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Noradrenergic Modulation of Lateral Geniculate Neurons: Physiological and Pharmacological Studies

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NORADRENERGIC MODULATION OF LATERAL GENICULATE NEURONS: PHYSIOLOGICAL AND PHARMACOLOGICAL STUDIES

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ABSTRACT

NORADRENERGIC MODULATION OF LATERAL GENICULATE NEURONS:
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The physiological actions of norepinephrine (NE) were examined in the rat dorsal lateral geniculate nucleus (LGNd) using extracellular single cell recording and microiontophoresis. Prolonged, low current iontophoretic applications of NE consistently elicited an increase in the firing rate of LGNd neurons which was delayed in onset and prolonged after cessation of the ejection. Sympathomimetic amines other than NE also activated LGNd neurons with varying degrees of effectiveness. On the basis of the relative potencies of a series of these agonists and the ability of iontophoretically or systemically administered α-antagonists to selectively block the facilitatory action of NE, it is concluded that NE acts via an α_1- ("postsynaptic") adrenoceptor. In contrast to NE, serotonin (5-HT) produced a suppression of the firing of LGNd neurons.

To examine the effects of NE on evoked activity, identified geniculocortical relay neurons (P-cells) were driven by electrical stimulation of the afferent visual pathway at the level of the optic chiasm. NE caused a marked facilitation of both the short latency (2-4 msec) and the delayed (70-230 msec) responses to such stimulation. The α-adrenoceptor antagonist phentolamine, which by itself had no consistent effect on evoked activity, strongly diminished the response to NE. 5-HT was a powerful depressant of electrically evoked activity; neither phentolamine nor the 5-HT antagonist

methysergide antagonized this response. Firing of LNGd units evoked by flashes of light was also facilitated by NE and depressed by 5-HT.

When afferent excitation from the retina was eliminated by enucleation of the eyes, many LGNd neurons ceased firing spontaneously. Silent neurons in enucleated animals generally did not respond to NE although the excitatory amino acid glutamate was still highly active. Under these conditions, NE enhanced the excitation produced by glutamate, suggesting that NE increases the general excitability of these neurons and that it acts in a "neuromodulatory" fashion. The γ-aminobutyric acid (GABA) antagonist picrotoxin, unlike NE, did not facilitate the activity of glutamate, indicating that the action of NE is not mediated by suppression of adjacent GABAergic interneurons.

Electrical stimulation of the locus coeruleus (LC), which contributes a dense noradrenergic innervation to the LGNd, mimicked the activating effect produced by locally applied NE. The onset of the response to 10 Hz trains was delayed by up to 20 sec and the increased rate persisted after the stimulation period (up to 20 sec). This effect was blocked by iontophoretic or intravenous administration of WB-4101. Silent cells in enucleated animals were not activated by LC stimulation, but, as with iontophoretic NE, LC stimulation did facilitate the excitatory action of glutamate. WB-4101 blocked both the facilitatory actions of LC stimulation and of iontophoretic NE.

It is concluded that NE, acting via an α_1-adrenoceptor, facilitates the excitability of LGNd relay neurons to afferent stimulation. The close similarity between the effects of locally applied NE and stimulation of the LC provide evidence that NE is a transmitter in the coeruleogeniculate

pathway. This pathway may serve to modulate the transmission of visual information from the retina to the striate cortex.

TO CAROL

... for her encouragement.

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ABBREVIATIONS

ACh	Acetylcholine
CARB	Carbachol
cAMP	Cyclic Adenosine Monophosphate
CLO	Clonidine HCl
EPI	Epinephrine
G	L-glutamic acid (glutamate)
GABA	γ-Aminobutyric acid
ISO	Isoproterenol
LC	Locus coeruleus
LGN	Lateral geniculate nucleus
LGNd	Dorsal lateral geniculate nucleus
LSD	D-Lysergic acid diethylamide
NE	Norepinephrine
OX	Optic chiasm
PGR	Perigeniculate part of nucleus reticularis
PHE	Phenylephrine
PHENT	Phentolamine mesylate
PIC	Picrotoxin
PSTH	Poststimulus time histogram
REM	Rapid eye movement
VC	Visual cortex
WB	WB-4101
4V	Fourth ventricle
5-HT	Serotonin (5-hydroxytryptamine)

PREFACE

The dorsal lateral geniculate nucleus (LGNd) is a major component of the mammalian visual system whose principal function is to relay information between the retina and the striate cortex. Since the work of Hernández-Péon and his co-workers in 1956, it has been recognized that there are extraretinal influences on the LGNd which regulate its responsiveness to optic stimuli. One pathway which is presumed to serve such a function consists of norepinephrine (NE)-containing neurons originating in the nucleus locus coeruleus (LC) of the pons. These neurons contribute a dense network of noradrenergic axons and terminals to the LGNd.

In 1974, two Japanese investigators, Yoshihisa Nakai and Shuji Takaori, demonstrated that electrical stimulation of the LC could facilitate the responsiveness of geniculocortical relay neurons to optic pathway stimulation. This finding was unexpected for two reasons. First, previous studies of the effects of LC stimulation on neurons in various other target areas had indicated that LC neurons exert a predominantly inhibitory action postsynaptically (Siggins et al., 1971; Segal and Bloom, 1974a; Sasa and Takaori, 1973). Second, local administration of NE using the technique of microiontophoresis had been reported by two research groups to depress the firing of LGNd neurons (Curtis and Davis, 1962; Phillis et al., 1967a).

However, one investigator, in direct conflict with these studies, reported that iontophoretic NE caused a facilitation of the activity of

geniculocortical relay neurons (Satinsky, 1967). This claim was supported by a recent careful reevaluation of the work of Phillis et al. (1967) in which one of the original authors noted that clear depressant effects could be obtained only with very large iontophoretic doses of NE and, in fact, that many geniculate neurons were excited by NE (Tebecis and DiMaria, 1972). Therefore, under appropriate circumstances NE could facilitate the firing of geniculate neurons and thus there appeared to be some correspondence between the effects of activation of the noradrenergic pathway to the geniculate, on the one hand, and iontophoretically applied NE, on the other. These observations raised a number of questions, all related to the central issue of establishing a transmitter role for NE in the coeruleogeniculate pathway:

1. Under what specific conditions does iontophoretic NE facilitate the firing of LGNd neurons?

2. Is the facilitatory effect receptor mediated and, if so, what are the pharmacological characteristics of the receptor?

3. Does iontophoretic NE act upon relay neurons directly or is the effectmediated by adjacent neurons (i.e., inhibitory interneurons)?

4. How is the facilitatory effect of LC stimulation related to the activation of spontaneous activity produced by iontophoretic NE? Are the effects mediated by pharmacologically identical receptors as must be the case if NE is a transmitter in the pathway?

5. Does NE act as a conventional excitatory neurotransmitter or does it interact specifically with other afferents to geniculate neurons in a modulatory fashion?

In this dissertation, I present the results of experiments designed to answer these questions. The LGNd was chosen as an area for study

because of the observation that geniculate relay neurons exhibit novel facilitatory responses to NE. However, the LGNd has a number of characteristics which make it a particularly useful model system for exploring the effects of NE in a target area for LC noradrenergic neurons:

1. The input-output relations and local circuitry of the LGNd are relatively simple and well defined.

2. In the rat, the LGNd consists of a virtually homogenous cell population; geniculocortical relay cells constitute 94% of the neurons within the nucleus. Moreover, the remaining neuronal class, I-cells, are distinguishable from relay neurons on the basis of their responses to orthodromic or antidromic stimulation.

3. The afferent and efferent pathways of the LGNd are readily accessible to stimulation. In addition, the input pathway can be easily activated physiologically by visual stimuli.

4. The noradrenergic innervation of the LGNd derives exclusively from the LC which, even in the rat, is a sufficient distance from the LGNd so that stimulation of the LC does not interfere with recording in the LGNd.

These considerations allowed a detailed physiological characterization of the action of NE in the geniculate. In the majority of the studies reported in this dissertation, I used the technique of microiontophoresis for local drug application in conjunction with single cell recording and brain stimulation. Initially, I found that iontophoretic application of NE, at low doses and for relatively prolonged periods, caused an increase in the spontaneous rate of virtually all geniculocortical relay neurons. This response to NE was characterized pharmacologically and the information obtained was used (1) to examine the way in which NE interacts with

afferent excitation converging upon geniculate relay neurons; and (2) to provide evidence for a transmitter role of NE in the coeruleogeniculate pathway.

PART I: INTRODUCTION

A. THE LATERAL GENICULATE NUCLEUS: ANATOMY

1. Gross Structure

The lateral geniculate nucleus (LGN) is a discrete mass of cells located superficially at the caudal end of the diencephalon. In lower mammals, such as the rat, the nucleus consists of a thick, gently curved sheet of grey matter which is divided into dorsal (LGNd) and ventral (LGNv) zones by a bundle of horizontally oriented fibers. The LGN is bounded by the zona incerta (inferomedially), the ventral nucleus of the thalamus (medially) and the lateral posterior nucleus (superiormedially).

2. Input-Output Relations: Classical Pathways

The axons of retinal ganglion cells constitute the major afferent pathway to the LGN. Unlike the LGN of higher mammals, no laminations representing the separation of contralateral and ipsilateral retinal projections are present histologically in the rodent LGN. In fact, the bulk of the rat LGN is innervated by crossed retinal fibers. However, Hayhow et al. (1962) have described minor zones of uncrossed retinal input, leading to the concept of "concealed" laminations which are present functionally but are not evident anatomically.

The efferent projection of the LGNd to the visual area of the rat cerebral cortex was originally described by Clark (1932), Waller (1934) and Lashley (1941). On the basis of retrograde degeneration studies,

these workers demonstrated that the LGNd projected to the striate cortex and that its field of termination was limited to this area. In addition, they showed that the neurons of the LGNv did not innervate the cortex, and thus provided the basis for a functional subdivision of the dorsal and ventral portions of the nucleus. In the following discussion, attention will be directed where possible to the LGNd which is the subject of the experimental studies presented in this dissertation.

