THE EFFECT OF INTRACEREBROVENTRICULAR NOREPINEPHRINE ON DENTATE GYRUS EVOKED POTENTIALS IN ANAESTHETIZED AND AWAKE, BEHAVING RATS

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BY

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Abstract: Exogenous application of norepinephrine (NE) or stimulation of endogenous NE release has been shown to potentiate the perforant path-evoked dentate gyrus population spike (popspike). *In vitro* studies (Lacaille and Harley, 1985) suggest beta receptor mediation of the enhancement. In contrast, an *in vivo* study (Winson and Dahl, 1985) implicates alpha receptors. The present study utilizes intracerebroventricular (icv) administration of NE (1, 10, 50, 100 μg) as well as alpha and beta agonists and antagonists in urethane-anaesthetized rats in order to examine *in vitro*-like bath application conditions in the intact preparation. The effect of icv NE was also assessed in awake, behaving animals. In the anaesthetized rat, NE as well as the beta agonist isoproterenol reliably potentiated the population spike (popspike) amplitude. The alpha agonist phenylephrine potentiated the popspike in a majority of experiments. The icv-induced enhancement was frequently long-lasting for the beta agonist isoproterenol and higher doses of NE (50,100 μg). Both alpha (phentolamine) and beta (metoprolol) antagonists blocked the NE induced potentiation in a majority of experiments. However, only the alpha antagonism was significant. EPSP slope changes were inconsistent. NE (10 μg) produced a potentiation in the awake, behaving rat which was virtually indistinguishable from that obtained with the same dose in the anaesthetized preparation. These results suggest

that the icv methodology is a reliable method of producing popspike potentiation by exogenous NE application *in vivo*. Secondly, the results suggest a possible role for alpha as well as beta receptors in the NE potentiation effect in the dentate gyrus. Finally, the results extend the finding of NE induced potentiation to exogenously applied NE in the awake animal.

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TABLES

FIGURES

LIST OF ABBREVIATIONS AND SYMBOLS USED

ACh	acetylcholine
APV	2-amino-5-phosphonovaleric acid
cAMP	cyclic adenosine monophosphate
CPP	3-[(+/-)-2-carboxypiperazin-4-yl]propyl-1-phosphonic acid
EPSP	excitatory post synaptic potential
GABA	gamma-amino-butyric acid
ICV	intracerebroventricular
ISO	isoproterenol, a beta-noradrenergic agonist
LC	locus coeruleus
LGN	lateral geniculate nucleus
LTP	long term potentiation
MET	metoprolol, a beta-noradrenergic antagonist
mRNA	messenger ribonucleic acid
NE	norepinephrine
PE	phenylephrine, an alpha-noradrenergic agonist
PGI	nucleus paragigantocellularis
PHENT	phentolamine, an alpha-noradrenergic antagonist
PKC	protein kinase C
Popspike	population spike

CONTENTS

General Introduction.

The hippocampus has been implicated in learning, memory formation and attentional processes in the mammalian brain (Isaacson and Pribham, 1975, 1986; O'Keefe and Nadel, 1978; Olton, 1983; Squire, 1986). Long-term potentiation (LTP) in the hippocampus, induced by high frequency stimulation, has been the most widely studied model of neuronal plasticity. Diffuse, modulatory inputs to the hippocampus have also been shown to be important in hippocampal functioning. In this regard, norepinephrine (NE) has been shown to produce both short and long-term enhancement of the dentate gyrus response to stimulation of the major hippocampal input, the perforant path, which originates in the entorhinal cortex. This thesis is concerned primarily with (1) establishing icv injection as a method of inducing potentiation with exogenous NE application (2) investigating the pharmacology of this NE potentiation <u>in vivo</u> and (3) extending the finding of potentiation to exogenous NE to the awake, behaving animal.

The perforant path, also known as the angular bundle, is comprised of a medial-lateral gradient of input (Steward, 1976). However, given the resolution of large stimulating electrodes, they may be thought of as composing two types of fibres. Fibres arising in the medial entorhinal cortex which are thought to carry visual and auditory sensory modalities

synapse in the middle layer of the dendritic tree of the dentate gyrus (Cragg, 1961; Krettek and Price, 1977). Fibres arising from the lateral entorhinal cortex carry olfactory and subcortical inputs, and synapse in the apical portion (outer-third layer) of the dendritic tree (Cragg, 1961; Krettek and Price, 1977). These dendrites arise from the granule cells in the dentate gyrus, the axons of which project to CA3 of the hippocampus. The hilar region between the two blades of the dentate gyrus contains a large variety of interneurons (Cajal, 1968; Amaral, 1978), most of which are presumably inhibitory (Anderson, 1975; Buzsaki and Eidelberg, 1981; Seress and Ribak, 1984; Scharfman et al., 1990; Schwartzkroin et al. 1990).

NE synapses in the hippocampus originate solely from the locus coeruleus (LC) via the dorsal noradrenergic bundle (Koda and Bloom, 1977). The predominant excitatory input to the LC arises in the medullary nucleus paragigantocellularis (PGI) (Ennis and Aston-Jones, 1986, 1987).

All studies of NE effects on neuronal plasticity in the dentate gyrus have involved stimulation of the perforant path at a low frequency while recording the evoked response at either the dendritic or granule cell layer. Two measures are taken as indices of the evoked response. The slope of the Excitatory-Post-Synaptic-Potential (EPSP) is taken as an index of activation of the dendritic tree; this reflects the

combined effect of the number of perforant path fibres activated by the stimulation, the amount of neurotransmitter released and the sensitivity of postsynaptic receptors (Lomo, 1971). The population spike (popspike) amplitude is taken as a measure of the number of cells that fire in response to the neurotransmitter activation. Using this basic paradigm, differences have emerged between effects of bath application of NE *in vitro* in the hippocampal slice and *in vivo* methods of NE application. In vitro, NE has been introduced by bath application (i.e. superfusion) (Lacaille and Harley, 1985; Stanton and Sarvey, 1985a). In the intact, anaesthetized animal, NE has been introduced by iontophoresis (Neuman and Harley, 1983; Winson and Dahl, 1985), via electrical or chemical activation of the LC (Dahl and Winson, 1985; Harley and Milway, 1986; Harley and Evans, 1988; Washburn and Moises, 1989; Harley, Milway and Lacaille, 1989; Klukowski, 1993), or via electrical activation of the PGI (Harley and Babstock, 1992).

NE Potentiation of Popspike Amplitude.

A common feature of NE application, regardless of method of application, is a potentiation of popspike amplitude, recorded at the granule cell layer, that is frequently long-lasting.

There were two initial reports of NE facilitation of popspike amplitude in the hippocampal slice. In one, ten minutes of NE (10 μM) superfusion produced a 31% enhancement that was long-lasting in one-quarter of experiments (Lacaille and Harley, 1985). In the other, thirty minutes of NE superfusion produced enhancement that persisted after a 30 min. wash with a concentration of 20 μM needed to produce half-maximal potentiation (Stanton and Sarvey, 1985a). The mean maximal increase was approximately 85% greater than control. Similar results had been reported in the anaesthetized animal. Neuman and Harley (1983) initially reported that 1-8 min. iontophoretic application of NE at the granule cell layer produced a potentiation of the popspike of a magnitude between 20 and 400% that was long-lasting at 39% of the sites. Similarly, Winson and Dahl (1985) reported long-lasting enhancement of the popspike amplitude in 3 of 8 preparations using 5 min. iontophoresis of NE. However, they also reported potentiation to the inactive isomer, d-NE, in 2 of 5 preparations.

Since these reports, popspike enhancement has been reliably found following activation of the LC to stimulate endogenous release of NE. The facilitation was approximately 40% greater than control and lasted more than 20 min. in 39% of animals following glutamate ejection in the LC (Harley and Milway, 1986). Harley and Evans (1988) replicated this

finding. Klukowski (1993) extended this finding to show that glutamate activation of the LC produced potentiation in the awake animal. Activation of the LC by electrical stimulation of the PGI produced a 39% increase in the popspike amplitude at a inter-stimulus interval between PGI and perforant path stimulation of 30 ms (range 20 - 50 ms). These effects were generally short-lasting (Babstock and Harley, 1992). Finally, electrical stimulation of the LC has produced popspike facilitation which may, however, be partly due to activation of fibres of passage (Winson and Dahl, 1985; Harley, Milway and Lacaille 1989; Washburn and Moises, 1989). Furthermore, it is possible that electrical stimulation causes an unphysiological release pattern of NE as well as promoting the release of peptides.

Overall, a clear pattern of NE facilitation of popspike amplitude in the dentate gyrus has emerged. The present study will introduce a new method of eliciting NE-induced long-lasting potentiation (NELLP) and extend it to the awake, behaving animal.

Pharmacology of NE Effects In Vitro.

While popspike enhancement has been consistently shown in different preparations, the pharmacology of NE effects on popspike amplitude with exogenous application differs markedly

between *in vitro* and *in vivo* studies. The pharmacology of NE effects was initially characterized in slice work. Lacaille and Harley (1985) reported that isoproterenol (ISO), a beta agonist, produced a larger facilitation of popspike amplitude than NE itself. Although the authors concluded that alpha agonists failed to mimic the effects of NE, PE, an alpha agonist, did facilitate the popspike on 29% of slices. However, in other studies, NELLP was demonstrated at a lower NE concentration if an alpha antagonist was added to the slice bath (e.g. Dahl and Sarvey, 1989). The facilitation in popspike amplitude seen in slices following NE superfusion has been prevented by prior bath application of timolol (beta antagonist) (Lacaille and Harley, 1985), propranolol (beta antagonist) and metoprolol (MET, beta-1 antagonist) (Stanton and Sarvey, 1985a). The alpha antagonist phentolamine has not effectively blocked the NE enhancement of popspike amplitude (Lacaille and Harley, 1985).

Pharmacology of NE Effects In Vivo.

The only pharmacological investigation of exogenous application of NE in vivo provides a complete contrast to the results obtained from slices. Winson and Dahl (1985) reported the opposite pharmacology following iontophoresis in the anaesthetized rat. With application at the cell body layer,

the beta agonist ISO decreased the popspike whereas the beta antagonist sotalol increased it. Conversely, the alpha agonist phenylephrine (PE) increased the popspike and the alpha antagonist prazosin decreased it. It is important to note that these effects occurred only during iontophoresis of the drug; long-term effects were not reported. Furthermore, the effects only occurred in a minority of preparations, so it is unclear whether the drugs are having an effect or there are methodological problems. Finally, the effect of ISO (beta agonist) could not be blocked by prior iontophoresis of the beta-antagonist sotalol. No attempt was made to block the effects of NE in the other iontophoretic studies (Neuman and Harley, 1983; Winson and Dahl, 1985).

Despite the lack of further <u>in vivo</u> pharmacological studies, a number of <u>in vivo</u> studies have examined the ability of a single antagonist (generally beta) to prevent the increase in popspike amplitude initiated by LC or PGI activation. NE effects on popspike amplitude following LC activation by glutamate ejection or PGI stimulation were blocked by a beta antagonist and this result was obtained with both peripheral injection of propranolol (Harley and Milway, 1986; Babstock and Harley, 1992) and local cannula application of propranolol or timolol in the dentate gyrus itself (Harley and Evans, 1988). A closer examination reveals that the result was usually an attenuation of the NE effect rather than a

complete blockage. Therefore, there may be room to demonstrate an effect of alpha antagonists on the NE potentiation.

NE potentiation following electrical stimulation of the LC was not attenuated by propranolol although glutamate activation effects were blocked (Harley, Milway and Lacaille, 1989). This was likely due to spreading activation of surrounding axons by the stimulation electrode since Washburn and Moises (1989) used half-maximal stimulation intensities and reported that the LC facilitation was blocked by peripheral injection of propranolol. While it appears clear that beta receptors are involved in NE effects on the popspike in vivo, the possible influence of alpha receptors has not been resolved.

NE Effects in Other Brain Areas.

Pharmacological evidence (Winson and Dahl, 1985) has already been presented for the involvement of alpha receptors in NE potentiation in the hippocampus. A second line of evidence that suggests a role for alpha receptors in NE potentiation originates from pharmacological studies of NE effects elsewhere in the brain.

Most in vivo reports of the pharmacology of NE effects outside the hippocampus consist of alpha-1 mediated enhancement of excitation and beta mediated enhancement of

inhibition. Waterhouse et al. (1981) reported that NE facilitated the responses of somatosensory cortical neurons to natural forepaw stimulation and iontophoresis of ACh (acetylcholine), in vivo. This effect was mimicked by the alpha agonist, PE, but not the beta agonist, ISO, and blocked by phentolamine (PHENT, alpha-1 antagonist) but not sotalol (beta antagonist) suggesting the effect was mediated by alpha receptors. Similarly, in vivo, Mouradian et al. (1991) applied drugs by iontophoresis into the rat somatosensory cortex and evaluated NE enhancement of single unit responses to ACh and GLU. The facilitatory action of NE was mimicked by PE and phorbol ester activation of protein kinase C (PKC) and blocked by PHENT. There was no evidence for a beta component since sotalol and propranolol (beta antagonists) failed to block the NE effect and a cyclic AMP analogue failed to mimic the NE effect. In the dorsal LGN (lateral geniculate nucleus), recorded in vivo, both iontophoretically applied NE and LC stimulation enhanced synaptic excitation, an effect blocked by an alpha antagonist (WB-4101) (Rogawski and Aghajanian, 1980). Waterhouse et al. (1982) performed in vivo recording from somatosensory cortical neurons in response to iontophoretic application of GABA (gamma-amino-butyric acid, an inhibitory neurotransmitter). NE and ISO, but not PE increased the GABA induced inhibition of neuronal firing rates whereas sotalol blocked the increased inhibition.

An *in vitro* report suggested a pharmacological profile in contrast to the above *in vivo* studies. Dodt et al. (1991) applied NE and various agonists to the perfusion media of cortical slices while recording intracellularly from somatosensory neurons. NE decreased the EPSP, which was mimicked by PE, and increased neuronal excitability to current injection, which was mimicked by the beta agonist isoprenaline. Finally, a contradictory *in vivo* report by Lehmenkuler and colleagues (1991) suggested that pressure ejection of NE reduced the negative CFP (cortical field potential) induced by NMDA. This appeared to be alpha mediated since it was mimicked by PE but not ISO.

To summarize, it appears that NE enhancement of excitation is mediated by alpha receptors whereas NE enhancement of inhibition is mediated by beta receptors in the cortex and LGN, *in vivo*. An *in vitro* study suggested the opposite picture of alpha mediation of inhibition and beta mediation of excitation. The suggestion of alpha mediated excitation in other brain structures highlights the importance of evaluating possible alpha mediation of the NE effect in the hippocampus. As well, the difference between *in vivo* and *in vitro* studies in the hippocampus and other brain structures points to the need to evaluate the pharmacology of the NE effect *in vivo*. Even if the hippocampus does prove to be different from other structures, the pharmacology will still

need to be established in vivo.

Alpha and Beta Receptor Distribution.

Taken together, the Winson and Dahl (1985) in vivo pharmacological study and evidence of NE effects on excitatory input elsewhere in the brain strongly suggest a possible role for alpha receptors in the hippocampus in enhancing the glutamatergic perforant path input. Furthermore, autoradiography studies show that both alpha and beta receptors are present in the hippocampus.

Rainbow et al. (1984) demonstrated that there were high levels of beta receptors in the hippocampus, 75-85% of which were beta-1. Young and Kuhar (1980) reported that alpha-1 receptor levels were low to moderate in the hippocampus although the caudal dentate gyrus and molecular layer of the dentate gyrus showed relatively higher levels. Finally, Rainbow and Biegon (1983) described low levels of alpha-1 receptors in the hippocampus except for the dentate gyrus where receptor density was closer to moderate levels. Actual densities reported were higher for alpha-1 receptors (150 +/- 14 fmol/mg protein; Rainbow and Biegon, 1983) than for beta receptors (41.6 +/- 4 to 53.4 +/-5 fmol/mg protein; Rainbow et al., 1984).

In Vivo vs. In Vitro Comparison.

The in vivo preparation contains much richer innervation than the in vitro preparation, as well as having tonically active inputs. While the non-specific beta antagonist propranolol has been shown to block or attenuate potentiation effects of endogenous NE release in vivo, no attempt has been made to reconcile reported differences in pharmacology between exogenous NE application in vitro and in vivo. In this study, we approach the question by using an in vivo preparation in combination with "bath-like" icv administration. Problems with this approach include effects on other structures but it avoids problems with disconnected circuitry in the slice as well as any effects caused by local iontophoresis of drugs.

EPSP Slope.

In addition to the primary purpose of investigating NE effects on the hippocampal popspike, this study will also examine other less critical issues such as NE effects on EPSP slope and latency measures.

While enhancement of the popspike has been ubiquitously reported in both in vitro and in vivo preparations, effects on the EPSP slope in the slice are unlike those reported in anaesthetized rats. In vitro studies have all reported NE

enhancement of EPSP slope (Lacaille and Harley, 1985; Burgard, Decker and Sarvey, 1989; Stanton and Sarvey, 1989). In vivo studies have reported predominantly increases (Harley and Milway, 1986; Harley and Evans, 1988), predominantly decreases (Harley, Milway and Lacaille, 1989) or an absence of any consistent effects (Neuman and Harley, 1983; Babstock and Harley, 1992; Washburn and Moises, 1989). Two in vivo studies have reported differential effects depending upon the area of the dentate gyrus involved. In one, the field EPSP was unchanged at the granule cell layer but decreased at the dendritic tree (Dahl and Winson, 1985). In the other, Winson and Dahl (1985) report different effects following NE iontophoresis depending upon the micropipette positioning. When NE was applied in the middle third of the dendrites, the EPSP was reduced. When NE was applied at the granule cell layer, changes in the EPSP were inconsistent.

