LOCUS COERULEUS-INDUCED POTENTIATION OF THE PERFORANT PATH EVOKED POTENTIAL IN THE DENTATE GYRUS OF THE AWAKE AND BEHAVING ANIMAL

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BY

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ABSTRACT

The effects of glutaminergic activation of the pontine nucleus locus coeruleus on the perforant path evoked potential in the dentate gyrus of the awake and behaving animal were investigated. Twenty-two male Long Evans rats received 150-450 nanolitre intracranial injections of .25-.5 M l-glutamate in the vicinity of the locus coeruleus. Injection sites localized to within 300 μm of the locus coeruleus reliably produced an enhancement (167% of control) of the population spike of the evoked potential. In 50% of these animals, a long-lasting (i.e. greater than 20 minutes) enhancement of the population spike amplitude was observed. Changes in the excitatory postsynaptic potential and population spike onset latency were variable. Injection sites in lobule centralis of the rostral vermis of the cerebellum also produced both short and long-lasting potentiation of the population spike. Behaviors recorded following glutamate application were not correlated with the production of an enhancement. Behaviors were sufficiently heterogeneous that a behavioral profile for locus coeruleus-activated animals could not be ascertained. Norepinephrine-mediated potentiation of granule cell responsivity may represent a neural substrate for the processing and storage of information.

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1. INTRODUCTION

The present experiments are directed at testing the occurrence of long-lasting potentiation of the perforant path (PP)-dentate gyrus (DG) evoked potential induced by activation of the locus coeruleus (LC)/norepinephrine (NE) system in the DG of the awake and behaving rat.

The PP-DG connection has been an important model system in our efforts to elucidate the biological substrates of learning and memory. This introduction will review the initial discovery of high-frequency long-term potentiation (LTP) at that synapse and the more recently observed NE-induced potentiation.

It is probably not coincidental that the PP-DG synapse was the first system to show a long-term functional change in synaptic efficacy as the hippocampus has long been known as a structure critically involved in the acquisition of new information in humans (Scoville and Milner, 1957). Numerous attempts to induce use-dependent changes in synaptic transmission, hypothesized as necessary for the acquisition of information, elsewhere in the nervous system, had failed. However, in 1971, Lomo reported such changes in the DG in response to stimulation of the PP (Lomo, 1971).

1.1 Perforant Path-Dentate Gyrus Synapse

The PP-DG connection is a subset of the hippocampal formation which is composed of 3 principle regions. They include the 1) DG, 2) Ammon's Horn (i.e. CA1, CA2, CA3 and CA4 or the hilar region) and 3) the subicular complex (presubiculum, subiculum and parasubiculum). The PP is a major afferent fibre pathway that originates from the medial layers of the entorhinal cortex (EC) and projects extensively to the hippocampus, including the DG (Hjorth-Simonsen and Jeune, 1972; Steward, 1976). The hippocampal fields appear to be anatomically linked in a tri-synaptic fashion beginning with the connection between the EC and the DG. The cells of the DG then project to CA3 via the mossy fibres and the cells of CA3 project to CA1 through the Schaffer collaterals. According to Andersen and colleagues, stimulation of the EC would result in the successive activation of 4 pathways (i.e. PP, mossy fibres, Schaffer collaterals and CA1 alvear fibres) in a direction transverse to the long axis of the hippocampus (Andersen, Bliss and Skrede, 1971b). Hence, the longitudinal spread of synaptic transmission could be recorded at multiple sites as a result of a single volley directed at the medial EC.

The DG consists of a dense, single layer of predominantly granular cells that are folded in such a manner as to present

a dorsal and ventral blade (Teyler and DiScenna, 1984). The apical dendrites of the granule cells project away from the dorsal blade into the molecular layer of the DG. The region between the blades is known as the polymorphous layer and contains neurons of the hilar field. PP fibres from the medial EC largely innervate the middle third of the ipsilateral molecular layer of the DG , making 'en passage' axo-dendritic connections with the apical dendrites of the granule cells. This circuit lends itself to extracellular analysis of field potentials due to its dipole-like structure and its laminar organization. Moreover, it represents the primary junction for the transmission of associational information from the medial EC.

Lomo (1971) reported that the extracellular field potential that was elicited by electrical stimulation of the PP while simultaneously recording in the molecular layer of the DG consisted of a negative wave of short duration with a biphasic spike in its trough. When the recording electrode was lowered into the cell body layer of the upper blade, the result was a slow positive wave with a negative compound spike superimposed upon it. The negative wave recorded in the molecular layer corresponded to the field excitatory postsynaptic potential (EPSP) caused by the PP volley and reflected the sum of synaptic depolarizations at the granule cell dendrites in response to PP stimulation (Andersen, Bliss

and Skrede, 1971a). In the cell layer recording, the negative compound wave represented the population spike (PS), while the positive wave upon which the PS was superimposed reflected the EPSP. The PS corresponds to the magnitude of unit discharges (i.e. the number of granule cells that are firing action potentials). Further, Lomo observed that the PS and EPSP increased with greater stimulus intensities delivered to the PP. However, the EPSP increased at current intensities that were not sufficient to fire cells in the granule layer and at higher intensities (i.e. above 11 Volts) the EPSP and PS appeared to dissociate as the EPSP levelled off while the PS continued to rise in amplitude. Overall, a large increase in the EPSP was required before cell discharge occurred.

1.2 Long Term Potentiation (LTP)

1.2.1 Acute Studies

Bliss and Lomo (1973) found that repetitive stimulation (e.g. 100 pulses/sec for 3-4 sec) of the PP resulted in a potentiation of the EPSP and PS of the evoked field potential recorded in the DG, that lasted for up to 10 hours. Of the 35 conditioning trains given to 18 animals, 4% showed long-lasting (i.e. >30 minutes) increases in PS, 57% showed long-term decreases in PS onset latency and 4.3% had a long-lasting

EPSP. With respect to the PS, 1) the amplitude increased dramatically during the conditioning train, 2) a brief period of potentiation of 1-2 sec followed the offset of the train, 3) this was followed by a depression in the PS lasting up to 1 minute and 4) a longer-lasting enhancement ensued. In contrast, the EPSP was depressed during the high frequency train, but quickly recovered at stimulation offset and increased beyond control levels. This period was followed by a gradual return to baseline. In many cases, the potentiation of the PS occurred independently from changes in the EPSP, implying an EPSP/PS dissociation. The duration of the effect suggests a long-term functional synaptic change which might provide a substrate for the storage of information.

The prolonged responsiveness of granule cells to high frequency activation of this mono-synaptic circuit has been consistently replicated both in the acute preparation and in the 'in vitro' hippocampal slice (e.g. deJonge and Racine, 1985; Bliss, Douglas, Errington and Lynch, 1986; Sarvey, Burgard and Decker, 1989). More importantly, it has been shown to be specific to the synapses that are activated (Alger and Teyler, 1976). LTP is presently used as a model system in the study of biochemical and physiological correlates of memory.

1.2.2 Chronic Studies

In order for LTP to be a representative model for the processing of learning and memory, it was important to demonstrate that this effect could be replicated in the awake and behaving animal. Bliss and Gardner-Medwin (1973) first showed that in unanesthetized rabbits, single high frequency volleys to the PP produced LTP 26% of the time and the LTP lasted from 1 hr to 3 days. Characteristics of the EPSP and PS were essentially similar to that observed in the acute, anaesthetized animal. However, high intensity stimuli delivered to the PP resulted in changes differing somewhat from those observed in the acute preparation. First, during the train, initial EPSP responses were depressed followed within seconds by a well defined EPSP and a small or absent PS. After 10 seconds, the entire potential was depressed for approximately 2 seconds after which multiple PS's occurred. Later researchers, however, have refined stimulus parameters for awake animals so that potentiation of both EPSP and PS was robust and lasted in some cases, up to several weeks (Douglas and Goddard, 1975; Robinson and Racine, 1985).

1.3 Mechanisms Underlying LTP

There have been significant advances in our understanding

of the mechanisms subserving LTP. Several lines of evidence suggest that the acidic amino-acid, glutamate (GLU) may be the excitatory neurotransmitter that is released from PP terminals onto granule cell dendrites (Herron, Lester, Coan and Collingridge, 1986; Collingridge and Bliss, 1987). The ligand is thought to bind with both N-methyl-D-aspartate (NMDA) and non-NMDA (i.e. quisqualate, kainate) receptors on the post-synaptic membrane. During low frequency stimulation of the PP, GLU affects granule cell excitability via the activation of non-NMDA receptors as NMDA receptors are believed to be suppressed via a magnesium-mediated block. During high frequency stimulation, the resulting post-synaptic depolarization effectively removes this block and NMDA receptors then participate in synaptic transmission (Collingridge and Bliss, 1987). The activation of NMDA receptors appears critical to the induction of LTP, based on findings that LTP produced by a high frequency afferent volley of the PP can be successfully blocked by 2-amino-5-phosphonovalerate (APV), an NMDA receptor antagonist (Morris, Anderson, Lynch and Baudry, 1986). Hence, LTP induction appears to depend on 1) the opening of the NMDA ionophore in response to glutamate binding and 2) post-synaptic depolarization that would allow voltage-dependent NMDA ionophores to carry ionic currents (Collingridge and Bliss, 1987).

A critical second messenger component in the expression of LTP appears to be the entry of postsynaptic calcium through NMDA channels (Lynch, Larson, Kelso, Barrionuevo and Schottler, 1983). In fact, calcium is thought to be the synaptic 'trigger' that activates a series of biochemical events that lead to the expression of LTP. This group demonstrated that the injection of the calcium chelator EGTA into postsynaptic neurons prevented the induction of LTP.

There appears to be considerable agreement as to the mechanisms that account for the induction of LTP. However, the maintenance of the non-decremental, long-lasting component of LTP cannot be explained by GLU-NMDA-Ca++ coupling. Maintenance of LTP may require the cooperation of pre- and postsynaptic activity. Bliss and colleagues assayed the molecular layer of the DG using push-pull cannulation following induction of LTP (Bliss, Douglas, Errington and Lynch, 1986). They found increased concentrations of both GLU and aspartate 1 hour after the delivery of the LTP-producing tetanus and further increases in glutamate but not aspartate concentrations by the second hour. Enhanced levels of GLU were maintained for at least 3 hours after stimulus delivery. Sustained increase in presynaptically-released excitatory amino-acids may provide a partial answer as to the mechanisms underlying the maintenance of LTP. This possibility would necessitate some retrograde process that could functionally link pre- and postsynaptic

systems in the induction and maintenance of LTP. Such a mechanism does not explain the EPSP/PS dissociation frequently seen in LTP. These effects would appear to require some enduring postsynaptic change in coupling of synaptic current to spike triggering.

The relatively long duration of LTP has prompted researchers to look for indicators of protein synthesis that may be able to support such plasticity. In DG of the anaesthetized animal, high frequency stimulation of the PP, is accompanied by the appearance of newly synthesized proteins in the extracellular space (Fazeli, Errington, Dolphin and Bliss, 1988). However, the evidence favouring a role for protein synthesis remains equivocal (Madison, Malenka and Nicoll, 1991).

1.4 LTP and LEARNING

The duration of LTP in chronic animals is part of the reason that LTP has been suggested as a substrate for certain types of learning and memory. Douglas and Goddard (1975) reported potentiation of EPSP and PS lasting up to 12 days with EPSP's continuing to show enhancement up to 2 months following initial saturation of LTP. In addition, they found that repeated stimulation of the PP over days led to higher levels of potentiation in the DG. The ability to produce

larger increases in granule cell excitability by repeatedly inducing LTP may serve as a model to mimic the consequences of repeated learning trials. A subsequent study revealed that repeated stimulation of the PP after LTP induction failed to increase the peak LTP or lower the threshold for LTP following the first stimulation regardless of whether or not LTP was allowed to decay to baseline (deJonge and Racine, 1985).

McNaughton and colleagues have attempted to evaluate the effect of LTP saturation on previously learned and newly acquired spatial memory (McNaughton, Barnes, Rao, Baldwin and Rasmussen, 1986). They found that LTP of the PP-evoked potential in the DG resulted in a significant impairment of the acquisition of new spatial information and in the ability to adapt to changes in previously learned spatial relationships. A subsequent study from this research group, demonstrated that learning impairments associated with LTP tend to recover concomitant with the decay of LTP (Castro, Silbert, McNaughton and Barnes, 1989). The rationale behind these experiments was that if LTP were involved in normal learning, then saturating the synapses artificially would prevent this normal participation in new spatial learning for which the DG is implicated. Along the same lines, Morris, Anderson, Lynch and Baudry (1986) reported that intraventricular infusion of the NMDA antagonist AP5 both suppressed LTP 'in vivo' and caused an impairment in place

learning but not visual discrimination learning.

Other studies have attempted to address the relationship between LTP and normal learning mechanisms in the DG by training animals in a conditioning paradigm and assessing training-induced changes in synaptic efficacy. Animals undergoing training in an operant conditioning task showed a gradual increase in PS amplitude over the training period whereas the PS for animals in a free-feeding control condition remained stable or decreased slightly over time (Skelton, Scarth, Wilkie, Miller and Phillips, 1987). EPSP measurements were not described. Similar findings have been reported during classical conditioning (Weiz, Clark and Thompson, 1984). This group observed that PS amplitude increased both during presentations of the conditioned stimulus (CS) (i.e. 85 dB tone) and between trials in the group that received pairings of CS with the unconditioned stimulus (UCS) (i.e. corneal air puff) whereas the PS amplitude of the group receiving unpaired presentations of stimuli did not significantly differ from baseline. Sharp, Barnes and McNaughton (1987) reported that exposure to a complex spatial environment is accompanied by an enhancement of synaptic efficacy in the DG. Briefly, exposure to a stimulus-rich setting for 11 consecutive evenings, 16 hrs/day produced a significant change in the PS amplitude over the baseline PS recorded in animals in the standard colony home cage. The field EPSP was not affected.

Finally, Barnes (1988) reported a striking correspondence between animals' ability to perform a spatial reference task and the characteristics of their subsequently induced LTP in that, animals performing poorly on the spatial task showed a faster decay of LTP. Despite the suggestive nature of these studies, it still remains to be shown that exposure or training-induced increases in granule cell responsivity are related to storage of information in any way. In summary, the evidence regarding the relationship between LTP and learning is suggestive but not definitive.

1.5 Does LTP Occur Naturally?

Electrophysiological studies have shed some light on the possibility that LTP may be generated by a combination of naturally occurring bursts of neuronal activity and behavioral states. One approach has been to investigate the role of the electroencephalographic (EEG) patterns in the hippocampus and their potential contributions to synaptic transmission. Hippocampal rhythmic slow activity (RSA) or theta occurs 'in vivo' and can produce synchronous discharge of populations of interneurons in the hippocampus. Interestingly, researchers have found that when stimulation trains delivered to stratum radiatum of CA1 were spaced at 5 Hz (i.e. within theta frequency range), the LTP that was produced in CA1 was found

to be optimal and stable (Larson, Wong and Lynch, 1986; Staubli and Lynch, 1987). Similarly, Greenstein, Pavlides and Winson (1988) reported that stimulation trains at 5 Hz were the most effective stimulus producing LTP in the DG. In fact, tetany delivered outside of the theta zone (i.e. at 2.9 Hz and 10 Hz) failed to produce LTP. Further inquiries into this effect led to the finding that the induction of LTP in the DG was greatest when trains of stimuli were applied at the peak of the theta rhythm whereas stimuli presented at the trough of the theta rhythm resulted in either a decrease or no effect on EPSP and PS measures. The notion that a natural oscillating mechanism may modulate synaptic efficacy is especially attractive given that theta waves have
been recorded in the DG during specific behavioral conditions such as running, sniffing, rearing, orienting and paradoxical sleep (Buzsaki, Grastyan, Czopf, Kellenyi and Prohaska, 1981). Also, it has been shown that lesioning the medial septum both eliminates theta rhythm and impairs memory for a previously learned spatial task (Winson, 1978).

In contrast to this view of theta-mediated regulation of plasticity is Buzsaki's two-stage model of memory trace formation (Buzsaki, 1989) in which he suggested that behaviors associated with the highest neuronal discharge frequency and largest number of synchronous population discharges are ones that would most likely modulate long-term synaptic plasticity.

Buzaki proposes that naturally occurring irregular sharp waves (SPW) that are intrinsic to the hippocampal circuitry induce a natural consolidation of previous environmental input during slow wave sleep, drinking and immobility (i.e. non-theta behavior). At these times, large populations of CA3 pyramidal cells and hilar neurons fire in a highly synchronous fashion. Conversely, SPW are not seen in association with theta-type behaviors. Briefly, Buzaki suggested that the PP may transfer information to the DG during theta-related behaviors and that this information remains in a labile form. Next, the interface between the end of a theta-type behavior and the onset of a non-theta behavior (e.g. slow wave sleep) would lead to SPW bursting of the environmentally-primed neurons via a trigger mechanism in CA3. This massive and synchronous discharge would culminate in long-term changes in CA1, where previously labile information relayed from the DG would be converted into a more enduring form. This model proposes that the probability of producing long-lasting changes in synaptic efficacy would be considerably reduced during active-waking behaviors.

Buzaki's model has received some support from Winson's work on neuronal gating mechanisms in the hippocampus (Winson, 1984). Hippocampal function along the tri-synaptic circuit is affected by the behavioral state in which the animal is engaged (Winson & Abzug, 1978). This is most evident in CA3 and DG and to a marginal extent in CA1 following stimulation

of the PP. The PS of the PP-evoked potential in the DG is larger during slow wave sleep than during a still alert state (i.e. waking immobility). In addition, the rate at which the PS increases as a function of increased stimulus intensity is also lower in the still alert condition. The remaining behavioral states, including awake/voluntary movement and paradoxical sleep, show variable response characteristics in the PS in that an intermediate number of granule cells respond during these two states with a high degree of variability between trials. In contrast, the EPSP of the PP-evoked potential is larger during the still/alert condition than in slow wave sleep. REM states were associated with lower EPSPs and higher EPSPs were commonly seen in the awake/voluntary movement condition. The authors conclude that transmission across the PP-DG synapse is gated such that information destined for the DG will be impeded during still/alert behavioral states, whereas information coming into the DG during slow wave sleep is likely to pass more efficiently through the tri-synaptic chain. Tonic inhibitory mechanisms active during still/alert states and tonic excitatory influences in effect during slow wave sleep are proposed as possible explanations for these differences. Taken together, there is some evidence to suggest that natural increases in the discharge of DG neuronal populations are less likely to occur during immobile/waking behavioral states.

Arguments have been put forth that suggest that LTP-like phenomena may occur in the natural environment (e.g. theta-gated bursting or non-theta sharp waves) but there is still limited evidence that LTP reflects a naturally occurring phenomenon.

1.6 Norepinephrine-induced Long-lasting Potentiation (NELLP)

More than a decade after the initial description of frequency-induced potentiation, a neurotransmitter-induced LTP at the PP-DG synapse was reported. Iontophoresis of norepinephrine (NE) at 30-150 nA for 1-8 minutes directly into the DG produced an enhancement of the PS of the PP-evoked potential at 76% of the sites tested (Neuman and Harley, 1983). The effect was short-term (i.e. <30 minutes) in 61% of the potentiated sites. Typically, in these instances, the PS returned to baseline levels within 6-30 minutes of the cessation of ejection current. More interestingly, the remaining potentiated sites all showed a long-lasting enhancement (i.e. >30 minutes) of the PS amplitude, one of which was monitored for 11 hours. Potentiation occurred up to 8 minutes after the termination of the iontophoretic current. Changes to the field EPSP were not typically seen. The authors argued that NE may alter EPSP/PS coupling thereby increasing the likelihood that cells will fire as opposed to augmenting

synaptic drive. These results were replicated by Winson and Dahl (1985) who observed long-lasting facilitation of the PS in 3 of 8 penetrations after iontophoretically applying NE for 5 minutes into the DG. Onset of effect ranged between 6.5-9 minutes after the end of NE release. EPSP effects were variable.

Additional support for NELLP has come from 'in vitro' experiments using the hippocampal slice preparation. Lacaille and Harley (1985) observed significant increases in the PP-evoked PS in the DG following bath application of 5-50 μM NE. Average time of onset was 4.3 minutes after NE application In some cases (i.e 24 % of slices showing increases), these effects persisted long after NE was washed out of the bathing medium. Increases in EPSP (i.e. mostly in slope) occurred in 53% of the slices. Also, decreases in the PS onset latency were observed in 74% of the slices.

Lacaille and Harley (1985) attempted to assess the adrenergic receptor type that mediated the enhancing effects of NE. The beta-adrenergic receptor agonist, isoproterenol (ISO) produced increases in the PS that were similar to those observed following perfusion with 10 μM NE. Conversely, the beta-adrenergic receptor antagonist timolol (TIM), reliably blocked the effects of NE when administered concurrently. Phenylephrine, an alpha-adrenergic receptor agonist, produced a weak and delayed potentiation of the PS that was unlike that

observed with 10 μM NE and more often produced a depression in PS, especially at higher concentrations. The alpha-adrenergic antagonist, phentolamine produced a smaller and more transitory enhancement of the PS whereas concurrent application with NE did not affect NE-mediated increases in PS amplitude. Hence, NE-induced enhancement of the PS appear to be mediated by beta-adrenergic receptors.

Stanton & Sarvey (1985a) found that 50 μM NE perfusion of the DG for 30 minutes produced long-lasting changes (i.e. at least 5 hours) in PS amplitude following NE wash out in all of the eight slices used. More interestingly, NE-mediated short-terms increase in PS were not blocked by the protein synthesis inhibitor emetine whereas NELLP was completely blocked. Both phases of NE-induced potentiation were reliably blocked by the beta-receptor antagonists, propranolol (PRO) and metropolol.

It appears that NE can produce both short and long-lasting enhancement of PS amplitude at the PP-DG synapse that; 1) may reflect independent processes, 2) are beta-receptor mediated and 3) support NE as an important modulating influence on synaptic efficacy in the DG.

The contribution of the hippocampal slice preparation to our understanding of mechanisms that alter hippocampal function cannot be overemphasized. Yet, implicit limitations of the slice work raise concern as to the generalizability of results to neural function in the awake animal. For example,

Stanton & Sarvey (1985b) reported that NE depletion by 6-OHDA resulted in a suppression of LTP following repetitive stimulation of the PP in the slice preparation. However, in vivo NE depletion by injections of the selective catecholaminergic neurotoxin, reserpine in the chronic preparation have been reported to enhance the LTP of the EPSP and decrease the LTP of the PS (Robinson and Racine, 1985). In the hippocampal slice where deafferentation of neural circuitry is a necessary outcome, the possibility exists that electrophysiological and pharmacologically-mediated changes in neuronal excitability in the DG may depend on this partial denervation.