Using modern anatomical methods, Ribak and Peters (1975) confirmed these early findings and were able to specify more precisely that the projection of the LGNd was primarily to area 17 [according to Krieg (1946)], with slight extension to the zones of transition between areas 17, 18 and 18a.

In addition to the efferent geniculocortical pathway, projections from the cortex to the LGNd have long been known to exist. The main source of these corticogeniculate fibers appears to be the primary visual area (Montero and Guillery, 1968).

3. Input-Output Relations: Brainstem Afferents

The anatomical basis for the brainstem influence upon LGNd activity has been elucidated only relatively recently with the introduction of retrograde tracing techniques and biochemical or histochemical approaches to studying neurochemically defined pathways. Using the horseradish peroxidase technique, it has been possible to demonstrate afferents to the LGNd from a number of brainstem areas. A complete study utilizing this method has not yet been carried out in the rat, so in the following discussion I will draw upon the data of Leger et al. (1975) and Gilbert and Kelly (1975) which were obtained in the cat, in addition to a preliminary report of Villar et al. (1979) in the rat.

Major projections to the LGNd originate in the raphe nuclei, especially in the n. raphe linearis and n. raphe dorsalis; in the "parabrachial area," i.e., in and around the brachium conjunctivum; and in the locus coeruleus (LC). The LC projection will be discussed in detail in a later section. It is of interest to note that the neurochemical identity of each of these pathways can be specified, although only with certainty in the case of the coeruleogeniculate pathway. Thus, the raphe projection is most likely serotonergic (Geyer et al., 1976; Kuhar et al., 1972; Moore et al., 1978); the "parabrachial" projection is probably cholinergic (Hoover and Jacobowitz, 1979); and the LC projection has been demonstrated to be noradrenergic (see Section C).

In addition to these pathways, Gilbert and Kelly (1975) found labeled cells in the mesencephalic reticular formation and in the periaqueductal grey after placement of peroxidase in the LGN.

4. Cell Types

A description of the morphology and organization of neurons in the rodent LGN on the basis of the Golgi impregnation technique was originally provided by Cajal (1911). More recent studies by Grossman et al. (1973) and Kriebel (1975) have verified and extended Cajal's basic description.

The neurons of the LGNd have generally been classified into two or three catagories based on their location and dendritic morphology. Following the most recent account of Kriebel, Type 1 neurons have multipolar perikaria (mean diameter 25 μm) which give off four to eight primary dendrites. These branch into secondary and tertiary dendrites, forming a "tufted dendritic pattern" noted by Ramón-Moliner (1968) to be characteristic of most thalamocortical relay neurons. The dendritic

processes extend in a radial fashion 108 to 120 μm into the neuropil. Type 1 neurons have short-stalked appendages with large terminal swellings on their dendrites which are believed to be the major site of retinal axon termination. These neurons are the most plentiful type and are distributed throughout the LGNd.

Type 2 neurons, on the other hand, are found only in the superficial zone of the middle third (anterior-posterior) of the LGNd. These neurons have a more limited dendritic field than do Type 1 neurons in addition to other subtle morphological differences. Both Type 1 and Type 2 neurons are believed to be geniculocortical relay neurons, although this point has not been demonstrated conclusively as their axons cannot be traced in the Golgi material.

Type 3 neurons have smaller perikarya (mean diameter 14 μm) than either Type 1 or Type 2 neurons and a less branched dendritic tree. The dendrites have a tortuous course throughout the neuropil with no apparent orientation to retinal or cortical afferents. The most characteristic feature of these neurons are bizarre multi-lobed dendritic appendages and short axons that ramify in the vicinity of the perikarya of origin. These features are typical of Golgi type II cells in other thalamic nuclei and, therefore, this cell is believed to be an intrinsic interneuron.

The actual percentage of interneurons within the LGNd has been a matter of some controversy (see LeVay and Furster, 1979). Comprehensive examination of this issue by LeVay and Ferster (1977, 1979) using the horseradish peroxidase technique suggests that about 25% of neurons within the cat LGNd do not project to the visual cortex. In the rat, the number is probably much smaller. Werner and Krüger (1973) found that I-cells constituted about 7% of neurons in Nissle-stained sections and

this was confirmed in physiological studies by Sumitomo and Iwama (1977) who found that 6.5% of neurons encountered had characteristics of I-cells (see Section B).

5. Local Circuitry

Numerous electron microscopic studies of the LGNd in various mammalian species have allowed the ultrastructural identification of the relay and intrinsic neuron processes, and the axon terminals of retinal and cortical origin. In addition, it has been possible to specify the precise synaptic arrangements in which these elements participate. A complete consideration of these findings is beyond the scope of the present discussion. Nevertheless, it is worth pointing out some of the characteristic features of the local circuitry in the LGNd.

Synapses within the geniculate are established either in the general neuropil or in so-called"synaptic glomeruli." The glomeruli are glial ensheathed zones containing a complex synaptic arrangement consisting of retinal and cortical axon terminals and interneuron processes (Guillery, 1969). A recurring unit, found within the glomerulus and, in some species, also in the surrounding neuropil is the synaptic "triad." These consist of a retinal axon terminal which is presynaptic to a relay cell profile, and has therefore been termed a "presynaptic" dendrite (for a consideration of these ultrastructural features in the rat, see Lieberman, 1973). The triad is presumed to be the anatomic substrate for "feed-forward" inhibition. Dendro-dendritic interactions may mediate all or most of the local inhibition within the LGNd, and it has even been suggested that the intrinsic interneurons may be anaxonal (Lieberman, 1973).

B. THE LATERAL GENICULATE NUCLEUS: PHYSIOLOGY

1. The Multineuron Response

The first investigations of impulse transmission through the LGN were performed by recording multiunit responses to electrical stimulation of the optic tract. In the rat, as in other vertebrates, the typical field response to a weak contralateral optic nerve stimulus consists of a small diphasic wave of about 0.8 msec duration (t_1) followed by a larger negative wave of up to 1.5 msec duration (r_1) (see Fig. 14) (Sefton and Swinburn, 1964). As the stimulus intensity is increased, a third wave (r_2) of slightly longer latency is seen. The exact latency of the field response depends upon the site of stimulation. With stimulation of the optic nerve at a point just behind the eyeball, Sefton and Swinburn (1964) observed a latency of 1.8-2.0 msec to t_1. However, with stimulation at the optic chiasm (OX), a more proximal site, Fukada (1973) found a mean latency of 0.7 msec.

The earliest response, t_1, is assumed to represent electrical activity in the lowest threshold, fastest conducting optic tract fibers; whereas r_1 and r_2 reflect postsynaptic activity. In a careful study comparing the field response with unit activity, Fukuda provided strong evidence that r_1 and r_2 represent the mass activity of two populations of relay neurons innervated by fast-conducting and slow conducting optic nerve fibers, respectively. The presynaptic component for a r_2 is rarely seen as it occurs during r_1 .

2. Single Unit Recording

Single neuron spike activity can be recorded extracellularly in the LGN either under resting conditions (i.e., spontaneous activity) or

with orthodromic or antidromic activation. The spike record consists of a positive-negative (sometimes positive-negative-positive) waveform in which the negative phase is often larger than the positive phase. Orthodromic responses can be fractionated into three components: the S-potential, the A-potential and the B-potential (Fig. 4). The S-potential, a small monophasic wave of relatively slow time course, corresponds to the excitatory postsynaptic potential. The A-potential, usually a positive wave, is considered to be the initial segment response and the B-potential, a positive-negative wave, is the soma-dendritic response.

3. Physiological Classification of Units

Burke and Sefton (1966) provided the first classification of LGNd units based upon their responses to orthodromic and antidromic stimulation. Two distinct groups of cells were described. P-cells, the most predominant neuronal type, respond to single shock stimulation of the optic pathway with a short-latency initial spike followed by grouped discharges of 2-5 spikes (with an interspike interval of 5 msec) occurring 160 msec or more after the initial response. The late bursts are repeated at regular intervals up to 5 or more times. These cells have been demonstrated to be geniculocortical relay neurons.

Burke and Sefton also reported a second class of cells which they believed to be intrinsic interneurons and were therefore called I-cells. These cells responded to orthodromic stimulation with bursts of about 10 spikes at a short latency; the bursts were then repeated at a constant interval.

In a careful reevaluation of Burke and Sefton's findings, Sumitomo and co-workers (1976) observed that the supposed I-cells were clustered,

not in the LGNd, but in the adjacent thalamic perigeniculate reticular nucleus. These perigeniculate reticular (PGR) cells did, in fact, inhibit P-cells and Sumitomo et al. provided evidence that they were responsible for the postexcitation inhibition P-cells characteristically exhibit.

In a later study, Sumitomo and Iwama (1977) were successful in recording from what were believed to be the true geniculate interneurons. These cells were fired once by single shock stimulation of the optic tract and never showed a late response. They were evenly distributed throughout the full extent of the LGNd and the frequency with which they were encountered corresponded well with the fraction of interneurons observed in anatomical studies: about 6% of the total neuronal population. Although their function is presently unknown, Dubin and Cleland (1977) have suggested that the intrageniculate interneurons are involved in precise, spatially organized inhibition, but that they do not participate in the post excitation inhibition of relay neurons, as do PGR cells.

Both the PGR and the intrinsic interneurons may utilize γ-aminobutyric acid (GABA) as their neurotransmitter. Curtis and Tebecis (1972) have found that bicuculline, a specific GABA antagonist, blocks the postexcitation inhibition of relay neurons which, as noted above, is presumably mediated by PGR neurons. Bicuculline also has effects on specific inhibition produced by light stimulation within selective regions of the receptive field (Morgan et al., 1975), a function presumably mediated by intrinsic interneurons. Recent ultrastructural studies based upon the specific labeling of GABAergic nerve terminals with ^{3}H-GABA or ^{3}H-diaminobutyric acid have provided additional

evidence that the intrinsic interneurons are GABAergic (Sterling and Davis, 1980).

