PARAGIGANTOCELLULARIS POTENTIATION OF PERFORANT PATH INPUT TO THE DENTATE GYRUS IS ATTENUATED BY LOCAL β- ADRENERGIC BLOCKADE BUT NOT BY LTP SATURATION

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BY

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ABSTRACT

In vitro work indicates that both frequency mediated potentiation (LTP) and norepinephrine mediated potentiation (NEP) in the dentate gyrus of the hippocampus are attenuated by NMDA receptor blockade and/or β-receptor blockade, suggesting both forms of potentiation converge on a single form of plasticity (Stanton, Mody, & Heinemann, 1989). However, *in vivo* work by Frizzell and Harley (Frizzell & Harley, 1994) indicates that locus coeruleus activated NEP can be induced when LTP has been occluded by intradentate application of the NMDA receptor antagonist ketamine. This suggests that *in vivo* NEP is independent of the LTP plasticity mechanism. The present study further explores the LTP/NE relationship.

Stimulation of the nucleus paragigantocellularis (PGi) with a 333 Hz train 30 ms prior to single pulse stimulation of the perforant path (PP) input to the hippocampus results in presumed adrenergic mediated short-term potentiation of PP-evoked potentials recorded in the dentate gyrus which is occluded by a systemic β-blocker (Babstock & Harley, 1992). This form of NEP was used in the present study. Using both timolol and saline filled recording pipettes placed in the dentate gyrus, it was demonstrated that PGi stimulation is an effective method of producing NEP, that PGi-induced

potentiation is locally mediated by β-receptors in the dentate gyrus and that LTP can be produced in the presence of intradentate application of β-blockers. Further, local β-blockade marginally reduces LTP induction but β-blockade has no effect on final LTP magnitude. An apparently independent PGi-induced potentiation persists following LTP saturation and following LTP saturation, PGi-induced potentiation can again be shown to be β-receptor dependent. These results support the hypothesis that there are two distinct, independent forms of plasticity which can be initiated in the dentate gyrus, β-mediated and NMDA mediated. Further, the changes induced by NE and high frequency stimulation appear to be additive.

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Plasticity in the brain has been of intense interest to those who seek to understand the physiological basis of learning and memory. Neuronal plasticity can be defined generally as the capacity of neurons to change, functionally and even structurally, in a relatively short period of time. Plastic changes have been observed in many cortical areas; plasticity within the hippocampus, an area important to spatial learning and memory formation, has been widely studied. In the hippocampus plastic changes in synaptic efficacy, changes which are the result of specific neural activity, have been hypothesized to underlie memory. At least two forms of synaptic plasticity exist in the dentate gyrus (DG) of the hippocampus: norepinephrine-induced potentiation (NEP) and frequency-induced potentiation, or, as it is more commonly referred to, long-term potentiation (LTP).

LTP and NEP have been studied using many approaches, including *in vitro* slice preparations, acute anaesthetized *in vivo* preparations, and chronic *in vivo* preparations. Each approach has contributed to a fuller understanding of plasticity in general, and to plasticity in the DG specifically. However, there are discrepancies between the findings from *in vivo* and *in vitro* work. *In vitro* work in the DG indicates that NEP and LTP are dependent on the same

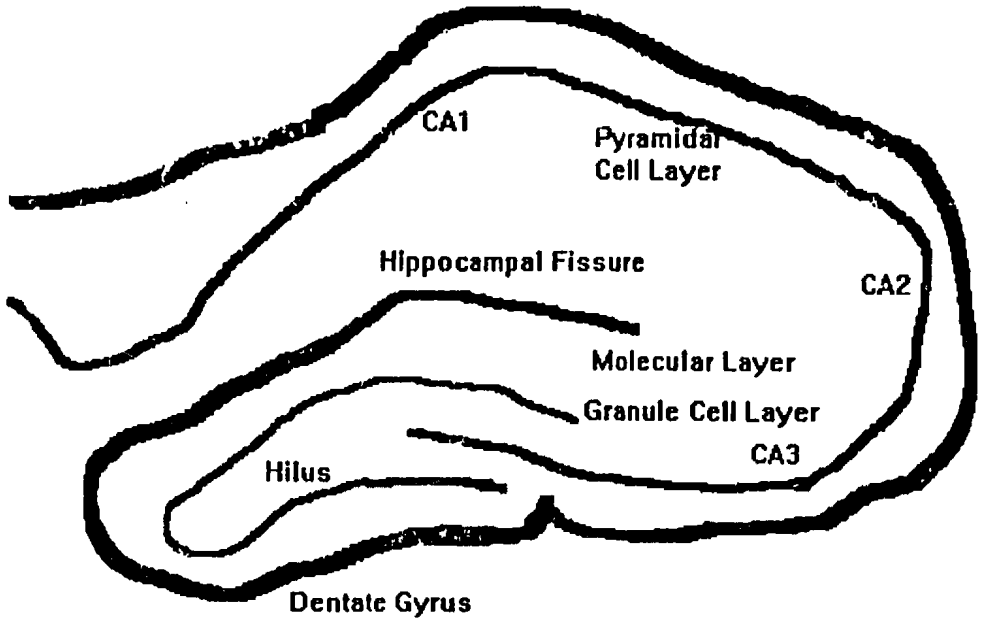
mechanism (eg. Burgard, Decker, & Sarvey, 1989; Stanton, Mody, & Heinemann, 1989). *In vivo* work has not yet clearly demonstrated if LTP and NEP are utilizing the same mechanism, if NEP promotes LTP by a separate mechanism, or if there are two separate and distinct systems producing potentiation. The present study will focus on some possible common mechanisms underlying NEP and LTP in the DG of the hippocampal formation *in vivo*; the aim of the study is to elucidate the degree of independence of the mechanisms and events which are responsible for the induction of plastic changes in the DG.

The Hippocampal Formation

The hippocampal formation encompasses four cortical regions: the DG, the subicular complex, the entorhinal cortex (EC) and Ammon's horn (Amaral & Witter, 1989). The hippocampus includes only the DG and Ammon's horn, not the subicular complex or the EC. The longitudinal axis of the hippocampal formation forms an elongated C-shape, extending from the septal nuclei rostrodorsally to the temporal lobe caudoventrally, and is referred to as the septotemporal axis (Amaral & Witter, 1989). The plane perpendicular to the long plane is referred to as the transverse plane (see Figure 1). The basic intrinsic circuitry of the hippocampal formation is that of unidirectional connections between the four cortical regions forming what is referred to as the trisynaptic

Figure 1

Transverse section of the hippocampus illustrating the various cell layers.



circuit: (a) the DG synapses with projections from the EC via the perforant path (PP), (b) the granule cells of the DG project via their mossy fibers to CA3 of Ammon's horn, and (c) CA3 pyramidal cells in turn give off collateral axons, termed Schaffer collaterals, which synapse within CA3 and in CA1 (Amaral & Witter, 1989). While this basic description of the intrinsic circuitry of the hippocampus may seem to describe a simple structure, there are reciprocal connections among the different areas of the hippocampal formation, as well as inputs to the hippocampus from various regions of the brainstem and the cerebral cortex, making it complex. The hippocampus is not functionally simple either, the role of the hippocampus appears to be one of integration of multimodal inputs; by integrating incoming information from the various inputs and relaying that information to regions of the cerebral cortex, the hippocampus is proposed to be vital for the formation of certain types of memories (see Squire, 1987 for review).

Dentate Gyrus Anatomy

The DG is composed of three main layers: the molecular layer, the granule cell layer, and the polymorph layer (for an extensive review of hippocampal anatomy see Amaral & Witter, 1989). The polymorph layer is also referred to as the hilus. The granule cells define the shape of the DG and are the

principal cell type of the DG (Amaral & Witter, 1989). The dendrites of the granule cells extend to, and synapse with, the PP terminals in the molecular layer. The axons of the granule cells, termed mossy fibers, collateralize in the polymorph layer then form synapses in the CA3 region with pyramidal cells (Amaral & Witter, 1989). Found in all areas of the DG, but in highest concentration in the hilus, are interneurons. There are many types of interneurons, some of which have inhibitory, others, excitatory influences on granule cell activity (Amaral, 1978).

Lamellar Hypothesis of Hippocampal Organization

Andersen, Bliss and Skrede (1971) proposed the lamellar hypothesis of hippocampal organization. The hippocampal cortex was thought to be divided along the septotemporal axis into parallel slices or lamella which functioned independently. Activation of a localized area of the entorhinal cortex resulted in activation of a lamella of tissue via four pathways (the perforant path, the mossy fibers, the Schaffer collaterals and the alvear fibers of CA1, all of which are oriented in the transverse plane) in succession. Activity in neighbouring lamellae could be modified by transverse inhibitory and excitatory connections, however the major flow of activity was proposed to be within a slice not between slices. This concept of the hippocampal

formation as a series of slices lead to the preparation used *in vitro*. Since the Andersen et al. (1971) paper was published, it has become clear that the lamellar hypothesis is an over-simplification of the functional organization of the hippocampal formation.

Amaral and Witter (1989) examined and summarized the data obtained from several studies of hippocampal organization and conclude that "aside from the mossy fibers, none of the intrinsic connections of the hippocampal formation is organized in a lamellar fashion". The hippocampal formation contains projections which run both in the septotemporal and the transverse planes, allowing for flow of information in both axes over much of the hippocampal formation, not just within a single narrow slice (Amaral & Witter, 1989). That activation of a portion of the entorhinal cortex does not result in activation of just one lamella points to the limitations of the *in vitro* slice preparation and the limitations of the conclusions which can be made from *in vitro* studies. The *in vitro* slice preparations are devoid of intrahippocampal projections along the septotemporal axes, a major set of connections, as well as cortical and subcortical connections.

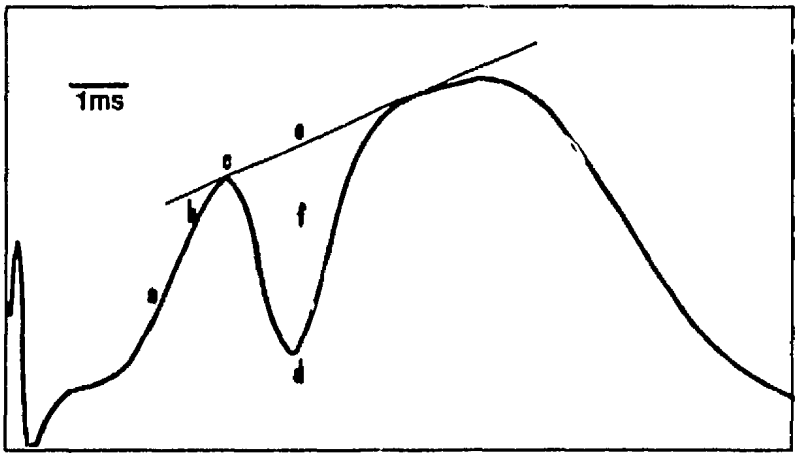
Evoked Potentials in the Dentate Gyrus

When the medial perforant path (PP) fibers are stimulated

with a single pulse, an evoked population potential can be recorded from the granule cells of the DG of the hippocampus (Andersen, Bliss, & Skrede, 1971; Lomo, 1971b). Activation of the PP-DG synapses causes depolarization of the dendritic membrane of the granule cells. Extracellular current flows from the cell body layer towards the dendrites, resulting in a negative potential in the synaptic region, and a positive potential in the cell body region, relative to a distant electrode (Bliss & Lomo, 1973). This depolarization is referred to as a population excitatory post-synaptic potential (pEPSP or EPSP) (Lomo, 1971b). Superimposed on the pEPSP is the population spike (popspike), a negative deflection maximal at the cell body layer, which reflects the synchrony of granule cell firing and the number of granule cells discharged (Andersen, Bliss, & Skrede, 1971; Bliss & Lomo, 1973; Lomo; 1971b). An increase in popspike size is said to reflect an increase both in the synchrony of and number of granule cells firing (Bliss & Lomo, 1973; Lomo, 1971a, 1971b). Typically, both the popspike and the pEPSP components are monitored when assessing treatment effects on hippocampal evoked potentials using at least two of the following measures (illustrated in Figure 2): (a) the pEPSP slope or amplitude (b) onset latency of the popspike, and (c) latency to popspike peak as well as size of the popspike, recorded as an (d) amplitude or (e) area measure or both.

Figure 2.

PP-evoked potential recorded from cell body layer. The population spike amplitude was measured as e-d, EPSP slope was measured as b-a/time, the onset latency was measured as the time from PP stimulus to c, the latency to peak was measured as the time from PP stimulus to d. "Area" was determined as the combined difference between the number of discrete points in the line forming the tangent between the two positive peaks and the number of discrete points forming the curve.



Long-Term Potentiation

Bliss and Lomo (1973) first described the phenomenon of LTP. They discovered that volleys of electrical stimulation of the PP fibers, arising in the medial entorhinal cortex and terminating at the granule cell dendritic molecular layer, resulted in a long-lasting increase in sensitivity of the population response recorded from granule cells of the DG to a single pulse to the PP. Volleys of stimulation were delivered at a rate of 10 to 20 Hz for 10 to 15 s, or 100 Hz for 3 to 4 s. Popspike amplitude and EPSP amplitude were found to increase while spike latency decreased following repetitive stimulation of the PP. These changes could persist for hours or days. In these experiments, LTP was arbitrarily defined as potentiation lasting for at least 30 minutes, a definition still employed. The authors concluded that there are two separate mechanisms involved in LTP, an increase in efficiency of synaptic transmission at the PP synapses and an increase in granule cell excitability. Bliss and Lomo noted that LTP was an artificially induced phenomenon, their work provided a major challenge: find a plausible connection between LTP and memory based tasks in the intact animal.

LTP has since been defined as the long-lasting increase in synaptic response following electrical stimulation of a input pathway to a cortical structure (Bliss, Errington,

Lynch, & Williams, 1990). While LTP can be induced in many cortical areas, it has been most extensively studied in the excitatory pathways of the hippocampus. LTP in the hippocampus has been the subject of such interest because its physiological properties appear relevant to learning theories (Bliss & Lomo, 1973). LTP is activity dependent, is quickly induced, can last for hours or days, and is associative and pathway specific (Bliss, Errington, Lynch, & Williams, 1990; Bliss & Lomo. 1973; Morris, Davis, & Butcher, 1990). As the present study is focused on potentiation in the DG, this review will be restricted to potentiation in the DG.