Neither the iontophoretic studies, which involve ejecting unknown concentrations of NE at arbitrary sites in vivo, nor the slice studies, which involve application of neurotransmitter to the bathing medium of a partially denervated preparation, directly probe for effects of normal, synaptic release of NE. To fulfil that requirement, it is necessary to activate the source of normal NE innervation of the DG, the locus coeruleus (LC).

The NE input to the DG arrives from a massively divergent afferent projection that arises from the LC (Koda and Bloom, 1977). More specifically, fusiform and multipolar neurons of the pars dorsalis of the LC project along the dorsal noradrenergic bundle (DNB), sending collaterals to the

molecular/granular layers of the DG and to a larger extent, the subcellular hilus (Haring and Davis, 1983; Oleskevich, Descarries and Lacaille, 1989).

Assaf, Mason and Miller (1979) initially reported that high frequency stimulation (i.e. 5 pulses; 15V, 300 Hz) of the DNB 40 msec before a low frequency test pulse was delivered to the PP, resulted in a brief increase in PS amplitude recorded in the DG cell layer that reached an approximate peak at 140% of control. No change was reported to occur in EPSP measures recorded in the same region. The effect was blocked by pre-treatment with 6-OHDA which effectively reduced hippocampal NE stores by 91% .

In a later study, Dahl and Winson (1985) observed that stimulation (6 pulses at 50 Hz) of the LC preceding single PP pulses also produced a short-lasting enhancement of the PS in 9 of 13 animals together with a decrease in the EPSP measured in the dendritic layer. The remaining animals showed a decreased PS accompanied by an earlier onset and a broadening of the PS. Repeated attempts to stimulate the LC resulted in progressively smaller enhancements in the PS amplitude. Pharmacological tests of NE-mediation were not attempted in these studies. More recently, Harley, Milway and Lacaille (1989) observed that 50 pairings of 333 Hz LC stimulation and .1 Hz PP stimulation where LC activation preceded the PP stimulus by 40 msec (i.e. as in Assaf et al., 1977 protocol),

produced a long-lasting facilitation (i.e. >30 min after LC stimulation offset) of the PS in the DG of 10 of 22 animals. In the remaining animals, LC conditioning stimulation produced short-lasting (i.e. <10 minutes) enhancement, a return to baseline after cessation of stimulation or a transient decrease in PS. Overall, changes to EPSP and PS onset latency were variable.

While the results of LC electrical stimulation on the PS of the DG are consistent with the effects of NE applied iontophoretically and in the slice, Harley et al. (1989) were not able to block their effects with an NE beta-adrenergic blocker. As other brainstem sites (e.g raphe nucleus) have also been shown to augment the PS of the DG (Winson, 1980) it is conceivable that current spread, produced by electrical stimulation in the vicinity of the LC, to other potentiating sites may account for these results. Yet, a study using parameters similar to those in Harley et al. (1989) with the exception of reducing PP current to elicit a PS of half-maximal amplitude, did find that PRO blocked the short-lasting enhancement of the PS produced by LC stimulation (Washburn and Moises, 1989).

This problem of stimulating fibres of passage is well illustrated in the classic studies of LC-NE effects on hippocampal unit activity. Segal and Bloom (1976) studied the effect of LC stimulation on hippocampal unit activity in awake

animals. While NE appears mainly inhibitory on hippocampal units and LC stimulation generates similar results, most units were inhibited at sites and current parameters that also evoked high rates of self-stimulation behavior. LC specificity of this stimulation is challenged by the evidence that the LC itself does not support self-stimulation behavior (Corbett, Skelton and Wise, 1977, Corbett and Wise, 1979) and therefore, other brain sites would presumably have had to be involved.

Goodchild, Dampney and Bandler (1982) had reported that GLU, delivered in discrete quantities, selectively activates cell bodies without stimulating fibres of passage that course through and around the locus of interest. Harley and Milway (1986) showed that selective activation of LC neurons using intracranial injections of nanolitre quantities of GLU in the vicinity of the LC (i.e. within 300 μm) produces both short and long-lasting enhancement of the PP-evoked potential in the DG. The average onset of effect was 1.1 minutes after the GLU pulse. Mean maximal increase in the PS was 140% and the potentiation lasted longer than 20 minutes in 37% of the animals tested. EPSP increases were observed in 13 of 20 animals with an average onset time of .8 minutes, post-injection. In those animals where the EPSP decreased, the change was earlier and shorter than the changes associated with EPSP increases. Changes in PS onset latency were variable and brief. Interestingly, multiple injections of GLU (i.e. up

to 3 in same animal) produced enhancements in PS amplitude that virtually mirrored that which was seen following the first injection. Significant decrements over multiple trials were not seen in contrast to what has been reported to occur with repeated electrical stimulation of the LC (Dahl and Winson, 1985). Most importantly, peripheral injections of PRO reliably reduced NE-mediated increases in the PS amplitude. In an elegant study, Harley et al. (1989) alternately stimulated the LC region with either electrical current or GLU applied at the same site and found that only the GLU-induced enhancement of the PS could be antagonized by PRO. Again, evidence has been presented that raises the possibility that potentiation of the PS in response to electrical or chemical stimulation in the LC region may reflect the activation of 2 separate neural systems.

The specificity of beta-receptor blockade in NELLP using glutaminergic activation of the LC GLU-mediated potentiation of the PS was evaluated by local intra-dentate pressure ejection of PRO and TIM (Harley and Evans, 1988). The beta blockers prevented the expression of NELLP but failed to attenuate NELLP once potentiated, implying again that induction and maintenance of long-lasting changes by NE, as with LTP, reflect the activity of distinctly different mechanisms.

Taken together, the evidence suggests that NE-mediated

alteration in granule cell responsivity represents a valid model of persistent heterosynaptic modulation of synaptic transmission.

1.7 Mechanisms Underlying NE-mediated Potentiation

In 1970, Kety hypothesized that forebrain NE was instrumental in the enhancement of cell firing in neurons that respond to affectively important environmental stimuli. He also proposed that NE could bring about a persistent facilitation in the transmission of those inputs that are affectively important. More recently, it has been suggested that NE-mediated facilitation of evoked activity represents a tuning mechanism through which NE improves the signal-to-noise ratio of neural transmission to areas that are innervated by NE terminals (Moises, Waterhouse and Woodward, 1981). This concept has received considerable support from the research literature (Olpe, Laszlo, Pozza, DeHerdt, Waldmeier and Jones, 1986; Moises, Burne and Woodward, 1990; Woodward, Moises, Waterhouse, Yeh and Cheun, 1991; Mouradian, Sessler and Waterhouse, 1991).

If NELLP indeed represents a mechanism by which important polysensory information coming across the PP is enhanced and possibly selectively processed into a more enduring form, how would this be accomplished? To date, there has been

considerable agreement that NE-induced potentiation of granule cell responsivity depends on beta-adrenergic receptor activation. How do these receptors influence granule cells? Gray and Johnston (1987) utilized voltage and patch clamping techniques to study NE effects on single granule cells in guinea-pig hippocampal slices. They found that 20 seconds after a 500 msec pulse of 10 μM NE was ejected directly onto exposed granule cells, an increase in the amplitude of the peak inward calcium current was seen. These effects were mimicked by the beta-adrenergic agonist, ISO, but not by the alpha2-adrenergic agonist, clonidine. Also, NE enhanced the amplitude and duration of calcium action potentials under conditions where potassium conductances were blocked. Certainly, augmenting calcium influx could lead to a number of post-synaptic events, one of which could be long-lasting synaptic change. In a complementary study, Haas and Rose (1987) observed that accommodation of cell firing in response to the injection of depolarizing currents and the amplitude and duration of the afterhyperpolarization (AHP) were reduced by both NE and ISO. The authors suggest that NE and ISO effectively blocked a calcium-activated potassium conductance that is believed to account for the AHP. The implications of this study are that NE can reduce the repolarization of granule cells thereby making depolarization by PP inputs more likely. However, the NE-mediated reduction of the AHP reflects

a short-term change and would not account for long-lasting changes in synaptic transmission.

Sarvey (1988) has speculated that NELLP may be mediated by a beta-receptor/adenylate cyclase coupling which leads to an increase in the synthesis of the intracellular second messenger, 3',5'-cyclic adenosine monophosphate (cAMP). Second, activation of cAMP may trigger a selective protein kinase, which through phosphorylation, would ultimately result in persistent changes in the cellular membrane. Stanton and Sarvey (1985a) investigated the hypothesis that NE can stimulate adenylate cyclase production of cAMP. Hippocampal slices were pre-incubated for 30 minutes with forskolin, an activator of adenylate cyclase, at a concentration that did not affect the PP-evoked potential. Subsequently, NE was added to the perfusate for 30 minutes and the result was an enhancement of the PS that was substantially greater than that observed in response to NE alone. This effect was blocked by both PROP and a selective antagonist of the beta 1-adrenergic receptor, metropolol. Interestingly, forskolin has also been found to increase calcium currents in the DG (Gray & Johnston, 1987) and reverse adenosine-induced inhibition of the PP-evoked PS in the dentate when added to PTA (Stratton et al., 1988). Hence, there is some evidence to support the contention that NE may act synergistically with PKC to modulate synaptic efficacy in the DG.

Recent efforts towards elucidating the mechanisms underlying NELLP have stressed the importance of NMDA receptors (Stanton, Mody and Heineman, 1989; Dahl and Sarvey, 1990). In the hippocampal slice, administration of 10 μM APV 15 minutes prior to, during and after perfusion of NE significantly attenuated the long-lasting potentiation of the PP-evoked EPSP and PS in the DG (Burgard, Decker and Sarvey, 1989). Interestingly, Lacaille and Schwartzkroin (1988), using intracellular recording, have shown that application of microdrops of NE near to the somata of granule cells results in a small depolarization accompanied by an increase in membrane resistance. Both effects were blocked by TIM. Such depolarization might make cells susceptible to NMDA-mediated change.

Finally, Lynch and Bliss (1986) observed that NE, like LTP, enhances the potassium-induced calcium-dependent release of radioactively-labelled GLU in slices of the DG by an average 35.2% and that ISO mimicked this effect. A partial block of the NE-induced increase in GLU release was seen following perfusion of PRO whereas both alpha 1 antagonist phentolamine and the alpha 2 antagonist yohimbine failed to block the NE effect. These studies lend support to the speculation that NE may 1) prime NMDA channels for the arrival of glutaminergic input from the PP and 2) facilitate the presynaptic release of GLU. The precise mechanism subserving

NE-induced potentiation remains to be determined.

1.8 NE and Behavioral Plasticity

There is considerable evidence demonstrating a noradrenergic role in plasticity in the immature cortex (Harley, 1987). In a series of elegant experiments, Kasamatsu (1983) reported that experientially-induced changes in ocular dominance in the visual cortex are dependent on the presence of NE during the critical experience. When kittens are exposed to visual experience in only one eye, the number of visual neurons responding to the experienced eye typically increases. This is accompanied by decreases in binocular neurons and in neurons that had initially been driven by the deprived eye. If NE levels in the visual cortex are reduced (i.e. by administering a neurotoxin) monocular deprivation does not produce functional changes in visuo-cortical neurons. Similarly, infusion of PRO directly into the kitten visual cortex suppresses the shift in ocular dominance accompanied by monocular deprivation (Shirokawa and Kasamatsu, 1986). Subsequently, it was shown that the resumption of spontaneous activity of endogenous NE a short time after cessation of PROP infusion was accompanied by a recovery of plasticity (Shirokawa and Kasamatsu, 1987).

NE has also been implicated in early olfactory learning

(Sullivan, Wilson and Leon, 1989). In a recent study, the hypothesis that NE may be involved in odour conditioning was investigated. Briefly, early learning about significant odours can be accomplished by the pairing of odours with reinforcing tactile stimulation. Apparently, the olfactory bulb is modified both functionally and anatomically in response to this kind of learning. In Sullivan's paradigm, 6 day old rat pups received a novel odour paired with stroking or odour paired with ISO or the beta-blocker PRO prior to odour and stroke or odour alone(Sullivan, McGaugh & Leon, 1991). The following day, the animals were given an odour preference test. Animals receiving either stroking or ISO exhibited an odour preference. Odour learning did not occur in those pups that received only saline prior to odour presentation. Olfactory learning did not occur in the group that received either PRO or TIM paired with stroking. They conclude that endogenous NE is necessary and sufficient for neonatal olfactory learning.

More recently, it has been shown that mitral-tufted cells in the olfactory bulb respond to rewarding brain stimulation delivered to the medial forebrain bundle/lateral hypothalamic area (Wilson & Sullivan, 1991). Further, odour learning can occur if odours are paired with such rewarding brain stimulation. Interestingly, systemic injection of PRO prior to stimulation/odour pairing effectively blocks olfactory

learning to that odour. Again, the acquisition of early olfactory learning appears to depend on the presence of NE and beta receptor activation.

The contribution of NE to learning in the immature and developing animal is substantial. In the adult, depletion studies have not suggested such a powerful role for NE in plasticity . However, some paradigms support the idea that NE continues to contribute to long-term modifications. Broyles and Cohen (1985) reported that during heart rate conditioning in the adult pigeon, lateral geniculate neurons alter their response characteristics upon the introduction of the CS (i.e. a light flash) which signals the onset of footshock (i.e. UCS). Lesions of the LC prevent the acquisition of the conditioned changes in lateral geniculate neurons. Further, they have also reported that LC activation can effectively be substituted for footshock in heart rate conditioning (Cohen, Gibbs, Siegelman, Gamlin & Broyles, 1982). Hence, NE appears to be instrumental for the conditioned changes in the lateral geniculate nucleus that accompany the classical conditioning of heart rate in the pigeon.

In adult cats, electrical stimulation of the LC for 2 hrs/day for 6 consecutive days during monocular experience alters the discharge characteristics of binocular neurons such that they respond primarily to monocular input (Kasamatsu, Watabe, Heggelund and Schoeller, 1985). The changes in

binocular function in adults persisted for up to 3 weeks.

Finally, Rosser and Keverne (1985) have demonstrated that depletion of NE from the medial olfactory striae or the accessory olfactory bulb six days prior to mating results in a 'pregnancy block' such that the pregnant female does not recognize the stud male's odour. As a result, the female mouse aborts the pregnancy. The authors explain that during mating, the female develops a memory or 'imprint' of the stud male's pheromones. Subsequent recognition of this imprint will not result in a pregnancy block. NE activity appears to be critical during the mating process when the memory for the male stud's odour is formed, since 6-OHDA-induced depletion of NE prevents this kind of learning.

1.9 NE Potentiation in the Awake Animal

NE has been reported to affect LTP in the awake and behaving animal. Robinson and Racine (1985) reported that systemic injections of reserpine (which depletes NE containing vesicles) three weeks prior to testing, resulted in decreases in EPSP and increases in PS amplitude both of which were significant in comparison to non-depleted animals. LTP of the EPSP was briefly enhanced in the NE-depleted group whereas LTP of the PS showed significantly less potentiation and shorter duration in comparison to non-depleted controls. The baseline

changes in PS particularly are the opposite of what would be produced if NE supported enhanced responsiveness, although the weak PS potentiation would be consistent with a role for NE enhancement of LTP. NE may alter PP-DG synaptic responses in the chronic animal but the researchers argue that the presence of NE is not necessary for the maintenance of LTP.

Dahl, Bailey and Winson (1983) reported that depletion of NE by 6-OHDA effectively eliminates the dependence of granule cell responsivity on behavioral state such that the magnitude of the EPSP and PS do not change as a function of whether the animal is still/alert or in slow wave sleep. The authors propose that when NE activity is high (i.e during a still/alert state), granule cell firing is suppressed. Conversely, during slow wave sleep (i.e. when NE activity is low), granule cell excitability is enhanced. It is suggested NE is part of a PP-DG gating mechanism which regulates information processing at the primary junction in the trisynaptic chain. But again, the predicted effects of NE are opposite to what has been seen both in the anaesthetized animal and the 'in vitro' experiments. These behavioral gating results from Winson's group appear to be at odds with studies of the circadian rhythm of synaptic excitability in the DG. Barnes, McNaughton, Goddard, Douglas and Adamec (1977) reported that the PP-evoked EPSP and PS of the nocturnal rat fluctuated with a circadian rhythm since both measures were

highest during the dark or active phase. However, behavioral state was not reported. In a subsequent study, Harris and Teyler (1983) also observed that LTP in the slice DG of the rat was more frequently observed in those animals that were taken from their housing colony and sacrificed during the dark period. Conversely, LTP of CA1 is more frequently observed and of greater magnitude in animals that are sacrificed during the light period. However, West and Deadwyler (1980) reported greater PS amplitude in the DG during the light period and no significant changes in the EPSP when recordings from light and dark periods were compared. Changes in PS amplitude were found to vary with circadian fluctuations and were independent of behavioral state.

These conflicting results suggest that more work needs to be directed at the natural "gating" effects on DG processing since it is not clear whether greater or less plasticity is possible during those periods when NE release is occurring.

Does selective activation of the LC produce an enhancement of the PP-evoked potential in the DG of the awake and behaving rat? Can the effect be long-lasting? The present study addresses these questions. NE-induced potentiation in the DG, both short and long-lasting, has been observed in the anaesthetized animal and in the hippocampal slice. In addition, the enhancing effects of NE on synaptic efficacy in other neural circuitry has been demonstrated (Moises, Burne

and Woodward, 1990; Dodt, Pawelzik and Zieglgansberger, 1991; Sessler, Mouradian, Cheng, Yeh, Liu and Waterhouse, 1991). Taken together, enhancement studies suggest that NE influences target neurons by promoting strong afferent input while reducing spontaneous, low level activity. The net result is described as an improvement in the signal-to-noise ratio and NE-mediated signal enhancement is hypothesized to be a physiological correlate for the modulation of attentional states.

Is such modulation demonstrable at the PP-DG synapse in the awake and behaving rat? To address this question, an attempt is made to use focal injections of the excitatory amino acid, GLU, into the LC of the awake and behaving rat while concomitantly monitoring the PP-evoked potential.

2. METHODS

Subjects

The subjects were 22 male Long Evans rats, obtained from Charles River Breeding Labs (Montreal, Quebec) and weighing between 450-675 g at the time of surgery. Animals were individually housed in Plexiglass cages and maintained on a 12:12 hr. light/dark cycle (8 a.m. on, 8 p.m. off). Food and

water were available ad libitum, except during testing periods. Testing was conducted between 12:00 p.m. to 12:00 a.m.

Surgical Procedures

Animals were anaesthetized with Avertin (1ml/100g body weight) and prepared for stereotaxic surgery. Each animal was fixed into a stereotaxic device with skull flat and fitted with a nose cup through which the animal received methoxyflurane to maintain the anaesthetic condition for the duration of the surgery. The inhalable anaesthetic was administered in a .5% concentration through a Pentec 2 dispenser (Cyprane Ltd.). The perforant path (PP) was stimulated with a bipolar twisted electrode (Plastic Products MS303/2) using a .2 ms square wave single pulse of 30 V every 10 sec delivered by an Ortec 4710 Dual Channel Stimulator. The PP electrode was cut diagonally so that tip separation in the dorsal/ventral plane was approximately 1 mm. The PP electrode was aimed at the following coordinates : 7.1 mm caudal to bregma; 4.2 mm lateral to the midline and 3.5 mm below dura. The recording electrode consisted of a single Teflon-coated stainless steel wire (diam., 127 μm) with a male Amphenol pin soldered onto the dorsal pole. Initial stereotaxic coordinates for the recording electrode were 3.5 mm posterior to bregma,

2 mm lateral to the midline and 1 mm below the dural surface. The recording electrode was then lowered until the evoked potential elicited by PP stimulation indicated that it was in the granule cell layer of the DG. Final depth was optimized by recording the PP-evoked potential and the recording and stimulating electrodes were cemented at their respective optimized depths. A metal wire with a male Amphenol connector was soldered to the head of one of four jeweller's screws which were fixed in the cranial bone.

A 22 gauge guide cannula (Plastic Products C313G) constructed of stainless-steel tubing of 0.7 mm in outer diameter and 15 mm in length, was chronically implanted above the locus coeruleus (LC) at an angle of 20 degrees from vertical in the rostral/caudal plane, approximately 4.8 mm caudal and 1.1 mm lateral to lambda. LC units were determined by identifying the location of the LC using the method of Cedarbaum & Aghajanian (1976). Briefly, a micropipette filled with 3 M NaCL was lowered into the anterior cerebellum to record unit activity. LC localization was obtained by monitoring characteristic patterns of unit activity that include 1) a relatively quiet zone corresponding to the fourth ventricle, 2) the presence of cells of the mesencephalic trigeminal nucleus just lateral to the LC that respond to passive jaw stretch and 3) putative LC neurons evidenced by tail pinch-responsive units. The identification of LC units

was followed by the withdrawal of the recording pipette and insertion of the outer guide cannula to a position approximately 1 mm above the LC. All implants and connectors were held in place by several layers of dental acrylic. A seven day recovery period followed during which time the animals were handled by the experimenter for 10 minutes/day. Testing commenced on the 8th day after surgery.

Testing Procedures

Habituation

The first day of testing involved a habituation session. Animals were placed on a raised platform covered with white plastic (138 cm x 77 cm x 5 cm) and were permitted to freely explore the environment for 30 minutes. During this time, PP electrodes were connected to shielded cable and coupled to the Ortec 4710 Dual Channel Stimulator via a Grass SIU5 Stimulus Isolation Unit. The recording and ground electrodes were fed into a World Precision Instruments DAM-70 differential amplifier by means of a ribbon connector. All connecting leads were channelled through a flexible suspension system in order to minimize torque on the acrylic cap. After 30 minutes, all leads were disconnected and the animal was returned to the home cage.