C. THE COERULEOGENICULATE NORADRENERGIC PROJECTION

1. Biochemical Determination of Catecholamines Within the LGN

Of the three types of catecholamine-containing neurons that are present in the rat b[illegible]jects to the LGN. This conclusion is [illegible]inations which demonstra[illegible]6 ± 20 ng/g); whereas [illegible]/g) (Kromer and M[illegible] to 1 and is [illegible]adrenergic cel[illegible] to se[illegible]72; Br[illegible]entricular nuc[illegible]ore and Bloom[illegible]

2. Or[illegible] the LGN

The ca[illegible] the pons and medulla are separated into [illegible]stem and the lateral tegmental system (Ungerstedt, 1971; Lindvall and Björklund, 1974; Swanson and Hartman, 1975). These neurons project widely throughout the forebrain, brainstem and spinal cord. Some brain regions, such as the hypothalamus, receive afferents from both noradrenergic systems (Lindvall and Björklund, 1974). However, the thalamus, with the exception of the paraventricular nucleus, appears to receive its noradrenergic innervation

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C. THE COERULEOGENICULATE NORADRENERGIC PROJECTION

1. Biochemical Determination of Catecholamines Within the LGN

Of the three types of catecholamine-containing neurons that are present in the rat brain, only the NE system projects to the LGN. This conclusion is based, in part, upon biochemical determinations which demonstrate a moderate concentration of NE in the LGN (356 ± 20 ng/g); whereas the concentration of dopamine is very low (64 ± 7 ng/g) (Kromer and Moore, 1980). The ratio of NE to dopamine is thus about 6 to 1 and is within the range expected for regions which contain only noradrenergic cells or axons. In these areas any dopamine present is believed to serve as a precurser in the biosynthesis of NE (Costa et al., 1972; Brownstein and Axelrod, 1974). With the exception of the paraventricular nucleus, epinephrine has not been described in the thalamus (Moore and Bloom, 1979).

2. Origin of the Noradrenergic Nerve Terminals Within the LGN

The catecholamine-containing neurons located in the pons and medulla are separated into two major groups: the LC system and the lateral tegmental system (Ungerstedt, 1971; Lindvall and Björklund, 1974; Swanson and Hartman, 1975). These neurons project widely throughout the forebrain, brainstem and spinal cord. Some brain regions, such as the hypothalamus, receive afferents from both noradrenergic systems (Lindvall and Björklund, 1974). However, the thalamus, with the exception of the paraventricular nucleus, appears to receive its noradrenergic innervation

exclusively from the LC (Lindvall et al., 1974; Kromer and Moore, 1980). Thus, when separate NE-containing cell groups located at various brain stem levels are ablated or their axonal projections destroyed, only lesions in the LC produce a significant decrease in the NE content of the lateral geniculate nuclei (Kromer and Moore, 1980). In correlative studies, injections of horseradish peroxidase restricted to the LGN resulted in the labelling only of the noradrenergic neurons located in the LC. Labelled neurons were found throughout the rostrocaudal and dorsoventral extends of both locus coerulei. However, the ipsilateral LC had a somewhat higher percentage of labelled cells (60%) than did the contralateral LC (40%). Both fusiform and multipolar neurons, which have been identified with Golgi methods (Swanson, 1976; Shimizu et al., 1978), were labelled indicating that the coeruleogeniculate projection arises from two morphological types of LC neurons (Kromer and Moore, 1980).

3. The Trajectory of Axons in the Coeruleogeniculate Pathway

Autoradiographic and fluorescence histochemical studies demonstrate that the ascending axons from the LC reach the LGN via the ipsilateral dorsal tegmental catecholamine bundle and the medial forebrain bundle (Ungerstedt, 1971; Lindvall and Björklund, 1974; Lindvall et al., 1974; Swanson and Hartman, 1975; Kromer and Moore, 1980). A small contralateral projection decussates in the pontine grey, the posterior commissure and the supraoptic decussation and joins the ipsilateral projections to the LGN. These fibers enter the dorsal lateral geniculate nucleus from the superior thalamic radiation, the thalamic reticular nucleus, and the lateral posterior nucleus.

Two major zones of entry have been distinguished. The majority of LC axons enter the rostral tip of the dorsal LGN by travelling along the lateral thalamus in the region immediately dorsal to the reticular nucleus. These fibers form a projection that parallels the longitudinal fiber system of the corticogeniculate axons (Montero and Guillery, 1968). In addition, long collaterals of these axons project at right angles to the parent axons and join the transverse corticogeniculate fiber bundles. A second group of fibers enter the medial dorsal LGN from the lateral posterior thalamic nucleus. These fibers send branches within both the longitudinal and transverse fiber systems where they intertwine with the axons that entered rostrally (Kromer and Moore, 1980).

4. The Noradrenergic Terminal Field Within the LGN

Using the Falck-Hillarp histofluorescence technique, early workers were able to demonstrate the existence of catecholamine-containing axons and terminals within the thalamus and LGN (Andén et al., 1966; Ungerstedt, 1971; Maeda and Shimizu, 1972; Maeda et al., 1973). However, it was not until the introduction of the more sensitive glyoxilic acid fluorescence method (Axelsson et al., 1973) that the abundance of the thalamic NE innervation was fully recognized (Lindvall et al., 1974). In fact, on the basis of studies using this new method, Lindvall and his co-workers (1974) concluded that "the thalamus stands out as one of the major projection areas of the locus coeruleus."

Even on the basis of the Falck-Hillarp formaldehyde method, it had been known that, within the thalamus, the anteroventral, paraventricular and dorsal lateral geniculate nuclei received a substantial catecholamine innervation (Fuxe, 1965). With the glyoxilic acid method, the complete

pattern of the thalamic noradrenergic projection could be specified, and it was found that virtually the entire dorsal thalamus is innervated (Lindvall et al., 1974; Moore and Bloom, 1979). The anteroventral nucleus was confirmed as having the greatest density of innervation within the dorsal thalamus followed by the anteromedial nucleus and the dorsal lateral geniculate nucleus which were regarded as having a "dense" innervation. The paraventricular nucleus, which is of different embyronic origin, is innervated by catecholamine fibers arising outside of the LC, some of which may be epinephrine containing (Moore and Bloom, 1979). The observations based upon histochemical fluorescence methods have been confirmed with an immunochemical technique utilizing an antiserum directed against dopamine-β-hydroxylase, an enzymatic marker for noradrenergic neurons (Swanson and Hartman, 1975) (Fig. 1).

The LC innervation of the LGN is comprised of a highly branched network of varicose axons. The projection does not appear to be topographically organized; instead, a single fiber may have collateral axons that arborize throughout large areas of the nucleus (Kromer and Moore, 1980).

The NE axons in the LGN are of the fine varicose type (Lindvall et al., 1974). Kromer and Moore (1980) distinguished two subpopulations of fibers. The first type are preterminal axons (approximately 0.5 μm in diameter) which enter the LGN from the myelinated fiber bundles of the superior thalamic radiation and zona incerta. These possess regularly spaced fusiform varicosities (0.5-1 μm in diameter). The second type of fiber is the predominant constituent of the terminal plexus within the dorsal LGN. These have varicosities which are larger (1-4 μm), more closely and irregularly spaced along the axon and more intensely

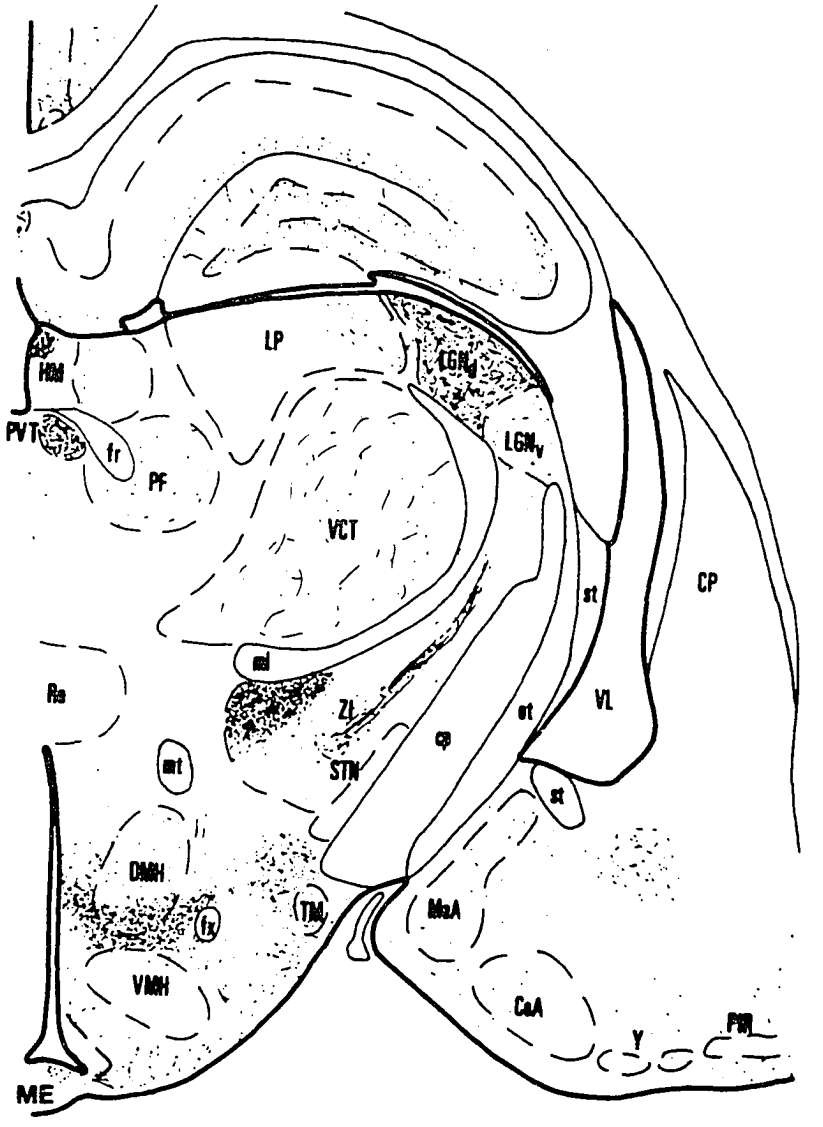

Fig. 1: Schematic illustration of dopamine-β-hydroxylase containing axonal processes in a frontal section through the LGNd as determined by the immunofluorescence technique. Note the high density of stained fibers in the LGNd. From Swanson and Hartman (1975); see this reference for key to abbreviations.

fluorescent than the fusiform varicosities of the preterminal axons. The entire LGNd contains a dense network of short terminal segments of these fibers in a highly branched pattern. On the basis of the glyoxilic acid fluorescence method, Kromer and Moore (1980) concluded that both geniculocortical relay neurons and intrinsic neurons may receive a noradrenergic input since varicose axons were observed to be closely apposed to the somata and dendritic profiles of all neurons within the nucleus. Moreover, the anatomical observations indicate that relay neurons may receive a particularly strong innervation as the branching patterns of many LC fibers located in the neuropil were observed to closely follow the dendritic arborizations of relay neurons as described in Golgi material (Grossman et al., 1972; Kriebel, 1975).

