EFFECTS OF IONTOPHORETICALLY ADMINISTERED NOREPINEPHRINE IN CAT SOMATOSENSORY CORTEX: MODULATION OF NEURONAL RESPONSES TO CUTANEOUS INPUT AND PROLONGED INCREASES OF GLUTAMATE-INDUCED EXCITATIONS

by

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To my father who would
have liked to see this work
completed and to my mother
who will appreciate it.

ABSTRACT

This work describes the neuromodulatory effects of norepinephrine (NE) on single neurons isolated in the somatosensory cortex of halothane-anaesthetized cats In 46 animals, a sample of 465 isolated neurons were characterized according to the presence or absence of peripheral input, of spontaneous activity and sensitivity to glutamate. The effects of NE were examined in 117 cases using two different paradigms: either the receptive fields of neurons displaying peripheral inputs were stimulated mechanically during NE administration or the effects of NE were studied on the response to iontophoretically-administered glutamate on neurons lacking a receptive field.

In the presence of NE, 54% (63/117) of the neurons were inhibited, 36% (42/117) displayed some increase in neuronal activity and 10% (12/117) were unaffected. Often, the inhibitory effects of NE were more important on the spontaneous activity than on the induced activity producing an increase in the signal-to-noise ratio in 74% (28/38) of neurons. In contrast, when NE produced some excitation, the signal-to-noise ratio was increased in only 38% (11/29) of those cases Inhibited neurons were located predominantly in the middle layers of the cortex while excited neurons were found in upper and lower layers These results suggest that NE has different functions in different layers of the somatosensory cortex

After the cessation of NE administration, the response to a peripheral stimulus was increased for more than 5 min in 22% (2/9) of neurons In contrast, long-lasting increases in the response to glutamate followed NE administration in 70% (31/44) of cases In this latter group, NE also produced long-lasting decreases

in the ongoing activity in 39% (9/23) of neurons and long-lasting increases in the signal-to-noise ratio in 59% (13/23).

Pharmacological evidence suggests that the inhibitions were mediated by α_2- and ß-receptors while the excitations and the long-lasting increases in neuronal activity were mediated by α_1-receptors.

RESUME

La présente thèse décrit les effets neuromodulateurs de la norépinephrine (NE) sur les neurones du cortex somatosensoriel de chats anesthésiés à l'halothane. Quatre cent-soixante-cinq neurones ont été étudiés dans 46 animaux. Les cellules ont été caractérisées par la présence ou l'absence d'une réponse périphérique, leur activité de base ainsi que par leur sensibilité au glutamate. Les effets de la NE ont été investigués dans 117 cas et deux paradigmes expérimentaux ont été utilisés. Dans le cas des neurones répondant à la stimulation périphérique, la NE était administrée pendant la stimulation mécanique du champ récepteur. En l'absence de champ récepteur, les effets de la NE étaient étudiés sur la réponse au glutamate.

En présence de NE, 54% (63/117) des cellules ont été inhibées, 36% (42/117) excitées alors que 10% (12/117) n'ont pas été affectées. Les effets inhibiteurs de la NE étaient fréquemment plus marqués sur l'activité spontanée que sur l'activité évoquée produisant ainsi une augmentation du rapport signal-bruit dans 74% (28/38) des cas. Par contre, le rapport signal-bruit n'a été augmenté que dans 38% (11/29) des cas où la NE produisait une excitation. Les neurones inhibés par la NE étaient localisés principalement dans les couches médianes du cortex alors que les cellules excitées se situaient plutôt dans les couches supérieures et inférieures. Ces résultats suggèrent que la fonction de la NE varie selon les couches corticales.

Dans 22% (2/9) des cas, la réponse à la stimulation périphérique a été augmentée pendant plus de 5 min suite à l'administration de NE. Un effet similaire a été observé sur la réponse au glutamate dans 70% (31/44) des cas. Dans ce dernier groupe, des inhibitions à long-terme de l'activité spontanée ont également

été observées dans 39% (9/23) des cellules et une augmentation à long-terme du rapport signal-bruit dans 59% (13/23) des cas.

Les données pharmacologiques obtenues, les effets inhibiteurs de la NE seraient médiés par les récepteurs α_2 et ß alors que les excitations et les effets à long-terme seraient dus à une action de la NE sur les récepteurs α_1

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

ACh	acetylcholine
ATP	adenosine triphosphate
cAMP	cyclic adenosine monophosphate
BEN	benoxathian
DAG	diacylglycerol
EPSP	excitatory postsynaptic potential
GDP	guanine diphosphate
GLUT	glutamate
G-protein	guanine nucleotide binding protein
GTP	guanine triphosphate
IDA	idazoxan
IP_3	inositol 1,4,5 triphosphate
ISO	isoproterenol
LTP	long-term potentiation
NE	norepinephrine
6-OHDA	6-hydroxidopamine
OXY	oxymetazoline
(S)RA	(skin) rapidly adapting
RF	receptive field
(S)SA	(skin) slowly adapting
SOT	sotalol
TIM	timolol
YOH	yohimbine

PREFACE

Statement of originality

The effects of iontophoretically-administered NE on the response of somatosensory cortical neurons to natural somatic stimulus and to iontophoretically administered glutamate have not been previously described in the cat. NE produced both inhibitory and excitatory effects. These effects are described in detail and are well documented. The time course of the effects on NE on both the spontaneous and peripherally evoked activities has been measured. The laminar distribution of the inhibitory and excitatory effects of NE was also examined to show that the effects of NE differ according to the layer in which a neuron is found. Finally NE produced long-lasting potentiation of the response to somatic stimuli and to glutamate and in some cases of the signal-to-noise ratio. These are novel observations that have significant implications for the understanding of the noradrenergic modulation of somatic information.

Assistance by others

During the initial 6 months of this project I was assisted by Ms. Laura Buyan who performed the surgery. In the subsequent 11 months, M. Serge Leclerc helped by performing an occasional surgery. I performed the remainer of the experiments, the data analysis and the thesis preparation. Dr. Dykes provided editorial assistance in the latter endeavour.

1. INTRODUCTION

This thesis describes the effects of iontophoretically administered norepinephrine (NE) on single somatosensory cortical neurons in halothane-anaesthetized cats It tests the hypothesis that treatment with NE may induce long-lasting changes in cortical neuronal excitability and describes the characteristics of the cells affected, including their laminar locations. These experiments were undertaken as part of an exploration of the mechanisms of neuronal plasticity in the somatosensory cortex.

In recent years, it has been shown that the somatosensory cortex of adult animals reorganizes following restricted deafferentation Reorganization occurs through a change in the excitability of neurons in the deafferented cortex. Since NE is a putative neurotransmitter in the cortex which has been implicated in long-lasting changes in neuronal excitability in other regions such as in visual cortex where it has been suggested to be a permissive agent for ocular dominance plasticity, it is a reasonable candidate for an active role in plasticity in the somatosensory system.

As a first step to test the hypothesis that the noradrenergic system might be involved in the plasticity of the somatosensory cortex the effects of iontophoretically administered NE were examined with particular attention being given to any long-lasting changes in the excitability of somatosensory cortical neurons. In this context, the introduction provides a brief review of the literature concerning the presence, the effects and the role of NE in the cortex. It does not attempt a comprehensive review of NE effects in the central nervous system nor concern itself with NE and plasticity in other structures such as the hippocampus (see Harley, 1987) but emphasizes only the aspects relevant to the present study.

1.1 Norepinephrine in the cerebral cortex.

1.1.1 Noradrenergic innervation.

In a recent review, Foote et al. (1983) related the detailed history of the growth of knowledge about the noradrenergic innervation of the neocortex. Only a brief overview of the historical events are presented here and the reader is referred to this excellent review for further details.

Fuxe (1965) was the first to show histological evidence of the existence of noradrenergic fibres in the cerebral cortex by using fluorescence techniques on freeze-dried tissue sections to visualize catecholamines (Carlsson et al , 1962, Falck et al , 1962; Falck and Owman, 1965). This technique, known as the Falk technique, was used in conjunction with lesions of brainstem structures to show that the soma of origin of the NE fibres innervating cortex fibres were located within the brainstem in well-localized NE-containing cell groups (Anden et al., 1966) These first studies were performed in rats and revealed only a very sparse catecholamine innervation of the neocortex where it was thought the fibres were largely restricted to the molecular layer.

Ungerstedt (1971), using a modified fluorescence technique combined with smear preparations and lesions, provided more evidence that the locus coeruleus was the source of cortical noradrenergic fibres. He suggested also that noradrenergic innervation of the rat cortex was achieved by a dorsal bundle of fibres that curved anteriorly through the septum, travelled caudally within the cingulum bundle, and furnished laterally directed branches that innervated the entire cortex.

Since Ungerstedt's study, anterograde transport and more sensitive histochemical procedures have been used to develop a more detailed description of the cerulocortical projection (Bowden et al., 1978; Jones and Moore, 1977; Lindvall and Björklund, 1974; Maeda and Shimizu, 1972; Svensson and Thorén, 1979). It now appears that the pathway through the septal region may be only one of 3 major routes that noradrenergic fibres follow as they enter the cerebral cortex. A lateral route through the ventral amygdalofugal pathway and an intermediate trajectory through the ventral caudate and around the rostral portion of the external capsule have also been described in the rat (Jones and Moore, 1977; Shimizu et al., 1974; Tohyama et al , 1974).

The introduction of the glyoxylic acid histofluorescence method (Bloom and Battenberg, 1976; Lindvall and Bjorklund, 1974) revealed that the catecholamine innervation of the cortex had been greatly underestimated in earlier studies. With this technique the noradrenergic innervation appeared more dense than that previously described (Battista et al., 1972) and was distributed over all six layers of the neocortex (Freedman et al., 1975; Levitt and Moore, 1978; Lidov et al , 1978a, b; Itakura et al, 1981) It is now clear that the locus coeruleus is the primary if not the only source of noradrenergic fibres in several neocortical regions of the rat (Divac et al., 1977, Gatter and Powell, 1977; Huang et al., 1975; Kievit and Kuypers, 1975; Mesulam et al., 1977; Mizuno et al., 1981).

The use of antibodies directed against dopamine-ß-hydroxylase, the final NE-synthesizing enzyme, has revealed an even denser noradrenergic innervation of the cortex in rats (Morrison et al , 1978). The same technique shows that the pattern of

noradrenergic innervation possesses a geometric orderliness and distinct laminar pattern of innervation through the lateral cortex. In contrast, the pattern of innervation in medial cortex varies regionally as does its density, changing with cytoarchitectonic boundaries (Lewis et al., 1979; Morrison et al., 1979).

Using the same antibody, Morrison and collaborators (Morrison et al, 1982) found that the NE innervation of primate cortex exhibited a greater degree of regional variation in density and pattern than that observed in the rat cortex although a strong tangential, intracortical trajectory similar to that observed in the rat was a dominant feature of the noradrenergic innervation of the primate brain. In the somatosensory cortex immunoreactive fibres were present in all 6 cortical layers. Layer I contained tangential, radial and oblique fibres. Radial fibres were also observed in layers II and III but in addition, short tortuous axon segments as well as long tangential fibres were observed. The innervation appeared dense and terminal-like in layers IV and V and included axon segments of varying length and orientation. Layer VI was characterized by a band of tangential fibres located dorsal to the white matter.

At an ultrastructural level, the proportion of noradrenergic terminals that appear to form synaptic contacts in the cortex varies greatly among different studies. Descarries and collaborators (Descarries et al., 1977; Lapierre et al, 1973; Séguéla et al., 1990) found that in rat frontal cortex very few terminals formed synaptic contacts. In single thin sections, only 5% of noradrenergic varicosities labelled with tritiated NE appeared to form synaptic complexes with their target cells (Descarries et al., 1977). In contrast, 50% of unlabelled terminals in the same sections made

typical synaptic contacts. Recently these initial findings were confirmed using immunocytochemistry with an antiserum against NE (Séguéla et al., 1990); only 7% of immunostained varicosities examined in single sections formed synaptic junctions. The data extrapolated from the examination of serial thin sections suggested that not more than 17 to 26% of the total noradrenergic varicosities actually formed junctional complexes. In comparison, 98% of unlabelled varicosities examined in serial sections appeared to form synapses. These data support the possibility of a diffuse release of cortical NE into extracellular space.

Others have found that a much larger proportion of NE terminals formed synapses. Molliver et al (1982) reported that 40% of dopamine-ß-hydroxylase positive varicosities formed synapses in the rat somatosensory cortex. In the deep layers of adult rat visual cortex, a large proportion of terminals labelled with tritiated NE were found to form synaptic junctions (Parnavelas et al., 1983, Parnavelas and McDonald, 1985). Papadopoulos and collaborators (Papadopoulos et al., 1987, 1989) used the same antiserum as Séguéla et al. (1990) and found that 25% of noradrenergic terminals in single section formed synaptic contacts in contrast to the 7% found by Séguéla et al. (1990).

The discrepancies between the different studies appears to come from the differences between the criteria used to define synaptic contacts. Séguéla et al. (1990) seem to have applied very rigorous criteria and despite that fact found that 98% of the unlabelled varicosities had characteristics of synaptic contacts, a fact suggesting that their estimation might be closer to reality.

1.1.2 Laminar distribution of noradrenergic receptors.

Noradrenergic receptors are present both in the periphery and in the central nervous system. The first evidence that there was more than one type of adrenoceptor was provided by Dale (1906) who suggested that adrenaline might be acting at two different 'myoneural junctions' to produce its excitatory and inhibitory actions. Ahlquist (1948) was the first to introduce the subclassification of adrenoceptors as α- and ß-adrenoceptors based of the differences in potency of various catecholamines in a variety of tissues. In, 1967, Lands and coworkers (Lands et al., 1967) proposed a subdivision of ß-adrenoceptors into $ß_1$- and $ß_2$-adrenoceptors based on the relative potencies of agonists. Their classification is still in use today.

Langer (1974) proposed that α-receptors should be classified according to their postsynaptic (α_1) or presynaptic (α_2) location. Berthelsen and Pettinger (1977) proposed a classification according to the function of the receptors, arguing that α_1-receptors are excitatory and α_2-receptors are inhibitory. The presence of α_1-, α_2-, $ß_1$-, and $ß_2$-adrenoceptors in the cerebral cortex is well documented and all 4 subtypes have been found in significant numbers in several different cortical areas (see Reader et al., 1988 for review). The laminar distribution of adrenergic receptors in various regions of the cortex has been visualized in autoradiographic studies using specific labelled ligands.

In the rat somatosensory cortex, the binding of the specific α_1-antagonist [^{3}H]prazosin appeared relatively high in layers I and V while low levels were found in layer VI (Rainbow and Biegon, 1983). The pattern appeared similar in the motor cortex, but the levels of binding were generally higher than in the somatosensory

cortex. In rat visual cortex, [^{3}H]prazosin binding was significantly higher in layer I and the lowest levels were found in layer II-III while layers IV to VI contained intermediate levels of binding (Schliebs and Godicke, 1988). Using a different ligand, Jones et al. (1985) found a denser band of labelling in layer V of rat frontal and parietal cortex corroborating the report of Rainbow and Biegon (1983). But in that case, labelling was present in two bands located above and below the soma of layer V pyramidal cells, corresponding to layers Va and Vb. In cat cerebral cortex, Palacios et al. (1987) reported that the laminar distribution of α_1-receptors was similar to that found in the rat with a characteristic dark band in layers I and V. In the ferret visual cortex, Goffinet and Rockland (1985) found a high level of α_1-receptors in superficial layers and in layer IV suggesting that there are variations between species (see also Palacios et al., 1987).

The distribution of α_2-receptors appeared similar to that of α_1-receptors in rat visual cortex (Schliebs and Godicke, 1988). High levels of [^{3}H]clonidine binding were found in layer I, low levels in layers II-III and intermediate levels in layers IV through VI. Using [^{3}H]p-aminoclonidine as a specific ligand, Young and Kuhar (1980) noted that in general, higher levels of α_2-receptor binding were found in the more superficial layers of the cerebral cortex.

Levels of ß-receptor binding appeared very high in layers I to III in cat visual cortex (Shaw et al., 1986). Low levels were found in layers IV and V while in layer VI binding was intermediate. In rat visual cortex, the density of ß-receptors was the highest in layers I and IV followed by layer II-III while the lowest levels were found in layers V and VI (Schliebs and Godicke, 1988). In the rat frontoparietal cortex,

receptors labelled with the mixed ß-antagonist [^{125}I]pindolol were found in about the same density in layer I, IV and VI (Rainbow et al., 1984). In that study, the indirect visualization of ß-receptors subtypes was made by the displacement of [^{125}I]pindolol with specific $ß_1$- and $ß_2$-receptors antagonists. The density of $ß_1$-receptors appeared to be higher than $ß_2$-receptors in layers I and VI while the same proportion of $ß_1$- and $ß_2$-receptors was found in layer IV. In ferret visual cortex high levels of $ß_1$-receptor binding were present in supra- and infragranular layers while layer IV corresponded to a band a very low density of binding (Goffinet and Rockland, 1985).

In the cat, the pattern of $ß_1$-receptors was similar to that of the ferret and $ß_2$-receptors binding were found in high densities in layers I to III and binding appeared evenly distributed in layers IV to VI (Aoki et al., 1986). Using an antiserum raised against ß-receptors that appeared to bind mostly to $ß_2$-receptors, Aoki et al. (1987) found in the rat somatosensory cortex a distribution similar to the distribution seen in the visual cortex. In rat somatosensory cortex, high levels of $ß_2$-receptors appeared to be specifically associated with the posteromedial barrel subfield (Vos et al., 1985)

In general, α- and ß-receptors appear to have different laminar distributions. While high densities of both types of receptors were found in layer I, $α_1$-receptors appear to be present in large amounts in layer V of sensorimotor cortex and ß-receptors were found in significant amounts in layers II-III while low levels of ß-receptors were consistently found in layer IV.

1.2 The effects of norepinephrine in the cerebral cortex and in other areas of the central nervous system.

1.2.1 Iontophoretic studies showing predominantly inhibitory effects.

Krnjevic and Phillis (1963b) studied the effects of several amines in the cerebral cortex of cats anaesthetized with allobarbitone, ether or chloralose and in a few cases in unanesthetized 'cerveau isolé' preparations. Epinephrine was found to depress both the spontaneous activity and the response to glutamate. They also noted that in contrast to dopamine, large amounts of epinephrine frequently produced a delayed excitation. NE was reported to produce effects similar to those observed with epinephrine but that it was a much weaker agent.

Foote and collaborators (Foote et al., 1975) reported the effects of NE on acoustically evoked activity in auditory cortical cells of awake monkeys. The responses were evoked by playing recordings of various vocalizations of the species studied. The iontophoretic administration of NE inhibited both spontaneous and acoustically evoked activities in all 28 neurons tested. In 75% of the neurons, the decrease induced by NE was more important on the spontaneous than on the evoked activity and the authors concluded that one possible role of NE might be to enhance the evoked activity to spontaneous activity ratio in the output of the neurons. This concept is now well known as the increase in signal-to-noise ratio produced by NE and it has been reported by a number of workers to occur in various regions of the brain.

Waterhouse, Woodward and their collaborators (Waterhouse and Woodward, 1980; Waterhouse et al., 1980, 1981) studied the effects of NE in the somatosensory

cortex of halothane anaesthetized rat. As in the auditory cortex of monkeys, a major effect of NE appeared to be its capacity to increase the signal-to-noise ratio by producing a more important inhibition of the spontaneous activity than of the peripherally-evoked activity. Further, in 12 of 41 (29%) neurons studied, NE produced a net increase in evoked activity but these excitations rarely exceeded 25% of the control response. In 27 of 32 (82%) neurons tested, NE also increased the postexcitatory depression of activity that immediately followed the response to peripheral stimulation. The excitatory effects of NE were observed in a larger proportion of neurons located in the lower layers than in the middle and upper layers while the other effects of NE appeared to be evenly distributed in all layers of the somatosensory cortex. The effects of NE usually lasted less than 5 min. The increase in signal-to-noise ratio appeared to be the result of an activation of α-receptors since the α-receptor agonist phenylephrine mimicked the effects of NE and because those effects could be reversibly blocked by the α-receptor antagonist phentolamine (Waterhouse et al., 1981, 1982). The increase in the postexcitatory inhibition was mimicked by the ß-receptor agonist isoproterenol and was blocked by the ß-receptor antagonist sotalol. Recently, Waterhouse et al. (1988) showed that, like the iontophoretic administration of NE, the electrical stimulation of the nucleus locus coeruleus 50 to 600ms before the stimulation of the receptive field enhanced the signal-to-noise ratio and the postexcitatory inhibition following tactile stimulation in the somatosensory cortex of halothane anaesthetized rats.

Although NE was usually inhibitory in rat somatosensory cortex in vivo, when somatosensory cortical slices were maintained in vitro, NE potentiated the responses

to glutamate in 51 of 54 (94%) neurons and to ACh in 6 of 9 (67%) cases (Mouradian et al., 1988). In 8 neurons, iontophoretically administered glutamate produced strong excitatory responses to pulses of glutamate in the presence of NE that were otherwise subthreshold. The potentiating effects of NE were blocked by the α-receptor antagonist phentolamine and could be mimicked by the iontophoretic administration of the protein kinase C activator, phorbol 12,13 diacetate, suggesting that the potentiation was mediated by an α-receptor linked to the intracellular activation of protein kinase C. The potentiating effects of NE were also observed when NMDA receptors were selectively activated (Mouradian et al., 1989).

In the visual cortex of cats and kittens anaesthetized with nitrous oxide and halothane, NE was found to inhibit 43 of 60 (72%) neurons responding to visual stimuli (Videen et al., 1984). In another 3 (5%) neurons an excitation was observed. In the visual cortex of urethane anaesthetized rats, NE depressed the ongoing activity in 60 of 64 (94%) cells and produced an increase in 6% of cases. In the presence of NE, the visually evoked activity was decreased in 97 of 118 (82%) and increased in 9% of neurons (Kolta et al., 1987; Kolta and Reader, 1989). The signal-to-noise ratio was increased by more than 20% in 20 of 37 (54%) neurons tested and it was decreased in only 8% (Kolta and Reader, 1989). The effects of NE lasted for an average of 3.5 min suggesting that most of the effects of NE lasted less than 5 min. In that study, the α_2-receptor agonists clonidine and oxymetazoline and the ß-receptor agonist mimicked the inhibitory effects of NE. But only the α_2-receptor antagonist idazoxan consistently blocked the effects of NE while the ß-receptor antagonist sotalol did not. The authors concluded that most of the inhibitory effects

of NE were mediated by α_2-receptors although some inhibition might have been ß-mediated. The mixed α_1-α_2-receptor agonist phenylephrine induced excitation more often (28% of 25 neurons) than any other drug tested and since the effects mediated by α_2-receptors was inhibition, it was suggested that the excitation was probably mediated by α_1-receptors (Kolta et al., 1987; Kolta and Reader, 1989).

Other studies have also reported that the predominant effect of NE in the cerebral cortex was an inhibition of the spontaneous activity (Olpe et al. 1980; Reader et al. 1979). In the hippocampus, NE has also been shown to produce mainly inhibitory effects (Curet and de Montigny, 1988a; Segal and Bloom 1974a, b) that appeared to be mediated by α_2-receptor when NE was administered iontophoretically and by α^1-receptor when the locus coeruleus was stimulated (Curet and de Montigny 1988a, b).

1.2.2 Iontophoretic studies showing predominantly excitatory effects.

In the studies reported above, the major effect of NE in the cerebral cortex was inhibition and excitatory effects were observed in only a few cases. In other studies the iontophoretic administration of NE appeared to induce mainly excitation. In the somatosensory cortex of halothane-anaesthetized rats, Bevan et al. (1977) found that iontophoretically administered NE produced an increase in the ongoing discharge in 66% of 194 neurons tested while depression in the activity was observed in 34% of cases. During the administration of the α-receptor agonists phenylephrine (n=78) and methoxamine (n=11) all neurons were excited while in the presence of the ß-agonist salbutamol (n=16) only inhibition was observed The excitatory effects of NE and of the α-receptor agonist were reversibly blocked by the α-receptor

antagonists phentolamine and phenoxybenzamine. The ß-receptor antagonist sotalol blocked the inhibitory effect of the mixed α-ß-receptor agonist isoprenaline, but in some cases, it could also antagonize excitatory responses to adrenoceptor agonists. The authors concluded that the excitatory effects of NE were α-mediated while ß-receptors mediated the inhibition although it was not clear if some of the excitation was not ß-mediated. In another study from the same laboratory, Szabadi et al (1977) found that, in halothane-anaesthetized cat somatosensory cortex, the ongoing activity was also increased in the presence of NE but that the probability of observing an excitation was negatively correlated with the level of spontaneous firing rate. For example, virtually all neurons displaying spontaneous activity lower than 5 impulses/s were excited by NE while less than 60% of the neurons with an ongoing discharge greater than 31 impulses/s were excited. Similar relationships were observed between the ongoing discharge and the excitatory effects of serotonin and mescaline.

Armstrong-James and Fox (1983) reported that NE enhanced the ongoing activity in a 8 of 36 neurons located 800μm or more below the pial surface in the somatosensory cortex of urethane-anaesthetized rats. In that study, the concentration of NE at the tip of the electrode was monitored continuously and excitation occurred only with very low concentrations of NE. In 6 cases higher concentrations reversed the effect to an inhibition. The effects of NE on peripheral input were not studied.

Excitations have also been observed in other brain regions. In the lateral geniculate nucleus of chloral hydrate-anaesthetized rats, the spontaneous activity was increased at least two-fold in 175 of 191 (92%) cells tested. The remaining neurons were unresponsive or displayed a smaller increase during the iontophoretic

administration of NE (Rogawski and Aghajanian, 1980). Some experiments were also performed in an unanesthetized 'cerveau isolé' preparation where the responses to NE were similar to those observed in anaesthetized animals, however in this preparation neurons appeared to be more sensitive to NE than in the intact animal. From the relative potency of various adrenergic agonists and antagonists, the authors concluded that the excitations were mediated by α_1-receptors. In chloral hydrate-anaesthetized rats, Baraban and Aghajanian (1980) iontophoretically administered NE to 44 neurons in the dorsal raphe nucleus and found that small amounts of NE produced mainly an increase in the spontaneous activity of dorsal raphe neurons while higher doses resulted in inhibition. The relative potency of adrenergic drugs suggested that the excitatory effects of NE were mediated by α_1-receptors. Evidence that inhibitory effects were mediated by α_2-receptor was reported in subsequent studies (Marawaha and Aghajanian, 1982; Freedman and Aghajanian, 1984). In the rat facial nucleus, Menkes et al. (1980) found that subthreshold pulses of glutamate induced a response in presence of NE and that this effect was mediated by α-receptor.

The responses to the electrical stimulation of lateral preoptic area were enhanced in a majority of neurons in rat lateral hypothalamus in the presence of NE (Sessler et al., 1988). Neurons with no or very low spontaneous activity were more likely to be enhanced than neurons displaying a high spontaneous discharge.

The differences between those studies showing predominantly excitatory effects of NE and those showing predominantly inhibitory effects are difficult to reconcile, but several things are consistent in both sets of studies. First, inhibition is

attributed to α_2- or ß-receptors and excitation is attributed to α_1-receptors. Second, excitation is more common in deeper layers than in the more superficial layers of cortex. One step towards the reconciliation of these sets of data is to suggest that the studies finding predominantly excitation may have selected their neurons from deeper layers than those showing predominantly inhibitory effects. Another step is to recognize that the distribution and relative proportion of the receptor types found on cortical neurons vary in different cortical areas and layers. Further, the levels of monoamines in cortex can vary as much as a factor of 10 due to seasonal changes (Kabani et al., 1990) and those changes might be paralleled by changes in receptors. Thus it is important to recognize that there are several receptors for NE in cortical tissues and that complex interactions among them may determine the net effect of NE release in cortex. In addition it must be recognized that variables related to the experimental design may influence neuronal response and that adequate controls must be performed.

1.2.3 Intracellular studies.

The ionic mechanisms of the response to NE have been extensively studied in rat hippocampal slice preparations. Segal (1981) found that the topical application (5 to 20nl droplets on the surface of the slice) of NE caused a slight hyperpolarization associated with a decrease in input resistance in CA1 neurons. A decrease in the spontaneous discharge and a reduction of the excitatory post-synaptic potentials (EPSPs) produced by the stimulation of the Schaffer collaterals were also observed. Since the hyperpolarization induced by NE was reduced in low Cl medium

and ouabain and low temperature also decreased markedly the response to NE, Segal (1981) concluded that two mechanisms were involved in the action of NE: the activation of a Cl conductance and the activation of a Na^+-K^+ pump. The action of NE appeared also to be independent of extracellular calcium concentration. The ß-agonist isoproterenol mimicked the effects of NE and the ß-antagonist sotalol produced a reduction of the neuronal response to NE suggesting ß-mediated effects of NE. The membrane permeant cAMP analogue, 8-Br cAMP, also mimicked the effects of NE suggesting that cAMP was the second messenger.

Madison and Nicoll (1982, 1986a, b) found that the accommodation of CA1 pyramidal cells was markedly attenuated during the bath application of NE in the rat hippocampal slice preparation. The number of action potentials observed with a given pulse of depolarizing current or of glutamate was markedly increased in the presence of NE. In many cases NE produced a slight hyperpolarization of membrane resting potential associated with a decrease in input resistance In some cases, the hyperpolarization was followed by a depolarization accompanied by a decrease in input resistance. The most striking effect of NE was to markedly reduce the slow calcium-activated potassium after-hyperpolarization that followed a train of action potentials (Madison and Nicoll, 1986a). The calcium current was not affected by NE suggesting that NE acted through a mechanism other than blocking the calcium channels. This effect of NE appeared to be mediated by $ß_1$-receptors since it was reversibly blocked by the $ß_1$-receptor antagonists and mimicked by a $ß_1$-receptor agonist while α- or $ß_2$-receptor drugs produced no effect. Furthermore, the α-receptor agonists phenylephrine and clonidine mimicked the hyperpolarizing effects

of NE while the ß-receptor agonist isoprenaline produced a depolarization. These results suggested that the hyperpolarization was α-mediated and the depolarization was ß-mediated (Madison and Nicoll, 1986a). In a companion paper, Madison and Nicoll (1986b) found evidence that the effects mediated by ß-receptors were linked to the intracellular production of cAMP. The extracellular administration of the cAMP analogue, 8-Br cAMP, mimicked the depolarizing effects of NE and also produced a reduction of the afterhyperpolarization without reducing calcium currents. The intracellular injection of cAMP also mimicked these effects while extracellular administration failed to produce such effects. These effects of NE mediated by ß-receptors have been confirmed by other studies on CA1 pyramidal neurons (Haas and Konnerth, 1983; Sah et al., 1985) and in granule cells of the dentate gyrus (Haas and Rose, 1987) in the rat hippocampal slice preparation.

More recently, Foehring and collaborators (Foehring et al., 1989) examined the effects of NE on large pyramidal neurons in layer V of cat motor cortex in a brain slice preparation. In that preparation NE usually caused a small depolarization while hyperpolarizations were seldom observed. Like the effect found in the hippocampus, NE reduced markedly the slow calcium-activated potassium after-hyperpolarization but the input resistance was not affected. In addition, NE also blocked the sodium-dependent portion of the slow after-hyperpolarization. The net effect of NE was an increase in cell excitability; neurons exhibited steady repetitive firing to depolarizing currents that were ineffective in the absence of NE. Results obtained with different noradrenergic agonists and antagonists suggested that as observed in the hippocampus, the reduction of the slow calcium-dependent potassium

current was mediated by ß-receptors.

The ionic mechanisms underlying the actions of NE mediated by α-receptors have been studied in more detail in other regions of the brain. In locus coeruleus neurons recorded in vivo, the activation of α_2-receptors with clonidine produced an hyperpolarization that was associated with a decrease in membrane input resistance (Aghajanian and VanderMaelen, 1982). In rat locus coeruleus neurons recorded in vitro, Williams et al. (1985) found also that clonidine, like NE, produced membrane hyperpolarization which was blocked by α_2-receptor antagonists. An increase in potassium conductance was suggested to be the mechanism underlying the hyperpolarization mediated by α_2-receptors in locus coeruleus neurons (Aghajanian and VanderMaeler, 1982; Egan et al., 1983, Williams et ., 1985). Hyperpolarizations mediated by α_2-receptors were also demonstrated in sympathetic (Brown and Caulfield, 1979) and parasympathetic (Nakamura et al., 1984) neurons.

The activation of α_1-receptors caused a depolarization associated with a decrease in membrane conductance, presumably to potassium ions in motorneurons in the facial nucleus (Aghajanian and Rogawski, 1983). Nakamura et al. (1984) recording intracellularly in parasympathetic neurons found that the depolarizations produced by NE were mediated by α_1-receptors since they were blocked by the α_1-receptor antagonist prazosin. In voltage-clamp studies of dorsal raphe neurons in vitro, the activation of α-receptors with the mixed α-agonist phenylephrine was shown to suppress both the resting potassium current and an early transient potassium current that resembled the previously described A-current (Aghajanian, 1985). This effect was blocked by the α_1-receptor antagonist prazosin suggesting that

it was α-mediated. Using the same preparation and pharmacological approach, Freedman and Aghajanian (1987) showed that the activation of α_1-receptors increased also the duration of the afterhyperpolarization possibly by increasing the duration of the calcium-dependent potassium current, but the calcium current was unaffected.

In summary, in vivo and in vitro studies yielded contradictory results. In both neocortex and hippocampus (Segal and Bloom, 1974a, b) in vivo, the inhibitory effect of NE appeared to be mediated by α_2- and ß-receptors while α_1-receptors appear as a candidate mediator of excitation. In vitro excitation appeared to be mediated by $ß_1$-receptor and inhibition by α-receptor. The only exception is the report of Segal (1981) who found that NE produced mainly ß-mediated inhibition in hippocampal slice. The fact that NE was administered topically might explain the difference from the other studies where NE was bath-applied.

1.3 Intracellular effectors of noradrenergic receptors.

All subclasses of noradrenergic receptors appear to use guanine nucleotide-binding proteins (G-proteins) as intermediaries in transmembrane signalling (see Gilman, 1987 and Stryer and Bourne, 1986). The pathway consists of 3 proteins: receptors, G-proteins and effectors. The receptor converts an external signal into a conformational change that initiates G-protein activation on the cytosolic face of the plasma membrane The binding of GTP appears to activate the G-proteins while the hydrolysis of GTP to GDP initiates its deactivation. The dissociation of GDP is slow in the absence of the excited receptor and is markedly increased when the

receptor is activated so that the G-protein is more often in its active state.

The ß-receptor is associated with a G-protein stimulating an adenylate cyclase. The activated adenylate cyclase will convert ATP to cAMP thus increasing intracellular cAMP. One of the roles of cAMP is to activate protein kinase A (Lefkowitz and Caron. 1988). In contrast, α_2-receptors are associated with an adenylate cyclase inhibitory protein and therefore the activation of α_2-receptors will reduce intracellular cAMP.

The effect of α_1-receptor activation appears also to be mediated via G-proteins and results in an increase in phosphatidylinositol hydrolysis in rat brain (Brown et al., 1984; Janowsky et al., 1984; Minneman and Johnson, 1984, Schoepp et al., 1984; Gonzales and Crews, 1985; Kemp and Downes, 1986). In this case, the G-protein activates a specific membrane-bound phosphodiesterase, the phospholipase C (Stryer and Bourne, 1987). The phospholipase C hydrolyses prosphatidylinositol 4,5-biphosphate into two intracellular signal molecules, diacylglycerol (DAG) and inositol 1,4,5 triphosphate (IP_3) (Nishizuka, 1984; Berridge and Irvine, 1984) DAG activates protein kinase C and IP_3 triggers the release of intracellular calcium ions into the cytosol. Recently, Crews and coworkers (Crews et al., 1988, Gonzales and Crews, 1988) found evidence that α_1-receptors also stimulate a calcium-dependent phospholipase C through the opening of calcium channels in neurons. In rat dorsal raphe neurons, IP_3 has been shown to mimic the increase in duration of the afterhyperpolarization produced by the activation of α_1-receptors while the activation of protein kinase C antagonized the receptor-mediated activation of the neurons (Freedman and Aghajanian, 1987).

1.4 Norepinephrine and plasticity.

1.4.1 Long-lasting effects of norepinephrine.

NE has been shown to produce long-lasting increases in neuronal excitability in several brain areas. Armstrong-James and Fox (1983) observed an increase in the spontaneous activity of 60% of neurons (n=90) located from 800 to 1400 μm under the cortical surface in rat somatosensory cortex that lasted for at least 3 min following the cessation of NE. In about 25% of these the increase lasted more than 20 min and in some cases spontaneous activity was still elevated an hour after ending the iontophoretic administration of NE. In contrast, long-lasting effects were not observed on the spontaneous activity of any neurons located above 800 μm.

NE produces long-lasting increases of neuronal excitability in the hippocampus and in other limbic areas (see Harley, 1987 for review). In the hippocampus, iontophoretically applied NE potentiated the population spike measured from the field potential in the dentate gyrus induced by the stimulation of the perforant path with biphasic constant current pulses at 0.1Hz (Neuman and Harley, 1983). The magnitude of the potentiation ranged from 20 to 400% and in 16 of 41 cases (39%) they were long-lasting (over 30 min to several hours) even if NE was administered only for a short period of time. The population EPSP was usually not increased unless high stimulating currents were used, suggesting that NE did not produce a potentiation of synaptic input to the dentate gyrus but rather brought a larger number of neurons to threshold (Neuman and Harley, 1983). These in vivo studies were confirmed by Winson and Dahl (1985) using a similar paradigm but, in that case, long-lasting increases were also observed when the inactive enantiomer d-NE

was used suggesting a non-specific effect. Long-lasting potentiation of the population spike of the dentate granule cells could be reproduced by activation of the locus coeruleus with pressure-ejected glutamate in the vicinity of the nucleus (Harley and Milway, 1986). Since propanolol attenuated the enhancement it was suggested that the effects were mediated by ß-receptors. The electrical stimulation of the locus coeruleus also produced long-term increases in the population spike in the dentate gyrus elicited by perforant path stimulation but in contrast to the activation with glutamate, the long-lasting effects were not blocked by a ß-antagonist suggesting that another ß-NE-independent system was involved (Harley et al., 1989).

In hippocampal slice preparations, bath application of NE for 10 min induced similar potentiations but only in 25% of the slices (Lacaille and Harley, 1985). The long-lasting potentiation could be prevented by timolol but not by phentolamine thereby suggesting that they were mediated through ß-receptors. In contrast to the in vivo preparations, the population EPSP was potentiated as well as the population spike in slice preparations (Lacaille and Harley, 1985). Using a similar paradigm, Stanton and Sarvey (1985) were able to produce long-lasting potentiation in every slice when NE was superfused for 30 min. The potentiation was blocked by the $ß_1$-receptor antagonist metropolol and by the preincubation of the slice with the protein synthesis inhibitor emetine suggesting that changes in protein constitution were necessary for the induction of long-lasting potentiation mediated by $ß_1$-receptors.

The best-known example of a long-lasting change in neuronal excitability is the enhanced responsiveness that follows tetanic stimulation of afferents to the hippocampus. This phenomenon, known as long-term potentiation (LTP), is also

affected by NE. Hopkins and Johnston (1984) recorded in the CA3 pyramidal cell layer during an experiment in which they activated the mossy fibre at a high rate in rat hippocampal slices. Stimulation parameters that did not produce LTP by themselves when paired with the superfusion of NE resulted in the induction of LTP. With a stronger stimulation that could induce LTP on its own, NE was found to increase the magnitude, the duration and the probability of induction of LTP. The ß-receptor agonist isoproterenol produced effects similar to NE on LTP. In a subsequent report, Hopkins and Johnston (1988) showed that the adenylate cyclase activator, forskolin and the intracellular injection of 8-Br cAMP both enhanced the probability of induction of LTP suggesting that NE enhanced LTP by stimulating the production of cAMP.

1.4.2 Functional plasticity.

Kasamatsu and Pettigrew (1976) were the first to suggest that NE was necessary for the ocular dominance shift observed in monocularly deprived kittens (Wiesel and Hubel, 1963). They found that the treatment of the visual cortex with 6-hydroxydopamine (6-OHDA), a compound which destroys NE terminals, prevented the ocular dominance shift. Following this initial experiment, it was shown that infusion of NE could restore plasticity to monocularly deprived kittens previously treated with 6-OHDA (Kasamatsu et al., 1979) and that the rate of recovery at the end of one week of monocular deprivation was also increased (Kasamatsu et al., 1981). Further, increasing cortical NE appeared to increase plasticity in the visual cortex even in the absence of monocular deprivation (Kuppermann and Kasamatsu,

1984). Other experiments suggested that these effects on the visual cortex plasticity were mediated via ß-receptors (Kasamatsu and Shirokawa, 1985a, b).

Several laboratories (Trombley et al., 1986; Daw et al., 1985; Adrien et al., 1985) attempted to reproduce these interesting observations with slight variations in the experimental design, but with mixed success. When 6-OHDA treatment was adjusted at levels that depleted most of the cortical NE, shift in ocular dominance still occurred but it was of a smaller magnitude than that reported by Kasamatsu and Pettigrew (1979). These authors suggested that some factor other than the depletion of NE was responsible for the loss of plasticity. Bear and Singer (1986) found that neither the destruction of the noradrenergic inputs with 6-OHDA injection in the dorsal noradrenergic bundle nor of the cholinergic input with N-methyl-DL-aspartate injections into the basal forebrain prevented the plasticity in the visual cortex. Only when both inputs were lost was the shift in ocular dominance blocked. Because both fibre systems travel in the cingulate gyrus, surgical lesions of the cingulate gyrus were also effective in depleting the density of acetylcholine esterase positive axons in the visual cortex and the level of endogenous NE. This procedure also prevented the ocular dominance plasticity. These experiments suggested that the integrity of either the noradrenergic or cholinergic system was sufficient for plasticity to occur This study also offered an explanation to rationalize the findings of Kasamatsu and coworkers since it was shown that iontophoretically administered 6-OHDA antagonized the enhancement of the visual response produced by ACh on cortical neurons suggesting that 6-OHDA could interfere with the action of ACh The current status of the hypothesis is that both NE and ACh are thought to gate the plasticity in the visual cortex of kittens.

