

NOREPINEPHRINE-INDUCED POTENTIATION IS NOT
BLOCKED BY THE LOCAL DIFFUSION OF THE NMDA
CHANNEL BLOCKER, KETAMINE, AT CONCENTRATIONS
THAT PREVENT TETANICALLY-INDUCED LONG-TERM
POTENTIATION IN THE DENTATE GYRUS IN VIVO

CENTRE FOR NEWFOUNDLAND STUDIES

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LYNN MARIE FRIZZELL



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LONG-TERM POTENTIATION IN THE DENTATE GYRUS
IN VIVO

BY

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An enduring increase in the size of the perforant path-evoked dentate gyrus response follows brief tetanic stimulation. This Long-Term Potentiation (LTP) is considered a cellular substrate of memory. A similar phenomenon, Norepinephrine-Induced Potentiation (NEP), results from exogenous application of NE or from endogenous NE release following Locus Coeruleus (LC) stimulation. It has been postulated that NE promotes LTP induction in a manner consistent with a role in arousal and attention. It has been documented both in vitro and in vivo that LTP induction requires the NMDA receptor and that NEP induction requires activation of the β -adrenergic receptor. In fact, it has been shown in the slice that LTP and NEP depend on both receptors. This suggests a codependence or synergy between NEP and LTP in the slice. However, the same has not been demonstrated in vivo. In fact, LTP is not eliminated by NE depletion in vivo, thus the β -receptor activation is probably not required. It was the aim of the present study to determine the role of the NMDA receptor in NEP in vivo. NEP was elicited by glutamate stimulation of the LC. Two recording pipettes were placed in the dentate gyrus. One pipette contained saline and the other, located less than 1mm away, contained the NMDA channel blocker, ketamine. Diffusion of ketamine significantly attenuated LTP as compared to that at the saline site. However, NEP was

either unaffected or enhanced as compared to that at the saline site. NEP in vivo does not depend on the NMDA channel. β -receptor activation, and the cAMP-mediated biochemical cascade triggered, appears to be necessary and sufficient for the induction of NEP. If NE promotes attention and memory through the β -receptor in the dentate gyrus, it does so independently of the NMDA receptor.

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Hebb (1949) proposed that an increase in the efficiency of synaptic transmission would constitute learning at the cellular level. More than 20 years after Hebb first presented his theory, Bliss and Lomo, in the anaesthetized rabbit and Bliss and Gardner-Medwin, in the awake rabbit, in a pair of 1973 studies, demonstrated a phenomenon which gave Hebb's theory substance (Gustafsson and Wigström, 1988). These studies showed that a brief high frequency train of stimulation, delivered to the input pathway, produced a significant and enduring increase in the efficiency of a group of synapses in the dentate gyrus of the hippocampus. After the tetanus, the granule cells of the dentate showed greater sensitivity to subsequent low frequency (single pulse) stimulation of that input pathway. The effect could be sustained for many hours. Bliss and Lomo arbitrarily defined 30 minutes as sufficient to differentiate this phenomenon from augmentation, facilitation and post-tetanic potentiation. Bliss and Lomo (1973), called this change in cellular behaviour long-lasting potentiation, now more commonly known as Long-Term Potentiation, (LTP).

While LTP can be produced elsewhere in the brain, nowhere is this form of synaptic plasticity as easily, reliably and dramatically produced as in the hippocampus (Collingridge and Bliss, 1987). In fact, LTP can be produced in any of the three synaptic areas of the hippocampal "trisynaptic circuit". Although the hippocampus

(HC) has been traditionally referred to as the trisynaptic circuit and been thought of as a simple relay station, it is now known that the HC is much more complex. In the words of Swanson et al. (1987), "while the hippocampal formation contains the simplest cortical fields...it receives, processes and transmits the most complex array of information of any cortical region". Thus, while the HC is structurally simple and the bulk of hippocampal throughput follows the trisynaptic loop, direct cortical input is made to all three areas of the HC and a great deal of intrinsic reciprocal innervation occurs among these regions, both within the local circuit and along the longitudinal axis (Amaral and Witter, 1989, Yeckel and Berger, 1990). Thus, converging within the HC is a complex array of information, including sensory information from primary and association cortices as well as modulatory inputs from subcortical sites such as the cholinergic basal forebrain, the serotonergic raphe nuclei, the dopaminergic ventral tegmental area and the noradrenergic locus coeruleus. Rather than acting as a simple relay station, the HC functions in the synthesis, modulation and elaboration of incoming information, before "relaying" it back to the cortex. Thus, while the cortex is the putative site for permanent memory storage, the HC is thought integral in forming the memories (Morris, 1990).

Given the proposed integrative function of the HC and the properties of LTP, it became a cellular model of memory (Morris, 1990). Initially, LTP attracted favour by virtue of the fact that it's properties fitted the predictions of Hebb, that it was discovered in the HC, a structure then known to be vital to new learning (Milner, 1972) and because the time course of LTP was such that it was "potentially useful for memory storage" (Bliss and Lomo, 1973). The HC has since proven critical for some kinds of learning and memory, especially spatial learning and memory. The principle cells of the HC fire in response to an animal's position relative to a constellation of external cues, (O'Keefe and Nadel, 1978), and the integrity of these cells is necessary for learning and memory of spatial maze tasks (McNaughton et al., 1989). Furthermore, preventing the induction of LTP prevents spatial learning (Morris et al., 1986). Such findings have made LTP the most widely accepted and exploited model of some kinds of learning and memory. An important ramification of the use of LTP as a cellular model of memory is that it has led researchers to uncover other putative modulators of hippocampal plasticity.

The focus of the present study is the modulator norepinephrine, (NE). Bringing NE to the forefront of research into hippocampal plasticity was Neuman and Harley's 1983 discovery of the phenomenon of norepinephrine-induced

long-lasting potentiation (NE-LLP) in the anaesthetized rat. As in high-frequency induced LTP (HFT-LTP), a long lasting change in the size of dentate granule cell responses was induced, but not by HFT electrical stimulation. Iontophoretic application of NE to the granule cell body layer produced a potentiation that, like HFT-LTP, outlasted the induction stimulation and was often greater than 30 minutes in duration.

As with LTP, norepinephrine-induced potentiation (NEP) is now recognized as an important player in hippocampal plasticity. The evidence, to be reviewed, shows that NE is released during aroused and attentive behavioural states. The effect of NE on sensory evoked unit and population responses is described as enhancement or facilitation which functionally means that the responses now have more physiological impact. Thus, theory holds that the role of NE is to prime or enable the HC to perform it's integrating and consolidating functions on the arousing, attention-getting input. The processes of NEP and LTP are thought to underlie these faciliitating and memory-making functions. Due to a number of discrepancies between the properties of LTP and NEP, it is not yet certain whether NE promotes other forms of plasticity, such as LTP, via the LTP mechanism, or if it promotes LTP by some other mechanism or finally, if NEP is a mechanistically distinct form of potentiation.

The aim of the present research was to investigate the degree to which the mechanisms of NEP and LTP converge in vivo. The impetus for this investigation was the finding that, in the in vitro preparation, NEP and LTP depend on the same mechanism (Burgard et al., 1989, Stanton et al., 1989). To date this has not been demonstrated in vivo. The purpose of the present experiment was to determine, in vivo, whether NEP depends on the LTP induction mechanism or is capable of inducing a potentiation independently of HFT-LTP.

The remainder of the introduction provides an overview of hippocampal anatomy and physiology, with particular attention to the dentate gyrus. The anatomy and physiology of the norepinephrine-containing nucleus locus coeruleus (LC) is reviewed with emphasis on suggested LC function; namely, a general role in mediating cortical arousal. Emphasis is placed on what is known of NE innervation of the dentate gyrus and on the effects of NE on dentate gyrus physiology. The effect of NE on unit responses, population responses and on the LTP of population responses will be examined. What is understood of NEP and LTP will be detailed as necessary. Since an in vivo analysis of the degree of convergence of mechanism between NEP and LTP is the aim of the present study, comparisons between LTP and NEP will conclude the introduction and set the stage for the present research. As the present work is an extension of in

vitro findings, differences between in vitro and in vivo preparations are highlighted throughout.

The term hippocampal formation includes four simple cortical regions, the entorhinal cortex (EC), the subicular complex, (which includes the pre- and parasubiculum and subiculum proper), the dentate gyrus and Ammon's Horn, (Amaral and Witter, 1989). The term hippocampus refers only to the latter two simple cortices. The hippocampal formation, in it's three dimensional extent, forms a C shape originating just caudal of the septum and terminating in the temporal lobe just caudal and medial to the amygdala. Thus the long axis of the hippocampal formation is descriptively called the septotemporal axis. (Amaral and Witter, 1989, Swanson et.al., 1987).

Investigators of the hippocampal formation often conceptualize it in the transverse plane, perpendicular to the septotemporal axis, as a lamella or slice. Figure 1 depicts a traverse section of the HC and illustrates the three synaptic regions and their main connections. Viewed transversely, the HC looks like two interlocking C shapes, the larger (more lateral) being the pyramidal cells of Ammon's Horn, including areas CA1 and CA3 and the smaller (more medial) being the granule cells of the dentate gyrus. Cells of the EC funnel cortical input into the HC via their axons, known collectively as the perforant path (PP), which

synapse onto the dendrites of the granule cells of the dentate gyrus. The granule cells, in turn, project their mossy fibres to synapse on the pyramidal cells of area CA3. Finally, the Schaffer collaterals of the CA3 pyramidal cells make synaptic contact with the pyramidal cells of CA1. Traditionally, it was thought that all hippocampal throughput followed this trisynaptic route and did so within a thin transverse section, and this belief led to the development of the in vitro preparation. While not disputing the usefulness of the slice preparation to certain inquiry, nor the usefulness of the term "trisynaptic circuit" to describe the main intrahippocampal connections, Amaral and Witter have taken exception to this classical view of the HC. They have shown that intrahippocampal projections, with the exception of the dentate mossy fibres, do not project primarily within a lamella, but in fact, innervate targets along a large portion of the septotemporal axis. Consideration of this point is crucial where comparisons are made between the findings yielded in vitro versus in vivo, since the slice preparation is devoid of those projections. For the present work, this consideration is central.

The first synapse of the classical hippocampal triad, the perforant path (PP)-dentate gyrus (DG) synapse, where LTP and NE-LLP were first discovered is the focus for the

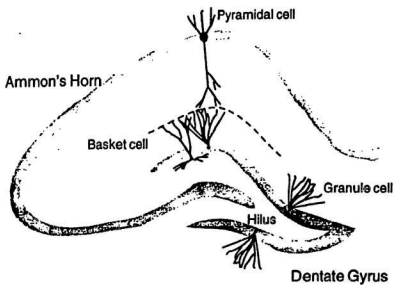


Figure 1: Transverse view of the hippocampus. Shading represents the cell layers and includes illustrations of the pyramidal cells of Ammon's Horn and the granule cells of the dentate gyrus.

present study. The perforant path (PP) fibres arise primarily from layer two cells of the EC, the source of cortical input to the HC. The EC is divided into lateral (LEC) and medial (MEC) portions, reflecting the preference shown for one part or the other by some cortical inputs. The EC receives primary sensory and multimodal associational information from olfactory bulb and areas that receive a direct projection from olfactory bulb, and from temporal, prefrontal, cingulate and insular regions. Since the presubiculum terminates exclusively in the MEC, inputs that travel via the presubiculum, such as that from visual cortex areas 17 and 18b also terminate exclusively in the MEC. Conversely, the bulk of olfactory input has been shown to terminate preferentially and directly in the LEC (Swanson et.al., 1987).

This medial/lateral informational divergence is also reflected topographically in the pattern of termination in the dentate gyrus. The PP fibres originating from the MEC synapse in the middle one third of the granule cell dendritic molecular layer while those arising in the LEC innervate the outer one third. Furthermore, a functional difference is also observable; LTP can be produced in the dentate gyrus in response to HFT stimulation of either the medial or the lateral PP (Bliss and Lynch, 1988) but, NEP is produced only in the medial PP. Cells responding to the

lateral PP exhibit NE-induced long-lasting depressions (Dahl and Sarvey, 1989). This is just one point at which the phenomena of LTP and NEP diverge.

The EC also serves as the ultimate output target of the HC. Output from the LEC reaches the entire extrahippocampal cortical mantle including the olfactory, temporal auditory and somatosensory cortices. Innervation of these areas from the MEC is present but sparse. Both parts of the entire EC innervate motor and medial prefrontal cortex. There are also, however, direct projections to cortical regions from subicular complex and Ammon's Horn and projections from Ammon's Horn that bypass the subicular complex to terminate in the EC (Swanson et. al., 1987). Thus the exceptions to the trisynaptic circuit theory and the lateral/medial topographical divergence discussed in relation to hippocampal input, also apply to hippocampal output.

The simplest of the cortical areas of the hippocampal formation, the dentate gyrus, contains three main layers; the molecular (dendritic) layer, the granule cell layer and the polymorph layer or hilus and two main populations of cells; the principle output cells, called the granule cells, and the local circuit interneurons. It is the bodies of the granule cells that define the C shape of the dentate. As mentioned, the granule cells receive input from the perforant path in a topographical manner where LFP and MPP

terminate in the outer and middle thirds of the molecular layer, respectively. Found in the inner third of the molecular layer are the terminations of hilar interneurons, granule cell recurrent collaterals and commissural fibres from contralateral interneurons. The collective output of the granule cells, the mossy fibres, make en passage excitatory synaptic contact with hilar interneurons before terminating in the CA3 area.

The interneurons of the dentate gyrus are scattered throughout the three layers but do concentrate in the hilus. The four main interneuron types are the GABAergic basket cells, the glutamatergic mossy cells, the somatostatin immunoreactive (-IR) aspiny cells which are also 90% GABAergic and the calretinin-IR spiny cells, for which the neurotransmitter remains unidentified.

The basket cells are found in the molecular layer but are more dense in the hilus and especially the subgranular zone of the granule cell layer (Ribak and Seress, 1983). Basket cells receive excitatory PP input directly in the molecular layer and respond to this input at a lower threshold than do the granule cells (Scharfman, 1991). Thus PP excitation can inhibit granule cells through a feedforward circuit before directly exciting them. Basket cells also receive recurrent excitatory input from the granule cell mossy fibre collaterals thus placing the

granule cells under feedback inhibitory control as well (Scharfman, 1991).