Frequency-Induced Potentiation is NMDA Receptor Mediated

LTP in the DG results when the PP is stimulated with a brief, high frequency electrical train (HFT). LTP induction is dependent on the activation of the post-synaptic NMDA receptor ionophore (Morris, Anderson, Lynch, & Baudry, 1986); activation of the NMDA ionophore is dependent on presynaptic and post-synaptic activity (Bliss, Errington, Lynch, & Williams, 1990; Morris, Davis, & Butcher, 1990). NMDA receptors are both voltage and chemically dependent. Opening of the NMDA ionophore requires the binding of glutamate to the recognition site and depolarization of the post-synaptic membrane (Collingridge & Bliss, 1987; Morris, Davis, & Butcher, 1990). When the post-synaptic membrane potential is

depolarized sufficiently, a magnesium ion, which blocks the NMDA receptor channel at the resting membrane potential, is removed from the channel (Collingridge & Bliss, 1987). If, concurrently, glutamate has been released presynaptically and glutamate binds to the recognition site, the channel will open. When the ionophore is open, calcium can pass through it and enter the cell (Collingridge & Bliss, 1987).

Calcium acts as a second messenger. An influx of calcium can trigger a cascade of reactions which result in the observed increase in cellular excitability (Collingridge & Bliss, 1987). An influx of calcium via the NMDA channel is required to trigger LTP (Lynch, Muller, Seubert, & Larson, 1988). Binding of glutamate to the NMDA receptor complex and post-synaptic membrane depolarization, the two required events for NMDA receptor ionophore opening, occurs in response to the high-frequency train stimulation to the PP used to induce LTP (Morris, Davis, & Butcher, 1990; Sarvey, Burgard, & Decker, 1989). NMDA receptor antagonists have been shown, both *in vivo* and *in vitro*, to prevent LTP induction but do not reverse LTP once it has been established (Collingridge & Bliss, 1987; Goldman, Chavez-Noriega, & Stevens, 1990; Morris, Anderson, Lynch, & Baudry, 1986). Chronic intraventricular infusion of the competitive NMDA antagonist D, L-AP5 has been demonstrated to prevent learning of spatial information needed to master the Morris swim task, as well as blocking the induction of LTP

(Davis, Butcher, & Morris, 1992; Morris, Anderson, Lynch, & Baudry, 1986).

Saturation of LTP

A behavioral approach to the question of the role of LTP in memory has been the LTP saturation methodology developed by McNaughton, Barnes, Rao, Baldwin and Rasmussen (1986). The approach is to deliver repeated HFT over a period of time to the PP to reach a ceiling of maximal frequency-induced change in the stimulated pathway, therefore removing the possibility of further potentiation and plastic changes. Animals are then tested on spatial tasks. Poor performance relative to controls would indicate that LTP induced change does underlie the acquisition of spatial memories. If LTP is involved in learning, and there is a limit to the amount of potentiation that can be induced, once that limit is reached, no further learning (or plastic changes) would be possible.

Studies using the methodology of McNaughton et al. (1986) were reviewed by Bliss and Richter-Levin (1993); serious problems with the method were identified. While the logic behind this approach seems sound, consistent positive results have not been obtained from these behavioral studies. The studies had used bilateral PP stimulation yet only one stimulating electrode was implanted in each PP. A single PP electrode would most likely not stimulate all PP fibers which

connect to the DG, as a result not all PP synapses would be saturated, so further plastic changes would still be possible and learning could still occur. Similarly, while recordings from the DG may indicate no further potentiation could be produced by PP stimulation, the recording electrodes sample only a fixed number of synapses in a limited area and other synapses could still be capable of further potentiation. It seems that it is functionally difficult in the whole animal to saturate all synapses in the DG and difficult to assess if all the DG granule cell connections have been saturated.

Neuromodulators

While much has been discovered about the mechanism of LTP, the debate over the biological relevance of LTP is far from resolved. The search for a plausible connection between potentiation in the DG and memory in the intact animal has led some researchers to investigate the role of monoamine containing cells, which project from nuclei in the brainstem to the DG (Kobayashi, Palkovits, Kopin, & Jacobowitz, 1974; Loy, Koziell, Lindsey, & Moore, 1980). Serotonin and NE are of particular interest as neuromodulators of cellular activity in the hippocampus, as there are large afferent projections to the DG and other regions of the hippocampus (Bliss, Goddard, & Riives, 1983; Loy, Koziell, Lindsey, & Moore, 1980). Again, since the focus of the present study is NEP and LTP in the DG,

the present review will be restricted mainly to NE and not serotonin.

NE Innervation of the Hippocampus

The major source of NE for many regions of the brain, including the cerebral and cerebellar cortices, is the locus coeruleus (LC) (Aston-Jones & Bloom, 1981a; Kobayashi, Palkovits, Kopin, & Jacobowitz, 1974). The LC is also the major source of hippocampal NE innervation (Loy, Koziell, Lindsey, & Moore, 1980). Fibers from the LC form the dorsal noradrenergic bundle, which the joins the medial forebrain bundle before entering the hippocampus (Loy, Koziell, Lindsey, & Moore, 1980). The densest LC-NE projection is to the hilar region of the DG, lower concentrations of axon terminals are seen in the molecular and granule cell layers (Loy, Koziell, Lindsey, & Moore, 1980; Stanton & Sarvey, 1985b; Winson & Dahl, 1985).

NE as a Modulator of LTP

Studies looking at NE as a modulator *in vitro* have uncovered a role for NE in LTP. Stanton and Sarvey (1985b) looked at the role of NE in LTP using the *in vitro* hippocampal slice preparation and NE depletion techniques. Slices from NE depleted rats did not differ from slices from normals in terms of baseline characteristics. However, NE depletion was found

in the DG, to reduce both the likelihood of LTP occurring and the increase in popspike amplitude following high frequency stimulation. The greater the degree of NE depletion, the greater the decrease in popspike amplitude increase (Stanton & Sarvey, 1985b). NE depletion had no effect on LTP in CA1. Similarly, β-receptor blockade in slices from normal rats was also found to reduce both the occurrence of LTP and the increase in popspike amplitude normally seen in response to HFT in the DG. When β-blockade effects were assessed on LTP in CA1 of normal slices, no reduction was observed. In the same 1985 paper, Stanton and Sarvey also reported a study which looked at the effects of serotonin depletion on LTP. Serotonin depletion had no effect on LTP in the DG nor in CA1.

The effects of NE depletion on LTP have also been studied *in vivo*. Bliss, Goddard and Riives (1983) reported that following bilateral NE depletion, baseline evoked potentials recorded from the hilus of the DG of depleted animals did not differ from responses recorded from control animals. As well the amount of HFT stimulation required to induce LTP did not differ between the two groups. However, the EPSP amplitude in the NE depleted group was half that of the control group following HFT delivery. No difference between control and NE depleted groups were seen in terms of EPSP slope or popspike amplitude following HFT delivery.

Robinson and Racine (1985) studied the effects of NE

depletion on DG LTP in the awake rat. NE depletion was found to result in a significant increase in baseline popspike and significant reduction in EPSP amplitude relative to pre-NE depletion values. Following HFT delivery, LTP of EPSP amplitude was found to be greater in NE depleted rats than in control rats, while the potentiation of spike amplitude was significantly less in the NE depleted group than the control group.

Depletion of NE has to be nearly complete before behavioral effects are seen (Dahl, Bailey, & Winson, 1983). Dahl et al. (1983) demonstrated when NE depletion is over 90%, there are changes in granule cell excitablity that can be seen between the awake but unmoving (still awake) and slow wave sleep behavioral states. In control animals, amplitudes of evoked action potentials recorded from the granule cells of the DG are greater when rats are in slow-wave sleep states than in still-awake states. When NE is depleted to a level greater than 90%, there is no difference between the amplitude of evoked action potentials recorded in the granule cell layer, indicating a role for NE in granule cell activity in different states. Dahl et al. (1983) predicted on the basis of these results that, in normal rats, NE release in the hippocampus during the still awake state should result in depression of the popspike recorded from the granule cell layer. However, when the LC was activated with glutamate in

unrestrained awake animals, the result was potentiation of the popspike not attenuation as was predicted by the depletion data (Klukowski & Harley, 1994).

While these depletion studies do in general indicate that NE plays a role in LTP, the results of the *in vivo* studies are somewhat conflicting. *In vivo*, Bliss, Goddard and Riives (1983) report in comparison to controls, NE depletion resulted in reduced EPSP slope following HFT and no significant change in spike amplitude while Robinson and Racine (1985) reported greater LTP of the EPSP and significantly less potentiation of popspike amplitude in depleted animals relative to non-depleted animals. Robinson and Racine noted, however, that when the measures were corrected for drug effects on the baseline PP-granule cell transmission, no difference in LTP was found between the depleted and non-depleted groups. Indicating that the observed effects may have been due to alterations in PP-granule cell transmission rather than changes in LTP. NE depletion studies use injections of 6-hydroxydopamine or reserpine into the dorsal noradrenergic bundle resulting in depletion of NE in all regions of the forebrain, which is not as specific to the hippocampus (Bliss, Goddard, & Riives, 1983; Dahl, Bailey, & Winson, 1983). As well, unless the depletion is almost complete, the loss of NE can be compensated for by an increase in tyrosine hydroxylase

activity and an increase in LC firing (Dahl, Bailey, & Winson, 1983). The possibility that the observed effects *in vivo* are due to changes outside the hippocampus can not be ruled out. Data from depletion studies must be interpreted with caution.

Cyclic AMP Levels and LTP

HFT stimulation of the PP input in the slice results in a 3 fold short-term increase in cAMP levels in the DG (Stanton & Sarvey, 1985a). NE depletion reduces this increase markedly and decreases basal cAMP levels (Stanton & Sarvey, 1985a). As well, NE bath application to normal slices results in an increase similar to that seen in normal slices in response to HFT stimulation. Unlike the HFT-induced increase in cAMP levels, the NE-induced cAMP increase lasts for more than 30 minutes. When NE depleted slices were tested with NE added to the bath, cAMP levels were also seen to increase dramatically (Stanton & Sarvey, 1985a). The cAMP levels were investigated, as cAMP had been proposed to act as a second messenger for NE β-receptor effects. Stanton and Sarvey (1985a) conclude from these data, as well as from their earlier findings (1985b), that NE stimulation of cAMP is involved in the expression of LTP.

Norepinephrine-Induced Long-Lasting Potentiation (NE-LLP)

In 1983, Neuman and Harley first described NE-LLP. When

studying the effects of *in vivo* iontophoretic application of NE in the DG on PP-evoked field potentials, it was discovered that an enduring potentiation of spike amplitude lasting 30 minutes or more could be produced by application of NE alone. The resulting potentiation, termed NE-induced long-lasting potentiation (NE-LLP) or NE-potentiation (NEP), is like LTP but tetanizing stimulation is not required to induce the potentiation. Following NE iontophoretic application to the granule cell layer of the DG, spike amplitude increased markedly. The appearance of the first notable increase in spike amplitude was varied, increases appeared between 30 s to 8 min following NE application. In 41 of 54 sites tested, potentiation of popspike amplitude was observed. In 16 of the 41 sites which displayed potentiation, long-lasting potentiation was observed. In these cases, the maximal increase in spike amplitude was typically attained within 30 minutes of NE application, however, further increases after the first 30 minutes were also observed. While in this first study, spike latency changes were not assessed and no reliable changes were seen in EPSP amplitude, later *in vitro* studies demonstrated reliable, long-lasting increases in EPSP size and decreases in popspike onset latency in response to NE (Lacaille & Harley, 1985; Stanton & Sarvey, 1987).

The NE effects on DG PP-evoked potentials were shown to be β-receptor mediated (Stanton & Sarvey, 1985b, 1985c;

Lacaille & Harley, 1985). The α-adrenergic receptor antagonists have no blocking or attenuating effects on NEP (Lacaille & Harley, 1985). NE appears to be important for the induction, rather than the maintenance, of LLP. In slice experiments, LLP has been shown to persist after NE washout and *in vivo*, LLP can be blocked by beta blockers if administered prior to NE application but once NE-LLP has been initiated, beta blockers have no effect (Harley & Evans, 1988).

NEP and Second Messenger Systems

β-Receptor activation results in activation of an intracellular second messenger system. NE-potentiating effects have been shown to be β-mediated, as will be discussed later, NEP most likely results from activation of the β-receptor. The β-receptor works via a G protein mechanism, not a direct ion channel. NE binding to a β-receptor recognition site results in activation of a G-protein associated with the intracellular membrane (Schwartz, 1985). G-protein activation in turn activates the enzyme adenylate cyclase, which converts ATP to cAMP. cAMP acts as a second messenger and has many intracellular effects, including initiation of phosphorylation of proteins associated with the membrane, and can therefore alter cellular excitability (Schwartz, 1985). As outlined earlier, NE and HFT both have the effect of increasing cAMP

levels (Stanton & Sarvey, 1985a).

NEP and LTP Induction Mechanisms are Synergistic *in Vitro*

Slice work by Stanton, Mody and Heinemann (1989), revealed that NE has an enhancing effect on HFT-induced LTP by enhancing calcium influx during NMDA receptor activation. These researchers also report NE induced LLP can be blocked by pretreatment with the NMDA antagonist APV. In fact, they reported that when APV is present *in vitro* all NE potentiation, short and long-term, is prevented. This finding is supported by the results of a study by Dahl and Sarvey (1990), which demonstrated that β-agonist in the bath will result in long-lasting increases in cellular excitability which can be blocked by the presence of APV (Sarvey, Burgard, & Decker, 1989).

The data from the work of Stanton and Sarvey's group *in vitro* has led them to conclude that NE induces LLP either by activating or acting synergistically with the NMDA receptor, as NMDA receptor antagonists block NE-induced LLP (Burgard, Decker, & Sarvey, 1989; Sarvey, Burgard, & Decker, 1989; Stanton, Mody, & Heinemann, 1989). Both NEP and LTP, *in vitro*, require the activation of the NMDA receptor and the β-adrenergic receptor. These results suggest, in the slice, that the mechanisms which induce these two forms of

plasticity, are either the same or synergistic.