1.5 Somatosensory system.

1.5.1 Cutaneous projections to the somatosensory cortex.

The somatosensory cortex is organized to receive, process and relay sensory information from the skin, muscles and presumably from the internal organs to higher centres of the nervous system. Mechanoreceptors located in the skin respond to mechanical displacements of the cutaneous surface with two general categories of responses: rapidly adapting (RA) and slowly adapting (SA). RA receptors respond transiently to a steady stimulus applied on the skin while SA receptors display a sustained response to such stimulation.

These sensory messages travel among primary afferent fibres whose cell bodies are located in the dorsal root ganglia of the spinal cord. Their axons make up the classic long fibre pathway projection via the dorsal columns to the dorsal column nuclei where they synapse on second order neurons. The neurons of the dorsal column nuclei project through the contralateral medial lemniscus to synapse in the ventroposterior complex of the thalamus. Thalamic neurons relay the cutaneous information to the primary somatosensory cortex, primarily in layer IV and in the bottom of layer III (Martin, 1985).

The somatosensory receptor sheet is represented in a topographical manner at each level in the central nervous system. Marshall and collaborators (Marshall et al., 1941) were the first to demonstrate the topographical organization of the somatosensory cortex by studying the pattern of evoked potentials recorded from the cortical surface. They illustrated the sites where cutaneous information arrived in the cortex from different body parts by a distorted map of the body that was said to

represent the somatotopic order of inputs to the primary somatosensory cortex Later, Mountcastle (1957), using extracellular recording technique for single cells showed that an individual cell in somatosensory cortex had a single, modality-specific receptive field on the skin surface and that adjacent cells had nearby receptive fields confirming the presence of the orderly somatotopy and establishing the concept of the cortical column which served one point on the body surface.

More recently, Kaas, Merzenich and their collaborators (Kaas et al, 1979; Merzenich et al., 1978) used fine grain mapping techniques to demonstrate that there were actually four independent and fairly complete maps of the body surface in primate somatosensory cortex, one within each of Brodmann's areas 3a, 3b, 1 and 2 with each area being dominated by input from a particular class of receptors. Areas 3a and 2 respond primarily to input from muscles while areas 3b and 1 respond to cutaneous input. Additional functional subdivisions are also found within each region, for example in area 3b there are separate strips for RA and SA submodalities (Sretavan and Dykes, 1983) and Merzenich et al. (1978) have argued for cortical divisions related to input from different parts of the body.

In the cat, the primary somatosensory cortex is located in the posterior sigmoid gyrus and is divided in 4 cytoarchitectonic areas like that of the monkey (Hassler and Muhs-Clement, 1964). Area 3a is located rostrally adjacent, to the cruciate sulcus while area 3b is on the exposed bank of the gyrus. Area 1 is found posterior to area 3b and area 2 is located on the posterior wall of the sigmoid gyrus delimitated by the ansate sulcus.

Dykes (1978) argued for multiple maps in cat somatosensory cortex whereas Felleman et al. (1983) suggested that there was only one. Sretavan and Dykes (1983) explored the representation of the forearm region in the cat somatosensory cortex showing that the somatosensory input to that region is organized in a manner similar to that described in the monkey; cutaneous inputs were found primarily in areas 3b and 1 while area 3a contained primarily input from muscle. This information was used in the present study to locate the cutaneous representation in the cat somatosensory cortex and to place the penetrations in the cortical region receiving input from the skin of the forearm and paw.

1.5.2 Plasticity in the somatosensory cortex.

For a long time the topographic organization of the representation of the body surface in the somatosensory cortex was though to be stable throughout adulthood. However, in recent years, it has been demonstrated that the ordered arrangement could be modified following restricted deafferentation procedures or by behavioural tasks (see Wall, 1988 for review).

The first evidence that somatosensory cortex could reorganize was provided by the work of Kalaska and Pomerantz (1979) who deafferented the forepaw of cats and kittens and then recorded in the primary somatosensory cortex weeks or months later. Detailed studies following restricted deafferentation of part of a limb soon showed that reorganization also occurred in primary somatosensory cortex of adult raccoon (Rasmusson, 1982; Kelahan and Doetsch, 1984), monkey (Merzenich et, 1983a, b, 1984) and rat (Wall and Cusick, 1984). From those studies it appears that

immediately after deafferentation most of the deafferented cortex is silent, although a few inputs from adjacent skin region can be found. In the following days and weeks. silent areas gradually develop responses to adjacent skin regions until a new, apparently stable, somatotopic order is found in the deafferented cortex. When larger areas of the cortex are deafferented, some of the deafferented cortex may remain silent.

More recently, the somatotopic organization has been shown to be remodelled in normal adult monkey trained to perform a task which required that a limited sector of skin be stimulated in a behaviourally motivated task (Jenkins et al , 1990) After several months of stimulation, the stimulated skin surface was overepresented in the somatosensory cortex demonstrating that neuronal plasticity might be an intrinsic attribute of the somatosensory cortical map and that the somatotopic order may be modified by use throughout the lifetime of the individual.

Today, the cellular mechanisms underlying the neuronal plasticity required for reorganization of the somatosensory map are unknown. Yet it is apparent from the work reviewed above that the excitability of certain neurons in the somatosensory cortex can be altered so that they will respond to previously subthreshold inputs The mechanisms underlying the plasticity of synaptic connections in the somatosensory cortex may involve neuromodulatory substances such as NE Recently it has been demonstrated that another neuromodulatory substance, ACh, produced long-lasting enhancement of somatosensory cortical neuronal excitability when it was either iontophoretically administered (Metherate et al, 1987, 1988b) or synaptically released by the electrical stimulation of cholinergic neurons located in the basal forebrain (Tremblay et al., 1990a, b).

1.6 Rationale.

There is substantial evidence that NE might play a role as a neuromodulatory substance in the somatosensory cortex. Although there is some information on the effects of NE in rat somatosensory cortical neurons as reviewed in the preceding sections, no data are available on the neuromodulatory effects of NE on the somatic input in the cat somatosensory cortex. Since, NE has been implicated in long-lasting changes in excitability in several regions of the brain it is a reasonable candidate for a substance that might be involved in the long-lasting changes in excitability seen in the somatosensory cortex. The present study was designed to study the effects of iontophoretically applied NE in cat somatosensory cortex and to explore the neuromodulatory effects of NE on neuronal excitation induced by peripheral inputs and by iontophoretically administered glutamate. The hypothesis that NE induces long-lasting changes in the excitability of single neurons was also carefully examined.

2.0 METHODS

2.1 The technique of microiontophoresis.

It was over 30 years ago that Curtis and Eccles (1958a, b) first used microiontophoresis to study the effects of drugs on neurons in the central nervous system. Since then, knowledge concerning the responsiveness of neurons in the presence of pharmacological substances in the central nervous system has expanded enormously, in a large part because of the use of this technique, which remains even today the best method available to mimic the synaptic release of a compound. Extensive reviews of the theoretical and methodological aspects of microiontophoresis are available (c f. Hicks, 1984, Krnjevic, 1971, Stone, 1985) The following section discusses only briefly the general principles of microiontophoresis and dwells more on the aspects relevant to the present study The interested reader is referred to the cited literature for a more detailed discussion of aspects beyond the scope of this study.

2.1.1 Principle of microiontophoresis.

The method of microiontophoresis is based on the fact that electric charges of the same polarity repulse one another. This principle is used to eject ionized substances in solution from a micropipette tip by applying a charge of the appropriate polarity For example, NE hydrochloride in solution at pH 4.0 is positively charged and will be ejected from an electrode by applying a positive charge to the opposite end. In contrast, negative current will move the ion away from the tip and prevent its free diffusion in the vicinity of the tip of the pipette The

former is referred to as the ejection current and the latter the retention current.

The molar flux (Q) of ions in solution produced by an electric current is given by:

$$Q = \frac{It}{FZ}$$

where F is the Faraday's constant, Z is the valency of the ion, I is the current and t is the transport number, i.e. the fraction of applied current carried by the particular ion. This equation reflects the amount of drug released from the micropipette by iontophoretic expulsion (Stone, 1985). Although this simple relationship should describe the ejection of ions from micropipettes by an applied current, a number of factors reduce the usefulness of the equation. For example, the transport number varies from pipette to pipette and complex interactions may occur between the solutions and glass at the tip of the electrode, making it difficult to estimate the actual amount of ion ejected. Because of these uncertainties and because of the generally linear relationship obtained between the flow of current and microiontophoretic release from a given micropipette barrel (Zieglgansberger, 1969), doses of a substance are generally reported in terms of the applied current rather than in terms of the moles of substance released.

Reproducibility of the amount delivered is usually good with a given barrel, however since retaining currents of 10-20nA are routinely used between ejection periods, the ions of interest are drawn away from the tip of the pipette to a degree proportional to the time that the retaining current was applied. Consequently, the first current pulses will eject less drug than the subsequent ones. Nevertheless, after these first few ejections, the amount of the substance ejected stabilizes, becoming

relatively constant for a given amount of current.

2.1.2 Advantages of the technique.

A chief advantage of the microiontophoretic method is that with multibarrel pipettes it is possible to examine the effects of several different drugs upon single neurons in vivo under conditions in which the drug ejected is limited to a small volume immediately surrounding the electrode tip, thus preventing effects on the whole of the nervous system or on other physiological processes (Aghajanian, 1972), a major disadvantage of systemic administration. Other advantages of microiontophoresis are that the blood-brain-barrier is avoided (Curtis and Eccles, 1958a, b) and a series of different compounds, both agonists and antagonists of neuromodulatory substances, can be tested rapidly and their effects compared with relative ease.

2.1.3 Disadvantages of the technique.

The absence of information on the actual concentration of drug at its receptor is usually considered one of the primary disadvantages of the microiontophoretic technique. Further, the drug concentration will vary along a space-time continuum, being less concentrated as it spreads farther from the tip of the pipette For example, it is impossible to know if the receptors for a particular drug are located on a nearby cell body or on distant dendrites and the ejection of the same amount of drug to two different cells might suggest that one is more sensitive than the other when in fact they are equally sensitive but have different geometries with respect to the electrode.

Thus it may become necessary to take into account other parameters of the drug response such as the delay to onset of a response to infer the proximity of the receptors to the pipette. Another problem with the technique is that a small cell must be approached more closely than a larger one to obtain a suitable signal-to-noise ratio. This may result in a higher drug concentration at the surface of the smaller cell. Further, if the electrode tip is very close to the cell membrane, the passage of current may modify the cell excitability giving false positive results. Current artifacts normally appear more quickly than true drug effects and can be distinguished from drug effects by passing an equivalent current through a barrel containing sodium chloride.

The pH of most drug solutions is adjusted to improve the chemical stability of the drug and/or to increase its ionization. For example, NE and related drugs are often used at the relatively low pH of 3.5-4.0. Therefore, there is a possibility that during the cationic ejection of ionized NE, the simultaneous ejection of hydrogen ions may influence the behaviour of the neuron under study. This issue remains controversial and there is even some disagreement about the effects of hydrogen ions themselves on neuronal firing (Bevan et al., 1973b; Krnjevic and Phillis, 1963a; Hewes and Frederickson, 1974) but nevertheless it appears that these effects may only be important for ejections of solutions of pH 2.5 or less (Krnjevic and Phillis, 1963a). When there are such concerns, artifacts attributable to pH may be tested by ejecting low pH HCl solutions in sodium chloride (Metherate et al., 1988a; Tremblay et al., 1990a).

2.2 Electrodes.

Low impedance glass microelectrodes (A-M Systems) containing a seven-micron diameter carbon fibre were used to map the somatosensory cortex to find areas responsive to stimulation of the skin of the forearm. The carbon fibre was inserted in the glass capillary using air suction and was then pulled in a vertical puller (Narashige, PE-2). The pulled electrode was filled with NaCl (3M) and the protruding carbon fibre was cut at its junction with the glass using microsurgical scissors under the microscope.

Seven-barrel microfilament capillary glass pipettes (A-M Systems) were pulled on the vertical puller and used for microiontophoresis. The tip of the pulled electrode was broken under the microscope to a final diameter of 10-13 μm. Then the pulled end of the pipette was bent in a microforge (C.H Beaudouin, model 647) at an angle of 15-30° with the elbow at least 4mm away from the tip. A single microfilament glass capillary (A-M Systems) was pulled and glued to the microiontophoresis electrode with light-cured dental adhesive (3M, #5502Y mixed with #7533L and #75335 or #9350 mixed with #7533L) that was polymerized with a high intensity visible light (3M, Visilux 2). The tip of the single electrode protruded 20-40μm beyond the multi-barrel electrode and was used to record single units during microiontophoretic experiments. It was filled with 2% Pontamine sky blue in NaCl 1M. After breaking the tip of the recording electrode slightly, the impedance at 1Khz was usually between 2 and 3.5MΩ.

2.3 Equipment.

2.3.1 Recording and data collection.

The electrode was held in a microdrive (Narashige) and linked with a single shielded cable to a preamplifier (Princeton Applied Research, model 113). The preamplified signal was led to an amplifier (Tektronix, AM 502) and filters were adjusted to provide a band-pass of 1 to 3KHz. The gain of the preamplifier was set at 10 and the amplifier usually at 2K. The amplified signal was directed to an oscilloscope (Tektronix model 5113 equipped with the 5A18N, 5A14N and 5B12N modules) via a voltage discriminator that consisted of a rate meter and an audio monitor (Winston Electronics, RAD-II-A). This equipment was sufficient for mapping the somatosensory cortex (see section 2.6.1).

During microiontophoresis, single action potentials were counted using the voltage discriminator and led to an interface (Cambridge Electronic Designs, model 1401) controlled by an IBM compatible computer employing a 80286 microprocessor. The data were stored on the hard disk of the computer for later analysis as spike trains and time interval histograms. All the software was written by Philippe Therien.

2.3.2 Microiontophoresis.

Several noradrenergic drugs have been tested in the course of these experiments. All solutions were made with deionized water containing 0.1% ascorbic acid to prevent their oxidation. The pH was adjusted with NaOH or HCl and the solutions were frozen immediately at -30° for later use. In each of the experiments, barrels were filled with l-NE hydrochloride (Sigma Inc.) 0.5M pH 4.0, dl-glutamate

(Sigma Inc.) 0.5M pH 8.0 and NaCl 0.9% pH 7.0 for current balancing. The remaining barrels could be filled either with oxymetazoline hydrochloride (Sigma Inc.) 0.1M pH 4.5, idazoxan (Reckitt & Colman) 0.01M in NaCl 0.9% pH 4.5, yohimbine hydrochloride 0.01M in NaCl 0.9% pH 5.5, dl-isoproterenol hydrochloride 0.2M pH 4.5, sotalol hydrochloride (Mead-Johnson) 0.1M pH 4.7, timolol maleate (Sigma) 0.1M pH 4.2, benoxathian hydrochloride (Research Biochemicals Inc.) 0.05 pH 4.0 and in most experiments one barrel was filled with an HCl solution pH 4.0 in NaCl 0.9% to control for H^+ ions and current effects.

A five-channel iontophoresis unit (Medical Systems Corp., BH-2 system) produced the currents for drug ejection and retention. Ejection currents ranged from 5-200nA and retaining currents were normally set between 10 and 15nA. Care was taken to ensure that the effects observed were due to drug administration and artifacts due to current or to the ejection of H^+ ions were controlled in two ways First, current balancing was used routinely throughout the experiments. Second, a pH 4.0 solution of HCl in 0.9% NaCl was administered using positive currents of the same magnitude as those used to administer the drugs allowing the control of both current and H^+ ions. Further, since the tip of the microiontophoretic electrode was 20-40μm behind the tip of the recording electrode it was less likely that the current produced by drug ejection would influence the firing rate than in some electrodes where the recording electrode is the same length as the ejection electrodes Finally, since current artifacts disappear rapidly upon removal of the current, effects outlasting the period of drug application were probably attributable to drug effects.

2.3.3 Tactile stimulation.

When a cell with a receptive field was isolated, quantitative stimuli were presented by positioning the tip of a tactile stimulator (Chubbuck, 1966) over the centre of the receptive field. Each stimulus consisted of a square indentation of the skin (0.5 to 1.0mm) on which was superimposed, 0.2s later, a 10-40Hz sinusoidal vibration that lasted 0.6s. The indentation was ended 0.2s after the end of the vibration so that the entire procedure lasted 1.0s. The indentation was controlled by a digitimer (Medical Systems Corp., model D4030) and the sinusoidal vibration was produced by a sweep generator (Interstate Electronics Corp., model F44). The digitimer was controlled by the computer and each sequence of the stimulation was stored in the computer simultaneously with the action potentials.

2.4 Animal preparation.

Experiments were performed on 46 adult mongrel cats of either sex. The animal was first anaesthetized in a closed environment containing halothane vapours. Once areflexic to the pinching of the forepaw, the neck was shaved and a tracheostomy was performed quickly while the animal was still breathing halothane vapours from a beaker containing cotton swabs soaked with halothane. Following this, the animal was ventilated artificially (Harvard Apparatus Respirator); the inspired air contained 2% halothane during the surgery and was lowered to 1-1.5% during recording sessions.

The animal was then placed on a thermostatically-controlled heating pad and body temperature maintained at about 37.5°C. Once the head was mounted in a

stereotaxic device, the head, the neck and left forearm were shaved. The skin over the cranium and the upper neck was incised along the midline and the temporalis muscles reflected to expose the bone after incision of their tendinous insertions The cisterna magna was opened to drain the cerebrospinal fluid and the lower torso was suspended with a vertebral clamp attached to a spiny process in the lumbar region to minimize brain movements. A drill bit was used to make a hole through the right frontal bone to expose the frontal sinus which was then filled with bone wax to prevent the leakage of liquid into the respiratory tract.

A craniotomy was performed over the right somatosensory cortex. Under a binocular microscope, a hole was carefully drilled through the bone down to the dura. Rongeurs were used to enlarge the craniotomy in order to prevent damage to the cortex caused by excessive vibrations and heating produced by the drill. The posterior and lateral margins of the opening generally corresponded to the curvature of the ansate sulcus where the posterior aspect of area 3b and part of area 1 represented the body at the level of the forearm.

Low melting point dental impression compound (Kerr) was used to form a well around the craniotomy. During this procedure, the exposed dura was covered with several layers of gaze soaked with warm saline to prevent damage caused by the nearby point of the soldering iron used to melt the compound The well was filled with warm Elliot's solution (Abbott Laboratories) to prevent cooling and drying of the exposed tissue. The dura was excised under the microscope with microsurgical scissors by elevating the meningeal tissue with forceps to prevent damage to the cortical surface as the dura was reflected.

2.5 Experimental paradigms.

2.5.1 Location of the forearm somatosensory cortex.

Prior to insertion of the microiontophoretic pipette, the somatosensory cortex was mapped using carbon fibre electrodes to find a region receiving inputs from the skin of the forearm that was easily accessible to the mechanical stimulator. Generally 5 to 15 descents in the suprasylvian gyrus were sufficient to find a suitable recording site having receptive fields in the region of the dorsal wrist or forearm.

2.5.2 Characterization of the neurons.

Following the mapping procedure, the carbon fibre electrode was replaced by a recording-microiontophoresis electrode assembly. Under the microscope, the tip of the electrode was placed about 200μm above the cortical surface and the artificial cerebrospinal fluid was replaced by a solution of 3% agar in artificial cerebrospinal solution at 37°. Once the agar had hardened, it was covered with artificial cerebrospinal solution to prevent drying and shrinkage of agar during the recording session

The electrode was advanced by small steps of 5-10μm and pulses of glutamate were delivered regularly to excite otherwise quiescent neurons. At the same time, the skin of the forearm, especially around the location of the receptive field region found in the earlier mapping, was stimulated by gentle tapping. Once a unit was isolated, its depth on the micrometer was noted and it was characterized by the presence or absence of a receptive field. When present, the receptive field was characterized by its modality and submodality in the following way: (1) a receptive

field was said to be located in the skin when the light touch of the skin with a hand-held, fire-polished glass probe and/or the flicking of a few hairs elicited a clear response over the background; these cutaneous receptive fields were further characterized as being either slowly adapting (SSA) or rapidly adapting (SRA) according to whether they responded or not to a sustained stimulus applied on their receptive field; (2) a receptive field was classified as being Deep when the neuron appeared to respond only to the stimulation of subcutaneous structures i.e. to the palpation of the underlying muscles or to joint movement and (3) in several cases receptive fields were classified as TAP when the unit required a light tapping of the skin to respond to every stimulus. In many cases the shape and size of the receptive fields were assessed manually with the glass probe and represented on a standard drawing of the cat forearm.

In the somatosensory cortex a large proportion of neurons do not display a receptive field (Dykes and Lamour, 1988a). Those neurons were assessed for the presence of a receptive field during the administration of subthreshold doses of glutamate. These neurons were classified as having no receptive field (NoRF) or as having a receptive field only in the presence of glutamate (Glut-RF).

Neurons were also characterized by the presence or absence of spontaneous activity. Since spontaneous activity was usually very low, the neuron was left unstimulated for at least one minute before a decision was made. In many cases where an ongoing discharge was present, the frequency of this activity was evaluated either by counting the action potentials with the window discriminator for at least 100s or by the use of data stored on the computer during the control period of a

series of somatic stimuli or pulses of glutamate. All neurons displaying an ongoing discharge of less than 0.1Hz were considered to be silent.

For many neurons, the threshold current of glutamate necessary to produce a response was determined by setting first a current that elicited a clear response and then by decreasing it in small steps until no clear response was observed. Then the current was slightly increased in steps until a response was detected over the background and this current was noted. In some cases glutamate failed to elicit a response even with currents up to 500nA suggesting that these units were lacking glutamate receptors and/or that they might have been fibres.

2.5.3 Effects of noradrenergic drugs.

For the quantitative assessment of the effects of noradrenergic drugs two different experimental paradigms were used depending upon whether or not the neuron had a receptive field.

2.5.3.1 Neurons displaying a receptive field.

For neurons displaying a receptive field the mechanical stimulator was placed over the centre of the receptive field and the skin indentation was set to produce a clear response to each stimulus presentation. The stimulus was presented at 7s intervals and the activity was recorded 2s before and 2s after the stimulus presentation. Prior to any drug administration, at least 30 such trials were recorded to establish the baseline of the response. Then NE was administered with currents ranging from 5 to 100nA until an effect on the response could be observed. If no

effect was observed within the first 10-30 trials, then the ejecting current was increased while the receptive field was stimulated. Following the cessation of NE ejection, time for recovery was allowed during which the stimulation was continued and the activity recorded.

Following recovery, other tests were performed with either NE or noradrenergic agonists or antagonists. To test antagonists, the antagonist was first ejected alone for varying periods of time and then NE was administered with the same current magnitude that had been effective before. Since, in the course of these experiments, it became clear that NE produced effects that outlasted its application for long periods of time, attempts to block these effects were performed by first applying NE in the presence of the antagonist and only later was testing NE alone to see if the result was different in the absence of the antagonist.

2.5.3.2 Neurons lacking a receptive field.

In the case of neurons without a receptive field a similar paradigm was used but the peripheral stimulation was replaced by glutamate pulses given at regular intervals to excite the neuron during the tests with noradrenergic drugs. Neuronal activity was recorded in trials lasting from 55 to 120s. The ongoing activity was recorded in the first 10 or 20s of each trial and then glutamate was turned on for 30 or 60s. The baseline response was established with at least 3 trials prior to the ejection of NE. The microiontophoretic channels were controlled by the computer during the tests. The administration of noradrenergic drugs was started at the beginning of each trial so that their effect on both spontaneous and glutamate

induced activity could be evaluated. In the case of antagonists, their administration was usually started one trial before NE and they were continuously ejected to ensure that they were already present when NE ejection was started.

2.6 Histology.

When a significant a number of neurons were isolated in one penetration, pontamine sky blue was ejected from the recording electrode in two locations 1000 or 1500μm apart along the electrode track. The dye was ejected by passing a current of negative polarity using a lesion producing device (Stoelting #58041) modified to deliver currents in the μA range in a stepwise manner: 5μA for 5min, 10μA for 5min and than 20μA for 10min at each location. During the ejection, the current was monitored continuously with a voltameter to make sure that the current was passing. In cases of blockage, reversing the polarity of the current several times was often sufficient to unblock the pipette. Following the dye ejection, two wires were inserted, one on each side of the penetration to facilitate its location during sectioning.

Prior to the perfusion the halothane concentration in the inspired air was raised to 4% for 10 to 15min. The animal was perfused through the ascending aorta with 0.9% saline followed by 10% buffered formalin. The brain was removed from the skull and placed in the same fixative for several days before being cryoprotected with 30% sucrose. Eighty μm thick sections were cut through the somatosensory cortex on a cryostat and mounted on gelatin-coated slides. The sections were Nissl-stained with cresyl violet using standard procedures and coverslipped.

Camera lucida drawings of the sections containing the dye spots were made at a 40x magnification and each neuron was attributed to its cortical layer and cytoarchitectonic area based on the cytoarchitectonic criteria of Hassler and Muhs-Clement (1964). The distance between the two dye spots was used to correct for shrinkage of the cortex during the histological processing. In some cases the dye could not be seen but electrolytic lesions were present.

2.7 Data analysis and significance of the data.

2.7.1 Data analysis of single neurons.

Data stored in the computer could be retrieved as spike trains and mean time histograms. The software counted the action potentials recorded over any time interval chosen by the user. Several trials could be averaged and displayed as time interval histograms. Such interval histograms were obtained during the control period, the drug treatments and the recovery period for each neuron. The number of action potentials recorded during the same periods were counted at the same time for both spontaneous activity and peripherally-or-glutamate induced activities. The number of action potentials counted during the drug administration and during the recovery period were expressed as a percentage of the activity observed during the control period.

There is no statistical test suitable for microiontophoresis that can be applied to a single neuron and authors have often used a minimal change in the neuronal activity to decide if a neuron was affected by a drug treatment (see Stone, 1985). In the present study, an excitation was considered to be significantly increased when the

neuronal activity was at least 1.3 time the activity observed during the control period, an increase of 30%. In the case of inhibition, the activity had to be at least 1.3 time less than during the control period, a decrease of at least -23%. Any change within the interval of -23% to +30% was considered not to be significant. These criteria have been applied systematically and whenever a neuron is qualified as excited, inhibited or not affected reference is made to these criteria. An antagonist was judged as efficient in blocking the effect of NE when a significant effect produced by NE alone was found to be not significant when NE was administered in the presence of the antagonist.

2.7.2 Statistical analysis.

Appropriate statistical tests were used whenever possible. The choice of the proper test to use was based on the theoretical arguments of Sokal and Rohlf (1981). To test for proportions differing from chance (i.e. a contingency table) the G-statistic was used whenever the size of the sample was large enough. In some cases, classes displaying similar characteristics were pooled (e.g. laminar distribution, distribution of the frequencies of spontaneous activity and of threshold current of glutamate) to meet the prerequisite sample size of the test. In the case of 2x2 tables of small sample size, the Fisher's exact test was applied using the tables of Siegel (1956). In other cases no statistical test was applied.

When the analysis of variance was used, the assumptions of that test were fulfilled. Statistical tests were complicated by the fact that the frequencies of spontaneous activity and the iontophoretic currents used for both NE and glutamate

were not normally distributed. Further, the variances of the samples were seldom homogeneous. After a logarithmic transformation, both spontaneous activity and glutamate currents appeared to be normally distributed and to have homogeneous variances, so the analysis of variance was performed on the transformed data. The geometric means are reported instead of the usual arithmetic means since they give a better representation of the data. The data for the activity evoked by cutaneous stimuli and the responses to glutamate were normally distributed and their variances were homogeneous. In those cases the data did not need to be transformed and statistical tests were performed on the original data and the arithmetic means were used.

3.0 RESULTS

The experiments are presented in 4 sections. The first section (section 3.1) concerns the general characteristics of the sample and is followed by the laminar analysis of the sample of neurons located in the histology (section 3.2) while the last two sections describe the effects of NE on somatosensory cortical neurons during its administration (section 3.3) and in the period following the cessation of NE (section 3.4).

3.1 The sample.

3.1.1 Origin.

Forty six adult mongrel cats have been used for this study and the responses of 465 neurons (see section 3.1.2) were examined in the primary somatosensory cortex along 66 electrode penetrations (Table 1). In 17 animals, the Pontamine sky blue ejected at the end of the penetration or the electrolytic lesions produced by the ejecting current was detected in the histological sections and 189 neurons were located in those sections containing the dye spots or the lesions (Figure 1). A relatively large mean of 7.0 neurons were isolated per penetration, suggesting that the electrodes used had excellent isolating capacities. In animals where all penetrations had few isolated neurons no effort was made to reconstruct the penetration from histology. Consequently Table 1 shows that almost twice as many neurons were found per penetration located in the histology compared to the sample not found in the histology.

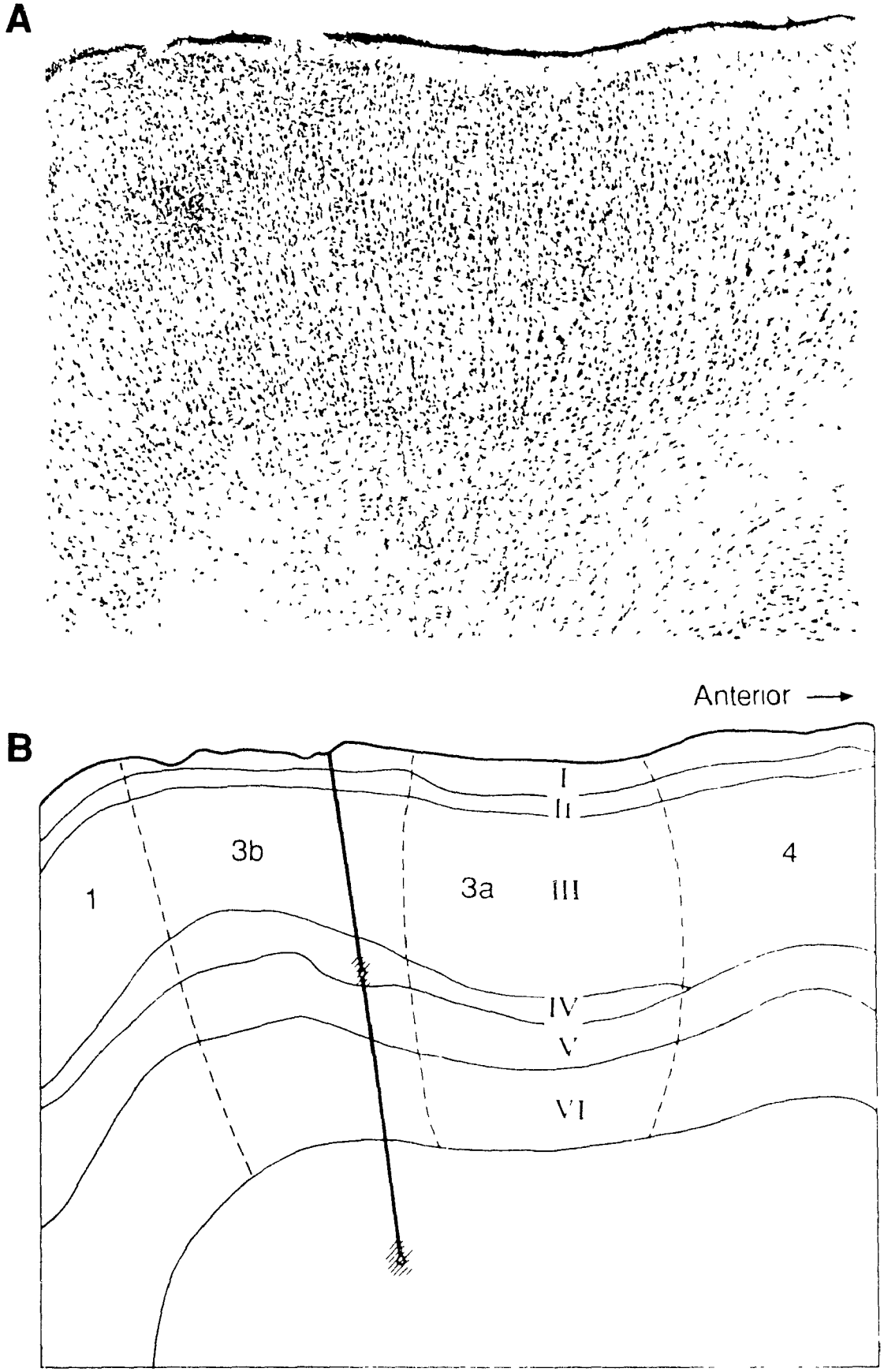
Table 1: Origin of the sample.

	Found in histology	Not found in histology	Total
Number of cats	17	29	46
Number of penetrations	17	49	66
Number of neurons isolated	189	276	465
Number of neurons per penetration	11.1	5.6	7.0

Figure 1: Cresyl-violet stained sagittal section through the somatosensory cortex and corresponding reconstruction.

A. Sagittal section through the somatosensory cortex. In that case, the Pontamine sky blue deposit was not seen but the electrolytic lesions produced by the current used to eject the dye could be seen in layer IV and in the white matter

B Corresponding reconstruction showing the trajectory of the electrode penetration. The known distance between the lesion was used to correct for shrinkage. The neurons were located relative to the micrometer reading of the lesions and assigned to a cortical layer.


A
Anterior
B
I
II
3b
1
3a
III
4
IV
V
VI
1 mm

3.1.2 Nature.

Actually over 500 single units were isolated in the somatosensory cortex. Of these, 465 (87%) were found to be sensitive to glutamate and were held long enough to be tested for the presence of a receptive field (Table 2A). Those units sensitive to glutamate could be considered as neurons since fibres lack glutamate receptors. This subset will constitute the core of the present report even though it is possible that some of the units unresponsive to glutamate might have been neurons (Schneider and Perl, 1988).

Only 23% of the neurons displayed a receptive field. These were subdivided further according to the modality of their peripheral input. Eighty-five appeared to receive input from the skin and of these, 82 were classified as skin rapidly adapting (SRA) whereas only three had the characteristics of skin slowly adaptating units (SSA) Only one neuron appeared to receive input from muscle receptors, responding to the flexion of the 5th digit; it was classified as serving subcutaneous receptors (Deep) In 20 cases the modality appeared uncertain because these neurons could have been classified as high threshold cutaneous neurons or low threshold neurons in deep tissue Since they responded to light tapping of their receptive fields they were classified TAP.

In the remaining 77% of the sample, no evidence of peripheral input was found in the absence of any drug but in 127 cases subthreshold amounts of glutamate ranging from 4 to 90nA (geometric mean=23.5nA) were sufficient to uncover receptive fields that had characteristics similar to those occurring in the absence of drugs; these neurons were classified as having a receptive field during glutamate

Table 2: Units isolated in cat somatosensory cortex (n=533).

A: Neurons sensitive to glutamate and assessed for the presence of a receptive field (n=465; 87% of the isolated units). To calculate the percentage of Glut-RF and No-RF neurons, the neurons that were not tested for the presence of a receptive field were proportionally distributed to these groups before the calculation (see text)

	Found in histology	Not found in histology	**Total**
With a Receptive Field:			
SRA	42 (22%)	40 (14%)	82 (18%)
SSA	1 (0.5%)	2 (0.7%)	3 (0.6%)
TAP	7 (3.7%)	13 (4.7%)	20 (4.2%)
DEEP	0 (0%)	1 (0.4%)	1 (0.2%)
Total:	**50 (26%)**	56 (20%)	**106 (23%)**
Without a Receptive Field:			
Glut-RF	49 (33%)	78 (35%)	127 (34%)
No-RF	61 (41%)	100 (45%)	161 (43%)
Receptive Field not tested with glutamate	(29)	(42)	(71)
Total:	**139 (74%)**	220 (80%)	**359 (77%)**

B: Other units (n=68; 13% of the isolated units).

Not sensitive to glutamate	13 (19%)
Lost while isolating	25 (37%)
Killed "en passant"	30 (44%)

iontophoresis (Glut-RF neurons). In the other 161 cases glutamate treatment failed to uncover peripheral inputs and these neurons were classified as lacking any receptive field (No-RF neurons). In 71 cases constituting 15% of the sample, no attempt was made to uncover a receptive field with glutamate. These were not considered a legitimate class since these cases were likely to be both Glut-RF and No-RF neurons Nevertheless these neurons had to be accounted for in the computation of the proportions of the Glut-RF and No-RF classes. To do this it was assumed that this group contained the same proportions of Glut-RF and No-RF neurons found in the overall sample tested for receptive fields with glutamate and they were distributed accordingly to the Glut-RF and No-RF groups, yielding a proportion of 43% of the neurons without any evidence of peripheral input and 34% with receptive fields uncovered by glutamate Consequently a grand total of 57% of the sample appeared to receive some input from the periphery

Table 2 also shows the number of neurons for each class found in the sample located in histology and in those not located in the histology The proportions do not vary much for any class of neurons between the two samples and no significant difference was found (G-test, $G_{adj} = 4.677$, degrees of freedom (df) = 3, $p > 0.1$; SSA and Deep classes were excluded from the test), suggesting that the two samples belong to the same population. Therefore it appears that no bias was introduced by locating some neurons in the histology and that the total sample represents the best estimate for the proportions of the different classes of neurons observed In the following sections, the neurons without a receptive field that were not tested for the presence of a receptive field with glutamate will not be considered for the reason

stated above, nor will the SSA and Deep neurons because they are relatively small samples of marginal importance. Thus, the results presented will refer to the remaining 390 neurons.

Relatively little information is available about the other 68 units presented in Table 2B and what is available is generally anecdotal in nature. Of these, 19% were found to be insensitive to glutamate and were suspected to be fibres whereas 37% were lost before any information could be taken and 44% were depolarized as the tip of the electrode impaled them.

3.1.3 Spontaneous activity

3.1.3.1 Proportion of spontaneously active neurons.

Of 340 neurons tested for the presence of ongoing activity, 42% were found to be spontaneously active (Table 3). Both those neurons found in the histology and those not found in the histology were much more often spontaneously active if they also had a receptive field 74% (n=71/96) compared to 29% (n=70/244) for the total sample, the proportions being significantly different (G-test, G_{adj}=53.837, df=1, $p<<0.001$). When comparisons were made within single classes of neurons, the only significant difference was found in the No-RF class (G-test, G_{adj}=6.483, df=1, $p<0.025$) where only 17% of those neurons found in the histology were spontaneously active compared to 37% in the sample not located in the histology (Table 3). It is not possible to determine whether the proportion of spontaneously active neurons has been underestimated in one sample or overestimated in the other, but in the Glut-RF group these proportions were reversed from the No-RF sample

Table 3: Proportions of neurons spontaneously active (adj, Williams correction; df, degrees of freedom).

	Found in Histology	Not found in Histology	Total
With a Receptive Field:			
SRA	29/39 (74%)	28/35 (80%)	57/74 (77%)
SSA	1/1 (100%)	2/2 (100%)	3/3 (100%)
TAP	3/6 (50%)	7/12 (58%)	10/18 (74%)
DEEP	0	1/1 (100%)	1/1 (100%)
Total	**33/46 (72%)**	**38/50 (76%)**	**71/96 (74%)****
Without a Receptive Field:			
Glut-RF	13/33 (34%)	15/66 (23%)	28/104 (27%)
No-RF	8/48 (17%)*	24/92 (37%)*	42/140 (30%)
Total	**21/86 (24%)**	**49/158 (31%)**	**70/244 (29%)****
Whole sample	**54/132 (41%)**	**87/208 (42%)**	**141/340 (42%)**

* Significantly different, G-test, $G_{adj} = 6.483$, $df = 1$, $p < 0.025$.

** Significantly different, G-test, $G_{adj} = 53.837$, $df = 1$, $p << 0.001$.

(although in this case no significant difference was found, G-test, $G_{adj} = 1.588$, df = 1, P>0.1) so that the two effects cancelled each other when the samples were pooled It seems reasonable to conclude that the significant difference in the No-RF group was the result of a type I error, that is the rejection of a true null hypothesis, leaving unaffected the major conclusion that cells with a receptive field are much more likely to be spontaneously active and that no overall difference was found between the sample found in histology and that not found in histology.