LC Activation

LC activation was carried out on the following day. Animals were coupled to the stimulation and recording equipment as previously described. Evoked potentials were amplified at a bandwidth of 1 Hz to 3 kHz and viewed on a Tektronix R5031 Dual-Beam Storage Oscilloscope. Voltage was adjusted until the population spike measure of the evoked potential was approximately half of its maximal amplitude. If changes were not seen in the evoked potential measures over the 15-30 minutes following the insertion of the inner cannula (28 gauge) to a depth of 1 mm below the guide cannula, this period was taken as the baseline. However, if changes in the evoked potential were seen, additional 15-30 minute samples were taken until a stable period was observed. Immediately after the baseline period, approximately 150-450 nl of .5 M glutamate (pH=7.4) was injected via the inner cannula. Initially, glutamate injections were delivered through a motor-driven drug infusion pump at a rate of 100 nl/sec. A subsequent loss of tissue integrity at the injection site was observed during histological analyses. Therefore, GLU concentration was reduced to .25 M and a foot pedal was coupled to the drug infusion pump in order to produce a slower delivery of GLU (i.e. approximately 2 nl/sec). Although tissue integrity improved, the electrical surging created by the

pulse-driven pump system often caused the computer to malfunction. As a result, this system was replaced by a hand-held 2 µl microsyringe (Dynatech) with the approximate rate of infusion at 50 nl/sec. Evoked potentials following GLU application were monitored until pre-drug baseline values recurred. If no change occurred the inner cannula was lowered in increments of .2 mm and GLU was again infused until either a significant change in the evoked potential was observed or a point 2 mm below the ventral point of the guide cannula had been reached.

In most of the testing sessions, behaviors and evoked potentials were recorded in a split-screen mode using Panasonic (WV-1500) and Hitachi (HV-16AU) cameras. However, it was found that fine distinctions in behavior were hard to discern due to poor picture resolution. Eventually, an RCA Camcorder/VHS recorder was utilized in place of the split-screen system.

Histology

At the end of the recording session, animals were deeply anaesthetized with pentobarbital after which the GLU injection site was marked by injecting a solution of red ink (Dynachrome II-DSR2) through the inner cannula. Brains were quickly removed and frozen at -20 C. Brain tissue was mounted in a

cryostat and sectioned at 35 μm through the LC. Alternate sections were either stained with cresyl violet or remained unstained and were subsequently used to identify the location of the red dye.

Statistical Analysis

The evoked potentials were digitized at 1 pt/75 μs with 12 bit resolution and stored on a microcomputer. Three parameters were measured on-line using a Forth-based software program. These measures consisted of the slope of the field excitatory post-synaptic potential (EPSP) slope, the amplitude of the population spike (PS) and the onset latency of the PS (See Figure 1). A mean for each of the parameters over consecutive 30 sec intervals (i.e. corresponding to 3 evoked potentials, one/10 sec.) was computed. In order to ascertain any differences between the period following GLU ejection and the control period, a two-tailed 95% confidence interval for the EPSP, PS and PS onset latency, was calculated. Initially, return to baseline for all parameters was assumed if 4 out of 5 consecutive averages did not fall outside the limits of the confidence interval for the preceding control period.

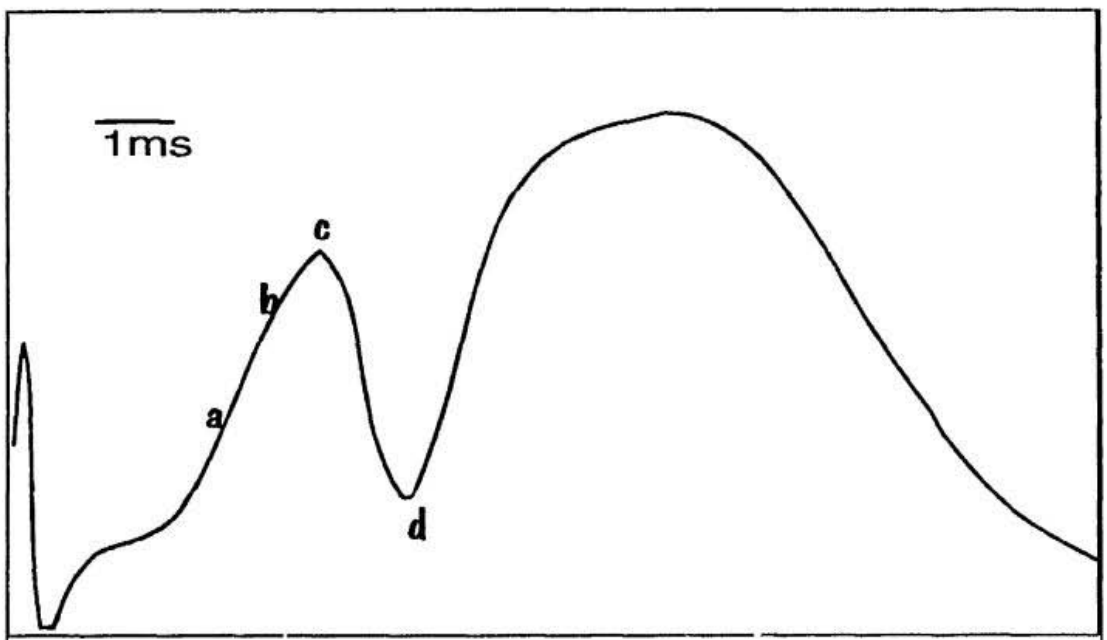

Fig. 1. An example of a dentate gyrus field potential evoked by stimulation of the perforant path. The following parameters can be measured.

1. EPSP Slope : The difference in mV between point b and point a divided by the difference in time.
2. Onset Of Population Spike : Point c.
3. Population Spike Height : Difference between points c and d.

3. RESULTS

3.1 Histology

Of the 22 animals tested, two were excluded from data analysis due to misplaced recording electrodes. The remaining animals comprised 3 groups. The first group had 12 animals with cannulae tips in or within a 300 μm boundary of the LC (LC group), Group 2 had 3 animals with injection sites in the rostral vermis (RV) of the cerebellum (RV group) and Group 3 consisted of 5 animals with cannulae localized to various sites along the dorsal/ventral axis of the cerebello-pontine region (See Figures 2 and 3). This latter group was used as a control (CTRL) group.

In 4 cases, no evidence of dye was found at the injection sites. Hence, precise placements were identified using serial tissue sections (35 μm) to recreate the entire injection cannula track.

3.2 Effect of LC Stimulation

All subjects from the LC group reliably showed a potentiation of the PS component of the PP evoked potential following injections of GLU . Effects on the field EPSP and onset latencies were inconsistent. Summary data are shown in

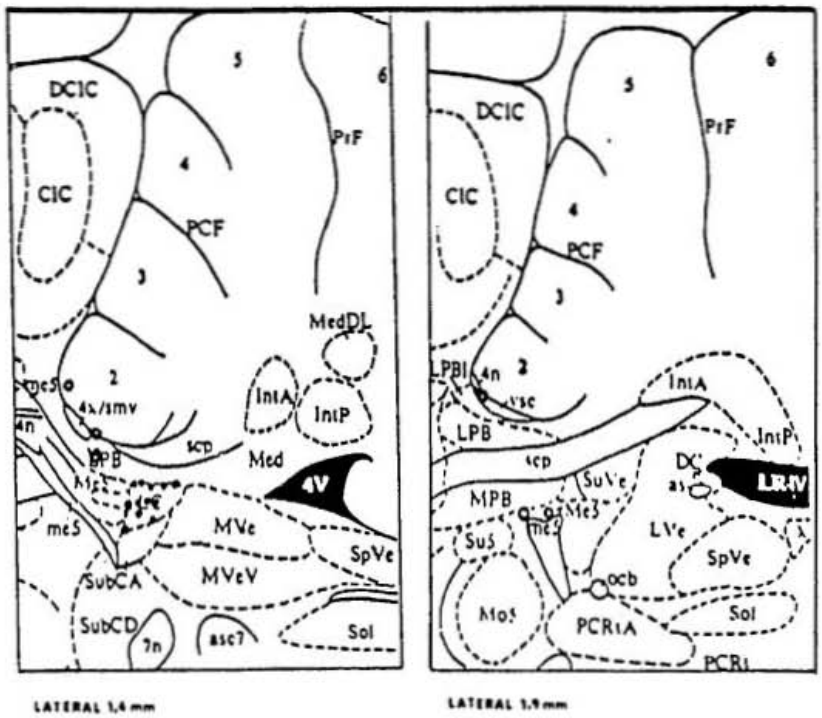

Fig. 2. Summary diagram of all cannulae placements (i.e. with the exception of the rostral vermis) in the cerebello-pontine region of the brain. Sites which produced a significant facilitation of the PS amplitude are indicated by the filled circles whereas sites negative for facilitation (i.e. beyond 300 um of the LC) are indicated by open circles. Sections presented have been adapted from the atlas of Paxinos and Watson (1986). Abbreviations: 2-6: cerebellar lobules, 4n: trochlear nerve or its root, 4V: 4th ventricle, 4x: trochlear decussation, 7: facial nu, as: acoustic stria, asc7: ascending fibers facial nerve, CIC: central nu inferior colliculus, DC: dorsal cochlear nu, IntA: interposed cerebellar nu, ant, LC: locus coeruleus, LPB: lateral parabrachial nu, LVe: lateral vestibular nu, Me5: mesencephalic trigeminal nu, me5: mesencephalic trigeminal tract, Med: medial cerebellar nu, Mo5: motor trigeminal nu, ocb: olivocochlear bundle, PCRt parvocellular ret nu, scp: superior cerebellar peduncle, Sol: nu solitary tract, Spve: spinal vestibular nucleus, Su5: supratrigeminal nu, SubCA: subcoeruleus nu, vsc: ventral spinocerebellar tract, x: nucleus x.

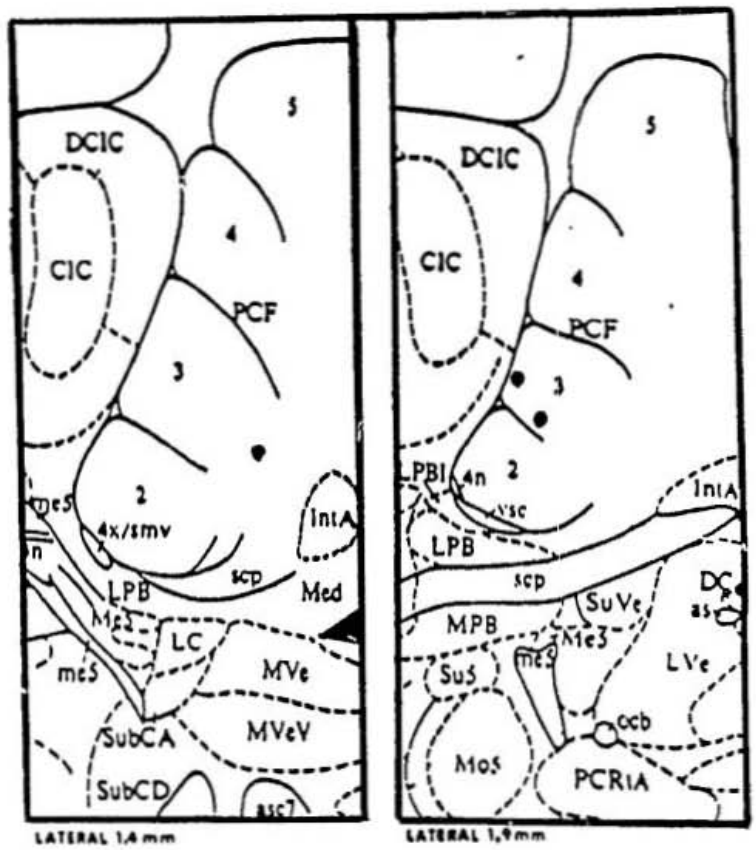

Fig. 3. Filled circles represent sites in the rostral vermis which produced a significant enhancement of population spike amplitude despite displacements of greater than 300 um from the LC. Abbreviations: 1-5: cerebellar lobules, 4n: trochlear nerve or its root, 4V: 4th ventricle, 4x: trochlear decussation, as: acoustic stria, asc7: ascending fibers facial nerve, CGPn: central grey pons, CIC: central nu inferior colliculus, cic: commissure inferior colliculus, DC: dorsal cochlear nu, DMTg: dorsomedial tegmental area, g7: genu facial nerve, IntA: interposed cerebellar nu, ant, LDTg: laterodorsal tegmental nu, LC: locus coeruleus, LPB: lateral parabrachial nu, Me5: mesencephalic trigeminal nu, me5: mesencephalic trigeminal tract, Med: medial cerebellar nu, mlf: medial longditudinal fasciculus, Mo5: motor trigeminal nu, MVe: medial vestibular nu, ocb: olivocochlear bundle, PCRtA parvocellular ret nu, alpha, scp: superior cerebellar peduncle, smv: superior medullary vellum, SPTg: subpeduncular tegmental nu, SubCA: subceruleus nu, alpha, SuVe: superior vestibular nu, vsc: ventral spinocerebellar tract.

Tables 1 and 2.

Considerable variability in all three measures was observed during baseline recording and particularly following injections of GLU. As a result, the termination of a significant effect was difficult to determine. The utilization of a discrete criterion to determine the return to control levels, such as 4 of 5 consecutive averages failing to exceed 95% confidence limits (Harley & Milway, 1986) seemed inappropriate based on the overall patterns of GLU-induced increase. Therefore, data will first be described as individual cases in order to 1) illustrate the complexity of the observations in the behaving animal and 2) utilize response patterns particular to each individual subject to determine duration of effect.

3.3 LC Group

Animals were subdivided based on the best estimate of duration. Subjects designated L1-L6 showed long-lasting potentiation of the PS (i.e. > 20 minutes) in response to injections of GLU. Animals L7-L12 were those that demonstrated a short-lasting potentiation of the PS (i.e. < 20 minutes).

TABLE 1. Baseline averages of evoked responses, maximum change, onset and duration of the effect for LC animals showing long-lasting potentiation of the PS following administration of GLU.

	SUB	MEAN	% CHANGE	ONSET	DURATION
EPSP	L1	9.09 mv/ms	-	N/A	N/A
ONSET		2.46 ms	-	N/A	N/A
PS		1.69 mv	353	3.5 min	26.0 min
EPSP	L2	3.03 mv/ms	125	3.5 min	36.5 min
ONSET		1.73 ms	-	N/A	N/A
PS		2.13 mv	154	1.5 min	26.0 min
EPSP	L3	7.07 mv/ms	-	N/A	N/A
ONSET		2.08 ms	-	N/A	N/A
PS		6.03 mv	156	3.5 min	42.0 min
EPSP	L4	N/A	N/A	N/A	N/A
ONSET		2.36 ms	-	N/A	N/A
PS		0.93 mv	141	2.0 min	79.0 min
EPSP	L5	4.71 mv/ms	-	N/A	N/A
ONSET		2.48 ms	-	N/A	N/A
PS		2.01 mv	236	3.0 min	39.0 min
EPSP	L6	0.57 mv/ms	-19.5	1.5 min	3.5 min
ONSET		1.95 ms	-	N/A	N/A
PS		1.74 mv	137	2.5 min	44.0 min

TABLE 2. Baseline averages of evoked responses, maximum change, onset and duration of the effect for LC animals showing short-lasting potentiation of the PS following administration of GLU.

	SUB	MEAN	% CHANGE	ONSET	DURATION
EPSP	L7	2.23 mv/ms	468	1.5 min	18.5 min
ONSET		1.73 ms	-	N/A	N/A
PS		1.52 mv	139	2.0 min	7.5 min
EPSP	L8	3.34 mv/ms	-	N/A	N/A
ONSET		1.94 ms	-	N/A	N/A
PS		7.78 mv	110	3.0 min	7.0 min
EPSP	L9	0.86 mv/ms	-	N/A	N/A
ONSET		1.83 ms	-	N/A	N/A
PS		2.07 mv	128	3.5 min	9.0 min
EPSP	L10	0.32 mv/ms	-	N/A	N/A
ONSET		1.91 ms	-	N/A	N/A
PS		0.14 mv	179	1.0 min	15.5 min
EPSP	L11	4.66 mv/ms	-	N/A	N/A
ONSET		1.94 ms	-	N/A	N/A
PS		3.06 mv	136	1.0 min	7.0 min
EPSP	L12	0.26 mv/ms	-	N/A	N/A
ONSET		1.83 ms	112	2.0 min	18.0 min
PS		0.43 mv	136	3.0 min	2.5 min

L1

Figure 4 illustrates the effect of .5 M GLU administered by drug infusion pump (DIP) on EPSP, onset and PS measures for a period of 30 minutes following a 10 minute control period. Maximal increase of PS reached 353% of the mean baseline amplitude of 1.7 mv and occurred 27 minutes post-injection (PI). The onset of a significant change was noted at 3.5 minutes PI and persisted for an additional 26 minutes. No significant changes were observed in either the EPSP or onset measures. This experiment was characterized by variability, especially in the PS amplitude following the GLU injection. However, since significant enhancements continued to be observed intermixed with baseline values, it is argued that potentiation lasted throughout. In addition, the pattern of pre-injection PS values (i.e. predominantly below 100% of control) was not observed PI. Yet, the duration of the enhancement was obscured by variability to the extent that the inner cannulae was reinserted to a more ventral site at minute 29.5 PI. PS enhancement continued for an additional 26.5 minutes although the animal did receive a 300 nl injection of GLU at 14 minutes following the insertion. No further change was noted subsequent to the last GLU ejection and recording was terminated at that point.

Notable behaviors accompanying the PS change included a

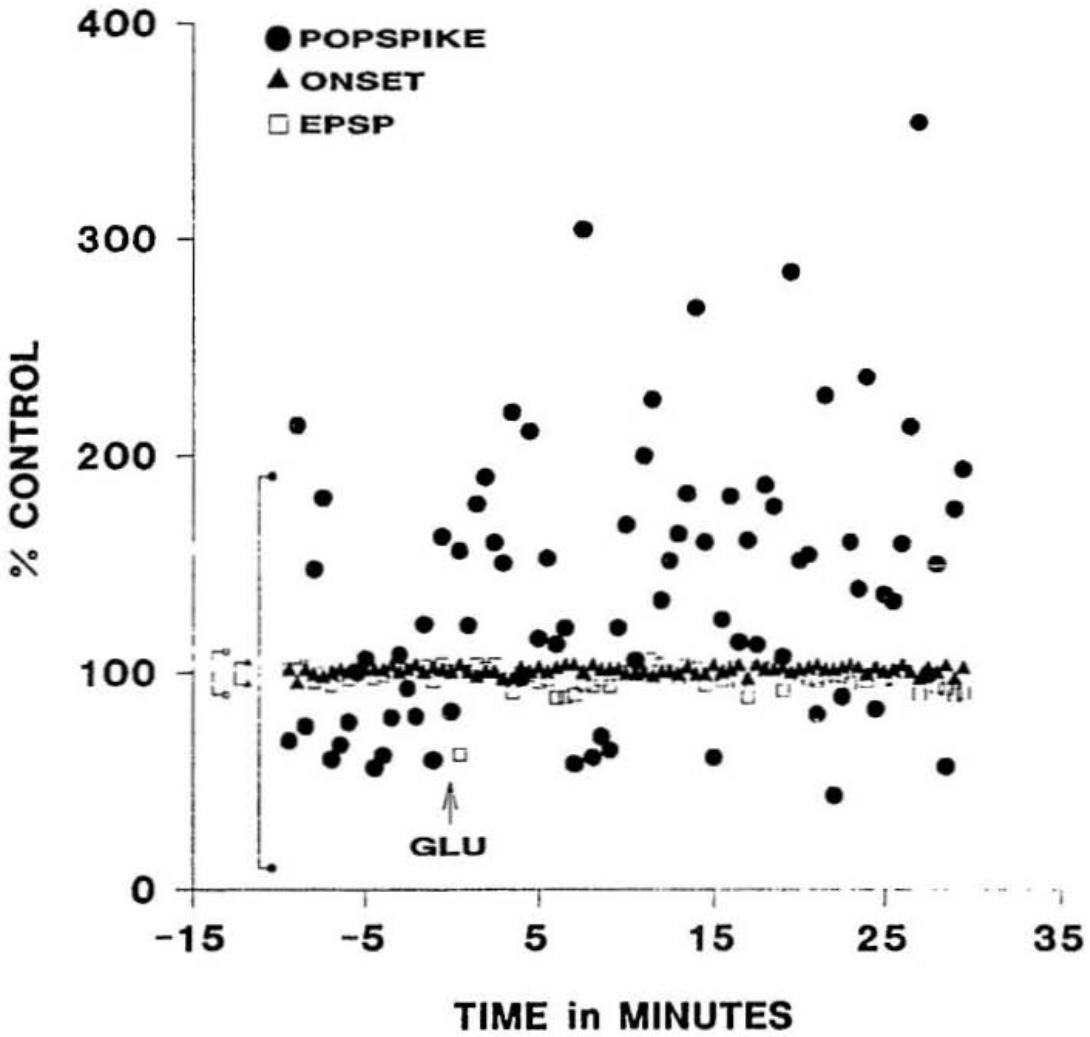

Fig. 4. Percentage change in EPSP slope, PS onset latency and PS amplitude for L1 following an injection of .5 M GLU within 300 um of the LC. Confidence interval (95%) of the control period are presented to the right of the Y axis.

flattening of previously erect posture and an ipsilateral (left) inclination from a horizontal position within 12 seconds of the injection. Facial fasciculation was noted within 5 seconds and persisted with intermittent bouts for the next 60 seconds. For the most part the animal remained in a still-alert state (SAS). At 104 seconds, the animal righted itself and began to sniff/orient. Posture remained low to the platform. At 136 seconds the animal resumed its left inclination but tried to right itself 4 seconds later. During this attempt, the animal lost its footing (i.e. most likely due to its own urine) and almost fell off the platform. The experimenter intervened to place the animal in a more central location. After this point, the animal resumed marginal mobility interspersed with longer periods of still alertness until the reinsertion of the cannula.

Histological analysis of L1 revealed considerable gliosis and tissue damage in and around the LC. As a result, L2-L6 received .25 M GLU which was administered more slowly using either a pulse-driven infusion pump (PIP) or a hand-held microsyringe (HHM).