The mossy cells of the hilus are glutamatergic and therefore excitatory (Scharfman, 1991). These interneurons receive direct PP input at molecular layer dendrites and also respond to this input at a lower threshold than do the granule cells (Scharfman, 1991). Since these interneurons are excitatory and send axons back into the granule cell dendritic layer, feedforward excitation is suggested. The evidence suggests, however, that the mossy cells probably act through other interneurons since their activity is associated with granule cell inhibition (Misgeld et al., 1992). Mossy cells also receive mossy fibre excitatory input and therefore may mediate feedback effects.

The somatostatin-IR aspiny cells, located in the hilus, have dendrites in the molecular layer and receive direct PP excitatory input as well as mossy fibre input (Leranth, 1990). Given that 90% are GABAergic, these cells probably inhibit the granule cells through feedforward and feedback mechanisms.

The calretinin-IR spiny cells do not project dendrites into the molecular layer and therefore probably do not receive direct PP input. These cells do receive excitatory mossy fibre activation and terminate on the granule cell dendrites and therefore are placed to mediate feedback

effects only. No transmitter has been identified, and it has yet to be shown whether this circuit is inhibitory or excitatory (Miettinen, 1992).

It is noteworthy that while basket cell innervation is concentrated near the parent cell, innervation of granule cells at distances along the septotemporal axis also exists. Mossy cells make no contact near the parent cell and in fact make contact only at distances several millimetres from the parent cell (Amaral and Witter, 1989). These are examples of the elements of dentate circuitry that are missing from the in vitro preparation and which demand consideration in comparisons made between findings yielded from in vitro versus in vivo preparations.

In sum, built into the dentate local circuitry is the means to regulate granule cell excitability through tonic inhibition (through spontaneous activation) and feedforward and feedback inhibition and excitation. Influences on these local circuit neurons have been proposed as the mechanism by which some putative neuromodulators may modify hippocampal cellular excitability and responsivity (Gustafsson and Wigström, 1988). In fact, it has already been demonstrated that disinhibition can produce a potentiation in the dentate gyrus of rat, in the slice, which is long-lasting (Burgard and Sarvey, 1991).

Unlike the cognitive cortical input, which funnels into the HC by way of the EC, brainstem and forebrain inputs, such as the noradrenergic locus coeruleus (LC), terminate directly and diffusely throughout the HC. It was due to this diffuse pattern of innervation that NE was first considered a modulator, purportedly involved in some generalized function.

The main source of NE in the CNS is the nucleus locus coeruleus. Forming a distinct cluster of neurons in the dorsal pontine brain stem at the ventrolateral edge of the fourth ventricle, the LC also contains peptides, such as neurotensin and vasopressin and neurotransmitter-related enzymes, such as dopamine- β -hydroxylase and tyrosine hydroxylase (Sutin and Jacobowitz, 1991). The LC is in a fibre rich area where axons of neighbouring modulatory neuron clusters course nearby (Foote et al., 1991). This fact was an important consideration in selecting the methodology of the present study.

The LC is thought to receive it's strongest afferent innervation from two other brainstem nuclei, the nucleus paragigantocellularis, (PGi) and the nucleus prepositus hypoglossi (PrH) (Aston-Jones et al., 1991a). The PGi provides a powerful, most probably glutamatergic, input to the LC and it is the PGi that activates the LC in response to some kinds of sensory stimulation, such as tail or

footpinch (Aston-Jones et al., 1991a). The PGI is also active in cardiovascular and respiratory functions and receives input from areas involved in autonomic actions which prompted the proposal that the LC may be the cognitive complement to sympathetic functioning (Aston-Jones et al., 1991b). In contrast to the PGI, the PrH provides GABAergic inhibitory input to LC (Aston-Jones et al., 1991b). In cat and primate this nucleus has been implicated in the control of eye movements, a role that has obvious importance to an attentional system, (Aston-Jones et al., 1991a, 1991b). The diffuse output from LC reaches sensory processing areas of the cortex, tectum, cerebellum and the dorsal horn of the spinal cord. There is, however, little LC innervation of the brainstem. Noncoerulean nuclei provide NE to brainstem and motor nuclei (Grzanna and Fritschy, 1991).

Within the dentate gyrus, NE innervation is densest in the hilus where NE terminals may form axosomatic synapses (Swanson et al., 1987) which suggests termination on interneurons. By terminating on interneurons, NE would be positioned to achieve modulatory effects through influences on the local feedback and feedforward circuitry. Norepinephrine terminals have also been reported in close apposition to dendrites in the molecular layer (Swanson et al., 1987) where NE can mediate direct effects on granule cells.

Curiously, the adrenergic receptor distribution pattern, in terms of density, bears little resemblance to the pattern of NE innervation (Crutcher and Davis, 1980, Booze et al., 1993). While α -receptors more closely correlate with NE terminal sites, in the HC more of the NE effects studied to date seem to be mediated by β -receptors (Segal et al., 1991). The α -receptor, like NE, is most abundant in the hilus and molecular layer. The β -receptors, however, are not most abundant in the hilus, but are found in the molecular layer. β -receptor numbers are reportedly equal in the dentate and Ammon's Horn, however the α_1 -receptors are twice as numerous in dentate as in CA1 (Crutcher and Davis, 1980). It is noteworthy that the IC-NE system is under autoregulatory control via the action of presynaptic α_2 -receptors, which exist on NE terminals. Antagonism of these receptors increases NE release while activation decreases NE release.

Since the β -receptor mediates most of the NE effects studied to date, it is important to consider how the β -receptor operates. The β -receptor is not associated with an ion channel but with a GTP-binding (G-) protein which activates an intracellular second messenger system. When NE attaches to the transmitter recognition site on the β -receptor, the G-protein is activated, in turn activating adenylate cyclase. Adenylate cyclase converts ATP to cAMP.

It is cAMP that accumulates intracellularly and acts as the second messenger that achieves NE effects. Second messengers in general, including cAMP in NE effects, can initiate phosphorylation of membrane-bound receptor proteins and ion channel proteins (Sarvey et al., 1989, Abrams and Kandel, 1988). By altering membrane associated receptors and channels, second messengers such as cAMP produce changes in cellular excitability and responsivity that underlie the actions of neuromodulators such as NE.

When is NE released? LC neurons are active during arousal, as defined behaviourally or by the presence of the waking EEG pattern. LC neurons are spontaneously active, (Williams et al., 1991) and this basal firing is altered by input, for example from the PGI and PrH. Increases (excitation) and decreases (inhibition) in LC activity correlate with behavioural state and environmental events (Aston-Jones et al., 1991a). Thus, activity of LC cells varies over the sleep-waking cycle, showing a continuum of increasing excitability corresponding to a continuum of increasing behavioural alertness or attentiveness (Aston-Jones et al., 1991b). The LC cells are active during waking, quiet during slow wave sleep and silent during paradoxical sleep. The continuum of LC activity is even seen within the waking state, increasing by degree with increasing attention to the environment. During the waking

state, automatic behaviours, such as grooming, are associated with a low level of activity in the LC. A stimulus that interrupts automatic behaviour and induces an orienting, or attending, response is associated with the greatest activation of the LC. The kinds of stimuli that best activate the LC are unexpected or are novel or are meaningful, in that they hold some environmental significance, (e.g. a stimulus that predicts reward), and "typically cause both a sympathetic and behavioural orienting response" (Aston-Jones et al., 1991a). Thus, the greatest release of NE denotes the presence, in the environment, of important, novel or unexpected stimuli. NE release indexes behavioural state.

Studies correlating LC firing and behavioural state have recently gained experimental support. Administration of the LC-activating cholinergic agonist, bethanechol, to rats under surgical levels of halothane anaesthesia produced activation of a majority of LC neurons and the appearance of a waking pattern in the hippocampal and frontal cortex EEG record. When bethanechol failed to induce LC activation no waking EEG appeared. It was concluded that LC activation was crucial in mediating cortical activation, however it was also speculated that LC-NE is sufficient but may not be necessary for such activation (Foote et al., 1991).

The correlational and experimental link between LC firing and behavioural state supports the early contention, based on little more than 'diffuse anatomy', that the LC-NE system is involved in the global function of mediating attention or arousal. It follows that the LC-NE system should also influence target functions that depend on arousal and attention to the environment, particularly those functions that depend on attention to novel or meaningful environmental events. The importance of such a function to the HC, a target structure involved in learning and memory, is obvious. The LC responds intensely to new and meaningful stimuli, which may because of their meaning and novelty, be worthy of remembrance. Perhaps the LC-NE system functions to prepare the HC for this important input, possibly priming or even mediating the mechanisms that underlie learning and memory. The evidence to date suggests induction of NEP may be the means by which the LC-NE system promotes learning and memory in the HC.

In their 1991 review, Sara and Segal present evidence supporting a role for the LC-NE attentional enhancement in enhanced hippocampal function. They proposed that the learning situation is optimal for LC activation because, in a learning situation, environmental stimuli are novel and the significance (or meaningfulness) of stimuli are changing. As stated, LC neurons are most active when

stimuli are new or meaningful or when a shift in attention is required. Accordingly, it has been shown in one experiment that in awake, chronic LC-recorded rats, in learning situations where a reward was first attached to a low tone and then reversed and associated with a high tone, the greatest LC activation was correlated with the shifts in CS-reward pairing. That is, LC activation was seen at the point where the low tone and reward were first paired and again when the reward was first unexpectedly withheld. The LC was activated again, immediately and vigorously, when the reversal occurred and the high tone was now paired with reward. This reversal task illustrates the elements of novelty and shifts in attention that activate the LC. Also, this experiment illustrates how the LC responds to the meaningfulness of a stimulus. The significance of the stimulus, and thus the propensity for the LC to fire, increased at the points where the relationship between the stimulus and food reward shifted, where something could be learned. A number of other investigations (reviewed in Sara and Segal, 1991) have shown that the LC activation that occurs in the learning situation is also important for the learning. Various pharmacological manipulations demonstrate that increasing LC firing facilitates learning in tasks that require shifts in attention, a response to novelty or successive discrimination reversals. These effects all

appear to depend on the postsynaptic β -adrenergic receptor. In fact, the facilitating effect is mimicked by β -agonists and is prevented by β -blockade. Insofar as the HC is involved in these kinds of learning tasks, this evidence suggests that the LC-NE system is important in promoting learning and memory in the HC. Study of the cellular effects of NE, to which the discussion will now turn, also supports this proposal.

In a series of early experiments in which the effect of applied or synaptically released NE on single CA1 cell firing was evaluated NE behaved like an inhibitory neurotransmitter, (Segal and Bloom, 1974a, 1974b, 1976). However, other research has shown that NE excites the inhibitory interneurons (Rose and Pang, 1989) thereby presumably increasing the inhibitory tone of the dentate. These effects can not be easily reconciled with the putative role of NE in promoting arousal, attention and learning. Current controversy surrounding the differentiation between the physiological signatures of the various interneurons questions interneuron excitation findings but also precludes an accurate report of NE effects on interneurons at this time, further research is needed. More recent investigations have also revealed that the effects of NE on granule cells are more complex than simple inhibition. It has been shown that in granule cells, the effect of NE

depends upon the receptor activated. At low levels, NE excites granule cells via the β -receptor, a result mimicked by β -agonists while at higher levels, NE, at the α_1 -receptor mediates inhibition, as do α_1 -agonists (Rose and Pang, 1989, Lacaille and Schwartzkroin, 1988). It is probable that, at the levels of NE administered in most experiments, the α_1 -receptor inhibitory effect masks the low dose β -mediated excitatory effect. Hence the early conclusion that NE acts as an inhibitory neurotransmitter. It has been suggested though, that the β -mediated excitation, at least in CA1 pyramidal cells, is probably the predominant effect seen with endogenous release (Mueller et al., 1982). At the very least it can be concluded that the net effect of NE on spontaneous cellular activity in the dentate may depend upon the balance of α - and β -receptor activation (Stanton et al., 1989).

The net effect of NE on stimulus-evoked neuronal activity also depends on the balance of α - and β -receptor activation and has been characterized as an increase in signal to noise ratio (Foote et al., 1991, McCormick et al., 1991, Harley, 1987). This relative increase appears to occur as a result of little or no suppression of the response signal against more suppression of the spontaneous background activity or as a result of enhancement of the signal against less or no enhancement of the background.

The net effect of this complex, modulatory action is to increase the physiological strength of the response (Foote et al., 1991). Foote et al., (1975) were the first to report this modulatory effect of NE. These researchers found that responses of squirrel monkey auditory cortex neurons to species-specific calls were inhibited by NE but less so than spontaneous background activity, yielding an increase in signal to noise ratio. A similar effect has been observed in neurons of rat somatosensory cortex which respond to electrical stimulation of the hindlimb with initial activation then an extended pause. Bethanecol activation of the LC, when paired with hindlimb stimulation, resulted in a suppression of the spontaneous activity and, to a lesser degree, of the activation and the pause. The net effect was an increase in the duration and overall magnitude of the activation against a suppressed background (Foote et al., 1991). It has also been observed that NE enhances the inhibitory and excitatory responses of cerebellar cells elicited by GABA and glutamate, respectively. This β -cAMP mediated effect well illustrates the modulatory nature of NE as distinct from straightforward inhibition or excitation (Woodward et al., 1991). The impact of NE modulation has also been illustrated by orientation detectors of the visual cortex. After NE, orientation detectors become more activated by optimal

stimuli, those closest to proper orientation, and less activated by stimuli that are less than optimal (Kasamatsu and Heggelund, 1982). This narrowing of the response reflects the strengthening of the focus on that stimulus, an effect useful to an attentional system. The evidence shows that the consequence of NE release in concert with sensory activation is to ensure the meaningful or novel stimulus-induced sensory signal more physiological impact. These findings support the contention that NE functions in drawing the animal's attention to meaningful or novel stimuli.

Anatomically and physiologically, in its correlation with behaviour and in its effects on responses in target cells, the LC-NE system does seem to subserve a generalized arousing, activating function (Foote et al., 1991). What follows is an analysis of the physiological effects of NE release on the response of a particular population of cells, the granule cells of the dentate gyrus. It is well documented that NE, like high frequency electrical stimulation, produces a potentiation of dentate granule cell population responses. The discussion will now turn to the physiology of the dentate gyrus evoked population response, the potentiation of this response, in the form of LTP and NEP, and how potentiation is measured and what it reflects.