3.1.3.2 Frequency of spontaneous activity.

The rate of ongoing discharge was measured in 107 of 141 (75.9%) spontaneously active neurons with frequencies ranging from 0.10 to 14.04Hz The probabilities of encountering cells having a given frequency is illustrated in Figure 2A for the sample found in the histology, the sample not found in the histology and also for the total sample No significant difference was found between the frequency distribution for those found and those not found in the histology (G-test, $G_{adj} = 5.637$, df = 4, p>0.1). All three distributions were strongly skewed to the lowest frequencies; 62% of the total sample had frequencies lower than 1.0Hz whereas only 20% had rates greater than 2.0Hz

The geometric mean of the ongoing discharge for the entire sample was 0.77Hz (Table 4A) and no significant difference was found between cells located and not located in histology for any class of neurons. Neurons displaying a receptive field were found to have significantly higher spontaneous rates than those lacking a receptive field (Table 4B). This difference appeared to be due to the fact that fewer

Figure 2: Distribution of the frequencies of spontaneous activity. The width of each class is 0.25Hz for frequencies below 2.1Hz and 2.0Hz for values above 2.1Hz. The number of neurons in each class was expressed as a percentage so that the distributions of the different samples could be readily compared

A. Samples of neurons located in the histology, not located in the histology and total sample.

B. Neurons displaying a receptive field and neurons lacking a receptive field

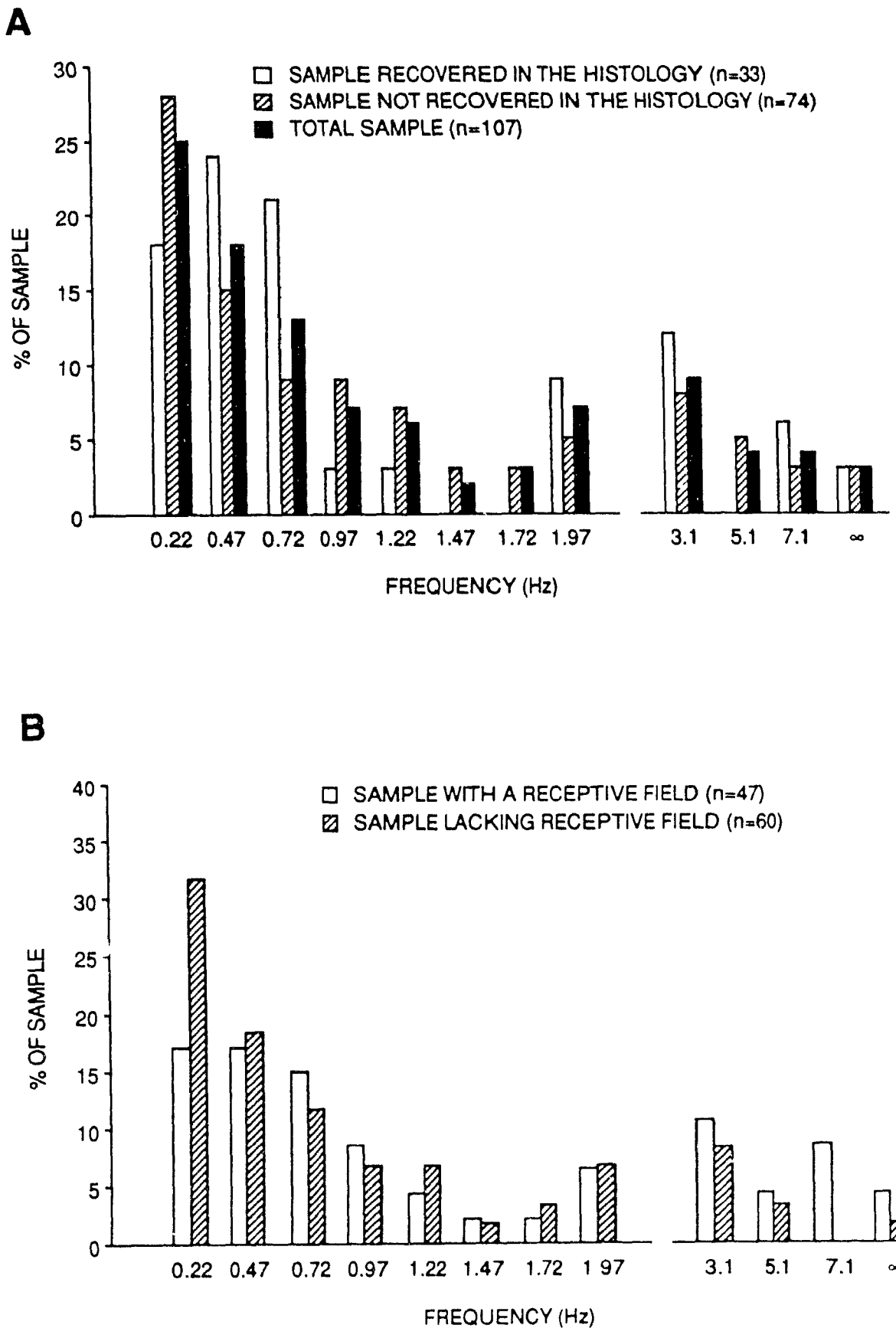

A
SAMPLE RECOVERED IN THE HISTOLOGY (n=33)
SAMPLE NOT RECOVERED IN THE HISTOLOGY (n=74)
TOTAL SAMPLE (n=107)
% OF SAMPLE
30
25
20
15
10
5
0
0.22 0.47 0.72 0.97 1.22 1.47 1.72 1.97
3.1 5.1 7.1 ∞
FREQUENCY (Hz)
B
SAMPLE WITH A RECEPTIVE FIELD (n=47)
SAMPLE LACKING RECEPTIVE FIELD (n=60)
% OF SAMPLE
40
35
30
25
20
15
10
5
0
0.22 0.47 0.72 0.97 1.22 1.47 1.72 1 97
3.1 5.1 7.1 ∞
FREQUENCY (Hz)

Table 4: Spontaneous activity.

A: Mean frequencies of spontaneous activity (Hertz).

	Located in the histology		
	Yes	No	Total
With a Receptive Field:			
SRA	1.18 (n=15)	0.97 (n=23)	1.05 (n=38)
TAP	0.55 (n=3)	1.31 (n=6)	0.98 (n=9)
Total with RF	**1.04 (n=18)**	**1.03 (n=29)**	**1.03 (n=47)**
Without a Receptive Field:			
Glut-RF	0.46 (n=8)	0.50 (n=17)	0.49 (n=25)
No-RF	1.01 (n=7)	0.67 (n=28)	0.73 (n=35)
Total without RF	**0.67 (n=15)**	**0.60 (n=45)**	**0.62 (n=60)**
Whole sample	**0.85 (n=33)**	**0.74 (n=74)**	**0.77 (n=107)**

B: Anova table (df, degrees of freedom; SS, sum of squares; MS, mean square; ns, not significant).

Source of variation	df	SS	MS	F,
RF vs without RF	1	1.3284	1.3284	5.046*
SRA vs TAP and Glut RF vs No-RF	3	0.4608	0.1532	0.583ns
Within subgroups	102	26.8503	0.2632	---
Total	106	28.6395	---	---

*$p < 0.01$

neurons displaying a receptive field had very low spontaneous discharge frequencies and more had higher frequencies whereas both distributions appeared similar in the midrange of frequencies (Figure 2B). Overall, the distributions were not significantly different (G-test, $G_{adj}=4.715$, df=3, $p>0.1$). No significant difference was found among SRA and TAP classes or among Glut-RF and No-RF classes (Table 4B) suggesting that the presence of a receptive field is a determining factor for the frequency of spontaneous activity but that the modality is not.

3.1.4 Sensitivity to glutamate.

The threshold current required to induce a discharge was determined for 301 neurons and the adequate currents ranged from 4 to 224nA. The distributions of the effective currents were skewed to the left (Figure 3A) and no significant difference was found between the neurons located in the histology and those not (G-test, $G_{adj}=9.340$, df=6, $p>0.1$). In the total sample, 65% of the neurons were driven with less than 50nA of glutamate and only 6.6% needed 100nA or more. The geometric means for the 4 classes of neurons ranged from 28.5nA to 37.1nA (Table 5A) but no significant differences were detected (Table 5B).

To test the hypothesis that spontaneously active neurons had lower thresholds of activation, glutamate currents used on spontaneously active neurons were compared to those used on silent neurons. Major differences were found at both tails of the current distributions (Figure 3B): 30% of spontaneously active neurons needed less than 20nA and only 9.1% needed more than 60nA whereas the picture was reversed for neurons having no ongoing activity: 9.1% were excited with less than

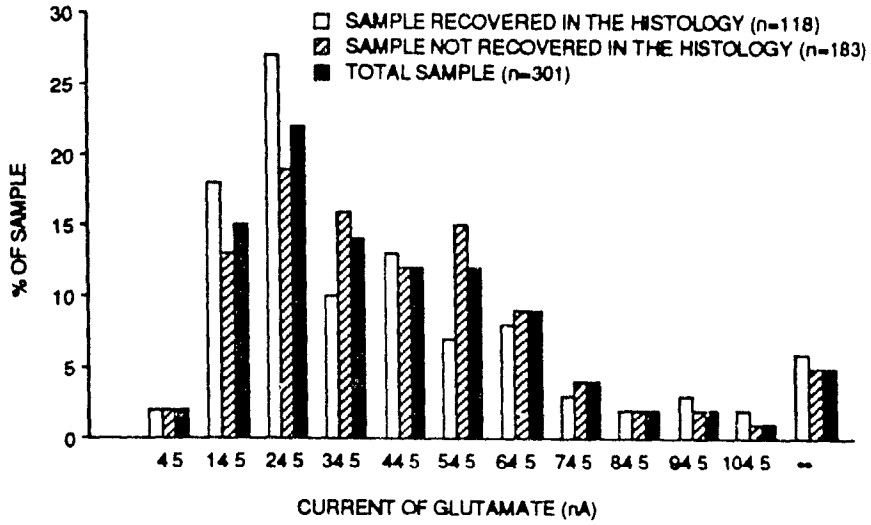
Figure 3: Distribution and the magnitude of the threshold current of glutamate required to activate neurons.

A Samples of neurons located in the histology, not located in the histology and total sample.

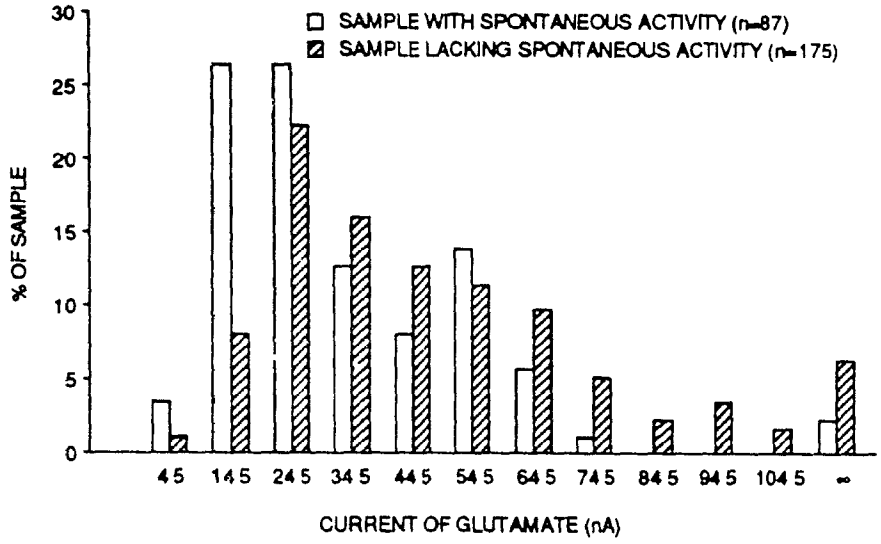
B Neurons displaying a receptive field and neurons lacking a receptive field.

C. Average threshold current of glutamate used on spontaneously active and silent SRA, TAP, GlutRF, NoRF and total sample. The currents of glutamate are in nA. The vertical bars represent the 95% confidence limits which were calculated for transformed data and than backtransformed (Sokal and Rohlf, 1981).

A


SAMPLE RECOVERED IN THE HISTOLOGY (n=118)
SAMPLE NOT RECOVERED IN THE HISTOLOGY (n=183)
TOTAL SAMPLE (n=301)
% OF SAMPLE
0
5
10
15
20
25
30
4 5
14 5
24 5
34 5
44 5
54 5
64 5
74 5
84 5
94 5
104 5
∞
CURRENT OF GLUTAMATE (nA)

B


SAMPLE WITH SPONTANEOUS ACTIVITY (n=87)
SAMPLE LACKING SPONTANEOUS ACTIVITY (n=175)
% OF SAMPLE
0
5
10
15
20
25
30
4 5
14 5
24 5
34 5
44 5
54 5
64 5
74 5
84 5
94 5
104 5
∞
CURRENT OF GLUTAMATE (nA)

C

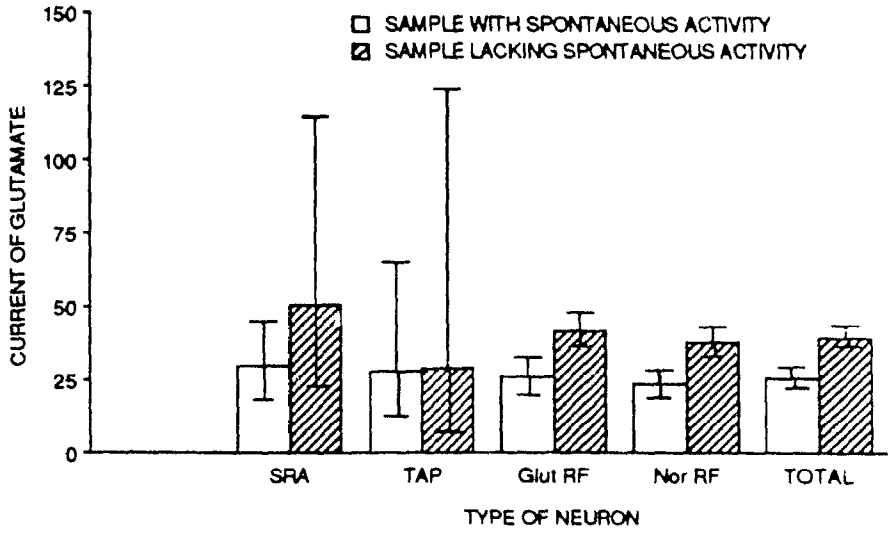

SAMPLE WITH SPONTANEOUS ACTIVITY
SAMPLE LACKING SPONTANEOUS ACTIVITY
CURRENT OF GLUTAMATE
0
25
50
75
100
125
150
SRA
TAP
Glut RF
Nor RF
TOTAL
TYPE OF NEURON

Table 5: Glutamate current required to drive neurons.

A: Mean current of glutamate (nA).

	Located in the histology		
	Yes	**No**	**Total**
With a Receptive field:			
SRA	28.6 (n=14)	40.4 (n=10)	33.0 (n=24)
TAP	24.7 (n=3)	30.1 (n=8)	28.5 (n=11)
Total	27.8 (n=17)	35.4 (n=18)	31.5 (n=35)
Without a Receptive Field:			
Glut-RF	35.9 (n=46)	37.9 (n=71)	37.1 (n=117)
No-RF	33.3 (n=57)	33.9 (n=92)	33.7 (n=149)
Total	34.5 (n=103)	35.6 (n=163)	35.5 (n=266)
Whole sample	33.4 (n=120)	35.6 (n=181)	34.7 (n=301)

B: Anova table (see abbreviations table 4B).

Source of variation	df	SS	MS	F_s
RF vs without RF	1	0.0693	0.0693	0.765ns
SRA vs TAP vs Glut-RF vs No-RF	3	0.1426	0.0475	0.524ns
Within subgroups	296	26.8376	0.0907	---
Total	300	27.0495	---	---

20nA and 29% with 60nA or more; the difference between the two distributions was highly significant (G-test, $G_{adj}=27.673$, $df=5$, $p<0.001$).

The geometric mean of current necessary to activate spontaneously active neurons did not vary much among the different classes of neurons, ranging from 23.4 to 29.8nA with an overall geometric mean of 25.6nA (Table 6A and Figure 3C). On average, 13.8nA more current was necessary to activate neurons that were not spontaneously active. More variations were observed among the different classes of silent neurons because of the small samples size for SRA ($n=6$) and TAP ($n=5$) neurons but when these two classes were pooled, the mean was very close to those observed for the Glut-RF and No-RF classes. There was a highly significant difference between the spontaneously active and the silent neurons whereas no difference was found between the four functional classes of neurons (Table 6B). No significant correlation was found between the glutamate currents and the rates of spontaneous discharge ($r=-0.231$, $p>0.05$, $n=72$) suggesting that ongoing discharges were not mistaken for glutamate induced activity.

It is interesting to note that when spontaneous and silent neurons were pooled as in Table 5, neurons displaying a receptive field were slightly more sensitive to glutamate than those lacking a receptive field (-4.0nA) whereas the situation is reversed for spontaneously active neurons and no difference is observed for silent neurons (Table 6A), suggesting that the difference in the pooled data was due to the fact that neurons displaying a receptive field were more often spontaneously active.

Table 6: Glutamate sensitivity of all classes of neurons (see text for classification).

A: Means of glutamate current with 95% confidence limits.

		SRA	TAP	All RF	Glut-RF	No-RF	All without RF	All sample
Spontaneously active neurons	Low. limits	18.9	12.1	20.3	20.3	19.2	21.0	22.2
	Means	**29.8**	**28.1**	**29.3**	**25.9**	**23.4**	**24.5**	**25.6**
	Upp. limits	47.0	65.0	42.2	33.0	28.6	28.5	29.5
	(n)	(16)	(6)	(22)	(28)	(37)	(65)	(87)
Non-spont. active neurons	Low. limits	22.3	6.8	20.5	35.9	33.3	35.8	35.8
	Means	**50.6**	**29.0**	**39.3**	**41.6**	**37.8**	**39.4**	**39.4**
	Upp. limits	114.7	124.3	75.3	48.2	43.0	43.3	43.4
	(n)	(6)	(5)	(11)	(70)	(94)	(164)	(175)

B: Anova table for glutamate current used on neurons with and without spontaneous activity (see abbreviations in table 4B.)

Source of variation	df	SS	MS	F_s
Sp Act vs Silent	1	2.0306	2.0306	25.179*
SRA vs TAP vs Glut-RF vs No-RF	3	0.1671	0.0557	0.691ns
Interaction	3	0.1947	0.0649	0.805ns
Within subgroups	254	20.4850	0.0807	---
Total	261	22.8775	---	---

*$p << 0.001$

3.2 Laminar analysis of the sample found in the histology.

In the previous section, the sample found in the histology and that not located in the histology have been compared for several parameters. Since they appeared quite homogeneous they could be confidently pooled to obtain better estimates however, the same statistics also suggest that the sample located in the histology is a fair representation of the whole sample and that conclusions drawn from it may be extended to the whole sample. In the present section, modality, glutamate sensitivity, and spontaneous activity are analyzed as a function of laminar location.

3.2.1 Cytoarchitectonic location of the sample.

The cytoarchitectonic and laminar locations of 189 neurons were determined from 17 penetrations recovered in the histology. Between 5 and 19 neurons were isolated per penetration. Only two neurons appeared to be located in the white matter. These were discarded leaving a sample of 187 neurons. Ten of the penetrations were located in area 3b comprising 59% of the sample whereas 27% of the sample was located in area 1, leaving only 14% of the sample in areas 3a and 2 (Table 7). No significant difference was found between the proportions of neurons with and without receptive fields among the different cortical areas (G_{adj}=4.757, df=3, p>0.1) so the neurons from the different areas were pooled.

Table 7: Cytoarchitectonic location of the penetrations and of the neurons found in the histology. Numbers between parentheses indicate the number of penetrations that entered more than one cytoarchitectonic area.

	Cytoarchitectonic Areas				
	3b	1	3a	2	Total
Number of penetrations:	10 (1)	2(4)	1	(3)	17
Neurons with RF:					
SRA	31	9	1	1	42
TAP	3	2	2	0	7
SSA	1	0	0	0	1
Total with RF:	35	11	3	1	50
Neurons without RF:					
Glut-RF	29	12	4	3	49
No-RF	31	20	6	3	61
RF not tested with glutamate	15	8	1	5	29
Total without RF:	75	40	11	11	137
Whole sample	110	51	14	12	187

3.2.2 Laminar distribution.

The laminar distribution of the sample located in the histology is shown in Figure 4A. The sample in each layer came from 7 to 12 penetrations (mean=9.0) suggesting that the sampling was quite even through the depth of the cortex. Indeed, 10 of the 17 penetrations covered the entire cortical depth, for 6 penetrations the sampling was located in the middle and lower layers whereas one penetration sampled only the upper layers. In one of the penetrations which was located in the posterior bank of the posterior sigmoid gyrus, 17 of the 19 neurons that were isolated were located in the middle of layer VI (midVI) almost doubling the sample of neurons in this layer. The remaining two neurons were located in lower layer VI (loVI) Because of this important bias, that penetration was removed from the laminar analysis, leaving a sample of 168 neurons with an average of 15.3 neurons sampled per layer. The smallest sample was found in the upper part of layer III (upIII) which contained 10 neurons and the biggest samples were located in layers IV and midVI with 18 neurons each (Figure 4A). The laminar distribution of the different classes of neurons are shown in Figures 4B, C and D.

3.2.2.1 Classes of neurons.

Prior to evaluating the probabilities of isolating neurons with and without a receptive field, the neurons that were not tested with glutamate for the presence of a receptive field were distributed to the Glut-RF and No-RF samples (see section 3.1.2) The probabilities of finding neurons with a receptive field are shown in Figure 5A and since the sample contained only 7 TAP and 1 SSA neurons, these were

Figure 4: Laminar distribution of neurons located in the histology. The vertical bars represent the mean number of neurons of all layers.

A All neurons located in the histology

B Neurons displaying a receptive field

C GlutRF neurons

D NoRF neurons

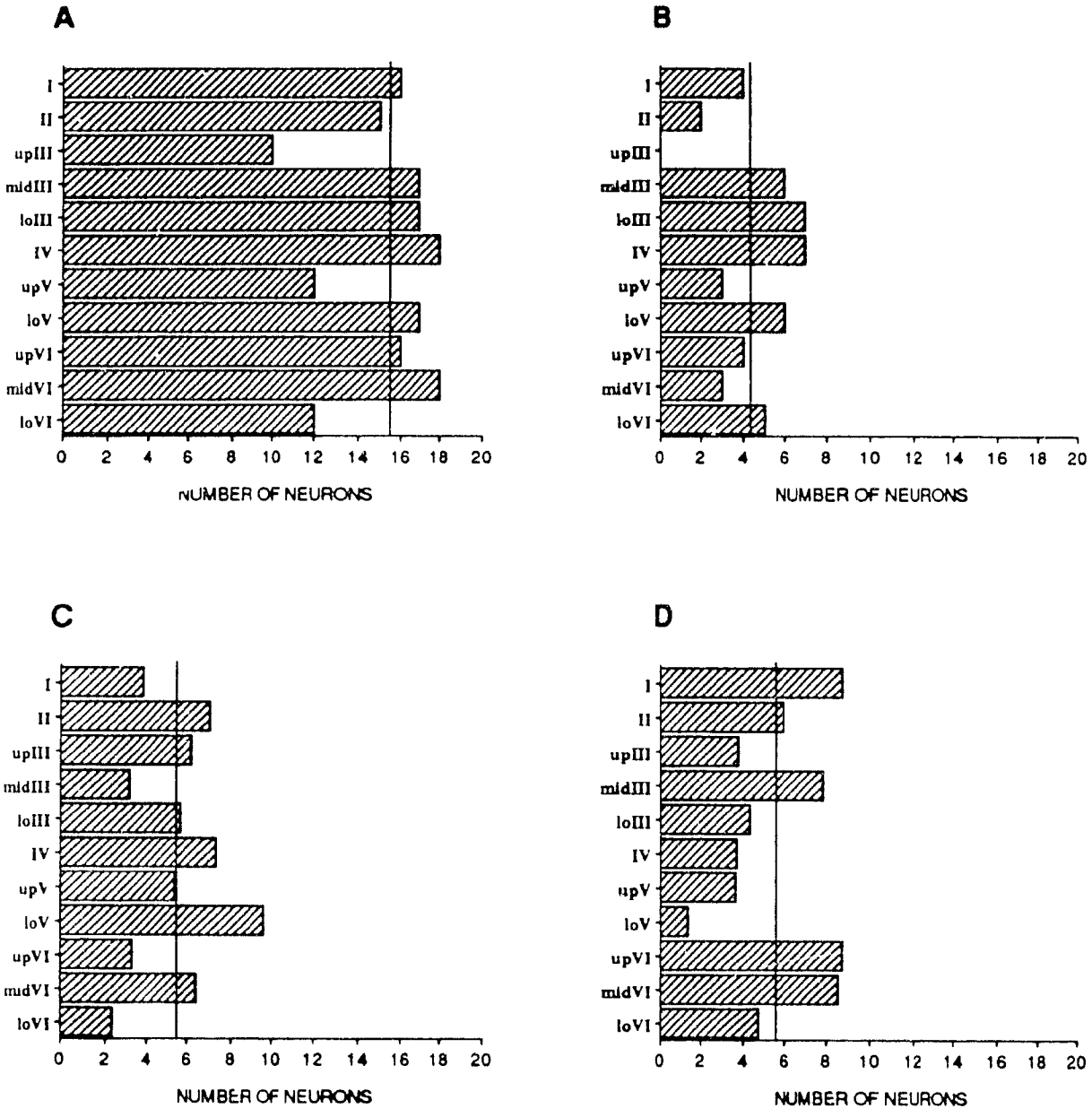

A
I
II
upIII
midIII
loIII
IV
upV
loV
upVI
midVI
loVI
0 2 4 6 8 10 12 14 16 18 20
NUMBER OF NEURONS
B
I
II
upIII
midIII
loIII
IV
upV
loV
upVI
midVI
loVI
0 2 4 6 8 10 12 14 16 18 20
NUMBER OF NEURONS
C
I
II
upIII
midIII
loIII
IV
upV
loV
upVI
midVI
loVI
0 2 4 6 8 10 12 14 16 18 20
NUMBER OF NEURONS
D
I
II
upIII
midIII
loIII
IV
upV
loV
upVI
midVI
loVI
0 2 4 6 8 10 12 14 16 18 20
NUMBER OF NEURONS

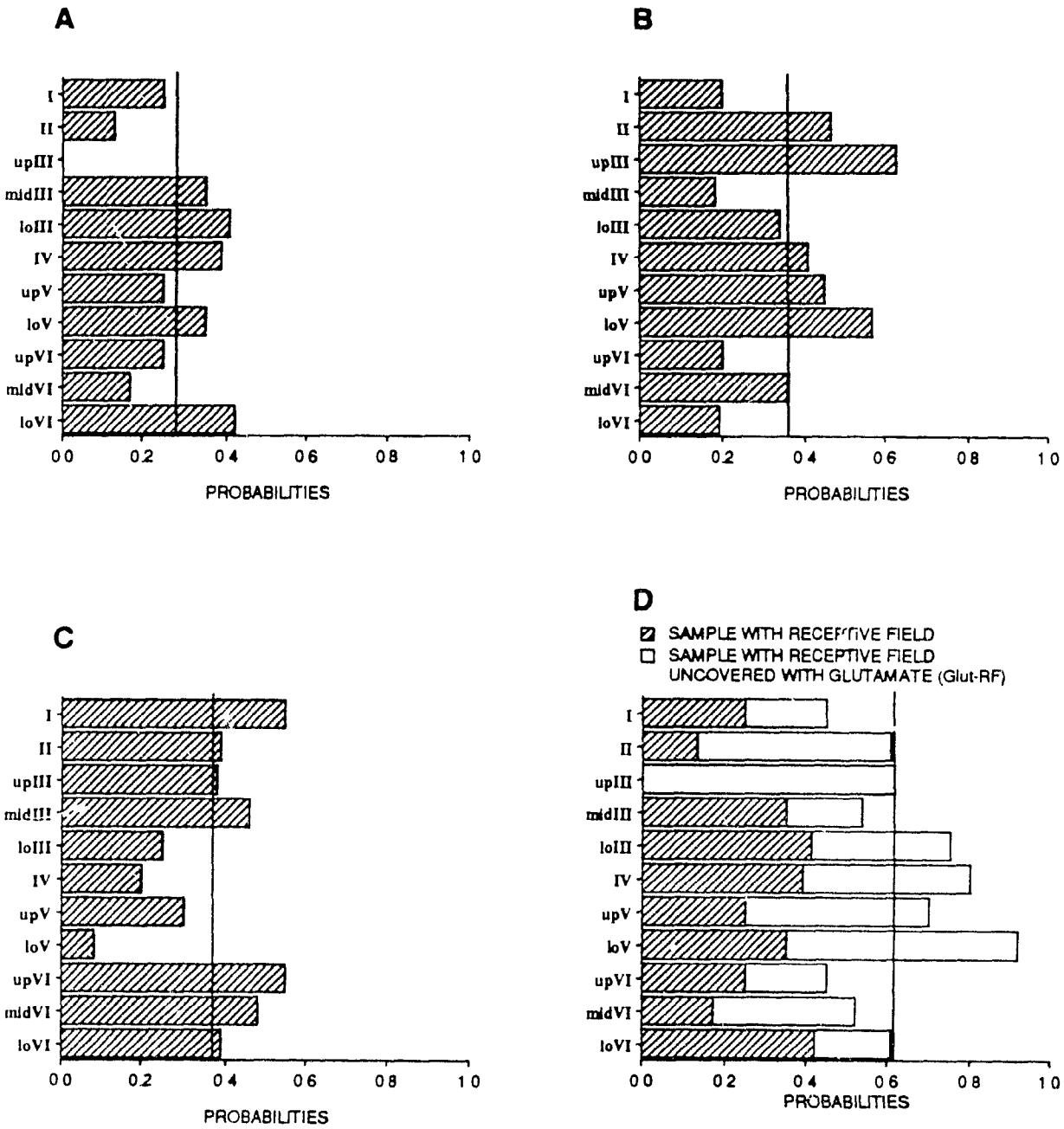
Figure 5: Laminar distribution of the probabilities of finding a class of neurons in each layer. The vertical lines represent the average probability of finding a cell in all layers.

A. Neurons displaying a receptive field.

B. GlutRF neurons.

C. NoRF neurons.

D. Neurons with evidence of somatic inputs (neurons displaying a receptive field and GlutRF neurons).


A
I
II
upIII
midIII
loIII
IV
upV
loV
upVI
midVI
loVI
00
0.2
0.4
0.6
0.8
1.0
PROBABILITIES
B
I
II
upIII
midIII
loIII
IV
upV
loV
upVI
midVI
loVI
00
0.2
0.4
0.6
0.8
1.0
PROBABILITIES
C
I
II
upIII
midIII
loIII
IV
upV
loV
upVI
midVI
loVI
00
0.2
0.4
0.6
0.8
1.0
PROBABILITIES
D
SAMPLE WITH RECEPTIVE FIELD
SAMPLE WITH RECEPTIVE FIELD UNCOVERED WITH GLUTAMATE (Glut-RF)
I
II
upIII
midIII
loIII
IV
upV
loV
upVI
midVI
loVI
00
0.2
0.4
0.6
0.8
1.0
PROBABILITIES

pooled with the SRA neurons, yielding 47 neurons displaying a receptive field. The highest incidences of neurons displaying a receptive field were found in the middle layers and in loVI whereas no neuron displaying a receptive field was found in upIII and low proportions were present in layers II and midVI. Glut-RF neurons were more often present in layers upIII and loV but they appeared very seldom in layers I, midIII, upVI and loVI (Figure 5B). In layers I and upVI, more than one half of the neurons gave no evidence of peripheral input whereas in the middle layers the absence of somatic input was less common (Figure 5C). Figure 5D shows the probabilities encountering neurons with a receptive field and Glut-RF neurons as a function of the layers; the probabilities of finding neurons with evidence of peripheral input were much higher in the middle layers than in the upper and lower layers.

3.2.2.2 Spontaneously active neurons.

The highest incidences of spontaneously active neurons were found in the middle layers; layers loIII and IV displayed the highest probabilities whereas in layers I and upVI neurons were often silent (Figure 6B). For neurons displaying a receptive field, the lowest probability of being spontaneously active (0.50) found in layers loV and up VI whereas almost all the neurons in layers loIII and IV were spontaneously active (Figure 6C). The majority of neurons without a receptive field were silent. Layers upVI and loVI contained only silent neurons and few spontaneously active neurons were found in layers I, loIII and IV (Figure 6D). The highest probability of finding spontaneously active neurons was found in layer upIII with an incidence of 0.50 followed by 0.43 in layer loV.

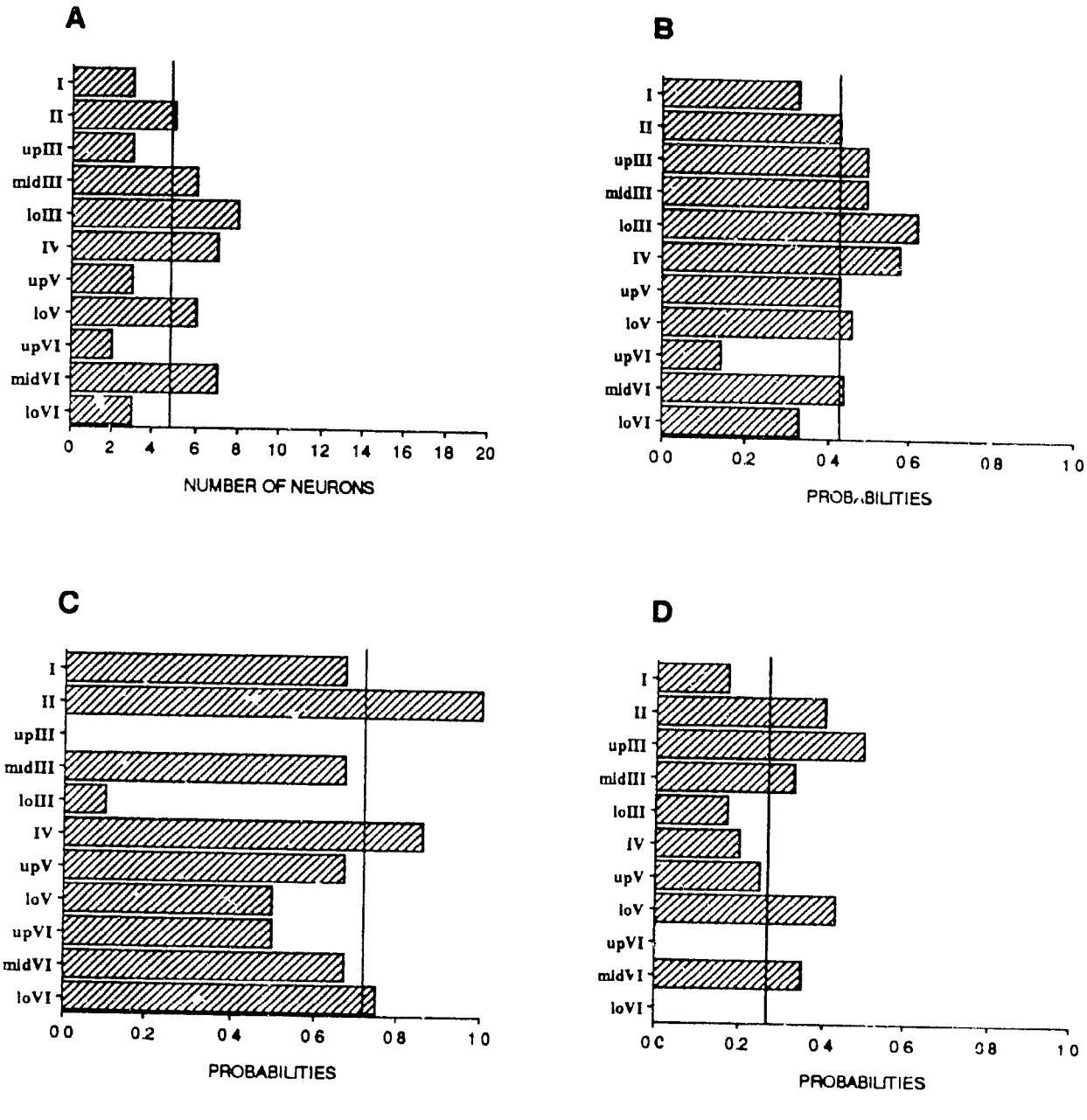
Figure 6: Laminar distribution of spontaneously active neurons.

A. All neurons located in the histology. The vertical lines represents the mean number of neurons in all layers.

B Probabilities of finding spontaneously active neurons in each layer. The vertical line represents the average probability of finding these across all layers.

C. Probabilities of finding spontaneously active neurons displaying a receptive field in each layer. The vertical line represent the average probability of finding these across all layers.

D Probabilities of finding spontaneously active neurons lacking a receptive field in each layer The vertical line represents the average probability of finding these across all layers.


A
I
II
upIII
midIII
loIII
IV
upV
loV
upVI
midVI
loVI
0 2 4 6 8 10 12 14 16 18 20
NUMBER OF NEURONS
B
I
II
upIII
midIII
loIII
IV
upV
loV
upVI
midVI
loVI
00 0.2 0.4 0.6 0.8 1.0
PROBABILITIES
C
I
II
upIII
midIII
loIII
IV
upV
loV
upVI
midVI
loVI
00 0.2 0.4 0.6 0.8 1.0
PROBABILITIES
D
I
II
upIII
midIII
loIII
IV
upV
loV
upVI
midVI
loVI
0C 0.2 0.4 0.6 0.8 1.0
PROBABILITIES

3.2.2.3 Discharge frequencies of spontaneously active neurons.

The rate of spontaneous activity was measured in 32 of 53 (60.4%) spontaneously active neurons. Because of the small sample size no measures were available for layers upV and loVI and there was only one measure for layers I, midIII and upVI. For the remaining layers, averages were obtained from 4 to 6 cells and the geometric means and 95% confidence limits are presented in Figure 7A. The geometric means for all the layers but two were within the confidence limits of the overall sample: layer midIII had an average above the upper limit and layer II was below the lower limit suggesting that those two layers might be different from the remainder of the sample.

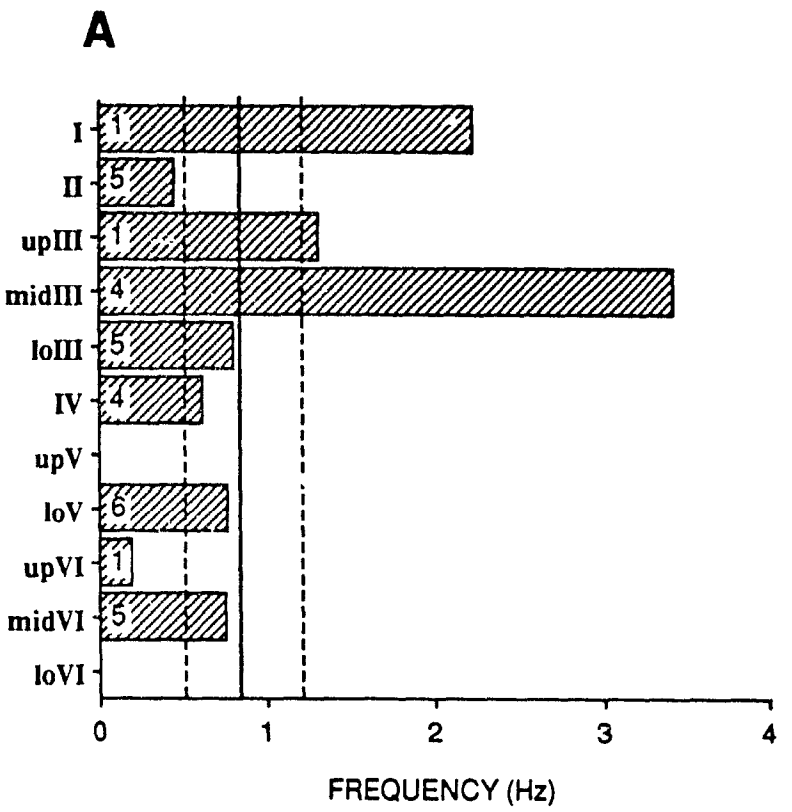
3.2.2.4 Sensitivity to glutamate.

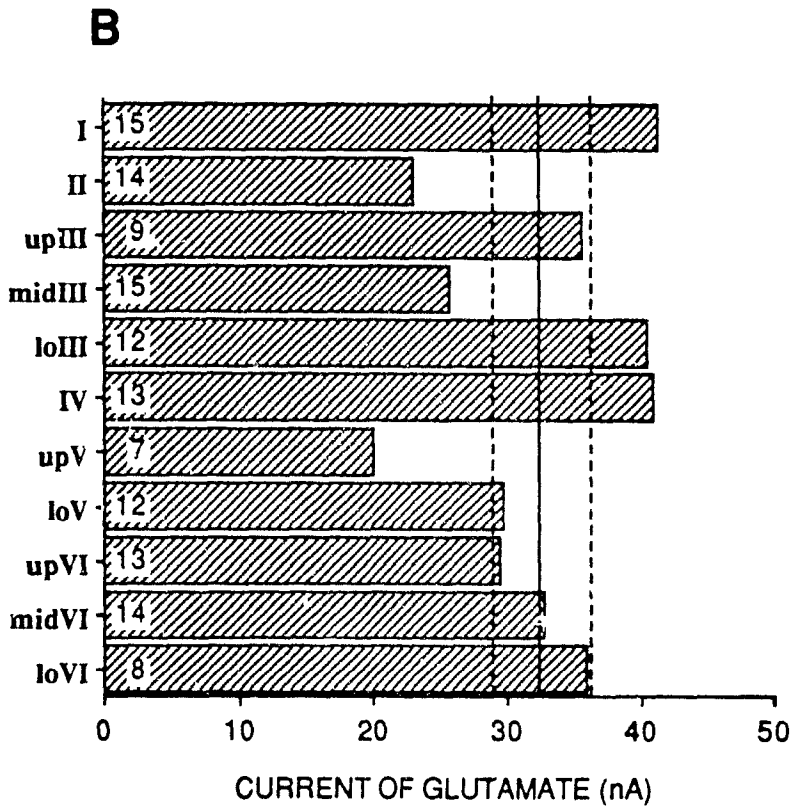
Figure 7B presents the average current of glutamate used to excite neurons as a function of their laminar location. Since no difference had been found previously between neurons with and without a receptive field (see section 3.1.4), the data from the two groups were pooled. The neurons located in layers I, loIII and IV were the least sensitive to glutamate and those located in layers II, midIII and upV were the most sensitive. The mean values for the remaining layers were within the confidence limits of the sample.

Figure 7: Laminar distribution of the geometric mean of the frequencies of spontaneous activity and of the threshold currents of glutamate. The vertical line in each histogram represents the geometric mean of the sample and the dotted lines the 95% confidence limits. The numbers in the histogram represent the number of neurons found in each layer.

A. Frequencies of spontaneous activity.

B. Threshold currents of glutamate.


A
I
II
upIII
midIII
loIII
IV
upV
loV
upVI
midVI
loVI
1
5
1
4
5
4
6
1
5
0
1
2
3
4
FREQUENCY (Hz)


B
I
II
upIII
midIII
loIII
IV
upV
loV
upVI
midVI
loVI
15
14
9
15
12
13
7
12
13
14
8
0
10
20
30
40
50
CURRENT OF GLUTAMATE (nA)

3.3 Effects observed during norepinephrine administration.

NE has been administered to 117 neurons in the cat somatosensory cortex using iontophoretic currents ranging from 5 to 100nA. The geometric mean of currents capable of producing a significant effect was 26.4nA. NE altered the excitability of almost all somatosensory cortical neurons during its iontophoretic ejection. In the presence of NE, inhibition was the most consistent effect observed, but during the minute following the cessation of NE ejection, the excitability of an important proportion of neurons increased and in most cases this enhancement lasted for as long as neurons were studied. This section concerns the effects observed during the ejection of NE while the following section (3.4) will deal with the effects that outlasted the presence of NE.

3.3.1 Neuronal responses to peripheral stimulation and to glutamate pulses.

The combination electrode used in the present study allowed a good isolation of single neurons in cat somatosensory cortex and multiunit activity was very seldom observed (Figure 8).