Overall, the consistent increases in EPSP slope seen in slices have not been replicated in anaesthetized rats. A possible explanation for this difference lies in the electrode positioning since most in vivo studies have recorded the EPSP at the granule cell layer whereas in vitro studies recorded in the dendritic layer. As demonstrated in Winson and Dahl (1985), the positive EPSP recorded at the granule cell layer may not reflect changes in the negative EPSP at the dendritic layer.

Popspike Onset Latency.

Popspike onset latency may be useful as an indicator of possible temperature modulation of the popspike. This may be important in the present study since temperature changes may be an alternate explanation of some of the effects of icv NE application. However, this measure has been infrequently reported. Lacaille and Harley (1985) reported a consistent decrease of 6% in the popspike onset latency following bath superfusion of NE in vitro. As with other measures, latency changes in vitro have not been consistently replicated in vivo. Two studies have found the absence of any consistent changes in onset latency following LC activation (Harley and Milway, 1986; Harley and Evans, 1988). Babstock and Harley (1992) gave the only in vivo report of decreased popspike onset latency following stimulation of the PGI.

Medial vs. Lateral Perforant Path Effects.

An important *in vitro* study suggests that NE differentially affects the medial and lateral perforant path inputs to the dentate gyrus (Dahl and Sarvey, 1989). A 30 min. perfusion with NE and PHENT (an alpha antagonist) produced a 103% and 43% potentiation of the popspike amplitude and EPSP slope, respectively, to medial perforant path stimulation. At

the same time, the popspike and EPSP elicited by lateral perforant path stimulation were depressed by 22% and 17%, respectively. Taken together with the reports of differing effects on the EPSP at different layers, the response to NE appears to be specific to type of input.

Rationale for Present Experiment.

The present study is concerned with demonstrating the feasibility of using intracerebroventricular (icv) NE and drug administration which was conceived of as an in vivo 'bath superfusion' of the hippocampus that is more similar to slice methods than iontophoresis. Secondly, the present study undertakes to investigate reported differences in the pharmacology of the NE potentiation that exists between in vivo and in vitro preparations. In this regard, the icv method avoids difficulties associated with precise location of NE infusion in the dentate gyrus, as well as iontophoretic charge effects, while allowing the investigation of the effects of various agonists and antagonists in the whole animal. Finally, the present study will attempt to demonstrate the existence of exogenous NE potentiation of the dentate gyrus population spike in the awake, behaving animal.

MATERIALS AND METHODS

EXPERIMENT 1 - ACUTE STUDIES

Fifty-two female Sprague-Dawley rats (obtained from Charles River Canada, Montreal) under urethane anaesthesia (1.5 g/kg) were maintained at normal rectal body temperature by means of a hot water blanket running underneath the animal. A metal guide cannula was implanted approximately 200 μm above the left lateral ventricle at stereotaxic coordinates 0.8 mm posterior to bregma, 1.5 mm lateral to midline and 3.1 mm ventral from dura. The cannula was held in position by dental cement attached to a skull screw. A bipolar metal electrode was directed at the perforant path using coordinates 7.1 mm posterior to bregma, 4.2 mm lateral to midline and 3.5 mm ventral from dura. Activation of the medial perforant path was confirmed by latency to popspike measures. (It was assumed that the popspike reflected medial perforant path activation whereas the EPSP was a mixture of medial and lateral perforant path activation). A tungsten electrode or glass pipette filled with pontamine sky blue (30-60 μm tip diameter) served as the recording electrode. It was directed at coordinates 3.5 mm posterior to bregma and 2.0 mm lateral to midline and positioned vertically in the dentate gyrus cell body layer according to electrophysiological criteria.

The perforant path was stimulated at .1 Hz with square wave pulses and the dentate gyrus evoked potential was digitized at 10 kHz and monitored on-line using software developed in this laboratory using Asyst. The sampling frequency was 1 point per 10 μs or 1 point per 20 μs. Parameter extraction was performed for 1) EPSP slope (sampled over a 300 microsecond interval beginning 750 microseconds before the first positive peak) 2) popspike amplitude (vertical change from first positive peak to first valley) and 3) popspike onset latency (from stimulus artifact to first positive peak). For all experiments except the highest dose of NE and each agonist the stimulation was decreased until the popspike was approximately 50-60% of maximal amplitude before any injections were performed. Data were combined into one minute means and a 95% confidence interval (t-test) was generated for the ten minute control period prior to icv injections. Evoked potentials were collected for 30 minutes post-injection. Effects were considered significant if at least three one minute means exceeded the confidence limits. Effects were considered long-lasting if they exceeded 20 min. in duration.

An injection cannula connected via plastic tubing to a 5 μl syringe was placed inside the guide cannula and projected approximately 0.6 - 1.0 mm beyond the guide cannula tip into the lateral ventricle. All icv injections consisted of a 2 μl

volume applied by unilateral freehand injection over 1-2 minutes. Drugs used in this experiment included the racemic mixture of norepinephrine (1, 10, 50, 100 μg), isoproterenol (10, 30, 60 g), phenylephrine (10, 50, 100, 300, 500 μg), metoprolol (10 μg) and phentolamine (30 μg). All drugs were dissolved in 0.9% saline except phentolamine which was dissolved in distilled water with the application of gentle heat.

During agonist experiments, one to four different drug dosages were applied per animal with a minimum 45 minute interval between infusions. No subsequent injection was given until the popspike amplitude had returned to baseline. Each drug dosage was given as the first injection for at least two different animals and the order of injections was varied. On two separate occasions, the same drug dosage was given twice in the same animal. Over the series of experiments, the highest dose of each drug was used initially and progressively lower doses were used until an ineffective dose was reached. During antagonist experiments, metoprolol was injected 30 minutes prior to application of isoproterenol (30 μg) or norepinephrine (10 μg). Phentolamine was injected 30 min prior to application of phenylephrine (50 μg) and 15 minutes prior to application of norepinephrine (10 μg). These time intervals were chosen in order to avoid short lasting effects which sometimes occurred following injection of either antagonist

alone. No other injections were performed during antagonist experiments.

Immediately prior to sacrifice, animals received a 2 μl injection of pontamine sky blue which was used during later histology to verify injection cannula placement inside the lateral ventricle. Dye perfusion of the lateral ventricle was verified by visual inspection immediately after brain removal. Cannula placement and electrode tracts were visualized using differentiated cresyl violet staining. In cases where electrode tracts were not visible, electrophysiological criteria were the only criteria of electrode placement.

EXPERIMENT 2 - CHRONIC STUDIES

Five female Sprague-Dawley rats (342 to 514 g; retired breeders, except for one) were anaesthetized with Avertin, supplemented as needed. (All rats were obtained from Charles River Canada, Montreal.) A guide cannula, tungsten recording electrode and bipolar stimulating electrode were implanted according to the coordinates listed in Experiment 1. A stainless steel ground screw electrode was positioned on the opposite skull surface and three other screws were placed around the electrodes. The assemblage was secured with dental cement and the wound was closed without suture. The dental cement was allowed to cover the edge of the skin. Animals

(with one exception) were given chloromycetin antibiotic at the end of surgery (1.5 g/kg, i.m.). Following surgery, animals were housed in cages with elevated roofs and paper towel bedding and allowed to recover for at least one week.

Animals were habituated to the Plexiglas recording box for at least one ten minute session and the appropriate stimulating current amplitude and direction were determined prior to recording. On recording days, animals were taken from the housing room and immediately placed in the recording box. Animals were stimulated for a specified length of time at the start of each session (two minutes for most animals) in order to ensure that proper stimulation was occurring. Perforant path evoked dentate gyrus potentials were then recorded for ten minutes at .1 Hz using Brainwave software. This was done over consecutive days to establish a baseline. On injection days, either norepinephrine (10 μg) dissolved in 0.9% saline or saline alone was injected at a volume of 2 μl over one to two minutes. For most animals, evoked potentials were recorded for 60 minutes following the injection and for two ten minute periods at 90 and 180 minutes after the injection. Animals were left in the box until the end of the recording sessions. Overall, each animal received one to three injections over a period of days or weeks. The order of injections was counterbalanced. However, not all animals received saline injections since some animals had to be sacrificed before the

second (saline) injection was given.

The behaviour of the animal was recorded at the time that each stimulation was delivered (i.e. every ten seconds). Behaviour in the interval between stimulations was not recorded. Behaviour was recorded manually by the experimenter and coded according to the following categories: N - No movement - eyes open (i.e. alert immobility), G - Grooming behaviour, H - Movement of the head and/or front paws without corresponding movement of the body axis, W - Walking or locomotion, S - No movement - eyes closed (i.e. sleeping), L - Rearing on hind legs. This categorization was designed to provide a gross description of movement associated with the recording sessions, particularly those in which the animal received an injection. The means of evoked potential parameters associated with each behaviour as well as the proportions of each behaviour that occurred were calculated prior to and following each injection.

Prior to sacrifice, the stimulating and recording electrode placements were lesioned by electrical current and pontamine sky blue was injected into the lateral ventricle under Avertin anaesthesia. Dye perfusion of the lateral ventricle was verified by visual inspection immediately after brain removal. Cannula placement and lesions were visualized using differentiated cresyl violet staining.

RESULTS

EXPERIMENT 1 - ACUTE STUDIES

Population Spike.

Table 1 shows the effect of saline (vehicle) and various doses of NE on the population spike amplitude in the dentate gyrus evoked by perforant path stimulation. Following saline injection, there were two small magnitude decreases and two long delay increases (which were presumably unrelated to injection since the delay was over 20 minutes). The net effect over eight animals did not vary more than one percent from baseline, however (see figure 13). NE reliably produced potentiation of popspike amplitude in 13 out of 14 injections at the three highest doses. The mean increase was 55.8%, 49.8%, and 46.5% at 10, 50, and 100 μg, respectively. These effects typically peaked at between 10 and 20 min post-injection. Long-lasting (greater than 20 min) effects were more often observed at the highest doses. No effects were observed at the 1 μg dose. The threshold dose for potentiation of the popspike amplitude is assumed to be between 1 and 10 μg. This information is presented graphically in Figure 1. The 1 μg dose was ineffective while the higher doses produced similar potentiation except that the highest doses exhibited

shorter delays to significant effects.

Table 2 and Figure 2 show the effect of ISO (beta-agonist) on popspike amplitude. The threshold for potentiation of the popspike was between 10 and 30 μg since no consistent effect was observed with the 10 μg dose whereas the popspike was always enhanced with both 30 and 60 μg doses (mean increase = 45.8% and 43.2%, respectively). The delay to significant potentiation was shorter with the 60 μg dose (mean = 7.2 min) than with the 30 μg dose (mean = 12.0 min). A total of 8 out of 10 injections at the effective doses resulted in long-lasting enhancement.

Table 3 and Figure 3 detail the effect of PE (alpha-agonist) on the population spike. The effect of PE was less consistent than the NE and ISO effects with 5 significant decreases and 6 injections which produced no significant effect. However, the most frequent effect observed was significant potentiation of the popspike amplitude which occurred following 15 of 26 injections. Furthermore, 11 of these 15 effects were long-lasting. The mean of the increases at 50, 100, 300, and 500 μg was 56.9%, 35.0%, 63.4%, and 50.2%, respectively. The threshold dose was probably above 10 μg since this dose produced 1 decrease and 1 no change and the two significant potentiations exhibited a long delay to significant effect (mean = 17.5 min). Surprisingly, the two doses which produced the clearest potentiation were 50 and 500

μg whereas the intervening doses of 100 and 300 μg were more inconsistent. This was demonstrated by shorter delays to significant effect (see Table 3) and a larger net deviation from baseline (see Figure 3). The investigator noted that a temperature drop of approximately 0.3 degrees Celsius occurred frequently with PE injection, despite attempts to maintain stable rectal temperature. However, this decrease was observed whether or not the popspike amplitude was increased or decreased.

Figure 4 illustrates three examples of long-lasting popspike amplitude potentiation which was followed for at least 80 min post-injection. The PE effect was typical in that the popspike amplitude returned to baseline within one hour. ISO and NE produced effects of varying duration with a minority staying elevated near maximal potentiation at the end of the recording period (which varied between 30 min and 2 hr).

EPSP.

The criterion for injection of saline or drug was a stable baseline of population spike amplitude for ten minutes prior to injection. The EPSP and popspike onset latency baselines were not considered in relation to the time of injection. Consequently, some injections were preceded by

drifting EPSP baselines and cyclical or drifting popspike onset latencies baselines.

The effect of saline and NE, ISO, and PE on EPSP slope are shown in Tables 1, 2, and 3, respectively. Saline and the dose of NE (1 μg) ineffective at producing popspike effects produced no clear effect on the EPSP. The NE doses effective at producing popspike potentiation produced 3 significant increases, 4 significant decreases, and failed to produce a significant change on 7 occasions. The selective beta and alpha agonists, ISO and PE, produced similar mixed results at doses above the threshold for popspike enhancement. The EPSP slope was significantly enhanced following 4 injections, significantly diminished following 3 injections, while remaining unchanged following 3 injections of ISO. There were 5 significant increases and 6 significant decreases following the injection of PE. The remaining 11 PE injections failed to produce a significant change in the EPSP slope. If the instances of variable control baselines are removed from the analysis, there is still no clear trend in EPSP changes.

Popspike Onset Latency.

Tables 1, 2, and 3 also contain the effect of the various agonists and vehicle alone on popspike onset latencies. Saline had little effect. With doses above the threshold for popspike

effects, NE increased the popspike onset latency on 8 of 14 occasions and PE increased the popspike onset latency on 15 of 22 occasions. In contrast, ISO produced a significant decrease of popspike onset latency on 8 of 10 occasions. A clear majority of these effects were long-lasting with all three drugs. If the instances of variable baseline are removed from the analysis the picture remains essentially the same. The effects of these three drugs on the popspike onset latencies were averaged for all doses which were effective at eliciting popspike changes and plotted as a percentage of the baseline in Figure 5. Clearly, NE and PE produced a similar magnitude increase in popspike onset latency whereas ISO produced a decrease which occurred over a similar time course. The mean of the maximal effect within 30 minutes of injection was approximately 6% for all three drugs.

Relationship Among Evoked Potential Parameters.

The types of EPSP and popspike onset latency changes were compiled separately for popspike increases, decreases, and absence of significant effects. Significant results are described only for above threshold doses (as determined by their effect on the popspike) of all three drugs.

When NE produced significant potentiation of the popspike, there was 1 EPSP slope increase, 3 decreases, and

there was no change on the remaining 5 occasions. The popspike onset latency was increased on 6 occasions and remained unchanged on the other 3 occasions. Looking at all three parameters simultaneously, on 3 out of 9 occasions the popspike amplitude and popspike onset latency were significantly increased at the same time that the EPSP slope was significantly decreased.

When ISO produced significant popspike potentiation, the EPSP slope was enhanced 2 times, was diminished 3 times, and underwent no significant change 3 times. The popspike onset latency was decreased on all 8 occasions. Looking at all three parameters simultaneously, on no occasion (out of a possible 8) was the popspike amplitude and popspike onset latency significantly increased at the same time that the EPSP slope was significantly decreased.

When PE produced significant popspike potentiation, the EPSP was decreased on 3/10 occasions while no change occurred on the remaining 7 occasions. The popspike onset latency increased 9/12 times, decreased once, and remained unchanged twice. When PE injection was followed by significant popspike amplitude decreases, both EPSP slope and popspike onset latency increased once and exhibited no change on the remaining 3 occasions. Finally, when PE had no significant effect on the popspike, the EPSP slope increased twice and remained unchanged once, whereas the popspike onset latency

increased twice and decreased once. Looking at all three parameters simultaneously, on 3 out of 17 occasions the popspike amplitude and popspike onset latency were significantly increased at the same time that the EPSP slope was significantly decreased. Conversely, there were no occasions (out of a possible 17 occasions) where the popspike amplitude and popspike onset latency were significantly decreased at the same time that the EPSP slope was significantly increased.

Figures 6, 7, and 8 illustrate individual examples of the effect of NE, ISO, and PE, respectively, on all three parameters of the evoked potential. In these particular examples, NE (50 μg) produced a significant increase in the popspike and popspike onset latency with no concomitant change in the EPSP. ISO (60 μg) produced a significant increase in the popspike amplitude, a significant decrease in the popspike onset latency, with a non-significant increase in the EPSP slope. PE (300 μg) produced a significant increase in the popspike amplitude and the popspike onset latency while at the same time producing a significant decrease in the EPSP slope. These effects were typical of the responses seen to these drugs except that the popspike changes were long-lasting which was generally typical of the higher doses only.

Beta Antagonist.

The ICV injection of the beta-antagonist Metoprolol in a 10 μg dose produced variable results on the popspike amplitude. Table 4 shows that there were 4 significant increases with a mean of 70.5% and 7 significant decreases with a mean of 25.1%. The remaining 4 MET injections produced no significant effect on the popspike amplitude. No clear trend was apparent despite the relatively large number of injections performed. The effect of MET on ISO and NE effects was only evaluated in experiments where there was no MET effect or the MET effect had decayed or plateaued and there was a stable baseline for ten minutes before the agonist injection.