When the medial perforant path is stimulated by single pulses the granule cells of the dentate respond with an

evoked population potential. This potential encompasses a number of measurable components which are used in the assessment of synaptic transmission and cellular excitability and of LTP or NEP. The three main components are the population excitatory post synaptic potential (pEPSP), the population spike (popspike) and the popspike onset latency (latency). The pEPSP "is a measure of the current which flows into the cells [the sink] in the synaptic region of the molecular layer and out of the cells [the source] in the cell body layer " (Bliss and Gardner-Medwin, 1973, p. 373). Thus during initial synaptic activation a positive going wave is recorded in the cell body layer. During cell firing, the sink and source reverse and so the recorded wave reverses. Thus, when recorded in the cell layer, the popspike appears as a negative deflection superimposed on the pEPSP. The popspike amplitude reflects the number and synchrony of cells firing and therefore reflects the strength of synaptic transmission and the excitability of the population of cells sampled. The latency reflects the rapidity with which the cells are brought to threshold and thus can also reflect synaptic strength and the excitability of the receiving population (Bliss and Lomo, 1973).

When synaptic efficiency is increased, such as might be expected when the input is new or meaningful and requires

remembrance, it is predicted that incoming current becomes larger and as a result it is also expected that more cells fire and perhaps do so more quickly and in greater unison. Thus, increases in synaptic efficacy and cellular excitability are reflected in increases in pEPSP and popspike amplitude and decreases in the latency. These are exactly the kinds of changes seen in LTP and NEP. Following HFT or LC stimulation the slope of the pEPSP and/or the amplitude of the popspike increase dramatically, and in LTP the latency decreases. The popspike increase is often greater than the change that can be accounted for by the pEPSP increase and in fact it can increase without any comparable change in the pEPSP. The fact that the pEPSP and popspike are differentiable and can be potentiated independently implicates independent mechanisms and suggests that perhaps both pre- and post-synaptic mechanisms are involved in LTP and NEP (Bliss and Lomo, 1973). Though simplified, what is currently known of the mechanisms of LTP and NEP will now be presented in brief.

Subsequent to the discovery of LTP, researchers sought a mechanism of LTP induction that acted essentially as a detector of coincidental pre- and postsynaptic activation in accordance with this "essentially Hebbian" induction rule; "A synapse will sustain an increment of potentiation if, and only if, it is active at a time when the region of dendrite

on which it is located is strongly depolarized" (Bliss and Lynch, 1988, p.12). It is now known that the key to this feature, the detector, is the N-methyl-D-aspartate (NMDA) receptor. The NMDA receptor is a unique member of the excitatory amino acid receptor group since it requires the satisfaction of two conditions before the channel opens. First, glutamate must bind the neurotransmitter recognition site and, unlike non-NMDA excitatory amino acid receptor subtypes, the post-synaptic membrane, on which the receptor is located, must be depolarized. Depolarization relieves a Mg^{2+} block that exists in the channel at resting membrane potential. Hence, the NMDA receptor requires coincidental pre- and postsynaptic activation before the channel can open. Due to the sustained activation achieved at the synapse in response to the LTP-inducing HFT stimulation, this coincidence can occur. Furthermore, because of this unique property, channels open only where this coincidence occurs and thus LTP is selective to the activated synapses. Another important feature of the NMDA receptor is that in addition to sodium and potassium, it permits significant Ca^{2+} entry. Researchers have shown that the NMDA-mediated accumulation of intracellular free Ca^{2+} is necessary for LTP induction and that in fact is the "critical trigger for LTP", (Nicoll et al., 1988).

When LTP is triggered the pEPSP and/or popspike can increase and the latency can decrease. However, not all three parameters will, necessarily, exhibit potentiation in any one experiment (Bliss and Lomo, 1973). Furthermore, if more than one of these indicators is present, the magnitude of changes in each are not necessarily comparable. According to Bliss and Lomo (1973), in their pioneering study in the rabbit, the latency decrease was the most reliable indicator of LTP. It is the current opinion of many, however, that the pEPSP increase as a reflection of synaptic events, is the truest indicator of LTP (Gutaffson and Wigström, 1988). With the HFT stimulation parameters in current use, changes usually occur in all three parameters. These properties of LTP hold true for the in vitro (Sarvey et al., 1989) and in vivo (Errington et al., 1987) rat dentate. It is also characteristic in vivo that the potentiation peaks and asymptotes within the first 30 minutes and invariably, though over hours, days and even weeks, decays. The pattern and intensity of tetanic stimulation used in an experiment, will, to a great degree, determine the magnitude of the potentiation.

Various pharmacological methods have shown unequivocally that blockade of the NMDA complex, either by receptor antagonists such as APV or CPP, or by channel blockers such as MK-801, PCP and Ketamine, prevents

tetanically-induced LTP in dentate and CA1, both in vitro and in vivo, (Collingridge and Bliss, 1987). Interestingly, Harris and Cotman (1986) have reported that APV does not prevent the induction of mossy fibre-LTP in area CA3, a NMDA receptor poor region.

NMDA blockade studies have also revealed that the processes underlying induction and maintenance are differentiable. Once LTP has been induced the involvement of the NMDA receptor ceases. If the NMDA receptor is blocked after induction LTP expression is not reversed. The processes turned on by NMDA-mediated Ca^{2+} influx are the same processes responsible for the expression and maintenance of LTP (Collingridge and Bliss, 1987, Muller et al., 1988). It is beyond the scope of this study to evaluate the many putative mechanisms of LTP maintenance, except to say that various pre- and postsynaptic mechanisms have been implicated and that an as yet unidentified retrograde messenger may coordinate these transsynaptic mechanisms.

Norepinephrine induces an LTP-like potentiation of the dentate pEPSP slope and/or popspike amplitude, that, unlike LTP, requires no concurrent HFT stimulation. Early on it was shown that electrical stimulation of LC could induce a potentiation of the popspike amplitude recorded in the somal layer of the dentate gyrus in the anaesthetized rat (Assaf

et al., 1979, Harley et al., 1982). Although the Harley et al. (1982) report indicated that the potentiation could be long-lasting, without proper pharmacological investigation the responsibility for the effects could not be unequivocally assigned to NE. In fact it has since been shown that electrical LC stimulation can produce a potentiation different from NEP, probably because of extracoeular stimulation (Harley et al., 1989, but see Washburn and Moises, 1989). Neuman and Harley's 1983 study was the first to illustrate that NE could induce a potentiation of the evoked population potential that mimicked LTP, in that it could be long-lasting, (>30 minutes). In the dentate gyrus of the anaesthetized rat, NE, iontophoresed for 1-8 minutes, produced a potentiation of the popspike amplitude recorded at 41 of 54 sites in the cell body layer. At 16 of 41 sites the potentiation was long-lasting. This phenomenon was termed NE-LLP, to differentiate it from HFT-induced LTP. NE-LLP developed gradually with a delay of 30 seconds to 8 minutes. Like LTP, NE-LLP peaked and then plateaued (to a mean of 130-140% of control), within the first 30 minutes and invariably showed decay (Neuman and Harley, 1983).

Based on earlier, preliminary work (Harley and Neuman, 1980), Neuman and Harley (1983) tentatively suggested that the β -receptor, and not the α -receptor, likely mediated NEP.

Lacaille and Harley (1985) were able to confirm this speculation and extend previous findings by demonstrating NEP in the dentate of the in vitro slice. A 10 minute bath application of the threshold level of NE (10 μ M) on the slice produced potentiation of the popspike amplitude (131% of control) in 74% of the slices tested, an increase comparable to that found with iontophoresis in vivo (130-140%, Neuman and Harley, 1983). In 24% of slices potentiation was long-lasting (>30 minutes) as compared to 40% in vivo (Neuman and Harley, 1983). In 53% of the slices, a pEPSP increase was reported (117% of control) and in 74% of slices a latency decrease was seen (94% of control), effects not reported in vivo (Neuman and Harley, 1983). In 2/38 and 3/38 slices, the pEPSP and latency effects were long-lasting. The pEPSP, latency and popspike changes were all mimicked by β -agonists and blocked by β -antagonists, but not by α -agonists and antagonists. In fact the increases were more reliably produced by the β -agonist isoproterenol than by NE. These investigations show that NE, in the dentate gyrus, can induce a potentiation of the popspike alone or together with the pEPSP which can be long-lasting, like LTP. Lacaille and Harley also indicated that both the short- and long-lasting NEP appear to depend on the β -adrenergic receptor, since no potentiation, short- or long-lasting, was induced in the presence of β -receptor blockers.

Stanton and Sarvey (1985b), using the in vitro preparation, as did Lacaille and Harley, were able to produce a more reliable NEP. Using 30 minute superfusion of $50\mu\text{M}$ NE, popspike potentiation (165% of control) was induced in all 8 slices tested. Moreover, in all 8 cases, NEP was long-lasting, compared with just 24% of cases with $10\mu\text{M}$, 10 minute exposure (Lacaille and Harley, 1985). The magnitude of potentiation during drug exposure was also larger with $50\mu\text{M}$, 30 minute (165% of control) than with a $10\mu\text{M}$, 10 minute superfusion (130-140%). Thus the likelihood of inducing a long-lasting popspike potentiation increases with prolonged NE exposure to a higher concentration of NE and may depend on the initial magnitude of the potentiation. While Stanton and Sarvey make no mention of the pEPSP or latency in this study, in a later experiment it was reported that 30 minute $50\mu\text{M}$ NE perfusion produced long-lasting NEP of the popspike and the dendritically recorded pEPSP (Stanton and Sarvey, 1987).

Returning to the in vivo preparation, Harley and Milway (1986) established another method for inducing long-lasting dentate NEP. Using pressure-ejection of 100-150 nL of 0.5M l-glutamate into the LC, in anaesthetized rats, an attempt was made to determine whether long-lasting NEP could result from endogenously-released, physiological levels of NE. This method of LC stimulation was chosen over electrical

since glutamate selectively stimulates cell bodies and spares fibres of passage (Goodchild et al., 1982). The results showed that glutamate, within 300 μ m of the LC, produced an activation of the LC which invariably resulted in potentiation of the popspike. Enhancement (141% of control) occurred with a mean delay of 1.1 minutes and lasted 8.4 minutes on average. In 37% of the trials, the potentiation was long-lasting (>20 minutes). In 13/20 animals the pEPSP increased (127% control) in conjunction with the popspike, at a mean delay of 0.8 minutes and lasting an average of 3.2 minutes. In the majority of animals, no change in popspike onset latency was reported. The percent enhancement, the proportion of effects maintained for the long term and the inconstancy of pEPSP effects, are comparable with results obtained from experiments utilizing local NE methods. Also consistent with earlier reports was the finding that the LC-induced enhancement of the popspike depended on the β -receptor. Glutamate ejection in the LC at 20, 40 and 60 minutes after 30 mg/kg i.p. propranolol injections, either produced greatly attenuated popspike potentiation or none at all. Interestingly, pEPSP changes were not blocked by propranolol but were shortened from 4.2 to 2 minutes in duration. Since the propranolol injection alone lengthened the popspike onset latency, it was possible that NEP attenuation or

blockade was secondary to a peripheral effect. As a result LC-NEP was tested against intradentate β -receptor blockers.

Harley and Evans (1988) utilized pressure ejection of β -blockers from pipettes located within the dentate, to test the role of β -receptors in LC-induced NEP. Prior to timolol or propranolol, popspike enhancement (115-200% of control) was evident in 14/14 experiments at a mean delay of 1.5 minutes. In 10/14 experiments a concurrent potentiation of the pEPSP slope (105-115% control) was reported. In half of the cases, NEP was long-lasting. Intradentate ejection of sufficient timolol or propranolol prevented LC-induced effects, both short- and long-lasting, on pEPSP and popspike. In agreement with the prior LC stimulation study, (Harley and Milway, 1986), but in contradiction to the slice findings (Lacaille and Harley, 1985), no effect on popspike onset latency was reported. The lack of latency effects also contrasts with LTP, in which latency decreases occur virtually invariably. The magnitudes of potentiation effects and latencies to their occurrences and proportions of long-term effects are, however, comparable to previous reports, both *in vivo* and *in vitro*.

Harley and Evans (1988) also reported that, as with the NMDA receptor in LTP, blockade of the β -receptor, after the induction of NEP, did not attenuate or reverse NEP. Thus, like the NMDA receptor in LTP, the β -receptor is essential

to the induction of NEP but is not needed for the maintenance or expression of it. This is consistent with the in vitro experiments where it has been repeatedly shown that NEP outlasts NE washout. NEP, once induced by β -receptor activation, persists in the absence of further activation.

Just as ongoing β -receptor activation is not needed for NEP expression and maintenance, a recent experiment shows that lasting LC cellular activation is also not necessary to sustain NEP, for the short or long term (Harley and Sara, 1992). Since glutamate-pressure ejection proved reliable in selective LC activation and β -dependent NEP induction, Harley and Sara investigated the effect of glutamate ejection on LC unit activity. Cellular activity was recorded from the same location as glutamate ejection, within the LC. For LC activation, pressure-ejection of 50-100nL of 0.5M glutamate was used in some experiments while in the remainder, 100nL microsyringe injections were made through implanted cannulae. Dentate evoked responses were also recorded from the anaesthetized rats. Glutamate ejection into the LC produced an immediate and dramatic increase in LC cellular activity. Harley and Sara reported that the effect was most easily observed as an audible 'rush' on the audio-monitor. This rush of unit activity was very brief, lasting no longer than 250-400 ms. The burst

was followed by an equally abrupt and dramatic silence, a pause in activity that lasted on average 4.6 mins before returning to baseline. With all 34 glutamate ejections, popspike potentiation was observed at a mean latency of 34 secs post-injection. The maximal potentiation seen was 158% of control. The mean duration of potentiation was 4.4 mins, although long-lasting NEP was also seen. NE-LLP occurred more often with cannulae than with pipette-ejected glutamate. Potentiation of the pEPSP was evident in 22/34 experiments at a latency of 15 secs. Popspike onset latency was typically unaffected, although an increase was reported in 5/34 cases. With the exception of latency increases, these results are consistent with previous reports.

Although the duration of the post-glutamate pause in LC activity (4.6 mins) and the mean duration of popspike potentiation (4.4 mins) were quite comparable, there was in fact no relationship between them in individual animals thus refuting any suggestion that LC inactivation induces NEP. Furthermore, injections of the α_2 -agonist, clonidine, which silences the LC for 3-5 minutes, produced no NEP (Harley and Sara, 1992).