To test the effects of NE on neuronal activity a baseline response had to be obtained so that any changes could be expressed as a deviation from control. NE was tested on 31 neurons while their receptive field was stimulated mechanically (Figure 9). Twenty-nine of these were spontaneously active; only two lacked this characteristic. The effects of NE were assessed on both the spontaneous activity and peripherically evoked response. In every case the stimulation of the receptive field produced a clear response over the spontaneous activity prior to the administration

Figure 8: Oscilloscope traces of neurons isolated in the somatosensory cortex. During the ejection of glutamate some neurons responded with long inter-spike intervals (A) or in short bursts (C). In a few cases the inter-spike intervals were less than one ms during a burst (D). In B is shown the discharge of a spontaneously active neuron during the mechanical stimulation of its receptive field. The upper trace shows the analog signal representing the displacement of the mechanical stimulator and the lower trace shows the action potentials. The cutaneous stimulation consisted of an indentation on which was superimposed 200ms later a vibration (20Hz in this case). The vibratory stimulus was applied for 600ms and the stimulator was removed from the skin 200ms later. The neuron responded to the indentation of the skin and also to the superimposed vibration but not to each cycle. The traces in A and D are a single sweep and 3 sweeps in B and C. The dotted line in A, C and D is the threshold for detection by the window discriminator. In B the trace from the window discriminator is located below the baseline trace.

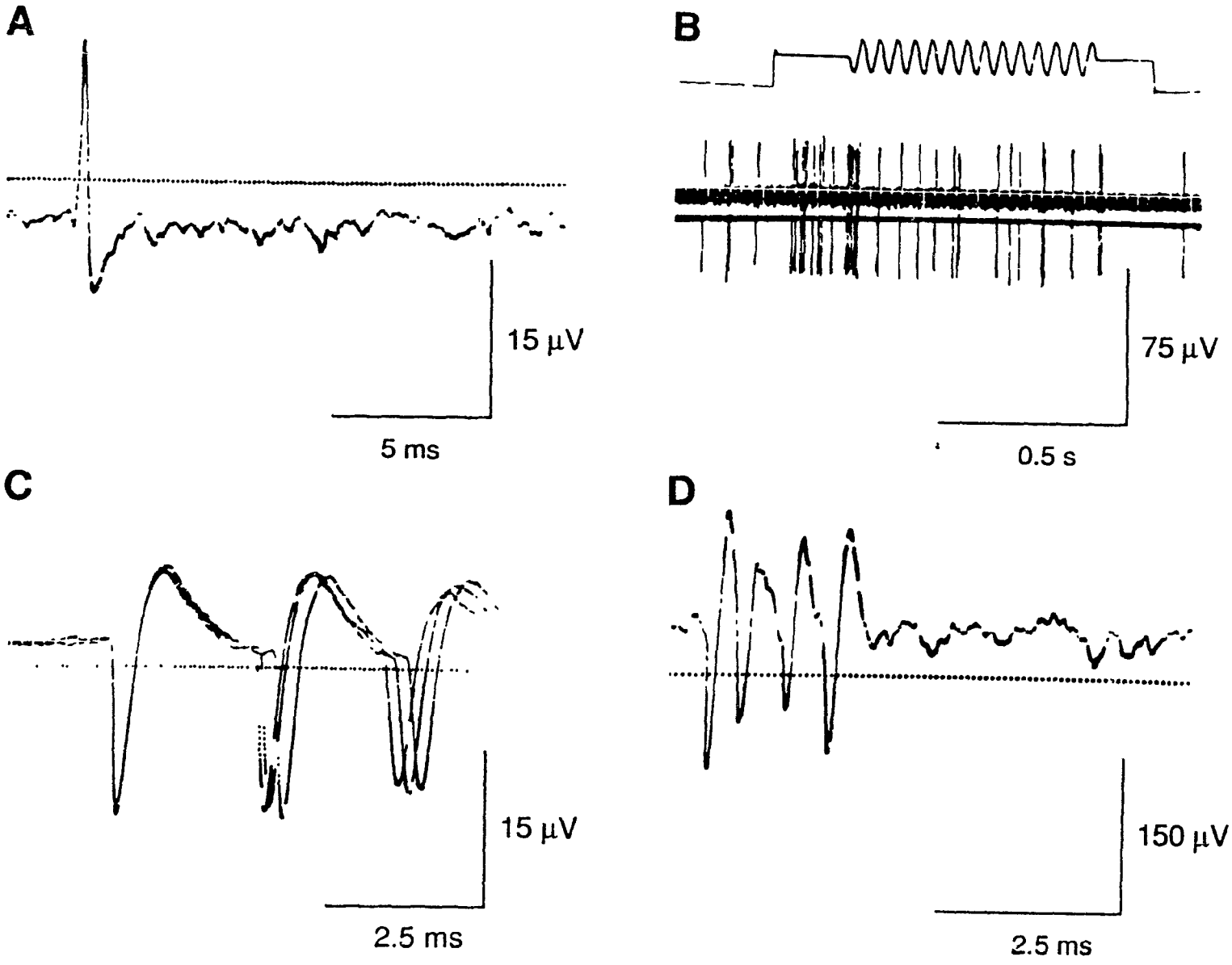

A
15 μV
5 ms
B
75 μV
0.5 s
C
15 μV
2.5 ms
D
150 μV
2.5 ms

Figure 9: Examples of receptive fields mapped on the forelimb for neurons isolated in the somatosensory cortex. The receptive fields A, C and D were drawn from different animals and those from B and E come from the same animal but from two different penetrations.

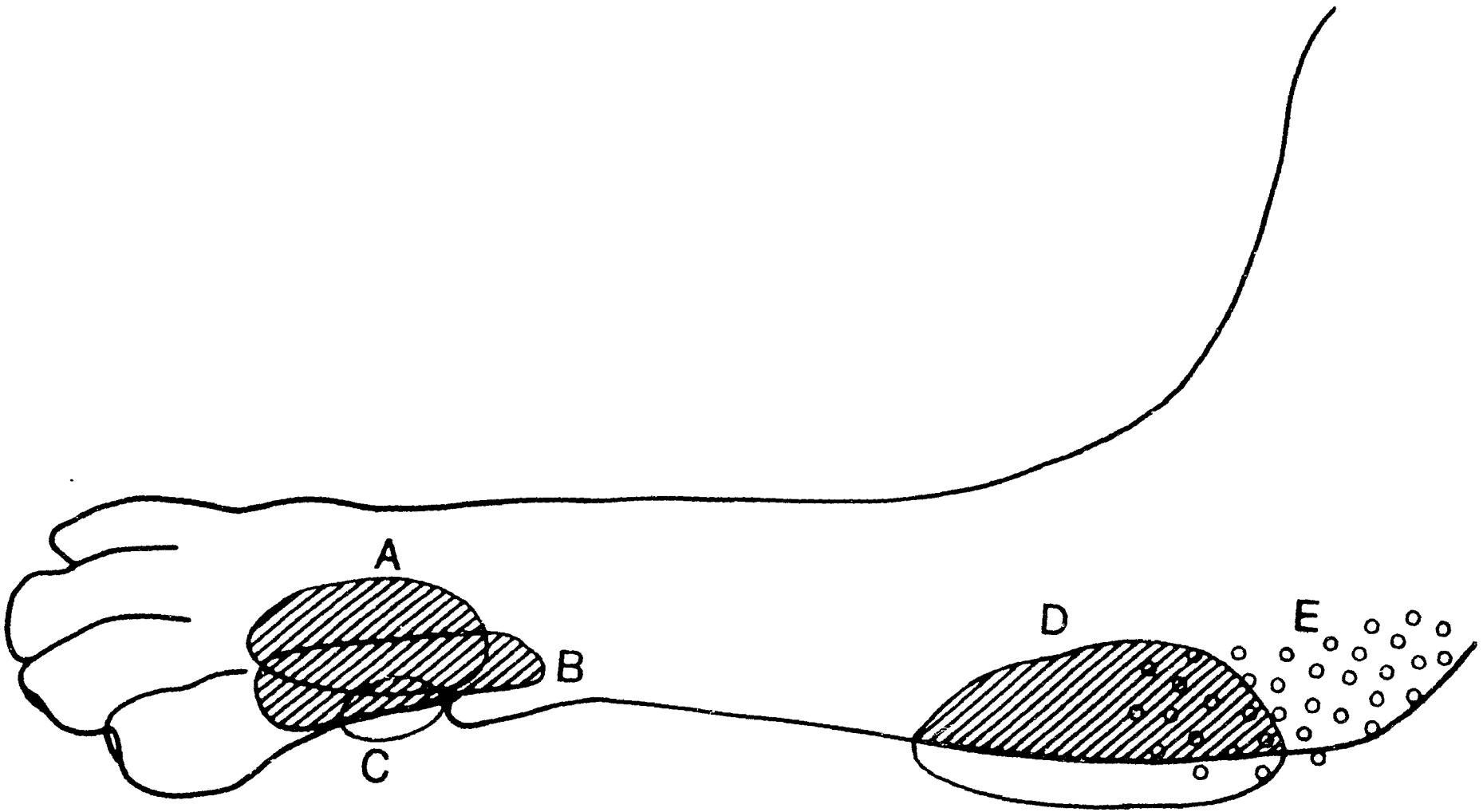
A. Receptive field with SRA modality of a neuron located in layer loV of area 3b The response of the neuron to the mechanical stimulation of the receptive field is shown in Figure 10A and B while the effects of NE are presented in Figure 16

B SRA receptive field of a neuron located in layer midIII of area 3b The neuron could not be isolated adequately to be studied. This receptive field was drawn from the same animal as the receptive field in E but during a different penetration that was located approximately 2mm lateral to receptive field E.

C. SRA receptive field of a neuron situated in the middle layers. The administration of NE to the neuron produced a decrease of both the ongoing activity and of the response to the peripheral stimulus. The signal-to-noise ratio was increased by an average of 41% during NE ejection.

D. SRA receptive field of a neuron located in layer IV of area 3b The effects of NE on this neuron are presented in Figure 20.

E. TAP receptive field of a neuron that was not located in the histology nor from the micrometer reading. The response of the neuron to the stimulation of its receptive field was not stable and it was not studied with NE.


A
B
C
D
E

of NE. During this period, the stimulus produced an average of 10 5 ± 1 3 action potentials per stimulus presentation (measured over 1 05s) In contrast, the geometric mean of ongoing activity was only 0 8Hz during the control period yielding a good signal-to-noise ratio An example of a recording generated as spike trains and time interval histogram of a neuron displaying a receptive field is shown in Figure 10A and B.

In 86 cases NE was applied to neurons lacking a receptive field while they were excited with iontophoretic pulses of glutamate (Figure 10C and D) Forty-two (49%) were spontaneously active and 44 (51%) were silent in the absence of glutamate. This sample included 33 Glut-RF neurons, 34 No-RF neurons and 19 neurons for which the presence of a receptive field with glutamate had not been tested. No significant difference could be found between the effects of NE on these 3 groups of neurons As a result, it was possible to pool the data and to present the effects of NE only for the pooled data. During the control period of spontaneously active neurons, the geometric mean of ongoing activity was 0.9Hz and the response to glutamate was 6 7 ±0 8Hz yielding a signal-to-noise ratio of 7 5 The magnitude of the response to glutamate for silent neurons was 6 9 ±1 1Hz prior to the administration of NE

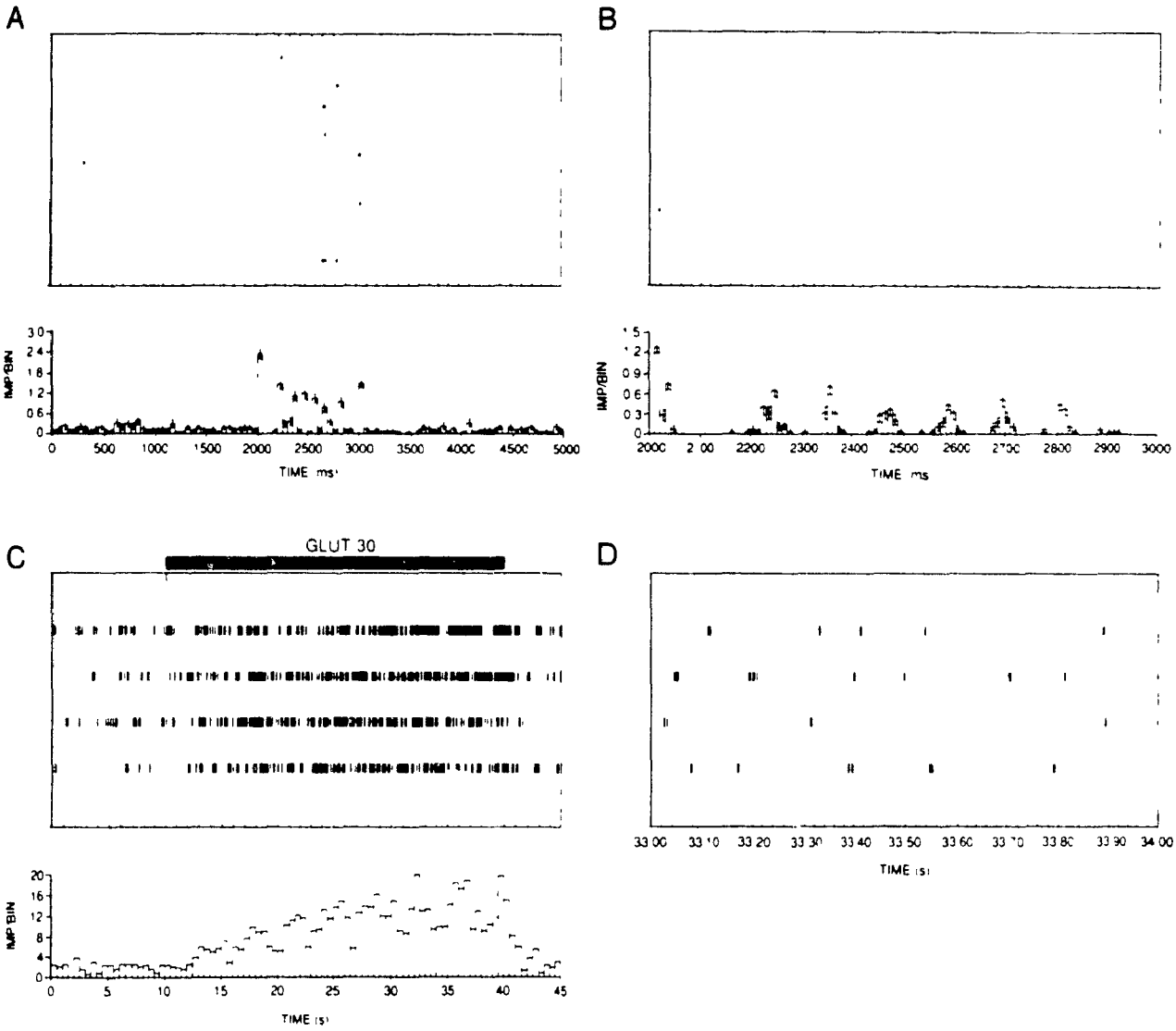
Figure 10: Computer generated spike trains and time interval histograms during the control period.

A Neuron having a receptive field which was stimulated mechanically 30 times during the control period. The activity was recorded for 5s and there was an interval of two s between each trial. The spontaneous activity was recorded for 2s prior to the stimulus presentation and the activity after the stimulation was not considered Each dot represent one or several action potentials. The neuron responded clearly to each stimulus presentation while the spontaneous activity was distributed randomly. The time interval histogram is the average of the 30 trials and the response to the stimulation is clearly seen over the background activity. Bin width 50ms

B The same neuron as in A but only the interval from 2.0 to 3.0s is shown on an expanded time scale (bin width, 10ms). At this scale most of the action potentials are represented by one dot. The stimulus was applied at 2.0s and a response was observed 10 to 20ms later When examined at higher temporal resolution, the latency appeared to be 16ms and a second peak in the histogram was observed at 38ms (not shown) The correspondence between the response and the vibration frequency is clearly seen, very little activity was present between the stimulus cycles confirming the rapidly adaptating nature of the input to this neuron.

C Spontaneously active neuron lacking a receptive field responding to 30nA of glutamate during the control period. The ongoing activity was recorded for 10s at the beginning of each run and glutamate was administered from 10 to 40s. Three to 4 s after the beginning of glutamate ejection the activity of the neuron started to increase and reached a plateau 15 to 20s later. As was typical of these cells, upon the termination of glutamate, the activity induced by glutamate disappeared within 2 or 3s which is a typical response to glutamate. This neuron responded in bursts and individual action potentials are rarely seen in the spike train at this scale. Bin width, 500ms.

D Spike train of the neuron shown in (C) from 33.0s to 34.0s at a higher temporal resolution showing individual action potentials as one dot each. During glutamate administration this neuron responded in small bursts of 2 to 5 action potentials with interspike intervals of 3 to 6ms.


A
B
C
D
GLUT 30
IMP/BIN
TIME (ms)
TIME (s)

3.3.2 General characteristics of the effects of NE.

During its ejection, NE affected 90% of the neurons studied leaving only 10% of the sample unaffected (Table 8). NE affected the neuronal excitability in 4 different ways while it was ejected from the micropipette. The most common effect of NE was an inhibition of both the spontaneous and peripherally or glutamate-induced activities; these pure inhibitory responses were observed in 54% of neurons tested with NE. Also, NE produced excitation and those responses could be divided into 3 different classes. First, in 15% of the sample, an excitation was observed at the beginning of NE ejection and it was followed by an inhibition causing the discharge to fall below the control level as NE ejection was continued, these responses were classified as biphasic. Second, in 19% of the cases, the excitation was not accompanied by an inhibition and those responses were classified as excitations because of their uniphasic nature. And third, a few neurons responded biphasically but with NE first producing a decrease in neuronal activity followed by an increase, these responses were classified as "other" and comprised only 2.6% of the sample. When an excitation was observed, only in a few cases were both the ongoing and evoked activities increased; usually the excitation affected only the ongoing activity or the evoked response and the other component usually decreased. In each case, NE was equally likely to produce an effect on the spontaneous activity as on the peripherally evoked response or the response to glutamate. Statistical tests showed that each of the effects produced by NE was independent of the type of stimulus used to excite the neuron. Despite the stringent criteria used to decide if a neuron was or was not affected by NE, only 10% of the neurons were classified as

Table 8: Neurons affected by NE (n = 117). No significant difference was found between neurons displaying a receptive field and lacking a receptive field ($G_{adj} = 1.190$, df = 2, $p > 0.5$).

Neuronal classes	Inhibition	Biphasic	Excitation	Other	No effect	Total
Receptive field	19 (61%)	6 (19%)	4 (13%)	0 (0%)	2 (6.5%)	31
Without a receptive field	44 (51%)	11 (13%)	18 (21%)	3 (3.5%)	10 (12%)	86
Total	63 (54%)	17 (15%)	22 (19%)	3 (2.6%)	12 (10%)	117

unaffected by NE (Table 8). Only two neurons (6.5%) displaying a receptive field and one spontaneously active neuron lacking a receptive field (2.4%) were not affected by NE. Nine neurons lacking spontaneous activity (20%) were not affected using current of NE ranging from 12 to 75nA with a geometric mean of 27.7nA applied for 2 to 6 runs. Data from one of the cells that did not respond to NE are illustrated in Figure 11. Each type of response to NE is described in detail below.

3.3.3 Inhibitory effects of norepinephrine.

3.3.3.1 Neurons displaying a receptive field.

In 19 (61%) of the 31 neurons displaying a receptive field, NE produced an inhibition when ejected with currents ranging from 10-100nA (geometric mean of 33.0nA). NE was applied for 1 to 10 runs (mean 3.0) of 10 stimulus presentations but a significant decrease in neuronal activity was observed during the first 3 runs in all cases.

In 15 of these 19 inhibited neurons, both the ongoing and evoked activities were significantly decreased whereas in 2 cases only the spontaneous discharge was inhibited and in one case only the evoked activity was affected. The response to stimulation of the receptive field of one neuron lacking spontaneous activity was also decreased. An example of inhibition of both spontaneous and evoked activity by NE in a neuron displaying a receptive field is shown in Figure 12 (see also Figures 18, 19, 22, 25 and 26).

Because the effects of NE developed gradually it was not immediately obvious what measure to use to best describe the magnitude of the response to NE.

Figure 11: Neuron lacking a receptive field not affected by NE. This neuron was not located in the histology. The baseline was established with 7 pulses of 50nA of glutamate (A). During the control period the average response to glutamate was 11.3Hz. NE was administered for two runs and no significant effects were observed (B). Following NE administration the response remained at the control level and 10 to 11 min later it was 98% of the control level (C). During the administration of a current of +40nA through a pH 4.0 solution for two runs the response was 96% of the control (D). The response remained at the control level during the following min (E). The experimental condition is indicated on the left of each panel and the magnitude of the response relative to the control is presented as a percentage on the right of each panel. G, response to glutamate; bin width, 600ms.

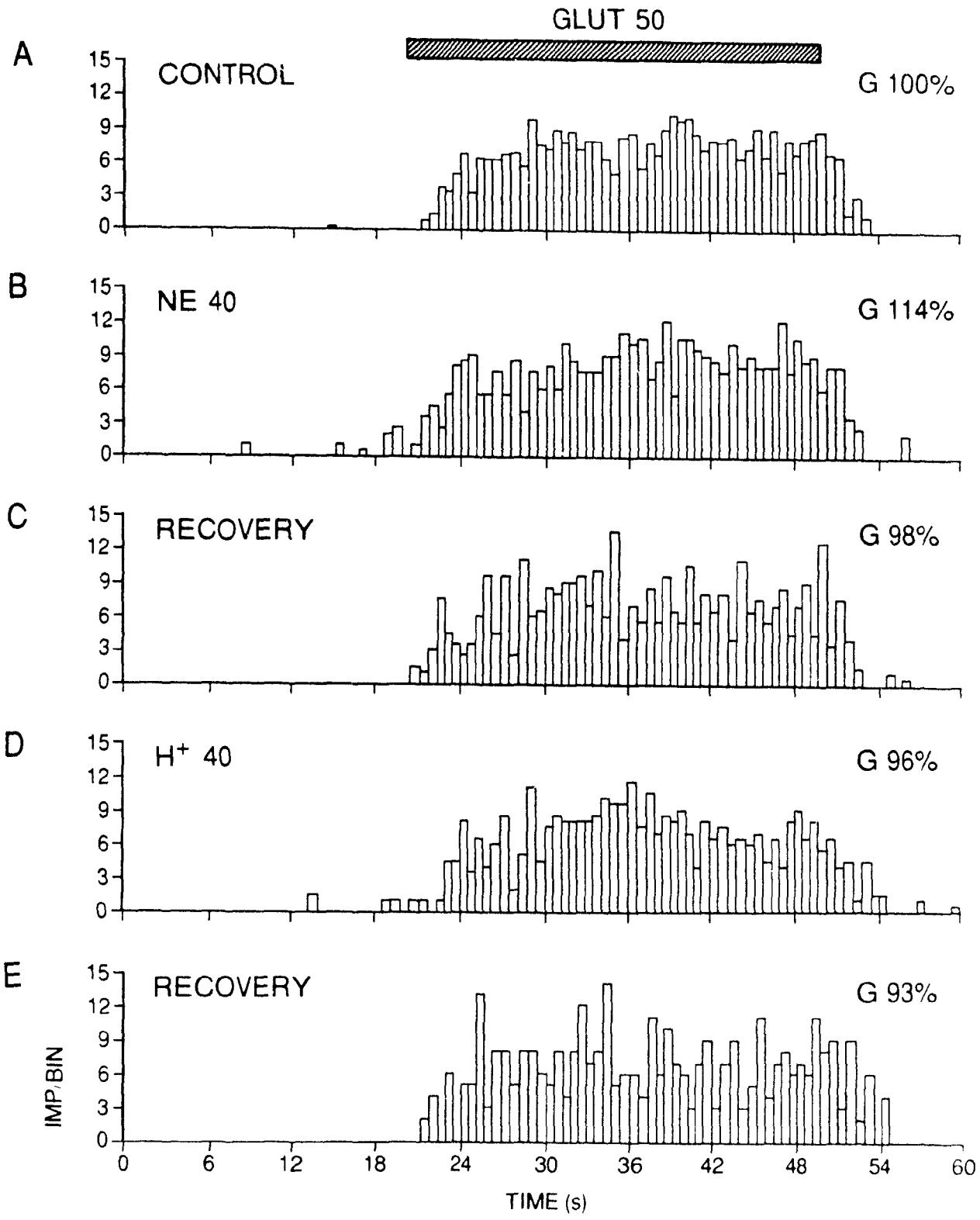
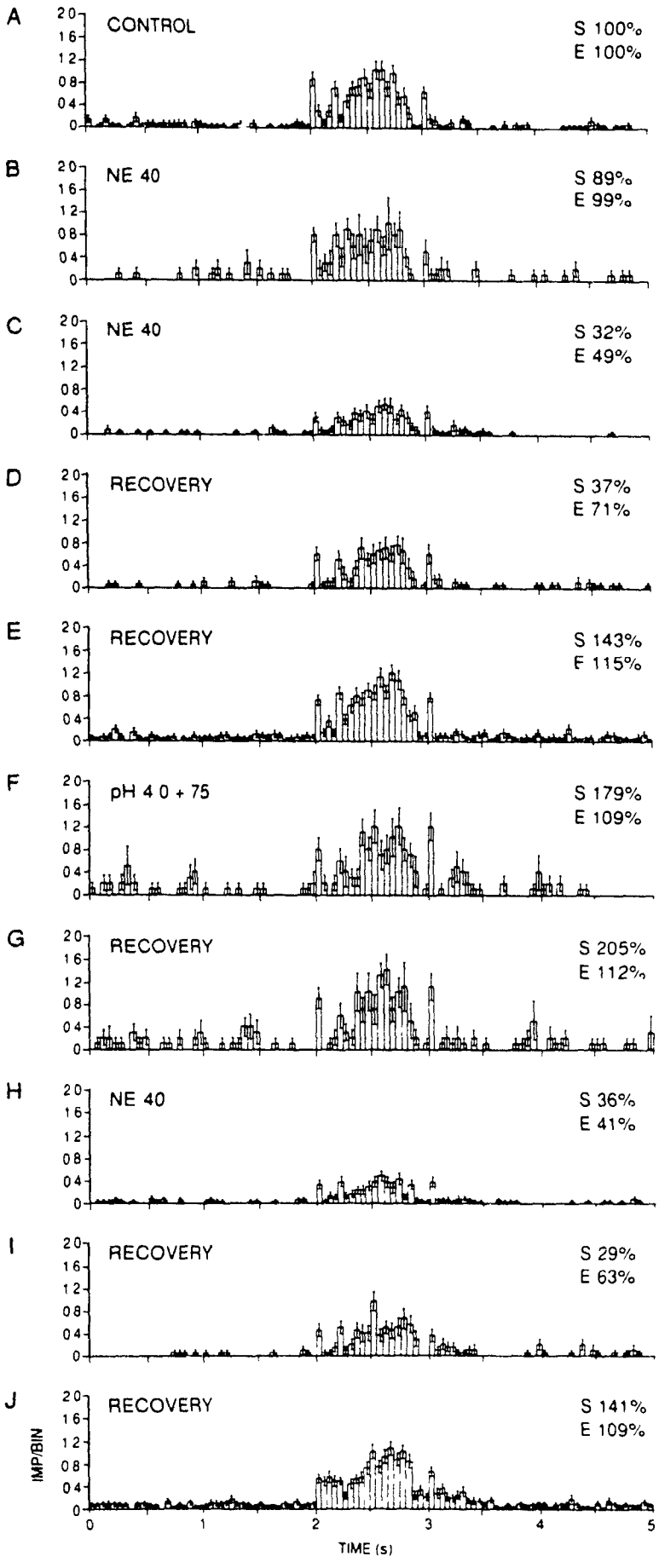

GLUT 50
A
CONTROL
G 100%
B
NE 40
G 114%
C
RECOVERY
G 98%
D
H+ 40
G 96%
E
RECOVERY
G 93%
IMP/BIN
0
3
6
9
12
15
0
6
12
18
24
30
36
42
48
54
60
TIME (s)

Figure 12: Neuron displaying a receptive field inhibited by NE. This neuron displayed a SRA receptive field and was not located in the histology. The baseline was established with 30 stimulus presentations (A). During the control period the ongoing activity averaged 0.95Hz and each stimulus presentation evoked 11.7 action potentials. NE 40nA was administered during 4 runs (B and C). During the first run both the ongoing and the evoked activities were not significantly affected (B) while during the next 3 runs the ongoing activity was reduced to 32% of the control and the evoked activity to 49% (C). The signal-to-noise ratio was increased by 53% during this last period. During the 2 first min following NE administration both the ongoing and evoked activities remained lower than the control level and the signal-to-noise ratio was further increased to 192% of the control level (D). Ten to 13 min following NE administration, the ongoing activity was 143% of the control level and the evoked activity 115% (E). Then ions H^+ were ejected with a current of +75nA for one run and produced no significant effect (F). Following one run of recovery (G) NE 40nA was administered again and produced effects similar to the first test and the average of the 4 last runs of NE administration is shown in (H). The recovery periods one to two min (I) and 5 to 10 min (J) after NE administration are shown. (See Figure 11; S, spontaneous activity; E, evoked activity; bin width, 50ms).


A
CONTROL
S 100%
E 100%
B
NE 40
S 89%
E 99%
C
NE 40
S 32%
E 49%
D
RECOVERY
S 37%
E 71%
E
RECOVERY
S 143%
E 115%
F
pH 4 0 + 75
S 179%
E 109%
G
RECOVERY
S 205%
E 112%
H
NE 40
S 36%
E 41%
I
RECOVERY
S 29%
E 63%
J
RECOVERY
S 141%
E 109%
IMP/BIN
20
16
12
08
04
0
0
1
2
3
4
5
TIME (s)

Consequently, both the average size of the response and the maximum effect of NE observed in a series of runs were compared to the control response as measures of the effects of NE. This examination suggested that both parameters were adequate indices of the effect of NE. Since discussing both measures would be redundant, they have been presented in the tables but they are discussed only in relation to Table 9. Subsequent tables show both the maximum and mean effects, however, only the maximum values are discussed in the text associated with the other tables.

Despite the fact that NE appeared to decrease spontaneous and evoked activities in an equal number of neurons, the magnitude of the decrease was more important on the spontaneous than on the evoked activity (Table 9A). The difference between spontaneous activity and evoked activity was 15.3% for mean effects and 20.8% when the inhibitory effects of NE reached their maximum. The distribution of these effects is shown in Figure 13A and B. The mean inhibitory effect of NE on the spontaneous activity was a decrease of 50% or more in 67% of the cases while on evoked activity it was less than 50% in 61% of cases. No significant difference was found between the two distributions ($G_{adj} = 1.816$, $df = 2$, $p > 0.1$) The maximal inhibitory effect on spontaneous activity was a decrease of more than 80% in 61% of the neurons. In contrast, the maximal decrease of evoked activity never reached that level and the two distributions were significantly different in this case ($G_{adj} = 8.608$, $df = 2$, $p < 0.025$) These results suggest that on average NE produced an increase in the signal-to-noise ratio. This issue will be discussed in section 3.3.9.

Table 9: Magnitude of the inhibitory effects of NE normalized as percent of control for spontaneous activity and peripherally evoked activity or response to glutamate. Mean effects were obtained by averaging the effects of the runs when NE was present for each cell. Maximal decreases were calculated by using only the run where the maximal inhibitory effect of NE was observed for each neuron.

A: Spontaneously active neurons displaying a receptive field (n = 18).

Mean effect		Maximal decrease	
Spont. Act.	Evoked Act.	Spont. Act.	Evoked Act.
-58.4 ±5.9%	-42.9 ±3.9%	-77.5 ±5.5%	-55.3 ±3.3%

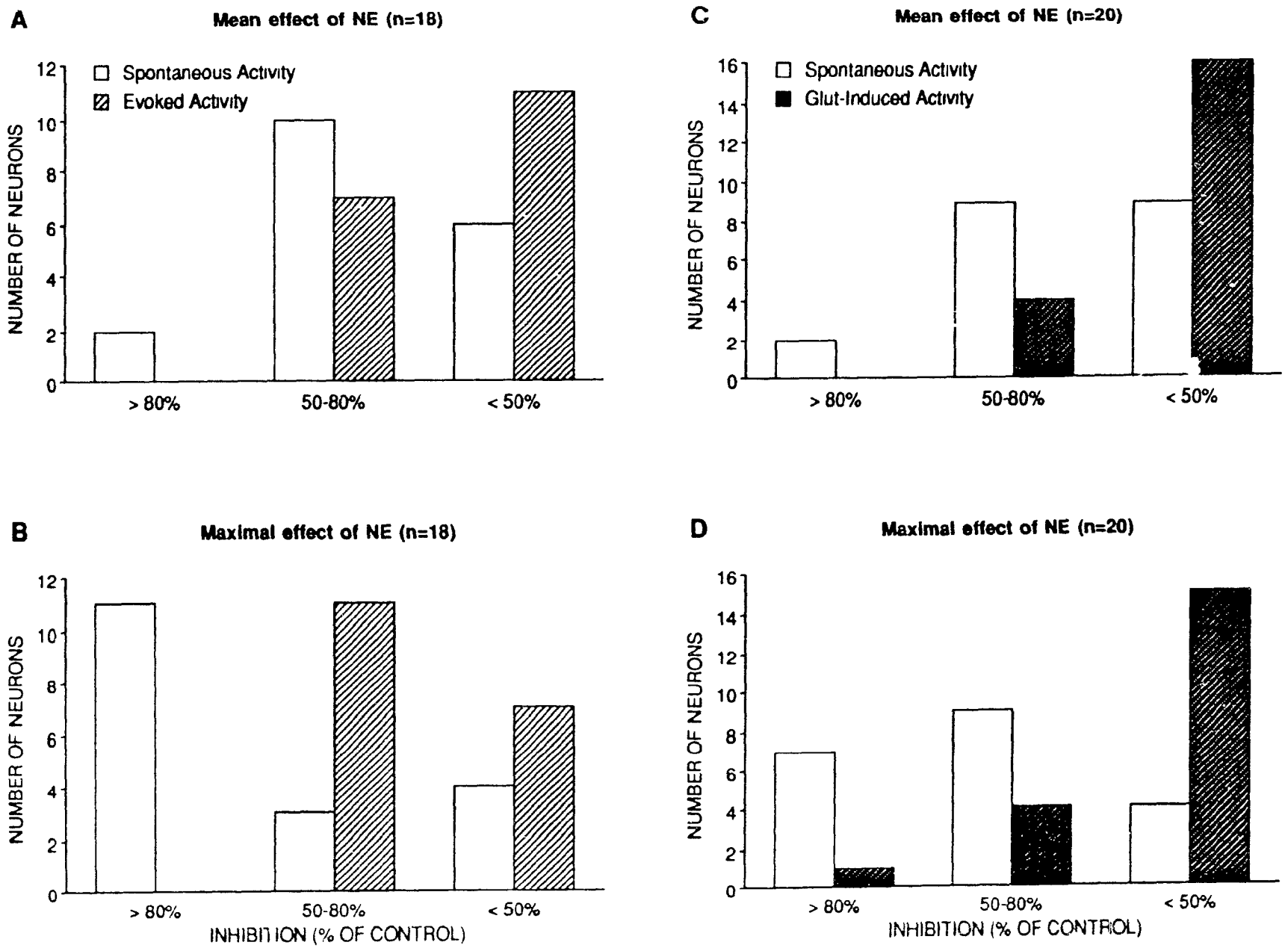
B: Spontaneously active neurons lacking a receptive field (n = 20).

Mean effects		Maximal decrease	
Spont. Act.	Response to glut	Spont. Act.	Response to glut.
-45.4 ±4.7%	-30.5 ±4.4%	-68.0 ±5.2%	-41.0 ±5.2%

C: Silent neurons lacking a receptive field (n = 24). Inhibitory effects of NE on the response to glutamate.

Mean effects	Maximal decrease
-50.6 ±4.3%	-64.2 ±4.2%

Figure 13: Mean and maximal inhibitory effects of NE on the spontaneous and induced activities. A and B, neurons displaying a receptive field; C and D, neurons lacking a receptive field.


A
Mean effect of NE (n=18)
Spontaneous Activity
Evoked Activity
NUMBER OF NEURONS
0
2
4
6
8
10
12
> 80%
50-80%
< 50%
B
Maximal effect of NE (n=18)
NUMBER OF NEURONS
0
2
4
6
8
10
12
> 80%
50-80%
< 50%
INHIBITION (% OF CONTROL)
C
Mean effect of NE (n=20)
Spontaneous Activity
Glut-Induced Activity
NUMBER OF NEURONS
0
2
4
6
8
10
12
14
16
> 80%
50-80%
< 50%
D
Maximal effect of NE (n=20)
NUMBER OF NEURONS
0
2
4
6
8
10
12
14
16
> 80%
50-80%
< 50%
INHIBITION (% OF CONTROL)

3.3.3.2 Neurons lacking a receptive field.

Eighty-six neurons lacking a receptive field were tested with NE. Of these 20 spontaneously active neurons and 24 lacking this characteristic during the control period were inhibited (Table 8). Both the spontaneous activity and the response to glutamate were decreased by NE in 18 cases whereas in 2 cases only the spontaneous activity was significantly inhibited while the response to glutamate remained unchanged. The iontophoretic current of NE used to produce these effects ranged from 5 to 100nA with a geometric mean of 24.3nA. A significant effect on either spontaneous activity or glutamate response was observed in the first run in all but one case where an effect was observed in the 4th run after the ejecting current of NE had been increased from 6 to 15nA. As had been observed for the neurons displaying a receptive field, the spontaneous activity was proportionally affected much more than the response to glutamate. The distributions of these effects are shown in Figure 13C and D and their magnitude is presented in Table 9B. The effects of NE on the response to glutamate of spontaneously active cells lacking a receptive field appeared to be less than the effect of NE on peripherally evoked activity of cells with a receptive field but the magnitude of the effects of NE on spontaneous activity appeared to be the same on both classes of neurons.

The response to glutamate of 24 (55%) silent neurons lacking a receptive field was inhibited with currents of NE ranging from 10 to 100nA (geometric mean of 33.5nA). NE was applied during 1 to 9 runs (mean 2.5) and in all but 5 cases a significant inhibition was seen in the 1st run. The magnitude of the effect on silent neurons was more important than it was on the response to glutamate of

spontaneously active neurons (Table 9C). This difference might be a consequence of the higher currents of NE used on neurons lacking spontaneous activity.

3.3.3.3 Time course of the inhibitory effects of norepinephrine.

When NE was administered continuously to neurons displaying a receptive field, the time course of the decrease could be evaluated easily since NE was administered continuously and the peripheral stimulus was repeated at short time intervals so that a measure of the neuronal activity was taken every 7s. To obtain a measure of the time course of the NE effect, the response of 14 neurons that were significantly affected during the first twenty stimuli following the beginning of NE ejection were recorded for 140s. The frequencies of spontaneous activity ranged from 0.1 to 13.5Hz during the control period. In order to give the same weight to all neurons whether they had low or high spontaneous activity, the data were normalized to percentages of the control period, and those values were used for the calculation. The averages for the spontaneous and evoked activity for the 5 last cutaneous stimuli of the control period and for the first 20 stimuli following the beginning of NE ejection are plotted in Figure 14A and B respectively.

The results obtained for the ongoing activity appeared to fluctuate a lot even during the control period (Figure 14A). The response clearly decreases over time but because of the variability, the time course of the effect could not be evaluated accurately. The variability can be explained by the fact that the ongoing activity was measured for only 2s in each trial and in many cases the frequency was below or close to 1Hz so that 1 impulse caused an important change in the mean frequency.

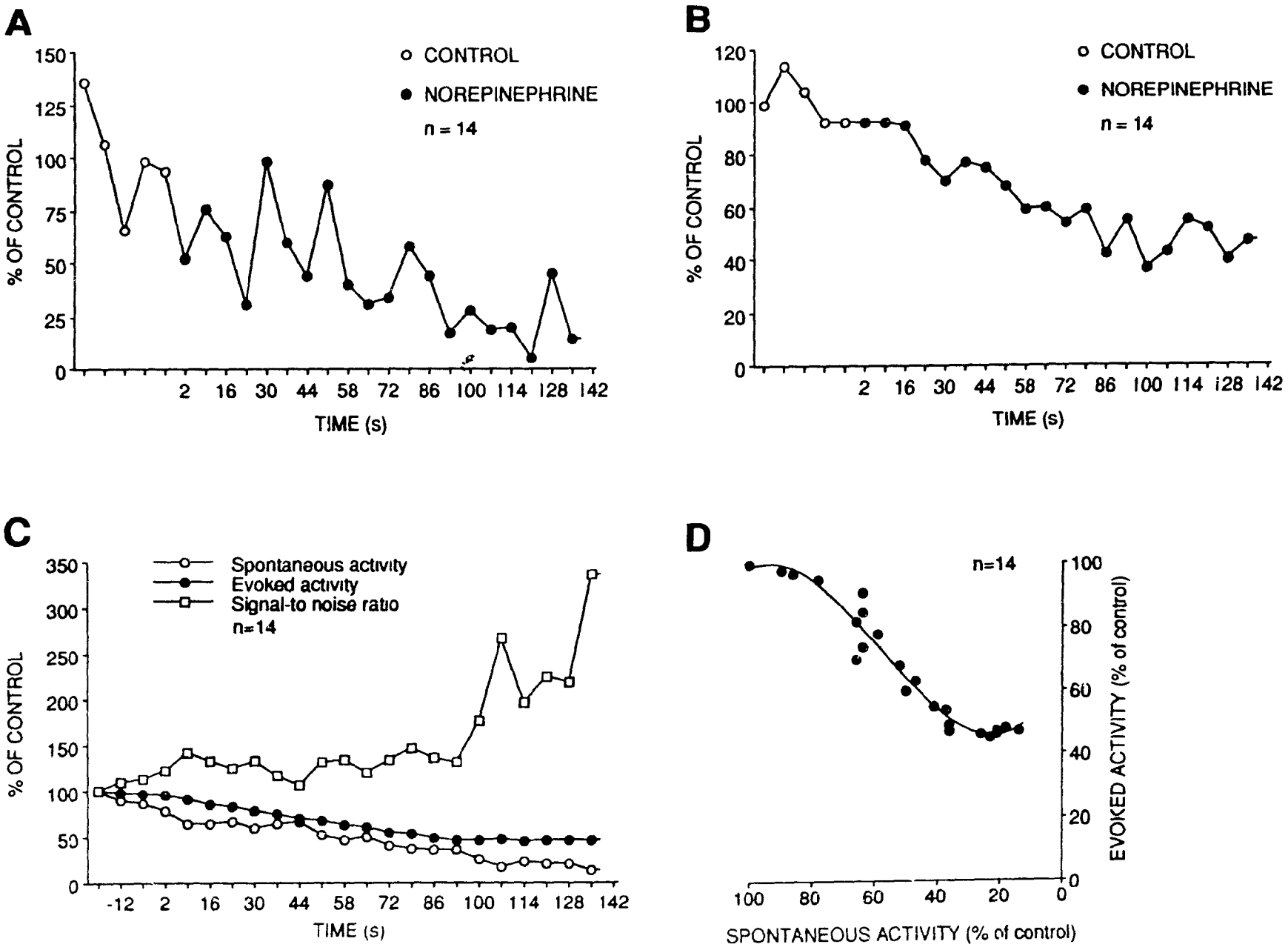
Figure 14: The average time course of the inhibitory effects of NE.