The effectiveness of MET as a beta antagonist was evaluated by giving ISO (30 μg) 30 min after MET. Whereas ISO alone produced significant long-lasting potentiation following all four injections, ISO only facilitated the popspike amplitude on 2 of 4 occasions following MET (Table 4). On those two occasions, the increase was of slightly smaller magnitude and somewhat longer delay to effect than without the antagonist. As shown in Figure 9, the mean maximal effect within 30 min. of ISO application was reduced by more than 50% by prior MET injection. This difference was significant ($p < 0.05$, Wilcoxon Rank Sum).

When injected 30 min. following MET injection, 10 μg NE produced a larger magnitude (mean = 75.5%), shorter delay to effect (mean = 4.7 min.), significant potentiation of the popspike amplitude (3 of 8 cases) than NE alone (Table 4). These increases were all long-lasting compared to an absence of long-lasting effects following NE alone. Conversely, the popspike amplitude exhibited significant depression in the remaining animals (5 of 8 cases), which was always long-lasting although of a much smaller magnitude (mean decrease = 36.6%). In comparison, NE alone caused significant enhancement of popspike amplitude following 4 of 4 injections at a dose of 10 μg. Figure 9 shows that the net effect of NE (10 μg) on the popspike following MET injection, averaging across all increases and decreases, was near zero compared to a net increase of 55.8% following NE alone. This difference was not significant, however ($p > 0.05$, Wilcoxon Rank Sum).

Alpha Antagonist.

The injection of PHENT (30 μg) produced a large magnitude (mean = 70.5%) long-lasting potentiation of popspike amplitude in 2 animals whereas in 4 other animals, PHENT injection produced no significant change (see Table 5). At 30 min. post-injection on the four occasions where there was no significant effect due to PHENT itself, PE 50 μg was injected. The net

effect of PE after PHENT was near zero whereas PE alone produced a mean maximal potentiation of over 20%, as indicated in Figure 10. However, this difference was not significant ($p > 0.05$, Wilcoxon Rank Sum).

Four subsequent injections of PHENT were monitored for 15 min without any significant change from baseline. At 15 min. following PHENT injection, NE (10 μg) was injected. Only one out of these four injections produced a significant potentiation of the popspike compared with all 4 NE (10 μg) injections which were not preceded by antagonist injection (see Table 5). Figure 10 shows that the net effect of NE after PHENT was a slight depression of the popspike amplitude compared to a mean increase of 55.8% following the same dose of NE alone. This difference was significant ($p < 0.05$, Wilcoxon Rank Sum).

Baseline Measures.

Appendix I contains the raw means for the popspike amplitude during the control period for various drug doses. Means were described separately for experiments with popspike increases and lack of increases (for drugs where popspike changes were variable). Baseline popspike amplitude did not appear to predict response to subsequent drug application since the baseline amplitude was larger in cases where the

popspike amplitude subsequently increased for some drugs and smaller for other drugs (only one small n result was significant).

Appendix II contains the raw means for the popspike onset latency during the control period for various drug doses. Again, means were described separately for experiments with popspike increases and lack of increases (for drugs where popspike changes were variable). Overall, popspike onset latency did not differ between cases where the popspike amplitude subsequently increased or decreased. However, with NE (10 μg, after MET) and PE (50 μg, after PHENT), cases with subsequent increases had a significantly longer delay to popspike onset than cases with subsequent decreases. However, whether this represents a real phenomenon is questionable since both these instances were small n and there was no overall trend.

EXPERIMENT 2 - CHRONIC STUDIES

Histology.

All five animals had clear lesions of the angular bundle through the stimulating electrode. Lesion-making attempts with the recording electrode failed. Thus vertical positioning was unknown. Electrode tracts verified medial-lateral placement in

the dentate gyrus. Two perforant path evoked-dentate gyrus profiles had the classic granule cell layer shape. Three other profiles had slightly different shapes. They were classified as granule cell layer profiles on the basis of their latencies to popspike onset and popspike peak, which were intermediate between the latencies exhibited by the other two profiles.

Pontamine Sky Blue injection prior to sacrifice revealed dye clearly through the lateral ventricles in three animals and dye slightly above the ventricles in 1 animal. On 1 animal, the cap was lifted immediately prior to sacrifice preventing verification of cannula placement. In some animals, one week had elapsed between the last ICV injection and sacrifice (and dye injection) and animals were not sacrificed until there was some visual or physiological evidence of skull cap lifting. Therefore, neither positive nor negative dye results are conclusive of cannula placement at the time of injection. Furthermore, the internal cannula may take varying tracks on successive injections since the guide cannula was much larger gauge than the internal cannula in order to facilitate placement of the internal cannula during recording sessions.

Population Spike.

The evoked potential was monitored for a 10 min.

baseline, and from 0 - 60, 90 - 100, and 180 - 190 min post-injection. The results were analyzed as 10 min. means. For equivalence to acute studies, the maximum effect on one min. means within 30 min. post-injection was also reported.

Saline injection failed to produce significant potentiation of the popspike within 30 min. or at any of the sampled ten minute intervals within 3 hr. (n=2). Table 6 lists the effect of saline on one min. means within 30 min. following the injection.

In contrast, NE 10 μg injection always produced significant potentiation of the popspike amplitude for at least two ten minute intervals (6 injections, 4 animals). This significant facilitation began within the first two ten minute intervals post injection on all but one occasion. There was only one ten min. mean that was significantly decreased and this was followed by a significant potentiation. Looking at one minute means, Table 6 shows that there were 4 long-lasting and 1 short-lasting significant increases within 30 minutes post injection (mean = 46.0%). There was 1 significant decrease (which was followed by a significant increase at 90 and 180 min.). Figure 11 plots the popspike amplitude at 10 min. intervals for all 6 NE 10 μg injections. Four strong long-lasting increases are clearly shown. Note that all significant increases returned to baseline except for the case where recording was terminated at 30 min. post-injection. (The

recording periods differ slightly as the recording period protocol was not adopted until after the first two injections.)

EPSP.

Following saline injection, there were two long delay (40 and 90 min) decreases in EPSP slope which persisted for two 10 min. intervals. NE (10 μg) produced mixed results on the EPSP slope. There were 3 significant increases, 1 significant decrease, and 3 biphasic effects (significant decreases followed by significant increases).

Popspike and EPSP together.

Ten min. means on which significant NE induced popspike facilitation was recorded were analyzed for corresponding changes in the EPSP. No correspondence between popspike and EPSP changes was found. Summing across all animals, during 40% of the time intervals which exhibited significant popspike facilitation, the EPSP slope was significantly increased. In contrast, the EPSP slope was significantly decreased 35% of the time and exhibited no significant change on the remaining 25% of the time. Figure 12 shows an example of the effect of NE on both parameters in a single rat. In this instance, there

was little change in the EPSP while the popspike was enhanced. However, when the popspike subsequently decreased, the EPSP increased at the same time.

Comparison Of Acute and Chronic Effects.

Table 6 contains the results for the effect of a NE 10 μg injection on popspike amplitude in both anaesthetized and awake animals. The results are analyzed as means of the maximum effect within 30 min post-injection. NE (10 μg) produced a mean maximal increase of 55.8% that was never long-lasting in 4 anaesthetized animals. NE produced a mean maximum increase of 46.0% that was long-lasting (greater than 20 min.) in 4 of 5 awake animals. The popspike amplitude was significantly depressed in one animal, a short-lasting effect.

Figure 13 plots the effect of NE 10 μg injection on the popspike amplitude, analyzed as ten min means. Saline failed to elicit any net change in the popspike amplitude in either anaesthetized or awake animals (n=8, n=2, respectively). The popspike amplitude was facilitated in both anaesthetized and awake animals to a similar degree (n=4, n=5, respectively).

Figure 14 plots the NE 10 μg effect on EPSP slope, analyzed as ten min. means. Again, saline failed to produce any net change. The EPSP slope was only slightly altered following NE injection. The net effect was a small magnitude

decrease in anaesthetized animals and a small magnitude increase in awake animals.

Population Spike and Corresponding Behaviour.

The behaviour of the rat was recorded while each evoked potential was elicited. This was done to determine if any popspike changes observed were correlated with a particular behaviour type. There was no consistent difference in popspike size associated with behaviour type following NE injection. Figure 15 shows one example of significant NE potentiation of the popspike. All popspikes were potentiated regardless of the behaviour type associated with the popspike. An alternative way of examining this is to restrict the analysis to the predominant type of behaviour exhibited in the ten minute control period. This analysis was performed in a subset of NE injections and showed that the mean popspike amplitude associated with no movement, eyes open was greater following the injection than during the control period to a similar degree as the overall effect. In one case, the potentiation of the popspike amplitude associated with no movement - eyes open was 28.3% compared with an overall increase of 23.9% during the first hour (figure 11, open circles). In the second, the potentiation associated with no movement - eyes open was 27.3% compared to an overall increase of 23.4% (figure 11, closed

triangles).

The popspike amplitude associated with each behaviour type was also examined over a period of days during the ten minute baseline recording period. Two such examples are illustrated in figures 16 and 17. Figure 16 shows that the popspike amplitude associated with no movement - eyes closed was lower than that associated with no movement - eyes open on 7 out of 10 days but this effect was not significant ($p > 0.05$, two-tailed, paired t-test). Figure 17 shows that the popspike amplitude associated with grooming was larger on two out of three baseline periods and larger following NE 10 injection (last three points) than the popspike amplitude associated with no movement - eyes open. An earlier period of recording in the same animal (not shown) exhibited lower popspike amplitude associated with grooming than with no movement - eyes open on 2 out of 3 days when both behaviours were present. Overall, the difference between popspike amplitude associated with grooming and no movement - eyes open was not significant ($p > 0.05$, two-tailed, paired t-test). Therefore it appears there was no clear association between behaviour and popspike amplitude during either the baseline period or subsequent to drug injection.

Norepinephrine Effects On Behaviour.

Table 7 details the effect of saline and NE 10 μg injection on the proportions of each behaviour type exhibited within 30 minutes of ICV injection. Behaviour was analyzed in ten min. bins. Following saline injection there was a decrease in no movement - eyes open and an increase in grooming and walking in the first ten min. and an increase in no movement - eyes closed in the last ten min. In the ten min. following NE injection, there was a larger decrease in no movement - eyes open, a decrease in grooming, a large increase in walking behaviour, and the appearance of standing on hind legs and chewing. Similarly to saline, there was an increase in no movement - eyes closed behaviour in the last ten min. bin.

Overall, it is unlikely that the behavioural effect of NE (particularly, the increase in walking) was responsible for the increase in popspike amplitude since there was no association between behaviour and popspike amplitude.

DISCUSSION

Agonists.

ICV injection of Norepinephrine reliably produced a potentiation of the perforant path-evoked potential in the dentate gyrus of the anaesthetized rat consistent with previous in vivo (Neuman and Harley, 1983; Winson and Dahl, 1985) and in vitro (Lacaille and Harley, 1985) reports. The enhancement was long-lasting in 6 of 13 experiments, more frequently so at higher doses. The beta agonist ISO mimicked the effect of NE and was more likely than NE to produce long-lasting enhancement (8 of 10 experiments). This agrees with the in vitro findings of Lacaille and Harley (1985) where ISO superfusion always produced an enhancement. It contrasts with Winson and Dahl (1985) who reported a decrease of popspike amplitude during iontophoretic application of ISO in vivo. The alpha agonist PE also caused a potentiation of popspike amplitude in a majority of experiments. However, the effect of PE was less consistent than that of ISO and included decreases in a minority of experiments. These findings, although less reliable than the beta enhancement, lend some credence to the earlier report of alpha induced potentiation following iontophoresis in vivo (Winson and Dahl, 1985). It is notable that one in vitro study which concluded that NE potentiation

was beta mediated did find enhancement following PE superfusion in 29% of experiments (Lacaille and Harley, 1985).

To summarize from the agonist results in anaesthetized rats, NE produced a potentiation of the popspike that was dose-dependent in duration but not magnitude. Both ISO and PE caused popspike potentiation although the ISO effect was more reliable, resembling the previous in vitro pharmacological study.

Antagonists.

Mixed results were obtained in experiments where antagonists were introduced prior to NE injection. NE produced an enhancement in only 3 of 8 experiments following MET (beta antagonist) injection. However, these increases were all larger and longer lasting than those following NE alone. Furthermore, the other 5 experiments showed decreases to NE injected after MET. On post hoc examination, it appears that two separate populations of results are occurring. Appendices I and II show that the popspikes that increased were smaller (not significant) and of longer latency (significant) than those that were blocked by prior application of MET. The overall difference between NE alone and following MET was not significant, however. In contrast, the alpha antagonist PHENT did significantly block the NE induced potentiation.

Previous *in vitro* studies have reported the enhancement effect of NE could be blocked by beta antagonists (Lacaille and Harley, 1985; Stanton and Sarvey, 1985a) but not alpha antagonists (Lacaille and Harley, 1985). As well, NE and PHENT produced a greater enhancement than NE alone (Dahl and Sarvey, 1989). Furthermore, potentiation by release of endogenous NE has also been antagonized through beta receptor antagonists (Harley and Milway, 1986; Harley and Evans, 1988; Babstock and Harley, 1992) although alpha antagonists have never been tried *in vivo*. However, the effect of the beta antagonist *in vivo* was often an attenuation of the NE effect rather than a complete block.

In this study, the failure of the beta antagonist to block NE potentiation in all experiments could be due to too low a dose or too long a delay between antagonist and NE administration since it failed to completely block ISO induced potentiation on all occasions (it did significantly antagonize the ISO effect, however). Higher doses or shorter delays were problematic since MET frequently had effects of its own and a delay was necessary to ensure that MET induced changes would not occur. The alpha antagonist more frequently blocked both PE and NE induced potentiation suggesting an effective dose and delay were employed.

To summarize, the present study presents preliminary evidence suggesting alpha antagonists may block the NE

potentiation effect, contrary to Lacaille and Harley (1985), whereas beta receptor antagonists may not do so as consistently as previously thought. Further in vivo pharmacological studies are clearly required. Given the variability in the present results with beta antagonists, conducting more experiments using the current protocol is of dubious value. A future elaboration of the current study might include examination of the effectiveness of MET using a narrower range of popspike sizes and latencies. The use of beta antagonists other than MET would also be helpful to evaluate the reliability of beta blockade of the potentiation with exogenously applied NE, in vivo.

NE Effects in Chronic Animals.

Intracerebroventricular administration of NE in the awake, behaving animal produced an enhancement of the population spike which was virtually indistinguishable from the effect of the same dosage in the anaesthetized animal. This result is encouraging since the bulk of NE studies have been done in the anaesthetized preparation. The present report of potentiation induced by exogenous NE application in the awake animal corresponds well with the report of Klukowski (1993) of potentiation of the popspike amplitude in awake animals following glutamate injection in the vicinity of the

locus coeruleus in order to stimulate endogenous NE release. While some of the icv injections produced a long-lasting (greater than 30 minutes) enhancement, no effect persisted for longer than 1 hour. It should be noted that only the lowest effective dose of NE from experiments in the anaesthetized rat was used. In the anaesthetized preparation, higher NE doses and the selective beta agonist ISO more frequently produced long-lasting potentiation. A future study employing these doses is needed in order to evaluate the length of time that the potentiation may last before decay. This is particulary relevant in reference to possible involvement of NE in cellular mechanisms of memory.

Mechanisms of Potentiation.

Any changes initiated by NE presumably involve second messenger activation. The beta-mediated NE effect has been documented as activating cAMP (cyclic Adenosine monophosphate). Stanton and Sarvey (1985b) showed that NE superfusion produced a 3-fold rise in cAMP levels in the dentate gyrus <u>in vitro</u>. Furthermore, another slice study by the same authors showed that the protein synthesis inhibitor, emetine, blocked the long-lasting facilitation induced by NE (Stanton and Sarvey, 1985a). Preincubation with forskolin, an adenylate cyclase activator, shifted the dose response curve

to the left and the potentiation was blocked by propranolol and MET leading the authors to conclude that the NE effect was mediated by beta-1 stimulation of adenylate cyclase. A presynaptic effect is also possible since NE has been shown to enhance a K^+ induced, Ca^{2+} dependant release of glutamate in the dentate gyrus in vitro (Lynch and Bliss, 1986). This effect was mimicked by isoprenaline (beta agonist) but not PE and was partially antagonized by propranolol but not PHENT.

The focus on beta mediation of NE effects has led to an absence of studies concerning NE effects on alpha receptor mediated second messengers. However, it is possible that alpha receptor stimulation of inositol triphosphate accumulation or effects on the beta mediated accumulation of cAMP may be involved in the NE potentiation phenomenon. In slices from cerebral cortex, NE caused a larger increase in cAMP than ISO and the addition of alpha-1 agonists elevated the cAMP response to ISO to the level seen with NE alone. In addition, alpha-1 agonists caused a large increase in inositol triphosphate accumulation (Atkinson and Minneman, 1991). Mailleux et al. (1991) showed high levels of inositol 1,4,5-triphosphate 3-kinase mRNA exist in granule cells of the dentate gyrus. Alpha mediated increases in inositol triphosphate might serve a helper function by increasing calcium levels after NE application, a possibility suggested by the shorter term nature of PE potentiation.

Summary of Pharmacology.