Harley and Sara concluded that NEP induction depends upon brief, intense activation of LC neurons and that a critical number of LC neurons must be recruited. This idea was supported by the observation that when NEP was induced,

there was always an audible rush of LC units upon delivery of glutamate and when there was no rush, there was no NEP. A glutamate-induced activation without concomitant NEP occurred when smaller amounts of glutamate were ejected. Larger volumes produced larger unit responses, more reliable NEP and more frequent NE-LLP. This was revealed when ejection of small glutamate volumes induced no NEP in a given location, but an increase in volume resulted in NEP in that same location. Also, the cannula method, which resulted in larger local volumes than pressure-ejection, produced larger and longer-lasting NEP. Based on these observations it was suggested that the likelihood of inducing larger and therefore longer-lasting effects depends on the number of LC units recruited. This is reminiscent of earlier in vitro findings showing that 10 minutes of 10 μ m NE superfusion produced NE-LLP 24% of the time (Lacaille and Harley, 1985) but 30 minute 50 μ m NE exposure produced a larger NEP that was long-lasting 100% of the time (Stanton and Sarvey, 1985b). Since longer lengths of exposure to higher concentrations of NE in vitro (Stanton and Sarvey, 1985b, Lacaille and Harley, 1985), and a larger number of activated LC units in vivo, all predict larger and longer-lasting NEP, and since NEP is β -dependent, it may be concluded that the magnitude and duration of NEP is, ultimately, determined by the degree, duration or number of

β -receptors activated. This is also supported by the subtractive effect of propranolol. In a dose-dependent fashion, propranolol, by decreasing the number of participating β -receptors, decreases the magnitude and duration of NEP. Large enough doses block NEP altogether.

The evidence seems to show that β -receptor activation is responsible for the induction only of NEP, be it short- or long-lasting. Given that the duration of the glutamate-induced LC cellular burst persisted just 250-400 ms, while popspike enhancement was 4.4 minutes in duration, ongoing LC activity, just like ongoing β -receptor activity, is obviously not responsible for NEP expression and maintenance. The duration of the effect seems to depend on the strength or duration of induction as supported by the findings that an increase in NE volume or in intensity of LC activation results in larger and longer NEP.

In summary, the results of NEP investigations suggest that activation of the LC, such as that provided by novel or noxious stimuli, produces a brief and intense LC cellular response which translates into a massive but brief influx of NE into the dentate. Given sufficient LC activation, β -adrenergic receptors induce potentiation in dentate cells. The degree of LC activation determines the magnitude and duration of NEP. It may also be that the numbers of β -receptors activated and the volume of cAMP accumulation also

influences the likelihood of inducing larger and longer effects. Continued NE release and β -receptor activation are not needed to sustain NEP. Perhaps β -receptor activation triggers NEP maintenance but β -receptor involvement ceases after induction, as does the NMDA receptor in LTP. It is not clear, just as in LTP, what the mechanisms are that maintain NEP. It is known only that β -activated cAMP accumulation may induce phosphorylation of proteins. The importance of protein synthesis to NEP is underlined by the finding that inhibitors which prevent protein synthesis also prevent the maintenance of the late phase of NEP (Stanton and Sarvey 1985b).

If, in the dentate gyrus, LTP is the cellular substrate of memory and the role of NE is to promote attention and memory, by priming or enabling the induction of a plasticity mechanism, then it might be expected that NE would promote LTP. In fact, Stanton and Sarvey and their colleagues have compiled convincing evidence from a series of in vitro experiments that NEP via the β -receptor and LTP via the NMDA receptor may actually act synergistically to promote dentate plasticity.

The first step taken in determining the role of NE in LTP was a study of the effect of NE depletion on the integrity of LTP. Stanton and Sarvey found that in slices prepared from NE-depleted animals the frequency and

amplitude of popspike LTP was greatly decreased (1985a, 1987) as was pEPSP LTP measured in the dendrites (1987). They concluded that without NE, HFT-induced LTP was "virtually eliminated" (Stanton and Sarvey, 1985a). Taking the experiment one step further, Stanton and Sarvey (1985a) examined the role of the β -receptor in the apparent NE modulation of LTP. By blocking the noradrenergic β -receptor, the same receptor that mediates NEP induction, the frequency and the magnitude of LTP induction was attenuated in a manner similar to NE depletion. Since NE activation of the β -receptor, through adenylate cyclase, promotes the conversion of ATP to cAMP, a three fold increase in dentate cAMP content following bath application of NE was expected (Stanton and Sarvey, 1985c). However, in this same study it was found that LTP-inducing HFT stimulation also produced a comparable 2.5 fold increase in cAMP levels. Moreover, in slices prepared from NE-depleted animals it was found that adding NE to the bath still produced cAMP increases, showing that the mechanisms underlying the increase were intact, however HFT-induced increases were abolished suggesting an LTP dependency on NE- β activation. Furthermore, in NE depleted slices, in which LTP could not be induced, the addition of forskolin, a direct adenylate cyclase activator, restored HFT-induced LTP (Stanton and Sarvey, 1985a). Finally, it was also demonstrated that preventing protein

synthesis, the likely consequence of cAMP accumulation, prevents the maintenance of NEP and LTP. By adding the protein synthesis inhibitor, emetine, to the bath, the late phases of both NEP and LTP were eliminated. While the maintenance mechanisms involved in LTP and NEP have not been identified, it has been shown that induction of both NEP (Lynch and Bliss, 1986) and LTP (Lynch et al., 1985) is followed by increased glutamate release. In sum, these findings led Stanton and Sarvey to conclude that the NE- β stimulated cAMP increase is a necessary step in the induction of HFT-LTP in the *in vitro* dentate gyrus. Thus the cooperation of the NMDA- and β -receptor-associated mechanisms, when activated by NE or HFT stimulation, appear to be sufficient and necessary for LTP induction *in vitro*.

The results of recent investigations lend support and insight into the means by which NE may promote LTP via the NMDA mechanism. Working in the slice, investigators have shown that bath applied NE (Lacaille and Schwartzkroin, 1988 and Stanton et al., 1989) and β -agonists (Lacaille and Schwartzkroin, 1988) induce granule cell depolarization. It has also been demonstrated that when NE or isoproterenol were applied to the dentate slice at levels sufficient to induce NEP, a reduction of the after-hyperpolarization was evident. It was speculated that NE- β activation reduces a Ca^{2+} -dependent potassium conductance that underlies the AHP.

The net effect is a prolonged depolarization (Haas and Rose, 1987). Through both of these effects, depolarization and AHP reduction, NE may enhance the opportunities for satisfaction of the pre-and post-synaptic depolarization coincidence required to trigger LTP.

It has also been demonstrated that NE enhances the influx of Ca^{2+} through the NMDA channel (Radhakrishnan and Albuquerque, 1989, Stanton et al., 1989) as well as through voltage-gated Ca^{2+} channels (Gray and Johnston, 1987, Fisher and Johnston, 1990). Stanton et al. (1989) have shown that NE at concentrations that induced NE-LLP in the slice also induced a facilitation of stimulus-evoked Ca^{2+} influx and potassium efflux. These effects were blocked by pretreatment with the NMDA receptor blocker APV. Furthermore, it was directly demonstrated that NE enhanced NMDA-induced changes in extracellular Ca^{2+} and potassium (K^+) concentrations while having no effect on non-NMDA-induced changes. Gray and Johnston (1987) patch clamp-recorded an isolated inward Ca^{2+} current in exposed granule cells from hippocampal slices. Pressure-ejected NE increased the size of the Ca^{2+} influx. This effect was mimicked by a β -agonist, a direct adenylate cyclase activator and by a cAMP analogue. Altogether these data offer convincing support for the notion that NE, via the β -receptor, may enhance the NMDA-mediated cascade of events

that induce LTP and thereby promotes LTP. By enhancing NMDA-mediated Ca^{2+} accumulation or by supplementing that accumulation through enhancement of voltage-gated Ca^{2+} influx, LTP likelihood and magnitude may be facilitated by NE. Note that through actions on voltage-gated channels NE could promote LTP without NMDA-receptor interaction.

It is noteworthy that just as NE may promote LTP by enabling NMDA activation and Ca^{2+} accumulation, other evidence shows that NMDA receptor activation enhances NE release. This suggests that the LTP mechanism may promote NEP induction (Craig and White, 1992, Raiteri et al., 1992, Pittaluga and Raiteri, 1992a and 1992b, Fink and Gothert, 1992).

The evidence shows that NE, via the β -receptor, not only promotes NMDA-mediated LTP but that NEP itself also depends on the NMDA receptor, at least in the slice. Just as LTP and NEP converge on the β -adrenergic receptor, so it has been discovered that NEP depends not only on the β -receptor, but also on the LTP induction mechanism, the NMDA receptor (Stanton et al., 1989, Burgard et al., 1989, Dahl and Sarvey, 1990). It was demonstrated that while bath applied NE induced reliable potentiation of the popspike and pEPSP in the dentate of the slice, coapplication of APV produced a dose-dependent reduction (1 μM) or blockade (10 or 30 μM) of NEP (Burgard et al., 1989 and Stanton et al.,

1989). Stanton et al. (1989) pointed out that NE produced no potentiation, neither short- nor long-lasting, in the presence of APV. Dahl and Sarvey (1990) also demonstrated that $1\mu\text{M}$ isoproterenol-induced NEP was completely blocked by NMDA antagonism; in the presence of $10\mu\text{M}$ APV, isoproterenol induced no short- or long-lasting effects.

In sum, the in vitro results suggest that dentate NEP and LTP converge on common induction mechanisms; the β -adrenergic and NMDA receptors. The evidence showing NE promotion of LTP and NMDA promotion of NE release suggest, as previously proposed, a synergy between NEP and LTP. This same thesis, however, has for the most part gone without investigation in vivo and preliminary evidence offers little support.

Many of the similarities between LTP and NEP that have been reported from slice studies; codependence on the β -receptor, NMDA receptor, cAMP increases, protein synthesis and NE, and increased glutamate release, have not been demonstrated in the whole animal. While NE depletion "virtually eliminates" LTP in vitro (Stanton and Sarvey, 1985a), the same has not been demonstrated in vivo. Bliss et al. (1983) found that although NE depletion decreased the magnitude of pEPSP LTP in the anaesthetized rat, popspike LTP was unaffected. Robinson and Racine (1985) reported the opposite in awake rats. They found that popspike LTP was

reduced but pEPSP LTP was actually larger in NE-depleted animals. Altogether, the data suggest that NE is not critical to the integrity of LTP in vivo as it is in vitro. The effect of β -receptor blockade on LTP induction has not been tested in vivo, but it would be predicted that if NE is not needed then the NE receptor should not be necessary. Finally, the role of the NMDA receptor in β -mediated NEP in vivo has not been investigated; that was the aim of the present study.

Sufficient differences exist between the phenomena of LTP and NEP to suggest that the two may be mechanistically distinct. As the preceding review shows, pEPSP and popspike increases and latency decreases are emblematic of HFT-LTP, yet in NEP only the popspike increase occurs invariably; pEPSP changes are typical but latency changes are rare. The fact that HFT stimulation produces NMDA-dependent LTP in the lateral perforant path while NE induces a depression there, suggests other important differences between NEP and LTP (Dahl and Sarvey, 1989).

Other recent in vitro studies also question the convergence between NEP and LTP. While investigating the role of muscarinic receptors in dentate plasticity, Burgard et al., (1993) proposed that if NEP and LTP converge on a common mechanism, muscarine should facilitate NEP as it had been shown to do for LTP. This proposal was not supported.

Bath application of muscarine together with a subthreshold concentration ($0.1\mu\text{M}$) of the β -agonist isoproterenol, did not produce NEP, as would be expected if muscarine acted as facilitator. In fact, muscarine completely blocked the induction of NEP by otherwise effective concentrations of isoproterenol ($1\mu\text{M}$). Burgard et al. (1993) concluded that "there is at least one fundamental difference between induction of NEP and LTP." Offering tentative support to this contention, Dahl and Li (1994) have shown that bath perfusion of a subthreshold dose of isoproterenol followed by a 30 minute wash and second low dose isoproterenol application induces a β -dependent NE-LLP. Unlike other in vitro findings, this NEP was not blocked by NMDA receptor antagonists. This β -mediated NEP did not require the participation of the NMDA receptor. However, in this same study it was found that NEP induced by a single, effective dose of isoproterenol was NMDA receptor dependent. It is not clear what is different about the single and sequenced applications and which better reproduces the effects of endogenous NE release.

Given the conflicting in vitro evidence, the differences in the characteristics of LTP and NEP both in vivo and in vitro, and the fact that the in vitro preparation is missing some of the vital circuitry present in the whole animal (Amaral and Witter, 1989), it is not

clear what the role of the NMDA receptor might be in NEP in vivo. It was the goal of the present research to clarify this issue. The present experiment tested the effect of NMDA receptor blockade on NEP, in the anaesthetized rat. Would NEP be intact, attenuated or completely blocked by the occlusion of the NMDA receptor?

In order to determine the role of the NMDA receptor in NEP, the task of the present investigation was to find a reliable means of blocking NMDA receptors/channels in the anaesthetized animal, which would not interfere with the concurrent recording/pressure ejection in the LC and the recording of perforant path evoked potentials in the dentate gyrus. Two different methods were tried; i.c.v. administration of APV and i.p. injection of ketamine, prior to success with the final method, local diffusion of ketamine.

Since Harley and coworkers have proven that, with accurate placement, glutamate pressure ejection into the LC induces a consistent, β -mediated NEP, that method was used in this experiment. A double barrel electrode was used which permitted glutamate ejection through one barrel after electrophysiological placement verification through the recording barrel. Testing for characteristic LC responses to tail- and footpinch aided in placement verification (Cederbaum and Aghajanian, 1976). Aside from the benefit of

accuracy, the simultaneous recording of LC units permitted monitoring of the continued effectiveness of the glutamate ejection. Through a stimulating electrode placed in the medial perforant path, eight trains of eight pulses at 400 Hz were delivered for LTP induction.