L2

This experiment was also characterized by variability in both the EPSP and PS measures (See Fig. 5). In contrast to the

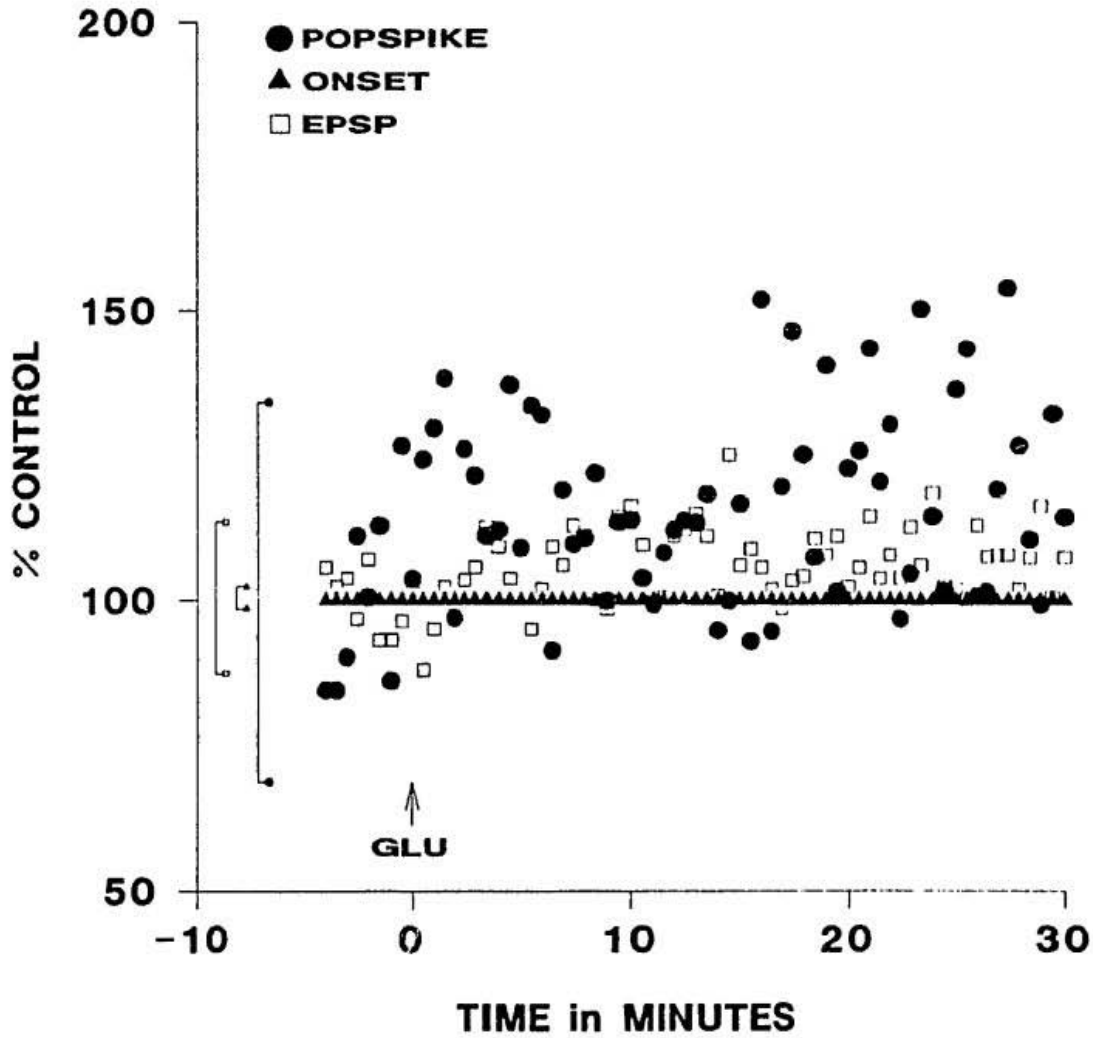

Fig. 5. Percentage change in EPSP slope, PS onset latency and PS amplitude for L2 following an injection of .25 M GLU within 300 um of the LC. Confidence interval (95%) of the control period are presented to the right of the Y axis.

previous case, the EPSP was also potentiated following injection of GLU (HHM). Recording instability during the first 30.5 minutes of the experiment determined the selection of a shorter control period (i.e. 5 minutes). Peak amplitude of the enhanced PS was 154% of control value of 2.13 mv at 27.5 minutes PI. Long-lasting duration was at least 26 minutes with onset of effect observed at 1.5 minutes. The PS was monitored for an additional 18 minutes prior to a more ventral displacement of the cannula site and found to be within control values. Potentiation of the PS appears biphasic with lower values occurring between minute 10 and 17 followed by an even larger increase in PS amplitude than that observed early in the recording.

The EPSP was enhanced to a maximal 125 % of the average value of 3.03 mv/ms. Onset of this effect occurred 2 minutes later than that observed in the PS and lasted approximately 44 minutes. Although significant enhancements of the EPSP were observed following cannula displacement, EPSP values were decreasing in general. Onset latencies remained unchanged.

From a behavioral perspective, the animal appeared anxious throughout the testing as evidenced by head shrugging and audible vocalizations in response to insertions of the inner cannula or connections of the recording and stimulating leads. GLU injection produced robust mastication (onset=6 seconds) followed by a SAS. The animal defecated within 10 seconds PI

and displayed an intermittent but pronounced fasciculation in the facial region that persisted for about 100 seconds. By 7.5 minutes PI, the animal was predominantly in SAS with some bouts of mastication. Reported times are approximate as video coverage of this experiment was not available.

L3

In the case of L3, only the PS was significantly augmented PI with an onset of effect at 3.5 minutes after the 10 minute control period and a peak effect of 156% of the control value of 6.03 mv (See Fig.6). The increase in PS amplitude lasted for the duration of the recording session (i.e. 42 minutes), although only a 30 minute period is shown. The maximal increase occurred at 13 minutes PI.

EPSP values appear to have decreased following GLU injection (HHM) but the apparent change was not found to be significant. Onset latencies were variable and not significantly changed. Testing was terminated as the animal appeared distracted by an irritation in the area of the acrylic cap.

Behavior elicited following GLU injection included one full ipsilateral rotation within 5 seconds of the injection. At the same time, the animal flattened its posture and respiration appeared more rapid and labored than during the

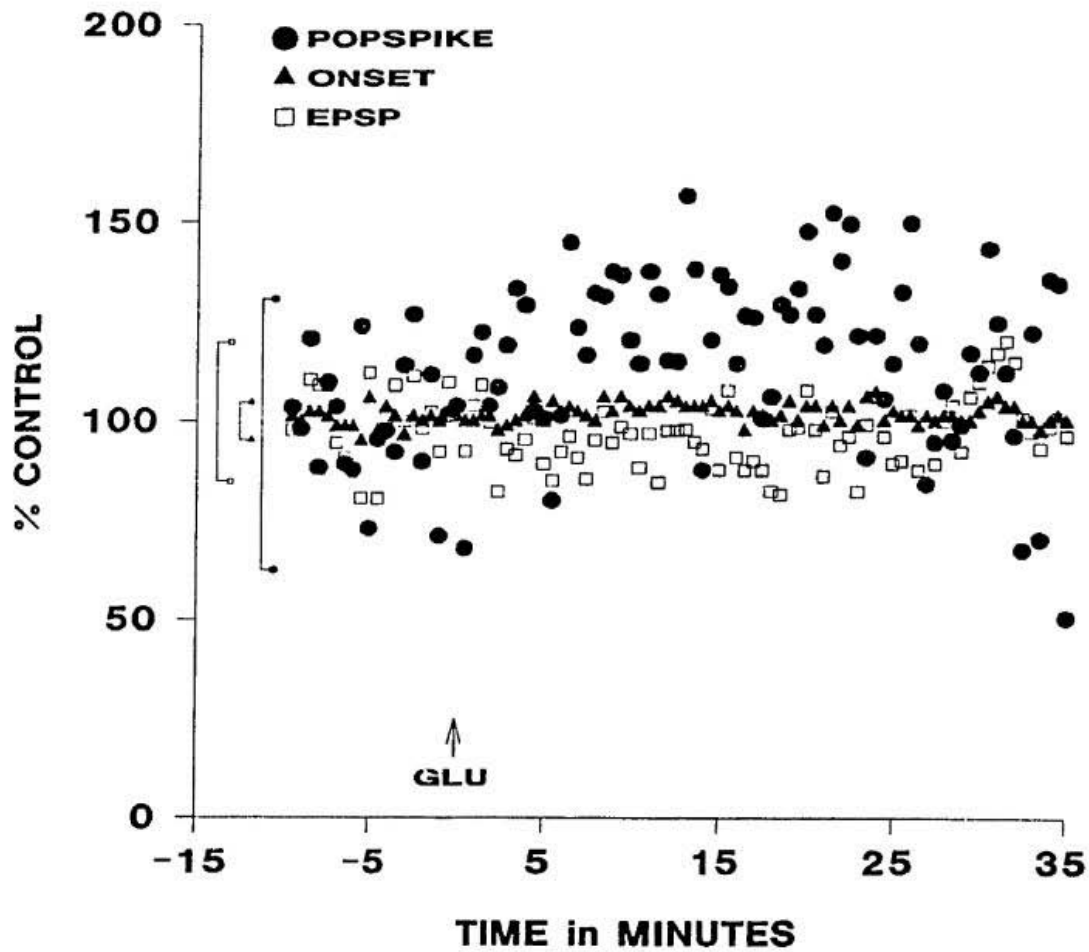

Fig. 6. Percentage change in EPSP slope, PS onset latency and PS amplitude for L3 following an injection of .25 M GLU within 300 um of the LC. Confidence interval (95%) of the control period are presented to the right of the Y axis.

baseline period. The animal defecated 10 seconds later in the midst of a partial ipsilateral rotation. This turn found the animal in a hunched position engaged in robust mastication which was elicited almost immediately following the injection. At 60 seconds, the animal appeared to be flexing its rectal muscles. For the first two minutes the predominant behaviors were a SAS and mastication. Mastication was pronounced in that it was both visible and audible. Facial fasciculation was noted at 160 seconds PI but was short-lived. Within 4 minutes of the injection the animal resumed a 'normal' posture and limited exploration which was similar to that observed during the control period.

L4

This experiment produced a long-lasting enhancement of the PS that was monitored for 79 minutes. Peak effect was 141 % of an average 2 mv PS and was observed at 80 minutes PI. Figure 7 shows the onset of significant change at 2 minutes. Despite the fact that the enhancement was not as dramatic (i.e. in terms of amplitude) as some of those previously reported, the increase was consistent over time and less variable.

Onset latencies were variable during both control and experimental periods and were not significantly different. Analysis of EPSP values was not possible due to difficulties

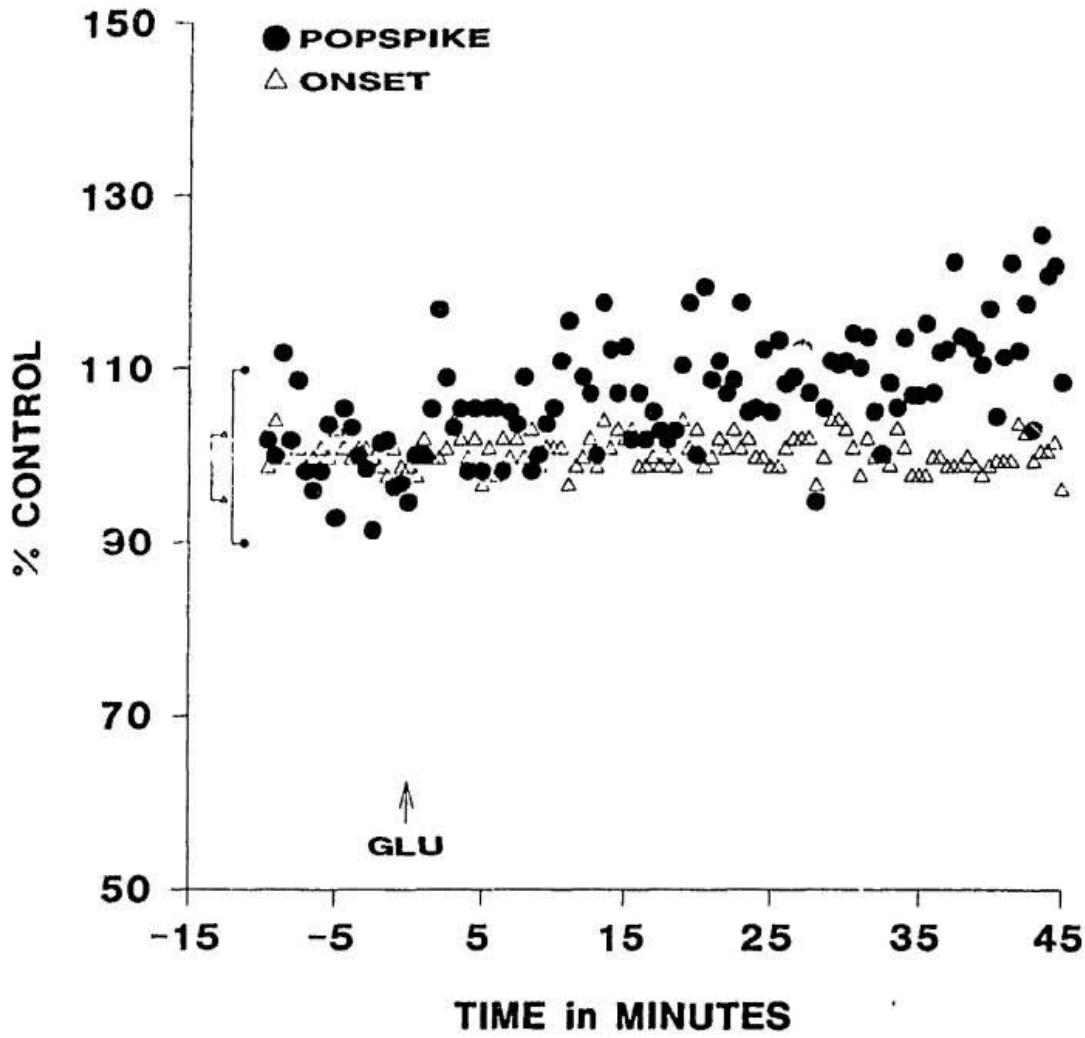

Fig. 7. Percentage change in PS onset latency and PS amplitude for L4 following an injection of .25 M GLU within 300 um of the LC. Confidence interval (95%) of the control period are presented to the right of the Y axis.

in the measurement of its extremely small amplitude.

Early onset behaviors (5-10 seconds) observed include urination and the more frequently observed episodes of mastication. In contrast to previous examples of LC activation, this animal maintained its normal posture and did not seem to incline towards any particular direction. Instead, within the first minute of the injection, the animal inched backwards to a corner position on the platform where it remained still and alert. At 60 seconds, a bout of fasciculation (facial) was noted. While in a still position, the animal was startled by a sound originating from outside of the testing room. The sound did not appear appreciably louder than the printer which triggers every 10 seconds and is located in the testing room. Finally, at 100 seconds, the animal began to actively explore the testing area. Subsequent behavioral data from this animal was not available.

L5

Figure 8 represents another illustration of long-lasting potentiation (i.e. 39 minutes) of the control PS amplitude of 2.01 mv that reached its peak effect, 236% of control, within 29 minutes of the GLU ejection (HHM). The PS was typified by variability during and following the 10 minute control period. In this instance, the onset of the increase was 3 minutes PI.

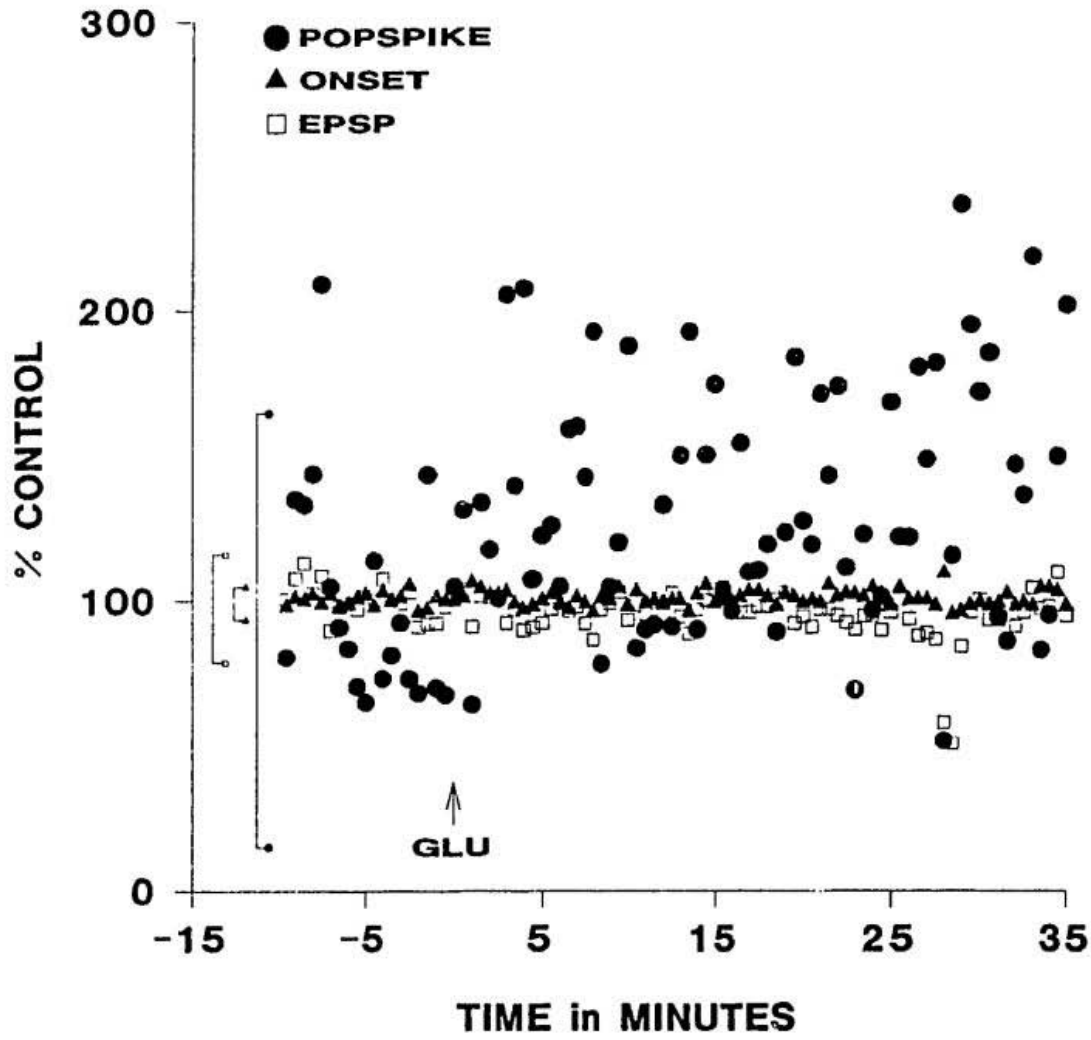

Fig. 8. Percentage change in EPSP, PS onset latency and PS amplitude for L5 following an injection of .25 M GLU within 300 um of the LC. Confidence interval (95%) of the control period are presented to the right of the Y axis.

This effect was defined as long-lasting due to the overall pattern of elevated spikes. The cluster of baseline PS values between 50-100% of control was not replicated at any time during the post-injection period. While a group of values in the control range is evident between minute 10-15 PI, it is followed by a clear increase in the PS which culminated in the maximal effect 14 minutes later.

EPSP and onset measure were not very stable but did not change significantly following the administration of GLU. While the EPSP was significantly depressed 28 minutes following the injection, it is difficult to extrapolate a relationship between this event and stimulation of the LC.

This animal's behavioral profile consisted of frequent mastication within the first 30 seconds, a flattening posture and a short-lived ipsilateral lean. Within 90 seconds of the injection of GLU, posture was corrected to an upright position and the animal began to groom. At 2.5 minutes PI, a one-half ipsilateral rotation was observed. This was followed by urination and a brief period (90 seconds) of exploration. Animal resumed SAS by 4.5 minutes PI.

L6

The final example of an enduring enhancement of the PS from the LC group is illustrated in Figure 9. Initially, this

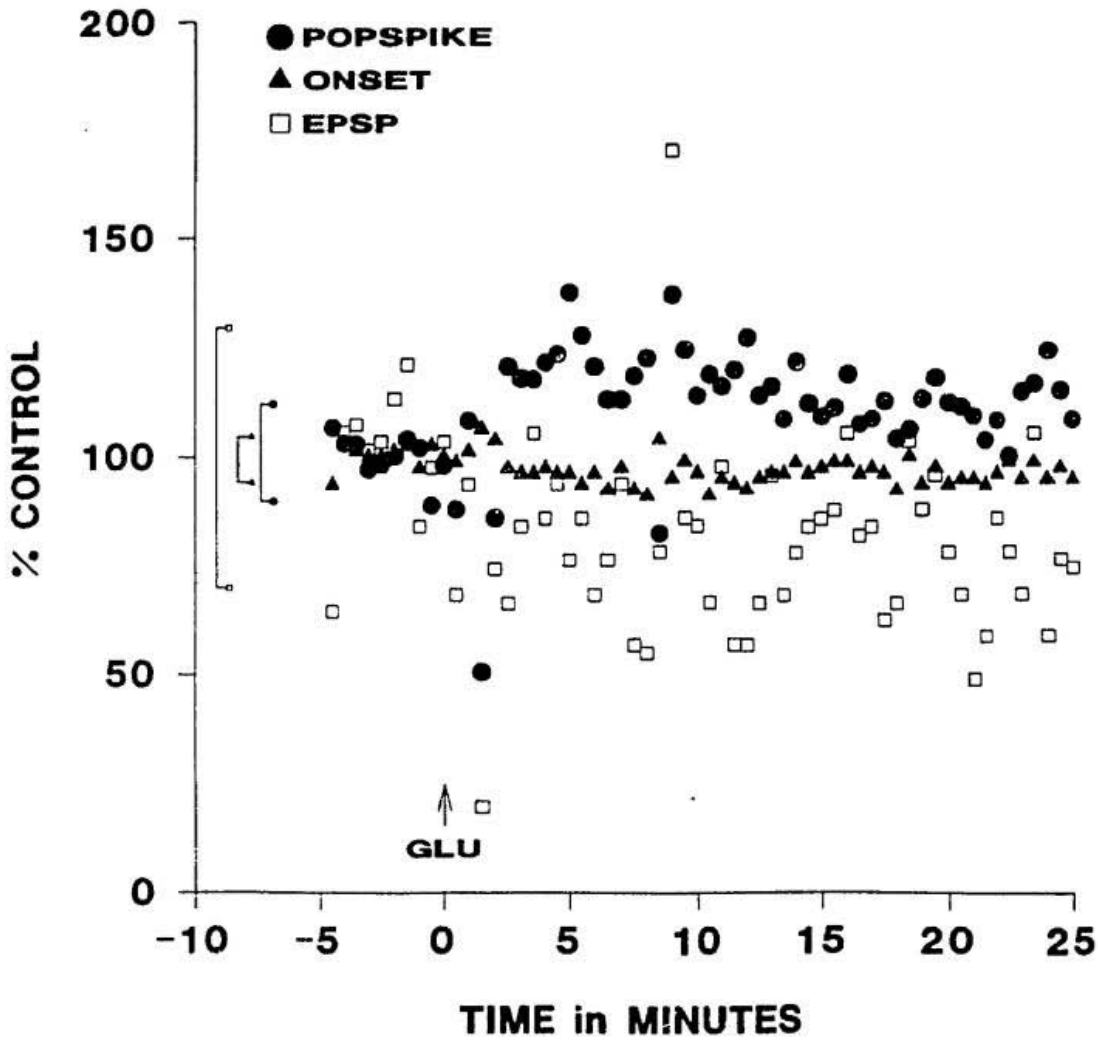

Fig. 9. Percentage change in EPSP slope, PS onset latency and PS amplitude for L6 following .25 M GLU application. Confidence intervals (95%) of the control period are presented to the right of the Y axis.

animal was tested with a GLU concentration of .125 M and demonstrated a marginal (e.g. 117% peak effect lasting 16.5 minutes) enhancement. Therefore, the animal was retested the next day using .25 M GLU. Similar to the case of L4, the potentiation that followed the 5 minute control period appears stable. A shorter control period was used due to the relative stability of the PS and the recording difficulties that preceded the sampling. A clear distinction can be drawn between control and post-injection PS values. A brief but significant depression in PS amplitude occurred prior to the onset (i.e. 2.5 minutes) of the significant and persistent enhancement. Behavioral anomalies were not observed during this decrease. Potentiation reached a maximal level of 137% above the average PS of 1.7 mv within 5 minutes of the injection (HHM) of GLU. The potentiation was monitored for a total of 42.5 minutes before a failure in the recording device led to termination of the testing session.