D. PHYSIOLOGICAL CHARACTERIZATION OF ADRENOCEPTORS ON CENTRAL NEURONS

1. The Technique of Microiontophoresis

The major physiological approach to studying pharmacological receptors for NE in the central nervous system has been with the technique of microiontophoresis. With this procedure the amine, its analogs or pharmacological antagonists can be applied in minute quantities directly into the immediate microenvironment of single neurons while their electrical activity is monitered. The technique has the following advantages over other modes of drug application. First, compounds, such as NE, which normally would not gain entry to the brain because of diffusional barriers or enzymatic degradation, are provided access to neuronal receptors. Second, the action of the drug is limited to neurons in the immediate vicinity of the delivery pipette, greatly

simplifying the interpretation of the response (although major interpretive difficulties still arise; see Bloom, 1974).

In iontophoresis, an electric current is used to expel drug molecules from a micropipette which is situated close to the neuron whose electrical activity is being recorded. The technique depends upon Faraday's law of electrolysis: $M = n \frac{IT}{ZF}$, where M is the number of moles of a given ion which will be released by passage of I amperes of charge of the same polarity as the drug molecule during each sec of ejection time, T. The number of ions delivered depends upon the valence of the charged ion, Z, and the Faraday constant, F. The exact relationship between the passage of charge ions depends on the complex factor n, known as the transport number, which varies for individual compounds due to differences in solubility, dissociation and polarity. The transport number of NE has been estimated to be approximately 0.29 (Bevan et al., 1979).

For drugs of low solubility or neutral charge, electro-osmosis may be the mode of drug delivery. In electro-osmosis, current carries the drug molecules passively within their hydration shells so that the drug is ejected with the bluk flow of small volumes of the solute from the pipette (Curtis, 1964).

Conventional glass electrodes can be used for iontophoresis providing their impedance when filled with the appropriate drug solution is low enough to allow passage of the drug ions without undue shunting of current. Typically, multibarrel microelectrode arrays are used so that more than one drug can be tested on an individual cell or the

interaction between two or more drugs can be examined. Between periods of drug ejection, a retaining current opposite in polarity to the ejecting current is usually applied to the drug barrels. This prevents unwanted diffusion of drug molecules from the pipette between tests.

One barrel in the pipette array is reserved for an electrolyte solution through which a balancing current is continuously passed. This current is equal in magnitude but of opposite polarity to the sum of the currents flowing through the other electrode channels and serves to prevent polarization of the tip and associated direct current artifacts.

In the central nervous system where visual identification of neurons is impossible, an electrode for detecting bioelectric potentials (usually extracellular action potentials) is fixed in close proximity to the iontophoresis pipettes. The recording electrode may be one barrel of the multibarrel array (generally the lower impedance center barrel) or another electrode cemented in parallel to the iontophoresis pipettes (see Fig. 2).

2. Microiontophoretic Studies of NE in the Spinal Cord and Brain

Neurons in virtually every part of the central nervous system have been found to respond to iontophoretically applied NE. The presence of receptors for NE on neurons in so many brain regions is not surprising as central noradrenergic projections innervate most parts of the neuraxis. Nevertheless, because responses even within a specific nucleus have been variable and often not reproducible, the usefulness of the iontophoretic technique for characterizing adrenoceptors on central neurons has been questioned. Possible technical reasons for these difficulties are discussed in reviews by Bloom (1974) and Szabadi (1979).

In general, however, experiments on identified homogenous cell populations have yielded more consistent findings which often correspond closely with the response to noradrenergic pathway stimulation. In the following discussion, I summarize the results of iontophoretic experiments in various central nervous system areas which are postsynaptic to noradrenergic neurons and indicate the degree to which the responses have been characterized pharmacologically.

3. Spinal Cord

NE was originally reported to hyperpolarize motoneurons in the anterior horn of the spinal cord (Engberg and Marshall, 1971). More recently, however, Engberg et al. (1976) concluded that this hyperpolarization was probably not mediated via specific adrenoceptors since a wide range of different agents (adrenergic agonists, antagonists and neuroleptics) had very similar effects. Moreover, Barasi and Roberts (1977) reported that NE increased the amplitude of antidromically evoked field potentials representing the activity of motoneurons, and concluded that the excitability of these cells was increased by NE. This hypothesis was confirmed in single cell recording studies by White and Neuman (1980).

In some early studies, spinal interneurons and Renshaw cells were also reported to be depressed by NE (Engberg and Ryall, 1966; Curtis et al., 1971). However, other workers found both excitatory and depressant effects (Weight and Salmoiraghi, 1966; Headley and Lodge, 1976).

4. Brainstem Reticular Formation

Unidentified neurons in the reticular formation have generally demonstrated both facilitatory and depressant responses to NE (Bradley and Wolstencroft, 1962; Holsi et al., 1971). Bradley et al. (1966)

reported that the excitatory responses could be blocked by chloropromazine, a neuroleptic with potent α-adrenoceptor blocking activity (Peroutka et al., 1977); whereas the depressant responses have been variously antagonized by mescaline (Gonzalez-Vegas, 1971) or other chemically related and unrelated compounds (Gonzalez-Vegas, 1971; Gonzalez-Vegas and Wolstencroft, 1971a,b).

5. Dorsal Raphé Nucleus

A number of studies have reported variable effects of NE on 5-HT-containing neurons in the dorsal raphé (Couch, 1970; Aghajanian et al., 1972; Haigler and Aghajanian, 1973; Svensson et al., 1975; Gallager and Aghajanian, 1976). Recently, however, Baraban and Aghajanian (1980) have found that uniform activation of firing is obtained with very low iontophoretic doses and that with higher doses an increasing proportion of depressant responses are obtained. The facilitatory effect of NE appears to be mediated via an α-adrenoceptor.

6. Locus Coeruleus

Noradrenergic neurons in the LC are uniformly depressed by iontophoretic NE (Svensson et al., 1975; Cedarbaum and Aghajanian, 1976). This effect is mimicked by clonidine, an α_2-adrenoceptor agonist, and other sympathomimetic amines with α-agonist activity. The α-blocker piperoxane is an effective antagonist of the response to NE but sotalol is not, suggesting that NE acts via an α-adrenoceptor. The rank order of agonist potencies resembles that of α_2- ("presynaptic") receptors on peripheral sympathetic neurons and other peripheral tissues (see Berthelsen and Pettinger, 1977).

7. Brainstem Motoneurons

NE facilitates the excitability of motoneurons in the facial nucleus (McCall and Aghajanian, 1979) and other brainstem motor nuclei (McCall and Aghajanian, personal communication). This effect is antagonized by piperoxane suggesting that it is mediated by an α-adrenoceptor.

8. Vestibular Nuclei

In the lateral vestibular nucleus, Yamamoto (1967) described a uniform excitatory action of NE. This observation has recently been confirmed by Kirsten and Sharma (1976). Yamamoto originally reported that the excitatory responses were insensitive to the α-adrenoceptor antagonist phentolamine, but could be blocked by the β-blocking drug dichloroisoproterenol. However, Kirsten and Sharma found that the excitatory responses were, in fact, abolished by phentolamine, while the β-adrenoceptor blocking agents sotalol and propranolol were without effect. The reason for this discrepancy is not apparent.

In contrast to the lateral vestibular nucleus, neurons in the medial vestibular nucleus are depressed by NE and this effect was not modified by phentolamine, sotalol or propranolol (Kirsten and Sharma, 1976).

9. Cerebellar Cortex

The spontaneous firing of Purkinje cells in the cerebellar cortex is uniformly depressed by NE (Hoffer et al., 1971). This response is mediated by a receptor with some characteristics of a β-adrenoceptor as it is antagonized by sotalol (Hoffer et al., 1971; Woodward et al., 1974) or dichloroisoproterenol (Freedman et al., 1975). However, the

neuroleptics fluphenazine and α-flupenthixol (Freedman and Hoffer, 1975) are also effective antagonists indicating that the receptor cannot be classified as strictly "β" by rigorous criteria.

Neurons in the cerebellar flocculus show mixed responses to NE (Yamamoto, 1967).

10. Hypothalamus

Neurosecretory and non-neurosecretory cells in the supraoptic nucleus of the hypothalamus exhibit uniform depressant responses to NE which are antagonized by sotalol (Barker et al., 1971; Sakai et al., 1974).

Neurons in the tuberal nuclei (consisting of the arcuate nucleus and median eminence) demonstrate mixed responses to NE although depressant effects predominate (Geller, 1976); sotalol blocks these depressant effects (Geller and Hoffer, 1977). Other hypothalamic nuclei have also been studied but specific antagonists have not been tested (see Szabadi, 1979).

11. Thalamus

Phillis and Tebecis (1976a,b) reported both excitatory and depressant responses to NE in the ventrobasal nuclear complex of the thalamus.

Medial geniculate neurons were found to have mixed responses with a predominance of depressant effects (Tebecis, 1967; 1970). A summary of previous studies in the lateral geniculate nucleus is presented in Section E.

12. Hippocampus

Hippocampal pyramidal cells are uniformly depressed by NE, an effect which is reportedly antagonized by sotalol (Stefanis, 1964;

Biscoe and Straughan, 1966; Segal and Bloom, 1974a).

13. Septal Nuclei

Only depressant responses to NE have been reported in this area, but no antagonists were tested (Herz and Gogolak, 1965; Segal, 1974).