A Spontaneous activity.

B. Peripherally evoked activity

C. Spontaneous activity, peripherally evoked activity and signal-to-noise ratio obtained from travelling means (see text).

D Decrease of the evoked activity plotted as a function of the decrease in spontaneous activity (values plotted are the travelling means of both variables).


A
CONTROL
NOREPINEPHRINE
n = 14
% OF CONTROL
TIME (s)
B
CONTROL
NOREPINEPHRINE
n = 14
% OF CONTROL
TIME (s)
C
Spontaneous activity
Evoked activity
Signal-to noise ratio
n=14
% OF CONTROL
TIME (s)
D
n=14
EVOKED ACTIVITY (% of control)
SPONTANEOUS ACTIVITY (% of control)

For the time interval tested, there was no spontaneous activity in several of the trials yielding 0% ongoing activity then when one or two action potentials were present in one trial the percentage was much greater than 100%. The curve is the result of these variations.

Evoked activity was much less variable and the time course could be evaluated more precisely (Figure 14B). The response began to decrease 16s after NE was turned on and it appeared to decrease almost linearly during the following 80s when a plateau was reached and the response did not decreased further. A significant decrease (-23%) was reached between 30 and 40s after the beginning of NE ejection. Over the 20 time periods studied during the ejection of NE, the spontaneous activity was decreased by an average of 56.5 ±5.7% and the evoked activity by 37.7 ±4.0% as compared to the control period. A significant difference was found between the decrease in spontaneous activity and the decrease in evoked activity (Wilcoxon's signed-ranks test, $T_s=24$, $n=20$, $p<0.01$).

To get a better picture of the time course of the effects of NE on the spontaneous activity, the values used for Figure 14A and B were averaged over 5 consecutive time periods starting with the control period. The averages were displaced by one time period to the right and were plotted as a function of the mean time of the 5 time periods used to obtain each value. The results of this process, shown in Figure 14C, yielded much smoother curves thereby giving a better estimate of the time course. The time course of the decline in evoked activity appeared to be similar to the curve without smoothing shown in Figure 14B, still reaching a significant level of inhibition around 35s and a plateau after 80s suggesting that the

averaging process did not displace the curve significantly to the right and that a better estimate of the time course of the ongoing activity could be obtained in this way.

The spontaneous activity appeared to decrease more rapidly than evoked activity as NE was turned on and a significant inhibition was reached after only about 7s. After that initial period, both the spontaneous and evoked activities appeared to decrease at the same rate for several seconds but the spontaneous activity appeared to reach a plateau about 20s later than the evoked activity The differences between the time course of spontaneous activity changes and the time course of the response to the stimulus was more obvious when the evoked activity was plotted as a function of the spontaneous activity (Figure 14D). Spontaneous activity decreased more rapidly at the beginning and then both spontaneous and evoked activities decreased at the same rate until evoked activity reached a plateau while spontaneous activity was still decreasing.

The time course of the inhibitory effects of NE on neurons lacking a receptive field could not be assessed because by the nature of the experimental paradigm NE was already present for 10-20s before glutamate ejection was started. Further, both NE and glutamate were ejected intermittently. Even if the precise time course of the inhibitory effect of NE on those neurons is not available it can be inferred from the data that the action of NE occurred within a few seconds since, despite the facts that NE was turned on just at the beginning of the recording of ongoing activity and that it was recorded for only 10-20s, NE still produced a significant decrease of the ongoing activity that was of the same magnitude as the decrease observed on

neurons displaying a receptive field. This suggests that the action of NE was at least as fast as it had been on neurons displaying a receptive field.

3.3.4 Biphasic effects of NE.

3.3.4.1 Neurons displaying a receptive field.

In 6 (19%) neurons displaying a receptive field, NE produced an increase of the ongoing and/or evoked activity that was followed by a significant decrease. These effects were observed when iontophoretic currents ranging from 10 to 50nA (geometric mean 28.0nA) were used. The excitatory effects were observed during the 1st or 2nd run and the decreases appeared in the subsequent runs. In 3 cases both the spontaneous activity and the response to somatic stimuli followed this pattern. In 2 cases only the ongoing activity was increased while the evoked activity was decreased in one case and not significantly changed in the other case. In the other case only the evoked activity was increased while the spontaneous activity was not altered. Overall, the spontaneous activity increased in 5 cases and the evoked activity in 4 cases. The magnitude of increase of the ongoing activity was proportionally more important than that of evoked activity (Table 10A). Following this excitatory phase, both ongoing and evoked activity decreased significantly in a fashion and to a level similar to that observed for neurons inhibited by NE. As observed with the inhibited neurons, in the biphasic cells the decrease in spontaneous activity was also more important than the decrease in evoked activity (Table 10A). Despite the fact that the percentage increase in ongoing activity was more important than the percentage increase in evoked activity, the absolute increase in the ongoing activity

Table 10: Magnitude of the biphasic effects of NE expressed as percent of control for spontaneous activity and on peripherally evoked response or response to glutamate (see table 9).

A: Spontaneously active neurons displaying a receptive field (n=6).

1. Spontaneous and/or evoked activities increased:

Mean effect		Maximal increase		Maximal decrease	
Spont. Act.	**Ev. Act.**	**Spont.Act.**	**Ev. Act**	**Spont. Act.**	**Ev. Act.**
-9.8 ±13.6	-23.0 ±5.3	+75.6 ±18.1	+41.3 ±9.5	-77.4 ±13.2	-65.1 ±6.5
n=5	n=4	n=5	n=4	n=5	n=4

2. Spontaneous and/or evoked activity not increased:

Mean effect		Maximal decrease	
Spont. Act.	Ev. Act.	Spont. Act.	Ev. Act.
-61.0	-21.5 ±12.0	-93.3	-38.5 ±2.1
n=1	n=2	n=1	n=2

B. Spontaneously active neurons lacking a receptive field (n=11).

1. Spontaneous and/or response to glutamate increased:

Mean effect		Maximal increase		Maximal decrease	
Spont. Act.	Glut. Resp.	Spont.Act.	Glut. Resp.	Spont. Act.	Glut. Resp.
+17.8 ±12.0	+14.5 ±9.0	+115.6 ±19.3	+47.3 ±9.4	-76.4 ±6.1	-15.2 ±19.9
n=9	n=4	n=9	n=4	n=9	n=4

2. Spontaneous and/or response to glutamate not increased:

Mean effect		Maximal decrease	
Spont. Act.	Glut. Resp.	Spont. Act.	Glut. Resp.
-43.0 ±17.0	-43.1 ±16.7	-83.0 ±17	-68.9 ±12.1
n=2	n=7	n=2	n=7

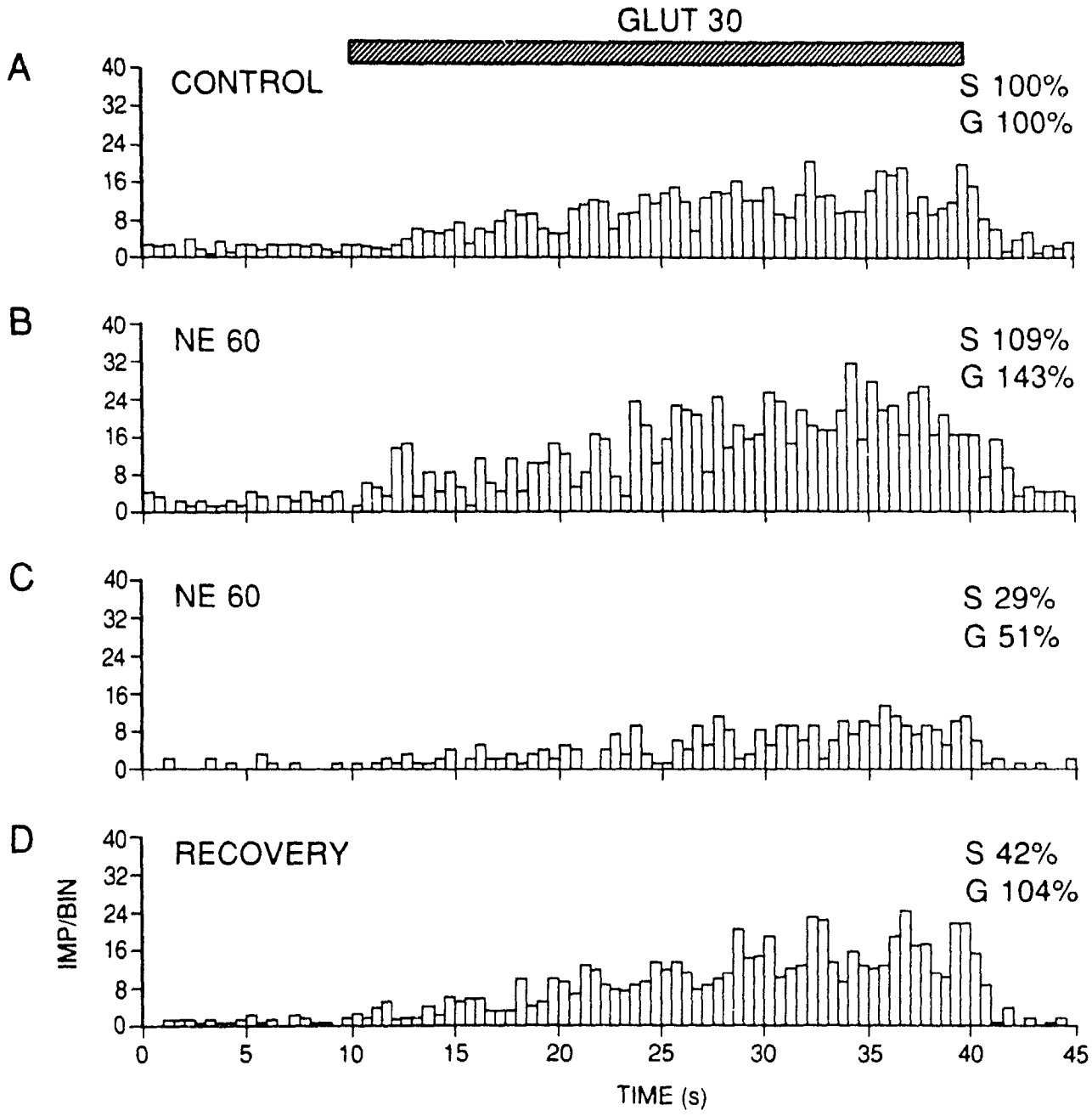
was only 0.38Hz (from 0.50 to 0.88Hz) whereas the absolute increase in the response to the stimuli was 3.36 action potentials per stimulus (from 8.15 to 11 51).

3.3.4.2 Neurons lacking a receptive field.

Biphasic responses were observed in 11 spontaneously active neurons lacking a receptive field while NE was ejected for 2 to 8 runs (mean 3 0) with current ranging from 5 to 50nA (geometric mean 28.1nA). In 10 of the 11 cases, the excitation was observed during the 1st run and it occurred in the 2nd run in the other case. In two cases a biphasic response occurred both in the ongoing activity and in the response to glutamate. In 7 cases it was observed only on the spontaneous activity while the response to glutamate remained at the control level in 3 of these cases and was significantly decreased in the remaining 4 cases In 2 neurons only the glutamate-induced excitation was biphasic while ongoing discharge was unchanged during the excitatory phase but decreased later. Overall, NE produced a biphasic effect on the ongoing discharge in 9 cases and on the response to glutamate in 4 cases. A biphasic response to NE is shown in Figure 15.

The magnitude of these biphasic responses are shown in Table 10B. Whereas the mean effect was the same for both ongoing activity and response to glutamate, the maximal increase was proportionally more important on spontaneous activity but in absolute terms it was increased by only 0.79Hz (from 0 68 to 1.47Hz) whereas the response to glutamate was increased by 3.91Hz (from 8 26 to 12 17Hz) The spontaneous activity decreased more than the response to glutamate during the inhibitory phase. This also occurred when the spontaneous activity and the response

Figure 15: Biphasic response of a spontaneously active neuron lacking a receptive field to NE. No receptive field was uncovered by glutamate in this neuron situated in the deeper layers based on the micrometer reading. The baseline was established with 4 consecutive pulses of glutamate (A). The frequency of spontaneous activity was 3.9Hz and the response to glutamate was 15.6Hz during the control period. NE 60nA was administered during 2 runs (B and C). During the first run, the ongoing activity was increased by 9% and the response to glutamate by 43% producing an increase in signal-to-noise ratio of 31% as compared to the control period (B). In contrast, during the second run, the spontaneous activity was reduced to 29% of the control period and the response to glutamate to 51% (C). In that case the signal-to-noise ratio was increased by 76%. During the 2 min following NE administration, the ongoing activity remained lower than the control while the response to glutamate had fully recovered and the signal-to-noise ratio was still 148% above the control level (D). (See Figure 11 and 12; bin width 500ms).


GLUT 30
A
CONTROL
S 100%
G 100%
B
NE 60
S 109%
G 143%
C
NE 60
S 29%
G 51%
D
RECOVERY
S 42%
G 104%
IMP/BIN
40
32
24
16
8
0
0
5
10
15
20
25
30
35
40
45
TIME (s)

evoked by glutamate were not increased (Table 10B.2).

Biphasic responses were not observed in silent neurons. In 5 of the 9 cases where an excitatory response was observed (see following section), NE was administered for only one run. In some of these cases biphasic responses might have been observed if NE had been administered for a longer period of time.

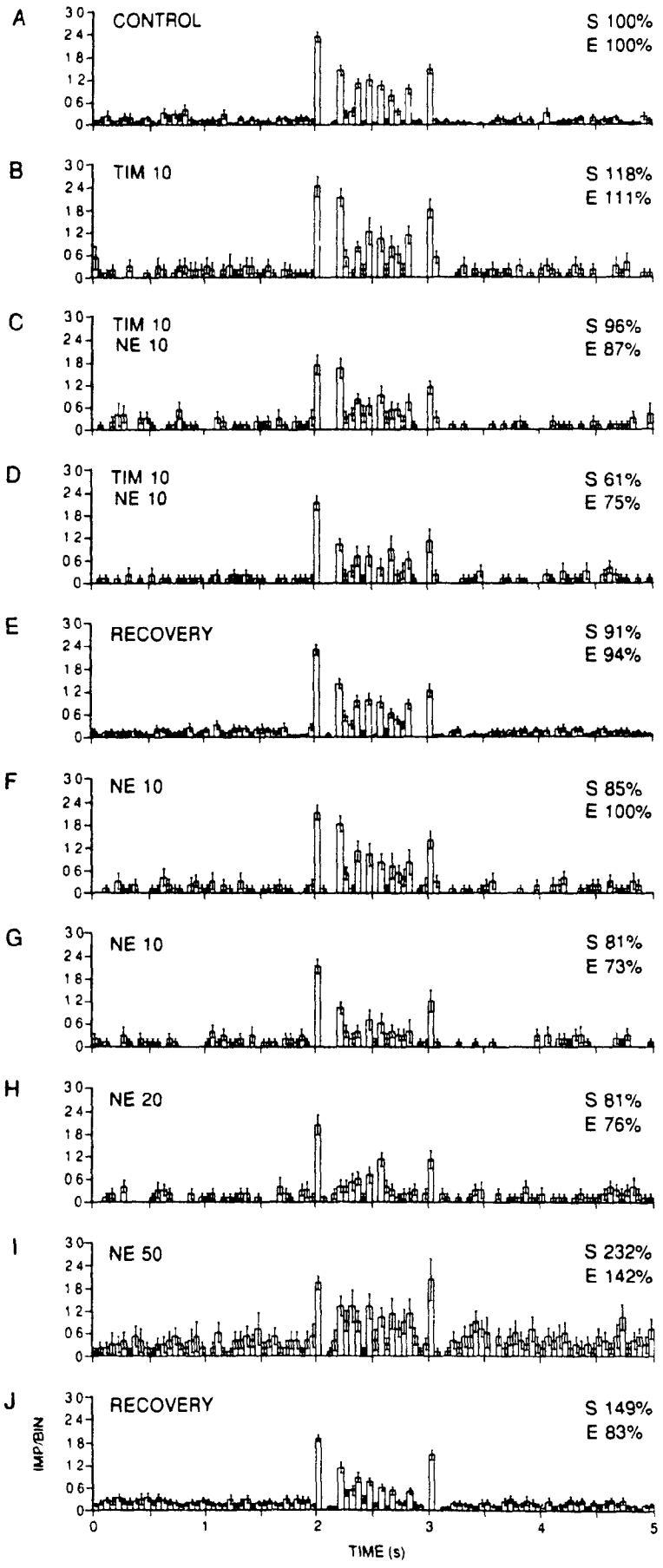
The time course of the biphasic as well as the excitatory effects of NE was not evaluated because of the small sample size and because these effects were seldom observed on both spontaneous and evoked activities in the same neuron.

3.3.5 Excitatory effects of NE.

3.3.5.1 Neurons displaying a receptive field.

The activity of 4 neurons with a receptive field increased in the presence of NE and these increases lasted for as long as NE was present. In one case, both the ongoing activity and the response to the peripheral stimulus were increased as the current of NE was increased from 20 to 50nA (Figure 16). In two cases only the ongoing activity was increased significantly. In one of these cases 10nA of NE was applied for only one run during which ongoing activity increased by 114% while evoked activity was unchanged and in the other case NE was applied for 4 runs (2 times at 50nA and 2 times at 100nA) and ongoing activity was increased (mean +84%) throughout while evoked activity was decreased (mean -54%) The last case was a silent neuron and the response to the stimuli increased progressively during 3 consecutive runs reaching a significant level (+32%) in the 3rd run. Overall, the spontaneous activity was increased 3 times and evoked activity 2 times. The

Figure 16: Effect of the ß-receptor antagonist timolol on a neuron displaying a receptive field that was excited by NE. This neuron displayed a SRA receptive field and was located in layer IoV of area 3b. The baseline was established with 30 stimulus presentations (A). During the control period the frequency of spontaneous activity was 2.7Hz and the stimulus evoked an average of 11.7 action potentials. Timolol was administered alone for one run and produced no significant effects (B). NE 10nA was added for two runs and in the first run (C) no significant effects were observed while in the second (D) the ongoing activity decreased to 61% of the control and the evoked activity to 75% producing a slight increase in the signal-to-noise ratio of 23%. During the 3 min following NE administration in the presence of timolol the ongoing activity averaged 91% of the control level and the evoked activity 94% (E). The same amount of NE was administered alone and produced an effect of lower magnitude than in the presence of timolol (F and G). During the second run (G), the ongoing activity was decreased by only 19% and the evoked activity by 22% as compared to the recovery period preceding the treatment (E) An attempt was made to increase the inhibitory effects of NE by increasing the iontophoretic current. Increasing the current to 20nA for one run produced no further effects (H) but with 50nA (I) the spontaneous activity increased by 132% and the evoked activity by 42% as compared to the control. During this period the signal-to-noise ratio was decreased by 39%. One to 5 min following NE administration, the ongoing activity was still enhanced by 49% and the evoked activity was in the range of the control level (J). (See Figure 11 and 12; bin width, 50ms).


A
CONTROL
S 100%
E 100%
B
TIM 10
S 118%
E 111%
C
TIM 10
NE 10
S 96%
E 87%
D
TIM 10
NE 10
S 61%
E 75%
E
RECOVERY
S 91%
E 94%
F
NE 10
S 85%
E 100%
G
NE 10
S 81%
E 73%
H
NE 20
S 81%
E 76%
I
NE 50
S 232%
E 142%
J
RECOVERY
S 149%
E 83%
IMP/BIN
30
24
18
12
06
0
0
1
2
3
4
5
TIME (s)

magnitudes of these effects are summarized in Table 11A for spontaneously active neurons. Again, the ongoing activity was increased proportionally more than the response to the stimuli.

3.3.5.2 Neurons lacking a receptive field.

Sustained excitation was observed in 9 spontaneously active neurons lacking a receptive field. NE was administered for more than one run in 7 cases (mean 3.4 runs). The increase was seen in the 1st run in 5 cases and in the 2nd and 4th run in the other 4 cases. The current of NE used on these neurons ranged from 15 to 50nA with a geometric mean of 24.1nA. In 2 cases both the spontaneous activity and the response to glutamate were significantly increased. In 3 cases where only the ongoing discharge was increased, the response to glutamate was decreased in 2 cases and unchanged in the other case. Only the response to glutamate was increased in 4 cases while the spontaneous activity was decreased in 3 of these cases and unchanged in another. No significant difference was found between the magnitude of the effects of NE on the spontaneous activity and on the response to glutamate (Table 11B). In absolute terms, the ongoing activity was increased by 1.02Hz (from 0.47 to 1.49Hz) and the response to glutamate by 7.26Hz (from 3.89 to 11.15Hz).

Nine neurons lacking ongoing activity were also excited using currents of NE ranging from 10 to 50nA with a geometric mean of 20.6nA. In 5 cases NE was applied during only one run producing a significant increase in the response to glutamate. In the 4 remaining cases, NE was administered for 3-8 runs and the increase was observed in the 1st run in 2 neurons and in the 3rd and 4th run in the

Table 11: Magnitude of the excitatory effects of NE expressed as percent of control (see table 9).

A. Spontaneously active neurons displaying a receptive field (n=3).

Mean effect		Maximal increase	
Spont. Act.	Evoked Act.	Spont. Act.	Evoked Act.
+76.3 ±29.7	+4	+141.3 ±16.8	+51
n=3	n=1	n=3	n=1

B. Spontaneously active neurons lacking a receptive field. (n=9).

Mean effect		Maximal increase	
Spont. Act.	Glut. Resp.	Spont. Act.	Glut. Resp.
+109.4 ±41.3	+115.8 ±65.0	+218.0 ±84.4	+186.6 ±61.4
n=5	n=6	n=5	n=6

C. Silent neurons lacking a receptive field. (n=9). Excitatory effects of NE on the response to glutamate.

Mean effect	Maximal increase
+159.6 ±66.7	+178 ±66.7

other cases. The mean increase of the response to glutamate was 150.6% and the maximal increase was 178.1% (Table 11C), that is a maximal increase of 11.36Hz (from 6.38 to 17.74Hz). Those increases did not appear significantly different than those observed on spontaneously active neurons lacking a receptive field (Table 11). An example of a neuron excited by NE is shown in Figure 24.

3.3.6 Other effects of NE.

In 3 cases neurons lacking a receptive field were initially inhibited and then the effects of NE changed to excitation. One of these cases was a spontaneously active neuron; while NE 20nA was applied during 4 runs its ongoing activity was decreased by 63% in the first run and then the discharge was increased in the following runs reaching a maximum of 159% over control. In contrast, the response to glutamate was decreased throughout the test with a mean decrease of 32.5%. The two other cases lacked spontaneous activity and the response to glutamate was first decrease by 40% and 53% and then increased by 72% and 157% respectively over the control period. In both cases the current of NE was increased between the inhibitory and the excitatory responses.

3.3.7 Iontophoretic current of NE required to produce inhibition and excitation.

Since several authors had suggested that lower concentrations of NE produce excitation and that higher concentrations result in inhibition, this hypothesis was tested on our data. Although this possibility was not an a priori hypothesis used to design a specific experiment, some aspects of the data provide some insight into this question. In 29 neurons the NE current was increased from low to a higher current during the test. In 21 of these cases the effect produced by NE was not changed by increasing the current of NE and in the other 8 cases the effect was reversed. In 16 of the cases in which the sign of the response did not change, the cell was only inhibited. The geometric mean of the initial current used on those neurons was 22.9nA. In these cases the current was increased to an average of 52.9nA. For the 5 cases where an excitation was observed, the current was increased from a geometric mean of 17.2nA to 57.4nA without converting any of the excitations to inhibitions. There was no significant differences between the inhibited and excited neurons for either initial or final currents.

In 5 of the 8 cases where the sign of the response changed, the neurons responded initially with an excitation while the geometric mean of NE current was 19.9nA. This reversed to inhibition when the current was increased to 49.0nA. The 3 other neurons displayed an initially inhibitory response which was followed by an excitatory response when the NE current was increased. These were first inhibited with a geometric mean current of 20.0nA and the excitatory response occurred with an average of 44.8nA. No significant difference was found between the two groups of neurons nor between the neurons showing no change in the sign of their response

and those which responded to the increase of current by reversing the sign of their response. Taken together these results suggest that, in the present study, NE produced inhibition and excitation at about the same concentration of NE and that low NE levels had no preferential excitatory effects.

The mean currents of NE used to produce inhibition and excitation were also compared. The effects of NE were grouped as decreases (solely inhibition) and increases (either biphasic or purely an increase) and the currents used to induce these effects were compared for both the spontaneous activity and peripherally evoked activity or response to glutamate (Table 12). In all classes of neurons, when an increase was observed lower currents of NE had been used. No significant differences were found between the currents used for those cases showing excitation and those showing inhibition for the ongoing activity but a significant difference was found for the induced activity. These findings show that excitation could be produced with lower currents of NE than those producing an inhibition. This is not to be considered as direct evidence that indeed excitation was produced with lower concentrations of NE as compared to those producing inhibition (see above) Nevertheless, this suggests that the excitations were not artifacts produced by the iontophoretic current or the relatively low pH of the solution of NE since they were observed with lower currents of NE than the inhibitory effects.

Table 12: Geometric means of iontophoretic current of NE used to produce inhibitory and excitatory responses.

A: Spontaneous activity (not significantly different, analysis of variance, $F_s = 1.375$, $df = 1, 63$, $p > 0.2$).

	Decrease	Increase	Difference
Spont. active neurons with a receptive field	33.5nA n=18	22.9nA n=8	-10.6nA
Spont. active neurons without a receptive field	24.4nA n=25	22.6nA n=14	-1.8nA
Total	27.8nA n=43	22.7nA n=22	-5.1nA

B: Evoked activity or response to glutamate.

	Decreased	Increased	Difference
Spont. active neurons with a receptive field	32.8nA n=19	26.0nA n=5	-6.8nA
Spont. active neurons without a receptive field	26.3nA n=25	20.9nA n=10	-5.4nA
Silent neurons without a receptive field	32.6nA n=24	20.6nA n=9	-12.0nA*
Total	30.2nA n=68	21.8nA n=24	-8.4nA**

* Significantly different, analysis of variance, $F_s = 4.504$, $df = 1, 90$, $p < 0.04$.

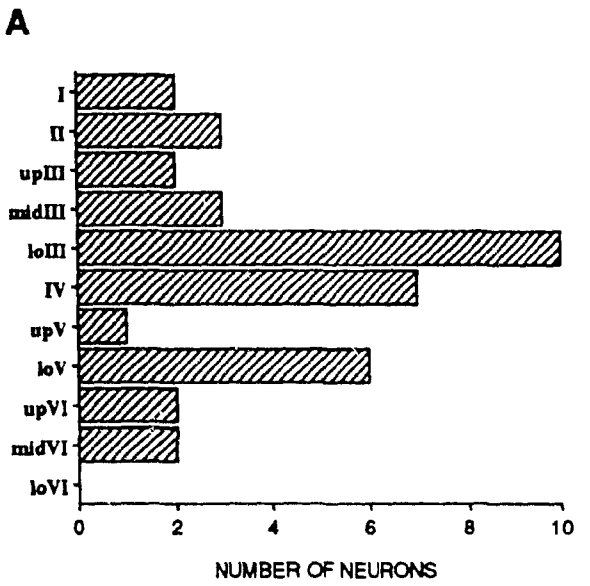
3.3.8 Laminar distribution of the effects of norepinephrine.

Thirty eight neurons studied with NE were recovered in the histology. Of these, 13 displayed a receptive field and 25 lacked this characteristic. Since NE appeared to produce similar effects in both groups, the data were pooled to produce the laminar distributions shown in Figure 17.

The laminar distribution of the whole sample (Figure 17A) was significantly different than that of the whole sample recovered in the histology (Figure 4A; $G_{adj} = 10.130$, $df = 2$, $p < 0.01$). Almost half (45%) of the neurons studied with NE were found in layers loIII and IV and another peak was found in layer loV (16%). None were found in layer loVI and the remainder of the sample (39%) was quite evenly distributed over the other layers so that each contained 1 to 3 neurons. The difference between this distribution and the total sample distribution is a subject to be explored in the discussion.

When the neurons were grouped according to whether they responded to NE exclusively by a decrease in activity or if some component of the response was excitatory, laminar differences were noted. Of the 20 neurons which had displayed solely inhibitory responses, 80% were located in layers midIII, loIII and IV and the rest was located rather uniformly above and below these layers (Figure 17B). The neurons that displayed an increase on a component of their response to NE were distributed in a contrasting way and only 22% of the neurons located in layer loIII while none was found in layers midIII and IV. Twenty eight percent were found in the upper layers and 50% in the lower layers (Figure 17C). A significant difference was found between the distributions of the effects of NE in upper (I, II and upIII),

Figure 17: Laminar distribution of the effects of NE. The distribution of all neurons tested with NE located in the histology (n=38) is shown in A. Neurons inhibited were primarily found in layers mid III to IV (B) while the excited neurons were found in upper and lower layers (C).


A
I
II
upIII
midIII
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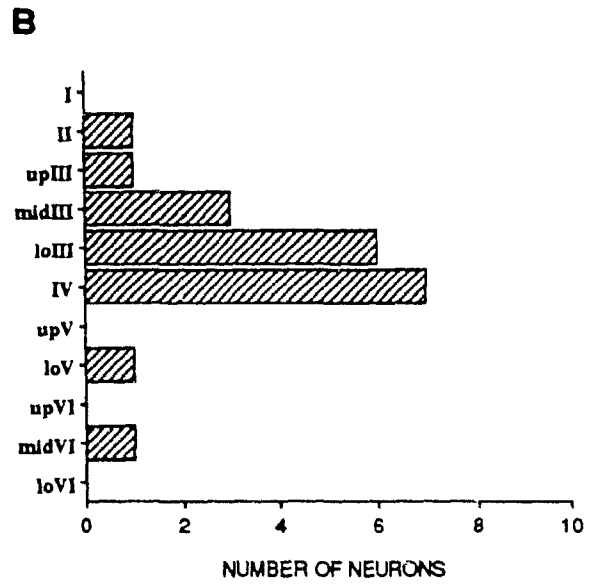
NUMBER OF NEURONS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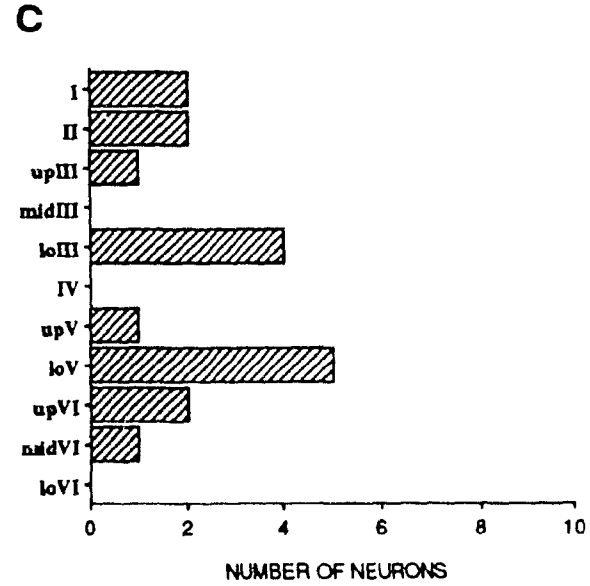
NUMBER OF NEURONS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NUMBER OF NEURONS

middle (midIII, loIII and IV) and lower (V and VI) layers (G_{adj} = 12.917, df = 2, $p < 0.005$) suggesting that NE produced different responses in different layers. The probability of finding neurons inhibited by NE in the middle layers was 80% as compared to 29% and 18% in the upper and lower layers respectively.

3.3.9 Effects of norepinephrine on the signal-to-noise ratio.

The effects of NE on the signal-to-noise ratio were quantified in 68 spontaneously active neurons that responded to NE administration. Of these, 27 could be excited by the peripheral stimulation of their receptive field whereas in 41 cases the cells did not display a receptive field and the effect of NE was estimated on the response to glutamate. It was found to be useful for the purpose of this analysis to divide the cells into those showing a decrease, those responding in a biphasic manner and those showing a sustained excitation. Further, since the response to NE varied over time, the signal-to-noise ratio also changed during the same period so the signal-to-noise ratio was calculated for the mean and maximal effects of NE. Both the mean and maximal effects of NE were considered to decide on the effects of NE on the signal-to-noise ratio of neurons (see table 14). As well, the time course of the change in the signal-to-noise ratio was documented for those neurons displaying a receptive field which were inhibited by NE.

3.3.9.1 Inhibited neurons.

The signal-to-noise ratio was increased in most of the neurons displaying a receptive field that were inhibited by NE. This occurred regardless of whether the mean or maximal effects of NE were used for the estimation but the magnitude of the increase in signal-to-noise ratio was much more important when the maximal effects were used (Table 13A). In the few cases where the signal-to-noise ratio was decreased, the magnitude of the effect was much less important than when an increase was observed. For example, with the maximal effects of NE, when increased, the signal-to-noise ratio was increased by a factor of 4.45 whereas in the cases where it was decreased, it was reduced by a factor of only 1.32. Note also (Table 13A) that the decrease in evoked activity was almost the same whether the signal-to-noise ratio was increased, decreased or not affected whereas the spontaneous activity varied much more among the 3 different effects observed An important inhibition of the ongoing activity corresponded with an increase in signal-to-noise ratio while a smaller inhibition resulted in a decrease in the signal-to-noise ratio Thus, the variations of the effects of NE on spontaneous activity appeared to be responsible in a large extent for the variations of effects of NE on the signal-to-noise ratio.

Overall, the signal-to-noise ratio was increased in 78% of the inhibited neurons displaying a receptive field while the signal-to-noise ratio was decreased in only two cases and was unaffected in only two cases also (Table 14A)

The time course of the increase in signal-to-noise ratio is illustrated in Figure 14C for the inhibited neurons displaying a receptive field; it appeared to be a two

Table 13: Changes in the signal-to-noise ratio produced by NE in relation to the observed effects on the spontaneous and evoked activities. The values are normalized as percent of the control period (S/N= signal-to-noise ratio).

A: Inhibited neurons.

Effects on S/N		Cells with a receptive field		Cells without a receptive field	
		Mean	Maximal	Mean	Maximal
Increased	Spont. Activ.	28.9 ±4.5	9.1 ±3.2	39.4 ±5.3	23.6 ±4.5
	Evok. Activ.	56.6 ±5.9	43.4 ±5.8	71.4 ±6.9	61.1 ±5.1
	S/N	195.7	478.0	181.2	259.0
	n	12	12	9	14
Decreased	Spont. Activ.	85.5 ±13.4	53	74	-
	Evok. Activ.	64.0 ±11.3	40	36	-
	S/N	74.9	75.5	48.6	-
	n	2	1	1	0
Not changed	Spont. Activ.	57.8 ±5.2	49.8 ±6.0	66.3 ±5.3	51.3 ±11.0
	Evok. Activ.	61.5 ±5.1	48.8 ±4.8	71.1 ±5.9	54.2 ±14.3
	S/N	106.4	98.0	107.2	105.5
	n	4	5	10	6

B: Biphasic neurons.

Effects on S/N		Cells with a receptive field			Cells without a receptive field		
		Mean	Increase	Decrease	Mean	Increase	Decrease
Increased	Spont. activ.	41.0 ±2.8	-	11.2 ±6.4	60.7 ±9.6	97.0 ±17.0	21.7 ±6.1
	Evok. activ.	72.5 ±12.0	-	43.2 ±9.0	112.7 ±14.6	142.0 ±1.4	70.3 ±15.1
	S/N	176.8	-	385.7	185.7	146.4	324.0
	n	2	0	5	3	2	7
Decreased	Spont. activ.	107.5 ±3.5	183.0 ±21.5	64	138.6 ±15.0	243.2 ±19.5	52
	Evok. activ.	74.0 ±5.7	114.3 ±14.5	47	37.0 ±14.6	57.0 ±17.5	31
	S/N	68.8	62.5	73.4	26.7	23.4	59.6
	n	2	4	1	5	6	1
Not changed	Spont. activ.	96 5 ±5.0	131.0 ±21.2	-	99.7 ±3.6	160.3 ±13.4	13.7 ±8.6
	Evok. activ.	86.0 ±1.4	149.5 ±23.3	-	111.0 ±2.6	145.0 ±13.1	11.3 ±7.1
	S/N	89.1	114.1	-	111.3	90.5	82 5
	n	2	2	0	3	3	3

C: Excited neurons.

Effects on S/N		Cells with a receptive field		Cells without a receptive field	
		Mean	Maximal	Mean	Maximal
Increased	Spont. Activ.	-	-	73.5 ±12.1	94.3 ±8.4
	Evok. Activ.	-	-	232.9 ±101.0	269.5 ±90.5
	S/N	-	-	316.9	285.8
	n	0	0	4	4
Decreased	Spont. Activ.	199.0 ±21.2	241.3 ±16.7	199.3 ±53.0	305.3 ±110.9
	Evok. Activ.	78.0 ±45.3	108.7 ±30.4	101.0 ±26.5	116.5 ±23.2
	S/N	39.2	45.0	50.7	38.2
	n	2	3	4	4
Not changed	Spont. Activ.	131	-	250	369
	Evok. Activ.	104	-	213	344
	S/N	79.4	-	85.2	93.2
	n	1	0	1	1

step process. After NE has been turned on, the faster decrease of the spontaneous activity as compared to the decrease in the peripherally evoked activity first produced an increase in signal-to-noise ratio of about 30% within 7 to 16s. Then the signal-to-noise ratio remained relatively stable during the interval of 70-80s when the spontaneous and evoked activities were decreasing at the same rate. As the decrease of the evoked activity reached a plateau, the ongoing activity continued to decrease causing a second increase in signal-to-noise ratio that was much more important than the initial one. This second step of the increase in signal-to-noise ratio explains why the ratio is much more important when the maximal response to NE is used to evaluate the signal-to-noise ratio as compared to the mean response (Table 13A).

The changes in signal-to-noise ratio calculated from the response to glutamate (n=20) were similar to those found with peripherally evoked activity (Table 13A) The only difference was that the signal-to-noise ratio was unaffected in proportionally more cases when the mean effect of NE was used. The effects of NE on the response to glutamate were about the same whether the signal-to-noise ratio was increased or unaffected. In contrast, the effects of NE on the spontaneous activity were between -60 to -75% when the signal-to-noise ratio was increased and only -34 to -49% when the signal-to-noise ratio was unaffected In the only case where the signal-to-noise ratio was decreased, this effect was due to both the relative resistance of the ongoing activity to NE and to the relative sensitivity of the response to glutamate to the inhibitory effects of NE. Overall, 70% of the 20 neurons tested with glutamate and inhibited by NE had their signal-to-noise ratio increased whereas a decrease was observed in 5% of the cases and the signal-to-noise ratio was

Table 14: Number and proportions of the effects of NE on the signal-to-noise ratio. To allow the effect of NE on the signal-to-noise ratio for each neuron, both the mean and maximal effects of NE on the signal-to-noise ratio were considered. In those cases where the signal-to-noise ratio was unaffected for both mean and maximal effects, a neuron was classified as unaffected. Whenever the effect on the signal-to-noise ratio had the same sign for both conditions or if it was not affected for the mean or maximal effect and was affected for the other it was classified according to the sign of the effect. The effect was classified as biphasic if the signal-to-noise ratio was first decreased and than increased. S/N, signal-to-noise ratio; Rev. biphasic, decrease followed by an increase.

A: Neurons displaying a receptive field.

Effects on	Classes of Neurons			
S/N	Inhibited neurons	Biphasic neurons	Excited neurons	Total
Increased	14 (78%)	2 (33%)	0 (0%)	16 (59%)
Rev. Biphasic	0 (0%)	3 (50%)	0 (0%)	3 (11%)
Decreased	2 (11%)	1 (17%)	3 (100%)	6 (22%)
Not affected	2 (11%)	0 (0%)	0 (0%)	2 (7.4%)
Total	18	6	3	27

B: Neurons lacking a receptive field.

Effects on	Classes of Neurons				
S/N	Inhibited neurons	Biphasic neurons	Excited neurons	Other neurons	Total
Increased	14 (70%)	5 (46%)	4 (44%)	0 (0%)	23 (56%)
Rev. Biphasic	0 (0%)	2 (18%)	0 (0%)	0 (0%)	2 (4.9%)
Decreased	1 (5.0%)	4 (36%)	4 (44%)	1 (100%)	10 (24%)
Not affected	5 (25%)	0 (0%)	1 (11%)	0 (0%)	6 (15%)
Total	20	11	9	1	41

unaffected in 25% (Table 14B).

3.3.9.2 Biphasic neurons.

The effects of NE on the signal-to-noise ratio were assessed on 6 biphasic neurons displaying a receptive field (Table 13B). During the excitatory phase, the signal-to-noise ratio was increased in none of the neurons and it was decreased in 4 of them (Table 13B). The reverse was observed during the inhibitory phase, where the signal-to-noise ratio was increased in 5 of 6 cases and decreased in only one case. As well as for inhibited neurons, the different effects of NE on the signal-to-noise ratio appeared to be dependent much more on the variability of the effects of NE on the spontaneous activity than on evoked activity. Overall, the signal-to-noise ratio followed a reversed biphasic pattern (decrease followed by an increase) in the 3 cases where both evoked and ongoing activities responded biphasically whereas an overall increase was observed in 2 cases and a decrease in one case (Table 14B).