EPSP amplitude decreased significantly to a minimum 19.5% of the control mean of .569 mV/ms. Onset of the effect preceded that of the PS by one minute and lasted for the duration of the experiment (i.e. 43.5 minutes). Changes in EPSP appeared multi-phasic and were clearly distinguishable from the control period. A brief but significant increase in EPSP was observed at 9 minutes PI. The onset measure was variable but did not change significantly.

Behaviors included mastication and facial fasciculation within a SAS. Short periods (i.e. 5 seconds) of exploratory behavior were interspersed with periods of immobility. At 5 minutes PI, the animal began to lick the platform for approximately 4 seconds.

PS amplitudes were averaged and combined for those animals in the LC group (L1-L6) that showed long-lasting enhancements following GLU injection and are presented in Figure 10.

L7

The outcome of this experiment was that enhancement of PS amplitude (i.e. using 5 minute baseline) was followed a short time later by depression of the same measure (see Figure 11). A 10 minute control period was sampled, but two recording potentials during the first half were attributed to artifact. Hence, only the latter 5 minutes was utilized. A 150 nl .5 M injection of GLU (DIP) produced a maximal potentiation of 139% of the control average (1.52 mv) at 6 minutes. Onset of the significant effect occurred within 2 minutes and persisted for an additional 7.5 minutes. PS amplitude appeared to be returning to baseline at 10 minutes PI but instead continued to decrease until a significant depression was observed 3.5 minutes later. The reduction in amplitude was brief as a return to baseline occurred by minute 20. The relative

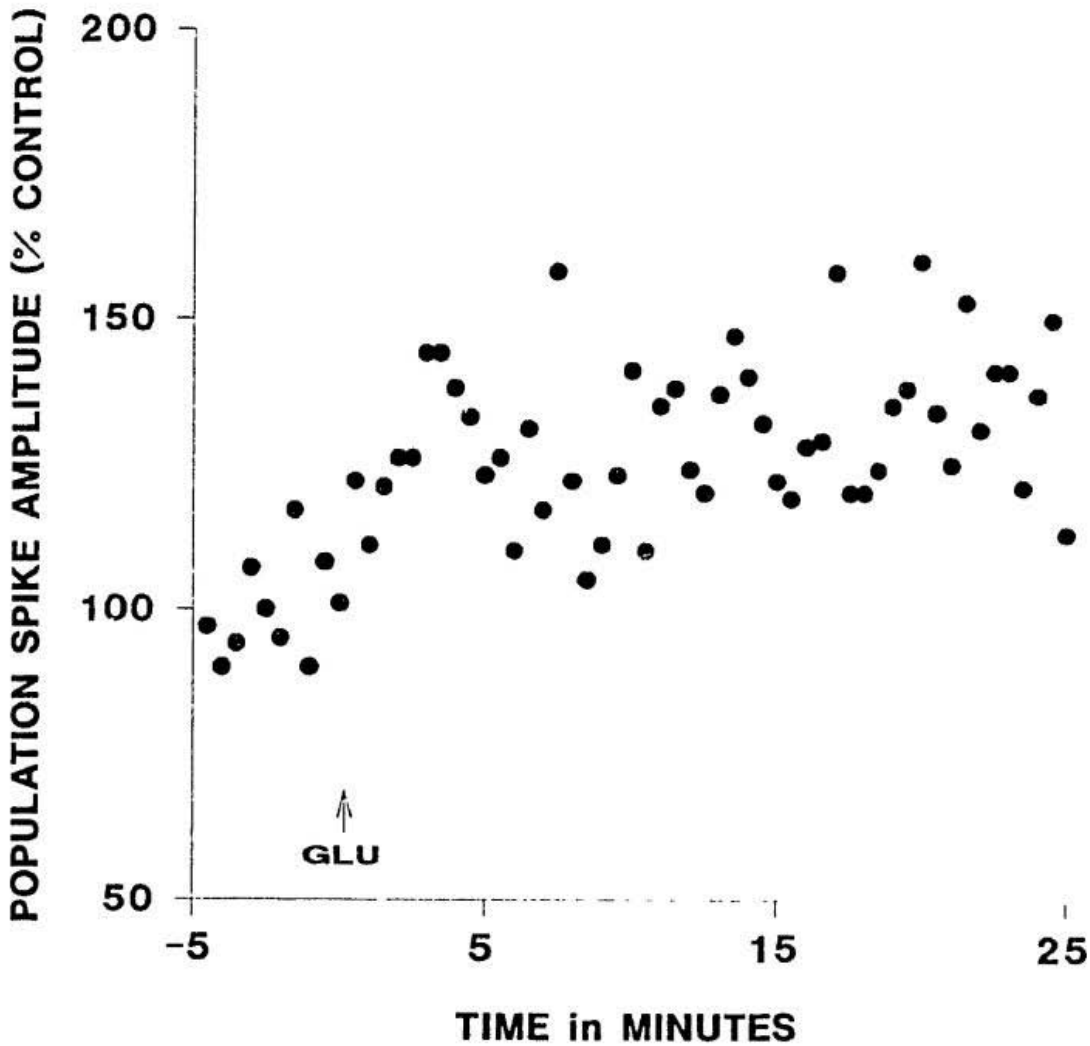

Fig. 10. Averages for 6 animals (L1-L6) showing long-lasting potentiation (i.e. >20 minutes) following .25-.5 M GLU administered within 300 um of the LC. Percent change in PS amplitude is presented as 30 second averages based on a 5 minute control period.

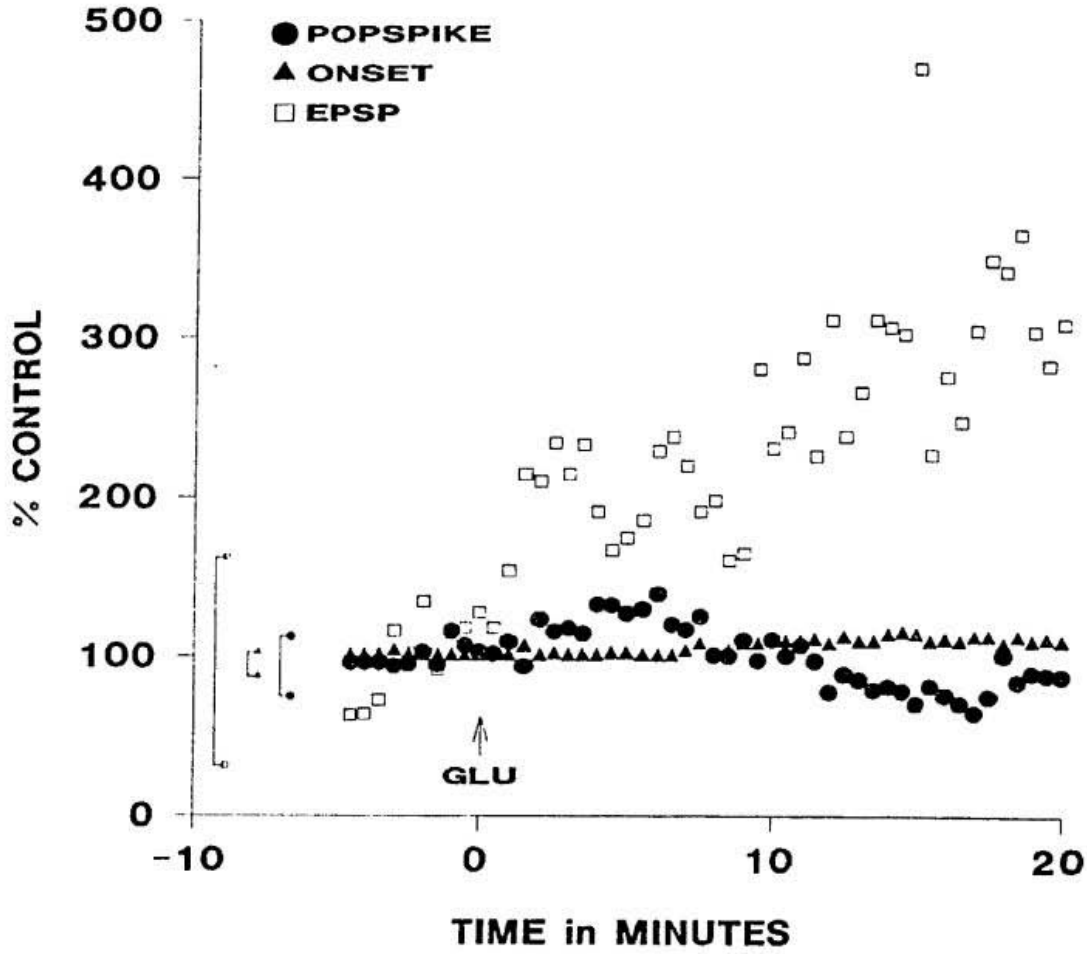

Fig. 11. Percentage change in EPSP, PS onset latency and PS amplitude for L7 following an injection of .5 M GLU within 300 um of the LC. Confidence interval (95%) of the control period are presented to the right of the Y axis.

stability of the PS measure, in general, supported the use of discrete criteria (i.e. 4 of 5 successive averages) to determine the cessation of the effect. A robust increase in EPSP was observed as well. Unlike the previous example of EPSP enhancement, the effect was relatively stable and achieved a maximal increase of 468% of 2.2 mv/ms. The increase was noted at 1.5 minutes PI and endured for approximately 60 minutes whereas the PS remained near control values (not shown in Fig. 8). Onset latencies did not change appreciably over the course of the experiment.

As in the case of L1, tissue damage was evident during the histological examination. This was the second of two animals in the LC group that received injections of a .5 M concentration of GLU.

Salient behavior that was recorded following GLU injection consisted of mastication and fasciculation in a SAS. In addition, the animal lowered its posture until it was flat to the ground. Then the animal made a partial ipsilateral rotation. By 4-5 minutes PI, the animal resumed its exploration (i.e. sniffing, rearing, locomoting) of the test platform. Approximate times for onset/offset of behaviors were not available for this animal.

L8

Figure 12 illustrates a short-lived increase in PS amplitude during a testing session with variability in all three measures. The peak effect reached 110% of control value (7.78 mv). Onset occurred within 3 minutes PI and the effect continued for the next 4 minutes. PS enhancement in response to the 450 nl injection of .25 M GLU was biphasic with a second increase observed at 12.5 minutes. Between increases, a return to control values between 7.5-11.5 minutes occurs. EPSP's were not significantly altered with the exception of a set of averages recorded at 2 minutes PI. Some decreases in EPSP amplitude were observed between 5 and 15 minutes after GLU (HHM) but did not reach significance. However, a decrease in PS values towards the end of the 20 minute sampling period was significant. Finally, onset latencies were not significantly different from those recorded during the five minute control period.

Behavioral data for this animal were not available.

L9

The enhancement of PS values following a 10 minute control period (depicted in Figure 13) was difficult to define in the context of duration of effect. The increase began at one

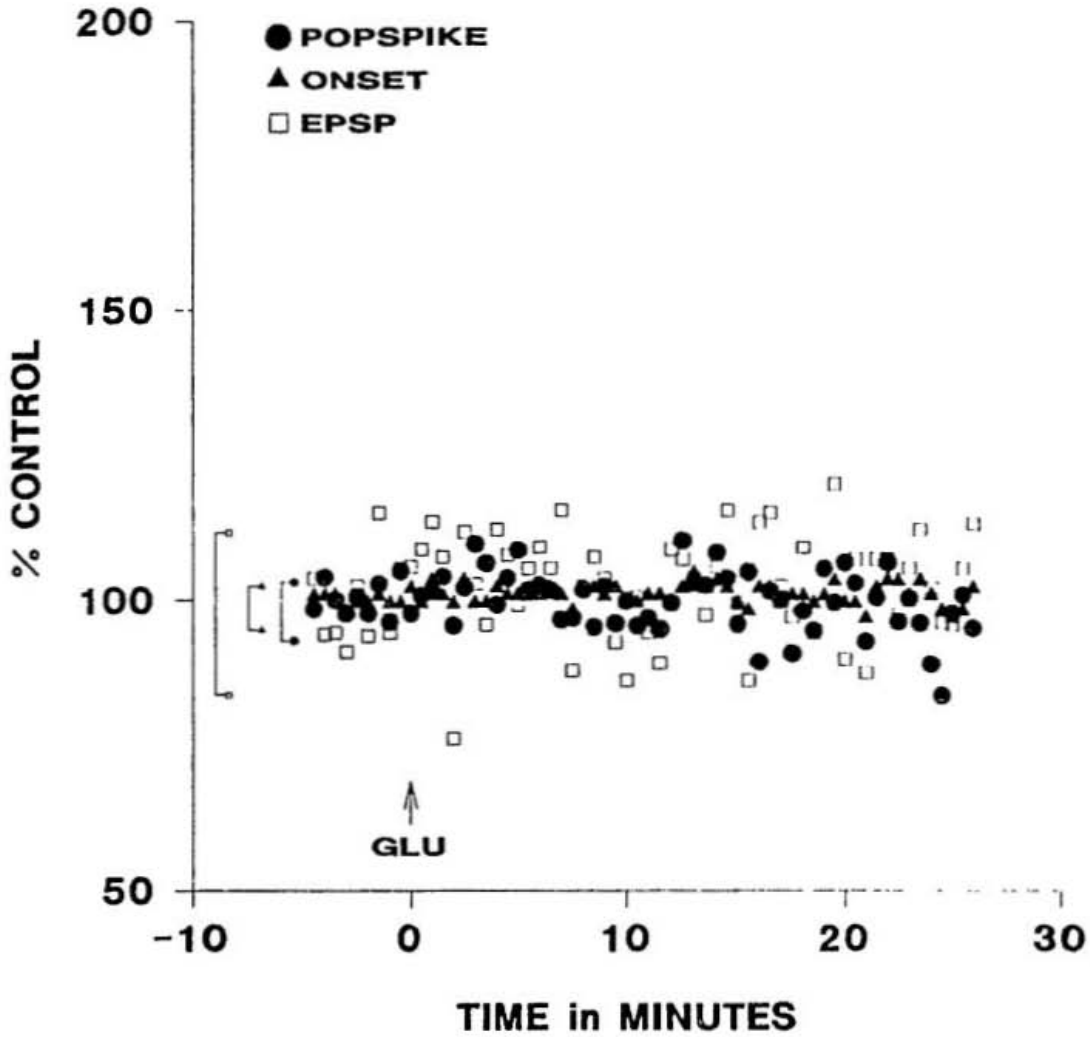

Fig. 12. Percentage change in EPSP, PS onset latency and PS amplitude for L8 following an injection of .25 M GLU within 300 um of the LC. Confidence interval (95%) of the control period are presented to the right of the Y axis.

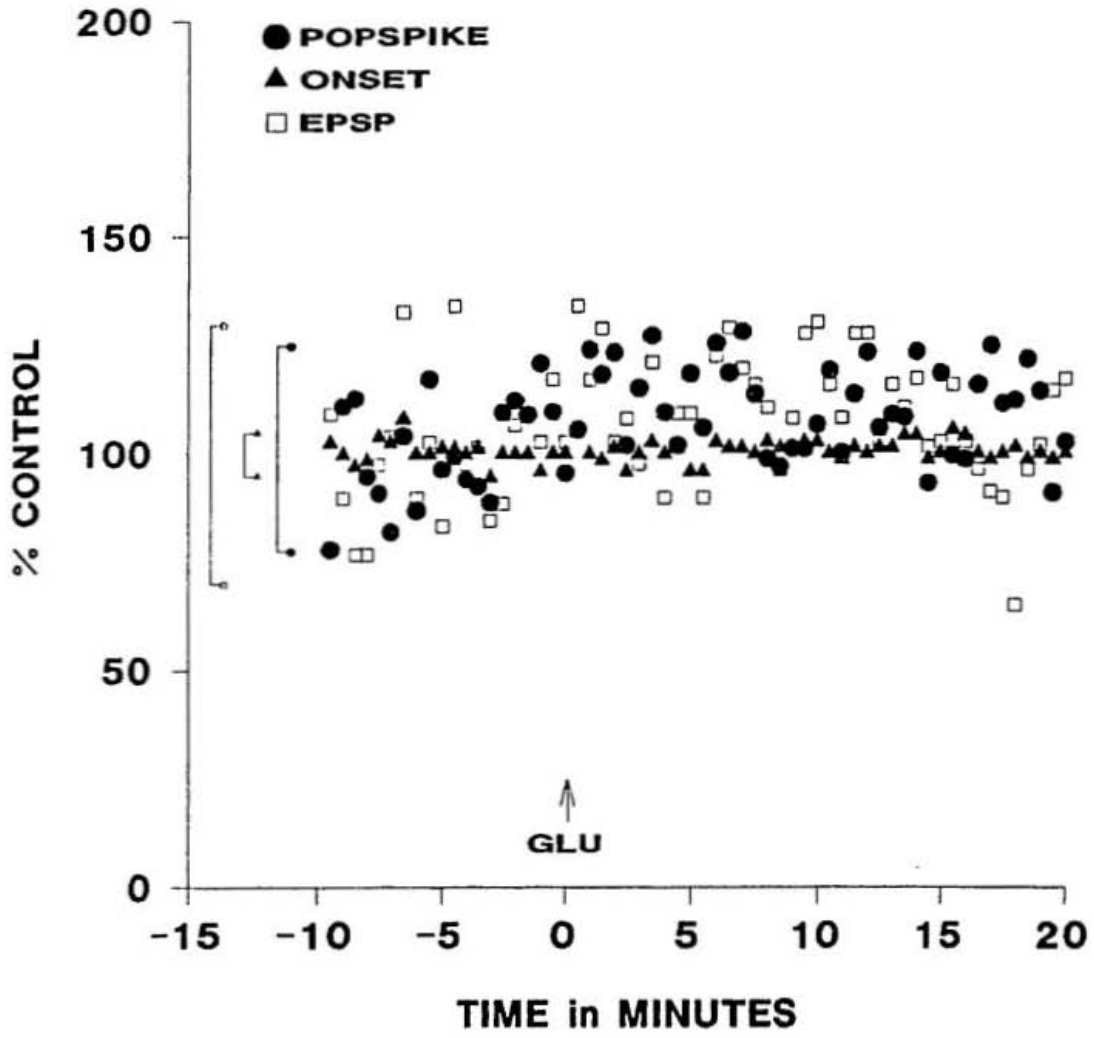

Fig. 13. Percentage change in EPSP, PS onset latency and PS amplitude for L9 following an injection of .25 M GLU within 300 um of the LC. Confidence interval (95%) of the control period are presented to the right of the Y axis.

minute following the injection of 150 nl of .25 M GLU (HHM) and reached its peak levels within 24.5 minutes. This would typically constitute a long-lasting potentiation. However, the increase was interspersed with a large number of values in the control range. It was unclear whether the PS continued to be potentiated or whether the baseline had shifted. When the 4/5 averages criterion was used to define termination, the duration was 9 minutes PI. If the higher baseline was taken as a real change, this would be another example of long-term potentiation. Significant changes in EPSP or onset latencies were not observed.

Injection of GLU was followed immediately by vigorous mastication, some orienting from a central position and erect posture. At 75 seconds, the animal constricted its forepaws until 122 seconds PI at which point forepaws were laid flush with the platform surface. Sixteen seconds later, the animal crossed its forepaws. A SAS persisted as did bouts of vigorous mastication. At approximately 5 minutes PI, mastication began to appear with protrusions of the tongue (4 seconds). Sniffing, orienting, grooming and limited motility resumed at minute 5.5.

L10

PS amplitude was significantly elevated over the mean

amplitude of .14 mv, one minute following a 350 nl injection of .25 M GLU (HHM) and remained elevated for an additional 15.5 minutes (See Figure 14). Peak effect was 179% of control and developed 13 minutes after the end of the 5 minute baseline period. Return to pre-injection values was both abrupt and clearly indicated. EPSP's and onset latencies did not differ from control values.

Behavior arising from LC stimulation included several ipsilateral rotations and typical facial activation (i.e. mastication and fasciculation). The circling behavior ended within 20 seconds of the ejection and the animal assumed a SAS. Grooming interrupted by mastication was observed at 120 seconds. At 4 minutes, the animal closed its eyes briefly then began to circle to its left. Active exploration resumed approximately 100 minutes later.