To summarize, results of the icv pharmacological investigation of NE potentiation of dentate gyrus popspikes suggest a possible involvement of both beta and alpha receptors. Alpha agonists frequently enhanced the popspike amplitude, although not as reliably as beta agonists or NE itself. However, alpha and beta receptor effects do not appear to sum since they both produced potentiation of the same magnitude as NE itself. As well, beta and alpha antagonists both blocked NE potentiation in a majority but not all experiments (only the alpha antagonism was significant, however). This picture of receptor mediation would bring NE effects in the hippocampus more in line with other brain areas where alpha receptors generally potentiate excitation. The suggestion of both beta and alpha mediated enhancement of the popspike lies somewhat intermediate between the findings of a previous <u>in vitro</u> study suggesting beta mediation (Lacaille and Harley, 1985) and a previous <u>in vivo</u> study suggesting alpha mediation (Winson and Dahl, 1985).

Temperature Effects in Acute Animals.

There are alternate explanations for some of the phenomena occurring in this study. It is possible that an

alpha mediated drop in temperature is responsible for the potentiation frequently seen. Body temperature was generally held constant. However, sharp drops in temperature often occurred after PE injections. This temperature drop was prevented from exceeding 0.3 °C by increasing the flow of hot water through the blanket underneath the animal. However, this might not have precluded a larger drop in brain temperature. Moser, Mathiesen and Andersen (1993) report that lower brain temperatures in awake rats produced larger popspikes, increased latency to popspike and decreased EPSP slope (positive slope). The converse was true of increased brain temperatures which would arise from increased motor activity. If the PE enhancement of popspike in the present study was due to an alpha mediated drop in brain temperature, we would expect a concurrent increase in latency and decrease in EPSP slope. In the cases where PE produced significant enhancement of the popspike, the EPSP decreased on only 3 of 10 occasions whereas the popspike onset latency was almost always increased (delayed). The EPSP changes do not support a temperature decrease whereas the latency changes are consistent with such a mechanism. Clearly, future studies should involve monitoring brain temperature. It should be noted, however, that the Dahl and Winson (1985) iontophoretic study presumably did not involve temperature changes but also reported an alpha mediated enhancement. As well, the blocking of NE enhancement

by an alpha antagonist is more difficult to reconcile by means of a temperature drop explanation. It should also be noted that the changes in brain temperature in the Moser et al. (1993) study were very large (about 5 °C). Furthermore, the magnitude of the latency changes (6%) was small in the present study relative to the change in popspike amplitude (approximately 50%) whereas the temperature induced changes in popspike amplitude and onset latency were of similar magnitude in the Moser et al. (1993) report.

Other Alternate Explanations in Acute Animals.

Another concern with intraventricular administration of drugs is the possibility of effects on non-hippocampal structures. Harley et al. (1993) performed icv injections of NE while recording popspikes from the hippocampus of anaesthetized rats employing similar methodology to this study. In vivo microdialysis revealed that NE levels increased markedly in the hippocampus following NE injection into the lateral ventricle. More importantly, substantial NE levels in the hippocampus appeared to be critical for NE potentiation to occur. When NE was injected into the medial ventricular system, NE levels were presumably elevated in non-hippocampal areas including the hypothalamus, but no potentiation of the popspike occurred. This supports the notion of direct effects

of NE upon the hippocampus although indirect effects of the various agonists cannot be ruled out.

This line of reasoning would tend to discount the involvement of blood pressure changes or the autonomic nervous system in the effect of NE on the dentate gyrus evoked potential. However, vasoconstriction or effects on the release of other neurotransmitters or neuropeptides could be involved in the NE induced potentiation within the hippocampus itself. The former explanation was not assessed and there is little experimental work which addresses the effect of vasoconstriction on the evoked potential. The latter explanation does not discount the present work since this study does not necessarily imply a direct cellular effect of NE on granule cell neurons. The effect may be due to effects on inhibitory neurons or the release of other substances.

Alternate Explanations in Chronic Animals.

In awake, behaving rats, an additional source of popspike variability is changes in behavioral state of the animal. Changes in behavioral state with removal of the animal from the home cage may occur. As well, animals might experience a drop in body temperature after prolonged immobility in the metal and Plexiglas recording chamber. In order to exclude the possibility that effects were occurring because of these

sources and not NE itself, recordings were taken from animals at a set time after removal from the home cage. Furthermore, animals were recorded after injection of saline at the same time as NE injection would occur without any resulting popspike amplitude changes.

Another source of behavioral interference with the popspike amplitude would occur if handling during the injection or the NE injection itself produced effects on behaviour which subsequently produced changes on the popspike. Hargreaves et al. (1990) have reported that popspikes were smaller during walking behaviour rather than during immobility. Winson and Azbug (1978) reported that the popspike was greater in magnitude during slow wave sleep than during the still, alert condition whereas the popspikes during REM sleep and voluntary movement were intermediate in amplitude and also more variable. Buzsaki et al. (1981) reported that the rank order of popspike amplitude during different behaviours was drinking > grooming > pressing > running, although only the difference between drinking and running was significant.

Behavioral effects due to handling or vehicle injection are unlikely to be responsible for the potentiation since no enhancement followed saline injection. Behavioral changes after saline were also relatively minor. NE itself appeared to cause large changes in behaviour; it mainly increased the

amount of locomotory behaviour. Is it possible that the NE induced behavioral changes are responsible for the popspike enhancement? This appears unlikely since an increase in walking or voluntary movement relative to immobility or the still, alert state should decrease the popspike according to Hargreaves et al. (1990) and Winson and Azbug (1978). This makes intuitive sense since an increase in activity would increase muscular activity thus increasing brain temperature which would have the opposite effect of depressing the popspike (Moser et al., 1993). This explanation also appears unlikely since the magnitude of popspike amplitude was not clearly associated with any particular behaviour. Popspike amplitude was elevated following NE injection during all types of behaviour. Finally, and perhaps most convincingly, the magnitude and time course of NE potentiation was virtually indistinguishable from the effect of the same dose in the anaesthetized animal where behavioral modification of the popspike was not possible.

Behavioral Effects in Chronic Animals.

The behavioral effects of icv NE in the awake animals consisted mainly of increases in locomotory behaviour and decreases in grooming and alert immobility (no movement, eyes open). In contrast, saline injections were followed by a

smaller decrease in the proportion of time spent in alert immobility and an increase in grooming. This corresponds well with previous reports that NE injection in the lateral ventricles or hippocampus produces an increase in exploratory behaviour. Herman (1973) reported that 10 and 50 μg icv doses of NE produced a significant increase in walking and decrease in immobility. Flicker and Geyer (1982) reported that 30 to 300 ng doses of a racemic mixture of NE continuously infused directly into the hippocampus over 40 minutes produced an increase in diversive exploration manifested as an increase in centre entries, hole poking and rearing behaviour without a corresponding increase in overall motor behaviour. Segal and Mandell (1970) reported that the racemic mixture of NE infused icv at a rate of 10 or 60 μg per hour for two hours produced an increase in general activity and locomotion without evidence of bizarre behaviour patterns such as circling or ataxia. It is interesting to note that some instances of ataxia, facial fasciculation, mastication and postural abnormalities were recorded in this study following NE injection in the awake animal. This may be because of the larger bolus received during two minutes rather than continuous infusion. Klukowski (1993) also noted the occurrence of postural asymmetries and facial fasciculation following glutamate injection in the area of the locus coeruleus. Weiss et al. (1986) reported a contrasting finding.

A 10 μg icv dose of the pure isomer of NE produced inactivity on a swim test without changing spontaneous activity levels. Oddly, both alpha and beta agonists caused inactivity on the swim test and a decrease in spontaneous activity. In addition to the correspondence between behavioral effects, these studies also used icv doses in the range of those employed here. This makes it possible to compare the effects of icv NE on electrophysiology with the literature concerning behavioral changes.

EPSP Slope.

EPSP changes were variable following NE and agonist infusions in the lateral ventricle in the anaesthetized preparation. There were a similar number of increases and decreases in EPSP slope while the most common effect was an absence of change. This contrasts with slice experiments where the EPSP was frequently facilitated (Lacaille and Harley, 1985; Burgard, Decker and Sarvey, 1989; Stanton and Sarvey, 1987) and agrees with most <u>in vivo</u> reports of inconsistent effects of NE on the EPSP slope (Neuman and Harley, 1983; Dahl and Winson, 1985; Winson and Dahl, 1985; Harley, Milway and Lacaille, 1989; Washburn and Moises, 1989; Babstock and Harley, 1992). Our results do not concur with the minority of <u>in vivo</u> studies which found EPSP increases on a

majority of experiments (Harley and Milway, 1986; Harley and Evans, 1988). Overall, it seems clear that the picture of EPSP modulation presented in the slice does not apply to the intact animal. This calls into question whether the picture of solely beta mediated popspike enhancement seen *in vitro* applies to the intact animal.

An alternate methodological explanation could be that slice studies involve more selective activation of medial perforant path fibres whereas *in vivo* experiments involve stimulation of mixed medial and lateral perforant path fibres. Dahl and Sarvey (1989) demonstrated that NE selectively potentiates medial perforant path evoked EPSP's while depressing EPSP's elicited by lateral perforant path stimulation. Work in progress in this laboratory concerning the effects of *in vivo* PGI stimulation on the popspikes selectively activated by either lateral or medial inputs should shed some light on this issue. It is also unclear if changes in the dendritic EPSP would be reflected in the positive EPSP recorded at the granule cell layer (Winson and Dahl, 1985, Dahl and Winson, 1985). This is an important consideration since, as stated in the introduction, most *in vivo* studies recorded from the cell bodies whereas *in vitro* studies recorded from the dendrites.

Popspike Onset Latency.

Changes in popspike onset latency were more consistent and notably included differential effects depending upon the receptor activated. NE and the alpha agonist PE produced a longer delay to the onset of the popspike whereas the beta agonist ISO caused a faster popspike onset latency to occur. The two reports of consistent latency changes have been decreases (Lacaille and Harley, 1985; Babstock and Harley, 1992) which is consistent with the effect of ISO in our anaesthetized animals. Whether the increases in latency following NE and PE infusion are due to a differential effect of alpha and beta receptors on popspike amplitude or due to an alpha mediated temperature decrease remains to be determined.

Relevance of NE Effects to Information Processing.

The question that naturally arises given the consistent NE enhancement of popspike amplitude in the dentate gyrus is what does this effect represent for normal processing in the mammalian brain? Winson and Dahl (1985) propose that the function of NE in the dentate gyrus is "modulation of granule cell excitability with behavioral state" (p. 505). According to this view, when the animal is alert the LC is more active causing more NE release in the dentate gyrus which would lead

to better information transfer through the hippocampus. Obviously, it would be advantageous to have better information transfer during exploration and food getting than during resting, etc. This could also explain why memory of dreams (which typically occur during periods of LC inactivity) or events during hypnosis is poor compared to normal functioning.

It has been suggested that NE may act to decrease spontaneous activity in the hippocampus while at the same time increasing evoked activity. i.e., NE may facilitate information transmission through the hippocampus by increasing the signal to noise ratio (see Harley, 1987).

The finding of simultaneous medial perforant path potentiation and lateral perforant path depression of evoked popspikes suggests another possible interpretation of NE's function in the dentate gyrus (Dahl and Sarvey, 1989). They conclude that "an NE-induced enhancement of medial PP activation would presumably strengthen multimodal input to granule cells over the concurrently depressed olfactory and subcortical input via the lateral PP." (p. 4779). "With respect to learning and memory, NE may selectively enhance specific intracortical circuits against a background of NE-induced suppression of neuronal activity." (p. 4779). The medial perforant path carries non-olfactory sensory information from the cortex whereas the lateral perforant path carries information from olfactory areas and sub-cortical

structures (Cragg, 1961; Krettek and Price, 1977). Lastly, NE released in the dentate gyrus may be an important component of memory formation in the mammalian brain through its direct enhancement of information transmission in the first stage of the hippocampal circuit which could promote the formation of more permanent changes in the cortex. The NE potentiation phenomenon might be incorporated into the Buzsaki theory of hippocampal functioning (Buzsaki, 1989; Buzsaki and Czeh, 1992) by temporarily strengthening connections of granule cells until the sharp wave stage during sleep, immobility, etc. Furthermore, it would act to sharpen the representation of novel stimuli and sharpen the signal to noise ratio to prevent the activation of a too diffuse net in CA3 of the hippocampus.

Summary and Future Experiments.

The results of this study support a role for beta receptor activation in NE potentiation of perforant path evoked-dentate gyrus population spikes. The results also suggest alpha receptors may promote potentiation. While temperature effects need to be more critically assessed, the effect of alpha antagonists supports this inference. The ability of either agonist to promote NE effects and the lack of evidence of summation effects suggests that both receptors

converge on the same mechanisms of plasticity. Given the suggestion of alpha receptor involvement in this study, further studies of LC and PGI activation should include the use of alpha as well as beta antagonists. As well, further *in vivo* studies of the pharmacology of NE potentiation are necessary.

Icv NE offers a promising route to study NE induced long-lasting potentiation in the *in vivo* preparation. Potentiation has been clearly demonstrated with exogenous NE application in the awake, behaving animal. A future study in the awake animal should infuse higher NE doses and the beta agonist, ISO, to assess the long term profile of popspike potentiation over hours or days. While progress has been made, further experiments are needed to clarify the role of NE in hippocampal information transmission.

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APPENDIX I

Popspike amplitude raw means and standard deviations for the ten minute control period prior to drug injections. Change in popspike represents significant deviations from baseline that occurred after drug injection.

Drug Dose	Change in Popspike	Mean (uV)	St. Dev. (uV)	n
saline	All	3298.0	2138.8	8
NE 1 ug	All	4770.1	1536.9	4
NE 10 ug	All	1889.9	440.5	4
NE 50 ug	All	2701.5	2049.0	4
NE 100 ug	All	3713.9	2798.4	6
ISO 10 ug	All	2447.3	445.2	4
ISO 30 ug	All	2864.9	2098.8	4
ISO 60 ug	All	4953.7	3331.2	6
PE 10 ug	All	3271.5	1010.5	4
PE 50 ug	All	1555.1	332.3	4
PE 100 ug	All	5435.5	1476.4	4
PE 300 ug	All	4360.5	2001.3	8
PE 500 ug	All	3760.1	1420.4	6
PE - all doses	Increase	3880.5	1780.4	15
	No Increase	3662.3	1946.1	11
MET 10 ug	Increase	4339.3	3095.7	4
	No Increase	5809.6	4026.9	11
	All	5417.2	3753.3	15
ISO 30 ug (after MET)	Increase	5081.8	3390.4	2
	No Increase	8991.6	5769.5	2
	All	7036.7	4474.7	4
NE 10 ug (after MET)	Increase	2032.2	806.2	3
	No Increase	6189.2	4100.8	5
	All	4630.4	3797.9	8
PHENT 30 ug	Increase	1394.0	2.0	2
	No Increase	5488.4	3241.9	4
	All	4123.6	3282.7	6
PE 50 ug (after PHENT)	Increase	7778.2		1
	No Increase	4007.8	2820.9	3
	All	4950.4	2976.4	4
NE 10 ug (after PHENT) *	Increase	7220.6		1
	No Increase	2155.6	1320.8	3
	All	3421.8	2752.6	4

* $p < 0.05$, two sample t-test (increases compared to no increases)

APPENDIX II

Popspike onset latency raw means and standard deviations for the ten minute control period prior to drug injections. Change in popspike represents significant deviations from baseline that occurred after drug injection.

Drug Dose	Change in Popspike	Mean (us)	St. Dev. (us)	n
saline	All	3350.8	239.2	8
NE 1 ug	All	3527.5	244.9	4
NE 10 ug	All	3346.3	295.8	4
NE 50 ug	All	3595.6	443.5	4
NE 100 ug	All	3594.3	198.0	6
ISO 10 ug	All	3412.3	216.3	4
ISO 30 ug	All	3654.8	476.4	4
ISO 60 ug	All	3479.6	435.0	6
PE 10 ug	All	3655.7	279.5	4
PE 50 ug	All	3498.3	631.5	4
PE 100 ug	All	3179.0	272.9	4
PE 300 ug	All	3492.0	491.9	8
PE 500 ug	All	3421.2	373.7	6
PE - all doses	Increase	3459.1	487.0	15
	No Increase	3446.2	348.8	11
MET 10 ug	Increase	3418.6	187.2	4
	No Increase	3458.6	321.3	11
	All	3447.9	285.6	15
ISO 30 ug (after MET)	Increase	3296.2	38.9	2
	No Increase	3310.7	119.3	2
	All	3303.4	72.9	4
NE 10 ug (after MET)*	Increase	3626.9	65.5	3
	No Increase	3325.5	133.1	5
	All	3438.5	188.9	8
PHENT 30 ug	Increase	3536.0	154.1	2
	No Increase	3556.3	178.6	4
	All	3549.5	154.9	6
PE 50 ug (after PHENT)*	Increase	3877.0		1
	No Increase	3507.3	36.3	3
	All	3599.8	187.2	4
NE 10 ug (after PHENT)	Increase	3429.0		1
	No Increase	3393.7	276.8	3
	All	3402.5	226.7	4

* $p < 0.05$, two sample t-test (increases compared to no increases)

APPENDIX III

Examples of evoked potentials recorded chronically from each of five awake, behaving rats (Experiment II).