This interdependence of NEP and LTP has not yet been established *in vivo*, in fact recent evidence suggests that NMDA receptor blockade does not prevent or attenuate NEP (Frizzell & Harley, 1994). The Frizzell and Harley study will be discussed further later in this paper.

The Locus Coeruleus (LC)

The LC, in the rat, is a small dense group of NE containing cells located in the dorsolateral pons. The LC, as mentioned, is the major source of NE innervation to the hippocampus (Loy, Koziell, Lindsey, & Moore, 1980). Activity of cells in the LC has been correlated with arousal and attention. Tonic cell firing discharge rate changes with the different stages of the sleep-wake cycle, with the highest rate during waking and the lowest during paradoxical sleep when tonic firing is almost absent (Aston-Jones & Bloom, 1981a). Cell firing also varies with behaviour in the awake state, the cell discharge rate decreases with grooming and drinking, and increases with orienting behaviours (Aston-Jones & Bloom, 1981a). LC neurons will fire in a phasic pattern in response to non-noxious auditory, visual and somatosensory stimuli (Aston-Jones & Bloom, 1981b) and in response to thermal stimuli (Hajós & Engberg, 1990). The magnitude of response of the LC to external stimuli varies with the level

of vigilance (Aston-Jones & Bloom, 1981b). The LC therefore can be said to play an important role in vigilance reactions to sensory stimuli. A high level of vigilance would be associated with a high rate of LC cell firing, and this state would likely occur when learning is occurring. When the firing rate of LC cells increases (as occurs in response to novel stimuli), increasing amounts of NE are released at target sites, and as a result, the responsiveness of target cells increases (Aston-Jones & Bloom, 1981b).

LC Stimulation and NEP

Endogenous release of NE in the hippocampus has been found to have potentiating effects on PP-evoked potentials in the DG (Dahl & Winson, 1985; Harley & Evans, 1988; Harley, Milway, & Lacaille, 1989). As NE innervation of the DG arises primarily from the LC, electrical stimulation of the LC has been used as a method of inducing an endogenous release of NE in the DG (Dahl & Winson, 1985). Short-term potentiation of spike amplitude and decrease in latency of the popspike can be produced using LC stimulation techniques (Dahl & Winson, 1985). Stimulation of the LC with a train of pulses prior to PP stimulation, results in potentiation of the popspike recorded from the cell layer of the DG (Assaf, Mason, & Miller, 1979; Dahl & Winson, 1985; Washburn & Moises, 1989). The optimal interstimulus interval (ISI) for producing

popspike potentiation from LC PP stimulation pairing is 35 ms with a 15 ms 333 Hz train delivered to the LC (Washburn & Moises, 1989). The effective ISI range is from 25 ms to 50 ms (Washburn & Moises, 1989). Antidromic latency from the forebrain to the LC ranges from 20 to 70 ms (Nakamura & Iwama, 1975).

Devauges and Sara (1991) used LC stimulation in behavioural tests of memory. Rats were trained in a complex maze, following which stimulating electrodes were implanted in the LC. Four weeks post-surgery, the rats were retested in the maze. When the LC was stimulated prior to the testing at four weeks following electrode implantation, no significant forgetting was observed, rats which received no LC stimulation priming had significantly more errors on the test than the LC stimulation group. The effect of LC stimulation resulting in memory enhancement was blocked by systemic propranolol, indicating β-receptor NE mediation of the effects.

Studies of electrical stimulation of the LC indicate that there may be both a β-receptor dependent mediating system and a β-receptor independent system involved in producing the potentiating effects seen in the hippocampus (Harley, Milway, & Lacaille, 1989; Harley & Milway, 1986). Systemic propranolol failed to block the effects elicited by LC electrical stimulation, indicating that β-receptor activation is not solely responsible for the effects (Harley, Milway, &

Lacaille, 1989). An alternate and more selective method of causing NE release in the DG is glutamatergic stimulation of the LC.

Glutamate ejection onto the cell bodies of the LC has potentiating effects in the DG, similar to that brought about by electrical LC stimulation (Harley & Evans, 1988; Harley & Milway, 1986). Electrical stimulation of the LC is likely to result in stimulation of fibers of passage making the technique less specific and therefore less can be said with regards to the effects of NE release alone in the DG . A single ejection of glutamate (100 to 150 nl) within 300 μm of the LC has been shown to reliably result in potentiation of popspike amplitude (significant increases were seen in all of 14 experiments) and EPSP slope (slope increases were observed in 10 of 14 experiments) (Harley & Evans, 1988). In one study, the result of glutamate-LC activation was long-lasting in 37% of the trials (Harley & Milway, 1986). Systemic injection of propranolol significantly attenuates or eliminates these glutamate induced effects (Harley & Milway, 1986). Intradentate ejection of the β-receptor antagonists, timolol and propranolol can also attenuate or block LC effects, if the β-blockers are administered prior to glutamate LC stimulation (Harley & Evans, 1988). β-Blockers administered once NE-LLP had been induced do not attenuate the potentiation, nor do β-blockers appear to have effects on

baseline evoked potentials (Harley & Evans, 1988). Following induction of LLP and then pressure ejection of β-blockers into the DG, repeated LC activation can result in significant depression of popspike amplitude, possibly an effect of α-receptor activation (Harley & Evans, 1988).

Paragigantocellularis Stimulation and Potentiation in the Hippocampus

Glutamatergic stimulation of the LC-NE is technically difficult, especially in the awake, free moving animal. This difficulty prompted Babstock and Harley (1992) to investigate the possibility of using electrical stimulation of the PGi, the major excitatory input to the LC, as an alternate method of β-receptor activation in the DG. Ennis and Aston-Jones had demonstrated in a series of papers that the LC receives major excitatory innervation from the PGi using both anatomical and physiological techniques (1986, 1987). Single pulses delivered to the PGi reliably resulted in activation of cells in the LC, with an onset latency of 11.7 $\pm$ 4.9 ms and a duration of 35.4 $\pm$ 4.3 ms (Ennis & Aston-Jones, 1986).

The Babstock and Harley 1992 study built on the evidence that PGi stimulation could excite cells in the LC, and that LC stimulation could result in potentiation of PP-evoked potentials in the DG, and demonstrated that stimulation of the PGi results in acute potentiation of popspike amplitude of PP-

evoked potentials. Stimulating the PGi with a 10 ms 333 Hz train, at an ISI of 30 ms prior to PP stimulus, results in maximal popspike potentiation. ISIs of 20 to 50 ms proved to be the effective range for evoking potentiation of spike amplitude. No consistent effects of PGi stimulation were seen on spike onset (in 4 of 11 experiments, an acute onset decrease was observed) or EPSP slope (in 2 of 11 experiments, an acute slope increase was seen) over control. An increase in baseline popspike amplitude was observed in 5 of 16 experiments, lasting for a minimum of 10 minutes. However when time between PGi stimulations was increased over 10 minutes, the baseline popspike amplitude tended to return to pre-manipulation levels.

Babstock and Harley (1992) demonstrated that systemic injections of propranolol had clear attenuating effects on PGi PP-evoked potentiation. Maximum attenuation was attained within 5 minutes of drug injection in 5 of 6 animals tested and persisted for at least 30 minutes. Systemic injections of β-blockers in this study did result in baseline changes in evoked potentials recorded from some of the animals: spike amplitude decreased in three experiments, EPSP slope decreased in three and spike onset latency increased in two. In two of the three experiments in which propranolol injection resulted in baseline decreases in spike amplitude, there had been a baseline increase in spike amplitude prior to drug injection

in response to PGi stimulation. It is not clear if these changes were due to effects in the hippocampus or if these changes in baseline were due to extrahippocampal changes, although previous work suggested local beta blockers did not alter baseline potentials. A study utilizing local β-receptor blockade would clarify these findings.

The Present Study

The focus of this project is the mechanisms underlying NE induced effects on evoked potentials in the DG of the hippocampus and LTP. The general question of the present study is: Are LTP and NEP mediated by separate mechanisms or are they mediated by the same mechanism? To address the general question of commonality of underlying mechanisms, four specific questions will be explored: (a) Can PGi induced acute potentiation be blocked by local DG application of the β-adrenergic receptor blocker timolol, as observed for direct LC activation? (b) If PGi potentiation is blocked by local β-blockade, then does the same local application of timolol have an affect on LTP induction and/ or; (c) on LTP saturation? And finally; (d) is PGi induced potentiation occluded by LTP saturation of frequency-induced potentiation? These questions, addressing the relationship between frequency-mediated and NEP within the DG will be investigated utilizing PGi stimulation techniques developed by Babstock and Harley

(1992), outlined above, combined with the LTP saturation methodology adapted from McNaughton et al. (1986) and a double pipette recording technique similar to that developed by Steward, Tomasulo and Levy (1990).

The Steward et al. (1990) study used simultaneous recording from two glass recording micropipettes placed in the DG, with a distance of approximately 0.5 to 1.0 mm between the two, one pipette filled with saline and the other filled with a drug. This method relies on local drug diffusion around the recording site and permits strong inferences about the drug effect independent of moment to moment variation in the state of the animal. Using this double pipette technique, Steward et al. demonstrated that the dentate commissural pathway has the capacity to express LTP.

The Steward et al. (1990) study illustrates the logic behind the two pipette methodology. Two recording pipettes were placed in the DG, one contained the GABA antagonist bicuculline and the other saline. Use of a large tip sized pipette and a high concentration of bicuculline ensured that diffusion of bicuculline could occur from the pipette. Local application of bicuculline had the effect of eliminating GABA mediated inhibition. GABA block was varified by the appearance of multiple population spikes only at the bicuculline recording site in response to PP stimulation, and of LTP in response to contralateral commissural stimulation

also only at the bicuculine site. Spread of bicuculline did not extend to the saline control site, as neither multiple spiking nor LTP was recorded from the saline pipette. The recording from the bicuculline pipette was stable over time, indicating a continuous local delivery of drug throughout the experiment.

The usefulness of the double pipette recording technique was later confirmed in studies by Tomasulo, Levy and Steward (1991) and by Frizzell and Harley (1994). The double pipette recording set-up, while more technically difficult, has many advantages over single pipette experiments. Since, in effect, each animal in the experiment was in both the control and experimental group, and the control and test experiments were conducted concurrently, concerns over the possibility that other physiological changes not of interest within the test group and/or extraneous variables are the source of effects are eliminated. Changes in temperature, level of anaesthetics, placement of stimulating electrode and ceiling effects should have equally affected both the control and experimental test sites. The only difference between the control and test sites was the presence of a drug in the test pipette, and so the effects of that drug are easily ascertained by comparisons of the magnitude of the effects at the two recording sites. This technique of local passive drug diffusion delivery also has advantages over iontophoresis

techniques. In experiments using iontophoresis, a high concentration of drug is initially delivered to a small group of cells, but with time the concentration decreases as the drug diffuses. Steward et al. (1990) noted that the recording from the bicuculline site was stable over long periods of time and did not affect the saline recording, indicating that diffusion out of the pipette provided a localized and continuous supply of drug.

The final protocol to be utilized in the current study is the LTP saturation method. While this method was developed for behaviourial testing, and as of yet has not been proven to eliminate behavioral plasticity, the hypothesis that saturation prevents plasticity has not been discounted and can be applied to acute *in vitro* recording studies. By delivering several sets of HFT to the PP in a relatively short period of time, it should be possible to reach a ceiling level of LTP within a group of granule cells, at which no more potentiation can be elicited by PP stimulation. Saturation of PP stimulation effects would indicate that NMDA receptor mediated effects are also maximized. By assessing both the degree of LTP and NE effects with the same recording pipette, it should be possible to assess LTP saturation and possible further potentiation from the same group of cells. Presumably, attaining LTP saturation in the group of cells sampled by the recording electrodes indicates that for that group of cells,

no further increases can be produced via the NMDA receptor complex. If NE effects can be produced, the effects must be via another mechanism.

Using the combination of PGi stimulation, LTP saturation and double pipette techniques allows for separate experiments which explore all four questions to be carried out consecutively in each experimental animal.

METHODS

Subjects

Fifteen female Sprague-Dawley rats, (Charles River Canada Inc., Montreal, Canada), weighing from 210 to 290 grams served as subjects. Each animal was anaesthetized with urethane (1.5 g/kg i.p.) and placed skull flat into a stereotaxic apparatus. Supplemental doses of urethane were delivered as required throughout the experiments to abolish foot and tail pinch responses. Core body temperature was monitored and maintained at 37±1°C using a rectal probe, heating blanket and noise free temperature control unit (FHC). Horizontal position between lambda and bregma was checked, and burr holes were drilled in the skull using a dental drill to allow placement of recording electrodes in the DG and placement of stimulating electrodes in the PP and PGi.

Electrode Placements and Timolol Application in Dentate Gyrus

Bipolar stimulating electrodes (SNE 100) were placed in the medial perforant path (7.1 mm posterior, 4.2 mm lateral to bregma and 2.5-3.5 mm below brain surface) and in the PGi (12.2 mm posterior, 1.4 mm lateral to bregma, 9.0-9.5 mm below

brain surface). Two glass micropipettes, which served as experimental and control recording pipettes, were positioned in the cell body layer of the DG (3.8 mm posterior and 2.0 mm lateral to bregma). The recording pipettes were placed into a Narishige holder which permitted a 1.0 mm range of movement, in three directions, of one pipette relative to the stationary other. In this holder, the pipette tips were held 700 - 1000 μm apart in the medial-lateral direction, from 0 - 1000 μm apart in the anterior-posterior direction and initially, less than 100 μm apart vertically. Depths of placement of the PP and the recording electrodes were determined by monitoring the PP-evoked population spike amplitude.

The control pipette was filled with 0.9% physiological saline (pH = 7.2). The experimental pipette contained 10, 20 or 50 mM timolol maleate (dissolved in 0.9% saline, pH ranged from 7.1 to 7.5). The inside tip diameters ranged from 40 to 50 μm and outer from 45 to 55 μm, the impedances of the pipettes ranged from 0.5 to 3.0 MΩ. Impedances of the experimental and control (or saline) pipettes used in the same experimental animal never differed by more than 1.5 MΩ and the tip diameter of pipettes used within an animal never differed by more than 5 μm. To ensure diffusion could occur from the experimental pipette, a tightly wound mediwipe was held to the pipette tip immediately prior to lowering the pipettes into the hippocampus. When viewed under a microscope, the mediwipe

could be seen to unwind when moistened.