L11

L11 exhibited a brief potentiation of the PS, following the 5 minute control period. Onset of the significant effect began one minute after the 450 nl ejection of .25 M GLU (HHM) and attained a maximal increase of 136% of the average PS (3.1 mv) (see Figure 15). The increase was determined to be 4 minutes in duration despite observations of another cluster of significant values at 7 minutes PI which is similar to the

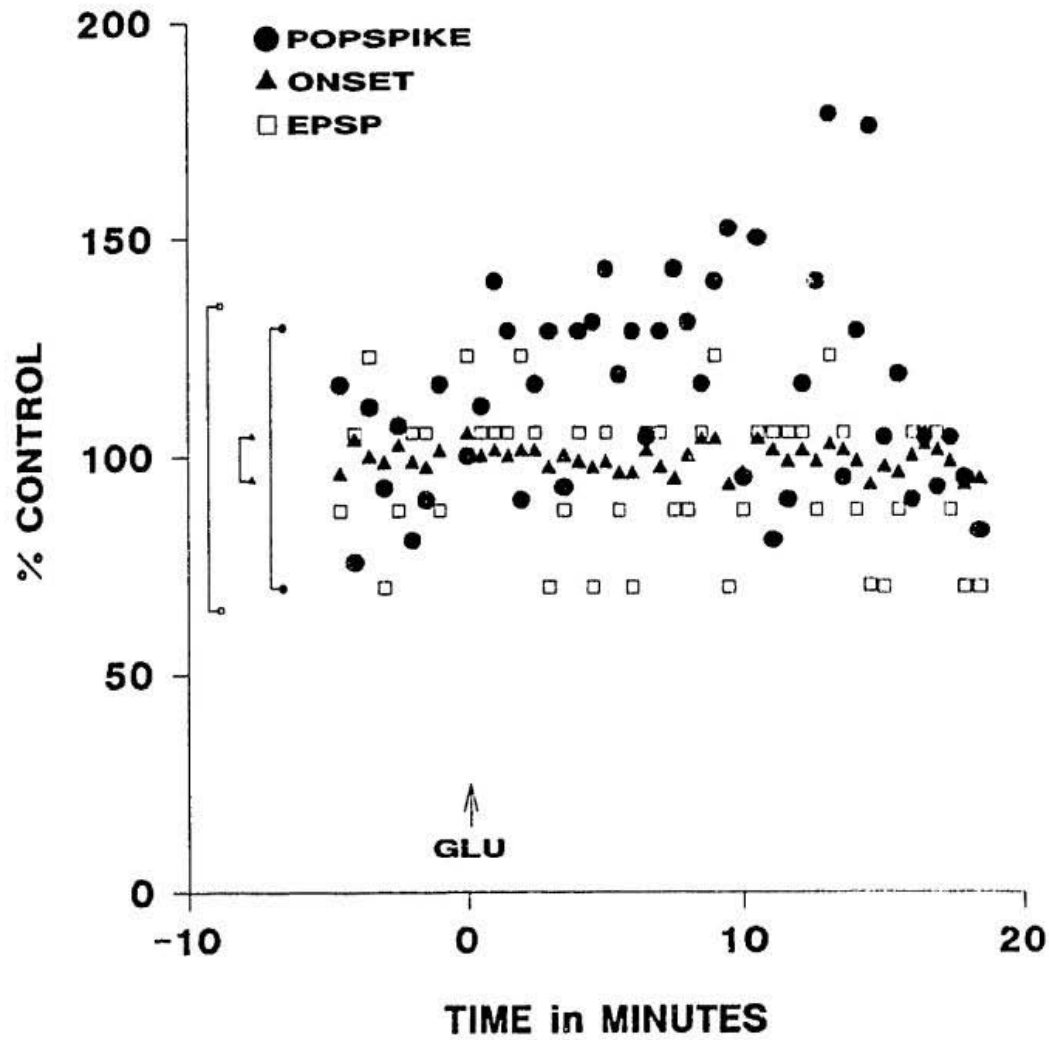

Fig. 14. Percentage change in EPSP, PS onset latency and PS amplitude for L10 following an injection of .25 M GLU within 300 um of the LC. Confidence interval (95%) of the control period are presented to the right of the Y axis.

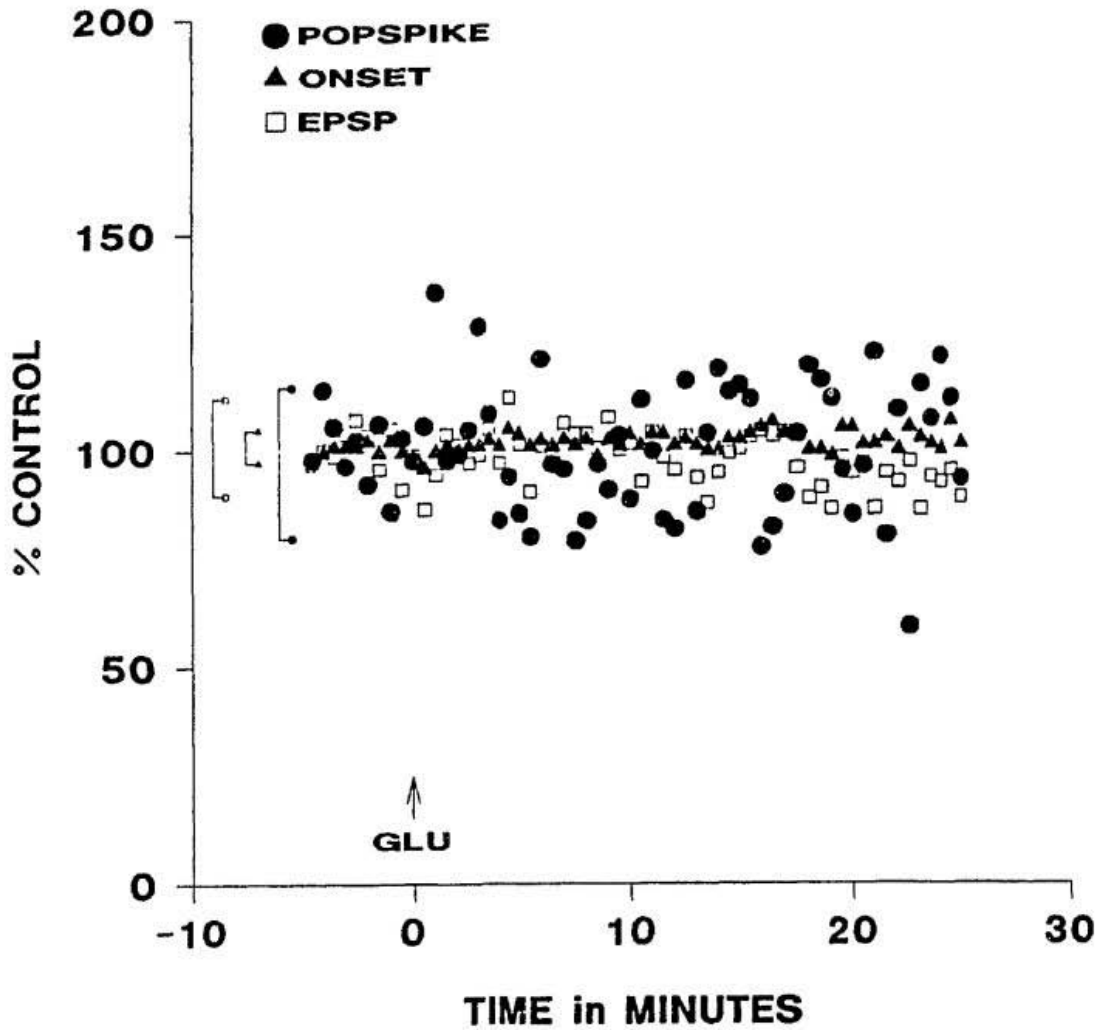

Fig. 15. Percentage change in EPSP, PS onset latency and PS amplitude for L11 following an injection of .25 M GLU within 300 um of the LC. Confidence interval (95%) of the control period are presented to the right of the Y axis.

biphasic patterns of L7 and L8. In general, PS amplitude became more variable after the injection as evidenced by both significant increases and decreases within a relatively short sample (e.g. 10-15 minutes PI). Significant changes in EPSP and onset measures were not observed.

The GLU injection was followed by early onset mastication (i.e. within 10 seconds PI) a hunched posture and still-alert behavior. Observations regarding the posture are different from those previously discussed as animal merely brought its hind legs closer to its forepaws as opposed to assuming a flat position. Moreover, normal posture was observed 70 seconds later. The animal remained in a SAS until minute 5 when it began to move around again.

L12

The final animal of the LC group presented a profile previously not seen as both the PS and the onset latencies were enhanced (see Figure 16) following the 5 minute baseline period. Maximal increase in PS amplitude was 136% of the control PS (.43 mv) at 3.5 minutes following the administration of 150 nl of .125 M GLU (HHM). The increase began at 3 minutes PI and concluded at 5.5 minutes.

Significant changes in onset latencies were observed at 2 minutes PI and outlasted the PS effect by 15.5 minutes. EPSP's

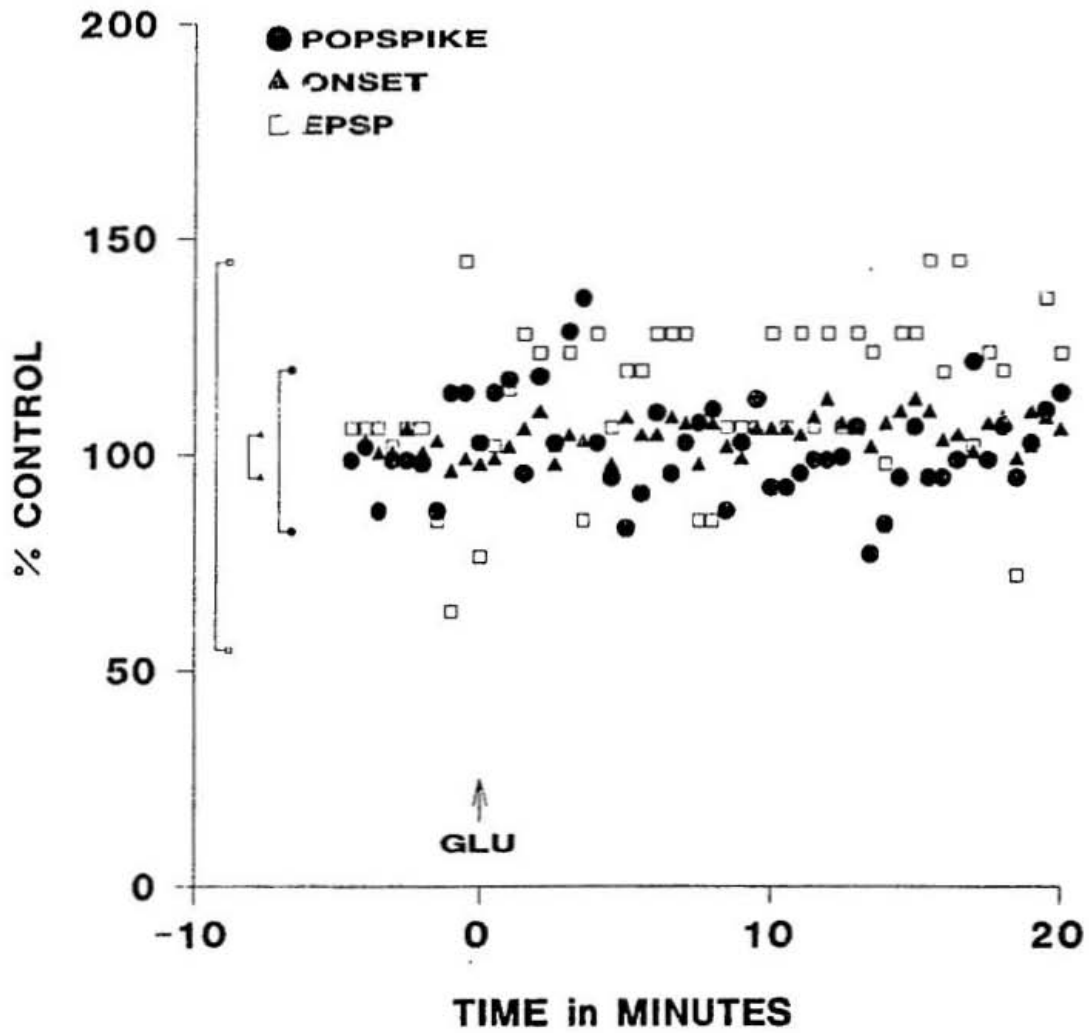

Fig. 16. Percentage change in EPSP, PS onset latency and PS amplitude for L12 following an injection of .25 M GLU within 300 um of the LC. Confidence interval (95%) of the control period are presented to the right of the Y axis.

were variable but not consistently altered.

Behavior elicited by GLU within the first 10 seconds included defecation and staggered movements. Animal assumed a horizontal posture by 60 seconds PI and became still and alert. During this time, the animal's vibrissae were fluttering in the absence of lateral snout movement. Active exploration resumed at 10 minutes following the injection which is considerably later than previously observed. However, partial attempts at motility (e.g. lateral snout movement and hind leg shifts) were seen during the SAS.

A summary graph of PS amplitudes averaged and combined over the 6 animals (L7-L12) that demonstrated short-lasting potentiation following GLU application are presented in Figure 17.

3.4 RV Group

Prior to the histological examination of animals R1-3, it was assumed that their cannulae would be found in the vicinity of the LC based on the potentiation observed during testing. Instead, all three were identified in lobulus centralis of the anterior lobe of the cerebellum.

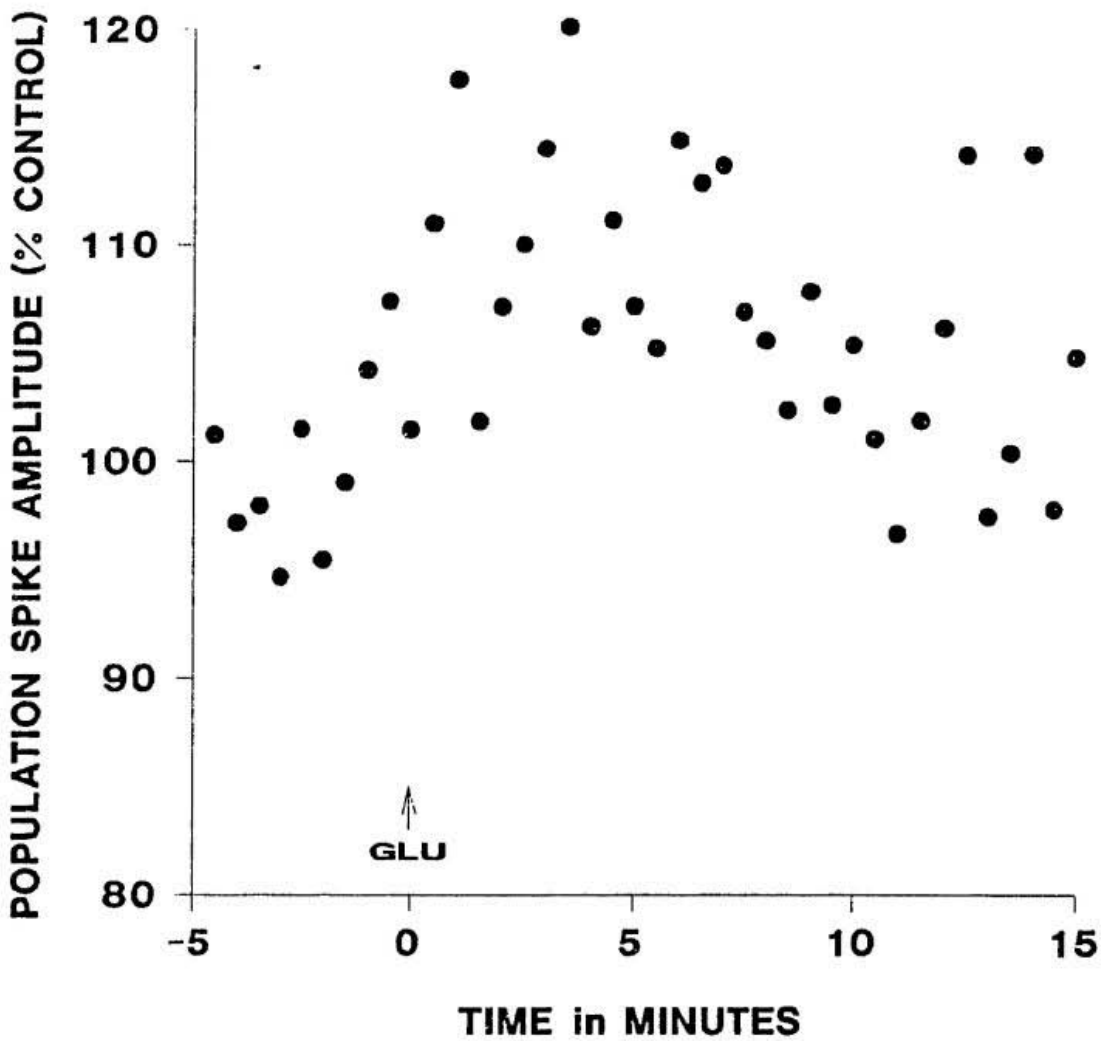

Fig. 17. Averages for 6 animals (L7-L12) showing short-lasting potentiation (i.e. <20 minutes) following .25-.5 M GLU administered within 300 um of the LC. Percent change in PS amplitude is presented as 30 second averages based on a 5 minute control period.

R1

In the case of R1, both the PS and EPSP were enhanced (see Figure 18) following the 10 minute control period. The peak effect for the PS was 123% of the mean amplitude (5.3 mv) and was attained at 26.5 min following the 10 minute control period. The peak effect for the PS was 123% of the mean amplitude (5.3 mv) and was attained at 26.5 minutes following the 150 nl injection of .25 M GLU (PIP). The effect began at 2.5 minutes PI and persisted for 26 minutes.

Onset of change and duration of the EPSP enhancement parallelled that observed with the PS amplitude. Maximal increase occurred at 26.5 minutes PI at a magnitude of 141% of the control value of 3.78 mv/ms. Significant changes in onset latencies were not evident. Essentially, these effects could not be differentiated from those reported in the LC group. Subsequent recording difficulties prevented the monitoring of PS and EPSP values beyond 28.5 minutes.

Unfortunately, a behavioral profile was not available for this testing session. However, the effects were replicated in a subsequent testing session later that evening, where the new control PS of 5.46 mv was further potentiated (onset=2 minutes) to a maximal 121% and lasted at least 155 minutes. Recording was terminated at that point. The behaviors elicited during the application of 150 nl GLU to the same site earlier

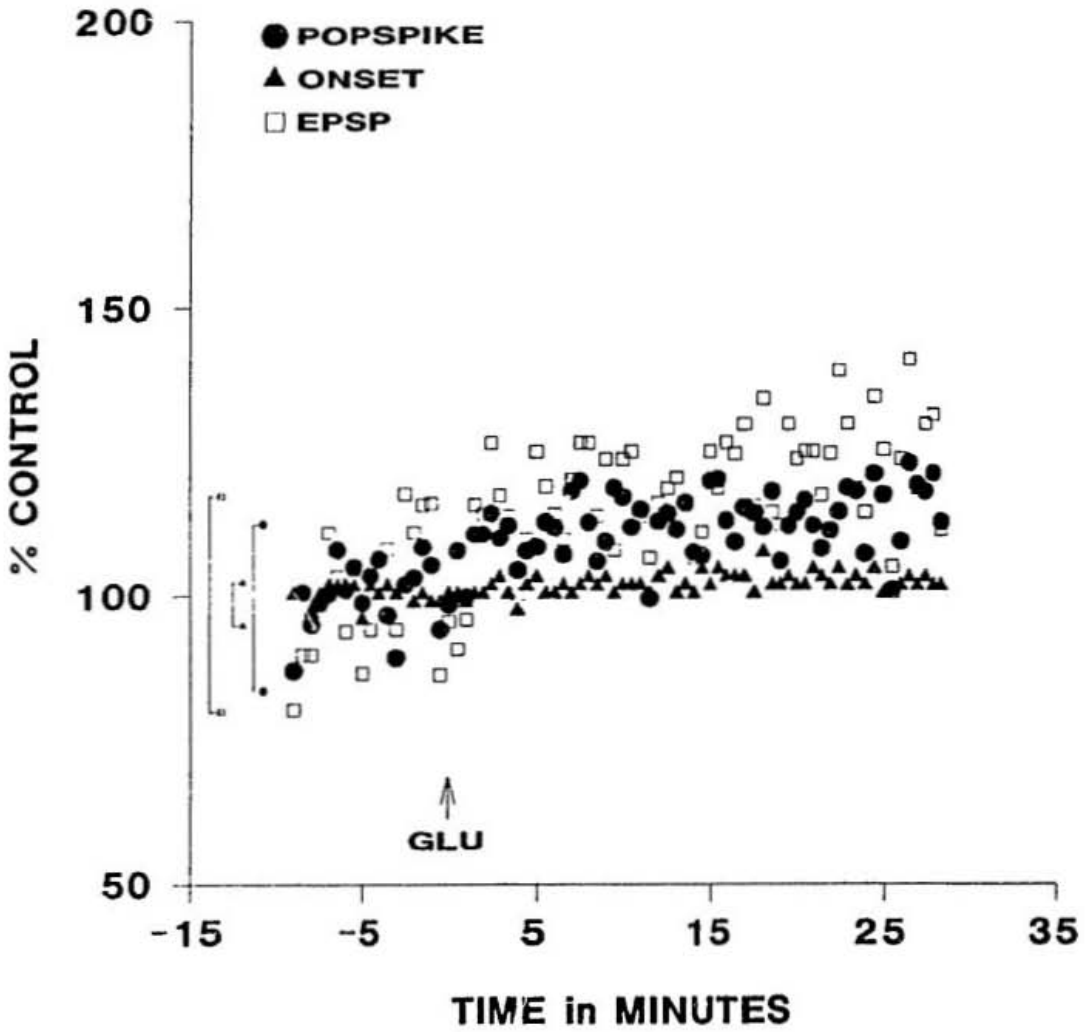

Fig. 18. Percentage change in EPSP slope, PS onset latency and PS amplitude for R1 following an injection of .25 M GLU into lobule centralis of the rostral vermis of the cerebellum. Confidence intervals (95%) of the control period are presented to the right of the Y axis.

the same day. Early onset behaviors included sniffing and mastication episodes. An increase in eye blinking was noted at this time. Animal assumed a SAS with a declined posture and forepaws crossed. Interestingly, the animal periodically lurched forward from a stationary position. While the animal did not resume active exploration for some time, the degree of sniffing behavior was pronounced during SAS. Unfortunately, precise onset times for these behaviors were not available but all were observed before 8.5 minutes PI.