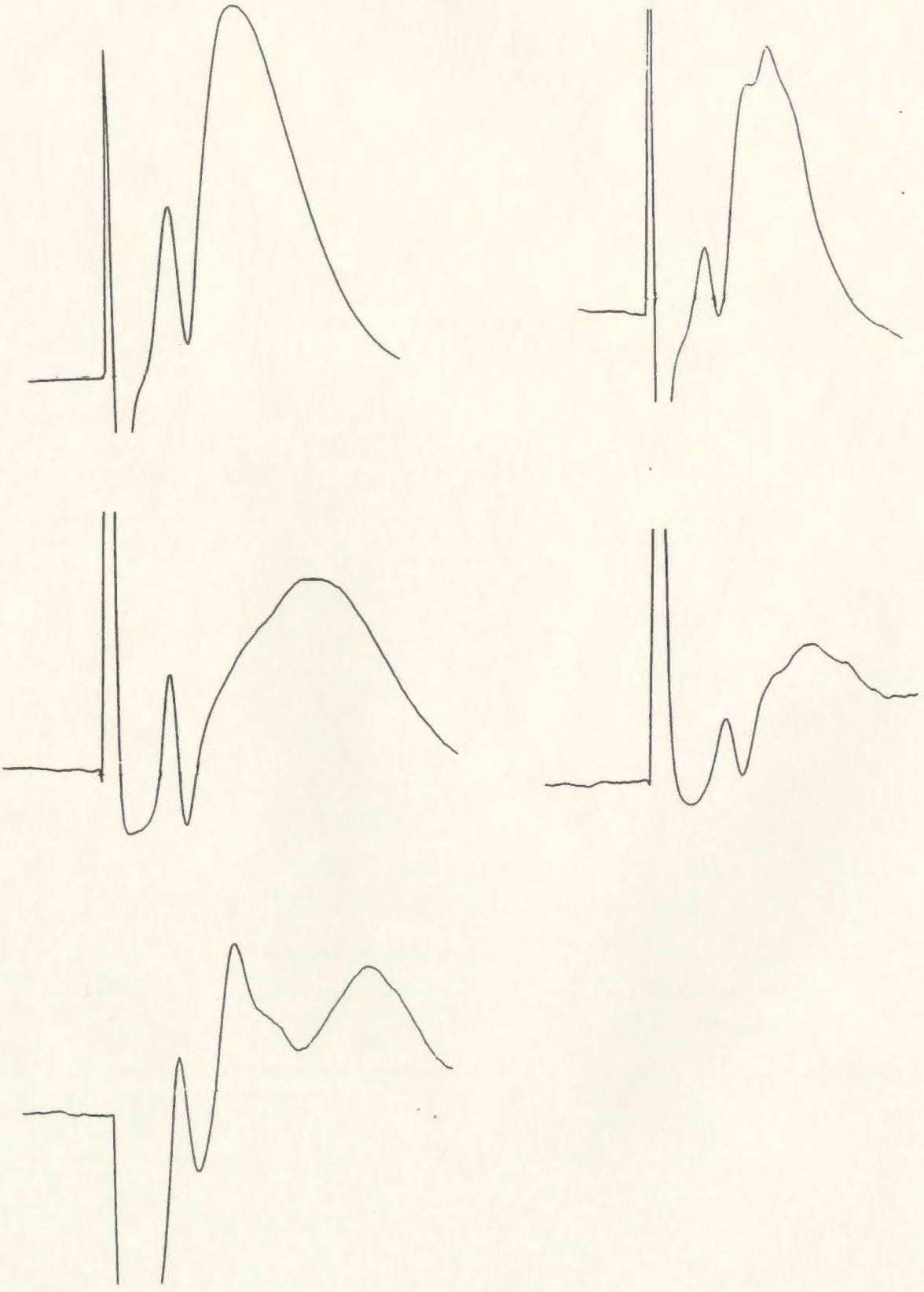


Table 1: The effect of saline and varying doses of NE on popspike amplitude, EPSP slope, and popspike onset latency following ICV injection in the anaesthetized rat. MEAN represents an average of the maximal change within 30 minutes post-injection that differs significantly from baseline. DELAY refers to an average of the time from injection until a significant effect. The number of significant effects of duration less than or greater than 20 minutes is listed. All doses are in ug.

		INCREASE				DECREASE				NO CHANGE
PARAMETER	DOSE	MEAN (%)	DELAY (min)	n<20 min	n>20 min	MEAN (%)	DELAY (min)	n<20 min	n>20 min	n/N
POPSPIKE	NaCl	43.9	21.5	1	1	16.4	6.0	1	1	4/8
	1					12.4	25.0	1		3/4
	10	55.8	9.3	4						0/4
	50	49.8	6.3	1	2					1/4
	100	46.5	4.5	2	4					0/6
EPSP	NaCl	18.0	6.8	1	3	11.6	11.0		2	2/8
	1					10.8	7.0	1	1	2/4
	10					16.0	1.5		2	2/4
	50									4/4
	100	13.3	11.0	1	2	12.0	3.0	1	1	1/4
LATENCY	NaCl					3.8	11.5		2	6/8
	1					2.5	16.0	2	1	1/4
	10	7.0	4.5	1	1					2/4
	50	6.1	5.7	1	2					1/4
	100	6.0	4.7	1	2	2.3	4.0	1		2/6

Table 2: The effect of varying doses of ISO (beta-agonist) on popspike amplitude, EPSP slope, and popspike onset latency following ICV injection in the anaesthetized rat. MEAN represents an average of the maximal change within 30 minutes post-injection that differs significantly from baseline. DELAY refers to an average of the time from injection until a significant effect. The number of significant effects of duration less than or greater than 20 minutes is listed. All doses are in ug.

		INCREASE				DECREASE				NO CHANGE
PARAMETER	DOSE	MEAN (%)	DELAY (min)	n<20 min	n>20 min	MEAN (%)	DELAY (min)	n<20 min	n>20 min	n/N
POPSPIKE	10	18.3	2.0		1	31.7	3.0	1		2/4
	30	45.8	12.0		4					0/4
	60	43.2	7.2	2	4					0/6
EPSP	10	9.9	15.5	1	1	11.2	3.0	1		1/4
	30	9.6	20.0		1	14.4	5.0	1	1	1/4
	60	13.3	5.7	1	2	41.8	2.0		1	2/6
LATENCY	10					5.5	13.3		3	1/4
	30	3.2	1.0	1		6.5	11.7		3	0/4
	60	5.5	7.0	1		6.3	3.2	1	4	0/6

Table 3: The effect of varying doses of PE (alpha-agonist) on popspike amplitude, EPSP slope, and popspike onset latency following ICV injection in the anaesthetized rat. MEAN represents an average of the maximal change within 30 minutes post-injection that differs significantly from baseline. DELAY refers to an average of the time from injection until a significant effect. The number of significant effects of duration less than or greater than 20 minutes is listed. All doses are in ug.

		INCREASE				DECREASE				NO CHANGE
PARAMETER	DOSE	MEAN (%)	DELAY (min)	n<20 min	n>20 min	MEAN (%)	DELAY (min)	n<20 min	n>20 min	n/N
POPSPIKE	10	43.6	17.5		2					2/4
	50	56.9	3.5		2	19.4	1.0	1		1/4
	100	35.0	11.7	3		15.6	1.0	1		0/4
	300	63.4	11.0	1	3	28.1	10.3		3	1/8
	500	50.2	5.3		4					2/6
EPSP	10	8.8	17.0	1		17.3	1.5	1	1	1/4
	50	34.6	1.0		1	16.8	14.0	2		1/4
	100									4/4
	300	62.4	4.0	1	1	16.1	3.0		2	4/8
	500	21.7	2.0	1	1	21.5	2.5	1	1	2/6
LATENCY	10	4.0	2.0	1						3/4
	50	5.4	9.0	1	2					1/4
	100	8.0	3.7	1	2					1/4
	300	5.5	4.2	1	5					2/8
	500	5.3	9.7		3	3.0	12.0	2		1/6

Table 4: The effect of MET (10 ug, beta-antagonist), ISO (30 ug, beta-agonist) alone and 30 min. after MET, and NE (10 ug) alone and 30 min. after MET on popspike amplitude following ICV injection in the anaesthetized rat. MEAN represents an average of the maximal change within 30 minutes post-injection that differs significantly from baseline. DELAY refers to an average of the time from injection until a significant effect. The number of significant effects of duration less than or greater than 20 minutes is listed.

Drug Condition	INCREASE				DECREASE				NO CHANGE
	MEAN (%)	DELAY (min)	n<20 min	n>20 min	MEAN (%)	DELAY (min)	n<20 min	n>20 min	n/N
MET	70.5	10.0	1	3	25.1	2.7	5	2	4/15
ISO ALONE	45.8	12.0		4					0/4
ISO AFTER MET	37.8	16.5		2					2/4
NE ALONE	55.8	9.3	4						0/4
NE AFTER MET	75.5	4.7		3	36.6	13.8		5	0/8

Table 5: The effect of PHENT (30 ug, alpha-antagonist), PE (50 ug, alpha-agonist) alone and 30 min. after PHENT, and NE (10 ug) alone and 15 min. after PHENT on popspike amplitude following ICV injection in the anaesthetized rat. MEAN represents an average of the maximal change within 30 minutes post-injection that differs significantly from baseline. DELAY refers to an average of the time from injection until a significant effect. The number of significant effects of duration less than or greater than 20 minutes is listed.

Drug Condition	INCREASE				DECREASE				NO CHANGE
	MEAN (%)	DELAY (min)	n<20 min	n>20 min	MEAN (%)	DELAY (min)	n<20 min	n>20 min	n/N
PHENT	98.2	6.0		2					4/6
PE ALONE	56.9	3.5		2	19.4	1.0	1		1/4
PE AFTER PHENT	45.2	1.0	1		61.2	7.0		1	2/4
NE ALONE	55.8	9.3	4						0/4
NE AFTER PHENT	37.8	10.0	1		63.8	1.0		2	1/4

Table 6: The effect of saline and NE (10 ug) on popspike amplitude following ICV injection in the awake, behaving (chronic) rat. The effect of NE (10 ug) on popspike amplitude in the anaesthetized (acute) rat is included for comparison. MEAN represents an average of the maximal change within 30 minutes post-injection that differs significantly from baseline. DELAY refers to an average of the time from injection until a significant effect. The number of significant effects of duration less than or greater than 20 minutes is listed.

		INCREASE			DECREASE			NO CHANGE
DRUG	DOSE	MEAN (%)	n<20 min	n>20 min	MEAN (%)	n<20 min	n>20 min	n/N
SALINE CHRONIC	2 ul				-32.7	1		1/2
NE CHRONIC	10 ug	46.0	4	1	44.9	1*		0/6
NE ACUTE	10 ug	55.8	4					0/4

* Followed by a significant increase at 90 and 180 min.

Table 7: The percent time spent engaging in various behaviours during ten minute intervals prior to and following ICV injections of NE (10 ug, n=5 animals, 7 injections) and saline (n=2) in the awake rat. Electrophysiological stimulation and recording were also taking place.

A) Norepinephrine (n=5).

Time Interval	No Movement	Head/Paw Movement	Grooming	Walking	Sleeping	Standing On Hind Legs	Chewing
Before	61.4	6.1	29.4	2.8	0.0	0.2	0.0
0 min After	26.7	10.2	2.1	53.4	0.0	4.9	2.6
10 min After	76.0	9.3	0.0	13.6	0.2	1.0	0.0
20 min After	64.1	8.3	4.0	5.5	18.1	0.0	0.0

B) Saline (n=2).

Time Interval	No Movement	Head/Paw Movement	Grooming	Walking	Sleeping	Standing On Hind Legs	Chewing
Before	70.8	8.4	12.5	5.8	0.0	2.5	0.0
0 min After	44.0	6.7	34.3	13.4	0.0	1.7	0.0
10 min After	90.8	5.9	0.9	1.7	0.9	0.0	0.0
20 min After	55.9	6.7	3.4	12.5	21.7	0.0	0.0

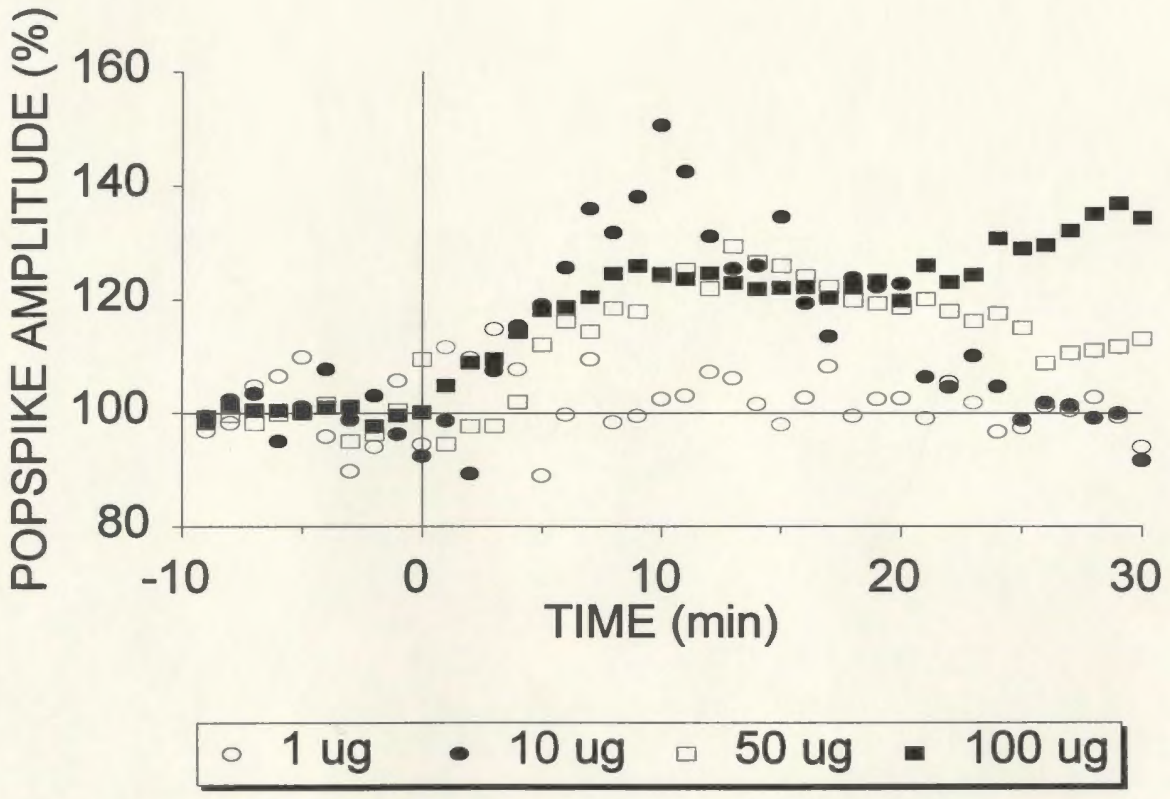


Figure 1: Mean popspike amplitude following NE 1 ug (n=4), 10 ug (n=4), 50 ug (n=4), and 100 ug (n=6) ICV injection in the anaesthetized rat at time zero. All results are plotted as a percentage of baseline.

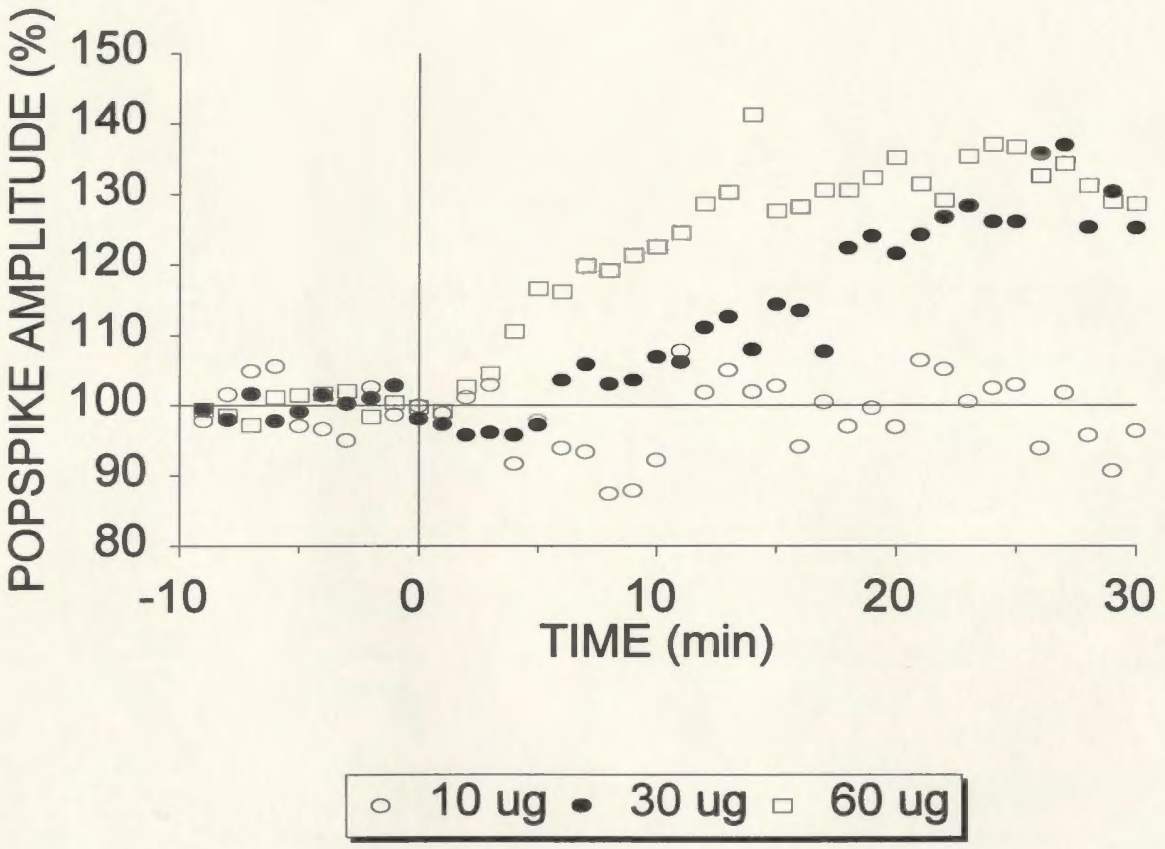


Figure 2: Mean popspike amplitude following ISO 10 ug (n=4), 30 ug (n=4), and 60 ug (n=4) ICV injection in the anaesthetized rat at time zero. All results are plotted as a percentage of baseline.