Perforant Path Stimulation and Dentate Gyrus Evoked Potential Recording

A 0.2 ms monophasic square wave pulse, 15 - 35 V, was delivered to the PP at 0.1 Hz via a dual channel stimulator (Ortec 4710) through a Grass stimulus isolation unit. PP stimulation strength was varied in each animal at a level sufficient to produce maximal popspike size. Signals from the two DG recording pipettes were coupled to separate Grass H1 Z probes (model HIP 5) and then were differentially amplified using a bandwidth of 0.1 Hz - 3 KHz (Grass P5 series preamplifiers, model P511 K). The resulting signals were displayed on a digital storage oscilloscope (Gould 420) and digitized on an IBM-PC compatible computer (1 point/10 μs). The data acquisition program was written in Asyst, a commercial package based on the language Forth (written by G. Carre). Once stable evoked potential recordings of similar waveform and magnitude were obtained from both recording pipettes, baselines of at least 60 population spikes were collected prior to the commencement of the experiments.

PGi Stimulation

The PGi was stimulated with 10 ms 333 Hz trains

consisting of 0.5 ms monophasic square wave pulses. Trains were applied at 0.1 Hz paired with PP stimulation for 6 stimuli (total time per trial block was one minute) with an interval of 30 ms between the start of the PGi stimulation and the PP stimulation. PGi stimulation was delivered at between 20 and 30 V via a dual channel stimulator through a Grass stimulus isolation unit. If little or no PGi-induced potentiation was observed during the initial stimulation pairings, the depth of the PGi stimulating electrode was adjusted to a level at which potentiation was seen and then six more trial blocks were conducted.

Data Collection

The dependent variables extracted on-line for each evoked potential from each electrode were: (a) population EPSP slope, measured in μV/ms; (b) latency to spike onset, measured in μs; (c) latency to the peak of the spike, measured in μs; (d) popspike amplitude, measured as the amplitude, in μV, difference from the first positive peak to the negative peak; and (e) "area" which is a measure of the combined differences in the number of discrete points between the curve of the spike and the tangent joining the two positive EPSP peaks. Figure 2 illustrates the measures monitored for each evoked potential. These measures were taken every 10 seconds and one minute means were calculated on-line. Latency measures are

calculated from the occurance of PP stimulation. Also calculated on-line were two-tailed 95 % confidence limits based on the means from 10 minute control periods, which allowed for assessment of potentiation during experimentation. Data were later analyzed using SPSSx and Quattro Pro for Windows software.

The oscilloscope used was equipped with a printer which provided a record of simultaneous traces from both channels of the oscilloscope as well as a reference trace from one of the channels. Printouts allowed for a visual record of waveform similarities between recording sites and for assessing waveform changes over the course of a recording session.

Experiment 1: Effects of PGi Stimulation on PP-Evoked Potentials and the Role of the β-Receptor

To evaluate the effects of PGi stimulation prior to PP stimulation on PP-evoked potentials and the effects of intradentate timolol on the reported potentiation of the popspike, a series of at least 6 trial blocks of the PGi and PP stimulation pairings were performed. There was a period of at least 10 minutes between each of the trial blocks, during which only the PP stimulation was delivered. If a difference between the experimental and control pipettes in the percentage increases over baseline of the spike amplitude was observed over the 6 trials following PGi stimulation then

Experiment 2 was conducted. If no difference was observed or the difference was judged (subjectively) to be small or unreliable, then the recording pipettes were removed from the brain, the drug concentration was increased and Experiment 1 was repeated. The final 3 trials of experiment 1, at the final drug concentration level, of PGi and PP pairings were used in the analysis. Once Experiment 1 had been completed, the drug concentration was not increased nor were the placements of the electrodes changed.

Experiment 2: Timolol Effects on LTP Induction and LTP Saturation

Eight trains of 4 pulses at 400 Hz (referred to as high frequency trains or HFT) were delivered to the PP to induce LTP. Stimulus strength as was the same as was delivered to evoke maximal popspike size in Experiment 1. Following the delivery of the trains, 30 minutes of data were collected to evaluate whether LTP had been induced and the degree to which it had been induced at each recording site. Data used in the analysis were from a ten minute control period immediately prior to the delivery of the HFT and the 10 minute period between 20 and 30 minutes after HFT delivery. Following the initial HFT used to induce LTP, at least two, and up to four more series of HFT (used to induce LTP) were administered to

ensure that LTP had been saturated (no further increase in population spike amplitude could be induced by delivery of HFT to the PP).

Experiment 3: Timolol and PGi Stimulation Effects following LTP Saturation

Once it was determined that LTP had been saturated, another series of 6 PGi-PP stimulation trial blocks were performed. These trial blocks were done to investigate the effect of PGi stimulation on evoked potentials following LTP saturation and to investigate the effects of direct timolol application in the DG on any possible PGi effects. The data from the first 3 trial blocks were used in analysis.

Experiment 4: PGi Effects following LTP Saturation with reduced PP Stimulation Intensity

Following the completion of Experiment 3, the PP stimulus intensity was reduced to produce popspikes that were approximately of the same size, based on spike amplitude, as those prior to LTP induction. Another HFT set was delivered, and if it appeared not to produce any further potentiation which would again indicate LTP had been saturated, another series of PGi-PP stimulation trials were done. This experiment was conducted for two reasons (a) to be sure that possible ceiling effects at the recording site did not obscure

potentiation magnitude and (b) to more accurately evaluate the changes in pre-LTP versus post-LTP PGi-induced potentiation. Comparing the effect of PGi stimulation on similar sized popspikes from the pre-LTP saturation and the post-LTP saturation conditions should reveal what, if any, effects LTP saturation has on the magnitude of PGi induced potentiation.

Histology

At the conclusion of the recording session, lesions for histological verification of electrode placements were made by passing approximately 8 μamps negative DC current through the recording electrodes and both of the stimulating electrodes for 5 minutes (using a Midgard C5 3 high voltage precision current source). The rat was then sacrificed by decapitation, the brain removed and frozen. For determination of electrode positions, 40 μm sections of tissue were cut on a cryostat microtome and consecutive slices were mounted on two sets of slides, one set for AChE-metachromatic Nissl staining (Paxinos & Watson, 1986) and the second for glycogen phosphorylase a staining (Harley & Bielajew, 1992).

Data Analysis

For all experiments, analysis was performed on each of the five evoked potential measures separately, except as

noted. Also, all ANOVAs done were completely within designs, that is, all animals received all treatments.

Experiment 1

Mean values for the six events in each PGi-PP stimulation trial were computed, as were mean values of the six evoked potentials immediately preceding the trial. These mean values, three control means and three stimulation means per pipette, per animal, were then used in 2x2 ANOVAs in which the control values for each pipette were compared, the PGi stimulation values were compared as were the interactions of PGi stimulation and pipette.

Experiment 2

LTP induction.

An initial t-test was done on the data for the evoked potential variables for the saline pipette of each animal to determine whether LTP had been induced. The means from each of the 10 minutes prior to the delivery of the first HFT (baseline period) were compared to the 10 means for the 10 minutes between 20 and 30 minutes after the delivery of the HFT. LTP was considered to have been induced if a significant increase over the baseline period was obtained in spike amplitude recorded from the saline electrode.

Magnitude of LTP induced.

The data for those animals which showed LTP were pooled and the data from the saline and timolol recording sites were compared in paired t-tests. This tested the effects of timolol on LTP. Here the data from 20 to 30 minutes after delivery of the initial HFT from the saline and timolol recording sites, expressed as percentage of control means, were compared to each other.

LTP saturation.

To determine if there was a difference in the LTP saturation between the timolol and saline recording sites both in terms of final values and in terms of the number of HFT sets required to reach saturation, two additional sets of paired t-tests were computed. For this part of the analysis, only spike amplitude and area were used. These t-tests were done comparing the data from the five minute control period prior to the initial delivery of HFT to the PP, to the means from the five minute period following the HFT. Then the means following the first HFT were compared to means from the five minutes immediately following the second HFT delivery. Finally the set of means following the second delivery were compared to the set of means following the final delivery of HFT. The means from the five minutes following HFT were used rather than the values from 20 to 30 minutes (as was used in

the analysis of the initial LTP induction) following the HFT as there was not always a full 30 minutes between the HFT deliveries.

Experiment 3

Experiment 3 was analyzed in much the same manner as Experiment 1. Means were computed for each of the evoked potentials in PP-PGi stimulation trials of Experiment 3 and for the corresponding control potentials. Using these means, 2x2 *ANOVAs* were then computed with the main effects being pipette and PGi stimulation and the interaction of pipette and PGi stimulation. Additionally, a set of 2x2x2 *ANOVAs* were also computed which involved comparing the data from Experiment 1 to the data from Experiment 3, looking at the effects of pipette (saline versus timolol), stimulation (control or PGi stimulation), and LTP saturation (pre-LTP or post-LTP saturation) as well as the two and three-way interactions.

Experiment 4

The analysis for Experiment 4 consisted of a set of 2x2 *ANOVAs* and a set of 2x2x2 *ANOVAs*. Means were again computed for each PP-PGi stimulation trial in Experiment 4 and for the control potentials. Then 2x2 *ANOVAs*, with the same main effects and interaction as in Experiment 1, were computed.

Finally, a set of 2x2x2 *ANOVAs*, combining the data from Experiment 1 with Experiment 4 were performed. As in Experiment 3, the main effects were LTP saturation, pipette, and PGi stimulation. Of main interest was the three-way interaction of LTP saturation, pipette and PGi stimulation.

RESULTS

Of the 15 animals in which the experiments were conducted, 7 were included in the final analysis. Of the eight animals not included, two were not included because of uninterpretable slope changes in the saline popspikes after delivery of the trains used to induce LTP and two were eliminated because of computer problems encountered during experiments. Four were eliminated on the basis of electrode placements, three because the stimulating electrode intended to be in the PGi was not accurately placed, and one because of an inaccurately placed recording electrode.

Histological Verification of Electrode Placement

The use of two staining techniques, AChE-metachromatic Nissl and glycogen phosphorylase a staining, allowed for relatively easy and accurate assessment of electrode placements. Glycogen phosphorylase a staining is more sensitive for identifying sections with maximal tissue disruption and tip termination than the AChE-metachromatic staining, while AChE-metachromatic staining provides more distinct structural information. Therefore provided by the two sets of slides afforded accurate interpretation of

electrode tip placements. Placements of PGi stimulating electrodes and the DG recording pipettes are illustrated in Figures 3 and 4. Placement of the stimulating electrode was found to be within the PGi in all cases. The recording sites were all found in the dorsal blade of the DG.

Waveform Similarity and Timolol Concentration

Prior to the commencement of Experiment 1, the waveforms recorded from the saline and timolol pipettes were judged to be similar. The control means were comparable (see Table 1) and no significant effects (see Table 2) were found for pipette in the *ANOVA*s used in the analysis of Experiment 1. This finding was supported by the results from *t*-tests done on the grouped data from the 10 minute control period prior to the initial HFT delivery. EPSP slope (saline X = 6321 μV/ms, timolol X = 5270 μV/ms) did not differ between sites ($t_{(6)}$ = 0.91, $p > .05$), nor did latency to spike onset (saline X = 3260, timolol X = 3199 μs; $t_{(6)}$ = 0.88, $p > .05$), latency to peak (saline X = 4112 μs, timolol X = 4056 μs; $t_{(6)}$ = 0.66, $p > .05$), spike amplitude (saline X = 3100 μV, timolol X = 2950 μV; $t_{(6)}$ = 0.16, $p > .05$) or area under the tangent joining the two peaks (saline X = 119, timolol X = 100; $t_{(6)}$ = 0.65, $p > .05$). The waveforms in Figure 5 are a sample pair taken from one animal, illustrating the initial similarities between the

Table 1

The effect of timolol on the potentiation of PP-evoked potentials produced by PGi stimulation prior to LTP induction. Mean values used in ANOVAs and *F* value reported for the interaction of Pipette and PGi Stimulation.
(* $p < .05$)

PARAMETER	SALINE N=7		TIMOLOL N=7		*F* VALUE
	Control	PGi	Control	PGi	
EPSP SLOPE (μV/ms)	6199.71	6374.24	5059.52	5230.14	0.0
LATENCY TO SPIKE ONSET (μs)	3229.86	3160.24	3159.24	3108.09	5.34
LATENCY TO SPIKE PEAK (μs)	4113.29	4101.62	4071.86	4027.62	5.53
SPIKE AMPLITUDE (μV)	3931.67	6162.57	4285.48	5517.62	6.13 *
AREA UNDER THE TANGENT	139.96	198.43	118.76	151.91	6.17 *

Table 2

PGi stimulation produces potentiation of PP-evoked potentials prior to LTP induction. *F* values for main effects and interactions from separate ANOVAs for each popspike variable monitored.
($p < .05$) * ($p < .01$) **

PARAMETER	PGi STIMULATION MAIN EFFECT *N*=7	PIPETTE MAIN EFFECT *N*=7	STIMULATION X PIPETTE INTERACTION
EPSP SLOPE	3.35	1.51	0.0
LATENCY TO SPIKE ONSET	46.34 **	1.11	5.34
LATENCY TO SPIKE PEAK	4.07	0.66	5.53
SPIKE AMPLITUDE	12.77 *	0.02	6.13 *
AREA UNDER THE TANGENT	15.78 **	1.51	6.17 *

Figure 3

Composite sketch of a sagittal section illustrating PGi stimulating electrode placements from all animals. Based on Paxinos & Watson, 1986. Abbreviations: 7, facial nucleus; LPGi, lateral paragigantocellularis nucleus; LRt, lateral reticular nucleus; MO5, motor trigeminal nucleus; MVPO, medioventral periolivary nucleus; sol, nucleus of the solitary tract; SPO, superior paraolivary nucleus; tz, trapazoid body.