14. Olfactory Bulb

Mitral cells in the olfactory bulb are depressed by NE (Baumgarten et al., 1963; Bloom et al., 1964; McLennan, 1971); whereas granule cells are excited (McLennan, 1971). The effect of NE on mitral cells was blocked by the α-antagonist dibenamine but not by dichloroisoproterenol (Salmoiraghi et al., 1964).

15. Neocortex

Cortical neurons have generally been found to show mixed responses to NE (Krnjević and Phillis, 1963; Johnson et al., 1969; Bevan et al., 1974; Fredrickson et al., 1971; Stone, 1973a; Bevan et al., 1978) although some authors have reported only depressant effects (Lake et al., 1972; Jordan et al., 1972). Szabadi and his co-workers have suggested that the excitatory and depressant responses are mediated by α- and β-adrenoceptors, respectively. Although there is some evidence in conflict with this view, in general, studies with various adrenoceptor agonists and antagonists support the concept. With regard to agonist drugs, Bevan et al. (1977) found that the α-agonists phenylephrine and methoxamine were exclusively excitatory while the β-stimulant salbutamol evoked only depressant responses. Isoproterenol caused depression at low doses and excitation at higher doses which the authors suggested was consistent with its strong β-agonist and weaker α-agonist activities.

The excitatory responses to adrenergic agonists were selectively

and reversibly antagonized by both α-adrenoceptor blocking agents (phentolamine, phenoxybenzamine) and β-adrenoceptor blocking agents (propranolol, sotalol). However, the doses of the β-agonists required were beyond those necessary for selective β-adrenoceptor blockade, and it was suggested that the antagonism might reflect the expression of α-blocking activity by the drugs. In previous studies, both α- and β-antagonists had been found to antagonize the excitatory effects of NE but doses were not controlled in these studies (Johnson et al., 1969; Bevan et al., 1974; Fredrickson et al., 1972).

The depressant response to NE is mimicked by isoproterenol (Bevan et al., 1977) and, in some studies, was antagonized by β-antagonists (Kostopoulos and Yarbrough, 1975; Stone, 1973b; Bevan et al., 1977).

16. Summary

This overview indicates that NE can have dual effects on the firing of central neurons depending upon the specific brain region and neuronal type involved. Although some conflicting data exists, the evidence is generally consistent with the idea that facilitatory effects of NE are mediated by α-type adrenoceptors whereas the depressant response most closely resemble a β-mediated effect.

E. SUSPECTED TRANSMITTER AGENTS IN THE LATERAL GENICULATE NUCLEUS

1. Serotonin

The earliest investigations of the response of LGNd neurons to microiontophoretically applied drugs was carried out by Curtis and Davis (1962). In an attempt to explain the observation of Evarts et al. (1955) that intracarotid injection of lysergic acid diethylamide (LSD) or bufotenine specifically depressed the response of LGNd neurons

to optic pathway stimulation, these workers used the newly developed technique of iontophoresis to test serotonin and a wide range of related indoles on field responses and evoked unit activity in the cat LGNd. They concluded that serotonin is a potent depressant of the synaptic firing of LGNd neurons but is much less active against activity evoked with the excitatory amino acid glutamate. Peripheral serotonin antagonists (methysergide, 2-bromo-LSD) or catecholamine antagonists (phentolamine, dibenamine) did not alter the response to 5-HT. Phillis et al. (1967a) observed similar effects of 5-HT in the geniculate but reported that glutamate and synaptically activated firing were depressed equally well. In a careful reevaluation of the findings of Phillis et al., Tebecis and DiMaria (1972) concluded that the initial observations of Curtis and Davis were essentially correct: glutamate activated firing was less sensitive than spontaneous or evoked activity.

Satinsky (1967) examined the effect of 5-HT on spontaneously active LGNd neurons. The discharge rate of most cells was slowed by 5-HT, although a few were excited. However, all physiologically identified geniculocortical relay neurons were depressed by the amine. Haigler and Aghajanian (1974a) and Aghajanian (1976) also observed depressant effects of 5-HT in the LGNd. Moreover, these authors found that 5-HT had a similar action in the LGNv. A recent study conducted by Torda (1978) has confirmed that 5-HT has a powerful depressant effect on spontaneous or evoked activity but is comparably weak against glutamate activated firing. The mechanism underlying the selective activity of 5-HT is not known (see also Tebecis, 1973).

2. Norepinephrine

In their original study of indole compounds, Curtis and Davis (1962) reported incidentally that NE and various phenylethylamine derivatives had a weak depressant effect on orthodromic field potentials in the LGN. Dopamine was the most active of the phenylethylamine compounds, but was only slightly more potent than NE. The potency of NE was rated 1 on a 1 to 12 scale where the potency of 5-HT was arbitrarily designated 12.

In contrast to the observations of Curtis and Davis (1962), Phillis et al. (1967) reported that NE and dopamine had "potent depressant effects on many LGN neurons." However, again in a reevaluation of this work, Tebecis and DiMaria (1972) confirmed the findings of Curtis and Davis. They reported that NE was a weak depressant of slightly over one-half of the neurons tested and that the remainder "were either excited (slow time course) or initially depressed and then excited." Moreover, it was found that a clear depression of firing could only be obtained with high (80 nA or greater) ejection currents. Again, dopamine was found to be more potent than NE. In another study, Satinsky (1967) found a predominant facilitatory effect of NE on spontaneously active cells. In particular, all antidromically identified relay neurons were activated by NE. Torda (1978) has also reported facilitatory effects of NE on LGN neurons.

The significance of these facilitatory effects of NE was strengthened by the observation of Nakai and Takaori (1974). These workers found that conditioning stimulation of the LC enhanced the amplitude of the orthodromic field potential in the cat LGN. There was a significant effect when the stimulation (trains of four 1 msec duration pulses at 200 Hz) was applied from 25-300 msec prior to the orthodromic shock and occurred

only when the stimulating electrode was actually within the LC. Evoked unit activity was also facilitated by prior LC stimulation and this effect was blocked by the catecholamine depleting agent reserpine or by the dopamine-β-hydroxylase inhibitor fusaric acid. The effect of reserpine could be reversed by L-dopa or by intraventricular injection of NE. These pharmacological observations suggested that the facilitatory effect was produced by the release of NE from LC neurons. One additional finding in this study was that certain "interneurons" (probably PGR cells; see Section B) were suppressed by LC stimulation. This led Nakai and Takaori to speculate that relay neurons were facilitated indirectly, due to suppression of inhibitory elements synapsing upon them.

3. Glutamate

Many investigators have found that L-glutamate produces an excitation of LGNd neurons (Curtis and Davis, 1962; Phillis et al., 1967a,b; Tebecis and DiMaria, 1971; Torda, 1978). Although the functional significance of this is not known, there is some evidence that the corticogeniculate pathway uses either glutamate or aspartate as its neurotransmitter (Lund Karlsen, 1978), whereas the retinogeniculate fibers probably do not (Tebecis, 1973; Lund Karlsen, 1978).

4. Acetylcholine

Because of the probable existence of a cholinergic pathway from the brainstem to the geniculate (Shute and Lewis, 1967; more recent studies: Brownstein et al., 1975; Hoover and Jacobowitz, 1979), a number of investigators have examined the effects of acetylcholine (ACh) and cholinergic drugs on geniculate neurons. In general, ACh excites these cells (Phillis et al., 1967a,b; Satinsky, 1967; Torda, 1978). Various

cholinergic agonists mimic this effect, carbachol being the most potent. The action of cholinergic agonists is antagonized predominantly by muscarinic blockers such as atropine or scopolamine (Phillis et al., 1967; Matsuoka and Domino, 1972).

Facilitatory effects of stimulation in the mesencephalic reticular formation are also blocked by either local (Phillis et al., 1967) or systemic (Matsuoka and Domino, 1972) administration of cholinergic antagonists, suggesting that a cholinergic pathway terminating in the LGNd is responsible for the effects of such stimulation. On the basis of their studies in the cat, Matsuoka and Domino (1972) concluded that the cholinergic system acts as a "facilitatory modulator" (see Section F) of lateral geniculate activity. This view is consistent with the findings of Curtis and Davis (1973) who noted in phenobarbitone anesthetised cats that the "excitation [due to ACh] was only apparent as a facilitation of the responses to synaptic stimulation or L-glutamate application. ACh was never observed to activate neurons in the absence of other, simultaneously applied, excitatory influences (Phillis et al., 1967b)."

5. γ-Aminobutyric Acid

Tebecis and DiMaria (1972) found that γ-aminobutyric acid (GABA) depressed spontaneous or evoked activity in the LGNd. Interestingly, the effect was qualitatively different from that observed with 5-HT in that GABA was more effective against amino acid induced excitation; whereas 5-HT was usually a more potent depressant of spontaneous or synaptically evoked activity (see above).

F. NOREPINEPHRINE AS A NEUROMODULATOR

1. The Concept of Neuromodulation

Largely on the basis of anatomical considerations, it has long been speculated that brain noradrenergic systems might exert postsynaptic actions with functional characteristics which are different from conventional ("mediating") pathways which transmit highly specified information with millisecond resolution. These proposed actions, termed "neurohormonal" or "neuromodulatory," were suggested by the fact that single noradrenergic neurons send axons to widely separated and functionally distinct central nervous system areas. Moreover, within each nucleus, the axons are highly collateralized and nerve terminals are distributed in a more or less uniform fashion without topographic specificity. Finally, there was a question as to the prevelance of specialized junctional zones between the terminal membrane of NE-containing neurons and their target cells, which encouraged speculation that released transmitter might have a generalized influence within the target nucleus. All of these considerations suggested to some investigators that noradrenergic neurons might be better suited for regulating the level of responsiveness of target neurons in a leisurly fashion than for mediating specific sensory or motor functions (Dismukes, 1977).

The first physiological explorations of this concept in the vertebrate nervous system were carried out by Foote et al. (1975) and Freedman et al. (1976, 1977) and later by Woodward and his collaborators (1979). These workers found that in certain cortical areas, NE exerted a differential influence on activity evoked by afferent synaptic inputs when compared with its effect on background activity. In general, background firing was depressed by NE more than was evoked activity,

resulting in an increase in the "signal-to-noise" ratio. These data were interpreted as indicating that NE improved the efficacy of synaptic inputs. The phenomenon was termed "modulatory" in a purely descriptive sense as its neuronal basis was unexplained (Woodward et al., 1979).