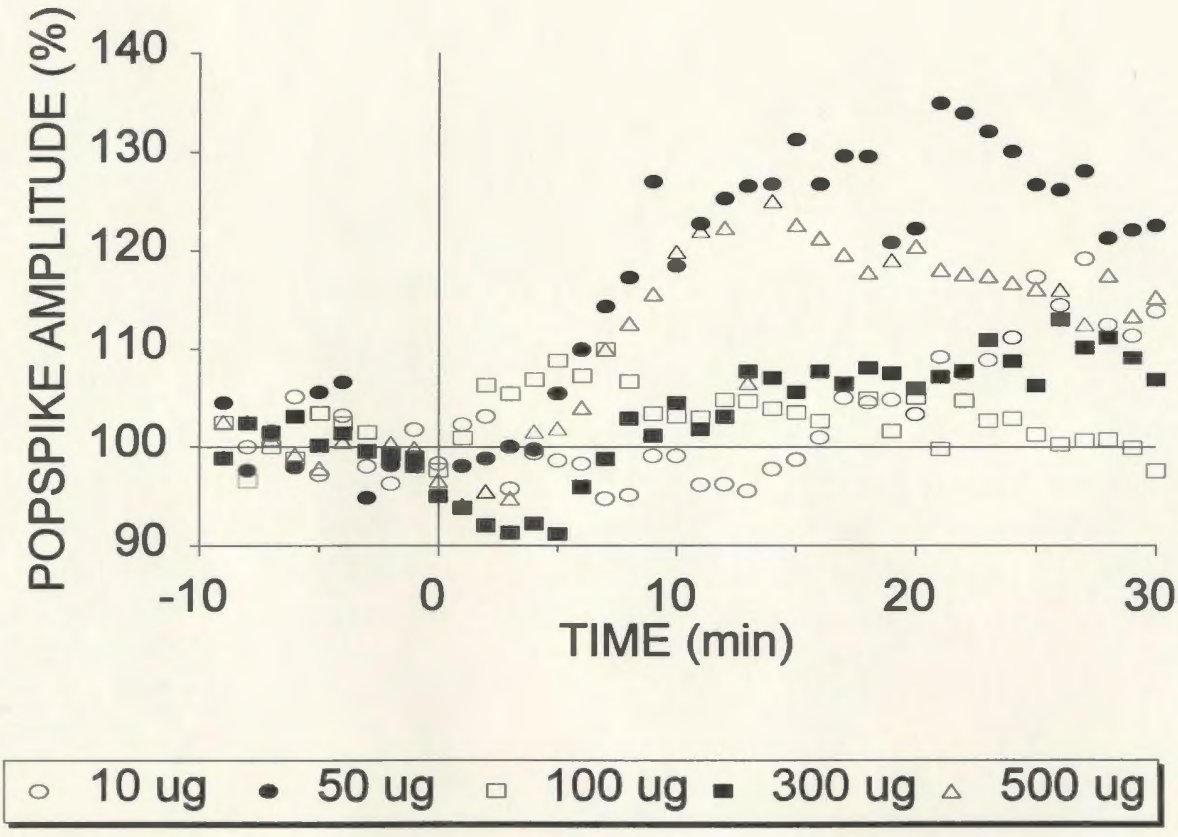


Figure 3: Mean popspike amplitude following PE 10 ug (n=4), 50 ug (n=4), 100 ug (n=4), 300 ug (n=6 animals, 8 injections) and 500 ug (n=6) ICV injection in the anaesthetized rat at time zero. All results are plotted as a percentage of baseline.

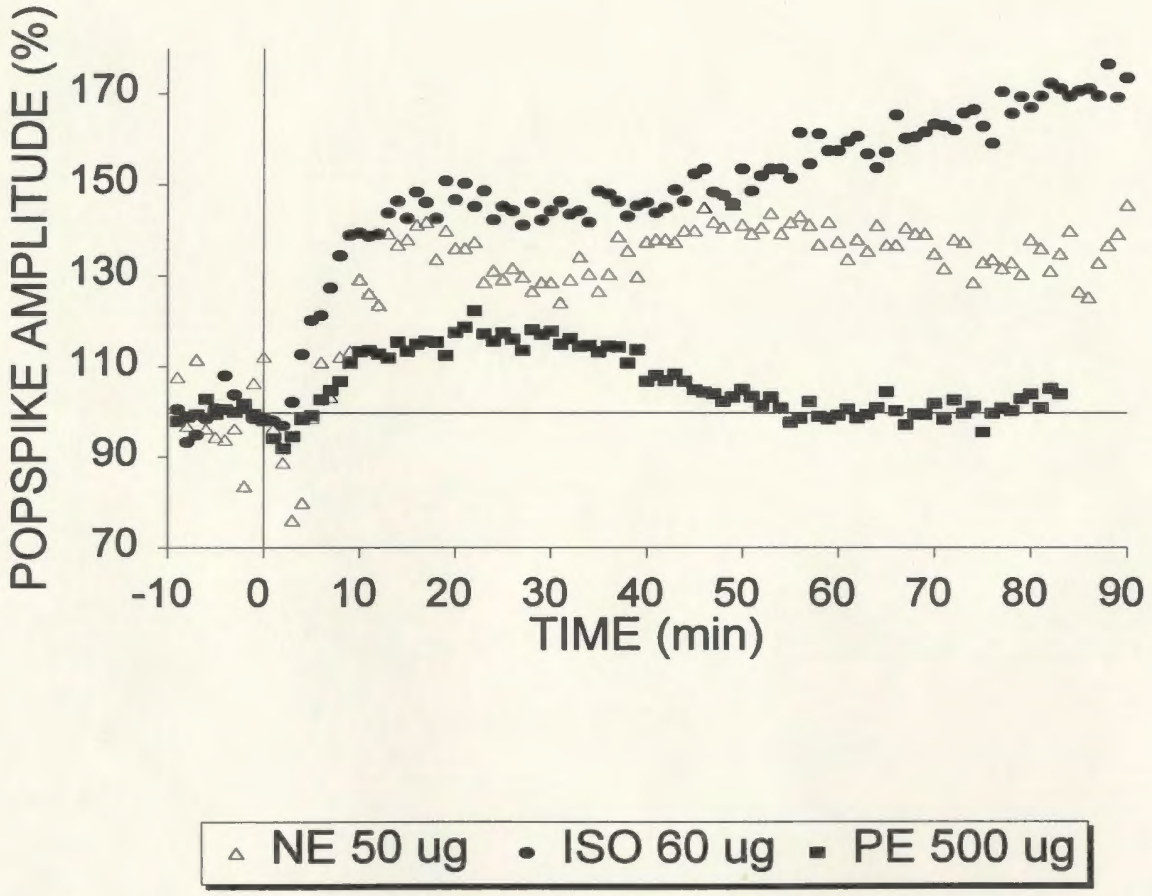


Figure 4: Examples of long-lasting potentiation of popspike amplitude following NE, ISO (beta-agonist), and PE (alpha-agonist) ICV injection in the anaesthetized rat at time zero. All results are plotted as a percentage of baseline.

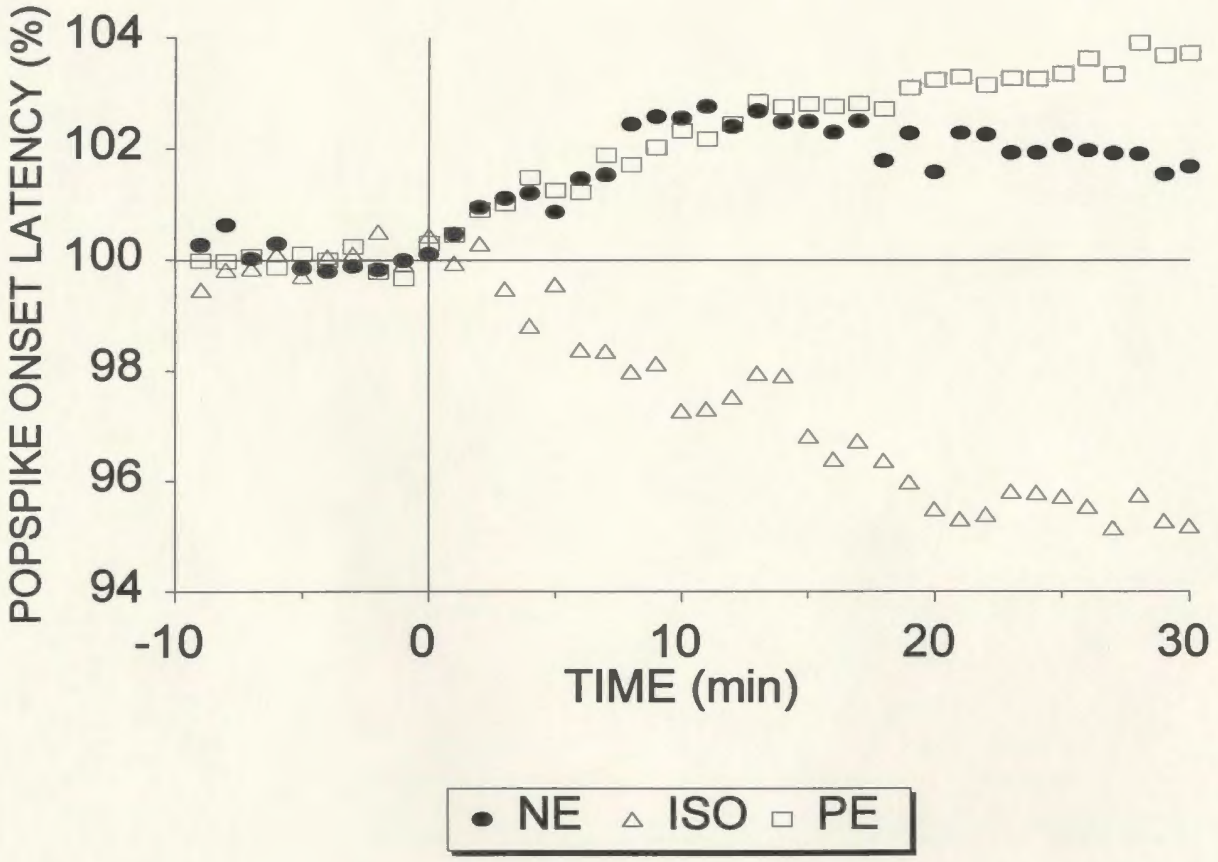


Figure 5: Changes in popspike onset latency following ICV injection of NE, ISO (beta-agonist), and PE (alpha-agonist) in the anaesthetized rat at time zero. Results are combined for all doses that produced significant potentiation of the popspike amplitude. All results are plotted as a percentage of baseline. (See also Tables 1 - 3.)

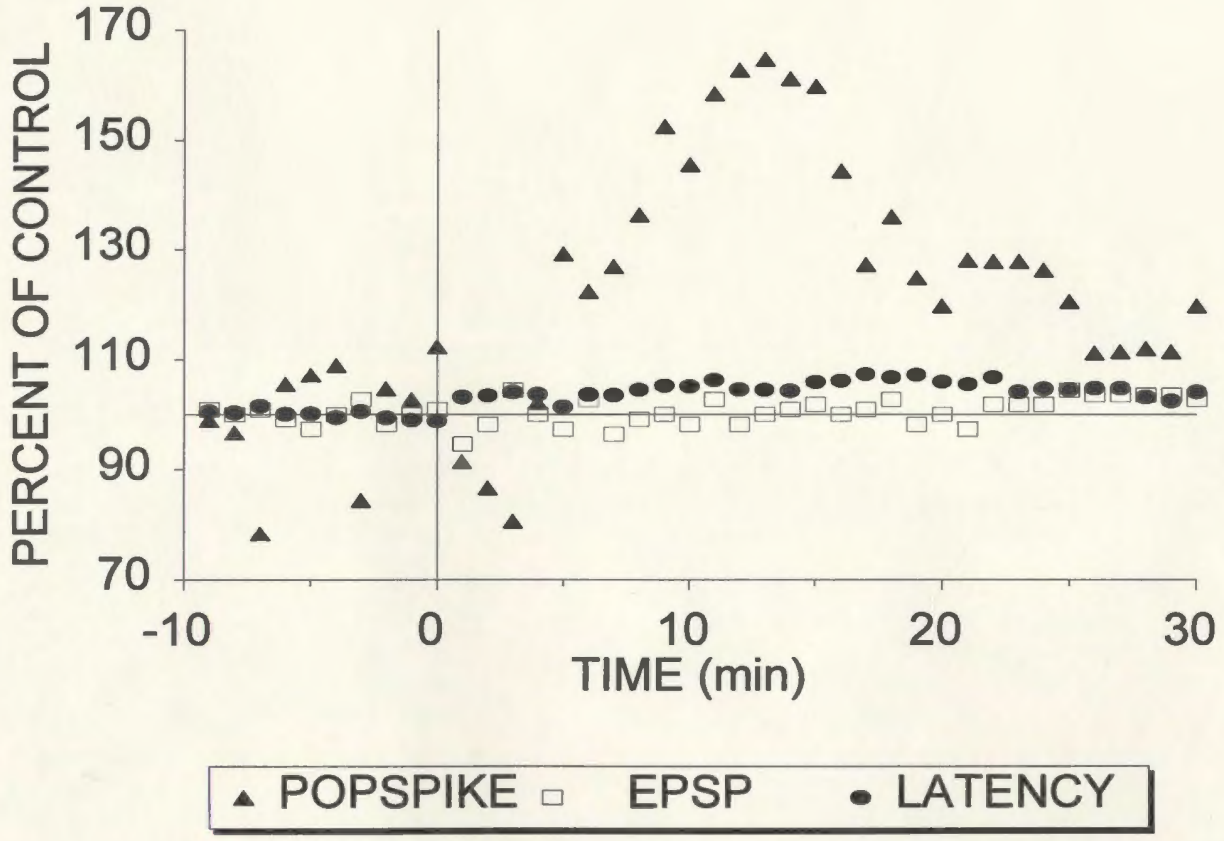


Figure 6: Example of the effect of a single NE ICV injection on popspike amplitude, EPSP slope, and popspike onset latency. All results are plotted as a percentage of baseline.

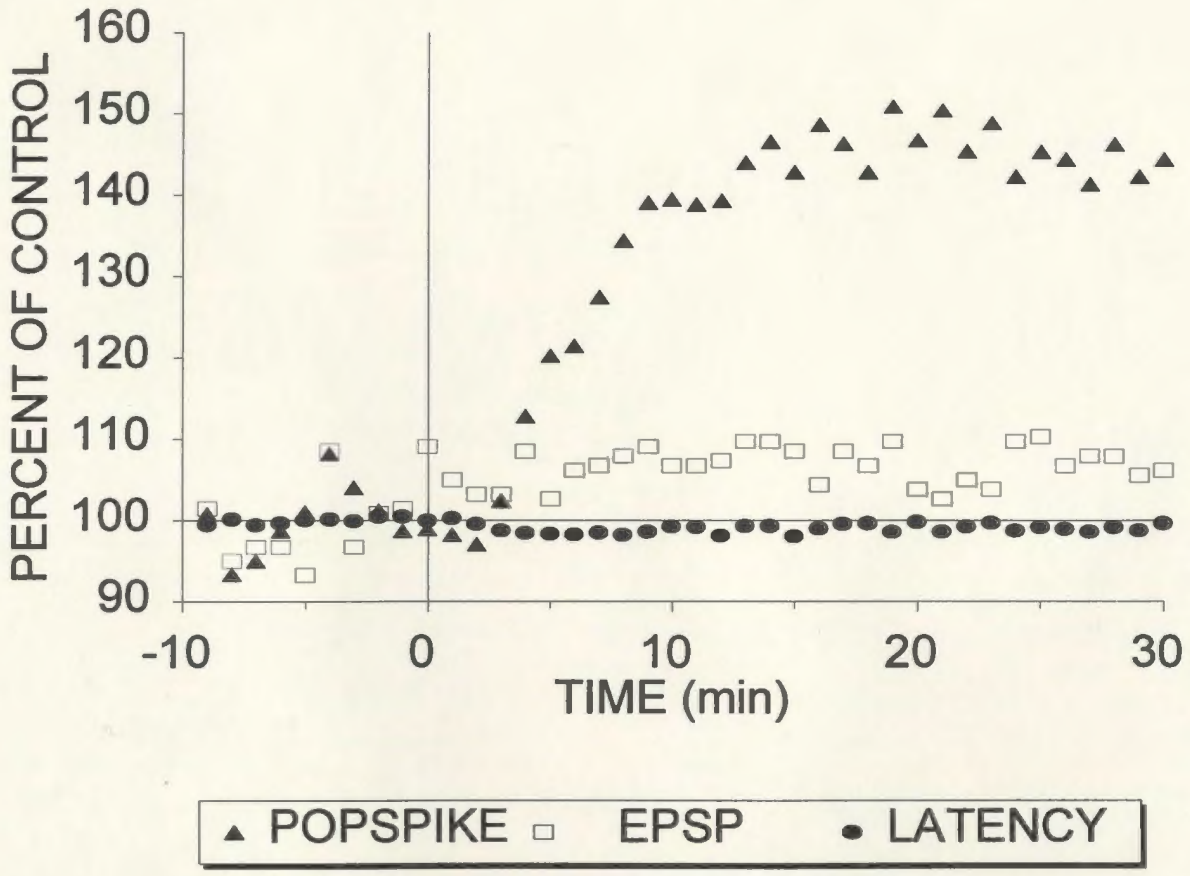


Figure 7: Example of the effect of a single ISO (beta-agonist) ICV injection on popspike amplitude, EPSP slope, and popspike onset latency. All results are plotted as a percentage of baseline.