For the 11 biphasic neurons responding to glutamate, the signal-to-noise ratio was decreased more often than it was increased during the excitatory phase (Table 13B). But during the inhibitory phase, the signal-to-noise ratio was increased in a majority of cases. When the signal-to-noise ratio was increased, the increases were more important during the inhibitory phase than during the excitatory phase. Also, the magnitude of the decreases were more important than those observed on neurons displaying a receptive field. In the present case, the different effects of NE on the signal-to-noise ratio appeared to be dependent on the effect of NE on both the spontaneous activity and the response to glutamate. When the mean effects of

NE were used, the effect of NE on the response to glutamate was the same whether the signal-to-noise ratio was increased or not affected whereas the effects of NE on the spontaneous activity was different between these two effects. In contrast, the decreases in the signal-to-noise ratio were observed when the response to glutamate was strongly inhibited and the ongoing activity excited. The same relationships between the effects of NE on the ongoing activity and the response to glutamate was also observed during the excitatory phase. During the inhibitory phase, the unaffected cases corresponded to very important decreases of both spontaneous activity and response to glutamate (Table 13B). Overall, reversed biphasic responses were observed in 2 cases whereas an increase was observed in 5 cases and a decrease in 4 cases (Table 14B). And again the effect of NE on the spontaneous activity seemed to be the primary determinant of the change occurring in the signal-to-noise ratio.

3.3.9.3 Excited neurons.

Only 3 spontaneously active neurons displaying a receptive field were classified as excited and in all cases the signal-to-noise ratio was decreased (Table 13C and 14A) since the ongoing activity was markedly increased in each case and in the only case where the evoked activity was increased also, the ongoing activity was proportionally more increased thereby still decreasing the signal-to-noise ratio. The magnitude of the decreases were over 50%.

In response to glutamate, NE produced an increase in the signal-to-noise ratio of 4 of the excited neurons. A decrease was also observed in 4 cases and one

neuron was not affected (Table 13C and 14B). The effect of NE in those cases appeared to depend on whether the spontaneous activity or the response to glutamate was increased the most. In the cases where the signal-to-noise ratio was increased, the spontaneous activity was not much affected whereas the response to glutamate was markedly increased (Table 13C). In contrast when the signal-to-noise ratio was decreased, the response to glutamate was only slightly increased and the ongoing activity was comparatively much more increased.

One neuron responded to NE first by a decrease of its ongoing activity that was followed by an increase while its response to glutamate was decreased producing a decrease in signal-to-noise ratio; this neuron has been included in Table 14B.

The effects of NE on the signal-to-noise ratio in response to peripheral stimuli and to pulses of glutamate are summarized in the last column of Table 14A and B. Overall, the proportions of increases and decreases in signal-to-noise ratio were about the same for the two classes of neurons. An increase was observed in almost 60% of neurons while a decrease was observed in less than a quarter of the sample.

3.3.10 Effects of noradrenergic agonists.

Noradrenergic receptor agonists were administered to 18 neurons and their effects were compared to those of NE administered to the same neurons. The α_2-receptor agonist, oxymetazoline was tested on 13 neurons and the ß-receptor agonist isoproterenol on 5 neurons.

3.3.10.1 The effects of oxymetazoline.

Oxymetazoline was administered to 13 neurons using iontophoretic currents ranging from 5 to 75nA. Neurons were affected with currents having a geometric mean of 16.4nA. The current of NE used on the same neurons ranged from 5 to 100nA with a geometric mean of 19.4nA.

In 4 cases oxymetazoline was applied on neurons while their receptive field was stimulated mechanically. It mimicked the effects of NE in 3 cases while both drugs produced a decrease of both spontaneous and evoked activities associated with an increase in signal-to-noise ratio. In the other case, oxymetazoline and NE produced both a biphasic response on spontaneous activity and a decrease of peripherally evoked activity. In that case, the administration of oxymetazoline resulted in a decrease in signal-to-noise ratio whereas in the presence of NE the signal-to-noise ratio was decreased during the excitatory phase and it was increased during the inhibitory phase. Overall, oxymetazoline produced the same effects as NE on neurons displaying a receptive field with the exception that there was no effect on the signal-to-noise ratio of the biphasic neuron (Table 15A) One case where oxymetazoline mimicked the effects of NE is shown in Figure 18.

In 5 cases oxymetazoline was tested on the response to glutamate of spontaneously active neurons lacking a receptive field. In 3 of these cases, NE produced a decrease of both ongoing activity and response to glutamate. Oxymetazoline produced the same effect in one of these cases and in the two other cases only the ongoing activity was significantly decreased while the response to glutamate was decreased but by only 21% and 14% in each case. The signal-to-noise

Table 15: Number of neurons affected by oxymetazoline. The effects of NE on the same neurons are indicated in brackets for comparison.

A: Neurons displaying a receptive field.

	Spont. Activ.	Evok. Activ.	S/N
Decreased	3 (3)	4 (4)	1 (0)
Biphasic	1 (1)	0 (0)	0 (1)
Increased	0 (0)	0 (0)	3 (3)
Total	4 (4)	4 (4)	4 (4)

B: Spontaneously active neurons lacking a receptive field.

	Spont. Activ.	Resp. to glut	S/N
Decreased	3 (3)	1 (4)	0 (2)
Increased	1 (1)	2 (1)	2 (3)
Not affected	1 (1)	2 (0)	3 (0)
Total	5 (5)	5 (5)	5 (5)

C: Neurons lacking spontaneous activity and a receptive field.

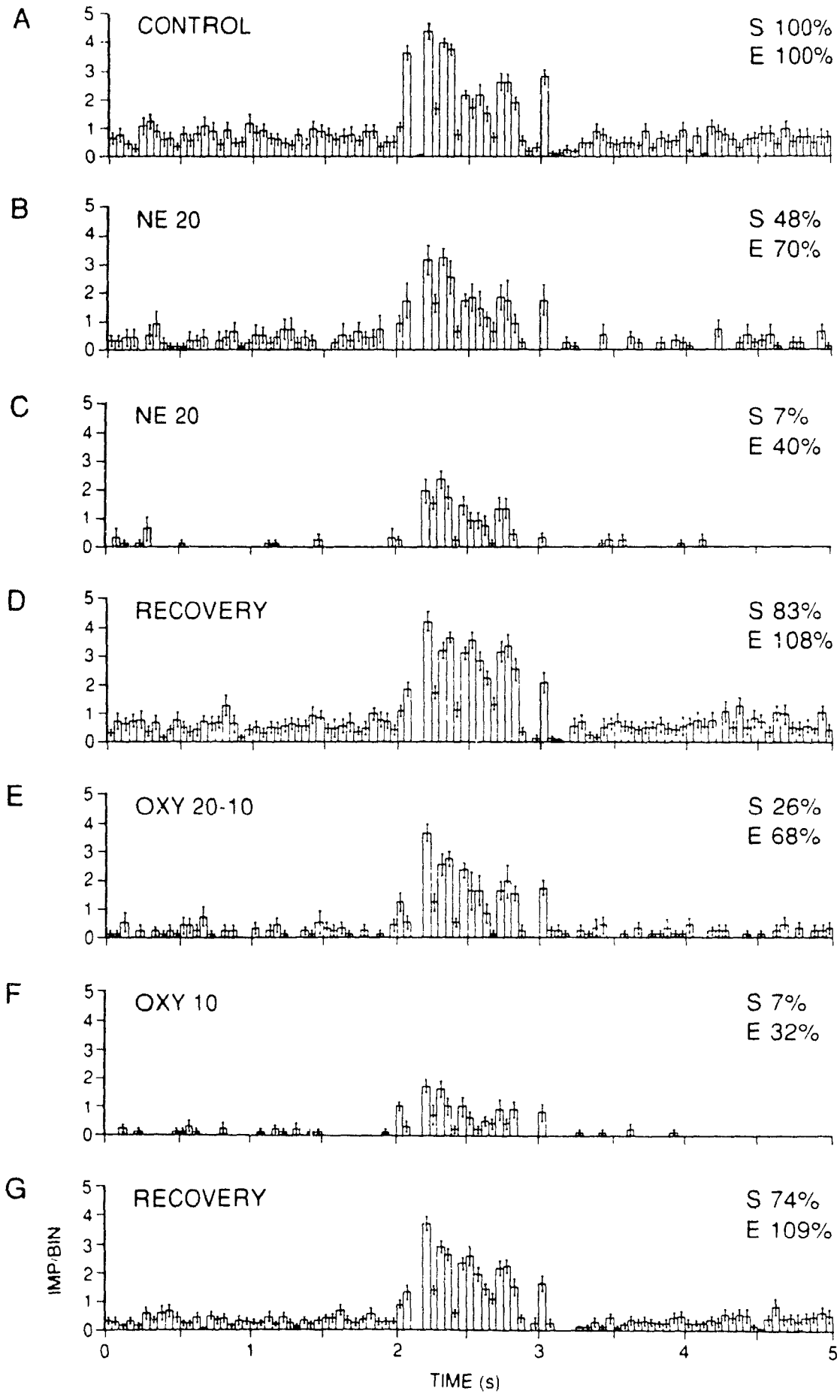
	Response to glutamate
Decrease	4 (3)
Other	0 (1)
Total	4 (4)

ratio was increased in 2 cases in the presence of NE and decreased in the other case. On the same neurons, oxymetazoline produced an increase in signal-to-noise ratio in one case and no change in the two other cases.

In one of the remaining two spontaneously active neurons lacking a receptive field both NE and oxymetazoline produced an increase of the response to glutamate while the ongoing activity remained at control level; both drugs also produced an increase in signal-to-noise ratio in that case. In the other case, both NE and oxymetazoline produced an increase of ongoing activity but the response to glutamate was decreased in the presence of NE and increased by oxymetazoline. So, with the exception of this latter case, oxymetazoline appeared to produce effects similar to NE but these effects of oxymetazoline were not significant on the response to glutamate in two cases (Table 15B).

In 3 of 4 neurons lacking spontaneous activity and a receptive field oxymetazoline mimicked the effects of NE by producing a decrease of the response to glutamate. In the other case, NE produced a decrease in the response during the first two runs and an increase in the subsequent runs whereas an inhibition was the only effect observed with oxymetazoline using the same iontophoretic current over the same number of runs (Table 15C).

Figure 18: Effects of the α_2-receptor agonist oxymetazoline on a neuron displaying a receptive field. This neuron had a receptive field that was classified TAP and it was not located in the histology. The baseline was established with 30 stimulus presentations (A). The mean frequency of ongoing activity was 13.5Hz and an average of 37.7 actions potentials were recorded at each stimulus presentation. NE was administered with a current of 20nA during two continuous runs of 10 stimulus presentation (B and C). During the first run, the spontaneous activity was decreased to 48% of the control period and the evoked activity by 70% while the signal-to-noise ratio was increased by 46% (C). In the second run the spontaneous activity was decreased further and represented only 7% of the control while the evoked activity was still 40% and this resulted in an increase in signal-to-noise ratio of 417% as compared to the control level. In that case both the ongoing and evoked activities were back to the control level within the following two min (D) and then oxymetazoline was administered for two runs and produced the same effect as NE (E and F). In the first run oxymetazoline was started with a current of 20nA which was decreased to 10nA after 5 stimulus presentations since it produced a very strong inhibition. During that run, the spontaneous activity was decreased to 26% and the evoked activity to 68% resulting in an increase in signal-to-noise ratio of 162% as compared to the period just prior to oxymetazoline ejection (E). In the second run, both the ongoing and evoked activity decreased further and the signal-to-noise ratio was increased by 357% (F). This was followed by recovery 3 min after oxymetazoline (G). (See Figure 11 and 12; bin width, 50ms).


A
CONTROL
S 100%
E 100%
B
NE 20
S 48%
E 70%
C
NE 20
S 7%
E 40%
D
RECOVERY
S 83%
E 108%
E
OXY 20-10
S 26%
E 68%
F
OXY 10
S 7%
E 32%
G
RECOVERY
S 74%
E 109%
IMP/BIN
TIME (s)

3.3.10.2 Effects of isoproterenol.

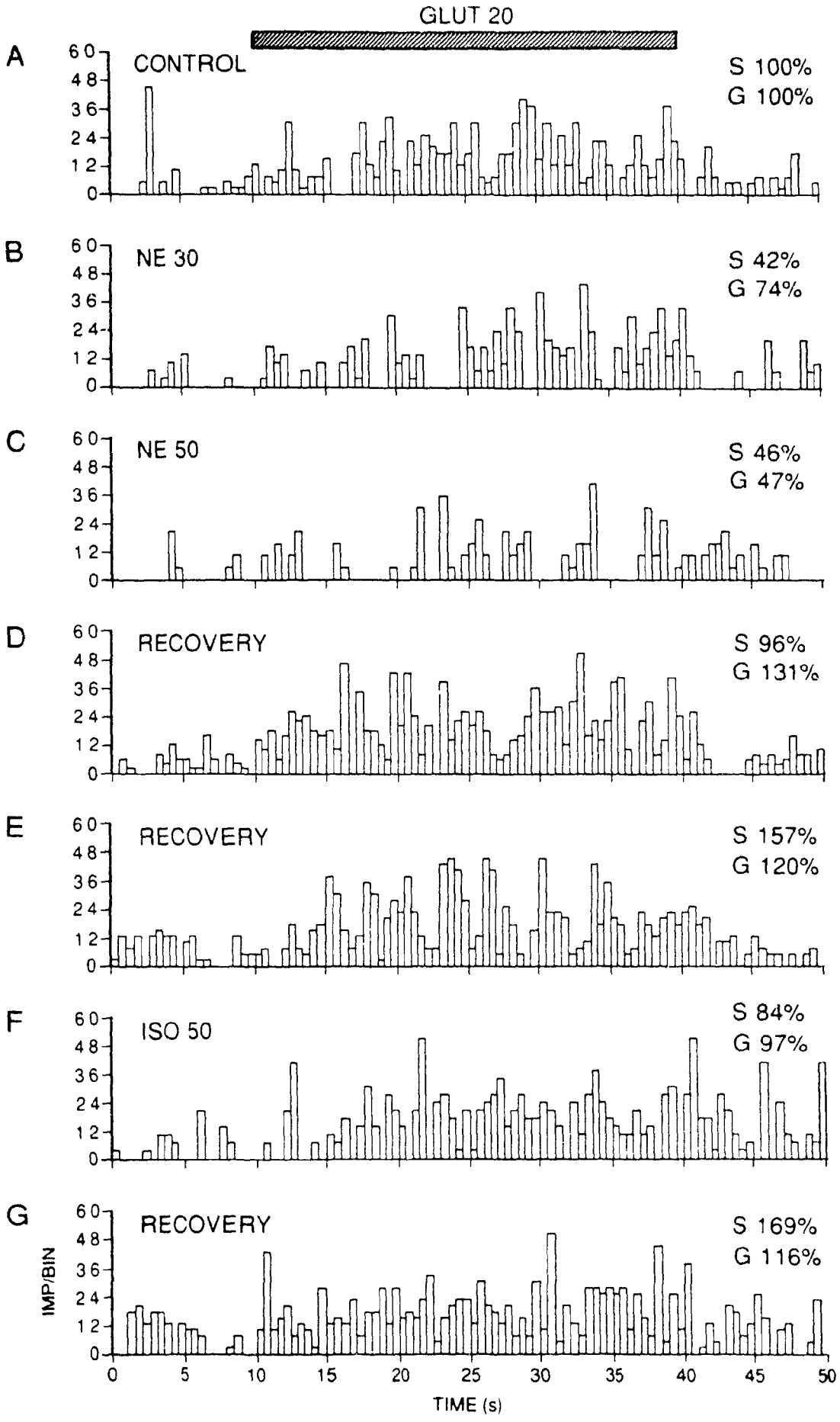
The ß-agonist isoproterenol was administered to only 5 neurons with iontophoretic currents ranging from 10 to 50nA with a geometric mean of 17.4nA. The current of NE applied to the same neurons ranged also from 10 to 50nA and the mean current was 17.8nA.

Isoproterenol was tested on peripherally evoked activity in only one case and it mimicked the effects of NE on that neuron. Both drugs produced a significant decrease of ongoing and evoked activities. The magnitude of the effects was more important on ongoing activity than on evoked activity resulting in an increase in signal-to-noise ratio.

Two spontaneously active neurons lacking a receptive field were tested with isoproterenol and in both cases it produced a decrease of ongoing activity and of response to glutamate. NE produced the same inhibitory effects on those neurons and one case is presented in Figure 19. Also both isoproterenol and NE produced an increase in signal-to-noise ratio in one case and a decrease in the other case.

On two neurons lacking ongoing activity and a receptive field, isoproterenol produced no significant effects and did not mimic the effects of NE. In one of these neurons the response to glutamate was decreased by 21% in the presence of isoproterenol while the ejection of NE produced first a decrease followed by an increase. In the other case, isoproterenol produced an increase of 11% in the response to glutamate while NE produced a decrease of 84%.

Figure 19: Effects of the ß-receptor agonist isoproterenol. This neuron lacked a receptive field even in the presence of glutamate. It was not located in the histology and could not be assigned accurately to a cortical depth from the micrometer reading. The baseline was established with 4 pulses of 20nA of glutamate (A). The response to glutamate averaged 3 1Hz and the frequency of spontaneous activity was 0.88Hz during that period. NE was then administered for two runs with a current of 30nA (B) and for 3 runs using 50nA (C). During the administration of NE 30nA the ongoing activity was reduced to 42% of the control period and the response to glutamate to 74% resulting in an increase in signal-to-noise ratio of 76%. With higher current of NE the ongoing activity was not further decreased while the response to glutamate was decreased to 47% and the signal-to-noise ratio returned to the control level One to 5 min after the cessation of NE, the ongoing activity was at the control level while the response to glutamate was increased by 31% during the same period (D) During the following 4 min the response to glutamate decreased to be only 20% more than the control level while the ongoing activity increased to 157% of the control level (E). Isoproterenol 50nA was then administered for 3 runs and the ongoing activity was decreased to 54% and the response to glutamate to 81% of the period preceding isoproterenol ejection (F) In the presence of isoproterenol the signal-to-noise ratio was increased by 50%. One to 4 min after the cessation of isoproterenol administration both the ongoing and glutamate induced activities returned to the level observed prior isoproterenol administration (G). (See Figure 11 and 12, bin width, 500ms).


GLUT 20
A
CONTROL
S 100%
G 100%
B
NE 30
S 42%
G 74%
C
NE 50
S 46%
G 47%
D
RECOVERY
S 96%
G 131%
E
RECOVERY
S 157%
G 120%
F
ISO 50
S 84%
G 97%
G
RECOVERY
S 169%
G 116%
IMP/BIN
0
12
24
36
48
60
0
5
10
15
20
25
30
35
40
45
50
TIME (s)

3.3.10.3 Effects of oxymetazoline and isoproterenol on the same neurons.

Of the neurons tested with noradrenergic agonists, oxymetazoline and isoproterenol were administered on the same neuron in 3 cases. In one case, both oxymetazoline and isoproterenol mimicked the effects of NE and produced a decrease of spontaneous activity and peripherally evoked activity with an increase in signal-to-noise ratio. In another case where NE produced a decrease of both spontaneous activity and response to glutamate with a decrease in signal-to-noise ratio; isoproterenol mimicked these effects of NE while oxymetazoline produced only a decrease of ongoing activity resulting in an increase in signal-to-noise ratio. The last case lacked spontaneous activity and the response to glutamate was decreased by both NE and oxymetazoline but was not affected by isoproterenol.

3.3.11 Effects of noradrenergic antagonists.

In order to determine which receptors were activated by NE, attempts to block the effects of NE on somatosensory cortical neurons were made using specific noradrenergic antagonists. The effects of NE in the presence of antagonists were compared to the effects of NE alone in 42 cases (Table 16). Specific antagonists to 3 different classes of noradrenergic receptors were used The α_2-receptor antagonists idazoxan and yohimbine were tested on 16 neurons, the ß-receptor antagonists timolol and sotalol were administered to 17 neurons and the α_1-receptor antagonist benoxathian was tested in 9 cases Examples of neurons tested with antagonists are presented in Figures 16, 22, 25 and 26.

Table 16: Numbers of neurons tested with noradrenergic receptor antagonists (n=43).

	Neurons with a receptive field	Neurons lacking a receptive field		Total
		Spont. Active	Lacking Sp. Act.	
α_2-antagonists:				
Idazoxan	2	9	2	13
Yohimbine	0	0	3	3
Total	2	9	5	16
ß-antagonists:				
Timolol	3	4	3	10
Sotalol	3	1	3	7
Total	6	5	6	17
α_1-antagonist:				
Benoxanthian	0	6	3	10
Total	8	20	14	42

3.3.11.1 Effects of α_2-receptor antagonists.

Idazoxan was tested on 13 neurons using iontophoretic currents ranging from 5 to 40nA (geometric mean 11.9nA) while NE was administered with currents ranging from 5 to 50nA (geometric mean 16.6nA). In all but one case, the antagonist was tested alone for at least one run prior to the ejection of NE.

On neither the spontaneous activity nor the evoked activity did idazoxan appear to antagonize the effects of NE consistently (Table 17A). On 7 of 11 spontaneously active neurons where NE alone produced a decrease of the ongoing activity, adding the blocking agent to NE blocked these effects of NE in only one case and a partial block was observed in another case. In the other cases where NE produced a different effect in the presence of the antagonist as compare to NE alone, no consistent effects were observed (Table 17A). When idazoxan was ejected alone, it produced a decrease of the spontaneous activity in 3 cases and no effect in 7 cases. In one case idazoxan alone was not tested.

In 10 of 13 cases tested with idazoxan, NE alone produced an inhibition of the evoked activity. These inhibitory effects of NE were blocked by idazoxan in only two cases. In one case where NE alone produced no effect on the evoked activity, the ejection of NE in the presence of idazoxan resulted in a decrease. The administration of idazoxan alone decreased the evoked activity in 2 cases and produced no effect in two other cases. Thus, this antagonist seldom showed any blocking of the effects of NE.

The most consistent effect of idazoxan was observed on the signal-to-noise ratio. In 6 of 8 affected cases the addition of idazoxan resulted in an increase in

Table 17: Effects of α_2-receptor antagonists (n=16). The effects of NE, of NE in the presence of the antagonist and of the antagonist alone are presented for spontaneous activity, peripherally or glutamate evoked activity and for signal-to-noise ratio. All the effects are relative to control and each row represents a single neuron. The cases where the effects of NE in the presence of the antagonist were different than the effects of NE alone are shown above the lines and those where no difference were observed below the lines. (↓), decrease; (↑), increase;(=), unaffected; (↑↓), biphasic; (↑↓), other; nt, not tested; IDA, idazoxan; YOH, yohimbine.

A: Effects of idazoxan (n=13).

Spontaneous activity			Evoked activity			Signal-to-noise ratio		
NE	**NE+IDA**	**IDA**	**NE**	**NE+IDA**	**IDA**	**NE**	**NE+IDA**	**IDA**
↓	=	↓	↓	=	=	↑	↑↓	↑
↓	↓↑	↓	↓	=	↓	↓	=	=
↑↓	↓	=	=	↓	=	↓↑	↑	↑
=	↓	=				↓	↑↓	nt
=	↓	=	↓	↓	=	=	↑	=
			↓	↓	=	=	↑	=
↓	↓	=	↓	↓	=	=	↑	↑
↓	↓	=	↓	↓	=	=	↓	↓
↓	↓	=	↓	↓	=			
↓	↓	=	↓	↓	=	↑	↑	=
↓	↓	↓	↓	↓	nt	↑	↑	=
↓↑	↓↑	nt	↓	↓	↓	=	=	↓
			↑	↑	=			
			=	=	=			

B: Effects of yohimbine (n=3).

Evoked activity		
NE	**NE+YOH**	**YOH**
↓	↑	↑
↑	=	↓
↓	↓	nt

signal-to-noise ratio as compare to NE alone (Table 17A): in 3 cases where NE alone produced no effect, the signal-to-noise ratio was increased when NE and idazoxan were ejected at the same time; in one case NE alone produced a decrease in signal-to-noise ratio while it was not affected by NE in the presence of idazoxan; in another case NE produced a decrease which was changed to a biphasic response and in the last case a reversed biphasic effect produced by NE became an increase in signal-to-noise ratio when both drugs were present. In the two other affected cases, the signal-to-noise ratio was decreased as compare to NE alone. Idazoxan administered alone produced an increase in signal-to-noise ratio in 3 cases and a decrease in one case; these changes in the signal-to-noise ratio were due to effects on both the spontaneous and induced activities. The signal-to-noise ratio was not affected in the remaining 6 cases while idazoxan alone was not tested in one case.

Yohimbine was tested on only 3 neurons lacking spontaneous activity and produced no consistent effects either (Table 17B).

3.3.11.2 The effects of α_1-receptor antagonist.

The α_1-receptor antagonist benoxathian was tested on 9 neurons lacking a receptive field with iontophoretic currents ranging from 1 to 5nA (geometric mean 1.9nA). In all cases the antagonist was tested alone and produced no significant effect in the range of currents used although it appeared to produce a significant decrease in both the spontaneous activity and the response to glutamate when higher currents were used. In all cases NE was first administered in the presence of the antagonist.

Six of the studied neurons were spontaneously active and 3 lacked this characteristic. In all cases NE produced a decrease in both the ongoing activity (mean -64.0 ±8.3%, n=6) and the response to glutamate (mean -53 6 ±6.3%, n=9) when it was administered in the presence of benoxathian. In contrast, when NE was administered alone the decrease in the spontaneous activity was less important than in the presence of benoxathian (mean -44 2 ±7.5%, n=6). The response to glutamate was significantly increased in 3 cases (mean +35.3 ±2.7%), not affected in 3 cases (mean -3.3 ±14 1%) and decreased in 3 cases also (mean -48.7 ±14.5%) suggesting that the excitatory effects of NE were mediated by α_1-receptors.

In the presence of benoxathian, NE produced an increase in the signal-to-noise ratio in 5 out of 6 cases (mean +77.6 ±24.3%) and a decrease in one case (-38%). When NE was administered alone, the signal-to-noise ratio was increased in 3 cases (mean +80 3 ±19.1%) and not affected in the other 3 cases (+12.7 ±10.0%).

3.3.11.3 Effects of ß-receptor antagonists.

The ß-receptor antagonist timolol was tested on 10 neurons using iontophoretic currents ranging from 5 to 50nA (geometric mean 20 7nA). The current of NE used to test timolol ranged from 10 to 30nA (geometric mean 16.8nA). In all cases the antagonist was tested alone prior to the administration of NE.

In 5 of 7 spontaneously active neurons the response of the ongoing activity to NE was modified in the presence of timolol (Table 18A). In one case the sign of the effect was reversed when NE and timolol were ejected together. In two biphasic

Table 18: Effects of ß-receptor antagonists (n = 17). TIM, timolol; SOT, sotalol; see legend of table 17 for other abbreviations.

A: Effects of timolol (n = 10).

Spontaneous activity			Evoked activity			Signal-to-noise ratio		
NE	NE+TIM	TIM	NE	NE+TIM	TIM	NE	NE+TIM	TIM
↓	↑	↑	↓	=	=	↑	=	↓
↑↓	↓	↑	↓	=	↑	↓↑	↓	↑
↑↓	↓	↑	↑	↓	↓			
↑↓	↑	↓	=	↓	=	↑	↑	↑
=	↓	=	=	↓	=	↓	↓	↓
			=	↓	=	↓	↓	↓
						=	=	=
↓	↓	↑	↓	↓	=	=	=	=
↑	↑	↑	↓	↓	=			
			↓	↓	=			
			↓	↓	↓			

B: Effects of sotalol (n=7).

Spontaneous activity

NE	NE+SOT	SOT
↓	=	↑
↑	↓	=
↓	↓	nt
↑↓	↑↓	=

Evoked activity

NE	NE+SOT	SOT
↓	↓	=
↓	↓	=
↓	↓	=
↓	↓	nt
↓	↓	nt
↑	↑	=
↑↓	↑↓	=

Signal-to-noise ratio

NE	NE+SOT	SOT
↑	↓	↓
↓↑	↑	=
↑	↑	nt
↓	↓	↓

neurons, the excitatory component was suppressed and in another case the inhibitory phase disappeared when NE was ejected in the presence of timolol. In one case where the spontaneous activity was not affected by NE, it was decreased in the presence of both NE and timolol. When administered alone, timolol produced an increase of the spontaneous activity in 5 cases and a decrease in one case while no change was observed in the last case.

Timolol appeared to block the inhibitory effect of NE on the evoked activity in two cases but had no effect in 4 other inhibited cases (Table 18A) In one excited neuron in the presence of NE alone, the evoked activity was decreased when both NE and timolol were present Three cases which were not affected by NE alone were inhibited when timolol was added to NE Timolol alone affected the evoked activity less often than the spontaneous activity, it produced a decrease in two cases and an increase in one case while in 7 cases no effects were observed

Despite the fact that timolol affected the response of the spontaneous and evoked activities to NE in a majority of cases, the effect of NE on the signal-to-noise ratio was altered in only 2 of the 7 cases studied (Table 18A) In the two affected cases, the addition of timolol appeared to produce a decrease in signal-to-noise ratio as compare to NE alone The administration of timolol alone produced a decrease in signal-to-noise ratio in 3 cases, an increase in 2 cases while 2 cases were not affected as compare to control.

Sotalol, the other ß-antagonist tested in the present study, affected the response to NE than timolol. Sotalol was administered with iontophoretic currents ranging from 10 to 75nA (geometric mean 39 2nA) and NE with currents ranging

from 15 to 100nA (geometric mean 41.7nA).

NE was tested in the presence of sotalol on 4 spontaneously active neurons and in two cases the response of the spontaneous activity to NE was changed as compare to NE alone but these effects were not consistent (Table 18B). In the 7 cases tested, the effect of NE on the evoked response was not modified by the presence of sotalol during the ejection of NE. Further, the administration of sotalol alone produced no effect on the evoked activity. The effect of NE on the signal-to-noise ratio was changed in two cases when sotalol was present.

So in summary, ß-antagonists did not consistently block the effects of NE.

3.4 Long-term effects of norepinephrine.

In addition to the effects produced in the presence of NE released from the iontophoretic pipette, some changes in neuronal excitability were observed to begin or to extend long after the administration of NE had stopped. Effects lasting beyond the period of administration were observed in all classes of cells studied. These effects were called short-term effects if they lasted for less than 5 min after the cessation of NE and long-term effects if they lasted more than 5 min. This time was chosen because in most iontophoretic studies in the cerebral cortex, the effects of NE were not reported to last consistently more than 5 min (see section 1.2 in Introduction). Furthermore, previous studies in this laboratory on the long-lasting effects of acetylcholine on somatosensory cortical neurons have used this criterion also (Metherate et al., 1988b).

Long-lasting effects were studied in 69 out of 117 neurons (59%) in which the action potential remained stable for some period of time after the tests with NE had been completed. In these cases it was possible to continue to monitor the level of neuronal excitability and to observe any effects of NE that began after the administration of NE had been terminated. In making such comparisons it is important to demonstrate that the 69 cases studied were not selected in a way that would produce a biased sample. To exclude this possibility the reasons that the other 41% of the 117 cells were not studied were explored carefully and they are listed in Table 19. In general, the reason for not studying a particular cell was simply a question of not being able to hold the cell for a period of time sufficient to obtain the necessary data, the average time that the excluded cells were studied was less

Table 19: Information concerning the 117 cells in the sample used to examine long-term effects of NE.

	Total time held (min)	Time held after NE (min)	Reasons to stop study
Type of cells			
Studied long-term n=69	58.0 ±3.1 (18 to 140)	12.9 ±1.0 (2 to 36)	other tests, n=58 too small, n=7 left, n=4
Not studied long-term n=48	26.2 ±2.2 (6 to 60)	4.8 ±0.6 (0 to 16)	too small, n=28 lost, n=12 unstable, n=4 depolarized, n=4

than 5 min following NE administration and the study was terminated because the cell was lost rather than because another study was undertaken. Thus there does not appear to have been any selection of neurons that was under the experimenter's control.

The first question is to establish by some objective measure whether or not long-term effects were produced by the prior administration of NE. This question is difficult to answer because tests for long-term effects, by their nature, are not amenable to the controls usually administered in iontophoretic studies. That is, it is not possible to establish a baseline response, administer the drug, and then to measure the baseline response again; long-term effects imply that the responses of the cell do not return to control values.

As a result, the observation of a long-term effect can only be related to its immediately preceding baseline, opening the possibility that a change in responsiveness may be simply a slow trend in the data due to some factor other than the administration of NE. Thus, the hypothesis that NE has no long-term effect is that some instability in the responsiveness of the cell produced data where the responses gradually increased in some cells, gradually decreased in other cells and in a third group there should be no change.

A test of the existence of a long-term effect of NE is to add all of the data from all of the cells. If the average response for the cells does not change then the net effect of NE on a population of somatosensory neurons would be negligible. That is the positive effects would be cancelled by the negative effects producing a mean that was not significantly different from the control value. however, pooling

the data in this way produced averages that were consistently larger than the control values by a significant amount when glutamate had been used to induced a neuronal response. The results were somewhat different in terms of magnitude and time course for the cells in each class and thus will be presented separately. The categories of neurons described in the present section remain the same as those employed in section 3.3, where four classes were identified: the majority of neurons responded by an inhibition of both the ongoing and the evoked activity. In 3 other classes either one or both of these aspects of the discharge pattern was enhanced by NE. However, those cells inhibited by NE and those with an excitatory component to the response appeared to have separate laminar locations. For this reason, the neurons with an excitatory component to their response were considered together and those inhibited were considered as a separate group in the present section.

3.4.1 Neurons displaying a receptive field.

The period following NE administration was examined in 15 neurons displaying a receptive field for periods ranging from 2 to 23 min (mean 9.7 ±1.6 min). Of these, 11 were inhibited in the presence of NE, 3 were excited and one was not affected. The effects observed within 5 min and more than 5 min after the cessation of NE are presented in Table 20 for the 14 neurons affected during NE administration.

In at least 80% of the inhibited neurons, both the spontaneous and evoked activities were back to the control level within 5 min following the cessation of NE (Table 20A). During that period, the ongoing activity was decreased in two cases and

Table 20: Short-term and long-term effects of NE on the spontaneous activity, the evoked activity and the signal-to-noise ratio of neurons with a receptive field.

A: Neurons inhibited during NE administration (n=11).

	Short-term (≤5min)			Long-term (>5min)		
	Sp. Act.	Ev. Act.*	S/N*	Sp. Act.	Ev. Act.*	S/N*
Increase	0 (0%)	1 (9.1%)	3 (30%)	2 (29%)	2 (25%)	0 (0%)
Decrease	2 (20%)	1 (9.1%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
No effect	8 (80%)	9 (82%)	7 (70%)	5 (71%)	6 (75%)	7 (100%)
Total	10	11	10	7	8	7

B: Neurons displaying biphasic response or excitation during NE administration (n=3)

	Short-term (≤5min)			Long-term (>5min)		
	Sp. Act.	Ev. Act.*	S/N*	Sp. Act.	Ev. Act.	S/N
Increase	2 (100%)	1 (33%)	0 (0%)	1 (100%)	0 (0%)	0 (0%)
Decrease	0 (0%)	0 (0%)	2 (100%)	0 (0%)	0 (0%)	1 (100%)
No effect	0 (0%)	2 (67%)	0 (0%)	0 (0%)	1 (100%)	0 (0%)
Total	2	3	2	1	1	1

* One neuron lacked spontaneous activity.

C: All neurons displaying a receptive field (n = 14).

	Short-term (≤5 min)			Long-term (>5 min)		
	Spont. Act.* (n = 12)	Ev. Act. (n = 14)	S/N* (n = 12)	Spont. Act.** (n = 8)	Ev. Act (n = 9)	S/N** (n = 8)
Increased	2 (17%) +88.5 ±12.0	2 (14%) +37.5 ±3.5	3 (25%) +35.3 ±6.7	3 (37%) +78.0 ±32.9	2 (22%) +48.5 ±3.5	0 (0%) -
Decreased	2 (17%) -46.0 ±19.8	1 (7.1%) -43.0	2 (17%) -54.5 ±2.1	0 (0%) -	0 (0%) -	1 (12%) -63.0
Not affected	8 (66%) -5.4 ±4.0	11 (79%) -5.2 ±3.3	7 (58%) +2.4 ±5.2	5 (63%) -9.2 ±5.2	7 (78%) -9.7 ±4.1	7 (88%) -4.0 ±5.1

* Two neurons lacked spontaneous activity.
** One neuron lacked spontaneous activity.

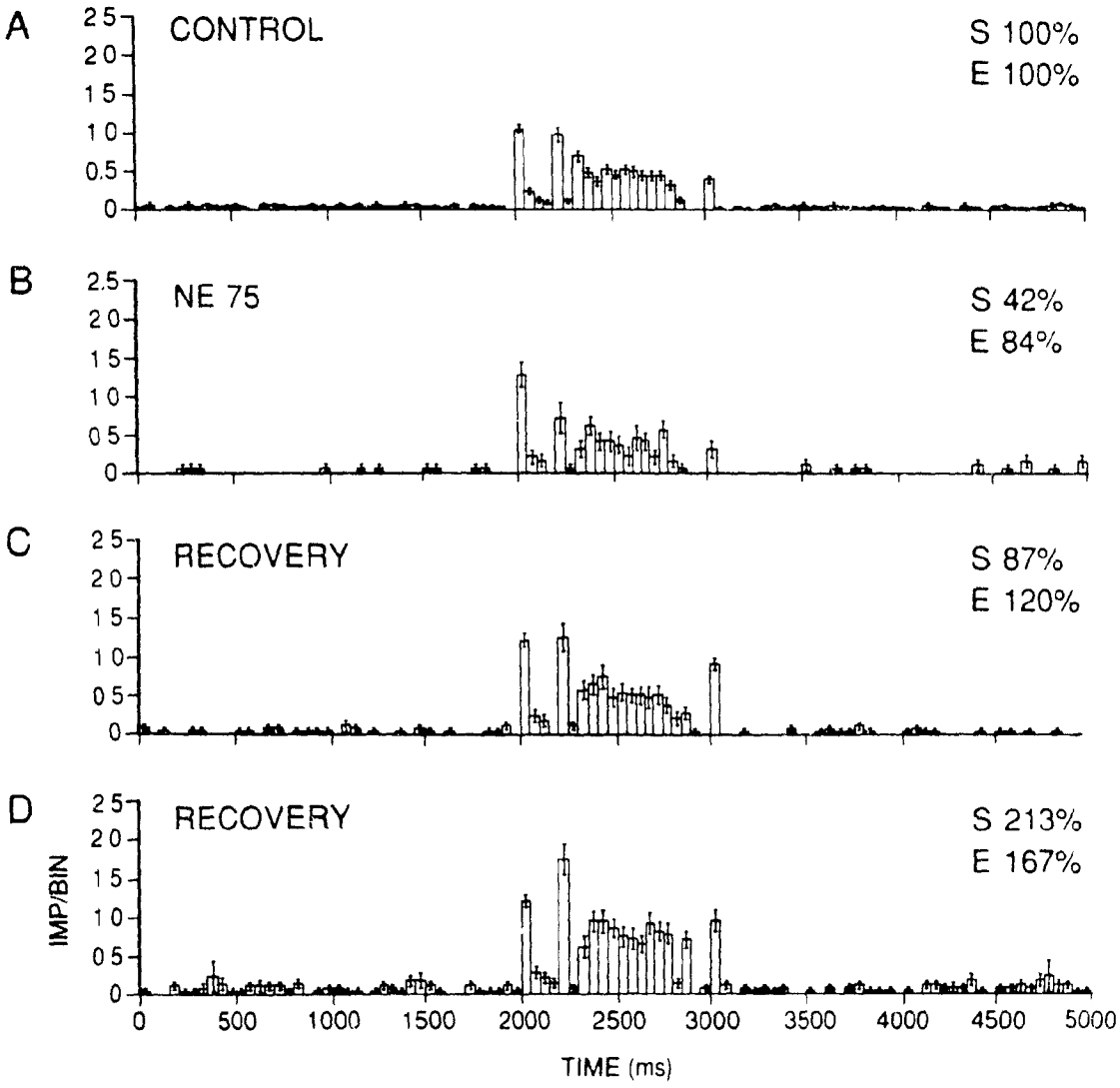
the evoked activity was decreased in one case and increased in another case. The signal-to-noise ratio had also returned to the control level in a majority of cases within 5 min after the cessation of NE.

For times greater than 5 min after NE, the spontaneous and the evoked activities were increased in two cases. These increases in activity were still present 13 and 23 min after the cessation of NE when other tests were performed on these neurons. The activity of the other neurons remained at the control level for the remaining period of time they were studied (6 to 11 min after the cessation of NE). In all the cases studied, the signal-to-noise ratio was not different from that observed during the control period. One case where both the spontaneous and evoked activities were enhanced for more than 5 min is shown in Figure 20.

The time course of the response of the inhibited neurons during and after the administration of NE is shown in Figure 21A. During NE administration, the decrease of the ongoing activity was more important than the one observed on the evoked activity resulting in an increase in signal-to-noise ratio. Following NE, the evoked activity recovered more rapidly than the spontaneous activity and during the first 3 min the signal-to-noise ratio was increased as compare to the control period. From the 4th min, both spontaneous and evoked activities were back to control level as well as was the signal-to-noise ratio.

Within 5 min of the cessation of NE, the ongoing activity was increased in the 2 spontaneously active neurons excited during NE administration (Table 20B). The evoked activity was enhanced in 1 out of 3 cases while in the 2 other cases the activity had already returned to the control level. The signal-to-noise ratio was

Figure 20: Long-lasting increase in spontaneous and evoked activity of a neuron displaying a receptive field. This neuron displayed a SRA receptive field and was located in layer IV of area 3b. The baseline was established with 90 stimulus presentations. During that period the ongoing activity was low at 0.55Hz and each stimulus presentation evoked an average of 8 action potentials (A). NE 75nA was administered during two consecutive runs reducing the spontaneous activity to 42% of the control period and the evoked activity to 84% resulting in an increase in signal-to-noise ratio of 100% as compared to the control (B). During the 3 min following NE administration the ongoing activity averaged 87% of the control period and the evoked activity 120% (C). In the following min both the ongoing and the evoked activities increased regularly and 12 to 14 min after NE administration the ongoing activity had increased by 113% and the evoked activity by 67% (D). The signal-to-noise ratio was decreased by 22% during this period. Following this other tests were performed on this neuron. (See Figure 11 and 12; bin width 50ms).


A
CONTROL
S 100%
E 100%
B
NE 75
S 42%
E 84%
C
RECOVERY
S 87%
E 120%
D
RECOVERY
S 213%
E 167%
IMP/BIN
TIME (ms)

decreased in two cases.