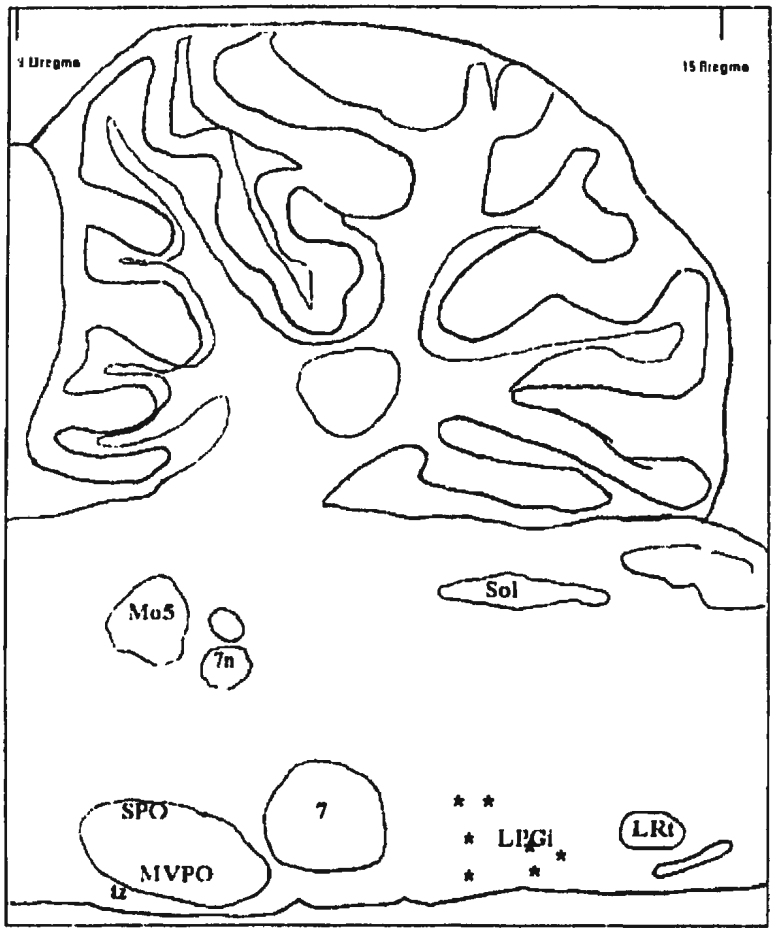


Figure 4

DG recording pipette placements. Filled boxes represent timolol placements, empty boxes represent saline placements. Representative sections, based on Paxinos & Watson, 1986. For cell layers, see Figure 1.

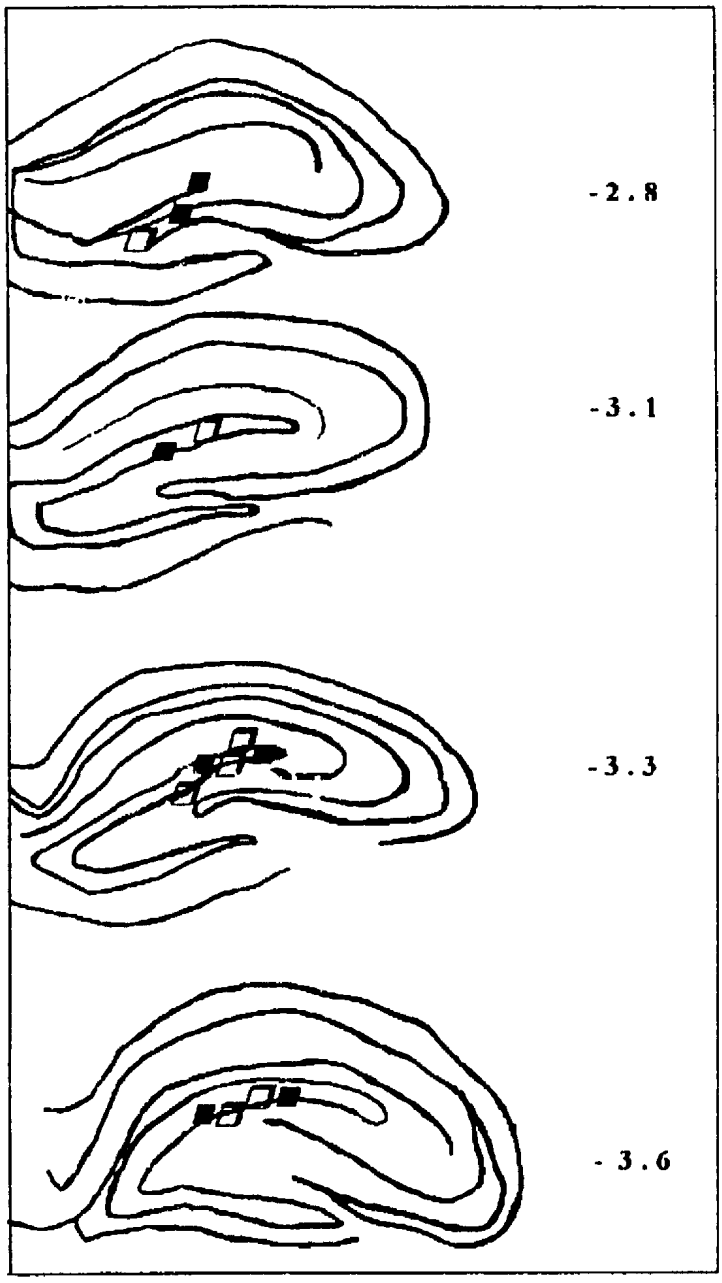
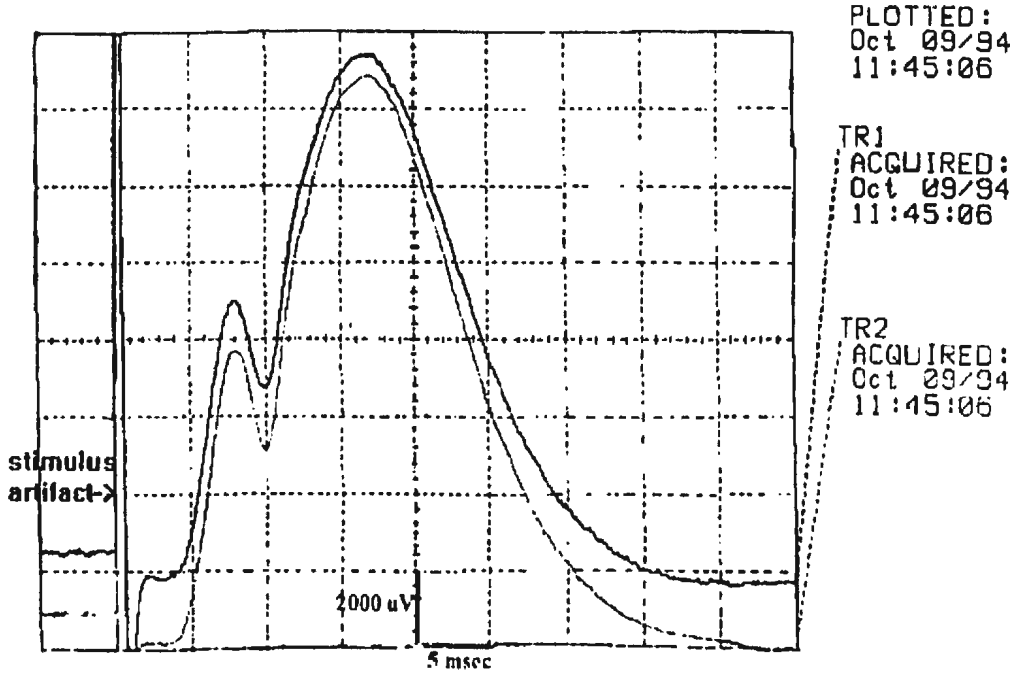


Figure 5

Sample pair of waveforms illustrating similarities between evoked potentials recorded from the saline and timolol pipettes. Upper trace recorded from saline pipette, lower from timolol pipette.



recordings and the saline and timolol electrodes. Final concentrations of timolol used was 20 mM in 5 animals and 50 mM in 2 animals. All data presented were obtained at the final drug concentration.

Experiment 1: Effects of PGi Stimulation on PP-Evoked Potentials and the Role of the β-Receptor

Stimulation of the PGi with a 333 Hz train 30 ms prior to PP stimulation was found to have acute potentiating effects on DG PP-evoked potentials in all 7 animals. At the saline recording site significant increases in popspike amplitude and area were seen as well as significant decreases in popspike onset latency relative to baseline. No significant changes were observed in EPSP slope or in latency to popspike peak in response to PGi stimulation. The average increase in popspike amplitude was 156.7 % (where baseline is 100%) and in popspike area the increase was 141.8 %, while latency to popspike onset was found to decrease to 97.8%. Direct application of timolol on cells of the DG was found to attenuate the effects of stimulation of the PGi on popspikes prior to LTP. PGi stimulation still resulted in significant increases in popspike amplitude and area over baseline, however, the increases (128.8 % and 127.9 % respectively) were significantly less than saline controls. The decrease in popspike onset latency (to 98.3% of baseline) was significant,

however it was not significantly different than that seen at the saline site. As outlined above, there were no significant differences in baseline values between the two pipettes (control mean values are presented in Table 1 and F values for main effects of pipette are presented in Table 2).

As summarized in Table 1, the popspikes recorded from the saline pipette differed from those recorded from the timolol pipette immediately following PGi stimulation. The saline popspikes were significantly larger in terms of spike amplitude ($F_{(1, 6)} = 6.13$, $p < .05$) and area under the tangent joining the two positive peaks ($F_{(1, 6)} = 6.17$, $p < .05$). No between site differences were revealed for slope ($F_{(1, 6)} = 0.0$, $p > .05$), latency to spike onset ($F_{(1, 6)} = 5.34$, $p > .05$) or latency to spike peak ($F_{(1, 6)} = 5.53$, $p > .05$).

Experiment 2: Timolol Effects on LTP Induction and Saturation

LTP induction.

To investigate the effect of timolol on LTP induction, the 10 means from the 10 minutes immediately preceding the HFT delivery were compared to the 10 means from 20 to 30 minutes after HFT delivery using paired t-tests. The mean values for each animal and each dependent variable, expressed as a

Table 3

Potentiation of PP-evoked potentials following delivery of an initial set of HFT for each animal. Values are expressed as percentage of the control means (set at 100) obtained immediately prior to HFT. S, saline recording site; T, timolol recording site. Note that all values were found to be significantly different from control ($p < .05$) in two-tail t-tests except the one marked with N.

ANIMAL / SITE		EPSP SLOPE	LATENCY TO ONSET	LATENCY TO PEAK	SPIKE AMPLITUDE	AREA
1	S	122.9	95.6	95.3	150.2	129.7
	T	119.9	95.7	96.4	136.3	135.2
2	S	125.9	98.2	97.1	119.2	112.0
	T	124.2	98.7	97.4	117.0	117.3
3	S	108.5	93.9	95.3	146.9	132.3
	T	108.1	94.5	95.2	123.2	132.3
4	S	104.8	92.9	93.7	117.2	106.7
	T	102.7	92.6	92.6	104.2N	106.6
5	S	96.9	94.4	95.7	145.7	122.0
	T	90.9	94.1	95.3	126.7	113.1
6	S	125.8	92.9	93.8	123.6	121.4
	T	107.3	92.3	92.5	132.1	118.4
7	S	108.4	92.6	95.2	142.4	130.0
	T	103.7	91.3	92.6	129.1	116.6

percentage of the control means, are summarized in Table 3. Significant changes from control levels were found for all variables ($p < .05$) in all but one animal. Data from the timolol recording site in one animal revealed there were nosignificant changes in spike amplitude ($t_{(9)} = -1.19, p > .05$). When the data from this animal were examined using one-tailed *t*-tests, spike amplitude still failed to reach significance.

Magnitude of LTP induction.

Induction of LTP following the initial delivery of HFT was found to be weaker at the timolol recording site than at the saline recording site, when the pooled data were examined. The mean increase of EPSP slope ($t_{(69)} = 6.96$, $p< .01$), spike amplitude ($t_{(69)} = 8.04$, $p< .01$) and area ($t_{(69)} = 2.33$, $p< .05$) were found to be significantly attenuated by the presence of timolol in the recording pipette. Along with the smaller mean values, the percent maximum change was less for potentials recorded from the timolol pipette. Greater decreases in latency to spike onset and in latency to spike peak were recorded at the timolol site than at the saline site. Means and maximum change from baseline, expressed as percent of control, are presented in Table 4.

Table 4

Effects of timolol on the magnitude of LTP induced following initial HFT. Mean and maximum change values are expressed as percentage of control values. Control values are set at 100. *t* values presented are from t-tests comparing the mean percentage increases above baseline for evoked potentials from the two recording pipettes. (* $p < .05$), ** $p < .01$)

PARAMETER	SALINE		TIMOLOL		*t* VALUE
	MEAN	MAX CHANGE	MEAN	MAX CHANGE	
EPSP SLOPE	113.33	135.82	108.11	127.71	6.96 **
LATENCY TO SPIKE ONSET	94.36	91.11	94.16	90.27	2.21 *
LATENCY TO SPIKE PEAK	95.15	91.48	94.59	90.21	4.04 **
SPIKE AMPLITUDE	135.01	173.16	124.11	141.94	8.04 **
AREA UNDER THE TANGENT	121.86	140.22	119.85	141.16	2.33 *

Table 5

Comparison of mean increases to initial control level following successive HFT in each animal and for both recording sites. Contol level set as 100% LTP saturation was achieved (no significant increase following HFT) at both recording sites in 6 of 7 animals. LTP saturation was not obtained in animal 3, see text.
(* $p < .05$, ** $p < .01$ D = significant decrease.)