R2

This testing session was characterized by variability, particularly in the PS measure (see Figure 19). However, the PS amplitude was significantly augmented following a 300 nl injection of a .25 M solution of GLU (HHM). Onset of the effect was observed at 5 minutes PI and was long-lasting (i.e. 42 minutes). Initially, low and variable baseline values could not be reliably sampled by the computer. Voltage was increased slightly at 10 minutes prior to the ejection of GLU but the first 3 minutes of the control period were not included in the analysis to account for PS stabilization in response to the change in voltage. Maximal increase was 241% of mean amplitude of .38 mv, at 23 minutes PI. Return to baseline was assessed as between minutes 8-20 due to small PS values.

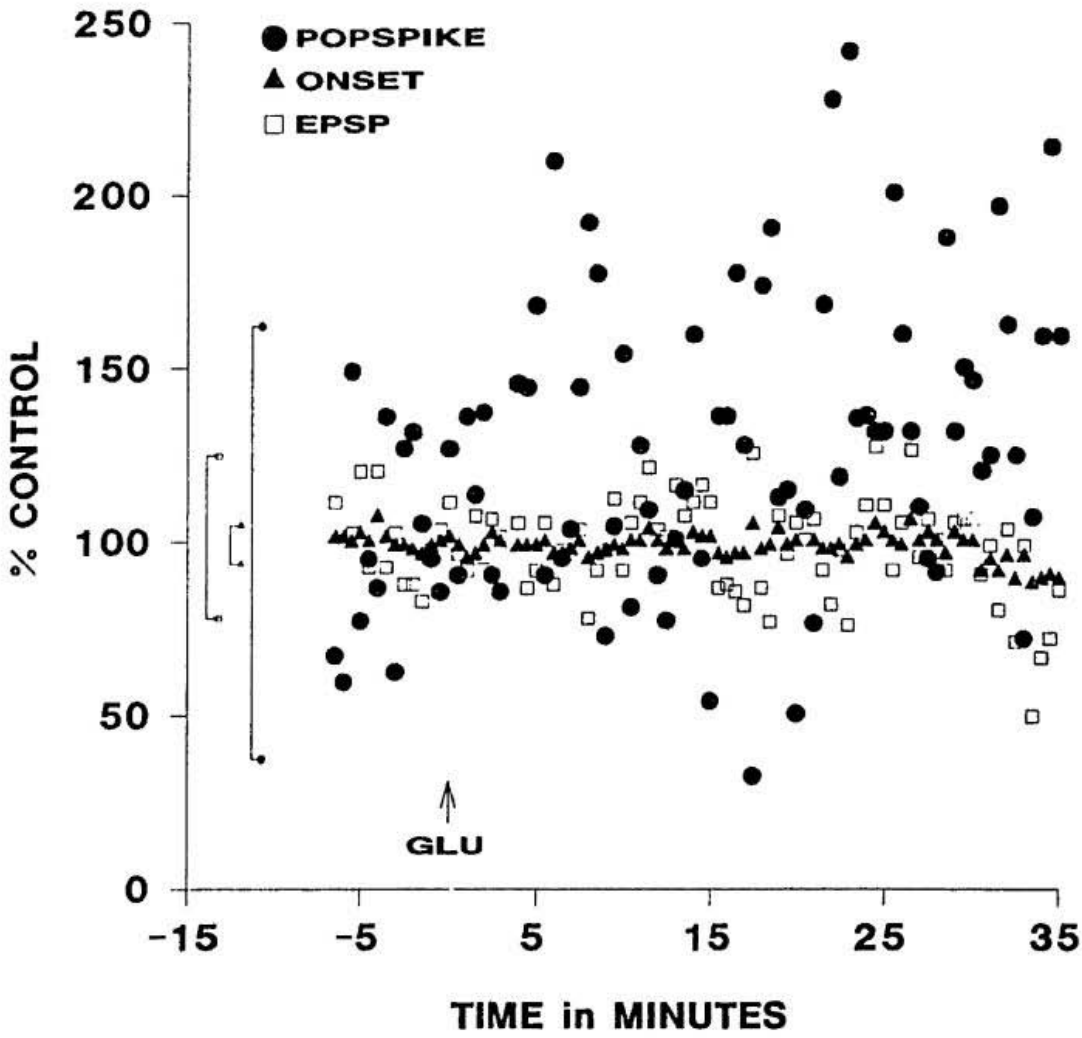

Fig. 19. Percentage change in EPSP slope, PS onset latency and PS amplitude for R2 following an injection of .25 M GLU into lobule centralis of the rostral vermis of the cerebellum. Confidence intervals (95%) of the control period are presented to the right of the Y axis.

However, another cluster of strong increases was recorded after 16.5 minutes. Moreover, low PS values were often accompanied closely in time with high PS values. Significant GLU-induced changes in the EPSP or onset latencies were not seen.

Behaviors noted in response to GLU ejection consisted of early onset (i.e. within 10 seconds) mastication and a SAS. Exploration resumed within 2.5 minutes but periods of stillness were accompanied by long (20 seconds) episodes of mastication and fasciculation.

R3

The final example of GLU-induced changes in the vermis was characterized by a relatively stable PS potentiation profile (see Figure 20). Onset occurred at 1.5 minutes following a 150 nl .25 M injection of GLU (HHM). The maximal increase (199% of control value of 1.18 mv) was observed within 9.5 minutes PI. Duration of the increase was 22.5 minutes.

In addition, EPSP's were significantly potentiated to a maximal 128% of average value of 3.75 mv/ms at 1.5 minutes PI and persisted for about 21 minutes. No significant change in the onset latencies was observed.

The behavioral profile was similar to that observed in many of the LC animals. The period immediately following the

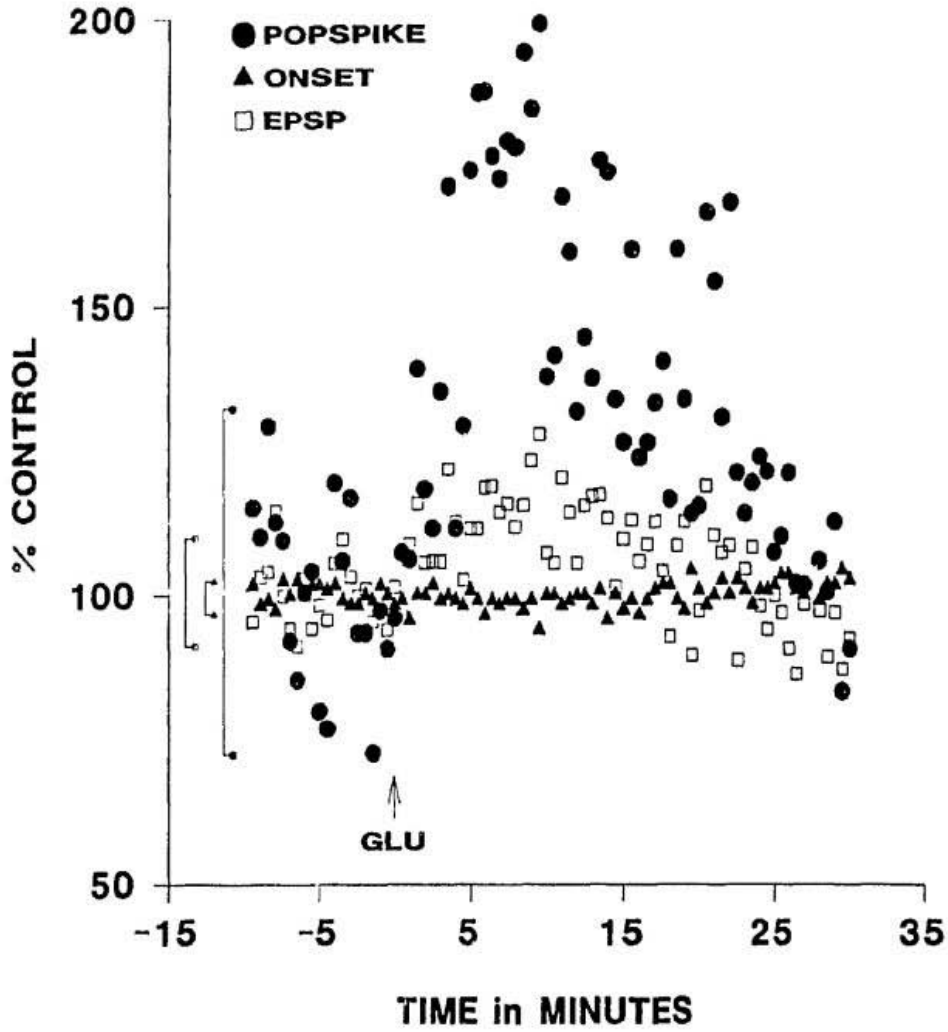

Fig. 20. Percentage change in EPSP slope, PS onset latency and PS amplitude for R3 following an injection of .25 M GLU into lobule centralis of the rostral vermis of the cerebellum. Confidence intervals (95%) of the control period are presented to the right of the Y axis.

injection saw the animal defecating, then assuming a still and alert position (i.e. within 10 seconds). At 2.5 minutes, the animal lowered its posture and laid its snout on the platform and began to sniff. Active exploration began 90 seconds later. The animal frequently masticated when it came to a full stop.

3.5 CTRL Group

Animals designated as the CTRL group (C1-5), failed to demonstrate potentiation of any of the three measures in response to GLU. It is important to note that there were many control sites in the LC and RV groups where GLU ejection did not affect the PP evoked potential. However, these data are not reported here. For the purposes of simplified analyses, the results of the CTRL subjects were grouped, averaged and are presented in Figure 21. Consistent with many of the previously described experiments, considerable variability occurred in individual animals. Characteristics of EPSP, PS onset latency and PS amplitude for the CTRL group will not be discussed here, but representative graphs for C2-C5 are included (see Figures 22-25). Data from animal C1 was not available.

Anatomical sites that did not result in short or long-lasting potentiation included the lateral parabrachial nucleus, the mesencephallic trigeminal nucleus and lobule 2 of

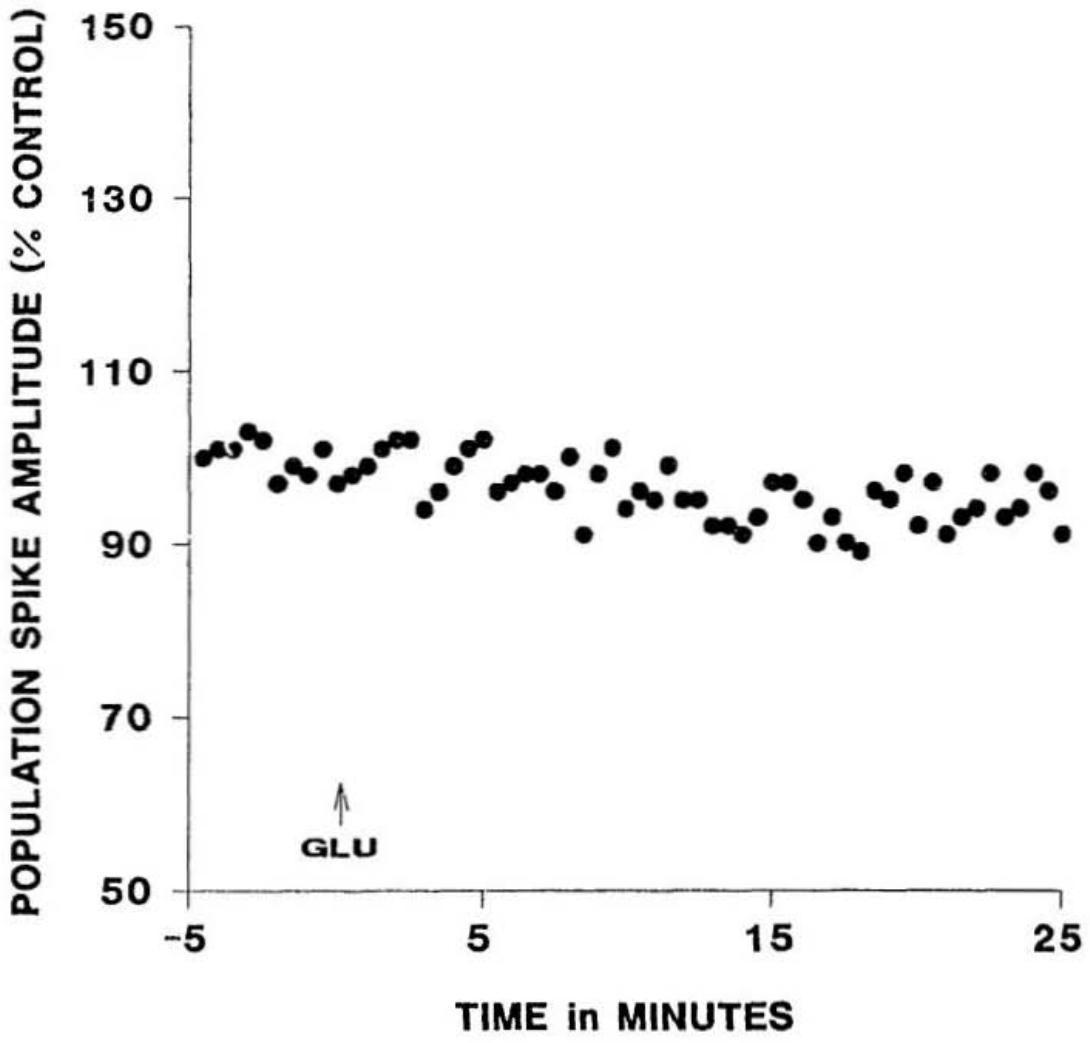

Fig. 21. Percentage change in PS amplitude averaged over 5 control animals (i.e. C1-C5) with placements beyond 300 um of the LC.

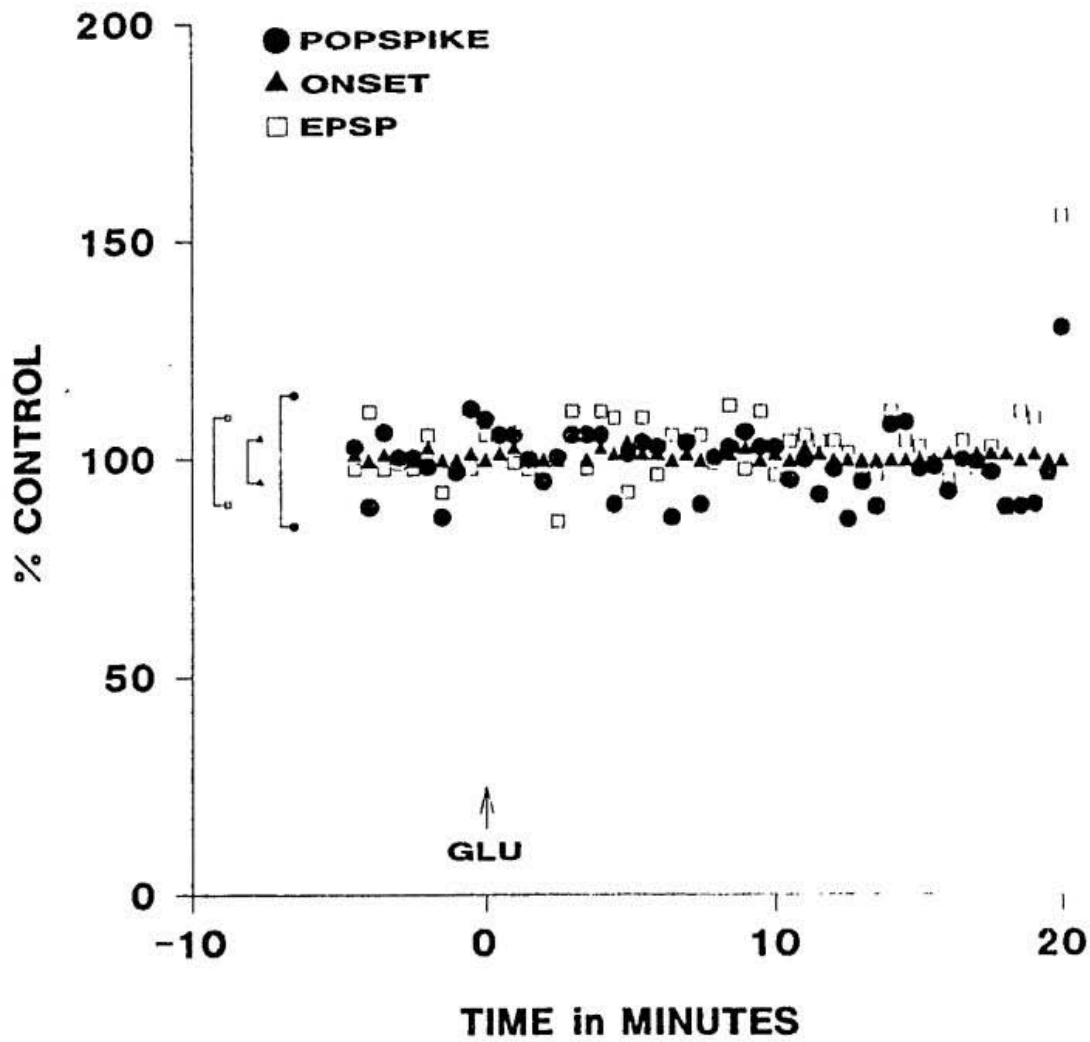

Fig. 22. Percentage change in EPSP slope, PS onset latency and PS amplitude for C2 following an injection of .25 M GLU beyond 300 um of the LC. Confidence intervals (95%) of the control period are presented to the right of the Y axis.

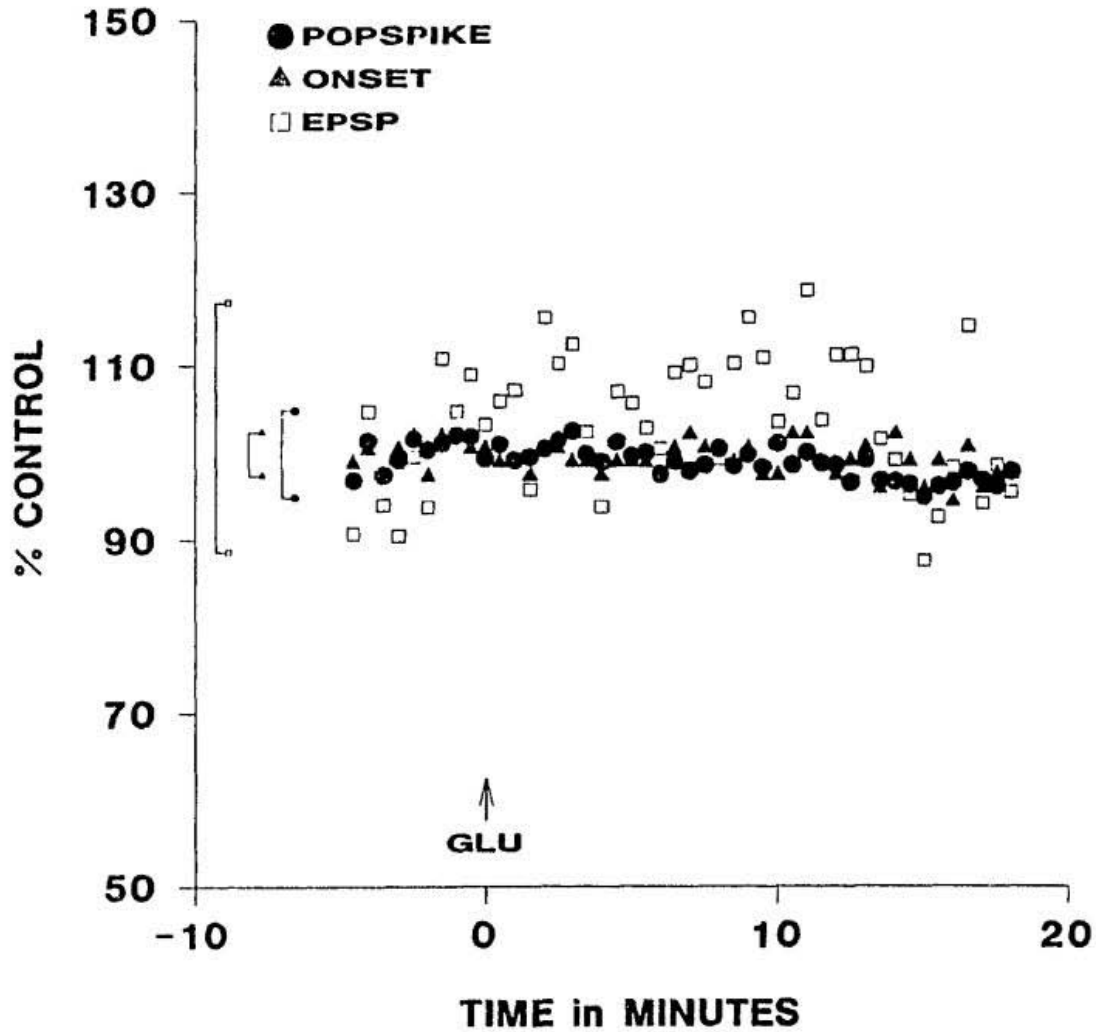

Fig. 23. Percentage change in EPSP slope, PS onset latency and PS amplitude for C3 following an injection of .25 M GLU beyond 300 um of the LC. Confidence intervals (95%) of the control period are presented to the right of the Y axis.