Studies with monoamines in invertebrates allowed the formulation of a more precise definition of the concept of modulation. This definition was based upon the distinction that Florey (1967) made between the nature of transmitter action and that of a hormone or "modulator substance." He proposed that "modulator substances can affect presynaptic neurons, and they can alter the tendency to spontaneous discharge." Although Florey was referring to blood born substances interacting with neuronal systems, Kupferman (1979) extended the definition to include neuronally released transmitter agents. In addition, he proposed a more rigorous distinction between conventional neurotransmitters which produce direct excitation or inhibition (by altering voltage-insensitive ionic conductances) and neuromodulators which have little effect on cellular activity in themselves, but instead alter a cell's responsiveness to synaptic events mediated by conventional transmitters. Kupferman suggested that neuromodulators may act via a wide range of cellular mechanisms, however, in the following discussion, I will consider only those cases where monoamines act to alter the excitability of postsynaptic cells.

2. Neuromodulation in Invertebrates

There are a number of examples where such modulatory effects have been demonstrated in invertebrates. In many cases, the target cell is muscle rather than nerve, and the transmitter is a monoamine other than

NE. Nevertheless, these examples provide a useful precedent for studies in vertebrates.

The original description of neuromodulation by a monoamine was in the anterior byssus retractor muscle of Mytilus. In this preparation, serotonin (5-HT) does not alter the membrane potential, but it does markedly decrease the threshold for spike generation, thus enabling the motor neurons which supply the muscle to cause a contraction (Hidaka et al., 1967).

5-HT produces a similar effect on the buccal musculature in Aplysia. It has been demonstrated that 5-HT can potentiate the excitation produced by stimulation of individual cholinergic motor neurons which project to the muscle. Moreover, stimulation of the serotonergic metacerebral cell which also innervates the muscle, results in similar modulatory effects, traditionally known as "heterosynaptic facilitation" (Weiss et al., 1978). Analagous observations were made in Planorbis by Berry and Pentreath (1976).

Monoamines other than 5-HT can also have modulatory actions in invertebrates. For example, neuromuscular contraction is enhanced by octopamine in the lobster (Evans et al., 1976) and locust (Evans and O'Shea, 1977) and by dopamine (Swann et al., 1978) in Aplysia.

3. Neuromodulation in Vertebrates

The membrane mechanisms underlying neuromodulatory actions produced by monoamines have been examined in several vertebrate systems as well. For example, myenteric neurons in the guinea pig small intestine respond to 5-HT with enhanced electrical excitability. This phenomenon is associated with a slow depolarization and an increase in input resistance (Wood and Mayer, 1979). All of these effects can be mimicked by

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Mor[illegible] in invertebra[illegible]ced by octopamine in [illegible]Evans and O'Shea, 1977) and [illegible]plysia.

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stimulation of fiber tracts within the plexus which are presumed to contain serotonergic axons.

A similar effect of serotonin has been observed on motoneurons in the rat facial nucleus (VanderMaelen and Aghajanian, personal communication). Iontophoretic 5-HT (or NE) cause a marked facilitation of the electrical excitability of these cells with membrane changes analagous to those observed in myenteric neurons. Although it has not been possible to directly activate the serotonergic pathway to these neurons, pharmacological agents, such as *p*-chloroamphetamine, which release 5-HT from serotonergic nerve terminals, mimic the facilitation of excitability, suggesting that activity in serotonergic neurons synapsing on the motoneurons can produce these modulatory effects.

Finally, in the rabbit superior cervical ganglion, dopamine causes an enduring facilitation of the slow depolarizing effect produced by ACh (in addition to a hyperpolarization due to an independent mechanism) (Libet, 1979). This modulatory action is somewhat unusual in that the response to a specific transmitter agent (ACh) is affected and this persists for an extended period of time.

In many cases where the modulatory effects of monoamines on post-synaptic muscle or nerve cells have been examined, the overall response is a facilitation of excitation. This general theme appears to hold for a wide spectrum of monoamine neurotransmitters. Except in the facial nucleus, NE has not previously been demonstrate to produce such excitability changes, although, as indicated at the beginning of this section, it has been shown to cause a relative enhancement of convergent inputs in a number of cortical areas.

PART II: METHODS AND MATERIALS

A. METHODS

1. Preparation of Animals

All experiments were carried out in male Sprague-Dawley rats (Charles River Laboratories, Wilmington, MA) weighing 230-340 g. Most animals were anesthetized with an intraperitoneal injection of chloral hydrate solution (350-400 mg/kg). Additional anesthetic injections (100 mg/kg) were given as necessary during the course of each experiment, however, the injections were spaced so as to keep the level of anesthesia relatively light. Under these conditions, there was an absence of spontaneous movement, although a withdrawal response could be elicited upon compression of the hind paw.

The animals were mounted in a stereotaxic apparatus (David Kopf Instruments, Tujunga, CA), a burr hole was drilled in the skull overlying the dorsal lateral geniculate, and the dura was carefully removed with a hooked needle. In some experiments, additional burr holes were drilled for placement of stimulating electrodes as described below. Core temperature was monitered with a rectal thermistor probe and maintained at 37 ± 1°C with a heating pad. All experiments were carried out under low level room light.

For systemic administration of drug solutions, a lateral tail vein was cannulated with a 25-gauge hypodermic needle (5/8 in). This remained in place throughout the experimental session and, when required, solutions were injected through the needle with a 1 ml tuberculin syringe.

To control for the effects of anesthesia, a small series of unanesthetized, decerebrate preparations were studied. In these animals, a pretrigeminal transection was made with a retractable wire knife (Sclafani and Grossman, 1969). This device consisted of a stainless steel wire "blade" housed in a 24-gauge stainless steel tube with a 30° bend at its lower end. The wire could be extended approximately 1 cm in a nearly perpendicular fashion from the housing. To perform the transection, animals were temporarily anesthetized with halothane. The head was placed firmly in a stereotaxic instrument; and after exposing the skull, a small elliptical burr hole was drilled just posterior and at the lateral border of the lambdoidal suture. The knife was lowered through the burr hole with the wire blade retracted. The blade was then extended, and the device was advanced slowly to the base of the skull. In the process, the brain was transected at a level just posterior to the inferior colliculus and anterior to the trigeminal nerve. The cut generally left some of the pyramidal tract fibers intact. The blade was then retracted, the knife was removed and a local anesthetic (mepivicaine HCl, 2%) was injected at all pressure points.

2. Extracellular Recording and Microiontophoresis

In some cases, extracellular action potentials were monitored with conventional 5-barrel micropipettes. These consisted of a central pipette for recording, surrounded by four peripheral barrels for drug or electrolyte solutions. The electrode was prepared in a Narashigi pipette puller adjusted to give a relatively blunt tip with a wide tip angle. The tip was broken back under microscopic control so that the center (recording) barrel, when subsequently filled with 2 M NaCl-2%

pontamine sky blue solution, gave an in vitro impedance of 2.8-4.5 MΩ. The tip diameter was typically 4-6 μm.

In other experiments, hybrid 6-barrel microelectrode assemblies were used. These consisted of a blunt (10-20 μm diameter) 5-barrel pipette to which a fine (1 μm) single barrel recording electrode was affixed with dental acrylic (see Wang and Aghajanian, 1977). The recording electrode extended 20-40 μm beyond the iontophoresis barrels (Fig. 2). This pipette assembly has the advantage of providing superior unit discrimination and uniformly large extracellular action potentials. The recording barrel, filled with the electrolyte-dye solution, typically had an impedance of 6-12 MΩ.

In both cases, the electrode barrels were filled with a few strands of fiberglass before pulling. This allowed the tips of the pipettes to fill rapidly by capillary action. Three of the iontophoresis barrels were loaded with drug solutions and the remaining barrel was always filled with 4 M NaCl. This was used to continuously pass a "balancing" current equal in magnitude but opposite in polarity to the sum of the currents in the other three channels. A retaining current of -10 nA was applied to the drug barrels between ejection periods except when glutamate was used, in which case the backing current was +10 nA.

The electrode assembly was positioned 4.0 mm anterior to lambda and 4.0 mm lateral to the midline and lowered vertically with a hydraulic microdrive (David Kopf Instruments) approximately 4.0 mm from the pial surfact until cellular responses to movement of a light beam across the visual field could be detected.