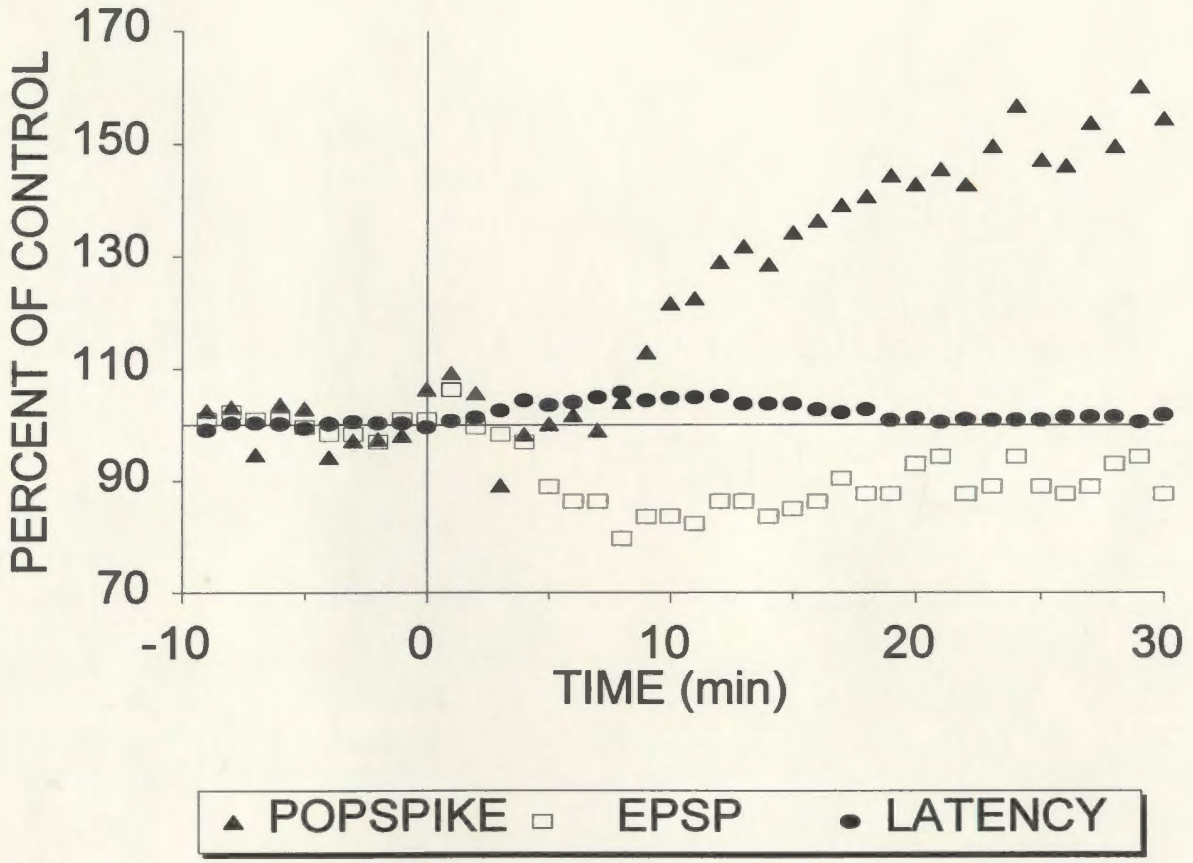


Figure 8: Example of the effect of a single PE (alpha-agonist) ICV injection on popspike amplitude, EPSP slope, and popspike onset latency. All results are plotted as a percentage of baseline.

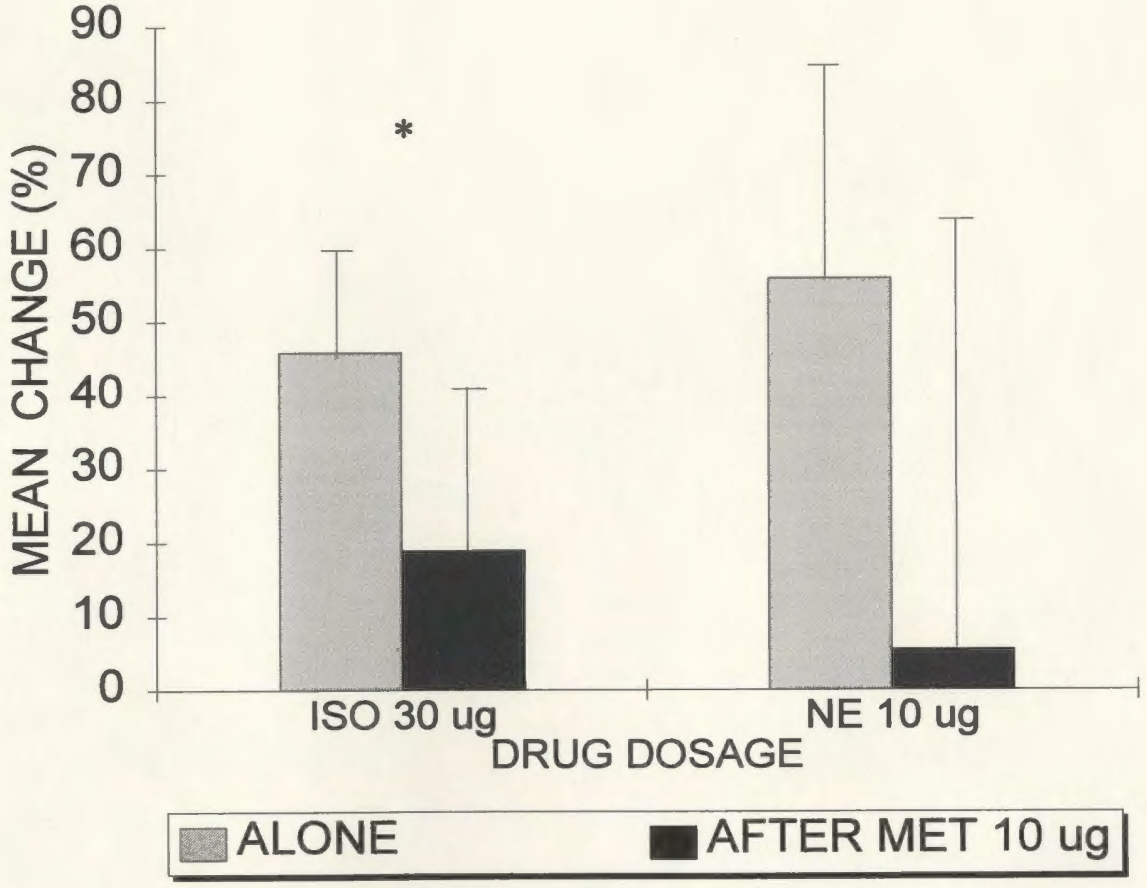


Figure 9: Comparison of the effect of ISO (beta-agonist) and NE, alone and following the injection of MET (beta-antagonist), on popspike amplitude in the anaesthetized rat. Means of the maximal effect within 30 min. of the ICV injection are presented. Vertical lines represent one standard deviation. * represents significant difference ($p < 0.05$).

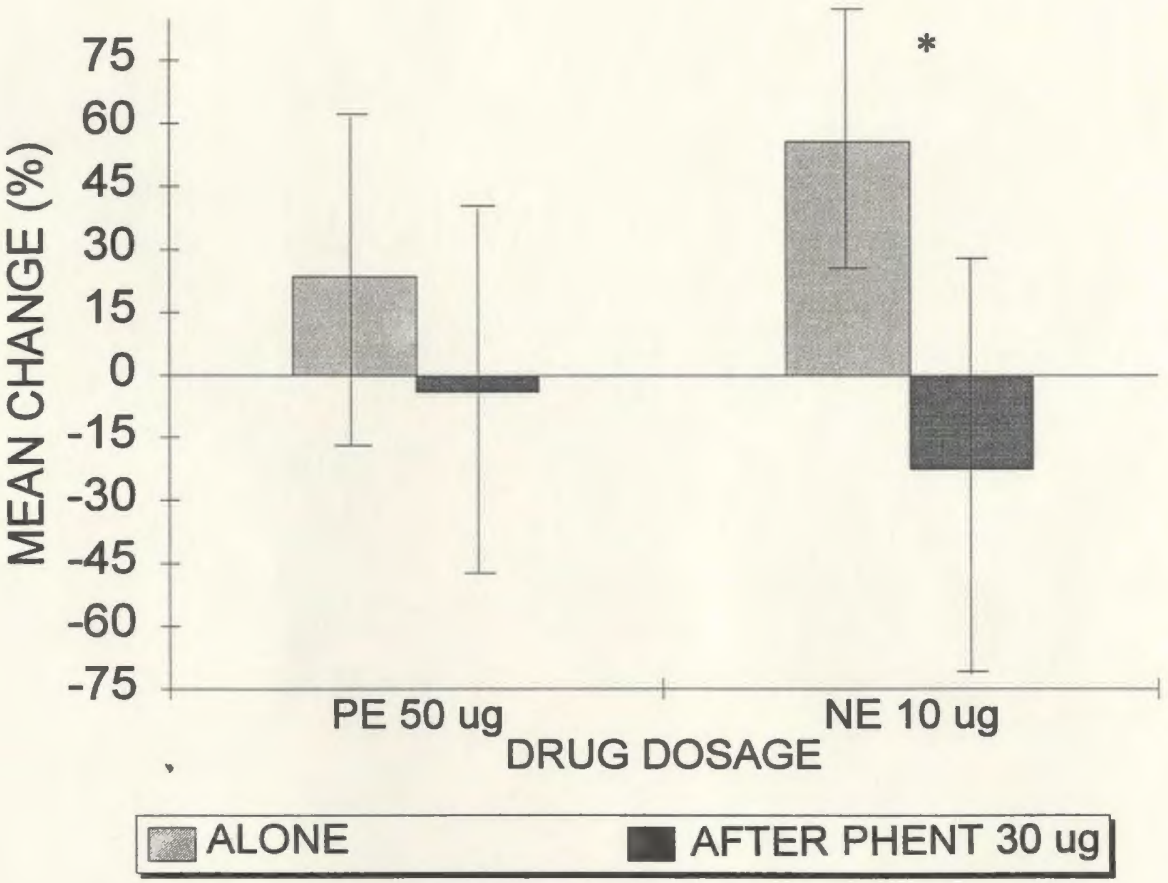


Figure 10: Comparison of the effect of PE (alpha-agonist) and NE, alone and following the injection of PHENT (alpha-antagonist), on popspike amplitude in the anaesthetized rat. Means of the maximal effect within 30 min. of the ICV injection are presented. Vertical lines represent plus or minus one standard deviation. * represents significant difference ($p < 0.05$).

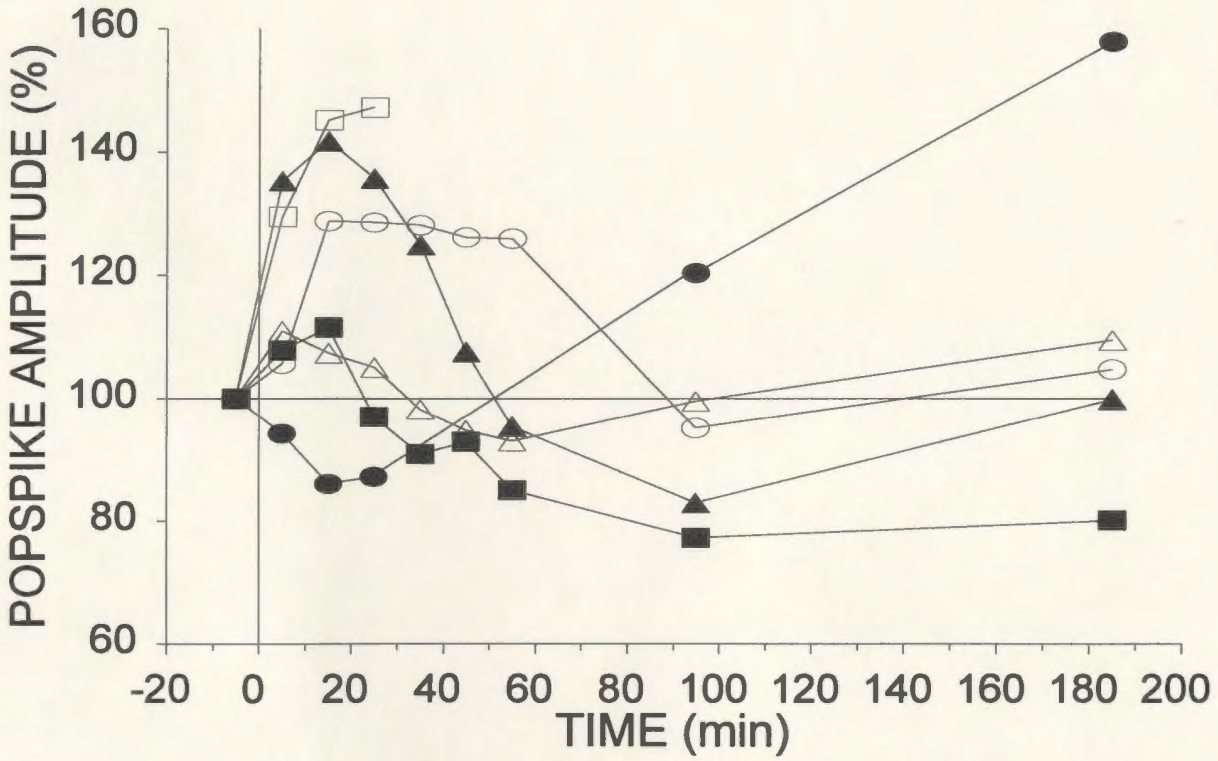


Figure 11: The effect of six individual NE (10 ug) ICV injections on popspike amplitude in the awake, behaving rat. Results were analyzed as ten minute means and plotted as a percentage of the ten minute baseline period (the first point in each series).

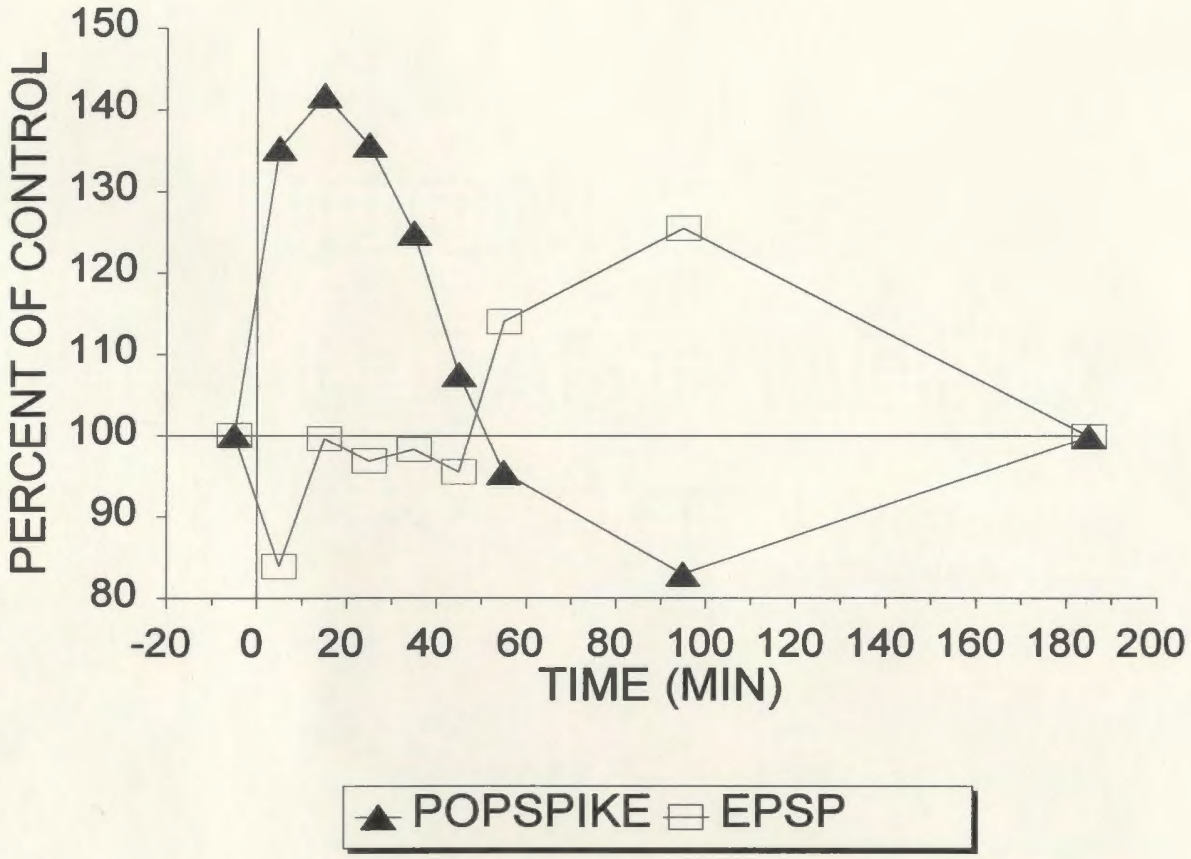


Figure 12: An example of the effect of NE (10 ug) ICV injection on popspike amplitude and EPSP slope in the awake, behaving rat. Results were analyzed as ten minute means and plotted as a percentage of the ten minute baseline period (the first point in each series).