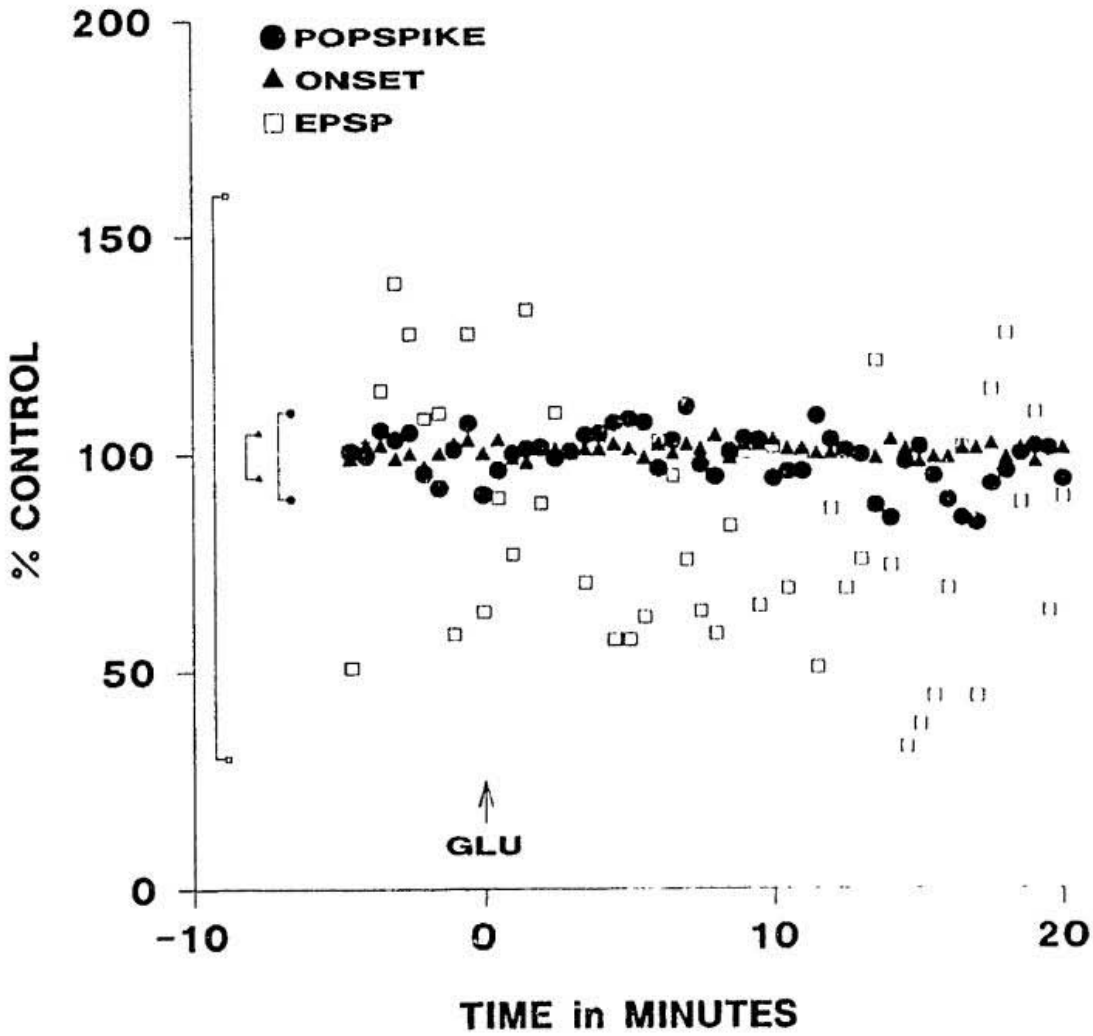

Fig. 24. Percentage change in EPSP slope, PS onset latency and PS amplitude for C4 following an injection of .25 M GLU beyond 300 um of the LC. Confidence intervals (95%) of the control period are presented to the right of the Y axis.

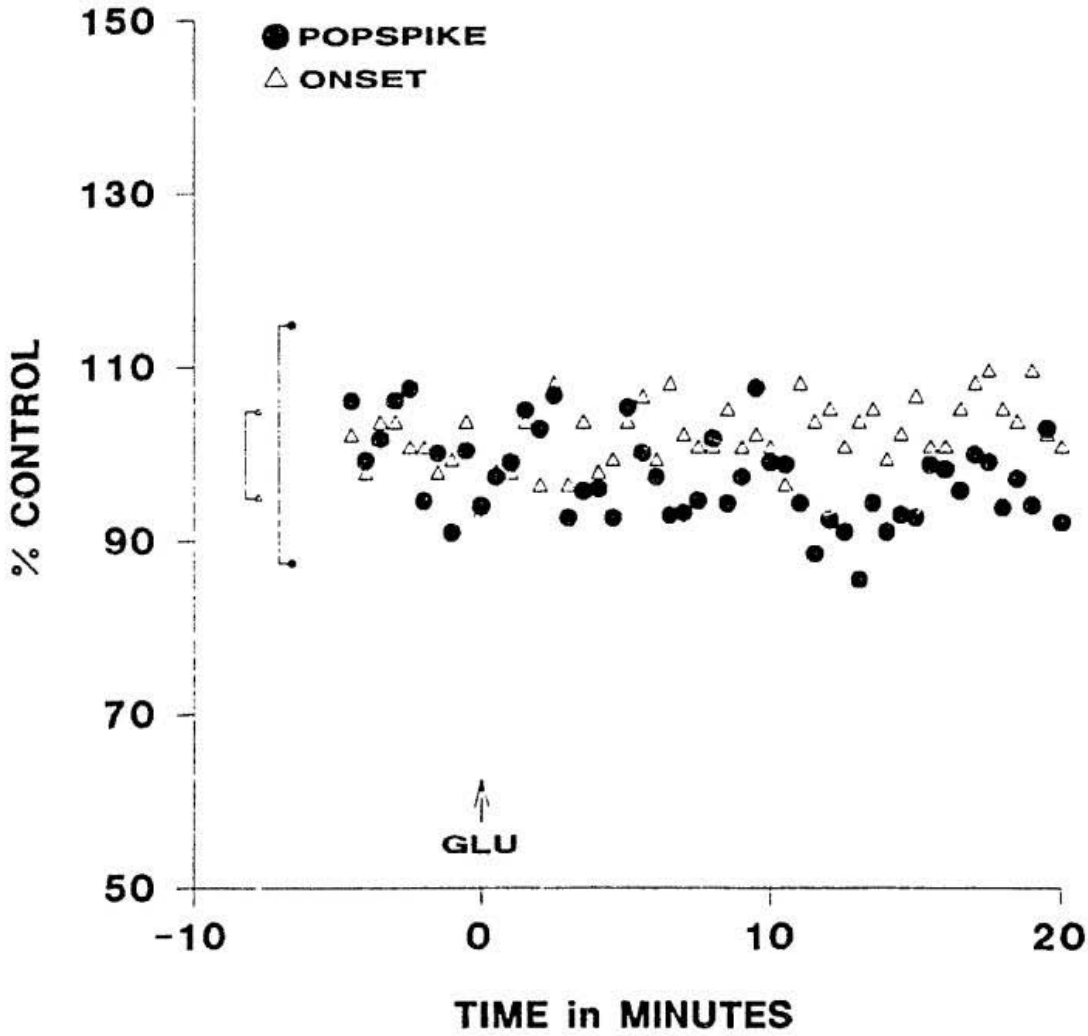

Fig. 25. Percentage change in PS onset latency and PS amplitude for C5 following an injection of .25 M GLU beyond 300 um of the LC. Confidence intervals (95%) of the control period are presented to the right of the Y axis.

the anterior vermis.

Generally, behaviors elicited by GLU injections in the vicinity of CTRL sites did not differ from those observed in the LC and RV groups.

In the case of C1, behavior arising following a 300 nl ejection of .5 M GLU included early onset fasciculation (facial), immobile sniffing and still alertness. Postural shifts were absent but lateral head movements were seen.

Animal C2 responded immediately to a 300 nl injection of .5 M GLU with robust mastication and facial fasciculation interspersed with the grinding of teeth. C2 remained in a SAS with the exception of a startle response to an 'unidentified' stimulus.

C3 showed a pronounced behavioral response to the ventral displacement of the cannula which included urination, defecation and eye blink. Mastication and a postural shift to a horizontal position followed shortly. However, upon administration of .5 M GLU (300 nl), changes in behavior were not apparent.

In the case of C4, a 350 nl ejection of .25 M GLU resulted in a prolonged SAS accompanied by frequent episodes of mastication. Brief fluttering of vibrissae was observed at 3 minutes PI.

Finally, C5 demonstrated a broad range of behaviors in response to 300 nl of .25 M GLU that included robust bouts of

mastication and facial fasciculation, rapid and deep respiration and teeth chatter. By minute 5 PI, the animal continued to breath heavily and began to lean on its left side.

4. DISCUSSION

The main findings of the present study are that GLU application in the vicinity of the LC does produce a potentiation of the PS amplitude in the DG of the freely moving animal and this potentiation is long-lasting in some cases. This result is consistent with a growing body of research implicating NE in the modulation of the PP-evoked potential in the DG (Harley, 1991; Stanton and Sarvey, 1985).

All animals with cannulae localized to within 300 μm of the LC showed potentiation of the PS. PS amplitude was enhanced to an average maximal 170% of control which is marginally higher than NE-mediated enhancements produced by GLU-activation of the LC in the anaesthetized animal (Harley and Milway, 1986) and NE perfusion of DG in the hippocampal slice (Lacaille and Harley, 1985) but similar to the average PS peak magnitude reported to occur following electrical stimulation of the LC (Harley et al., 1989).

NE-induced potentiation of the PS typically develops

somewhat gradually following application of NE into the DG or activation of the LC (Neuman and Harley, 1983; Harley and Milway, 1986). Our findings are consistent with this observation as average onset was 2.5 minutes following termination of GLU injection into the LC. Recently, Harley and Sara (1992) observed that pressure ejection of GLU (50-100 nl .5 M) directly into the LC produces a burst of activity in LC cells lasting 250-400 ms followed by a quiescent period for the next 4.6 minutes. Potentiation of the PS in the DG was delayed relative to the burst by 30 sec. Despite the delay between LC activation and the development of PS enhancement, LC neuronal bursting appears to be the critical trigger.

Both short and long-lasting increases in PS have been observed following iontophoresis of NE into the DG, NE perfusion into the DG of the slice preparation, LC electrical stimulation and LC-GLU activation in the anaesthetized animal. In the awake and behaving animal, GLU-induced enhancement of the PS was long-lasting in 50% of the animals and short-lasting in the remaining animals. The percentage of animals showing NE-induced long-term increases is greater in the chronic animal than in either the anaesthetized animal (Neuman and Harley, 1983) or the hippocampal slice using threshold concentrations of NE (Lacaille and Harley, 1985). In contrast, Stanton and Sarvey (1985a) reliably produce long-lasting increases in the PS in the slice preparation but they apply

higher concentrations of NE for a longer period of time. In our study, brief LC activation is sufficient for the potentiation of the PS to occur and the development of the long-lasting component does not appear to depend on prolonged NE activity (Harley and Sara, 1992). The reasons for this observation are not presently well understood.

Duration of potentiation of PS amplitude ranged from 4-79 minutes. Long-lasting increases in PS were not assessed for absolute duration or decay characteristics. In some cases, continuous recording of long-lasting changes in PS was terminated due to either mechanical error, excessive variability in the PS or time constraints. It would be important to conduct time course studies of NELLP in the chronic animal to 1) draw comparisons with LTP-induced changes in PS and 2) to evaluate the role of behavioral state and circadian rhythm in the modulation of granule cell excitability.

In 6 animals, potentiation of the PS appeared to be characterized by oscillations over the duration of the increase. NE-mediated multi-phasic enhancement of the PS has been observed in response to both bath application of NE in the hippocampal slice (Lacaille and Harley, 1985) and following GLU ejection into the DG of the anaesthetized rat (Harley and Evans, 1988). The greater frequency of multiphasic potentiation in awake animals may be attributed to

a number of factors including modulation of PS by DG interneurons responding to behaviors that elicit theta activity in the DG. Briefly, Buzsaki et al. (1981) reported that PP-evoked PS amplitude in the DG of awake animals was decreased when animals were either running or lever pressing (i.e. during behaviors associated with high amplitude, high frequency theta). Hargreaves, Cain and Vanderwolf (1990) observed significant differences in PS amplitude as a function of whether animals were in an 'immobile' or 'movement' behavioral state and argue that motor behavior must be controlled at the time of recording in order to make valid inferences about learning-induced changes in synaptic efficacy in the DG. Tonic hippocampal activity in the anaesthetized animal is not subject to neuronal modulation as a function of behavior, hence it is more likely to remain stable.

In our study, modulation of the evoked potential by specific behaviors was observed infrequently, however, the experimental design did not address the assignment of behavior into discrete groupings for analysis. The onset of different behavioral states did not appear to predictably affect the PS. The mechanism(s) underlying oscillations in PS in the chronic animal remains to be determined.

Consistent with previous findings of EPSP and PS onset latency effects following GLU injections, EPSP and PS onset latency measures were significantly different from control

period values in only 27% and 8% of the animals respectively. This result is compatible with EPSP/PS dissociation reported to occur in LTP studies (Bliss and Lomo, 1973) and further implies that presynaptic release of NE in the DG does not preferentially enhance synaptic currents at the granule cell dendrites but rather increases the probability that cells will discharge. NE-mediated improvement of the EPSP/spike coupling has received considerable attention as a possible mechanism to account for NE-induced changes in granule cell responses (Harley, 1991; Richter-Levin, Segal and Sara, 1991). In the slice preparation, Lacaille and Harley (1985) observed that the antidromically evoked PS in the DG was not enhanced in the presence of NE whereas the orthodromically evoked PS was enhanced. The absence of EPSP/spike coupling in the antidromic model is likely to account for these results. Taube and Schwartzkroin (1988) have reported that the LTP in CA1 consists of 2 independent components which include a synaptic component and an EPSP/spike coupling component. Taken together, these results suggest that NELLP produced by selective LC activation preferentially improves the EPSP/spike coupling and reflects predominantly postsynaptic processes.

Finally, Sara & Bergis (1991) have recently reported that NE-mediated potentiation of the PP-evoked potential can be produced in the awake, behaving rat following systemic

administration of the alpha-adrenergic antagonist, idazoxan. Idazoxan's mechanism of action is to inhibit alpha2 autoreceptors located on noradrenergic cell bodies and presynaptic terminals causing an increase in the firing rate of NE-containing neurons and enhanced NE release from synaptic boutons. The authors suggest that idazoxan's ability to augment the PS in the DG is attributable to its action on LC-NE neurons. These findings provide additional support that NE-mediated enhancement of PP-evoked PS in DG is not restricted to anaesthetized and slice preparations.

Is NELLP a Naturally-Occurring Phenomenon?

Winson proposed that the PP-evoked PS and EPSP in the DG are modulated by the behavioral state of the animal and that this modulation is dependent on the presence of NE (Winson and Abzug, 1978; Dahl, Bailey and Winson, 1983). They predicted that increasing NE release would result in a reduction in PS amplitude and an increase in the EPSP. Our results do not support this prediction, as we reliably observed enhancement of the PS following GLU-induced activation of LC-NE neurons. As previously mentioned, PS enhancement occurred regardless of the behavioral state that the animal was in at the time of injection (see later section on behavior). Subsequent studies

by Winson's group also failed to support this hypothesis as they found that both LC activation and NE iontophoresis into the DG produced potentiation of the PP-evoked PS (Dahl and Winson, 1985; Winson and Dahl, 1985).

The NELLP in the adult behaving animal seen here to some extent parallels Kasamatsu's findings in the adult cat (Kasamatsu et al., 1985). In Kasamatsu's work, LC stimulation produced an enhancement of visual neuronal responsivity to the open eye and a suppression of responses to the initially closed eye. Therefore, in both cases, enduring plastic changes result from an increase in the availability of NE by LC activation. Ultimately, we are left to address the question of whether NELLP represents a neural process that is available to the animal during naturalistic behavior.

Part of the answer lies in determining the functional significance of the LC-NE system. Aston-Jones and Bloom (1981a,b) employed single and multiple unit recording techniques to assess LC activity within and between behavioral states and in response to the presentation of polysensory stimuli. Tonic LC neuronal discharge varied with the sleep/wake cycle, being highest during waking, considerably lower during slow wave sleep and virtually silent during paradoxical (REM) sleep. Cell firing during waking decreased during grooming and consumption periods. Phasic bursting followed by prolonged suppression of discharge was observed in

response to spontaneous background sensory stimuli in the environment. Further, systematic exposure to sensory stimuli including tone pips, light flashes and touch revealed biphasic bursting patterns similar to that which was observed in the spontaneous condition. Similarly, Harley and Sara (1992) observed that pressure ejection of GLU into the LC also produces a brief bursting of LC cells followed by an extended period during which firing is suppressed. More importantly, this burst of impulses is followed by a potentiation of the PP-evoked PS in the DG. In view of the similarity between GLU-induced triggering of multiple spikes in LC and sensory-induced phasic bursting of LC neurons, it is conceivable that naturally occurring sensory stimuli can precipitate a rapid increase in LC firing which may subsequently affect hippocampal function. This speculation is strengthened by the finding that long-lasting changes in granule cell responsivity do occur in animals exposed to an enriched and complex environment (Sharp et al., 1985). Moreover, NE depletion in the neonatal rat has been found to attenuate improved maze learning in animals reared in an enriched environment (Mohammed, Jonsson and Archer, 1986) and reverse the increases of hypothalamic and cortical dopamine that accompany enriched rearing (O'Shea, Saari, Pappas, Ings and Stange, 1983). The results of the neonatal studies suggest that forebrain NE may play a permissive role in behavioral and neurochemical changes

that are concomitant with rearing in enriched environments. Whether robust activation of LC-NE neurons is a critical factor in the expression of naturally-occurring long-lasting facilitation in the DG remains to be determined.

Cerebellar-mediated Potentiation

Cannulae sites identified beyond a 300 μm boundary of the LC failed to potentiate the PP-evoked potential with the exception of 3 sites that were clustered in lobule centralis of the rostral vermis of the cerebellum. Potentiation of the PS, two of which were long-lasting, occurred in all cases whereas the EPSP was enhanced in two animals but only one was long-lasting. Average maximal PS amplitudes and onset of significant effect were similar to those observed following LC activation.

The cerebellum has been implicated predominantly in memory for motor patterns (Lalonde and Botez, 1990) but a growing body of evidence suggests that the cerebellum may play an important role in associative memory underlying classical conditioning of the nictitating membrane and long-term depression of Purkinje cell response to stimulation of the vestibular nerve (Thompson, 1986).

The cerebellar vermis also appears to be involved in long-term habituation of the acoustic startle response (Leaton and Supple, Jr., 1986) and in the acquisition of heart rate conditioning (Supple Jr. and Leaton, 1990). Maiti and Snider (1975) observed that electrical stimulation of the anterior vermis can suppress seizure discharges produced by hippocampal kindling. Further, Heath, Dempesy, Fontana and Myers (1978) reported that hippocampal unit activity was inhibited by vermal stimulation. These results suggest that the two brain regions may be functionally connected via a mono- or more likely a multisynaptic pathway. However, considerable work will be required to determine the exact nature and function of cerebellar-induced long-lasting facilitation of hippocampal synaptic transmission.

LC-Vermal-Control Animals and Behavior

Behavioral observations following GLU injections were remarkably similar across most animals such that discrete groups could not be distinguished on the basis of the behaviors that were elicited by drug administration. Predominant behaviors included robust mastication, prolonged periods in a still-alert state, postural asymmetries and facial fasciculation. In most instances, the full spectrum of these behaviors occurred within the first minute following the

injection and were no longer evident by 5 minutes PI. These behaviors have been observed following electrical stimulation in the area of the LC (Segal and Bloom, 1976) and cerebellum (Ball, Micco and Berntson, 1974).

It is likely that the diffusion of GLU up the cannulae and through the cerebello-pontine region was extensive enough to activate multiple sites (e.g. mesencephalic trigeminal nucleus and cerebellum) which would have contributed to the behavioral profile that we observed.

Interestingly, exploration was limited predominantly to stationary sniffing and orienting within the first 5 minutes of the GLU ejection. These periods were generally brief. A resumption of active exploratory behavior (i.e. locomotion, rearing) was usually concomitant with the recovery of 'normal' posture.

In summary, LC-induced potentiation of the PS was not associated with a predictable array of specific motor behaviors. Many of the behaviors elicited in the LC group were similar to those observed in RV and CTRL groups.

The present results suggest that selective chemical activation of the LC-NE system produces long-lasting potentiation of the PP-evoked PS in the DG of the awake and behaving adult rat. This is consistent with previous reports of NE-induced prolonged increases in granule cell responsivity in anaesthetized animals and in the hippocampal slice. This

effect is probably mediated by postsynaptic beta-adrenergic receptors (Stanton and Sarvey, 1985a), although we did not attempt to block NE-induced potentiation in the present study. A definitive answer to this issue would require blockade studies in the awake animal.

Does NELLP represent a possible neural substrate for the processing and storage of information in nature? Several lines of evidence make this contention appealing. First, GLU injections into the LC (Harley and Sara, 1992) and presentation of a variety of non-noxious sensory stimuli (Aston-Jones and Bloom, 1981b) both produce brief but vigorous bursting of NE-containing neurons, the former leading to persistent increases in responsiveness in DG granule cells. Second, exposure to an enriched and complex environment produces long-term enhancement of synaptic strength in the dentate (Sharp et al., 1985). It is therefore plausible that sensory-evoked LC activation may be instrumental in the expression of exposure-induced changes in granule cell responsivity.

We did see specific behaviors that were associated with GLU-induced activation in the area of the LC. However, injection spread likely stimulated adjacent sites thereby confounding the behavioral pool from which we had hoped to extract information as to behaviors that may signal the onset of robust LC-NE activity. This task may be further complicated

by the reported lack of a correlation between any specific motor behaviors and LC discharge (Aston-Jones and Bloom, 1981a).

Perhaps a more precise behavioral profile may be teased out by using smaller quantities of GLU, although reduced volumes have been reported to affect the PP-evoked PS either marginally or not at all (Harley and Sara, 1992).

Surprisingly, GLU activation of the rostral vermis also produced potentiation of the PP-evoked potential. To our knowledge, this form of plasticity has not been previously demonstrated in the cerebellum. These results may ultimately lend support to the role of cerebellar systems in higher-order learning and memory.

Aston-Jones, Chiang annd Alexinsky (1991) have proposed that the LC-NE system functions to control vigilance and promote the extraction of salient features from the environment thereby facilitating the expression of adaptive behaviors in response to environmental input. Consistent with this notion are the NE-mediated enhancement studies which ascribe the improvement in signal-to-noise ratio of synaptic transmission to the LC-NE system (Woodward, Moises, Waterhouse and Yeh, 1991). The NELLP model fundamentally extends these theories as LC-NE induced hippocampal plasticity appears to be a neural substrate for the prolonged, enhanced processing of information that may be critical to adaptation in the natural

environment.

Future efforts towards characterizing NELLP in the freely moving animal can include 1) determination of the receptor specificity of the NE effect, 2) time course studies examining the temporal profile of NELLP (i.e. duration and decay), 3) a closer examination of the relationship between LC activation, behavioral state and evoked potential parameters and 4) attempts to identify and utilize natural stimuli capable of eliciting robust LC-NE activity coupled with voltammetric or microdialysis-based assessment of NE release in the PP-DG synapse. Hopefully, these inquiries can lead to the development of a naturally occurring NELLP paradigm.

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