ANIMAL	PIPETTE	HFT	SPIKE AMPLITUDE		AREA	
			MEAN	t	MEAN	t
1	SALINE	1	134.2	7.7 **	118.4	7.0 **
		2	163.3	8.7 **	127.3	5.4 **
		3	159.3	1.4	122.1	3.5 *D
	TIMOLOL	1	118.1	8.1 **	119.5	7.2 **
		2	139.4	12.2 **	134.0	12.0 **
		3	137.9	0.7	131.3	1.6
2	SALINE	1	110.3	2.3 *	106.6	2.4 *
		2	140.0	10.7 **	128.0	13.C **
		3	142.5	0.9	130.0	1.6
	TIMOLOL	1	95.5	0.7	95.6	0.8
		2	157.4	12.7 **	137.5	13.6 **
		3	163.1	1.0	139.0	1.5
3	SALINE	1	157.6	12.8 **	144.7	10.1 **
		2	180.8	5.5 **	165.6	5.6 **
	TIMOLOL	1	131.1	10.3 **	125.4	9.8 **
		2	140.2	3.5 **	131.8	3.5 **

4	SALINE	1	110.5	2.8 **	102.7	0.8
		2	116.2	2.2 *	100.8	1.5
		3	105.4	3.9 * D	101.2	0.3
	TIMOLOL	1	97.7	0.8	101.0	0.4
		2	103.7	3.3 **	103.1	2.1 *
		3	95.5	4.3 * D	101.4	1.9
5	SALINE	1	158.1	7.7 **	126.4	6.3 **
		2	186.5	4.4 **	130.7	1.5
		3	195.4	1.6	131.2	1.5
	TIMOLOL	1	135.7	7.3 **	133.9	2.3 *
		2	146.5	3.3 **	180.0	0.8
		3	145.4	0.8	195.9	0.2
6	SALINE	1	134.4	7.9 **	121.7	7.5 **
		2	133.3	0.3	130.3	3.1 **
		3	128.5	1.0	128.5	0.5
	TIMOLOL	1	129.2	13.5 **	112.7	6.1 **
		2	139.2	5.1 **	119.0	3.1 **
		3	134.0	2.5 * D	114.4	2.4 * D
7	SALINE	1	216.2	18.3 **	177.6	18.7 **
		2	230.1	2.5 *	181.5	1.1
		3	230.6	0.1	178.7	1.2
	TIMOLOL	1	171.5	16.8 **	145.2	16.6 **
		2	186.6	3.7 **	155.3	4.0 **
		3	186.0	0.1	155.4	0.1

LTP saturation.

Analysis using *t*-tests (see Table 5) revealed that LTP saturation was achieved for both recording sites in 6 of 7 animals. In the one animal in which LTP saturation was not reached with 2 sets of HFT, there was a third HFT delivered, however, insufficient time between the HFT delivery andcommencement of Experiment 3 was allowed, making analysis of LTP saturation with those data questionable. In those animals in which LTP saturation was reached, an average of 1.8 HFTs were required. The number of HFTs required to reach LTP saturation did not differ significantly between pipettes.

Experiment 3: Timolol and PGi Stimulation post-LTP Saturation

Following LTP saturation, PGi stimulation still produced significant potentiation of popspikes and PGi effects themselves were still attenuated by the application of timolol. *F* values for main effects from the series of *ANOVA*s done in the analysis are summarized in Table 6. The post-LTP saturation control recordings from the two pipettes did not differ significantly for any of the five measures. However, when the PGi was stimulated significant changes emerged. Overall, PGi stimulation resulted in a significant decrease in the latency to spike onset ($F_{(1,6)} = 14.87$, $p < .01$), and in

significant increases in spike amplitude ($F_{(1,6)}$ = 6.06, p < .05) and in the area ($F_{(1,6)}$ = 17.53, p < .01) measures.

As before LTP saturation, significantly larger popspikes were recorded from the saline pipette when the PGi was stimulated than those recorded from the timolol pipette. These between pipette differences are evident in Table 7. The mean increase in spike amplitude recorded from the saline site was 30 % above baseline, while the corresponding increase recorded from the timolol site was 8 % above baseline ($F_{(1,6)}$ = 7.68, p < .05). Similarly, the mean increase in area was 20 % above baseline from the saline site, while the mean increase recorded from the timolol was 15 % ($F_{(1,6)}$ = 16.19, p < .01). No significant differences between the two recording sites were found for EPSP slope or the two latency measures following PGi stimulation.

Data from Experiment 1 and Experiment 3 were combined and analyzed in 2x2x2 *ANOVAs*. ANOVAs were done to see if the magnitude of PGi effects was different between the pre-LTP and post-LTP saturation conditions. Mean spike amplitude values for each condition are plotted in Figure 6, mean area values in Figure 7 and slope mean values in Figure 8. As in the separate 2x2 *ANOVAs* analysis, no significant main effects for Pipette were found. Significant main effects of PGi stimulation were found for latency to spike onset, spike

Table 6

The potentiation of PP-evoked potentials produced by PGi stimulation is not occluded by LTP saturation. *F* values for the main effects from within *ANOVA*s for each popspike variable. (* $p<$ .05, ** $p<$ .01)

PARAMETER	PGI STIMULATION MAIN EFFECT *N*=7	PIPETTE MAIN EFFECT *N*=7
EPSP SLOPE	0.66	1.77
LATENCY TO SPIKE ONSET	14.87 **	1.96
LATENCY TO SPIKE PEAK	1.27	1.09
SPIKE AMPLITUDE	6.06 *	0.28
AREA UNDER THE TANGENT	17.53**	1.73

Table 7

The effect of timolol on the potentiation of PP-evoked potentials produced by PGi stimulation following LTP saturation. Mean values used in *ANOVAs* and *F* value reported for the interaction of Pipette and PGi stimulation. (* $p < .05$, ** $p < .01$)

PARAMETER	SALINE *N*=7		TIMOLOL *N*=7		*F* VALUE
	Control	PGi	Control	PGi	
EPSP SLOPE (μV/ms)	6503.57	6528.29	5023.81	5120.38	1.32
LATENCY TO SPIKE ONSET (μs)	3026.67	2983.33	2925.52	2900.19	5.16
LATENCY TO SPIKE PEAK (μs)	3857.00	3855.67	3798.10	3776.48	1.95
SPIKE AMPLITUDE (μV)	5816.24	7589.43	5757.24	6198.00	7.68 *
AREA UNDER THE TANGENT	181.48	218.29	148.91	170.53	16.19 **

Figure 6

Effect of timolol and LTP on PGi induced potentiation of PP-evoked popspike amplitude. Mean control values were obtained from the six events preceding PGi-PP stimulation, PGi means were from six consecutive PGi-PP stimulation events. *N* = 7

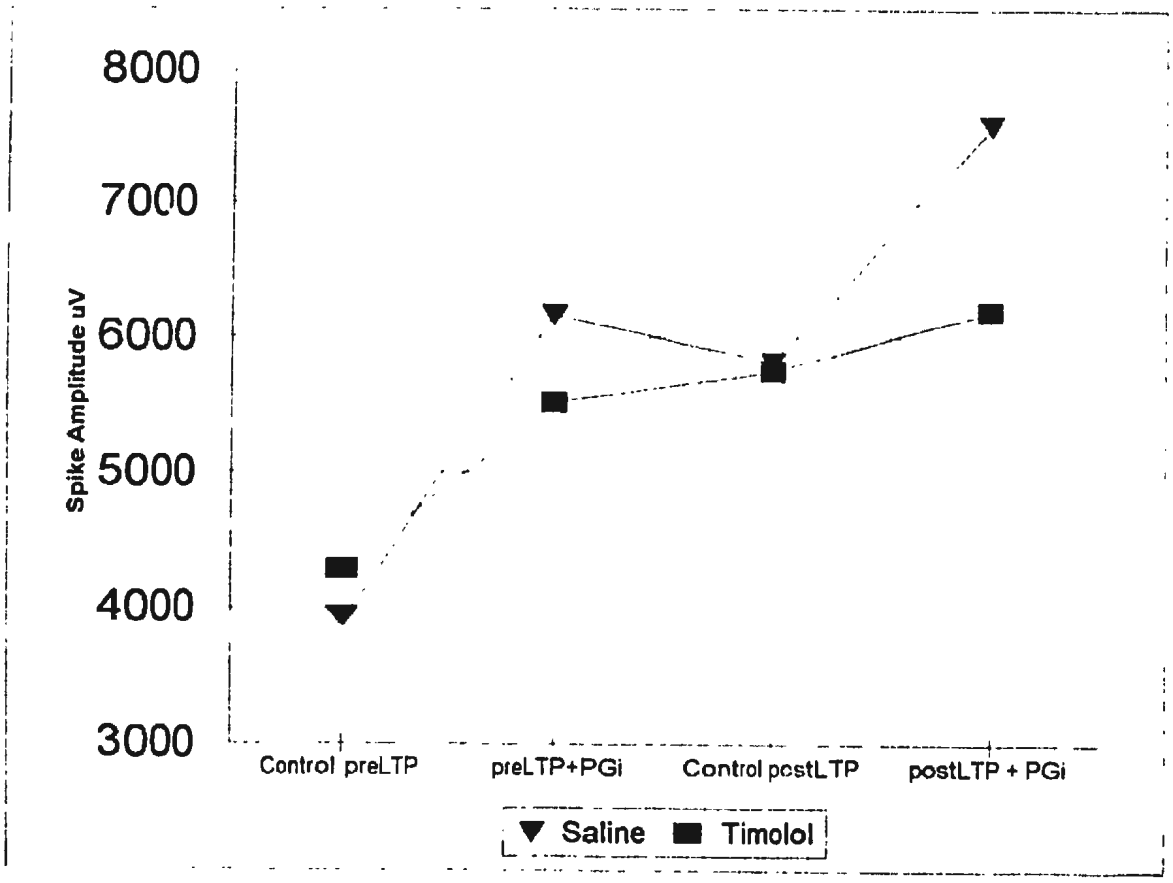


Figure 7

Effect of timolol and LTP on PGi induced potentiation of PP-Evoked popspike spike area. Mean control values were obtained from the six events preceding PGi-PP stimulation, PGi means were from six consecutive PGi-PP stimulation events.
***N* = 7**

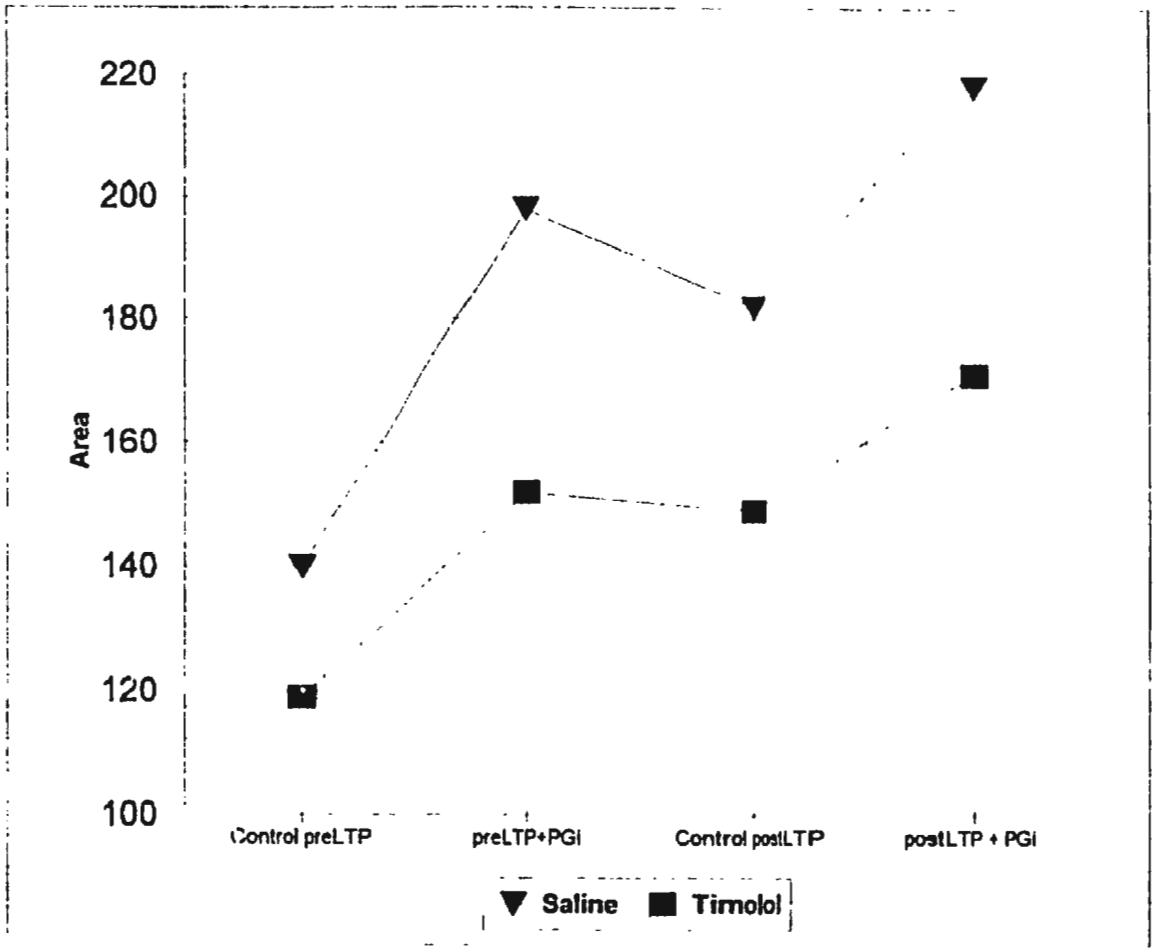


Figure 8

Effect of timolol and LTP on PGi-induced potentiation of PP-evoked potential EPSP slope.
N = 7

