M* CTRLPOP 2@ D->S M/ . DROP ." %." CR ;
 7
 8 : 95%CONFIDENCEINTERVAL ( Must be done after )
 9             ( AVERAGECONTROL; measures & prints the )
10             ( 95% confidence interval of control period )
11   SETSUMSQUARES SUMSQUARES STANDARDDEVIATION 95%INTERVAL
12 PRINTER HOR132 .95%EPSP .95%ONSET .95%POP HOR80 CONSOLE ;
13
14 -->
```

```
15

Screen # 47
 0 ( La fin caline de bine )
 1: CONTROL ( n1  n2  CONTROL : Averages blocks of 3 means )
 2            ( and prints its 95% confidence interval for )
 3            ( control period FIELD POTENTIAL n1 to n2 )
 4      SETCONTROL SUMCONTROL AVERAGECONTROL PRINTCONTROL
 5      95%CONFIDENCEINTERVAL ;
 6
 7 -->
 8
 9
10
11
12
13
14
15

Screen # 48
 0 ( Sums of squares & standard deviations pop. spike )
 1: SUMSQUARESPOP ( sum of squares of means of blocks of 3 )
 2                ( for pop. spike only )  DO 0 I 2@L
 3  0 I 0C - 2@L D+ 0 I 18 - 2@L D+ 3 M/MOD ROT DROP CTRLPOP
```

```
 4    2@ D- D->S DUP M* SUMPOP 2@ D+ SUMPOP 2! -24 +LOOP ;
 5 : STANDARDDEVIATIONPOP ( For pop. spike only )
 6     SUMPOP 2@ LENGTH @ M/MOD ROT DROP SQUAREROOT SDPOP ! ;
 7 -->
 8
 9
10
11
12
13
14
15
```

```
Screen # 49
 0 ( 95% confidence interval for pop. spike ) DECIMAL
 1 : 95%INTERVALPOP ( Mean +/- T.975[s.d.] for pop. spike )
 2   ( only ) CTRLPOP 2@ T.975 LENGTH @ 1- 201 MIN 2 * + @
 3      SDPOP @ M* 100 M/ S->D ROT DROP 2DUP CTRLPOP
 4      2@ D+ POP+95% 2! D- POP-95% 2! ;
 5 : 95%CONFIDENCEINTERVALPOP ( Calculate & print 95% )
 6     ( confidence interval for pop. spike only )
 7     SETSUMSQUARES SUMSQUARESPOP STANDARDDEVIATIONPOP
 8    95%INTERVALPOP PRINTER HOR132 .95%POP HOR80 CONSOLE ;
 9 : CONTROLPOP ( Same as CONTROL but for pop. spike only )
10      SETCONTROL SUMCONTROL AVERAGECONTROL PRINTCONTROL
```

```
11      95%CONFIDENCEINTERVALPOP ;
12
13 -->
14
15

Screen # 50
 0 ( Means as % of control for pop. spike only ) HEX
 1 : MEANBLOCK3POP ( Calculate means for blocks of 3 for )
 2                  ( pop. spike only )
 3      0 T @ 2@L 0 T @ 0C - 2@L D+ 0 T @
 4      18 - 2@L D+ 3 M/MOD MEANPOP 2! DROP 1 POPCOUNT +! ;
 5
 6 : MEAN%CONTROLPOP ( Calculate & print means as % of )
 7                    ( control for pop. spike only )
 8      SPACE POPPOINT @ 4 .R ." -" POPPOINT @ 2+ 4 .R
 9      4 SPACES
10      0 5 .R 5 SPACES
11      0 5 .R 5 SPACES MEANPOP 2@ D->S 2710 M*
12   CTRLPOP 2@ D->S M/ 5 .R DROP 2 SPACES ." *" 3 POPPOINT
13      +! POPCOUNT @ 3 MOD IF ELSE CR ENDIF ;
14 -->
15

Screen # 51
```

```
 0 ( %CONTROL for pop. spike only )
 1: %CONTROLPOP ( Same as %CONTROL but for pop. spike only )
 2     DECIMAL PRINTER HOR132
 3     SET%CONTROL %HEADING DO I T ! MEANBLOCK3POP
 4     MEAN%CONTROLPOP -24 +LOOP CR HOR80 CONSOLE ;
 5 DECIMAL ;S
 6
 7
 8
 9
10
11
12
13
14
15
```

END

16 10 85

FIN