The medial perforant path-evoked population potential was recorded before and after the delivery of LC-NE or HFT stimulation in order to assess and measure NEP and LTP induction. Comparison of the magnitude of LTP induced with and without NMDA antagonists present, by revealing the degree of LTP attenuation, indicated the degree of NMDA receptor blockade. NMDA receptor blockade, sufficient to attenuate LTP, had to be indicated in order to assess the role of the NMDA receptor in NEP. A review of the pilot studies, in which the attempts were made to achieve NMDA receptor/channel blockade, and the problems encountered serves to illustrate the rationale and the advantages of the final selected methodology.

The first method attempted involved intracerebroventricular, (i.c.v.), injections, through an implanted stainless steel 30 gauge cannula, of the NMDA receptor antagonist AP5 ($2\mu\text{L}$, $5\mu\text{g}/\mu\text{L}$). The difficulty with this method was in placing the cannula into the ventricle such that the antagonist could spread adequately to the dentate gyrus where needed. It has been observed that

i.c.v. delivery of NE, (also in 2 μ L volumes) often (3/4 animals) resulted in an insignificant rise in NE levels in the dentate, as measured by microdialysis, indicating that i.c.v. delivered NE did not reach the dentate. As a result, the NE delivered in this manner did not reliably produce NEP. Also, NE spreading throughout the ventricular system was not an indication of NE spread to the dentate. Only when i.c.v.-delivered NE produced a significant rise in dentate NE levels, was NEP apparent (Harley, personal communication). It was probably a similar problem that precluded consistent blockade of LTP with i.c.v. delivery of AP5. Without complete and consistent blockade of the NMDA receptors their role in NEP could not be adequately and accurately assessed. The few results that were obtained from the i.c.v. studies are presented next.

Of seven animals in which NEP was induced by glutamate LC stimulation (n=2) or i.c.v. NE (n=1) and in which AP5 was delivered via the i.c.v cannula, an LTP control conducted upon completion of the NEP experiments indicated NMDA receptor blockade (n=2) or apparent attenuation (n=1) in three animals. In these three animals, however, there was no significant difference ($t=1.59$, $p>0.05$) in the magnitude of NEP on the popspike induced before or after the AP5 ejection. This result suggests that NMDA receptor blockade did not prevent the induction of NEP where LTP was

precluded. However, in the four remaining experiments, in which LTP was not tested or not noticeably attenuated or blocked, post-AP5 NEP was considerably smaller than pre-AP5 NEP in 3/4 cases. In the absence of a proper LTP control the data seem to suggest that NMDA receptor blockade attenuates NEP. If AP5 delivery was inadequate to achieve LTP attenuation then NMDA receptors were not blocked, yet it appears that NEP was attenuated. This suggests two possibilities: 1. NEP is blocked at a lower threshold than LTP or 2. there was no blockade at all and second NEP effects are simply not as large as first effects. These data illustrate another problem with this and the following method; by comparing first and second NEP effects, a ceiling effect may appear as blockade.

The next attempted method was chosen in order to eliminate the inaccuracy of i.c.v. delivery. NMDA antagonists were given through i.p. injections. Following the protocol of Desmond et al., (1991), the non-competitive antagonist, ketamine, was used. Desmond et al. found in animals anaesthetized with 1.0g/kg urethane, supplemented as needed, ketamine HCl given as a 30mg/kg bolus and three supplemental 10mg/kg doses at 15 minute intervals reversibly attenuated or blocked LTP induction. In this pilot, urethane and ketamine were used at the same doses and concentrations and with the same protocol, but it proved

quite unsatisfactory for continued use. Although LTP attenuation was indicated, the anaesthetic properties of the ketamine proved troublesome, drastic decreases in baseline responses frequently occurred and in a number of cases the animals died prior to completion of data collection. Even when i.p. ketamine blockade worked, the possibility existed that observed effects were due to systemic action. In four animals that survived the protocol and in which NEP was induced and i.p. ketamine was given, an LTP control conducted upon completion of the NEP experiments indicated complete NMDA channel blockade in all four animals. Unlike LTP, NEP was not completely blocked after ketamine since significant popspike NEP was evident in three of four animals. There was, however, a significant difference ($t = 3.37$, $p < 0.05$) in the magnitude of popspike NEP induced prior to ketamine (mean = 141%) versus that induced post-ketamine (mean = 121%). These data suggest that systemic ketamine, sufficient to completely block LTP, attenuated but did not block NEP. However, again, these data may simply reflect a ceiling effect.

The possibility that second NEP effects might be consistently smaller than first effects precludes a proper evaluation of the role of NMDA receptors in NEP in a sequentially designed experiment. Due to the nature of NEP, i.e., the necessity for very accurate placement of the LC

pipette (Harley and Milway, 1986) and, given accurate placement, the need for the activation of a critical number of LC neurons to induce NEP (Harley and Sara, 1992), glutamate ejections often fail to induce effects. As a result it was necessary in these experiments to establish NEP prior to NMDA receptor blockade to ensure accurate placement and sufficient glutamate volume. This meant that the role of NMDA receptors in NEP was always tested on a second ejection. Although Harley has consistently reported repeated effects of LC-GLU ejections on dentate popspike, up to as many as 10 times (Harley and Milway, 1986, Harley and Evans, 1988), this was dependent upon a return to baseline between ejections. Due to the very nature of NEP, a return to baseline is not assured. Working in the CA1 slice, Heginbotham and Dunwiddie (1991) reported that a second NEP was never as large as a first, even with a return to baseline and even with the intensity of stimulation adjusted to artificially reestablish baseline. Since it seems that the magnitude of LTP or NEP depends on the initial size of responses, as indicated by the practice of many investigators to select half-maximal potentials for baseline, the potential existed in these pilots, for a ceiling effect to masquerade as LTP blockade. Given that attenuation and not just complete block of NEP would still be an interesting effect, it was important that second

effects be reliably comparable to first effects. In the same vein the sequential nature of the experimentation introduced other problems such as changes in baseline, changes in level of anaesthesia, and changes in the degree of NMDA blockade; changes that occur over time. The final method selected provided reliable NMDA receptor attenuation and eliminated the sequential nature of the experimentation and all the problems that resulted from that protocol.

The selected method was inspired by the work of Steward et al. (1990) who investigated the ability of the commissural-dentate gyrus synapse to support LTP given the elimination of the strong inhibition that normally obstructs LTP at this synapse. Steward's group relied on passive diffusion of bicuculline from the tip of a large bore glass pipette to create a localized disinhibition. By placing two pipettes into the dentate gyrus, one with bicuculline and the other with saline, approximately 0.5-1.0 mm apart, Steward et al. created an elegantly simple study in which a comparison of the responses recorded on the two pipettes revealed the effects of the bicuculline. The appearance of multiple perforant path-induced spiking during baseline recording and of LTP in response to contralateral, commissural tetanic stimulation, on the bicuculline pipette only, suggested that local diffusion of bicuculline created significant yet restricted disinhibition, sufficient to

uncover a capability for LTP. On the saline pipette, no multiple spiking and no LTP appeared indicating no spread of the bicuculline to this nearby control pipette. Thus, while introducing problematic stereotaxic constraint, this double pipette method was adopted in the present study.

A pilot study of 6 animals was conducted to evaluate the utility of the Steward diffusion method in antagonizing a significant number of NMDA receptors and to determine the dose of antagonist needed to produce the effect. For the pilot, only one pipette, containing various concentrations of ketamine, was placed in the dentate cell layer. A stimulating electrode was positioned in the medial perforant path through which 0.2 ms pulses of 10-20 V were delivered at 0.1 Hz for eliciting half maximal evoked potentials. After collection of a 10 minute baseline, (60 evoked potentials), eight trains of eight pulses at 400 Hz were delivered through this same electrode. The effect of tetanic stimulation on the evoked potential recorded through various concentrations of ketamine was followed for a 30 minute post-tetanus period. The results are presented in Table 1 showing that whereas concentrations of 18mM (n=2) and 90mM (n=1) were insufficient, 181mM (n=3) ketamine produced an attenuation or complete block of LTP. Since diffusion of ketamine through a large bore tip antagonized enough NMDA receptors to attenuate or prevent LTP induction,

the double pipette method of Steward et al. was adopted in order to evaluate the effect of ketamine on locus coeruleus-induced potentiation.

The major advantage of the double pipette method is that the responses recorded through the drug pipette could be compared to those recorded at the same time, in response to the same stimulation, at a nearby saline control site. That the control study and experiment happened at the same time and in about the same place, eliminates concerns about ceiling effects, changes in the degree of pharmacological blockade and changes in baseline. Except that the two pipettes sampled different populations of cells, the only other difference between the two was that one contained an NMDA blocker and the other did not. Any differences in effects recorded on the two sites could be accounted for by local NMDA receptor blockade, and not, for example, by changes in temperature, level of anaesthetic, or ceiling effects. These should affect both recordings. Thus the effect of ketamine on LTP and NEP could be assessed by simply comparing the magnitude of the effect on the two pipettes.

TABLE 1

**The Effect of Increasing Concentrations of Ketamine
on the Magnitude of LTP of the Popspike as
Compared to a Saline Control:
A Pilot of 6 Animals**

SALINE		KETAMINE	
3M	18mM	90mM	181mM
MEAN PERCENT (%) POTENTIATION ON POPSPIKE			
307.10	281.33	152.26	107.48
n=1	n=2	n=1	n=2

Methods

Subjects:

Twenty three female Sprague-Dawley rats, (Charles River Canada Inc., Montreal, Canada), weighing from 205 to 322 grams served as subjects. Each animal was anaesthetized with urethane (1.5 g/kg i.p.) and placed skull flat into the stereotaxic apparatus. A circulating water blanket maintained rectal temperature at 36.5-38°C.

Recording and Ketamine Application in Dentate Gyrus:

A bipolar stimulating electrode (Kopf model NEX-100X) was aimed at the medial perforant path (7.2 mm posterior, 4.2 mm lateral to bregma and 3.0-3.5 mm below brain surface) and two non-capillary glass pipettes were positioned into the dentate gyrus (3.5 mm posterior and 2.0 mm lateral to bregma). The pipettes were placed into a Narishige holder which permitted a 1 mm range of movement, in three directions, of one pipette relative to the stationary pipette. In this holder, the pipette tips were held 500-1000 μ m apart in the anterior-posterior and medial-lateral directions and less than 100 μ m apart vertically. Depths depended on maximal similarity between the two evoked potentials, typically 2.8-3.8 mm below brain surface. The control pipette was filled with either 3 M saline or 2%

pontamine sky blue in 0.5 M sodium acetate. The outside tip diameter ranged from 15 to 35 μm and the impedances were 0.1 to 2.0 M Ω . The experimental pipette contained either 181 mM or 362 mM of the NMDA receptor channel blocker Ketamine HCl (Ketalar, Parke-Davis; Ketamine HCl, Sigma, dissolved in 0.9% saline). The outside diameter of these tips were larger, from 40-70 μm , to permit diffusion of the antagonist. Impedances of the ketamine pipettes ranged from 0.1 to 1.0 M Ω . Since the ketamine solution is colourless, a tightly wound mediwipe was held to the pipette tip, which could be seen, under a microscope, to unwind when moistened, thus ensuring diffusion could readily occur from the pipette tip. Some control pipettes were filled with pontamine sky blue in an attempt to mark the area of diffusion around the tip.

Recording and Glutamate Ejection in Locus Coeruleus:

A double barrel, non-capillary glass pipette was placed in or near the locus coeruleus, at 4.9 mm posterior and 1.1 mm lateral to the peak of lambda. The pipette was angled 20° posterior from vertical. One barrel was filled with 0.5 M l-glutamic acid in distilled H₂O and was connected by tubing to a Neuro Phore BH-2 pressure ejection system. With this system, a 30 psi nitrogen pulse was delivered to the glutamate-containing barrel, ejecting a drop of glutamate.

The spherical volume of the drop was calculated from the diameter as read under a microscope. Duration of ejection controlled the size of the drop and was adjusted to achieve a spherical volume of 73-244 nL. The actual volume in brain has been estimated from intracerebral injection to be about 68% of that estimated from the drop diameter in air (Goodchild, 1982). The second barrel was filled with 2% pontamine sky blue in 0.5 M sodium acetate. Recording through the pontamine barrel aided in localization of the Locus Coeruleus. The tip size of each barrel was between 10-20 μm and the impedance through the recording barrel ranged from 2-6 M Ω . The top of the recording barrel was cut back to allow placement of the tubing on the glutamate barrel.

Spontaneous unit activity recorded through the LC pipette was differentially amplified at a band width of 600 Hz-3 kHz, displayed on the oscilloscope and coupled to an audio unit. Depth profiles of unit activity through the cerebellum and upper pons were constructed for the purpose of locating the LC but this activity was not stored. The LC is known to respond to a tailpinch with a distinctive brief rush of firing, increasing from 1.2 Hz as high as 10 Hz for about 100-500 ms, followed by a brief pause (Cederbaum and Aghajanian, 1976). Tests for the tailpinch response were conducted to physiologically verify placement. Also helpful

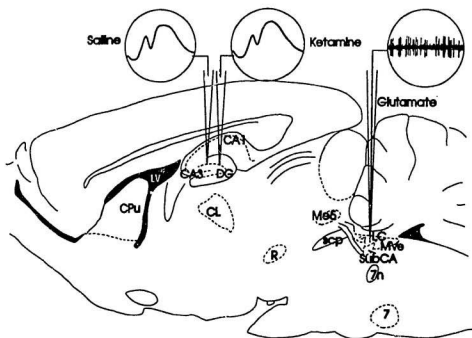


Figure 2: A sagittal view of the rat brain illustrating the location of the Locus Coeruleus recording/ejecting pipette and of the two dentate gyrus recording pipettes. The similarity in the dentate gyrus evoked potentials is also evident.

in locating LC is the proximity of the mesencephalic trigeminal nucleus to the LC. These cells run the lateral and anterior border of the LC and respond to manipulations of the jaw thus providing another physiological test useful in achieving placement accuracy (Harley and Milway, 1986). The LC was encountered at 5.0-5.8 mm below brain surface. The double barrel set-up permitted testing for glutamate responses (visual and auditory inspection of response to glutamate pulse). Responses to glutamate were always tested somewhere in the upper cerebellum to ensure sufficient ejection. This testing was important since the tips sometimes became blocked. On occasion it was necessary to withdraw the pipette, after LC localization, and break back the tip to achieve the desired drop volume. Figure 2 illustrates the overall setup.