Only one excited neuron was studied for more than 5 min after NE. Its spontaneous activity was increased and its evoked response not different from control. During that period, the signal-to-noise ratio was decreased. The profile of the response of these neurons is not shown because of the heterogeneity of the responses observed during NE administration and also because of the small sample size during the recovery period

When inhibited and excited neurons were pooled, it appeared that in a majority of cases both the spontaneous and evoked activities returned to control level within 5 min following NE and the activity was increased in a few neurons (Table 20C) When increases were observed, they were proportionally more important on the spontaneous activity than on the evoked activity. Some neurons did not recover from the inhibitory effects of NE during that period. In those cases, the decrease was about 50% of the control for both the spontaneous and evoked activities. In over half of cases, the signal-to-noise ratio was not different than it was during the control period In 25% of the neurons it was increased by an average of 35.3% while it was decreased by 54.5% in 17% of cases

In neurons studied for more than 5 min after the administration of NE, the proportion of neurons with increased activity was slightly higher than had been observed within 5 min The magnitude of the increases was the same as within 5 min of the cessation of NE. No significant decreases as compared to the control period were observed. In over 60% of the neurons there was no evidence of a long-term increase in activity. The signal-to-noise ratio was decreased in only one case (12%)

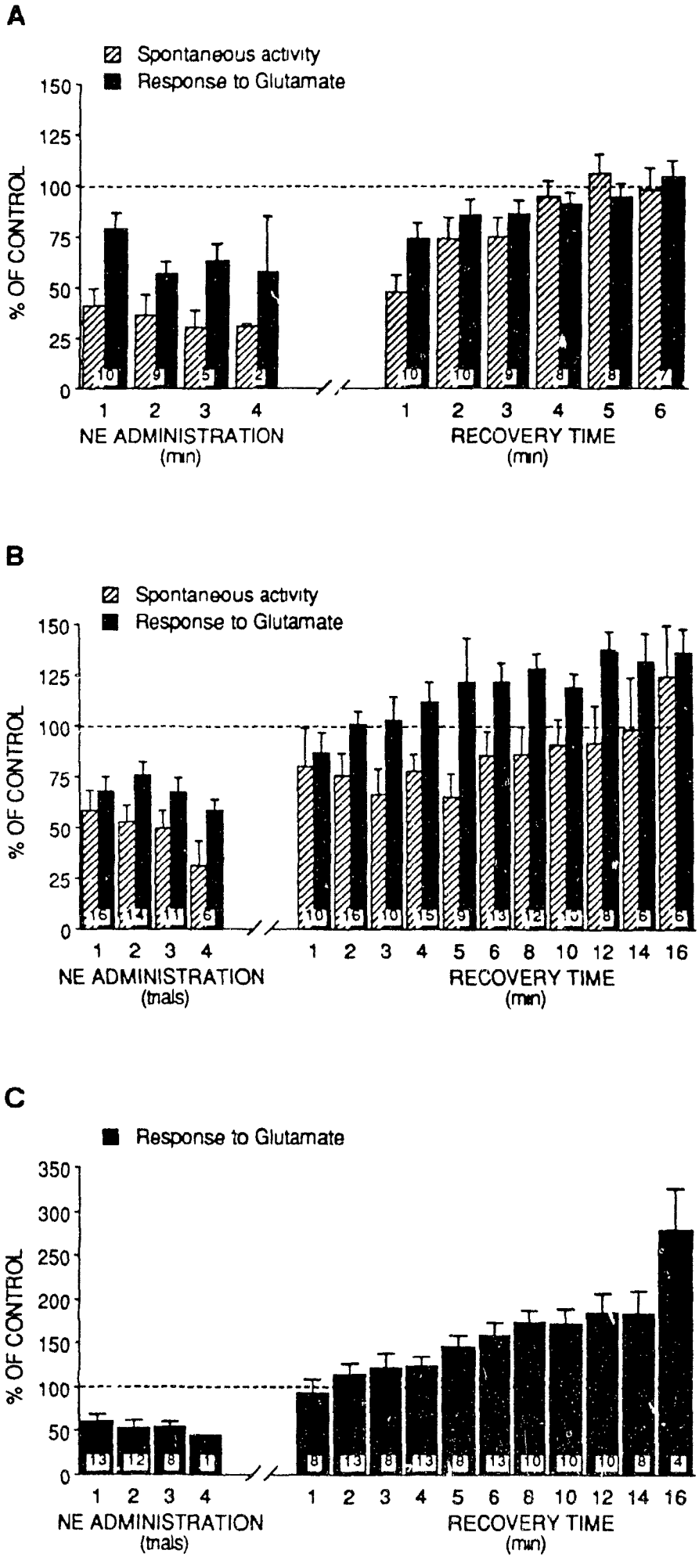
Figure 21: Time course of the responses to NE during and after the administration of NE for different classes of neurons inhibited in the presence of NE. In B and C the number of neurons are lower at times 1, 3 and 5 min because for some neurons glutamate pulses were given at 2 min intervals. Vertical bars represent the standard error of the mean.

A Neurons displaying a receptive field and inhibited by NE

B Spontaneously active neurons lacking a receptive field and inhibited by NE

C. Neurons lacking spontaneous activity and a receptive field and inhibited by NE

Note the difference in the vertical scale from A and B.


A
Spontaneous activity
Response to Glutamate
% OF CONTROL
150
125
100
75
50
25
0
10 9 5 2
10 10 9 8 8 7
1 2 3 4
NE ADMINISTRATION
(min)
1 2 3 4 5 6
RECOVERY TIME
(min)
B
Spontaneous activity
Response to Glutamate
% OF CONTROL
150
125
100
75
50
25
0
16 14 11 6
10 16 10 15 9 13 12 10 8 6 6
1 2 3 4
NE ADMINISTRATION
(trials)
1 2 3 4 5 6 8 10 12 14 16
RECOVERY TIME
(min)
C
Response to Glutamate
% OF CONTROL
350
300
250
200
150
100
50
0
13 12 8 11
8 13 8 13 8 13 10 10 10 8 4
1 2 3 4
NE ADMINISTRATION
(trials)
1 2 3 4 5 6 8 10 12 14 16
RECOVERY TIME
(min)

and was not affected as compared to the control period in 7 (88%).

In summary, long-lasting effects were seldom observed in neurons displaying a receptive field and the overall tendency was a return to the baseline within a few min following the administration of NE.

3.4.2 Neurons lacking a receptive field.

Fifty-four neurons lacking a receptive field displayed a stable action potential following the administration of NE long enough for study. Half of those were spontaneously active.

3.4.2.1 Spontaneously active neurons.

The period following the ejection of NE was examined in 27 spontaneously active neurons lacking a receptive field for 3 to 28 min (mean 13.4 ± 1.4 min) following the administration of NE. In 16 neurons NE had produced only inhibition during administration and within 5 min of the cessation of NE both the spontaneous activity and the response to glutamate returned to the control level in about half of them (Table 21A). In the rest of the cells, the response to glutamate was either increased (25% of the cases) or decreased (in 19% of cases) with respect to the control. In contrast, the spontaneous activity was greater than the control in only 6% of neurons and lower in 44% of cases. As a result, the signal-to-noise ratio was increased in 63% of the inhibited neurons in the first 5 min following the end of NE treatment.

More than 5 min after NE, the proportion of cases with an enhanced response to glutamate had increased to 54% while the spontaneous activity was

Table 21: Short-term and long-term effects of NE on spontaneously active neurons lacking a receptive field.

A: Neurons inhibited during NE administration (n=16).

	Short-term (≤5min)			Long-term (>5min)		
	Sp. Act.	Ev. Act.	S/N	Sp. Act.	Ev. Act.	S/N
Increase	1 (6.3%)	4 (25%)	10 (63%)	3 (23%)	7 (54%)	7 (54%)
Decrease	7 (44%)	3 (19%)	0 (0%)	4 (31%)	0 (0%)	2 (15%)
No effect	8 (50%)	9 (56%)	6 (37%)	6 (46%)	6 (46%)	4 (31%)
Total	16	16	16	13	13	13

B: Neurons displaying biphasic response, excitation and other response during NE administration (n=11).

	Short-term (≤5min)			Long-term (>5min)		
	Sp. Act.	Ev. Act.	S/N	Sp. Act.	Ev. Act.	S/N*
Increase	5 (45%)	5 (45%)	5 (45%)	5 (50%)	7 (70%)	6 (67%)
Decrease	5 (45%)	2 (18%)	5 (45%)	5 (50%)	0 (0%)	2 (22%)
No effect	1 (9.1%)	4 (36%)	1 (9.1%)	0 (0%)	3 (30%)	1 (11%)
Total	11	11	11	10	10	9

* One neuron was excluded because the current of glutamate was decreased 7 min after the administration of NE.

C: All spontaneously active neurons lacking a receptive field (n=27).

	Short-term (≤5 min)			Long-term (>5 min)		
	Spont. Act. (n=27)	Glut. Act. (n=27)	S/N (n=27)	Spont. Act. (n=23)	Glut. Act (n=23)	S/N** (n=22)
Increased	6 (22%) +154.3 ±33.7	9 (33%) +84.1 ±24.5	15 (56%) +108.2 ±25.3	8 (35%) +66.5 ±15.2*	14 (61%) +57.8 ±8.1*	13 (59%) +182.1 ±42.9
Decreased	12 (44%) -47.2 ±5.0	5 (19%) -38.8 ±9.6	5 (19%) -47.0 ±8.3	9 (39%) -53.3 ±5.9	0 (0%) -	4 (18%) -47.7 ±10.0
Not affected	9 (33%) -3.4 ±3.7	13 (48%) +7.6 ±2.8	7 (26%) +3.6 ±6.2	6 (26%) -4.7 ±4.2	9 (39%) +9.0 ±4.7	5 (23%) +12.6 ±6.4

* Two neurons were excluded from the average because the current of glutamate was decreased 7 min after the administration of NE in one case and in the other case the ongoing activity was 977% of control

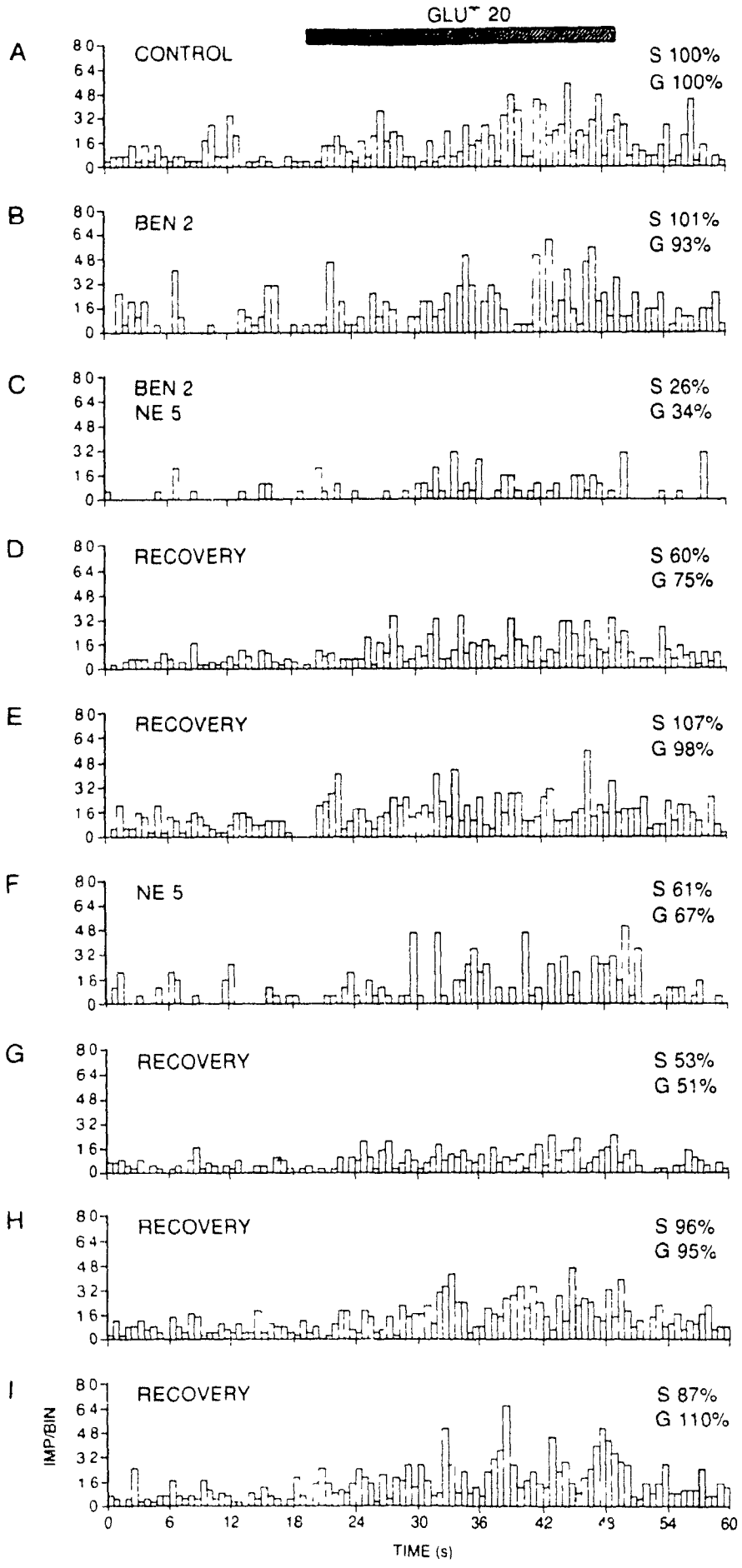
** One neuron was excluded from the average because the current of glutamate was decreased 7 min after the administration of NE.

enhanced in only 23%. In contrast, the spontaneous activity remained lower than during the control period in 31% of the neurons and in 46% the level of ongoing activity was not different than it had been during the control period. In over half of the neurons the signal-to-noise ratio was increased as compared to the control period and it was decreased in only 15% leaving 31% unchanged. Figure 22 shows an example of a neuron that was not affected following NE administration.

In all cases where the response to glutamate was seen to be enhanced for more than 5 min, it remained enhanced for the duration of the period studied (6 to 20 min). The spontaneous activity tended to return to control; in two of 3 cases the increase in the ongoing activity was back to the control level 12 and 14 min respectively after the end of NE administration. In the cases where the ongoing activity was depressed more than 5 min after the cessation of NE, these effects also remained for as long as the cells were studied, until 8 to 15 min after the administration of NE.

The time course of the neuronal activity during and after NE administration to those inhibited neurons appears in Figure 21B. During the administration of NE, the ongoing activity was decreased much more than the response to glutamate producing a significant increase in signal-to-noise ratio. During the recovery period, the ongoing activity recovered very slowly from the inhibitory effects of NE and it was not before 6 to 8 min after the cessation of NE that the average reached the control level. In contrast, the average response to glutamate reached the control level after only two min and it kept growing after that for the period of time presented. In neurons studied for 12 min or more, the mean spontaneous activity

Figure 22: Inhibitory effects of NE that was not followed by a long-lasting increase in the response. This neuron had no receptive field uncovered by glutamate and from the micrometer reading it appeared to be situated in the middle layers. The baseline was established with three pulses of 20nA of glutamate (A). During the control period the frequency of the ongoing activity was 1.23Hz and the response to glutamate averaged 1.9Hz. The α_1-receptor antagonist benoxathian was administered alone for 2 runs and produced no significant effect (B). NE was added for 3 runs and during the two last runs the ongoing activity was decreased to 26% of the control period and the response to glutamate to 34% (C). During the same period the signal-to-noise ratio was increased by 31%. During the 5 min following the administration of NE in the presence of benoxathian, the ongoing activity averaged 60% of the control and the response to glutamate 75% so that the signal-to-noise ratio was increased by 25% during that period (D). Six to 10 min after the treatment the ongoing activity was 107% of the control level and the response to glutamate 98% (E). NE was administered without the antagonist for 3 runs and produced a much less important inhibition than in the presence of benoxathian. During the last two runs the spontaneous activity was decreased to 61% of the control period and the response to glutamate to 67% resulting in no significant change in the signal-to-noise ratio (F). During the 5 min following NE the ongoing activity averaged 53% of the control level and the response to glutamate 50% (G). But 6 to 10 min (H) and 11 to 15 min (I) after both the ongoing activity and the response to glutamate were back at the control level. (See Figure 11 and 12, bin width, 600ms.


GLU 20
A
CONTROL
S 100%
G 100%
B
BEN 2
S 101%
G 93%
C
BEN 2
NE 5
S 26%
G 34%
D
RECOVERY
S 60%
G 75%
E
RECOVERY
S 107%
G 98%
F
NE 5
S 61%
G 67%
G
RECOVERY
S 53%
G 51%
H
RECOVERY
S 96%
G 95%
I
RECOVERY
S 87%
G 110%
IMP/BIN
0 16 32 48 64 80
0 6 12 18 24 30 36 42 48 54 60
TIME (s)

remained in the range of the control level while the response to glutamate was significantly increased.

The recovery period for 11 neurons excited in the presence of NE was studied. Within 5 min of the cessation of NE, in 46% of the neurons the ongoing activity and the response to glutamate were increased (Table 21B). The ongoing activity decreased during that period in the same proportion of neurons and a decrease in the response to glutamate was observed in only 18% of cases. In 45% of the neurons the signal-to-noise ratio was increased, decreased in 45% and only one case was unaffected.

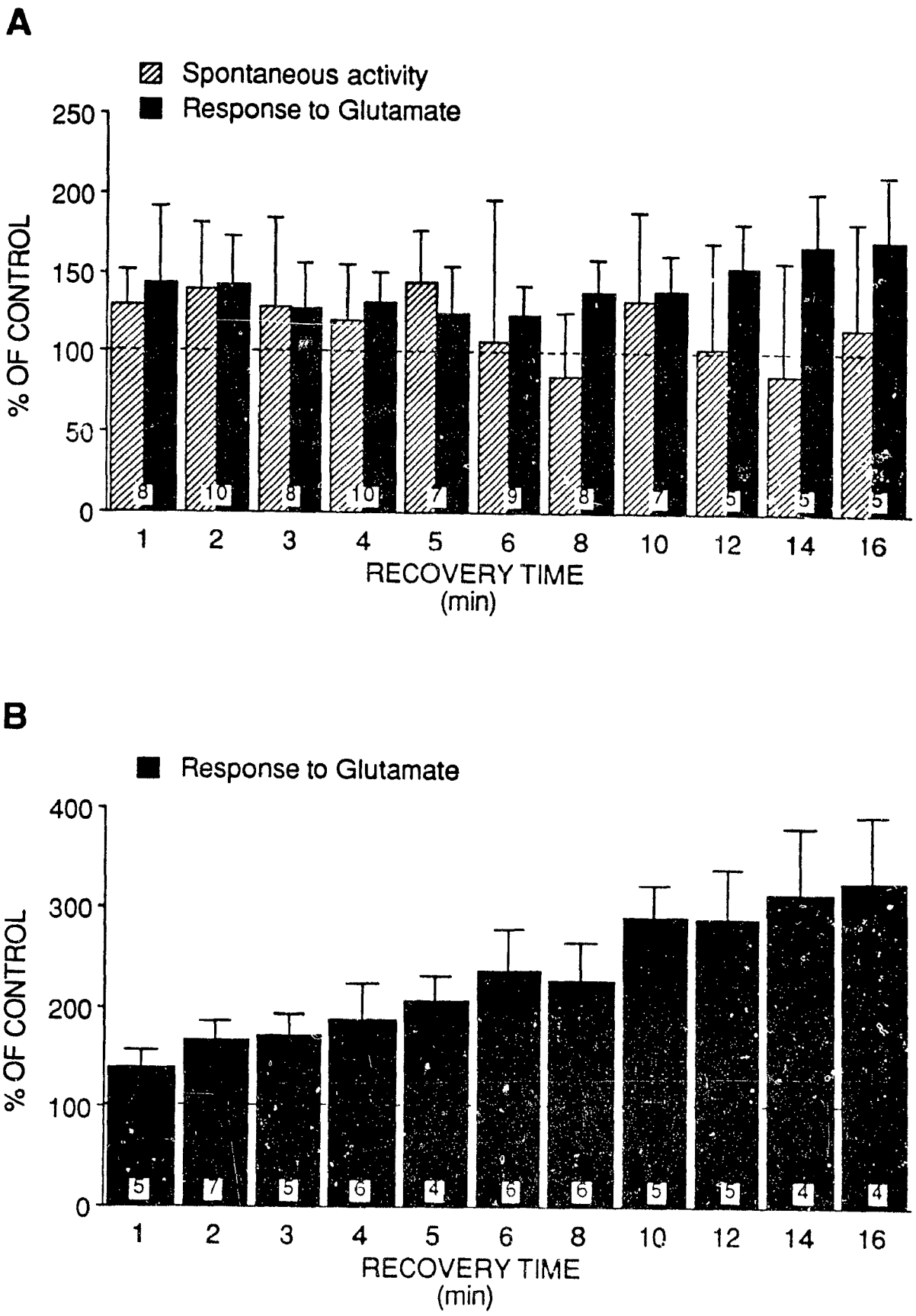
After 5 min, 70% of the responses to glutamate were increased while the others were not different than the control level. During the same period the ongoing activity was increased in 50% of the cases while the other half of the sample displayed a decrease in their spontaneous activity. In all cases these long-term effects lasted for as long as the recovery period was studied (7 to 30 min)

The average time course of the changes in excitability following NE is shown in Figure 23A Only the recovery period is shown because of the heterogeneity of the responses observed in the presence of NE. During the 5 first min following the administration of NE, the average of both the spontaneous activity and the response to glutamate increased above the control level while more than 5 min after the cessation of NE the ongoing activity decreased to the control level while the response to glutamate remained above the control level and increased further These opposite effects on the ongoing activity and on the response to glutamate resulted in an overall long-term increase in the signal-to-noise ratio.

Figure 23. Recovery period for excited neurons in the presence of NE. The number of neurons are lower at times 1, 3 and 5 min because for some neurons glutamate pulses were given at 2 min intervals. Vertical bars represent the standard error of the mean.

A. Recovery period following NE administration of spontaneously active neurons lacking a receptive field and excited by norepinephrine.

B. Recovery period following NE administration of neurons lacking spontaneous activity and a receptive field that were excited by NE. Note that the vertical scale is different in A and B and also from Figure 21.


A
Spontaneous activity
Response to Glutamate
% OF CONTROL
250
200
150
100
50
0
8
10
8
10
7
9
8
7
5
5
5
1
2
3
4
5
6
8
10
12
14
16
RECOVERY TIME
(min)
B
Response to Glutamate
% OF CONTROL
400
300
200
100
0
5
7
5
6
4
6
6
5
5
4
4
1
2
3
4
5
6
8
10
12
14
16
RECOVERY TIME
(min)

From the pooled data, it appeared that the response to glutamate increased significantly in a higher proportion of neurons than did the ongoing activity (Table 21C). Within 5 min of the termination of NE, the increase in the spontaneous activity was much more important than was the response to glutamate. More than 5 min after NE, the magnitude of the enhancement of the spontaneous activity was much less important than it was within 5 min of the administration of NE. Furthermore, two neurons with significantly increased ongoing activity during the short-term period had significantly decreased activity more than 5 min after the administration of NE. More than 5 min after NE, the spontaneous activity was even decreased in a slightly higher proportion of neurons than it was increased. The average increase in the response to glutamate seemed to have diminished more than 5 min following NE as compared to less than 5 min. This was due to the fact that 5 neurons that were not increased within 5 min after the cessation of NE and that were more than 5 min after were increased by an average of only 43.9 ±4.8%, this resulted in a relative decrease more than 5 min after NE administration and this was not the result of a diminution in the responses to glutamate. For the majority of neurons (61%) the response to glutamate increased and in no case was it decreased as compare to the control level.

The signal-to-noise ratio was increased in over half of the neurons during the short-term period as well as during the long-term period but the magnitude of the increase was much more important more than 5 min after the administration of NE. This was due to the fact that overall the spontaneous activity was lower more than 5 min after NE than within 5 min following the administration of NE while the

response to glutamate was increased overall (see also Figure 21B and 23A).

When compared to the long-term effects observed on neurons displaying a receptive field, the ongoing activity in neurons lacking a receptive field was increased in about the same proportion of neurons but it was decreased in an important proportion of neurons while decreases were not observed for neurons displaying a receptive field. The response to glutamate was increased in a much larger proportion of neurons than was the response to peripheral stimulus. These differences between the long-term effects observed on neurons displaying a receptive field and those lacking one explain why the signal-to-noise ratio was increased in a majority of neurons lacking a receptive field and this was not observed in neurons displaying a receptive field.

3.4.2.2 Neurons lacking spontaneous activity.

Twenty seven neurons lacking spontaneous activity displayed stable action potential during the recovery period. In the presence of NE, 13 were inhibited, 9 were excited and 5 were not affected. The effects of NE were studied on the response to glutamate for periods ranging from 2 to 36 min during the recovery period.

During the 5 min following NE administration, the responses of the majority (62%) of the inhibited neurons returned to the control level while in 38% of cases the response was increased as compare to the control (Table 22). In contrast, more than 5 min after NE administration, 69% of the responses were increased and only 31% remained at the control level. In none of the cases was the response to

Table 22: Short-term (≤5min) and long-term (>5min) effects of NE on neurons lacking a receptive field and spontaneous activity (n=22).

	Inhibited during NE administration		Excited during NE administration		Total	
	Short-term	**Long-term**	**Short-term**	**Long-term**	**Short-term**	**Long-term**
Increase	5 (38%)	9 (69%)	8 (89%)	8 (100%)	13 (59%) +86.4 ±17.0*	17 (81%) +125.0 ±20.0*
No effect	8 (62%)	4 (31%)	1 (11%)	0 (0%)	9 (41%) -4.4 ±3.8	4 (19%) -6.0
Total	13	13	9	8	22	21

* One neuron was excluded because its response to glutamate was more than 10 times the control within 5 min after the termination of NE and more than 15 times more than 5 min after NE.

glutamate lower than during the control period for both time periods. In all but one case the long-term increase lasted for as long as the cell was studied (7 to 28 min). In one case the response to glutamate returned to the control level, this occurred after 14 min of recovery and that neuron was lost 8 min later without displaying any instability in the action potential or other sign of injury before it was lost.

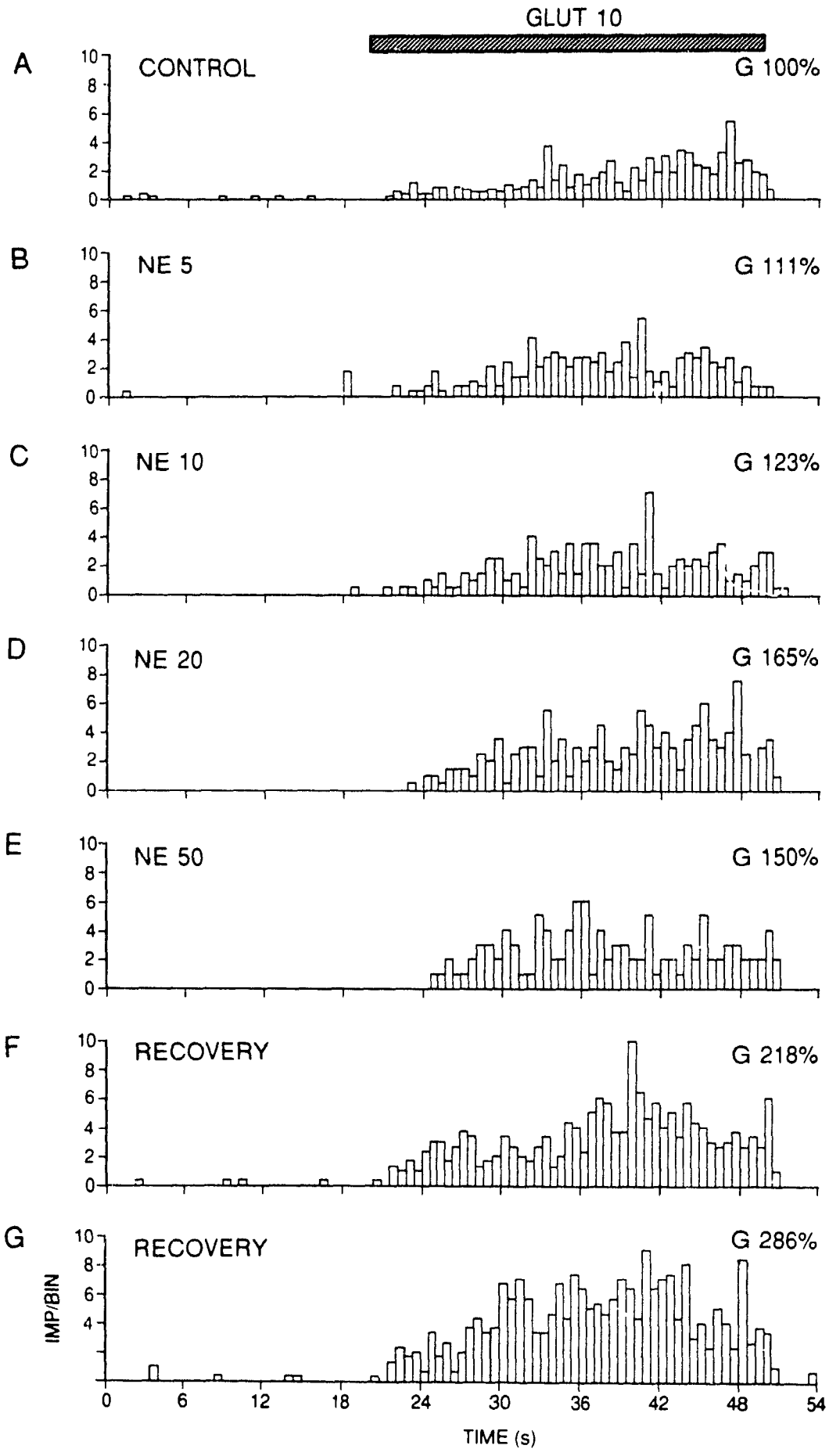
The time course of these responses to NE is presented in Figure 21C. During NE ejection the response to glutamate was decreased in manner similar to that observed on spontaneously active neurons. One min after NE administration, the response to glutamate was already back to the control level and it was significantly increased after 5 min. The response increased further to almost 200% of the control within 14 min and was almost 300% in the four neurons studied for 16 min.

For neurons excited during the administration of NE, increases in the response to glutamate were observed in 89% of the cases within 5 min of the cessation of NE and in all the cases studied for more than 5 min (Table 22). In none of the cases did the response to glutamate return to the control level for the period of time studied (8 to 36 min). A neuron excited in the presence of NE that displayed a long-lasting increase in the response to glutamate is shown in Figure 24

The time course of the recovery period showed that even in the first min following NE administration, the response was already above the control level (Figure 23B). In the following min, the response to glutamate increased further and was more than 300% of the control after 14 min (Figure 23E).

Of the 5 neurons that were not affected during NE administration, 4 were also unaffected following NE administration for periods ranging from 2 to 11 min.

Figure 24: Long-lasting increase in the response to glutamate of a neuron lacking a receptive field excited during NE administration. This neuron had a receptive field uncovered in the presence of glutamate. It was not located in the histology The baseline was established with 5 pulses of glutamate during which the response averaged 2.4Hz (A) The spontaneous activity was smaller than 0.1Hz (0.07Hz) and the neuron was considered to be lacking spontaneous activity. NE was administered for 7 consecutive runs with increasing iontophoretic currents ranging from 5 to 50nA (B, 3 runs; C, 2 runs; D, 2 runs; E, 1 run). The response to glutamate increased as the current of NE was increased. Note that despite the increase in the response to glutamate during the ejection of NE, the spontaneous activity did not increase and instead completely disappeared. Upon the cessation of NE the response to glutamate further increased one to 3 min after NE administration (F) and was even bigger 17 to 19 min following NE administration (G). No significant change in the rate of spontaneous activity was observed during that period. (See Figure 11 and 12; bin width, 600ms.


GLUT 10
A
CONTROL
G 100%
B
NE 5
G 111%
C
NE 10
G 123%
D
NE 20
G 165%
E
NE 50
G 150%
F
RECOVERY
G 218%
G
RECOVERY
G 286%
IMP/BIN
0
2
4
6
8
10
0
6
12
18
24
30
36
42
48
54
TIME (s)

In one case the response to glutamate began to decrease 3 min after the cessation of NE and remained low for the next 5 min when other tests were performed on that neuron.

In summary, the responses of more than 80% of the 21 neurons affected by NE significantly increased over long periods of time. This proportion was higher than that observed for neurons displaying a receptive field and for spontaneously active neurons lacking a receptive field. The magnitude of the effects was also more important than in the two other classes of neurons (Table 22).

3.4.3 Amounts of norepinephrine necessary to produce long-term effects.

The long-term effects of NE were examined in 55 neurons that responded to the administration of NE. Both excitatory and inhibitory long-term effects were observed. The hypothesis that long-lasting effects were produced by amounts of NE different than when the activity returned to the control level was tested. To obtain an indication of the amount of NE administered to each neuron, the iontophoretic current used to eject NE was multiplied by the number of runs for which NE was ejected. The resulting indexes were transformed to logarithms to perform statistical tests.

In 41 enhanced neurons, the index ranged from 10 to 425nA.run with a geometric mean of 78.7nA.run. In the cases where no long-lasting increase was observed, the indexes ranged from 15 to 250nA.run while the geometric mean was the same as for excited neurons, 78.7nA.run.

Long-term decreases in the spontaneous activity were observed in 9 neurons lacking a receptive field. In this case the indexes ranged from 15 to 250nA.run with a geometric mean of 74.6nA.run. For the remaining spontaneously active neurons lacking a receptive field the indexes ranged from 20 to 300nA.run with a geometric mean of 62.3nA run. No significant difference was found between the two classes of neurons ($t_s = 0.518$, $df = 21$, $p > 0.5$).

In conclusion, no evidence was found that the amount of NE administered is a determining factor for the production of long-lasting effects or for the type of effect observed following NE administration.

3.4.5 Laminar distribution of long-term effects.

Nineteen of the 55 neurons studied for more than 5 min following NE administration were recovered in histological sections. Inspection of the responses suggested that the likelihood of observing a long-lasting effect was different among cortical depths. Of 13 spontaneously active neurons, 9 were found in the middle layers and 4 in the upper-lower layers (Table 23A). The spontaneous activity of each of the 4 neurons found in either the upper or lower layers was decreased or increased more than 5 min after the administration of NE. In contrast, only 4 of the 9 neurons found in the middle layers still showed an effect 5 min after NE was stopped. Similarly, a larger proportion of neurons in the upper-lower layers showed a long-lasting increase in the evoked activity than those found in the middle layers.

Because the sample recovered in the histology was small, it was not possible to perform a statistical analysis, however, when those neurons not located in the

Table 23: Laminar distribution of the long-term effects of NE.

A: Neurons recovered in histological sections.

	Spontaneous Activity				Evoked activity			
	Increased	Decreased	Unaffected	Total	Increased	Decreased	Unaffected	Total
Middle	2	2	5	9	6	0	6	12
Upper-lower	2	2	0	4	5	0	2	7
Total	**4**	**4**	5	**13**	**11**	**0**	**8**	**19**

B: Neurons not located in histological sections.

	Spontaneous Activity				Evoked activity			
	Increase	Decrease	Unaffected	Total	Increased	Decreased	Unaffected	Total
Middle	2	0	2	4	4	0	4	8
Upper-lower	6	3	2	11	12	0	6	18
Total	8	3	4	15	**16**	**0**	**10**	**26**

C: Neurons located and those not located in histological sections.

	Spontaneous Activity				Evoked activity			
	Increase	Decrease	Unaffected	Total	Increased	Decreased	Unaffected	Total
Middle	4	2	7	13	10	0	10	20
Upper-lower	8	5	2	15	17	0	8	25
Total	12	7	9	28	27	0	18	45

histological sections were assigned to upper, middle or lower layers on the basis of the micrometer reading that indicated their depth below the pial surface, tests could be performed. Twenty six neurons could be located with some accuracy and they had a distribution similar to that obtained with neurons located in histological sections: the spontaneous and induced activities increased or decreased for most neurons (9 of 11) in the upper and lower layers while in the middle layers this occurred in only 50% of the neurons (Table 23B).

When both the sample located in histological sections and the sample located with the micrometer readings were pooled (Table 23C), the proportion of neurons located in the upper or lower layers that was either increased or decreased was 87% (13 of 15) while it was only 48% (6 of 13) for the neurons located in the middle layers. These proportions were significantly different (Fisher's exact test, $p < 0.05$, Siegel (1956)).

In contrast, induced activity was increased in only 68% of neurons found in upper or lower layers and it was 50% for the neurons in the middle layers suggesting that the long-term effects on induced activity were independent of their laminar location ($G_{adj} = 1.450$, $df = 1$, $p > 0.1$), leaving the long-term effects on the spontaneous activity in the upper and lower layers as the only significant effect correlated with laminar location.

3.4.6 Effects of noradrenergic receptor agonists on long-term effects.

3.4.6.1 Effects of oxymetazoline.

In 7 out of 13 cases where the α_2-receptor agonist oxymetazoline was tested the action potential was stable following NE and oxymetazoline administration. Five of those were spontaneously active. In all 7 cases, the induced activity returned to the control level within a few minutes following the administration of oxymetazoline but NE produced a long-term increase in 3 of those cases.

Of the 5 spontaneously active neurons, 3 returned to the control level following oxymetazoline administration. In one of these NE produced a long-term increase in the spontaneous activity while the other two returned to control. In two neurons lacking a receptive field the ongoing activity remained below the control level for the 5 and 9 min the recovery periods which were studied for those neurons were studied following oxymetazoline. In one of these cases NE also produced a long-term decrease in the spontaneous activity while in the other case the spontaneous activity returned to the control level after NE.

In summary, no evidence was found that the selective activation of α_2-adrenoceptors induced long-term increase in the ongoing or the induced activities but an action of NE on these receptors might have been responsible for the long-term decrease in the ongoing activity observed for neurons lacking a receptive field.

3.4.6.2 Effects of isoproterenol.

On 3 stable neurons where the ß-receptor agonist isoproterenol had mimicked the effects of NE, the activity returned to the control level within 2 to 4 min

following the ejection of isoproterenol. Long-term effects were not observed even though in one of these neuron NE subsequently produced a long-term increase in the response to glutamate.

3.4.7 Effects of noradrenergic receptors antagonists on long-term effects.

3.4.7.1 Effects of α_2-receptors antagonists.

The recovery period following the administration of NE in the presence of idazoxan or yohimbine was examined in 11 stable neurons. In 9 cases NE was first administered alone In 4 of the 6 cases where a significant increase in activity was observed following the administration of NE, a further increase was observed when NE was administered in the presence of an α_2-receptor antagonist In the 3 other cases NE produced no long-lasting effects either when administered alone or in the presence of an antagonist

In two cases NE was first administered in the presence of an antagonist and no long-term effects were observed nor were they observed when NE was later administered alone. In summary, α_2-receptor antagonists never blocked a long-term effect of NE.

3.4.7.2 Effects of ß-receptors antagonists.

Fourteen neurons tested with the ß-receptor antagonists sotalol or timolol were studied for more than 5 min into the recovery period. In 7 cases, NE was first administered in the absence of an antagonist. In 3 cases NE produced a long-term increase in activity. In one of these there was a further increase when NE was

administered in the presence of an antagonist. In 4 cases, NE ejected alone or with an antagonist produced no long-lasting effects.

In 7 neurons, NE was administered first in the presence of the antagonist. An increase in activity was observed in 3 cases following this treatment and the activity was further increased in one case following the administration of NE alone. No long-lasting effects were observed in the other cases.

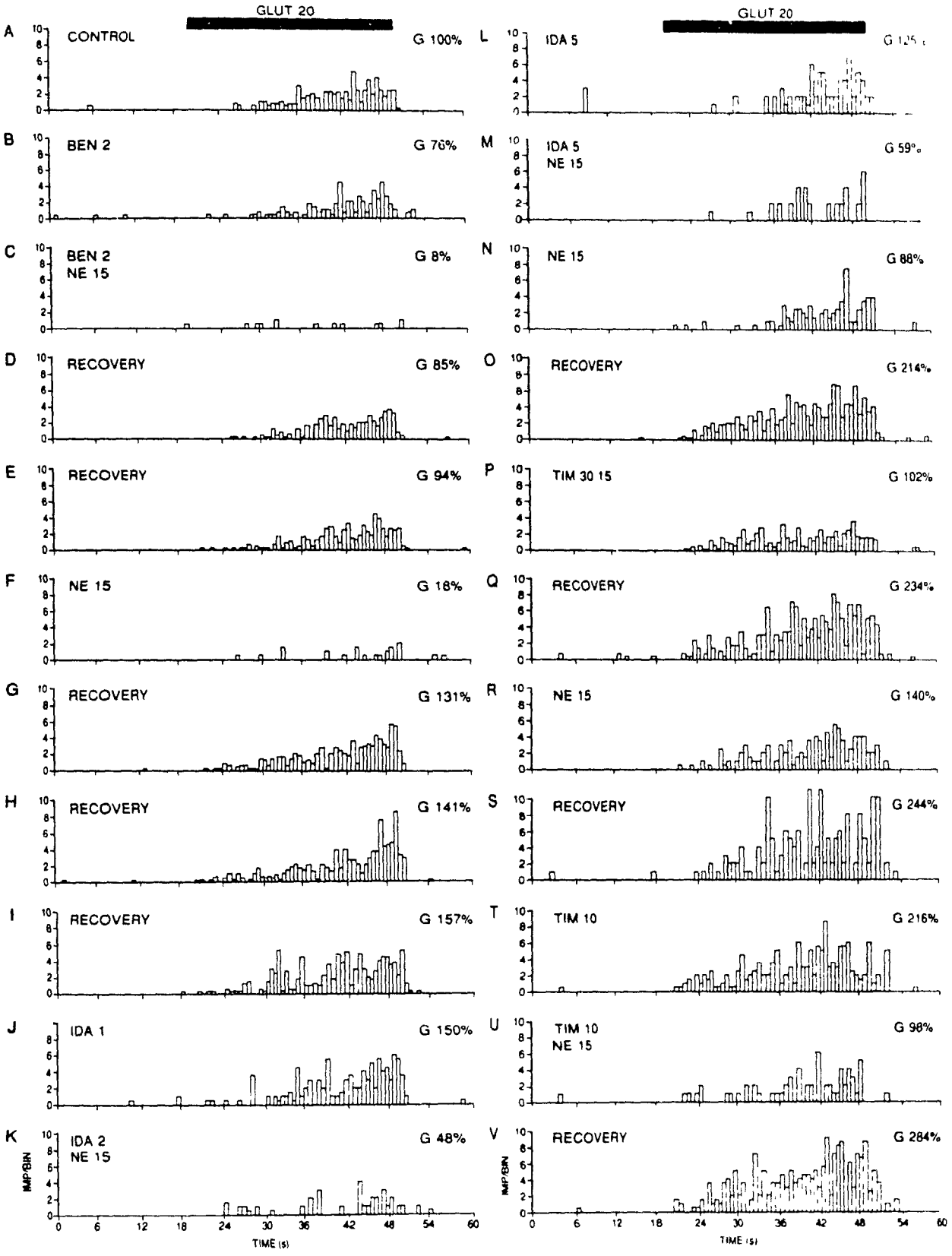
3.4.7.3 The effects of α_1-receptor antagonist.