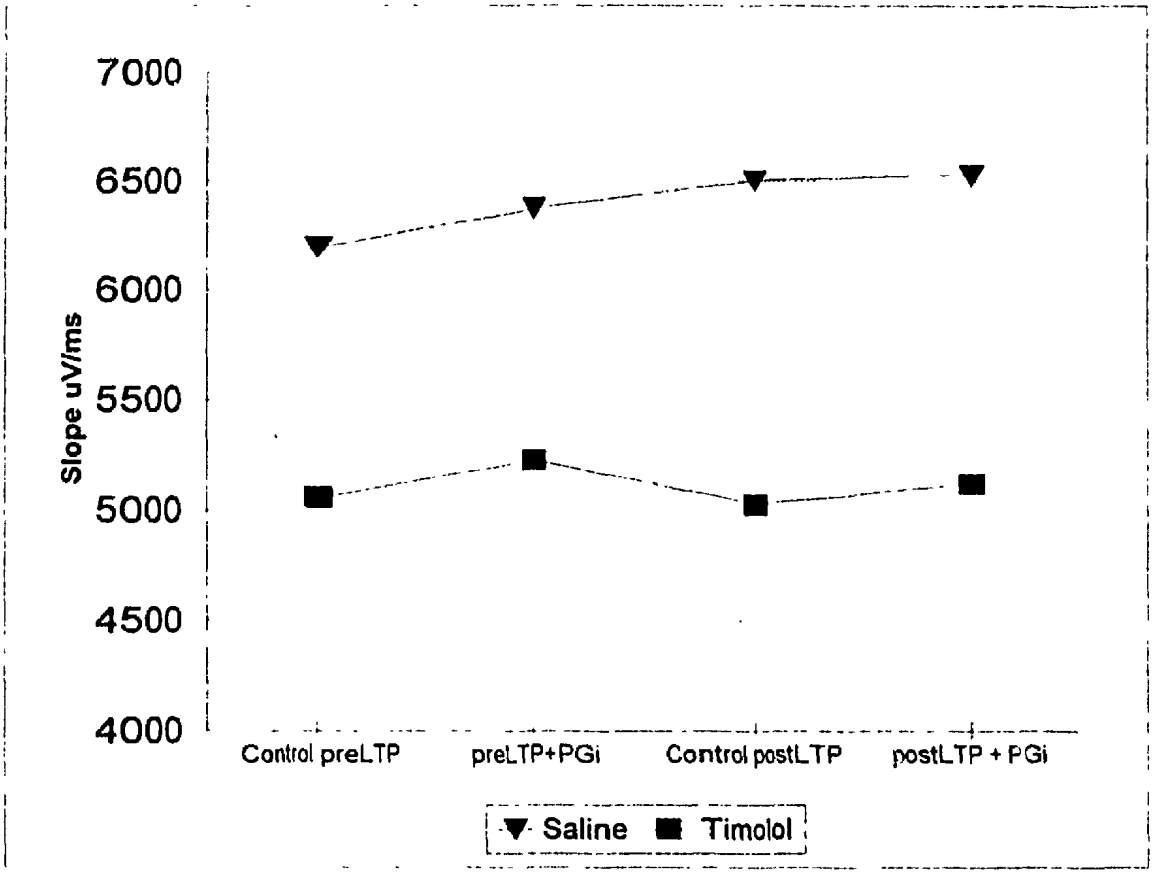


Table 8

The effects of timolol and LTP saturation on potentiation of PP-evoked potentials produced by PGi stimulation. *F* values reported for main effects and interactions from 2x2x2 within ANOVAs. (* $p < .05$), ** $p < .01$)

	EPSP SLOPE	LATENCY TO SPIKE ONSET	LATENCY TO SPIKE PEAK	SPIKE AMPLITUDE	AREA
LTP SATURATION MAIN EFFECT	0.06	21.42 **	24.68 **	14.11 **	6.90 *
PGi STIMULATION MAIN EFFECT	1.92	50.17 **	3.34	11.56 **	19.51 **
PIPETTE MAIN EFFECT	1.71	1.72	0.87	0.15	1.65
SATURATION X STIMULATION INTERACTION	21.80 **	4.95	2.16	2.20	3.68
SATURATION X PIPETTE INTERACTION	0.58	0.54	0.47	0.59	0.58
STIMULATION X PIPETTE INTERACTION	0.03	8.26 *	5.55	15.18 **	10.24 *
SATURATION X STIMULATION X PIPETTE INTERACTION	17.33 **	0.00	0.56	0.26	1.35

amplitude and area (*F* values from this analysis are presented in Table 8). There were also significant interactions of PGi x Pipette for spike amplitude and for area (see Figures 6 and 7). There were significant main effects for LTP saturation for all measures except EPSP slope. Of interest was the lack of significance for the three way interaction of LTP saturation x PGi stimulation x pipette, indicating that LTP saturation had the same effect on both pipettes and the attenuation of PGi induced potentiation seen at the timolol site was due to β-blockade alone.

Experiment 4: PGi Effects post-LTP Saturation with Reduced PP Stimulation Intensity

When the PP stimulus intensity was decreased to a level producing evoked potentials of a similar spike amplitude as were recorded from the saline sites prior to any manipulations, and then a final HFT set was delivered to the PP, it was evident that LTP saturation does not occlude PGi potentiating effects. *ANOVA*s revealed significant main effects for PGi stimulation of spike amplitude ($F_{(1, 6)} = 9.31$, $p < .05$), area ($F_{(1, 6)} = 10.87$, $p < .05$) and latency to spike onset ($F_{(1, 6)} = 31.62$, $p < .01$) (see Tables 9 and 10). There was also a main pipette effect for area ($F_{(1, 6)} = 10.35$, $p < .05$), indicating that the baseline popspikes were not well

matched for area. Interestingly, in this last experiment, there was no significant stimulation x pipette interaction, indicating no between site differences with PGi stimulation.

Table 9

The effect of timolol on the potentiation of PP-evoked potentials produced by PGi stimulation following LTP saturation with PP stimulation reduced. Means used in *ANOVAs* and *F* values reported for the interaction of Pipette and PGi stimulation. Note none of the F values were significant.

PARAMETER	SALINE *N*=7		TIMOLOL *N*=7		*F* VALUE
	CONTROL	PGi	CONTROL	PGi	
EPSP SLOPE (μV/ms)	5409.57	5504.10	3885.19	4038.43	0.35
LATENCY TO SPIKE ONSET (μs)	3201.43	3124.00	3192.43	3126.91	0.62
LATENCY TO SPIKE PEAK (μs)	4017.81	4026.43	3998.48	4013.10	0.00
SPIKE AMPLITUDE (μV)	3855.24	6791.85	3899.86	5580.71	4.64
AREA UNDER THE TANGENT	147.19	220.90	106.38	149.14	3.41

Table 10

The potentiation of PP-evoked potentials produced by PGi stimulation is not occluded by LTP saturation when PP stimulus intensity is reduced. *F* Values for main effects and interactions from *ANOVA*s for each variable. (* $p < .05$) ** ($p < .01$)

PARAMETER	PGi STIMULATION MAIN EFFECT	PIPETTE MAIN EFFECT	STIMULATION X PIPETTE INTERACTION
EPSP SLOPE	1.11	3.55	0.35
LATENCY TO SPIKE ONSET	31.62 **	0.00	0.62
LATENCY TO SPIKE PEAK	0.01	0.01	0.0
SPIKE AMPLITUDE	9.31 *	0.25	4.64
AREA	10.87 *	10.35 *	3.41

DISCUSSION

PGi-stimulation has been demonstrated to be an effective method of evoking NEP in the DG, which is significantly attenuated by local β-adrenergic receptor blockade. Local β-receptor blockade was shown to weaken the initial induction of LTP, but does not reduce the final magnitude of LTP once LTP saturation has been obtained. Further, PGi-induced NEP persists following LTP saturation and is attenuated by β-blockade. These results, when considered in detail with the findings of earlier work, lead to the conclusion that *in vivo* PGi-induced NEP and frequency-induced plasticity are distinct and additive phenomena.

β-Blockade did not Affect Baseline Potentials

There were two concerns about baseline shifts in the present study which will be addressed prior to a more comprehensive discussion of the implications of the results. First, there was a concern that timolol would spread outwardly and have a significant effect on the cells contributing to the potentials recorded by the saline pipette. The popspikes recorded from the two pipettes were matched in terms of shape, size and spike onset at the beginning of the experiment when it is unlikely that any significant β-blockade would have been

obtained, at the timolol or at the saline site, by simple passive diffusion. Care was taken in matching the baseline waveforms, as it was felt that if the baseline values were not comparable, then any changes seen over time would not be interpretable. Once well matched popspikes were obtained, normally within 10 minutes, then baseline data of a minimum 60 pairs (typically 120 pairs) of popspikes were collected prior to the commencement of Experiment 1. Also, the data presented in this study for Experiment 1 were from the final three PGi-PP stimulation pairings, thus ensuring that there had been an adequate period of time (a minimum of 40 minutes) for the drug to diffuse out from the tip. Steward et al. (1990) saw maximal effects of bicuculline at around 15 minutes into testing which remained stable for many hours. The rate at which timolol and bicuculline diffuse outward may well differ but it was felt in the current study that a minimum of 40 minutes for diffusion would be sufficient. The comparisons indicate that the baseline waveforms were comparable and stable over time, as was found by Frizzell and Harley (1994), using ketamine in the second pipette.

The magnitude of potentiation observed in response to PGi stimulation at the saline site in Experiment 1 (popspike amplitude increased to 157 % of baseline popspike amplitude) is comparable to the potentiation seen in both the Babstock and Harley studies (1992, 1993) (which report potentiation of

spike amplitude as 139 % and 172 % of baseline respectively) and in control experiments conducted in the current study, indicating that little if any of the drug had diffused to the saline tip region. It is possible that timolol had an effect on the cells which encircled the saline pipette, however, the concentration of timolol that may have affected the cells contributing to the potentials recorded at the saline site would have been much less than the concentration of timolol immediately surrounding the timolol pipette.

There was also a concern that timolol would have an effect on the baseline potentials at the timolol site. This concern arose from the fact that there were no data prior to application of timolol to use as a reference in monitoring for any subtle effects timolol may have had on baseline. Timolol had no apparent effects on baseline activity in the current study. The two potentials were matched with relative ease by adjusting the dorsal-ventral placements, and no adjustment of the PP stimulation strength was required. The matching of the potentials remained effective throughout the course of the experiments, with no drifting of the timolol evoked potential. When the PP stimulus intensity was decreased, it was difficult to match the potentials again, however this occurred many hours into the experiment. This lack of effect of β-blockade on baseline potentials is in agreement with the data from β-blockade (Dahl & Sarvey, 1989; Harley & Evans, 1988; Lacaille

& Harley, 1985) and NE depletion studies both *in vivo* and *in vitro* (Bliss, Goddard, & Riives, 1983; Harley & Evans, 1988; Stanton & Sarvey, 1985).

However, there have been some reports of β-blockers producing baseline effects, Dahl and Winson (1985) report iontophoretic application of the β-antagonist sotalol in the granule cell layer enhances the population spike in a minority of trials with no effect on EPSP slope. As well, Babstock and Harley (1992) reported a depression of baseline following systemic propranolol injections. The most likely cause of the shifts in the Babstock and Harley study were effects on the cardiovasculature. Propranolol has much more membrane anesthetic effects than timolol (Hahn, Oestreich, & Barkin, 1986). Harley and Evans (1988) utilized intradentate infusion of both propranolol and timolol. They noted no significant differences between the two drugs in terms of blocking of LC-induced potentiation and found no effect on baseline by application of either drug, supporting the suggestion that the shifts in baseline observed by Babstock and Harley (1992) were due to extrahippocampal changes.

Local β-Receptor Blockade was Achieved

Passive diffusion of timolol out of the recording tip did appear to result in a significant β-receptor blockade which was highly localized. β-blockade was revealed when the

records from the two recording pipettes were compared. As predicted, there was significantly less PGi-induced acute potentiation of PP-evoked potentials recorded from the timolol pipette, implying that a significant blockade of β-receptors in the immediate area surrounding the timolol pipette had been achieved. PGi-induced potentiation was found to persist following LTP saturation and again, PGi-induced effects were attenuated by β-blockade. That PGi-induced potentiation was recorded following LTP saturation from both pipettes and was significantly attenuated on the timolol record is a further indication that the diffusion of the drug was highly localized to the area surrounding the drug pipette, as was found by Steward et al. (1990) and by Frizzell and Harley (1994).

PGi Stimulation Produces β-Receptor Mediated NEP

PGi stimulation was found to be a reliable, effective method of producing NEP in the DG, confirming the work of Babstock and Harley (1992, 1993). Babstock and Harley demonstrated that stimulation of the PGi results in a short-term potentiation of PP-evoked potentials recorded from the DG, most likely via PGi activation of LC neurons. This potentiation appeared to require β-adrenergic receptor activation, as evidenced by attenuation of PGi effects following i.p. propranolol. In the present study, it was shown that the PGi produced potentiation does require local

intradentate β-receptors for full expression. In addition, the present study is the first to find *in vivo* PGi-induced NEP can be induced following LTP saturation, a finding which supports the conclusion of Frizzell and Harley (1994): NEP can be induced independently of NMDA receptor mediated LTP.

Short-lasting NEP was consistently found to be the result of PGi stimulation, which was attenuated by β-receptor blockade. In Experiment 1, PGi stimulation was found to result in a popspike amplitude increase in all animals, with a mean popspike amplitude increase at the saline site to 156.7 % of baseline (comparable to 139 % and 172 % reported by Babstock & Harley, 1992 and 1993, respectively). The potentiation was attenuated by β-blockade to 51% of the potentiation at the saline site (timolol site mean potentiation was 128.8 %). This finding is comparable to Babstock and Harley (1992) who reported that 15 minutes following i.p. propranolol injection, potentiation of spike amplitude with PGi stimulation was 59% of pre-drug potentiation. Popspike area was found to be 156.7 % following PGi stimulation, Babstock & Harley, (1992) report area as 143.7 % of baseline.

As in the current study, Babstock and Harley (1992, 1993) did not find PGi-PP induced changes in EPSP. This lack of EPSP effect was attributed to poor isolation of the medial EPSP. When the medial PP was stimulated at a level which

evoked popspikes, the lateral PP probably was co-activated. Co-activation of the medial and lateral PP would result in cancellation of the potentiation and depression effects of the two pathways. In the current study, the popspike was medial, appearing on the rising phase of the EPSP. Medial and lateral PP potentials will be discussed in more detail later.

In the current study PGi stimulation resulted in a small but significant reduction of latency to popspike onset, which was not consistently seen in the Babstock and Harley studies. PGi stimulation was found to result in an onset latency decrease to 98.1% ($p < .01$) of baseline on both the saline and drug pipettes. No difference between the saline and drug pipettes were seen with respect to onset latency decrease. That timolol had no effect on the this decrease in onset latency indicates that the effect is not β-receptor mediated. There are direct projections from the PGi to the entorhinal cortex, and to the DG, CA1-CA2 and CA1-subicular regions of the hippocampus, which are not NE and, in all likelihood, not adrenergic (Zagon, Totterdell, & Jones, 1994). It is possible that the observed decrease in onset latency occurs as a result of direct stimulation of these cells in the PGi or that the decrease is the result of PGi-induced activation of other non-adrenergic inputs to the DG. That this effect was not consistently observed by Babstock and Harley can be attributed to variation in PGi stimulation electrode placement,

stimulation levels or to other differences in methodology and analysis.