Data Acquisition and Analysis:

A 0.2 ms monophasic square wave pulse, 10-20 V, was delivered to the perforant path at 0.1 Hz, (Ortec EG&G 4710 dual channel stimulator). Each dentate recording pipette was coupled to a Grass H1 Z Probe which conveyed the evoked potentials for differential amplification at a bandwidth of 1 Hz to 3 KHz (Grass P5 series A.C. Pre-amplifier). Evoked potentials were displayed on a cathode ray storage oscilloscope and digitized on an IBM-PC compatible computer

(1 point/10 μ s). The data acquisition and analysis programs were written by G.P. Carre, in Asyst, a commercial package based on the language Forth. For each evoked potential a number of measures were extracted online, including the pEPSP slope, the popspike amplitude, measured as the amplitude difference from the first positive peak to the negative (popspike) peak, and the latency to the popspike peak. These measures were taken every 10 seconds and means of six, representing one minute, were calculated. A period of 10 minutes constituted a control period. The two-tailed 95% confidence limits were calculated around these 10 means. Thus an LC- or HFT-induced effect was considered significant if the measures fell outside the confidence limits and judged no longer significant when two consecutive means (twelve data points, two minutes), remained inside the confidence limits. At least ten minutes of data was collected after LC or LTP stimulation but usually collection continued for 30 minutes. At the completion of LC activation experiments an LTP control was conducted in each animal to assess whether ketamine diffusion from the experimental recording pipette was sufficient to produce NMDA receptor channel blockade. In one animal two such LTP control trials were conducted. Eight trains of eight pulses at 400 Hz were delivered to the perforant path to induce LTP.

Since the saline pipette served as the control site and the ketamine pipette served as the NMDA-blocked, experimental site a comparison of the relative magnitudes of LTP on the two pipettes revealed the efficacy of ketamine-induced NMDA receptor blockade. Similarly the role of the NMDA receptor in LC-induced effects could be assessed by comparing the magnitude of NEP on the two pipettes. Paired t-tests were performed to evaluate the significance of any difference between parameters measured at the saline and ketamine sites.

Control period responses recorded on the two pipettes were compared to determine their comparability prior to any manipulation. Averages of the slope of the pEPSP ($\mu\text{V}/\text{ms}$), the amplitude of the popspike (μV) and the latency (ms) were calculated from the 10 minute control periods, prior to LTP or LC stimulation. Paired t-test assessment of the significance of any between-site differences in mean baseline measures revealed the effect of ketamine on low-frequency activity. It was expected that there would be no difference prior to LTP or NEP induction.

For paired t-test, between-site, comparisons of the magnitudes of LTP and NEP, all one minute mean pEPSP slopes, popspike amplitudes and latencies were converted, by Asyst, to a percent (%) of baseline. The maximal percent increase or decrease within the first five minutes following HFT or

LC stimulation was determined from each of the NEP and LTP experiments, for each of the pipettes. Thus, a group mean maximal (pEPSP and popspike) or minimal (latency) percent potentiation was calculated for each measure recorded on each pipette (saline or ketamine) for each experiment (NEP or LTP). A significant difference in LTP magnitude recorded at the ketamine and saline sites was taken to indicate significant NMDA receptor blockade. A between-site comparison of the magnitude of NEP would reveal the role of the NMDA receptor in NEP.

Photographs of the evoked potentials were taken with an oscilloscope-mounted polaroid camera in some animals, while in others computer-generated traces were produced, thus providing a visual means of assessing similarity in shape of the ketamine and saline recorded waveforms.

Locations of the two dentate recording pipettes and the LC ejection pipette were marked by pressure ejection of pontamine dye, the ketamine was aspirated and replaced with the dye for this purpose. This was not done in those animals in which an attempt was made to assess diffusion, in which case the pipette tract sufficed for placement verification. Brains were removed and blocked so that the brainstem and cerebellum could be mounted sagittally, the anterior portion coronally and frozen. Sections were taken at 40 μ m and checked for dye markings before staining in

0.1% cresyl violet. Electrophysiologically identified locations were verified after staining.

Results

Of 23 animals in which the double pipette experiment was conducted, 11 animals were included for analysis based on satisfaction of three criteria. First, stable evoked potential recordings of similar magnitude and waveform were obtained on both the saline and ketamine pipettes. Secondly, LTP on the ketamine pipette was significantly attenuated as compared to that on the saline pipette, indicating significant NMDA channel blockade. Finally, significant NEP was evident on either or both the pEPSP slope and popspike amplitude, on one or both pipettes. Of the 12 animals not included in the analysis, three were excluded for lack of comparability between evoked potentials, three more were omitted since LTP was either absent or comparable on both pipettes and an additional two animals were eliminated because the LC pipette was not accurately placed. Finally, four more animals were excluded because computer problems or the death of the animal precluded completion of the experiment. In the 11 animals, 25 NEP experiments were conducted followed by an LTP control

trial at the conclusion. In one animal two LTP controls were performed.

Ketamine effects on baseline responses:

The waveforms illustrated in Figure 2, taken from one animal, represent the degree of similarity in the recordings from the two pipettes prior to any manipulation. As evident in Table 2, prior to HFT stimulation in the LTP control studies, average pEPSP slopes on the saline (11039 $\mu\text{V}/\text{ms}$) and ketamine (11361 $\mu\text{V}/\text{ms}$) pipettes and average popspike amplitudes on the saline (7475 μV) and ketamine (6204 μV) pipettes did not differ. Statistical analysis verified the comparability of the baseline pEPSPs, ($t_{(df=10)} = -0.40$, $p > 0.05$), and popspikes, ($t_{(df=10)} = 1.16$, $p > 0.05$). Similarly, the average latency to the popspike peak recorded on the saline pipette (4096 μsec) did not differ from that on the ketamine pipette (4125 μsec) prior to LTP induction ($t_{(df=10)} = 0.03$, $p > 0.05$).

Since LTP and NEP experiments were both carried out in each animal, Figure 2 also represents pre-NEP waveform similarity. Prior to NEP experiments, as detailed in Table 2, baseline mean slope of the pEPSP recorded on the saline (10076 $\mu\text{V}/\text{ms}$) and ketamine (10588 $\mu\text{V}/\text{ms}$) pipettes did not significantly differ ($t_{(df=23)} = -1.28$, $p > 0.05$). The saline popspike (6702 μV) and ketamine popspike (6355 μV) were also

TABLE 2

The Effect of Ketamine on Baseline Evoked Potential
Parameters and Magnitude of Potentiation
as Compared to Saline Control

COMPARISON	SALINE MEAN	KETAMINE MEAN	T VALUE ($p < 0.05$) *
LTP BASELINE			
EPSP SLOPE ($\mu\text{V}/\text{ms}$)	11039	11361	0.40
POPSPIKE (μV)	7475	6204	1.16
LATENCY (ms)	4096	4125	0.03
POST-LTP			
EPSP MAX % INCREASE	123.08	108.54	3.18*
POPSPIKE MAX % INCREASE	218.93	128.60	3.73*
LATENCY MIN % DECREASE	89.0	92.0	1.79*
NEP BASELINE			
EPSP SLOPE ($\mu\text{V}/\text{ms}$)	10076	10588	1.28
POPSPIKE (μV)	6702	6355	1.17
LATENCY (ms)	4034	4050	1.30
POST-NEP			
EPSP MAX % INCREASE	111.47	111.45	0.08
POPSPIKE MAX % INCREASE	139.67	148.25	2.11*

comparable ($t_{(df=23)} = 1.17$, $p > 0.05$) prior to NEP. Finally, baseline latencies recorded on the saline (4034 μ sec) and ketamine (4050 μ sec) pipettes also revealed no between-site differences in baseline ($t_{(df=23)} = 1.3$, $p > 0.05$).

Ketamine effects on LTP:

In all 12 LTP trials, in the 11 animals, significant attenuation or blockade of pEPSP and popspike increases and of latency decreases was evident on the ketamine pipette as compared to the saline pipette. This between-site difference is illustrated in Figure 3. The range of LTP magnitudes, expressed as a percent of control, also reveals this difference. The maximal post-HFT values on the saline pEPSP ranged from 105.00% - 162.62% whereas on the ketamine pEPSP values ranged from 99.73% - 120.48%. The maximal post-HFT values on the saline popspike ranged from 125.82% - 383.99%, whereas on the ketamine popspike values ranged from 63.08% - 182.18%. Finally, maximal decreases (minimal percents) in saline latency ranged from 81% - 94%, while the ketamine latency decreases ranged from 86% - 97%.

Means of the 12 maximal (pEPSP and popspike increases) or minimal (latency decreases) percents were compared using paired t-tests confirming the between-site difference. As summarized in Table 1, the group average of the maximal potentiations seen on the saline pEPSP was 123.08%,

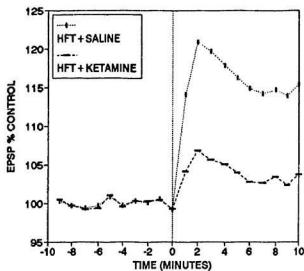
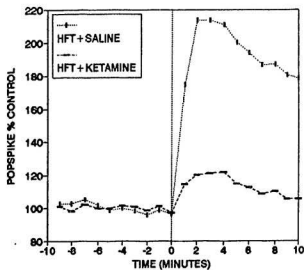
A**B**

Figure 3: The magnitude of LTP evident on the pEPSP (A) and popspike (B), at the ketamine site, is significantly attenuated compared to that at the saline site.

significantly larger than the 108.54% evident on the ketamine pEPSP, ($t_{(df=10)} = 3.18$, $p < 0.05$). The average maximal LTP on the saline popspike was 218.93%, significantly larger ($t_{(df=10)} = 3.73$, $p < 0.05$) than the 128.60% potentiation produced on the ketamine popspike. The average LTP on the saline latency (89%) was just significantly larger than the magnitude of LTP on the ketamine latency (92%), ($t_{(df=10)} = 1.79$, $p = 0.05$, one-tailed test).

The durations of HFT effects also differed on the two pipettes. This would be expected, since LTP was often not induced on the ketamine pipette or because the effect induced was much smaller thus returning to baseline sooner. In two of the 12 cases, LTP, defined as greater than 30 minutes in duration, was not induced on either pipette and only short term potentiation (STP) was seen. These experiments were still included since the criterion for inclusion was induction of an effect and that this effect was significantly larger on the saline than on the ketamine pipette; duration was not of concern. The duration of HFT-induced effects on the saline pEPSP ranged from 3 - >30 minutes with 7 of the 12 cases showing LTP. On the ketamine pEPSP, effects lasted from 0 - >30 minutes, in 4 cases there was no effect at all and in 4 others, LTP was induced. On the saline popspike, effects lasted from 7 - >30 minutes and

in 9 of 12 cases LTP was induced. On the ketamine popspike, effects ranged in duration from 0 - >30 minutes, LTP was induced only twice and in 5 cases there was no effect at all. In no case did an effect on the ketamine pipette outlast that on the saline pipette. LTP (>30 minutes) was evident on the latency in 11 of 12 trials on the saline pipette and in 10 of 12 trials on the ketamine pipette.

Ketamine effects on NEP:

In all 25 NEP experiments there were significant pEPSP effects evident on both pipettes. In 20 of the NEP trials, significant popspike enhancements were induced on one or both of the dentate pipettes. Thus, in five experiments in which there was a significant pEPSP enhancement, there was no concomitant popspike increase of significance. Of the 20 cases where there were significant popspike enhancements, in all but two cases these enhancements appeared on both pipettes. While there appeared to be no difference in the magnitude of NEP on the saline versus ketamine pEPSPs, it appeared that the magnitude of NEP was slightly larger on the ketamine popspike than on the saline popspike. This between-site difference is evident in Figure 4. The range of NEP magnitudes also reveals this difference. The 25 post-LC maximal values on the saline pEPSP ranged from 105.79% - 127.76%, similar to the range seen on the ketamine

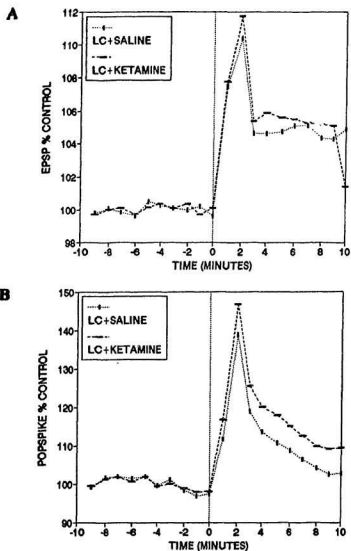


Figure 4: There is no between-site difference in NEP of the pEPSP (A) but NEP of the popspike (B) is significantly enhanced by ketamine.

pEPSP, from 106.16% - 127.34%. The 20 post-LC maximal values on the saline popspike ranged from 103.68% - 166.01%, which is noticeably smaller than on the ketamine popspike, where values ranged from 122.26% - 194.87%. Paired t-tests, detailed in Table 1, confirmed no difference between the magnitude of NEP on the saline versus ketamine pEPSP, ($t_{(df=23)} = 0.08$, $p > 0.05$). It was also confirmed that the magnitude of NEP on the ketamine popspike was larger than that on the saline popspike ($t_{(df=23)} = -2.11$, $p < 0.05$). In 17 of 25 NEP experiments there was no effect of LC stimulation on latency and this was true on both pipettes. In 4 of 25 NEP experiments comparable, brief (2-4 minute) increases in latency were seen on both the saline (mean= 103.25%) and ketamine (mean= 103.43%) pipettes. In the final 4 experiments long-lasting (>30 minute) decreases were seen on both the saline (mean= 97.90%) and ketamine (mean= 97.85%) pipettes. In these four animals NE-LLP of the latency was associated with NE-LLP of the pEPSP, present on both pipettes.

The durations of NEP effects were comparable on the pEPSP but less so on the popspike, in keeping with the effects seen on the magnitude of popspike NEP. On the saline popspike, NEP lasted on average 5.55 minutes with none of the 20 cases of NEP lasting longer than 30 minutes. The duration of NEP on the saline popspike ranged from 0 -

19 minutes, whereas on the ketamine popspike, NEP effects lasted from 1 - >30 minutes. The mean duration of NEP on the ketamine popspike was 10.55 minutes, nearly twice that of the saline popspike, and in 3 of the 20 cases, NEP lasted greater than 30 minutes. In the case of the saline pEPSP the duration of NEP was from 1 - >30 minutes on both pipettes. The mean duration of NEP on the saline pEPSP was 13 minutes and there were 7 cases of NEP effects outlasting 30 minutes. The mean duration of NEP on the ketamine pEPSP was 11.72 minutes, with 6 cases of NEP lasting greater than 30 minutes.