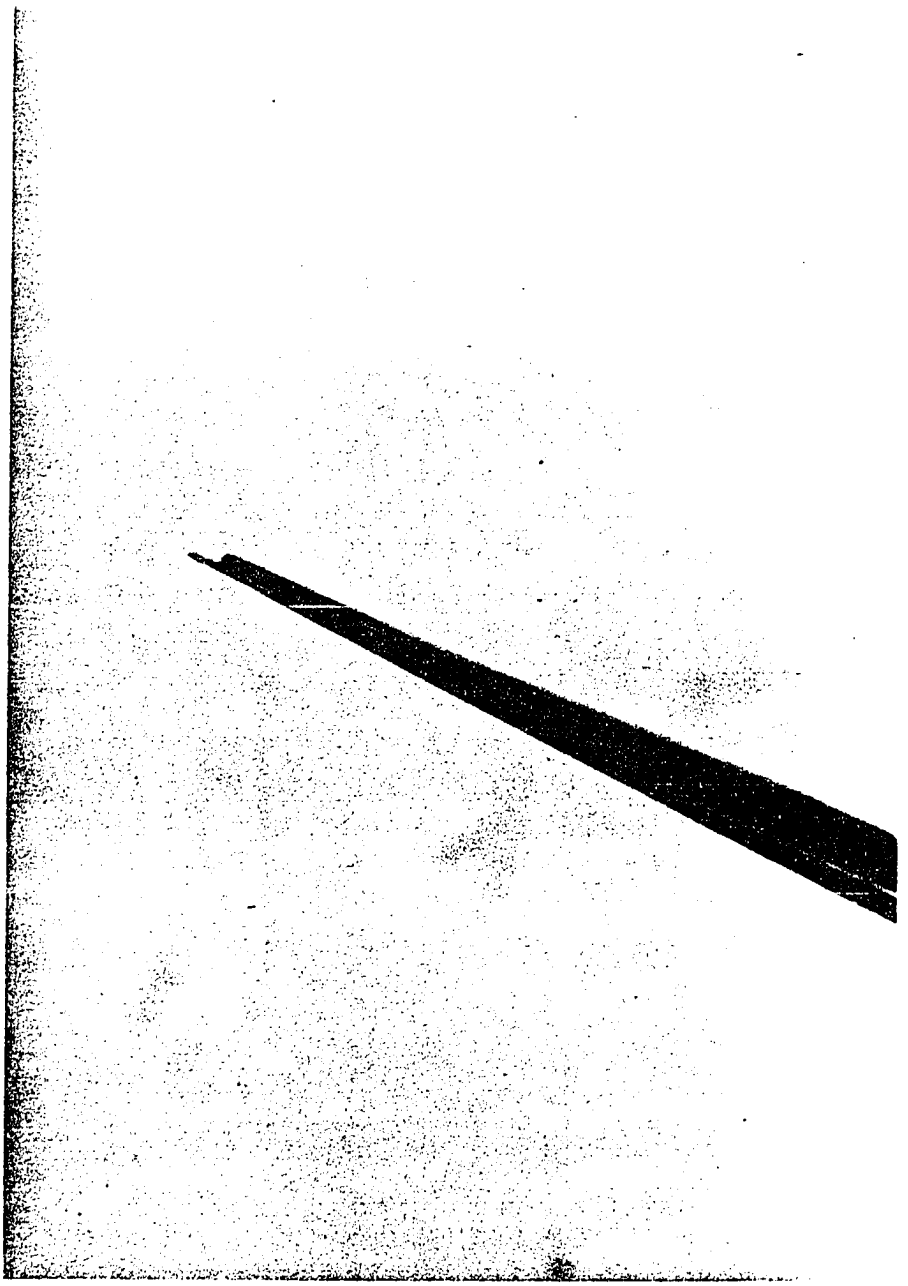


Fig. 2: Photomicrograph of a 6-barrel micropipette assembly used for simultaneous extracellular recording and drug ejection. The tip of the recording electrode extends 20 μm ahead of a multi-barrel array which carries drug and electrolyte solutions for microiontophoresis.

3. Amplification and Data Analysis

Electrode signals were led through a high-input impedance preamplifier (W-P Instruments Model 725) and displayed on a storage oscilloscope. The stored spike records were continuously monitored to assure counting of single units and to confirm that drug applications produced no diminution in spike amplitude suggestive of local anesthetic effects. Units were isolated and stimulation artifacts were excluded with the voltage window feature (Fig. 4) of a window discriminator (designed and constructed by V. Lipponen). The pulse output of the window discriminator triggered a rate counter with a period of 10 sec. By plotting the analog output of the rate counter with a graphic recorder (Gould Brush Model 220), a continuous average rate record was produced. The filtered output of the oscilloscope also drove an audio monitor.

For experiments employing brain stimulation, poststimulus time histograms (PSTH) were produced with a Nicolet 1072 Signal Averager. Actual spike activity was recorded either with a mercury vapor lamp oscillograph or by photographing the screen of the storage oscilliscope.

4. Brain Stimulation

In some experiments, stimulating electrodes (Rhodes Medical Instruments) were placed in the optic chiasm (OX), visual cortex (Model SNE-100) or locus coeruleus (Model NE-100). The electrodes consisted of a stainless steel wire surrounded, except at the tip, by tubing of the same material. The inner contact and an uninsulated portion of the outer tubing formed the two poles of a "concentric" bipolar electrode.

For placement in the OX, the electrode was positioned at bregma and lowered vertically until its tip was just above the base of the skull; it was then cemented with dental acrylic to three small machine screws embedded in the frontal and parietal bones. In a few animals, a stimulating electrode was positioned in the occipital cortex (area 17) or optic radiation with a micromanipulator. The stereotaxic coordinates were roughly 1.5 mm anterior to lambda and 3.5 mm lateral to the midline (ipsilateral to the recording electrode) [based on the atlas of Krieg (1946)]; however, the exact position and depth was determined empirically by maximizing the antidromic field potential in the LGNd.

LC stimulating electrodes were placed 1.1 mm lateral and 1.0 mm posterior to the lambdoidal suture and lowered 6.0 mm from the pial surface. These were also cemented in place to machine screws.

5. Histological Verification of Recording or Stimulating Sites

At the conclusion of each experiment, the recording site was marked by passing a 20 μA negative current through the recording barrel for 20-60 min. This resulted in the deposition of a discrete spot of dye at the site of the electrode tip (Fig. 3).

To verify the position of the stimulating electrodes, lesions were made by passing a current of 20 μA for 20 sec (anodal for inner contact).

The animals were deeply anesthetized and perfused through the heart with 10% buffered formalin solution. Brains were then removed and 50 μm-frozen sections were cut, stined with Cresyl Violet and counterstained with Neutral Red. In some cases, sections through the stimulating electrode sites were stained with Cresyl Violet alone.

Fig. 3: Histological section through the dorsal lateral geniculate nucleus (LGNd) showing typical recording electrode placement. The dark spot (arrow) marking the site of the electrode tip was produced by iontophoretically ejecting Pontamine Sky Blue dye. The electrode tract is visible passing through the hippocampus just dorsal to the LGNd.

6. Determination of Agonist Potencies

In order to determine the relative potencies of adrenergic agonists in activating LGN neurons, each drug was compared with NE as a standard. It was not possible to utilize the latency to 50% activation (T_{50}) as a measure of agonist potency since ejections of sufficient duration to produce a plateau at maximal activation in some cases generated a response of such long duration that comparisons between drugs was impractical. Therefore, agonists were compared on the basis of the iontophoretic currents necessary to produce an equal activation. Upon encountering a cell, the response to a one min iontophoretic pulse of NE was determined. If necessary, the current was adjusted to give a robust activation. The cell was then tested with one min pulses of the agonist drug. Successive pulses of increasing current were applied to the cell until the response equalled or exceeded that produced by NE. The degree of activation was defined as the maximum rate obtained per 10 sec epoch during the 90 sec period following the onset of the ejection. The maximum rate usually occurred at the end of or immediately following the pulse of drug. The response produced by the agonist and that produced by the test dose of NE were compared graphically. For each cell, a log dose-response curve was constructed where the dose was taken to be the iontophoretic current of the one min drug pulse. The agonist current producing an activation equal to that of the test dose of NE was estimated from the graph. Care was taken to utilize only the linear portion of the "S"-shaped curve. The ratio I_{NE}/I_A was then calculated where I_{NE} was the current of NE applied to the cell and I_A was the current of the agonist estimated to produce an equivalent

degree of activation. This ratio provides an indication of the relative potencies of the agonist drugs under the assumption of similar transport numbers. This assumption was not verified experimentally, although two of the compounds (NE and dopamine) showing very different potencies have been reported to share similar transport numbers (Bevan et al., 1978).

B. MATERIALS

Drugs used for iontophoresis and intravenous or intraperitoneal administration were all of the highest purity obtainable from the sources noted.

For iontophoresis, solutions were prepared in distilled water and adjusted to their final pH with either HCl or NaOH. The following solutions were used, all 0.1 M and pH 4.0, unless otherwise indicated: acetylcholine chloride (Calbiochem, La Jolla, CA); carbamylcholine chloride (carbachol; Aldrich Chemical Co., Milwaukee, WI); clonidine HCl (Boehringer-Ingelheim, Ltd., Elmford, NY); dopamine HCl (Calbiochem, La Jolla, CA); L-epinephrine D-bitartrate (Regis Chemical Co., Morton Grove, IL); L-glutamic acid, monosodium salt, 0.5 M, pH 8.0 (Sigma Chemical Co., St Louis, MO); L-isoproterenol D-bitartrate (Regis); methysergide maleate, 0.01 M, pH 4.4 (Sandoz Pharmaceuticals, East Hanover, NJ); magnesium chloride, 1 M; L-α-methyl norepinephrine (Winthrop, Rensselear, NY); norepinephrine bitartrate (Regis); L-phenylephrine HCl (Sigma); picrotoxin, saturated solution (Sigma); piperoxane HCl (Rhône-Poulenc, Paris); phentolamine mesylate, 20 mg/ml (Ciba Pharmaceuticals, Summit, NJ); serotonin creatining sulfate monohydrate, 0.04 M (Regis); WB-4101 (2-[(2',6'-dimethoxy)phenoxyethylamine] methylbenzodioxane; WB Pharmaceuticals, Ltd., Backnell, Berkshire, UK).

For systemic administration, drug solutions were prepared in 0.9% sodium chloride.

All solutions were prepared fresh or stored frozen (in airtight tuberculin syringes) at -30° C.

PART III: EXPERIMENTAL STUDIES

The experimental results presented in this dissertation are oragnized into four major sections. The first section deals with the characterization of the adrenoceptor on LGNd neurons based upon the ability of NE and other sympathomimetic amines to increase the spontaneous firing rate of these neurons. The second section considers the interaction between NE and the major synaptic input to the LGNd by exploring the effects of NE on activity evoked by electrical stimulation or by light. In this section a comparison is made between NE and serotonin (5-HT), another monoamine present within axons which innervate the geniculate. The third section presents studies demonstrating that NE acts as a "neuromodulator" rather than as a conventional excitatory transmitter. Data is also provided which indicates that NE can facilitate the excitability of relay neurons by a postsynaptic mechanism and that the effect is not mediated by adjacent interneurons. In the fourth section, a comparison is made beteen the effects of iontophoretically applied NE and electrical stimulation of the locus coeruleus.