The recovery period following the administration of NE in the presence of the α_1-receptor antagonist benoxathian was examined in 9 neurons lacking a receptive field. In all cases NE was first administered in the presence of the antagonist. Six of the studied neurons were spontaneously active and 3 lacked this characteristic.

Following the administration of NE in the presence of benoxathian, 8 to 15 min were allowed for recovery prior to the administration of NE alone. During that period, a significant increase in the response to glutamate was observed in 2 cases while in 7 cases the response returned to the control level. Following the administration of NE alone, further increase was observed in the 2 already increased neurons, in 3 cases no long lasting- effects were observed and an increase was observed in 4 additional neurons suggesting that benoxathian had blocked the long-lasting increase in the response to glutamate in these latter cases. An example of the antagonist effect of benoxathian is shown in Figure 25. The α_2-receptor antagonist idazoxan and the ß-receptor antagonist timolol were also tested on that neuron. One case that was not antagonized by benoxathian is presented in Figure 26.

Twenty three to 26 min after the administration of NE the response to glutamate had further increased and was 157% of the control and the response frequency was 3.2Hz (I). Idazoxan was first administered alone for two runs and produced no significant effect on the response to glutamate (J). NE 15nA was added for two runs and the response to glutamate decreased by 69% as compared to the period preceding idazoxan administration (K). NE was turned off and idazoxan current was increased to 5nA for the next run in order to see if a higher current would blocked the inhibitory effect of NE (L). When NE was turned on for one run it produced a significant decrease again (M). Then idazoxan was turn off and NE was left on for two additional runs and the response to glutamate remained decreased (N). Following this prolonged administration of NE the response to glutamate further increased to 214% of the original control with an average frequency of 4.4Hz (O). The ß-receptor antagonist timolol was administered alone during 4 runs (P). The ejection was started with 30nA and was decreased to 15nA during the following runs because it produced a decrease in the response to glutamate. Since even with the lower current of timolol the response was inhibited it was turn off. After recovery (Q) NE was tested again for two runs (R) and after one run of recovery (S) timolol 10nA was administered for two runs producing no significant effect (T). When NE was added for one run (U) it produced a decrease of 58% as compared to the recovery period just before the administration of timolol (S). This test was followed by recovery (V) and the neuron was abandoned. (See Figure 11 and 12; bin width, 600ms).

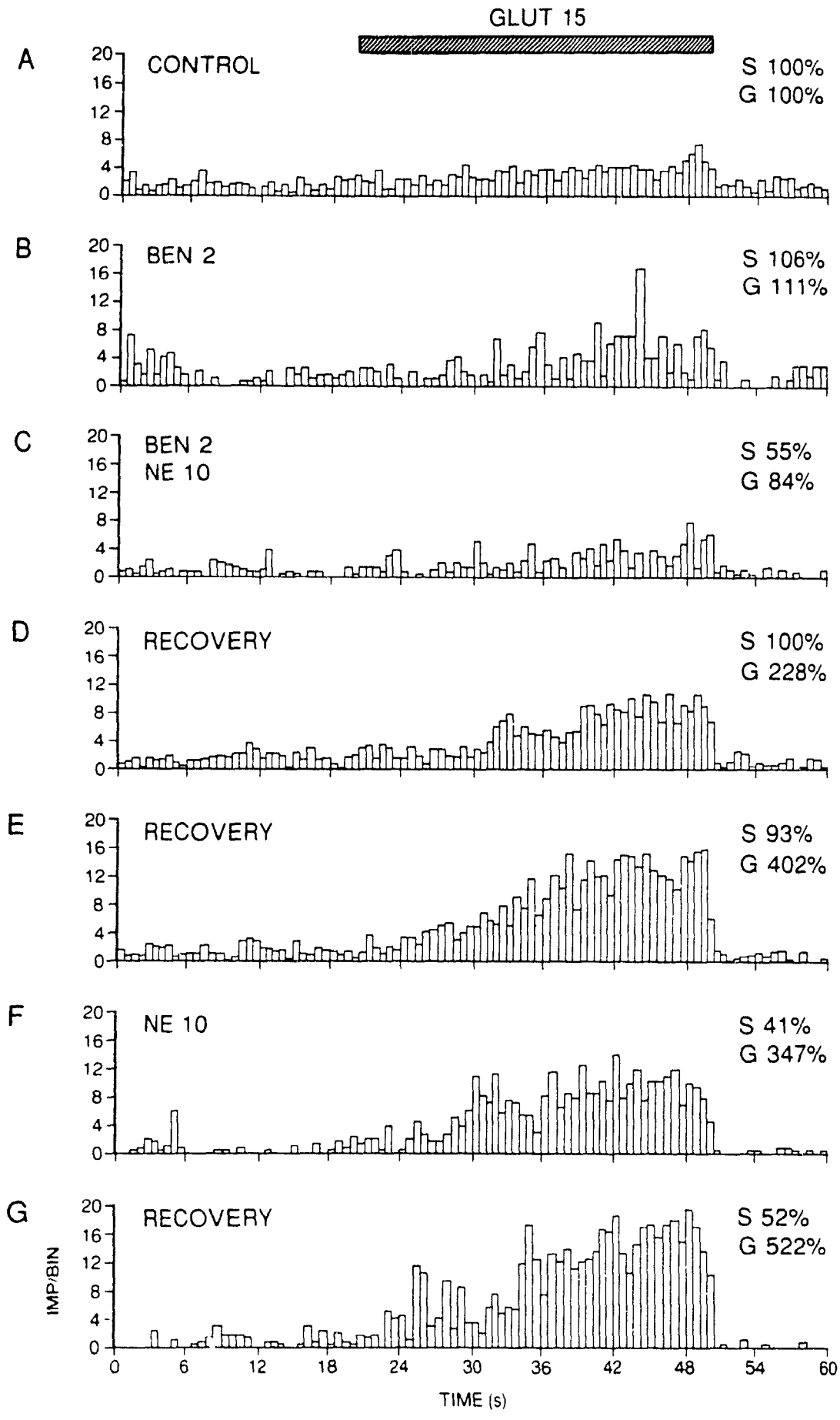
Figure 25: Long-lasting effects of NE blocked by the α_1-receptor antagonist benoxathian. This neuron lacked a receptive field and was classified as NoRF since no receptive field was uncovered by glutamate. It was not located in the histology but from the micrometer reading it was probably situated in the deeper cortical layers. Spontaneous activity was lower than 0.10Hz so it was considered as lacking spontaneous activity. During the control period, the baseline was established with 4 consecutive pulses of 20nA of glutamate administered from 20 to 50s in each run (A). Glutamate induced an average of 2.0 action potentials/s during that period. The α_1-receptor antagonist benoxathian was administered for 3 runs and produced a decrease in the response to glutamate (B). When NE 15nA was added for 2 runs the response decreased further to 8% of the control response (C). Recovery periods 1-5 min (D) and 6-10 min (E) following the administration of NE in the presence of benoxathian are shown and the response to glutamate was 85% and 94% of the control during these two periods. The same amount of NE was administered alone producing a decrease of the response to glutamate 18% of control (F). One to 5 min after the administration of glutamate the average response to glutamate was increased by 31% as compared to the control (G) and by 41% 5 to 10 min after (H). Note that despite the fact that the response to glutamate was increased, the ongoing activity was not affected.


GLUT 20
A
CONTROL
G 100%
B
BEN 2
G 76%
C
BEN 2
NE 15
G 8%
D
RECOVERY
G 85%
E
RECOVERY
G 94%
F
NE 15
G 18%
G
RECOVERY
G 131%
H
RECOVERY
G 141%
I
RECOVERY
G 157%
J
IDA 1
G 150%
K
IDA 2
NE 15
G 48%
IMP/BIN
TIME (s)
GLUT 20
L
IDA 5
M
IDA 5
NE 15
G 59%
N
NE 15
G 88%
O
RECOVERY
G 214%
P
TIM 30 15
G 102%
Q
RECOVERY
G 234%
R
NE 15
G 140%
S
RECOVERY
G 244%
T
TIM 10
G 216%
U
TIM 10
NE 15
G 98%
V
RECOVERY
G 284%
IMP/BIN
TIME (s)

The spontaneous activity was significantly increased in only one out of 6 cases following the treatment with NE in the presence of benoxathian. In that case no further increase was seen when NE was administered alone. Following the administration of NE alone, a significant increase in the ongoing activity was observed in two other cases suggesting that the increase had been previously blocked by benoxathian in those cases. The response to glutamate of these two neurons had not been significantly affected following the administration of NE alone or in the presence of benoxathian.

Overall, long-lasting increases in the response to glutamate were observed in 6 neurons and these were blocked by benoxathian in 4 cases (67%). Long-term increases in the spontaneous activity were blocked in 2 out of 3 cases (67%). Further, benoxathian also blocked the increase in the response to glutamate during the administration of NE in 3 out of 3 cases (100%). These results suggest that the increase in activity observed both during the administration of NE and following the cessation of NE were produced by the action of NE on α_1-adrenoceptors.

Figure 26: Long-lasting increase in the response to glutamate not blocked by the α_1-receptor antagonist benoxathian. This neuron was located in layer II of area 1 and had no receptive field even in the presence of glutamate. The baseline was established over 6 consecutive pulses of 15nA of glutamate (A). The frequency of ongoing activity was 2.5Hz and the response to glutamate averaged 2.8Hz over that period. The α_1-antagonist benoxathian was administered alone during 2 runs and produced no significant effect on either the spontaneous activity or the response to glutamate (B). When NE 10nA was added for 3 runs, the ongoing activity was decreased to 55% of the control period and the response to glutamate to 84% producing an increase in signal-to-noise ratio of 53% as compared to the control period (C). Fourteen min were allowed for recovery before the administration of NE alone. During the first 7 min the ongoing activity was not different from control but the response to glutamate and the signal-to-noise ratio were both increased to 228% of the control period (D). During the following 7 min the response to glutamate increased further and reached 402% of the control period while the ongoing activity was slightly decreased (E). The administration of the same amount of NE alone produced the same effects as what was observed in the presence of benoxathian (F). As compared to the period preceding the administration of NE alone, the ongoing activity was decreased by 56% and the response to glutamate by only 14% producing an increase in signal-to-noise ratio of 95%. During the 3 min following NE administration the ongoing activity remained low while the response to glutamate increased further (G). The signal-to-noise ratio was increased by 904%. (See Figure 11 and 12; bin width 600ms).


GLUT 15
A
CONTROL
S 100%
G 100%
B
BEN 2
S 106%
G 111%
C
BEN 2
NE 10
S 55%
G 84%
D
RECOVERY
S 100%
G 228%
E
RECOVERY
S 93%
G 402%
F
NE 10
S 41%
G 347%
G
RECOVERY
S 52%
G 522%
IMP/BIN
0
4
8
12
16
20
0
6
12
18
24
30
36
42
48
54
60
TIME (s)

4.0 Discussion

4.1 Sample.

Four hundred sixty five single units were found to be sensitive to glutamate in the present study suggesting that they were neurons. Only 13 units (2.4%) were insensitive to glutamate even with iontophoretic currents of up to 500nA. Although some of these might have been neurons (Schneider and Perl, 1988) the majority were probably axons. Since they constituted only a marginal proportion of the sample they will not be considered further and only those neurons sensitive to glutamate will be discussed.

Only 23% of the neurons isolated had demonstrable input from the periphery in the absence of any drug. Small amounts of glutamate uncovered somatic input in 34% of the sample while in 43% no evidence of somatic input was found even when they were partially depolarized. This suggests that most neurons in the somatosensory cortex lack a receptive field. This was found in several other microiontophoretic studies in both cats (Dykes and Lamour, 1988a, Tremblay et al, 1988; Tremblay et al, 1990b) and rats (Dykes and Lamour, 1988b; Lamour et al., 1988). But in other studies (Dykes et al., 1984; Metherate et al, 1988a) more than half of the neurons studied displayed a receptive field. The reasons for those differences in the proportion of the sample displaying a receptive field are not clear but they are likely to be related to the kind of anaesthetic used, the type of recording electrode and the presence of certain excitatory drugs in the iontophoretic pipette rather than to fundamental differences in the cortex studied. While anaesthesia could account for a low a proportion of neurons displaying a receptive

field, even in unanesthetized paralysed cats about half of the neurons were found to lack a receptive field (Dykes and Lamour, 1988a). On the other hand, the presence of bicuculline in the microiontophoretic pipette has been shown to increase the probability of finding neurons displaying a receptive field (Lamour et al., 1988) and might explain the higher proportions found in some studies (Dykes et al., 1984). Metherate et al. (1988a) found almost twice as many neurons with a receptive field as compared to the present study and this can be accounted for by the fact that they used carbon fibres electrodes which might be more likely to isolate smaller cells found in the middle layers than the glass pipettes used in the present study (see also section 4.3). Thus we prefer a small sampling bias of the electrodes used in the respective studies as an explanation for these differences. The role of those neurons lacking a receptive field in the processing of somatic information is not clear and this issue will be discussed later in relation with the effects produced by NE on those neurons (but see also Dykes and Lamour, 1988a).

4.2 Spontaneous activity and sensitivity to glutamate.

Both the proportion of spontaneously active neurons and the rate of spontaneous activity found in the present study are in the range of those reported in other iontophoretic studies from this laboratory in cat somatosensory cortex under various anaesthetics (Metherate et al., 1988a; Tremblay et al., 1988, 1990a). In the present study the probability of finding a spontaneously active neuron was increased to 74% if it displayed a receptive field while only 29% of neurons lacking a receptive field were spontaneously active. Furthermore, the rate of spontaneous activity was

higher in neurons displaying a receptive field as compared to those lacking one. This suggest that inputs from the periphery might be an important factor in the generation of ongoing activity and that neurons displaying a receptive field have an excitatory-to-inhibitory input ratio which is higher than neurons lacking one. In anaesthetized cats, both classes of neurons appear to receive strong tonic inhibitory input from GABAergic neurons that masks the expression of weaker peripheral excitatory inputs (Dykes et al., 1984; Hicks and Dykes , 1983). In the presence of the GABAergic antagonist bicuculline, neurons with a receptive field display a larger receptive field while an enlarged receptive field is uncovered in neurons lacking a receptive field. In contrast, glutamate administration did not enlarge receptive field size and uncovered receptive fields of normal size. Furthermore, in rat, bicuculline uncovered receptive fields more often than glutamate (Lamour at al , 1988) These data suggest that, at least some of the neurons lacking a receptive field receive an important input from the periphery and that a large part of this excitatory input is shut down by inhibition suggesting that neurons lacking a receptive field are less spontaneously active because they receive stronger inhibitory inputs If input from the periphery was the critical factor in the generation of spontaneous activity, the rate of spontaneous activity and the proportion of spontaneously active Glut-RF neurons would have been somewhere between No-RF neurons and neurons displaying a receptive field. Conversely, No-RF neurons tended to be more spontaneously active than Glut-RF neurons. Furthermore, spontaneously active neurons displayed lower threshold of activation to the iontophoretic administration of glutamate that may be the result of a lower inhibitory input

4.3 Laminar distribution of the sample.

In the present study, the distribution of isolated neurons appeared quite uniform in all cortical layers. In previous iontophoretic studies where glutamate was used to excite otherwise quiescent neurons, the result was a more even sampling of neurons throughout the somatosensory cortex than had been true in earlier studies (Mountcastle, 1957; Towe et al., 1964; Morse et al., 1965) but still more neurons were in the middle layers than in the upper and lower layers in both cats (Dykes et al., 1984; Metherate et al., 1988b; Tremblay et al., 1988) and rats (Dykes and Lamour, 1988c). In contrast, Tremblay et al. (1990a) using the same anaesthetic and recording electrode as in the present study found a distribution that most closely resembled that reported here. While the difference in anaesthetic might account for the difference in sampling it appears that the electrode type might also be a determining factor. In the course of the present experiments, it was noted that at depths between 600 to 1000μm although usually some background neuronal activity could be recorded when the receptive field was stimulated, often those neurons could not be isolated well enough to be studied. In previous studies, carbon fibre electrodes were used and it was usually at these depths that many neurons could be isolated, suggesting that carbon fibre electrodes were more suitable to isolate the small neurons found in the middle layers.

As well as in previous studies in cat somatosensory cortex, probabilities of finding neurons displaying a receptive field were increased in the middle cortical layers but a significant proportion was also found in the upper and lower layers. In the somatosensory cortex, the bottom of layer III and layer IV receive denser

thalamic innervation than lower layers (Jones, 1975). Further, the dendritic arbours of most pyramidal neurons of layer III, V and VI extend in the layers where the densest thalamic innervation is found and are in a position to receive thalamic inputs (Hendry and Jones, 1983). In that context, it is interesting to note that a high proportion of No-RF neurons were found in layers I and VI while Glut-RF neurons were concentrated in layers II, upIII and V, suggesting that the probability of finding demonstrable somatic input decreased as the neurons were located farther from the middle layers.

As expected, higher proportions of spontaneously active neurons were found in layers containing high proportions of neurons displaying a receptive field. This distribution is not different from that found by Tremblay et al. (1990a) in cats and by Dykes and Lamour (1988c) in rats. The distribution of spontaneously active neurons for the different classes of neurons is more difficult to interpret because of the small size of the sample in some layers. The most striking feature is the low proportion of spontaneously active neurons displaying a receptive field found in layer loIII as compared to layers midIII and IV. The reason for this is unknown but neurons in layer loIII displayed a high rate of spontaneous activity. Neurons lacking a receptive field were more likely to be spontaneously active in upper and lower layers than in the middle layers.

Layer IV and the bottom third of layer III contain the higher densities of GABA immunoreactive terminals (Hendry et al., 1987) suggesting that neurons located in these layers are more strongly inhibited than in other layers. Neurons located in layers loIII and IV were also less sensitive to glutamate than neurons

found in the layers just above or below perhaps because of the strong GABAergic inhibition.

4.4 Effects observed during norepinephrine administration.

Many of the effects of NE observed in the present study were similar to and confirmed previous studies in various sensory cortices including monkey auditory cortex (Foote et al., 1975), rat somatosensory cortex (Waterhouse and Woodward, 1980; Waterhouse et al., 1980, 1981), rat (Kolta et al., 1987; Kolta and Reader, 1989) and cat (Videen et al , 1984) visual cortex. In those studies NE had been found to have mainly an inhibitory action on both the ongoing activity and on afferent input. Excitatory effects were seldom but consistently observed. The inhibitory effects of NE were usually more important on the spontaneous activity than on the evoked activity resulting in an increase in the signal-to-noise ratio in a majority of neurons. The present study confirms each of these earlier findings in the cat somatosensory cortex but provides also several new insights on the effects and the role of NE in sensory processing. First, NE appears to be able to produce both excitation and inhibition in some neurons while in other neurons it produced only inhibitory effects. Second, inhibited and excited neurons were located in different laminae of the somatosensory cortex and NE produced different effects on the signal-to-noise ratio of inhibited and excited neurons suggesting that the role of NE is not the same in different laminae of the cortex. Third, our pharmacological studies suggest that the excitatory effects of NE are mediated by α_1- receptors in somatosensory cortex. Fourth, the time course of the inhibitory effects of NE on neurons displaying a

receptive field was documented, showing that the effect of NE on the signal-to-noise ratio is dependent on the magnitude of the inhibitory effect produced by NE. And fifth, NE was found to produce a long-lasting increase in the neuronal excitability of a large proportion of somatosensory cortical neurons. These results will be discussed in the following pages with emphasis on the original findings of the present study.

4.4.1 Effects of norepinephrine on neuronal excitability.

Early iontophoretic studies in cats and rats suggested that NE produced mainly excitatory effects in somatosensory cortex (Bevan et al., 1977; Szabadi et al. al., 1977). These results were not confirmed in later studies in rat somatosensory cortex where NE appeared to produce mainly depressant effects on neuronal excitability (Waterhouse and Woodward, 1980; Waterhouse et al., 1980, 1981). Studies in monkey auditory cortex (Foote et al., 1975) and rat (Kolta et al., 1987; Kolta and Reader, 1989) and cat visual cortex (Videen et al., 1984) also confirmed the inhibitory effect of NE in sensory cortices. In the present study, we also found that NE depressed the activity of a majority of neurons (54% of 117 cells) in the somatosensory cortex of halothane-anaesthetized cats. The proportion of neurons classified as excited by NE was higher than those reported in these latter studies (36% vs 0 to 29%). This difference appears to result from the manner in which the effects of NE were classified. Excitations observed in this study were classified into 3 classes: biphasic, excited and other. In biphasic neurons (15% of the sample), the excitation was transient and it was followed by a significant inhibition. In those neurons classified as other (2.6% of sample), the excitation was observed only after an initial inhibition. Further, in spontaneously active neurons, only in 8 of 31 cases

did NE produce an excitatory effect on both the spontaneous and induced activities. In the other cases only one component of the neuronal activity was increased and the other component was usually decreased. So, in most neurons classified as excited in the present study, NE also produced important inhibitory effects and probably many of those would have been classified as inhibited in earlier studies. They were classified as excited here because they appeared to form a class of neurons different from those that displayed only inhibition. This working hypothesis was confirmed later when it was discovered that the excited classes were found in different cortical layers than the inhibited neurons (see below).

The fact that NE could produce both inhibitory and excitatory effects on the same neuron suggests these effects are mediated by different subtypes of noradrenergic receptors present on the same neuron. Four subtypes of noradrenergic receptors have been shown to be present in the cerebral cortex (reviewed by Reader et al., 1988). In rat somatosensory cortex, Bevan et al. (1977) were the first to argue that the excitatory effects of NE were mediated by α-receptors while inhibition was mediated by ß-receptors. Waterhouse et al. (1981, 1982) also suggested that the activation of ß-receptors mimicked some of the inhibitory effects of NE in the rat somatosensory cortex while the effects of NE on the signal-to-noise ratio were attributed to the activation of α-receptors. More recently, Kolta and Reader (1989) found that the inhibitory effect of NE were mediated by both α_2- and ß-receptors. They found that α_2-receptor agonists were more efficient in increasing the signal-to-noise ratio than was a ß-receptor agonist. Furthermore, the inhibitory effects of NE could be blocked with α_2- and ß-receptors antagonists. The pharmacological results

in the present study are in close agreement with those of Kolta and Reader (1989) since the inhibitory effects of NE were mimicked by α_2- and ß-receptors agonists suggesting that the inhibitory effects of NE in cat somatosensory cortex are also mediated by both α_2- and ß-receptor subtypes. The presence of both types of receptors on the same neuron would explain why the inhibitory effects of NE were not blocked by either of the specific receptor antagonists used in the present study. The fact that the specific α_1-antagonist benoxathian (Melchiorre et al., 1984, 1988) consistently blocked the excitatory effects of NE suggests that α_1-receptors mediate the noradrenergic excitation in the somatosensory cortex and that it is likely the only excitatory noradrenergic receptor present. Furthermore, benoxathian was administered at very low currents because with higher currents it produced a significant inhibition suggesting that NE might exert an α_1-mediated tonic excitatory effect on somatosensory cortical neurons. Such tonic excitatory effects mediated by α_1-receptors have been observed in the dorsal raphe and dorsal lateral geniculate nuclei (Baraban and Aghajanian, 1980; Marawaha and Aghajanian, 1982) and have also been described for the cholinergic system in the cat visual cortex (Sato et al., 1987a, b).

4.4.2 Effects of NE on the signal-to-noise ratio.

Foote et al. (1975) were the first to note that NE exerted a stronger inhibition on the spontaneous activity than on the afferent input. They suggested that the role of NE was to enhance the specific afferent inputs from sensory organs over the spontaneous activity. This effect of NE has now been observed in several regions of

the neuraxis and in several sensory cortices, the proportion of neurons showing an increase in signal-to-noise ratio in the presence of NE ranged from 40 to 78% in those studies (Foote et al., 1975; Kolta and Reader, 1989; Videen et al., 1984; Waterhouse and Woodward, 1980). In the present study, 59% (n=27) of the neurons responding to somatic stimuli and 56% (n=41) of those responding to glutamate had their signal-to-noise ratio increased by NE. These proportions increased to 78% and 70% respectively when only inhibited neurons were considered and decreased to only 22% and 43% in excited neurons. Only few inhibited neurons (11%) showed a decreased signal-to-noise ratio in the presence of NE while in over 40% of the cases the signal-to-noise ratio of excited neurons was decreased. This strongly suggests that NE plays a different role on different classes of neurons and in different layers of the somatosensory cortex.

The classical view of processing of the peripheral information in the somatosensory cortex is that the information is relayed by the thalamus mostly to layer IV and to the bottom of layer III. Then it is relayed to the superficial layers and from there to the lower layers (Jones, 1984). In the present study, inhibited neurons were located mostly in the layers that receive the denser thalamocortical input and it seems likely that the role of NE in those layers would be to increase the responses to peripheral input of those neurons relative to other inputs. In the upper and lower layers, fewer thalamocortical input are found and likely, an important part of the input of those neurons does not come from the periphery but from corticocortical connections. Furthermore, these cells often send their projections outside the cortex. The role of NE on those neurons might be to increase their

excitability so that their output is increased when NE is released in the cortex.

The magnitude of the increases in signal-to-noise ratio were relatively important and in inhibited neurons it increased as the magnitude of the inhibitory effects of NE increased but not as a linear relationship. In the first seconds of the administration of NE a relatively modest increase in the signal-to-noise ratio of 30-50% was observed which remained stable for about one min. A second much more important increase was observed when the decrease in the evoked activity reached a plateau but the spontaneous activity continued to decrease. One question is whether or not one or both of these effects are physiologically relevant. The first increase in the signal-to-noise ratio occurred when the evoked activity was decreased by approximately 10 to 15% while the second occurred when the evoked activity was reduced by more than 60%. Despite the important increase in the signal-to-noise ratio at the second step, the decrease in the excitatory input itself was so large that this decrease might be more relevant than the further increase in the signal-to-noise ratio. To resolve this issue it will be necessary to know the effects of these two variables on their target. Another consideration is that the level of ongoing activity was relatively low in this study and the relevance of a change in the signal-to-noise ratio might be questioned since it is the fluctuation in what is already a very small signal.

4.5 Long-lasting effects of norepinephrine.

Long-lasting increases in neuronal excitability produced by NE have been seldom reported in the cerebral cortex. In iontophoretic studies of neuronal excitability driven by afferent input to sensory cortices, the effects of NE have been reported to be mainly inhibitory and to usually last less than 5 min (Foote et al., 1975; Kolta et al., 1989; Videen et al., 1984; Waterhouse and Woodward, 1980). The only exception was the report of Armstrong-James and Fox (1983) on neurons having a demonstrable receptive fields in rat somatosensory cortex. They found that the spontaneous activity of 60% of the neurons located in the deeper layers was increased significantly for at least 3 min following NE iontophoresis; in about 25% of those cases the increase lasted more than 20 min to hours.

In the present study, long-lasting effects following the administration of NE were observed in both neurons responding to peripheral stimuli (37% on spontaneous activity and 22% on evoked activity) and those excited with iontophoretic pulses of glutamate (35% on spontaneous activity and 61% on the response to glutamate; 81% of silent neurons). In over 70% of cases these effects developed within 5 min of the cessation of NE administration. Long-lasting effects were studied for periods over 30 min following the cessation of NE and in most cases there were no signs of decay over the periods of time studied suggesting that the increase could have lasted for a much longer period. Another important feature of these effects was that a long-lasting increase in the signal-to-noise ratio was observed in 59% of the spontaneously active neurons excited with glutamate. Long-lasting enhancements could not be mimicked by α_2- or ß-receptor agonists nor

blocked by α_2- or ß-receptors antagonists but were antagonized by a specific α_1-receptor antagonist suggesting that they were α_1-mediated.

Although these effects were observed both in neurons responding to peripheral stimuli and those excited with glutamate, important differences were observed between the two classes. First, long-lasting effects were observed much more often when the neuron responded to glutamate (70%) than on the response to a peripheral stimulus (22%). Second, a long-lasting decrease in the spontaneous activity was observed in 39% of neurons excited with glutamate but was never produced by NE in neurons activated from a receptive field. The magnitude of the long-lasting increase in neurons lacking both a receptive field and spontaneous activity was over 2 times that observed on the two other classes of neurons.

4.5.1 Specificity of the effect.

The first issue is to establish that the long-lasting effects were produced by the prior administration of NE and was not the result of other factors such as damage due to the proximity of the electrode or the leakage of some compounds from the iontophoretic pipette. Several lines of evidence suggest strongly that those effects were not artifactual in neurons excited with glutamate.

First, if the long-lasting effects were artifactual, the same proportion of neurons should have been affected in each class of cells. This was clearly not the case since large differences were observed between the proportion of long-term effects observed in neurons responding to peripheral stimulation and those responding to glutamate. Different proportions and magnitudes were also observed

between spontaneously active and silent neurons lacking a receptive field (see above). Second, non-specific effects would have affected both the spontaneous and evoked activities in the same way. This may be the case in neurons responding to a peripheral stimulus where both components were affected in about the same proportion of neurons, but, in contrast for spontaneously active neurons lacking a receptive field, a significant proportion of neurons displayed a long-lasting decrease in their ongoing activity while the response to glutamate was increased resulting in long-lasting increases in the signal-to-noise ratio of a majority of neurons. Furthermore, the response to glutamate in 81% of silent neurons increased for long periods of time without evidence of an increase in the level of ongoing activity. Thus, NE produced a different effect on the ongoing activity than on the response to glutamate suggesting a specific action rather than a generalized increase in neuronal excitability. Third, random artifactual effects producing a decrease in some cases, and an increase or no effect in other cases would have cancelled each other upon averaging. Although the long-term effect disappeared when the response of inhibited neurons responding to peripheral stimulus were averaged, in none of the other groups of neurons responding to glutamate did this happen and the overall trend was a clear increase in the response to glutamate. Fourth, long-lasting increases in the response to glutamate were blocked by a specific α_1-receptor antagonist arguing again for a specific action of NE (see below).

The recovery period following NE treatment of neurons responding to a peripheral stimulus was studied for shorter periods of time than neurons responding to glutamate (see Figure 21 and 23) because generally, the two groups of neurons

behaved in a different way following the administration of NE. In most cases, neurons responding to a cutaneous stimulus returned to the control level within 2-3 min following the cessation of NE and displayed a stable response during the following min suggesting that NE had produced no long-term effects. In contrast, often the response to glutamate kept growing in the min following the cessation of NE and in those cases neurons were studied until the activity returned to the control level or more often until the response had stabilized for several min at a new, higher level of excitability, at which time other tests were performed. In several cases NE was administered more than once on the same neuron. In those cases, NE might be administered either alone or in the presence of an antagonist. Following this treatment, neurons displaying a receptive field generally returned to the control level as had been observed previously. In contrast, a further increase in the response to glutamate was often observed in neurons lacking a receptive field (c f. Figure 25).

In conclusion, long-lasting effects on the response to a peripheral stimulus were 3 times less common (22% vs 70%) than those observed on the response to glutamate. While several facts suggest that the long-lasting increase in the response to glutamate were specific effects produced by NE, long-lasting effects on neurons displaying a receptive field might be a non-specific increase in neuronal excitability. But this statement has to be interpreted carefully, since in cat somatosensory cortex, Metherate et al., (1988b), using a paradigm similar to that used in the present study, found that ACh induced long-lasting increase in the neuronal excitability of 29% of neurons displaying a receptive field and 34% of neurons in response to glutamate. Thus, it is possible that NE, like ACh, affects only a small proportion of neurons

displaying a receptive field but that it is a much more powerful agent on neurons lacking a receptive field.

4.5.2 Factors involved in long-lasting effects produced by NE.

NE was tested on two different classes of neurons, each responding to a different stimulus during NE administration. An important question is to know if the differences observed between those two classes reflect the intrinsic properties of the neurons or the difference between the stimuli used. An easy way to answer that question would have been to compare the effects of NE on the response to receptive field stimulation and on the response to glutamate on neurons displaying a receptive field to see if one input was more likely to induce a long-lasting effect than the other. Unfortunately, this was not done in the present study and one can only speculate.

During the administration of NE, excitatory and inhibitory effects were observed in about the same proportion of cases in each class of neurons suggesting that the same population of adrenergic receptors were present on both classes of cells. In contrast, the magnitude of the excitatory effects produced by NE was over 3.5 times more important on the response to glutamate than on the response to somatic stimulus suggesting that more α_1-receptors may have been present on those cells. The response to glutamate of excited neurons exhibited an increase in the probability of induction and in the magnitude of long-lasting effects as compared to inhibited neurons. However, this does not explain why long-lasting enhancements were observed on a much larger proportion of inhibited neurons lacking a receptive

field than those displaying a receptive field although the inhibitory effects of NE were of about the same magnitude on both classes of neurons. This suggest that the incidence of long-lasting effects was related to the stimulus used.

However, there was a negative correlation between the probability of induction of a long-lasting enhancement of excitability produced by NE and the evidence of excitatory inputs to the neurons. Neurons displaying a receptive field were those that presumably received the stronger excitatory inputs because of the presence of a somatic input. They also had a high probability of being spontaneously active and, when present, a higher rate of spontaneous activity. Nevertheless, they were less likely to be enhanced than neurons lacking a receptive field. In contrast, neurons lacking both a receptive field and spontaneous activity were the neurons displaying the highest probability of being enhanced and the biggest magnitude of the enhancement. Spontaneously active neurons lacking a receptive field behaved intermediately. Thus, in the present study, NE appeared to enhance preferentially the excitability of neurons for which the least excitatory inputs could be found. The role of NE on these neurons might be to enhance their excitability so that their response to their presumably weak input resulted in a more important output. Neurons that were not enhanced by NE might have had their excitability already increased to a maximum and could not be further enhanced.

Another possibility is that other receptors present on the cell membrane need to be activated concomitantly with α_1-receptor to observe a long-lasting change in excitability. These receptors might have been activated during glutamate iontophoresis but not by the stimulation of the receptive field. An obvious candidate

receptor for such role is the n-methyl-d-aspartate (NMDA) receptor which has been suggested to play an important role in the induction of LTP in the hippocampus (reviewed in Collingridge, 1987; Cotman and Monhagan, 1988; Sarvey, 1988). Further, the long-lasting increase in neuronal excitability induced by NE in the dentate gyrus is prevented by NMDA receptor antagonists (Burgard et al., 1989) suggesting that its coactivation with noradrenergic receptors was necessary to induce long-lasting effects.

The activation of the NMDA receptor produces a significant influx of calcium directly through the ligand receptor-channel complex. The NMDA receptor is voltage dependent and is activated only upon sufficient depolarization of the membrane. One possibility is that the depolarization produced by the stimulation of the receptive field was not sufficient to activate NMDA receptors while the relatively long iontophoretic pulses of glutamate were sufficient. This is also supported by the fact that neurons excited by NE displayed an increased probability of induction as well as a larger magnitude of long-lasting effects suggesting a relationship between the degree of depolarization of the membrane and long-lasting effects.

Another possibility is that NMDA receptors are not present at thalamocortical synapses. In that respect it is interesting to note that in adult cat visual cortex, NMDA receptor seems to participate in the visual response of single neurons only in layers II and III but not in layers IV, V and VI (Fox et al., 1989) suggesting that this receptor is not always activated by thalamic afferents.

4.5.3 Possible cellular mechanisms.

Pharmacological data presented in the this study suggest that the long-lasting potentiations were dependent on the activation of α_1-adrenoceptors. Specific α_2- and ß-adrenergic drugs failed to mimic or to block the long-lasting effects In contrast, long-lasting effects on the response to glutamate were blocked by a specific α_1-antagonist 4 out of 6 cases on the response to glutamate and 2 out of 3 cases on the spontaneous activity. This is the first study involving this adrenergic receptor in long-lasting increase in neuronal excitability. NE has been shown to produce long-lasting increases in neuronal excitability in the dentate gyrus of the hippocampus but these effects appeared to be mediated by ß-receptors (Winson and Dahl, 1985, Harley and Milway, 1986; Lacaille and Harley, 1985; Stanton and Sarvey, 1985). The increase in LTP induced by NE appear also to be mediated by ß-receptor (Hopkins and Johnson, 1984, 1988). Also the excitatory effects of NE in the hippocampus have been shown to be mediated by ß-receptors (Haas and Konnerth, 1983, Haas and Rose, 1987; Sah et al., 1985; Madison and Nicoll, 1986a). On the other side, ß-receptor appears to mediate inhibition in the cerebral cortex (Bevan et. al., 1977; Kolta and Reader, 1989; Waterhouse et al., 1981, 1982; and the present study (but see also Foehring et al., 1989) while the excitatory effects appear to be mediated by α_1-receptors. Despite the fact that long-lasting effects appear to be mediated by a different noradrenergic receptor in the hippocampus and in the cerebral cortex, they are mediated by the receptor mediating NE excitation in both structures. Furthermore, low levels of α_1-receptor binding are found in the hippocampus particularly in the dentate gyrus, as compared to the cerebral cortex where much

higher levels are found even in the cortical layers containing the lowest densities of α_1-receptors (Jones et al., 1985; Rainbow and Biegon, 1983). NE has also been shown to induce long-lasting depression in the dentate gyrus mediated by ß-receptor (Dahl and Sarvey, 1989). Thus it is possible that α_1-receptors mediate the long lasting decrease in the spontaneous activity in the cerebral cortex.

Intracellular studies suggest that the activation of α_1-adrenoceptors suppressed both the resting potassium current and an early transient potassium current that resemble the previously described A-current (Aghajanian, 1985; Aghajanian and Rogawski, 1983; Nakamura et al., 1984). In addition, this receptor mediates an increase in the duration of the afterhyperpolarization possibly by increasing the duration of the calcium-dependent potassium current but without affecting the calcium current (Freedman and Aghajanian, 1987). Such long-lasting changes in the membrane properties would explain the effects that were observed in the present study. The decrease in resting and early transient potassium current would result in an increase in the neuronal excitability while the increase in the afterhyperpolarization would suppress the weaker inputs that generate the ongoing activity but without affecting significantly the stronger input.

The activation of α_1- receptors produces an increase in phosphatidylinositol hydrolysis in rat brain (Brown et al., 1984; Crews et al., 1988; Gonzales and Crews, 1985; Janowsky et al., 1984; Kemp and Downes, 1986; Minneman and Johnson, 1984; Schoepp et al., 1984) and generates two intracellular signal molecules, DAG and IP_3. While DAG activates protein kinase C, IP_3 increases the intracellular concentration of calcium by triggering its release from intracellular stores. The activation of protein

kinase C and the increase in intracellular calcium concentration appear to play an important role in LTP observed in the hippocampus (reviewed in Linden and Routtenberg, 1989; Sarvey, 1988) and such processes could be responsible for the long-lasting changes observed in the present study. Furthermore, the activation of the cholinergic muscarinic receptor which also increases phosphoinositol hydrolysis (reviewed in Nahorski, 1988) has also been shown to mediate long-lasting increases in the excitability of cat somatosensory cortical neurons (Metherate et al., 1987, 1988b; Tremblay et al., 1990a, b).

4.6 Functional considerations.

At the end of this analysis we are left with the question of what role NE plays in somatosensory cortex. NE produces inhibition in many cells, excites some and often enhances the signal-to-noise ratio. Yet in other cells it produces no effect. To identify a functional role for NE in the cortex from this constellation of characteristics is difficult; the effect on a given cell cannot be predicted; the test must be done before one knows to which class a cell belongs.

To speculate about functions one must refer to the behavioural literature. There is a consensus that more NE is released during waking than during sleep and that NE release is highest during enhanced levels of arousal (Foote and Morrison 1987). This is not to imply that NE produces high level intellectual activity by suppression of neuronal activity, rather, one must presume that the released NE affects only certain classes of cells. We have already reviewed the literature indicating that the NE released may be rather diffuse and not limited to traditional

synaptic sites (Séguéla et al. 1990). Thus, it seems likely that the control of NE effects may depend on the nature of the postsynaptic membrane, that is, they will be determined by the nature of the receptors found on somatosensory neurons; the nature of the effect and the class of neurons affected will depend upon the nature of these receptors. For example, in a study of orbitofrontal cortex of behaving monkeys, Aou et al. (1983) found that generally only those neurons involved in a delayed response task were sensitive to iontophoretically administered NE; 74%(n=32) responded to NE while only 17%(n=41) of those not involved in the task were affected by NE. The NE effects on the cells modulated during the animal's behaviour could be described even more precisely; those cells affected by NE were generally those inhibited during the time that the monkey pressed a bar or during the time that the monkey was eating the reward. Further, the bar press-dependent activity was shown to be mediated by ß-receptors and those cells depressed during eating behaviour were shown to be affected through α-receptors by blocking the effects with the appropriate antagonists. Thus, the effects of NE were determined by which cells had receptors for NE and the effects in different behavioural states were differentiated by which class of receptors was present.

Waterhouse et al. (1988) and Mouradian et al. (1988) have argued that NE could play a gating role by showing that the presence of small amounts of NE may allow otherwise ineffective inputs to produce action potentials in some cells. Again the selectivity of this effect will depend upon the type of receptors found on the cell surface, however, receptors for NE on a given cell will allow the locus coeruleus to act as a gate for generation of postsynaptic activity from inputs that would have

otherwise been ineffective.

It is clear that we will need to have more information about the distribution of the several types of receptors for NE on cortical neurons before we can go very far towards building a picture of the functional role of NE in somatosensory cortex. This is equally true for the long-term effects produced by NE. These effects seem to be dependent upon the presence of α_1-receptors, thus, whether or not these effects are expressed in a particular cell will depend on the presence of the requisite receptors. If a cell does have α_1-receptors then the role played by NE will depend upon which cortical system that cell is part of. The non-random laminar distribution of the long-lasting effects demonstrated here suggests that some cortical functions are likely to be more plastic than others, but again much more information is required to determine which functions those may be.

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