Physiological and Histological Verification:

Recording through the cerebellum, on the descent to the LC, is characterized by distinctive active and inactive zones, reflecting the cell and fibre layers. If this layered pattern was not seen a more lateral site was used. In the cerebellum, though not documented, observation showed that cells typically responded to glutamate stimulation with a very brief burst of activity, a few seconds at most, followed by a longer period of silence, on the order of 2 or more minutes. This characteristic response is similar to that seen in the LC as observed here and documented by Harley and Sara (1992). Histological verification of locus coeruleus placement was available for 10 of the 11 animals,

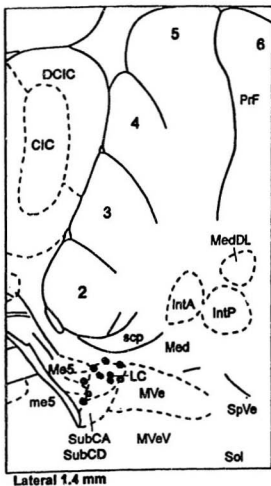


Figure 5: The 10 available sites from the 11 animals are illustrated. All recording/ejecting sites were within 400 μ m of the Locus Coeruleus.

(one brain was damaged when the microtome malfunctioned), Figure 5 illustrates these placements relative to LC. All placements were within 400 μ m of the LC. The proximity of the two dentate recording pipettes ranged from approximately 500 μ m to 1000 μ m apart. Unfortunately, diffusion of the pontamine dye, unlike with pressure ejection, could not be visualized on the sections, possibly because these tips were typically smaller or, more likely, simply because the dye density was insufficient.

Discussion

Steward et al. (1990) concluded from their double pipette study that a significant number of GABA receptors were blocked by the local diffusion of bicuculline methiodide. This conclusion was supported by the appearance of perforant path-induced multiple population spiking during baseline recording and tetanus-induced LTP at the commissural-dentate synapses, on the bicuculline pipette only. Since no multiple spiking and no LTP were evident on the saline pipette, positioned 0.5-1.0 mm away, Steward et al. also concluded that the blockade of GABA receptors was highly localized.

In the present study it was found that, as bicuculline diffusion produced significant GABA receptor blockade in the

Steward study, so the diffusion of high concentrations of ketamine produced a localized blockade of a significant number of NMDA receptors. Blockade was indicated by the significant reduction in the magnitude of LTP produced on the ketamine pipette as compared to that on the nearby saline pipette. In the absence of pharmacological manipulation, in twelve experiments, eight trains of eight pulses at 400 Hz delivered to the perforant path produced LTP (>30 mins) of the pEPSP (n=7/12), the popspike (n=9/12) and of the popspike peak latency (n=11/12). Where LTP was not seen, short term potentiation (STP) was. In contrast, at the ketamine site, significant attenuation of pEPSP and popspike increases and to a lesser extent of latency decreases were apparent. A complete block of potentiation, STP or LTP, was evident on the pEPSP in four of 12 cases and on the popspike in five of 12. It appeared that the latency change was the most resistant to NMDA channel antagonism since at least a short-lasting effect was evident in all 12 trials. When pEPSP and popspike increases were both completely blocked (n=3/12), latency decreases were still present on the ketamine pipette although the magnitude of the decrease was significantly less than that on the saline pipette. These data agree with previous findings. As shown in the past with perfusion and iontophoresis in vivo and bath application in vitro (Collingridge and Bliss, 1987),

and as demonstrated here with restricted, local application by diffusion through a large-bore pipette, NMDA receptor antagonists attenuate or prevent the induction of LTP on pEPSP, popspike and latency in the dentate gyrus.

It is noteworthy that the occurrence of the latency decrease in the absence of concurrent pEPSP and popspike changes is not unprecedented. In fact, it was Bliss and Lomo (1973) in their pioneering LTP study who reported that the latency decrease was the most common post-tetanic result, perhaps indicating the strength of this particular change. If latency decreases are the most robust of the LTP indicators it might be predicted, as indicated by the present results, that a higher level of ketamine would be necessary to block the change.

Dentate pipettes were purposely placed to achieve maximal similarity in the shape and size of the recorded waveforms since it was expected that the initial size of responses might determine the degree of potentiation produced. This was confirmed by regression analysis with both popspike NEP ($r = 0.34$, $p < 0.05$) and LTP ($r = 0.68$, $p < 0.05$). Comparability of the two responses in terms of potential for plasticity was necessary since a between-site comparison of LTP was used to determine NMDA receptor blockade. If responses had differed at the outset, a consistent difference in potential for potentiation could be

erroneously interpreted. Due to the purposeful matching of initial responses no between-site differences should have been present at the time of placement however, there were also no differences in baseline response sizes over time, as the ketamine diffused from the pipette. The lack of between-site differences in pEPSP, popspike and latency, prior to LTP or NEP experiments suggests that the NMDA channel plays no detectable role in normal synaptic transmission in the dentate gyrus in vivo, an opinion held by many (Collingridge and Bliss, 1987). However, it has been reported that i.p. ketamine does decrease responding, establishing a new baseline within 15 minutes. Thus, it may be that in the present study, by the time two evoked potentials were located, matched and then finally recorded, the new baseline was established.

Glutamate activation of the locus coeruleus (LC) produced reliable potentiation of dentate responses, evident in popspike and pEPSP increases. The overall average maximal popspike increase (140%) recorded at the saline site, in the present study, was comparable to that reported previously with the LC method (141% and 158%, Harley and Milway, 1986 and Harley and Sara, 1992, respectively), with intradentate NE (130-140%, Neuman and Harley, 1983) and with in vitro bath application (131%, Lacaille and Harley, 1985). The mean maximal pEPSP increase at the saline site (111%)

was also comparable to that found previously with LC stimulation (127% and 105-115%, Harley and Milway, 1986, Harley and Evans, 1988, respectively) and with bath applied NE (117%, Lacaille and Harley, 1985). Unlike LTP, consistent latency decreases are not characteristic of NEP. This study concurs with prior findings on this point (Harley and Milway, 1986, Harley and Sara, 1992). In four experiments where latency decreases were seen, all were long-lasting and were accompanied by NE-LLP of the pEPSP. Most cases of pEPSP NE-LLP were, however, unaccompanied by latency changes.

The reliability of the NEP effects reported here differ somewhat from that reported by others. In the present study, popspike increases were evident in only 80% of the 25 LC experiments conducted. It is not clear why popspike increases were not produced in the other five experiments since others have reported glutamate ejection within the LC invariably produces popspike increases (Harley and Milway, 1986, Harley and Evans, 1988 and Harley and Sara, 1992). The percent of successful popspike effects reported here does fall within the range of that reported in vitro with brief (10 min) bath application of a low (10 μ M) NE concentration (74% of slices, Lacaille and Harley, 1985). In contrast, pEPSP increases occurred in all 25 NEP experiments. Other investigators of pEPSP changes reported

fewer pEPSP increases with LC stimulation (65%, 71% and 65%, Harley and Milway, 1986, Harley and Evans, 1988 and Harley and Sara, 1992, respectively) or bath NE application (53%, Lacaille and Harley, 1985). In one in vitro study, however, pEPSP increases were induced invariably, however this effect was demonstrated with molecular layer evoked potential recording (Stanton and Sarvey, 1987).

The fact that pEPSP increases were invariably induced, unlike the findings of most other investigators, may be explained by a better isolation and therefore selective activation of the medial perforant path (MPP). Since NE produces NEP in the medial path and depression in the lateral path (Dahl and Sarvey, 1989) and since any perforant path-evoked pEPSP may be a mixture of LPP- and MPP-evoked activity (McNaughton and Barnes, 1978) the net effect of NE on the pEPSP results from NE-MPP potentiation minus NE-LPP depression. Thus, with selective stimulation of MPP, as might be more easily achieved in vitro (Stanton and Sarvey, 1987), pEPSP responses would be potentiated and not depressed by NE. Given the variety of strains, sexes and sizes of animals used in NEP experiments a comparison of perforant path placements is difficult. However, popspike peak latency may serve as a good indicator of relative MPP/LPP stimulation since MPP-evoked popspikes peak much earlier than do LPP-evoked popspikes. Thus the latency of a

popspike may reflect to some degree the relative contributions of each pathway. Harley and Sara (1992) who reported pEPSP increases in only 65% of LC experiments also report that in a clonidine experiment which involved four animals, the mean popspike peak latency was approximately 4.6 ms. In this study where pEPSP increases were seen in 100% of LC experiments, the mean latency over all 25 experiments was 4.0 ms. This difference in latency may reflect better relative isolation of the MPP in this study than in the Harley and Sara investigation. A couple of single experimental illustrations in Harley and Evans (1988) also support this idea. In one experiment in which latency was 3.4 msec NE-LLP of the pEPSP was evident whereas in another where latency was 4.7 msec no increase in pEPSP slope was observed.

The pEPSP and popspike increases seen in LTP are always more robust than those evident in NEP, within a subject. This NEP/LTP difference does not itself suggest a mechanistic divergence but may merely reflect a quantitative difference; tetanic activation is a stronger stimulation than NE release. However, the fact that popspike peak latency decreases occur invariably in LTP (12/12 experiments) and rarely (4/25 experiments) in NEP suggests that NEP and LTP are mechanistically distinct.

The main purpose of this experiment was to clarify the role of the LTP mechanism, the NMDA receptor, in NEP. In direct contrast to the in vitro findings which support the hypothesis that the NMDA receptor underlies the induction of NEP (Burgard and Sarvey, 1989, Sarvey, 1988, Stanton et al., 1989), in the present in vivo study, it was found that blockade of a significant number of NMDA receptors did not attenuate NEP. The magnitude of NEP, of the pEPSP and popspike, was not attenuated on the ketamine pipette as compared to the saline pipette. This suggests that in the in vivo preparation the induction of NEP does not depend on the NMDA receptor.

Not only did the presence of ketamine not attenuate NEP of the popspike, but, in fact it appeared that the presence of ketamine enhanced the popspike increase which was significantly larger at the ketamine site than at the saline site. Given that ketamine has been shown to increase NE availability by decreasing NE reuptake systemically in ferret myocardium (Cook et al., 1992), it is certainly possible that the same effect occurred here. If ketamine did inhibit NE reuptake the net effect would be prolonged exposure to a larger volume of NE. As presented in the introduction, a prolonged exposure to a higher concentration of NE produces larger and longer lasting popspike NEP than does a shorter exposure to a lower concentration (Lacaille

and Harley, 1985, Stanton and Sarvey, 1985b). More robust LC stimulation, which presumably produces a larger NE release, also induces larger and more enduring popspike NEP (Harley and Sara, 1992). Hence, ketamine, by prolonging the synaptic presence of NE appeared to mimic the effect of more robust LC stimulation. However, it also appears that prolonging NE exposure is advantageous to cellular excitability only, since ketamine produced no facilitatory effect on pEPSP NEP, an effect which is consistent with the previously mentioned investigators who also reported effects of enhanced NE exposure on popspike only (Stanton and Sarvey, 1985b and Harley and Sara, 1992).

Prolonged exposure to NE may also result from α_2 -adrenergic receptor blockade, given that the release of NE from LC terminals is under autoinhibitory control. Blocking LC autoinhibition would prolong LC stimulation-induced NE release, the effect of which may be similar to the effect of inhibited NE reuptake. In a study utilizing electrical activation of the LC, idazoxan blockade of α_2 receptors resulted in the facilitation of popspike NEP "in some cases" but as in the case of ketamine, facilitation of pEPSP NEP was not reported (Washburn and Moises, 1989). Again an effect on cellular excitability is suggested.

As briefly alluded to in the introduction it is interesting that in LTP, and perhaps also in NEP, two

independent plasticity events seem to occur (Taube and Schwartzkroin, 1988). One is synaptic plasticity, which accounts for pEPSP change as well as popspike change, insofar as pEPSP potentiation accounts for that in the popspike. The other is plasticity of what is called pEPSP-spike coupling (E-S coupling). Taube and Schwartzkroin, working in the CA1 of the in vitro slice, recording dendritic pEPSP and somal layer popspike, found that the potentiation of the pEPSP did not correlate well with the degree of potentiation in the popspike. A pEPSP of a given size elicited a larger popspike after potentiation than prior to induction. They theorized that in addition to synaptic plasticity, which accounted for pEPSP increases and that portion of the popspike increase that could be predicted from the pEPSP increase, E-S coupling potentiation also resulted. This is a common finding in LTP experiments both in CA1 and dentate (Bliss and Lomo, 1973). In the present investigation the LTP data collected at the saline site, when subjected to regression analysis, revealed that the magnitude of LTP on the pEPSP served as a significant predictor ($r = 0.63$, $p < 0.05$) of the magnitude of LTP on the popspike. This suggests, according to the reasoning of Taube and Schwartzkroin (1988), that in this study in the in vivo dentate gyrus, tetanic stimulation produced a synaptic effect.

The change in the popspike was predicted by that in the pEPSP.

Applying the theory of Taube and Schwartkroin to NEP in the in vivo dentate gyrus, regression analysis revealed that at the saline site, the magnitude of pEPSP NEP significantly predicted the degree of popspike NEP ($r = 0.38$, $p < 0.05$). Furthermore a popspike increase was never observed in the absence of a pEPSP increase. As in the LTP experiments, this suggests that norepinephrine induced plasticity at the synapse and this modification produced pEPSP and popspike increases. The change in the popspike is predictable from the pEPSP change.

However, the facilitatory effect of ketamine (and idazoxan, Washburn and Moises, 1989) on popspike but not pEPSP, suggest that some E-S coupling must have been involved and that this is the component effected when NE exposure is prolonged. This is supported by the fact that the predictability of popspike increases from pEPSP increases drops to nonsignificance on the ketamine pipette. Regression analysis showed that popspike LTP and NEP magnitudes were predicted by the degree of pEPSP potentiation at the saline sites. However, at the ketamine site (where LTP was greatly attenuated or absent), NEP of the popspike was no longer predicted by that of the pEPSP ($r = 0.22$, $p > 0.05$). This suggests that if ketamine enhances

NE exposure by inhibiting NE reuptake, the effect is felt by the E-S coupling mechanism and not by the synapse per se. Future experiments concentrating on the effect of LC stimulation on pure MPP-elicited and LPP-elicited responses should help clarify whether NEP is predominantly an E-S coupling potentiation or whether in fact synaptic plasticity is disguised by the subtractive effect of NE-induced depression on the lateral component. Much more work is needed to understand the mechanisms that mediate synaptic plasticity and those that mediate E-S coupling potentiation.