Complete blockade of PGi-NEP was not obtained in the current study, only significant attenuation, which was consistent over time. Babstock and Harley (1992) reported that maximal attenuation of PGi-NEP was obtained within 15 minutes of injection of β-blocker and as time passed, the effectiveness of blockade decreases. The difference in duration of effect by the β-blocker can be attributed to the different modes of drug delivery in the two studies. Systemic injections, the mode of drug delivery used by Babstock and Harley, would result in delivery to the hippocampus (in addition to many other regions of the brain and body) of high concentration of drug for a short period of time following injection which would decrease in concentration as the drug was metabolized. The method used in the current study ensured a consistent level of β-blockade in the region immediately surrounding the tip of the drug pipette.

PGi-Induced NEP Persists following LTP Saturation

Following LTP saturation, PGi stimulation again resulted in short-lasting potentiation of PP-evoked potentials. While the potentiating effects were not as great as were observed prior to LTP saturation when the spikes were smaller, the same trends were seen under both conditions. Overall, PGi

stimulation resulted in significant increases in popspike amplitude and area, and a small yet significant decrease in popspike onset latency. Again, there were significant reductions in the PGi-induced increases in popspike amplitude and in area in the potentials recorded from the timolol pipette, while values from the timolol pipette did not significantly differ from the saline pipette values for the decrease in popspike onset latency. At the saline site, the average increase in amplitude was to 130.5% and in area to 120.3% of baseline, the average decrease in onset latency was to 98.5%. The corresponding changes observed at the timolol site with PGi stimulation were spike amplitude 107.7%, area 114.5% and onset latency 99.1%. This again confirms the β-receptor dependence of PGi stimulation effects and supports the hypothesis of LC-NE mediation of PGi potentiation. The results of Experiment 3 also support the idea that *in vivo*, unlike what is seen *in vitro*, NEP is independent of NMDA channel induced plasticity.

PGi-Induced Effects Compared to LC-Induced Effects

Harley and Milway (1986) observed that glutamate ejection in the LC resulted in significant increases in population spike amplitude (mean percentage increase of 41%), with long lasting effects seen at 37% of sites tested. Variable effects were seen on EPSP slope and on spike onset latency.

Potentiation of spike amplitude lasted an average of 8.4 minutes after glutamate ejection. Harley and Evans (1988) also looked at LC-induced potentiation of PP evoked potentials and reported significant increases in popspike size and increases in EPSP slope in 71 % of experiments, lasting for several minutes to more than 30 minutes. No effect on spike onset latency was observed. Intradentate ejection of β-blockers had no effect on previously induced long-lasting effects and resulted in significant attenuation of LC effects.

In the current study it was observed during experimentation, based on the 95 % confidence intervals calculated on-line, that maximal increase in potentiation occurred 30 ms following PGi stimulation and potentiation did not outlast PGi stimulation. It could be that if the PGi stimulus intensity had been increased or if frequency and time of stimulation was increased, long lasting changes may have been induced, as was reported in Babstock and Harley (1992). They reported that long lasting potentiation resulted when the interval between repeated PGi-PP pairings was relatively short. Optimal potentiation was obtained with a 333 Hz train lasting 10 ms, with a stimulus intensity of 25 to 35 V, as was used in the current study.

NE Promotes LTP Induction

Since *in vitro* data indicates that the β-receptor plays a role in LTP induction, it was thought that in the current study, β-blockade would result in a decrease in the magnitude of LTP induced. Initially, induction of LTP was found to be weakened by the intradentate application of timolol. However, following repeated HFT delivered to reach LTP saturation, the magnitude of LTP achieved was not reduced at the timolol recording site. This finding suggests that while initially the β-receptor promotes induction of LTP, NMDA receptor activation via electrical stimulation alone is sufficient to produce maximal LTP *in vivo*.

Stanton and Sarvey (1985) reported that slices which are NE depleted do not differ from control slices in terms of DG evoked potential baseline characteristics, however, both the likelihood of LTP induction and magnitude of LTP induced in NE depleted slices is greatly reduced. These are results which suggest, as the present results do, that NE promotes LTP. But the present results and the results from *in vivo* depletion studies also suggest that LTP can occur without NE (Bliss, Goddard, & Riives, 1983; Dahl, Bailey, & Winson, 1983; Robinson & Racine, 1985).

The data from the current study are also consistent with a report by Hopkins and Johnston (1984). In CA3, NE was found

to permit stimuli ineffective in producing LTP to be effective on a non-NMDA form of LTP. When NE was added to the bath, tetanic stimuli which in control slices did not produce LTP, induced long lasting synaptic enhancement. With bath application of NE, magnitude and duration of LTP was enhanced. Addition of timolol (100 nM) or propranolol (10 to 100 nM) to the bath reversibly blocked both NE enhancement of LTP and induction of LTP. NE can facilitate non-NMDA as well as NMDA frequency dependent plasticity (Hopkins & Johnston, 1984).

The present data suggests that NE-mediated plasticity operates via mechanisms which are relatively independent of NMDA mediated plasticity. Interestingly, another difference between NE and NMDA mediated plasticity had earlier been identified by Dahl and Sarvey (1989). NE has selective effects on the medial and lateral divisions of the PP. While both pathways evoke LTP in response to HFT stimulation, when stimulated with NE, the medial PP potentials are potentiated and the lateral potentials are depressed (Dahl & Sarvey, 1989; Pelletier, Kirkby, Jones, & Corcoran, 1994). Dahl and Sarvey (1989) found addition of the α-receptor antagonist phentolamine and NE or the β-receptor agonist isoproterenol to the *in vitro* bath resulted in enhancement of population spike amplitude and EPSP slope of medial PP evoked potentials and long-lasting depression (LLD) of spike amplitude and EPSP slope of lateral PP evoked potentials. These effects were

prevented by the addition of propranolol (Dahl & Sarvey, 1989).

In vivo, PGi-induced NE release onto the DG has also been shown to have pathway specific effects (Babstock & Harley, 1993). PGi stimulation prior to PP stimulation depressed the lateral PP evoked EPSP while the medial PP evoked potential was potentiated. The lateral PP was selectively activated by stimulating the lateral olfactory tract (LOT). When the LOT was stimulated 5 - 20 ms following PGi stimulation, the amplitude and area of the DG EPSP was decreased by an average of 32.8%, EPSP slope decreased by an average of 32.6 % and the latency from stimulation to peak of the EPSP was increased in 5 of 9 animals by 6.1 to 7.2 %. All observed PGi-LOT EPSP effects were eliminated 20 minutes following i.p. injection of propranolol. Direct stimulation of the PP resulted in an evoked potential that was mostly, but not completely, medial. PGi pre-stimulation was found to result in NEP of the medial PP popspike area and amplitude. PGi stimulation that enhanced the medial PP potentials, significantly depressed the LOT EPSPs in all animals tested, confirming the *in vitro* finding of NE selective modulation of PP evoked potentials (Dahl & Sarvey, 1989).

Pelletier, Kirkby, Jones and Corcoran (1994) reported on *in vitro* studies of selectivity of plasticity of the medial and lateral PP DG evoked potentials, and described a number of

observations which can be compared to the results of the present study. Pelletier et al. observed that 20 μM metoprolol and not 1 μM metoprolol, in the absence of tetanic stimulation, resulted in LLD of medial PP potentials and LTP of lateral PP potentials. In the present study, no shifts in baseline were observed with concentrations of timolol that resulted in effective β-blockade. In the Pelletier et al. study, tetanic stimulation resulted in potentiation of both the medial and lateral potentials. With the subsequent application of isoproterenol, medial PP potentials showed further potentiation while lateral PP potentials did not. However, tetanic stimulation followed 30 minutes later by further tetanic stimulation resulted in further potentiation of slope of both the medial and lateral potentials and further increase in medial PP potential amplitude, indicating that LTP had not been saturated by the initial delivery of tetanic stimulation. Pelletier et al. also noted that tetanic stimulation following wash of isoproterenol resulted in further potentiation of the medial PP potentials and the lateral PP potentials returned to baseline levels.

Pelletier's et al. (1994) result that magnitude of isoproterenol-induced potentiation was not effected by prior LTP induction support the findings of the present study, that NEP and LTP are independent and additive processes. However, as they note, LTP saturation had not been obtained in their

study prior to testing the effects of the NE agonist. Testing slices for LTP saturation prior to NE or NE agonist application is indicated by the findings of both the Pelletier et al. study and the present. Pelletier et al. also found indications that β-receptors play a role in LTP induction. Initial HFT stimulation following β-blockade did not result in significant changes, indicating β-blockade attenuated LTP induction, however, further tetanic stimulation did result in significant increases.

LTP Saturation was Achieved

With repeated HFT stimulation of the PP, saturation of the PP-DG synapses can be achieved. This was seen in the analysis of Experiments 2 and 4 of the current study. Analysis of Experiment 2 revealed that in 6 of the 7 animals tested, LTP was saturated. When, in Experiment 4, the PP stimulus intensity was decreased, it was shown in all animals that further HFT stimulation had no further potentiating effects, arguing against ceiling effects as the reason for observed saturation in Experiment 2 and confirming that LTP had been saturated.

Experiment 4, as noted earlier, was conducted for two reasons: 1) to ensure that possible ceiling effects at the recording site did not obscure potentiation magnitude and 2) to evaluate the changes in pre-LTP versus post-LTP PGi-induced

potentiation. While it appears clear from the data that LTP was saturated and PGi prestimulation produced potentiation, the data are less clear with respect to the magnitude of PGi induced potentiation. Comparing the effect of PGi stimulation on similar sized popspikes from the pre-LTP saturation and the post-LTP saturation conditions should have revealed what, if any, effects LTP saturation has on the magnitude of PGi-induced potentiation. It proved to be very difficult to match the post-LTP waveforms recorded at both sites within an animal to the pre-LTP waveforms. Reducing PP stimulation did reduce the size of both popspikes but not at the same rate on both pipettes and it was difficult to match the potentials to each other and to the pre-LTP levels. In fact, the analysis did reveal a main effect of pipette for area, indicating the potentials were not matched for this dependent variable.

While it was made clear when the PP stimulus intensity was reduced that LTP had been saturated in Experiment 3, and PGi stimulation continued to produce further potentiation following LTP saturation, any conclusions made from Experiment 4 with respect to differences in magnitude of PGi effects would be tenuous at best. As in earlier portions of the experiment, PGi stimulation resulted in an acute small yet significant decrease in latency to spike onset and in increases in spike amplitude and area. The data demonstrated no between site differences in response to PGi stimulation,

indicating no magnitude difference between potentiation seen at the saline or timolol sites. This lack of attenuation at the timolol site may be the result of a number of factors including poorly matched popspikes and possibly decreased effectiveness of β-blockade. This portion of the study was the last performed in each animal, typically occurring 5 to 7 hours following placement of the recording pipettes. It is possible that with time the drug in the pipette had begun to oxidize and blockade had been decreased. Further experimentation, including a control experiment, similar to Experiment 1 but extended over a period of hours to see if the effectiveness of the timolol blockade decreases, would help to elucidate the effects observed here.

NEP can be Induced Independent of NMDA Receptor Induced Plasticity

Following LTP saturation, PGi-stimulation resulted in potentiation, suggesting that PGi-induced potentiation is not supported by the same NMDA plasticity mechanism that produces LTP. Once saturation of the PP input synapse had been reached, no further NMDA receptor induced plasticity can be induced. That LTP saturation had been obtained in Experiment 3 was also confirmed by Experiment 4, when further HFT PP stimulation did not result in potentiation.

That PGi stimulation results in β-mediated NEP of DG evoked potentials following LTP saturation lends support to the conclusions of the Frizzell and Harley (1994) study. Frizzell and Harley were the first to study the role of the NMDA receptor in NEP of PP-evoked potentials in the DG *in vivo*. They concluded that NMDA receptor blockade does not prevent NEP. This was determined in an experiment utilizing a double pipette set-up, much like the set-up in the present study. By placing a solution containing a high concentration of the NMDA channel blocker ketamine in one pipette and saline in the other, Frizzell and Harley were able to determine that local diffusion of ketamine out from a pipette containing a high concentration of ketamine is sufficient to prevent or significantly attenuate the induction of LTP. Frizzell and Harley then proceeded to test the effects of ketamine on NEP. By stimulating the LC with glutamate, release of NE in the DG was produced, which resulted in NEP at both recording sites. Interestingly, there was a significantly greater enhancement of the popspike amplitude seen at the ketamine site than the saline and the effect of LC stimulation was longer lasting on the ketamine site. The authors proposed that this is due to a decrease in NE re-uptake caused by ketamine. Following LC activation, the effectiveness of the NMDA receptor blockade was evaluated by delivery of HFT to the PP. Again, ketamine was found to either significantly attenuate or abolish

completely LTP induction.

Frizzell and Harley (1994) found NEP was induced at sites where LTP was blocked, which clearly indicates that NE can invoke plastic changes independently of NMDA dependent mechanisms. In order for frequency mediated plastic changes (LTP) to be induced, NMDA channel activation is necessary. The Frizzell and Harley study was the first to indicate that there is NMDA-independent NEP *in vivo*. The results of the present study support the conclusions of Frizzell and Harley, NEP was found to persist after LTP saturation had been achieved, indicating that *in vivo* NEP is additive of NMDA mediated plasticity. It appears that, unlike what is observed *in vitro*, either mode of induction can produce independent plastic changes in the DG.

DG NEP is distinct from LTP

The results of the present study indicate that while *in vivo* NE promotes induction of LTP, maximal levels of LTP can be obtained when β-receptors are blocked. This suggests that NE acts as a facilitator of NMDA receptor mediated potentiation *in vivo*, but it does not appear to be required for NMDA mediated potentiation, unlike what is observed *in vitro*. It also appears that *in vivo* NEP can be additive with frequency mediated potentiation, arguing that NEP and frequency-mediated potentiation are distinct. The role of NE

appears to be more than a neuromodulator of NMDA current, as it appears capable, under *in vivo* conditions, of evoking a potentiation which is distinct from NMDA receptor mediated potentiation.

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