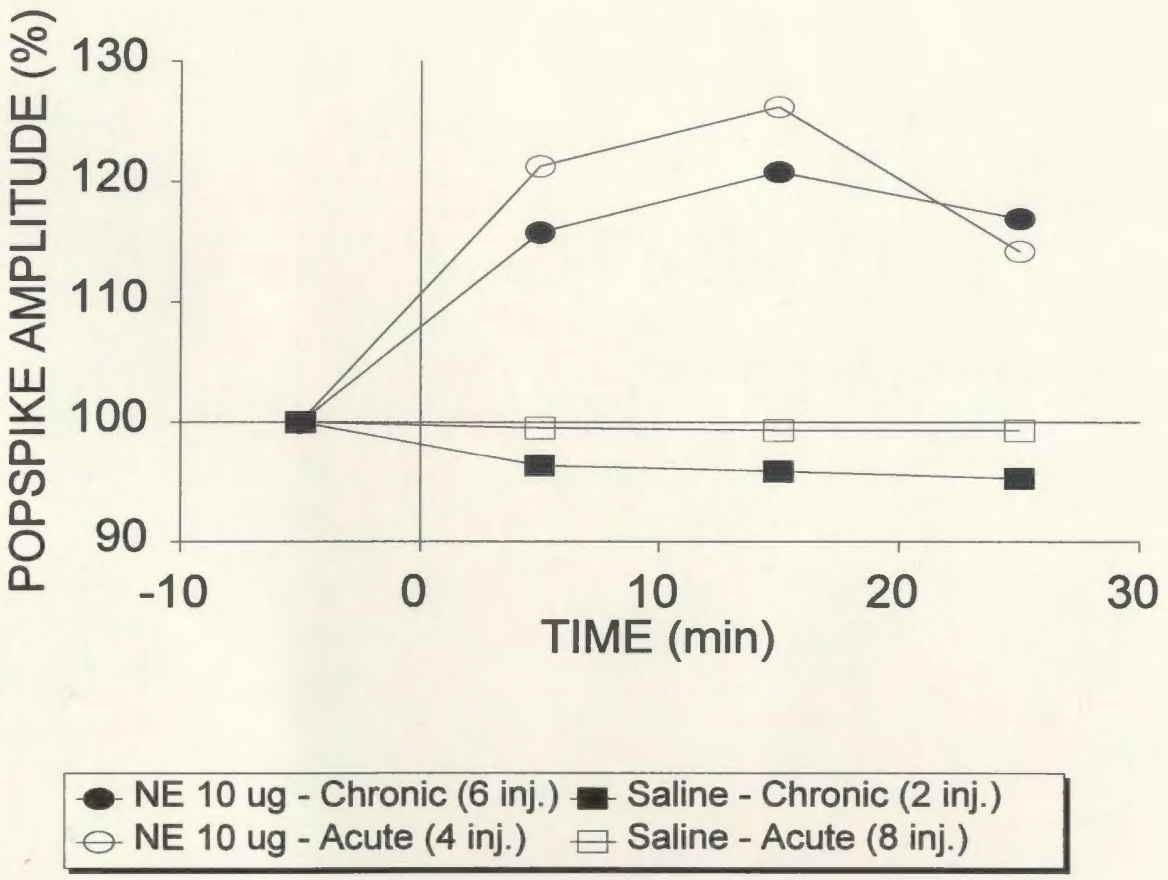


Figure 13: Comparison of the effect of saline and NE (10 ug) ICV injection on popspike amplitude in anaesthetized and awake, behaving rats. Results were averaged as ten minute means and plotted as a percentage of the ten minute baseline period (the first point in each series).

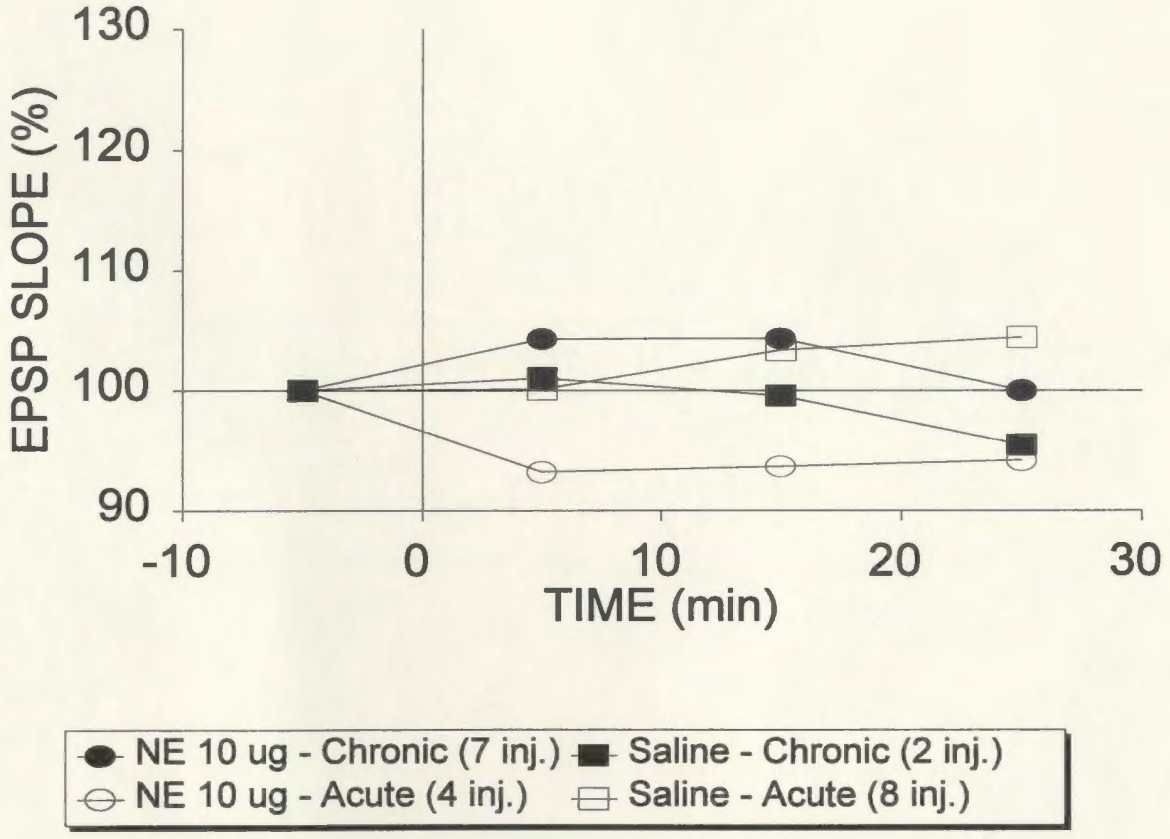


Figure 14: Comparison of the effect of saline and NE (10 ug) ICV injection on EPSP slope in anaesthetized and awake, behaving rats. Results were averaged as ten minute means and plotted as a percentage of the ten minute baseline period (the first point in each series). Note: Y-axis is on same scale as Figure 13.

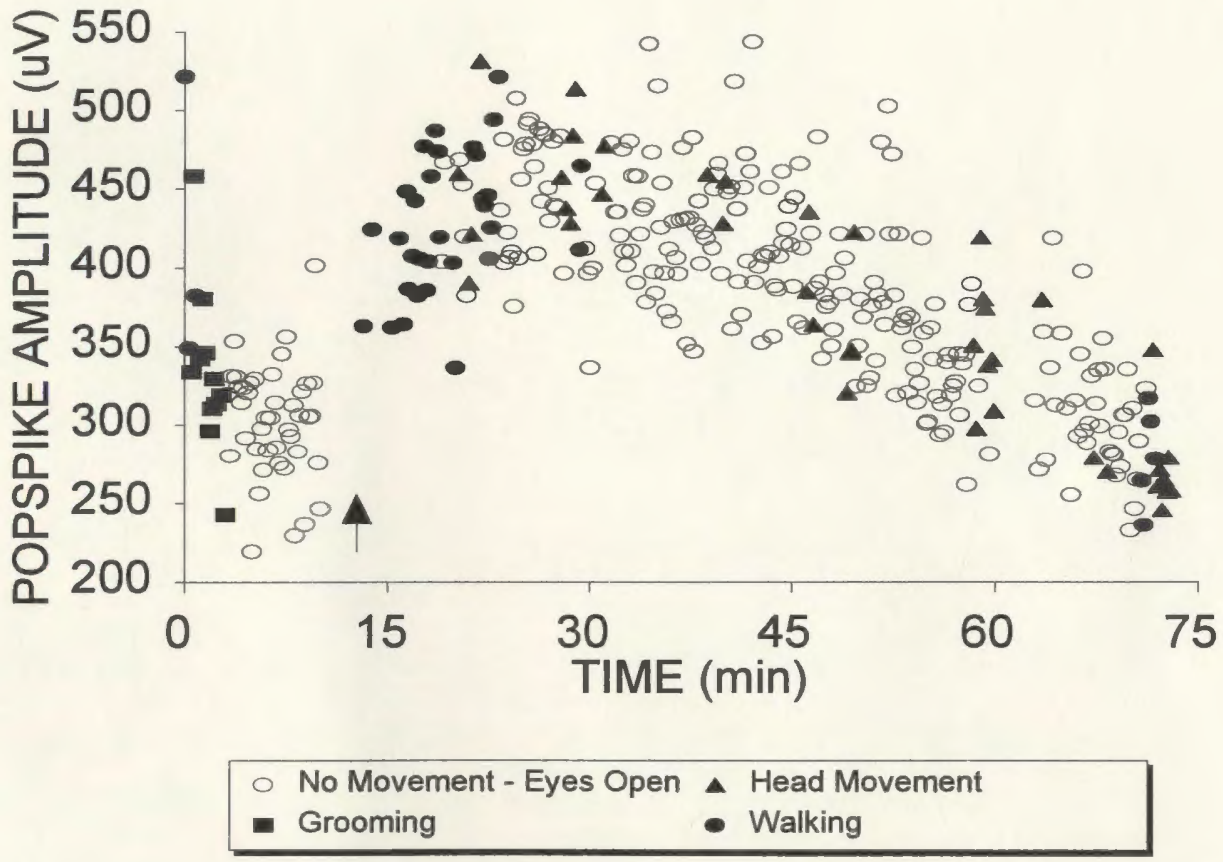


Figure 15: Example of the effect of NE (10 ug) ICV injection on popspike amplitude in an awake, behaving rat. The amplitude of individual popspikes is plotted separately for each category of behaviour. Injection was carried out at the end of the ten-minute baseline period (see arrow).

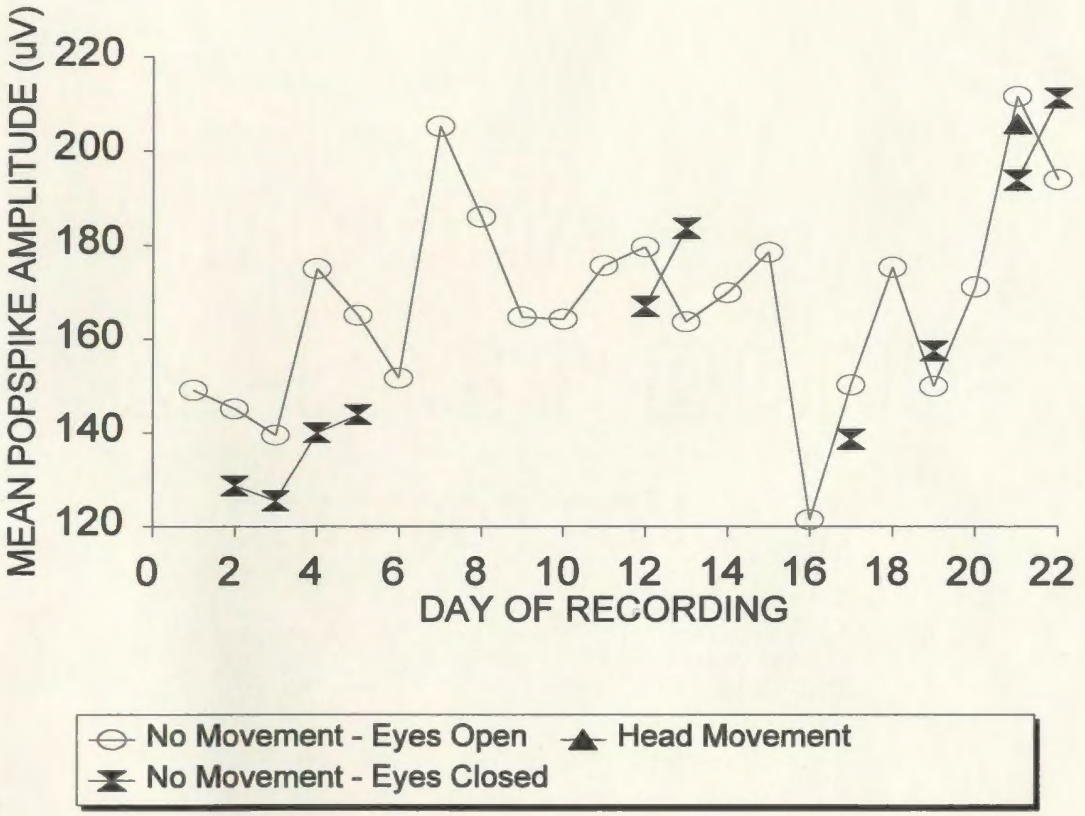
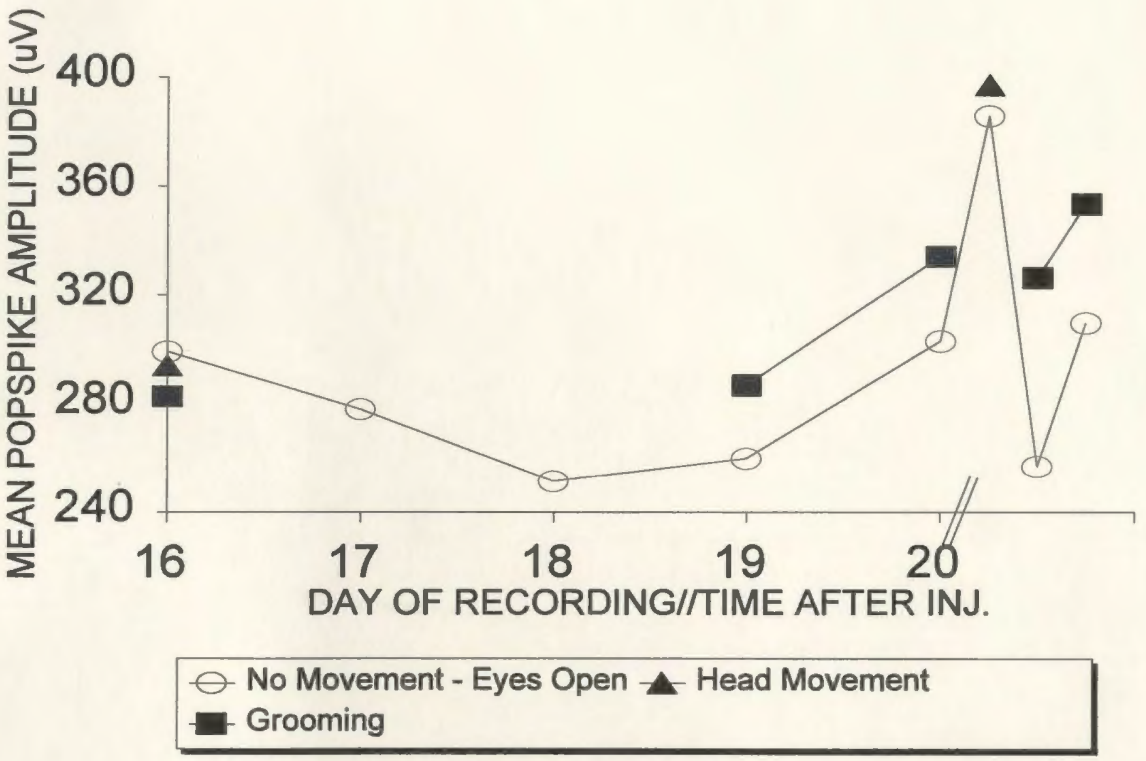


Figure 16: The mean popspike amplitude associated with different behaviours observed in the first ten minutes of recording over successive days in an awake, behaving rat.



Note: Last three points are 0-60, 90, 180 min after ne10 inj.

Figure 17: The mean popspike amplitude associated with different behaviours observed during recording over successive days in an awake, behaving rat. Note that the x-axis is discontinuous. The last four points represent the baseline period and three time periods following NE (10 ug) ICV injection on the same day.