The present findings show that LTP and NEP do not converge on the NMDA receptor, in vivo. Such is not the case in vitro. Why then the difference? There are a number of probable explanations. Firstly, as detailed in the introduction, there are elements of dentate circuitry that are entirely absent in the in vitro slice. The excitatory mossy cell efferents and many of the inhibitory basket cell connections would be disconnected by the slice preparation (Sharfman, 1991). However, the basic circuitry that mediates NEP must remain intact since the same popspike and pEPSP changes, of comparable size and duration, take place in vitro and in vivo. It might be speculated that the missing circuitry is serviceable but not necessary to NEP and LTP induction. Thus, without the additional effect of NE on these elements, LTP and NEP become interdependent, as

shown in vitro. NE (or β -receptor activation) and HFT stimulation (which produces NMDA receptor activation) must cooperate to induce plasticity in the slice. As speculated by Harley, (1991), since LTP is more difficult to elicit in the slice than in vivo, facilitating factors such as NE may be required for reliable dentate slice LTP induction. In vivo, it appears that either mechanism alone is sufficient to induce plasticity. The NMDA receptor is sufficient but not necessary for dentate plasticity in vivo.

Alternatively, it may be the case that the mechanisms activated by bath applied NE and those activated by endogenous NE release are not entirely the same. Perhaps the true response of a population of dentate granule cells to natural NE release is better simulated by in vivo activation of the source LC than by bath application of NE in vitro. Stimulation of the LC with exogenous glutamate produces an endogenous and therefore physiological level of NE release at the synapses. With bath application of NE in the in vitro slice, the observed actions of NE may occur at physiologically irrelevant levels and locations. The bath administered level of NE is not necessarily physiologically relevant and is free to act even at synapses where it may not normally do so, while LC activation elicits NE release throughout the system (Foote et al., 1991). This implies that bath applied NE fails to activate all the normal

mechanisms or activates extra-synaptic mechanisms, such that a codependence between NEP and LTP is created. This possibility is supported by the fact that, in the hippocampus, the sites of NE release do not strongly correlate with the locations of noradrenergic receptors (Crutcher and Davis, 1980). Hence bath applied NE could readily activate adrenergic receptors not normally activated by synaptic NE release.

Recent in vitro findings suggest another alternative. Rather than assuming that because of indiscriminate adrenergic receptor activation the role of the NMDA receptor in NEP in the slice is different from that in vivo, it may be the pattern of the activation of these adrenergic receptors that differs in vitro versus in vivo. Dahl and Li (1994) working in the dentate slice, have shown that the β -agonist isoproterenol given at a subthreshold 75nM dose produced short-term popspike potentiation that did not outlast a 30 minute wash. However, a large 1 μ M dose produced an NMDA receptor-dependent NE-LLP. Interestingly, sequential applications of 75nM isoproterenol, separated by a 30 minute washout, produced NE-LLP. Furthermore this NE-LLP was blocked by metoprolol but not by APV NMDA receptor blockade. Possibly sequential low dose isoproterenol-induced β -receptor activation in vitro, more closely mimics endogenous, in vivo, NE release. Perhaps NEP and LTP would

not have to cooperate in vitro if NE were presented in a pattern that resembles that of natural release.

Although the NMDA receptor is not vital to NEP induction, NE may still promote LTP induction by facilitating calcium influx through voltage-gated Ca^{2+} channels (Gray and Johnston, 1987). By enhancing intracellular calcium accumulation NE can enhance the calcium-dependent cascade of biochemical events that lead to the establishment of LTP, all independently of the NMDA receptor channel. In addition, these data do not refute the possibility that NEP and LTP may converge on common mechanisms of expression and maintenance. Since protein synthesis inhibitors prevent the late phase of both phenomena (Stanton and Sarvey, 1985b, Sarvey, 1988) it is possible that saturation of one potentiation would occlude the maintenance of the other, an event that would take place post-induction.

This study is the first to investigate the role of the NMDA receptor in NEP in the in vivo dentate gyrus and therefore the first to find that NMDA receptor blockade does not interfere with NEP induction or maintenance. It is necessary to acknowledge, however, that ketamine, as an NMDA noncompetitive antagonist has been classified as use-dependent (MacDonald et al., 1991). Thus, if low-frequency activation is insufficient to open NMDA channels

(Collingridge and Bliss, 1987) it could be suggested that the ketamine did not enter the channels and therefore could not block NEP. However, in this study and in the pilot, ketamine blocked LTP, even when not preceded by NEP-inducing stimulation. It might still be argued, though, that during LTP-inducing tetanic stimulation, unlike during LC activation, sufficient NMDA receptor stimulation would occur to permit ketamine entry at that point in time. Thus, LTP attenuation could occur where NEP was intact. However, it might be expected that if such was the case, Ca^{2+} would also enter and some level of LTP would still occur, that is attenuation would be expected (Maren et al., 1991). Again, a complete block of LTP was evident in some animals and in these same animals, NEP was intact. There is other evidence to suggest that ketamine blocks the channel even with low frequency stimulation. In cultured mouse hippocampal cells it was found that bath-applied ketamine often depressed NMDA-activated currents on the first agonist application as compared to a control (MacDonald et al., 1991). Also, in cultured rat hippocampal cells, ketamine, unlike another channel antagonist (MK-801) produced a non-use-dependent block of the NMDA channel, reducing NMDA-induced current fairly efficiently and at short latency (Halliwell et al., 1989). Even in studies where tetanic stimulation has been used, the use-dependency of ketamine has been questionable.

In the urethane anaesthetized rat, while investigating the involvement of the NMDA receptor in tetanically-induced paroxysmal dentate discharges, Stringer and Lothman (1988) found that both ketamine and MK-801 blocked this activity but only MK-801 required an initial discharge to do so. Finally, in the anaesthetized rat, it was found that ketamine, but not MK-801, blocked LTP induction at the perforant path-dentate gyrus synapse just ten minutes after i.p. administration, leaving the authors to conclude that ketamine, unlike MK-801, is fast-acting and is not use-dependent (Maren et al., 1991). With the ongoing diffusion of ketamine used in the present study, ketamine block should also be irreversible. Given the complete LTP block obtained in some experiments and in the pilot study, it appears that ketamine did block the NMDA channel.

Though the first to demonstrate NMDA-independent NEP in vivo, this study is not the first to present an NMDA-independent form of plasticity mediated by the NE- β pathway, either in rat hippocampal NEP or other systems.

In CA1 of the in vitro slice, a β -mediated, NMDA-independent potentiation has been described, which the authors call δ AP (Heginbotham and Dunwiddie, 1991). Like dentate NEP, a 30 minute bath application of 500nM isoproterenol induced an enhancement of the popspike that outlasted the wash. δ AP was blocked by timolol, but not by

APV pretreatment. Timolol application did not reverse BAP once induced. Furthermore, popspike potentiation was mimicked by forskolin and cAMP analogues. Further investigation (Dunwiddie et al., 1992) revealed that BAP was accompanied by a prolonged depolarization of the pyramidal cells and a reduction in the Ca^{2+} dependent potassium conductance that mediates the AHP. These effects were also mimicked by forskolin and cAMP analogues. Cellular depolarization and reduced potassium conductance also accompany dentate NEP (Lacaille and Schwartzkroin, 1988, Stanton et al., 1989, Haas and Rose, 1987). Thus, BAP may be the result of enhanced cellular excitability (E-S coupling) mediated by the cAMP biochemical cascade, synaptic enhancement is not indicated since pEPSP changes were never observed. Thus while the cAMP-mediated potentiation of CA1 is very similar to that in dentate, dentate NEP has, primarily or additionally, a synaptic effect. Given the lack of NMDA receptor involvement in either BAP or NEP, they are more similar to one another than to LTP. Further elucidation of BAP processes in vitro and an exploration of BAP capability in vivo, should contribute to understanding of dentate NEP. In particular it would be interesting to understand what part of the dentate β -cAMP pathway diverges from that of CA1 to produce enhancement of the synaptic component of the population response. As indicated by

invertebrate studies, the same biochemical pathway can mediate both synaptic and cellular excitability potentiation (Klein et al., 1986, Schuman and Clarke, 1990).

While NE is not involved, in the invertebrate aplysia, NMDA-receptor independent, cAMP-mediated plasticity has been firmly established (Abrams and Kandel, 1988, Hawkins et al., 1993). Two kinds of cAMP-dependent learning have been studied in aplysia; sensitization and associative classical conditioning. Sensitization occurs when a response, (e.g. gill withdrawal), is strengthened to a previously neutral stimulus (e.g. siphon touch) following a particularly threatening stimulus (e.g. noxious neck or tail stimulation). In classical conditioning aplysia learns to withdraw its tail to siphon touch when siphon touch is repeatedly preceded by tail shock. Both forms involve presynaptic facilitation where the pairing of sensory neuron activation (siphon touch) with modulatory neurotransmitter (noxious stimulation), which invades the sensory neuron terminal, enhances future transmitter release from that sensory neuron terminal. The authors call this a pre-modulatory coincidence, as opposed to the Hebbian pre-post coincidence.

Both these forms of learning depend on cAMP and serotonin (5-HT) initiates the biochemical cascade which underlies this plasticity. The 5-HT activated pathway is,

however, very similar to that in NEP. As NE does in dentate, 5-HT acts via a G-protein to stimulate adenylate cyclase to produce cAMP. cAMP acts as a second messenger, activating protein kinase-mediated phosphorylation. The result is a reduction in a potassium conductance which yields a prolonged action potential and therefore an enhanced Ca^{2+} influx. In sensitization cAMP has the additional effect of enhancing a voltage gated Ca^{2+} influx. The enhanced Ca^{2+} accumulation that results from these actions produces the facilitated neurotransmitter release that underlies sensitization and associative learning in aplysia. These effects are reminiscent of those in NEP, where reduced potassium conductance (Haas and Rose, 1987) and enhanced voltage-gated Ca^{2+} influx (Gray and Johnston, 1987) have also been observed.

In aplysia sensitization and associative learning can both be short- or long-lasting and either effect depends on the 5-HT-cAMP pathway. However, long-lasting plasticity has additional requirements for altered gene expression and morphological change. This is similar to NEP where NE-SLP and NE-LLP are both blocked by β -antagonists but only NE-LLP is blocked by protein synthesis inhibitors (Stanton and Sarvey, 1985b). As stated, it has also recently been observed in aplysia, that the one biochemical pathway may produce both synaptic enhancement and cellular excitability

increases (Schuman and Clarke, 1990, Klein et al., 1986). Perhaps the cAMP pathway can account for both the synaptic and pEPSP-spike (E-S) coupling enhancement sometimes observed in NEP. The similarities between dentate NEP and sensitization and associative learning in aplysia suggest that NEP may be less like pre-post coincidental NMDA-mediated LTP and more similar to the presynaptic modulatory mechanism observed in invertebrate learning.

The importance of cAMP plasticity to learning is well illustrated in drosophila learning mutants, dunce and rutabaga. In these mutants, the cAMP biochemical pathway is dysfunctional, imparting an inability for plasticity. It is thought that this is the reason for the memory deficits observed (Zhong and Wu, 1991). Interestingly, it has also been shown in the mammalian visual system that both developmental and adult experience-dependent plasticity, depend on the NE- β pathway (Kasamatsu, 1991). In kittens, monocular deprivation induces a shift in ocular dominance columns such that cells come to respond only to the open eye. NE acting via the β -receptor is necessary for this plasticity. NE depletion prevented this shift and subsequent direct injection restored it. Recovery from monocular deprivation was also inhibited by NE depletion and facilitated by NE. In adults, this shift does not normally occur, binocular vision remains intact after monocular

deprivation, except in the presence of NE. Adult exposure to NE seems to extend developmental plasticity. Revealing its true modulatory nature, NE modifies the ocular dominance shift regardless of direction. Kasamatsu concluded that " β -receptors occupy a key step in the biochemical cascades underlying the regulation of visuocortical plasticity".

Altogether, these mammalian and invertebrate findings, in addition to the present results, support the ability of cAMP to behave as a substrate for plasticity, independent of the NMDA receptor. These results suggest that cAMP may be both necessary and sufficient for some kinds of learning.

In the introduction, which reviews the history of inquiry into the role of NE in the dentate, the theory was presented that NE, which is released during aroused and attentive states, functions as a neuromodulator. As a modulator, NE operates in an indirect fashion, mediating effects that are distinct from straightforward inhibition and excitation. The evidence shows that the overall effect of NE on cellular activity depends on the balance of α -inhibitory and β -excitatory activation. As a neuromodulator, it is implied that NE achieves its effects by modifying the functioning of other mechanisms. This has been demonstrated in many modalities, including the visual, olfactory and cerebellar cortices, where NE modifies cellular responding to improve the signal to noise ratio of

the sensory signal. As a true modulator, NE makes inhibitory signals more inhibitory and excitatory signals more excitatory. Due to NE co-release, these sensory signals have more physiological impact. As the research also shows, NE release occurs during learning situations, which are arousing and attention-getting, for the purpose of promoting the learning. It was postulated that NE promotes learning by enhancing LTP, the memory substrate of the hippocampus, via the NMDA receptor. This theory received great support in vitro where, in fact, it was found that LTP was induced only in the presence of NE. Furthermore, it was also found that NEP in the slice depended on the LTP induction mechanism, the NMDA receptor. In the present study, however, it has been shown that blockade of the NMDA channel in vivo, which obstructed LTP induction, did not hinder the induction of NEP. LC-induced potentiation does not require the NMDA channel, unlike LTP. Thus it appears that NEP is a phenomenon distinct from LTP at least in terms of induction. Although NE does modulate cellular responding, and LTP, perhaps both in vitro and in vivo, its role may surpass the restricted bounds of the "neuromodulator" role such that, if necessary, it can induce potentiation via its own mechanisms. Sufficient evidence, from various systems, has been presented to support the contention that NE, via the β -cAMP pathway, is both

necessary and sufficient for some kinds of neuronal plasticity.

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