A. CHARACTERIZATION OF THE ADRENOCEPTOR ON LGNd NEURONS

1. General Characteristics of Units Studied

Most LGNd units exhibited extracellular action potentials having a positive-negative wave shape of approximately 1.5 msec duration (Fig. 4A). The spontaneous firing rate varied among units and some had an erratic or

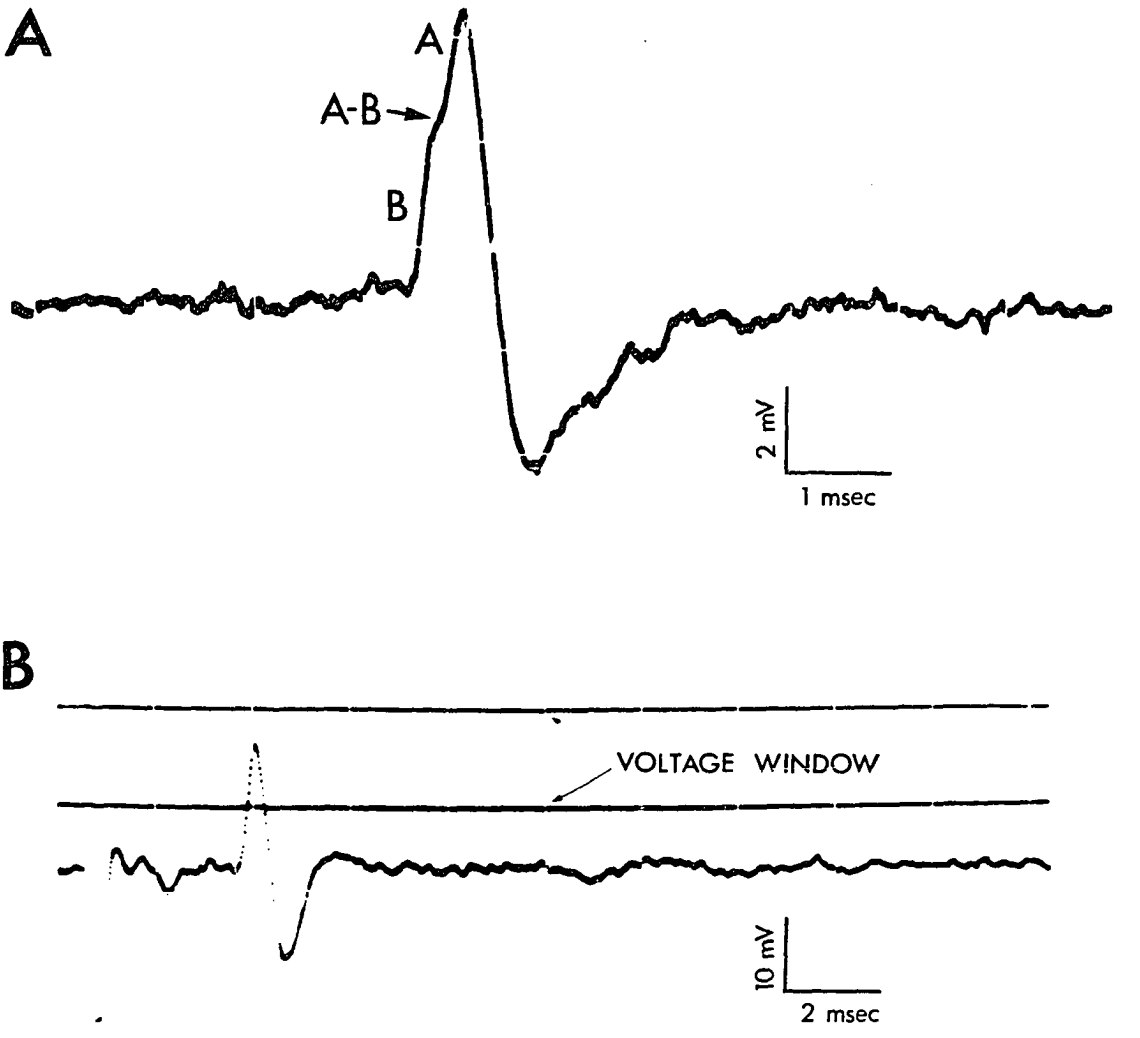


Fig. 4: A, Storage oscilliscope tracing of a spontaneous extracellular action potential from a geniculocortical relay neuron. The initial segment (B) and soma-dendritic components (A) are indicated. "A-B" marks the inflection point. B, Action poential evoked by an optic chiasm shock. The spike amplitude falls within the boundaries of the window discriminator. The oscilliscope beam is chopped electronically to produce three traces.

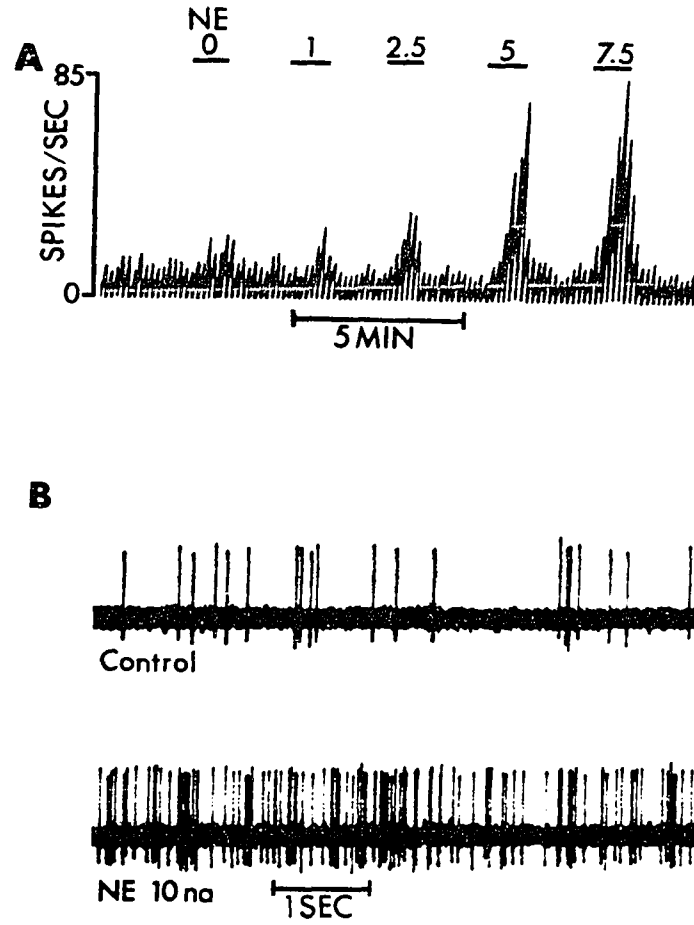
oscillatory firing pattern even under conditions of constant illumination. Action potentials occurred singly or in bursts of up to 4 or 5 spikes. All cells responded with a change in firing pattern to movement of a light beam across the visual field.

2. Response to Norepinephrine

Microiontophoretic application of NE with conventional 5-barrel pipettes produced a powerful activation of the majority of LGNd neurons studied. In a representative sample of 191 spontaneously active cells, 175 responded to 1-30 nA, one min pulses with at least a two-fold increase in spontaneous firing rate. The remainder were unresponsive or showed less than this degree of activation. Most cells were maximally activated with currents of NE in the 5-15 nA range. There was a variable latency to the onset of the response, usually 20-50 sec, and the activation persisted for 20-60 sec following the pulse of NE. No pure depressant responses to NE were observed although frequently with supramaximal ejection currents a transient depression (<40 sec) occurred prior to the facilitation in rate. In other cases, with excessive ejection currents, there was a period of depression (or simply a loss of responsiveness) following an initial activation. Glutamate was able to produce an excitatory response when ejected during this period of depression (4 cells).

The degree of activation was related to the magnitude of the ejection current in a dose dependent fashion within a relatively narrow range of currents (Fig. 5A). Higher currents tended to decrease the latency to full activation and prolong the period of enhanced rate but generally produced no further increase in maximum rate. Fig. 5B shows an oscillographic record of the spike discharges of an LGNd unit before and during

Fig. 5: Activation of LGNd neurons by microiontophoretic application of norepinephrine (NE). A, Varying the iontophoretic current of NE produces a dose-dependent increase in the magnitude of the activation. Cellular activity is expressed as an integrated rate histogram with a period of 10 sec. In this and subsequent figures, periods of drug ejection are indicated by bars above each record; numbers refer to the iontophoretic current in nanoamperes. B, Extracellular action potentials of a single unit before (upper) and during (lower) the iontophoretic application of NE (10 nA). Reproduced directly from an oscillographic record. Negativity is upward.



the iontophoretic ejection of NE. Note that the extracellular spike amplitude is constant and that the pre-drug bursting pattern is maintained although the overall rate is markedly increased by NE.

Deepening the level of chloral hydrate anesthesia tended to decrease the spontaneous firing rate of LGNd neurons. There was a corresponding reduction in the responsiveness of the cells to NE and, in some cases of particularly deep anesthesia, the effect was completely abolished. For this reason all experiments were conducted in lightly anesthetized animals.

3. Responses to Sympathomimetic Amines

A series of sympathomimetic amines were compared with NE for their ability to activate LGNd neurons (Table I). Several agonists mimicked NE but their potencies varied. Epinephrine was the most potent agonist tested. In the majority of cells, epinephrine gave a somewhat greater degree of activation than that produced by equal current pulses of NE (13 of 15 cells; Fig. 6A).

The α-agonist phenylephrine was as effective as NE or epinephrine in activating LGNd neurons but it was less potent and the responses tended to be more prolonged (9 cells; Figs. 6B,C). α-methylnorepinephrine was similar in potency to phenylephrine; the duration of the responses were comparable to that produced by NE (9 cells) Dopamine was less potent than the other agonists and in some cases it was not possible to achieve degrees of activation equivalent to that produced by NE even with currents up to 50 nA (5 cells).

Eleven cells in chloral hydrate anesthetised animals were tested with isoproterenol at currents ranging from 3-50 nA. None of the cells responded with activation or depression (Fig. 6A).

TABLE I

Relative Potencies of Adrenergic Agonists In Activating Lateral Geniculate Neurons

Agonist	Number of Cells Tested	Potency Ratio*
Epinephrine	8	1.9 ± 0.2
Norepinephrine		1
Phenylephrine	6	0.38 ± 0.06
α-Methylnorepinehrine	8	0.34 ± 0.08
Dopamine	5	0.21 ± 0.04
Clonidine	12	†
Isoproterenol	22	< 0.09 ± 0.02§

*Ratio between magnitude of control NE current and current of agonist producing equivalent activation. For details of test procedure and calculations, see Methods and Materials. Data expressed as mean ± S.E.M.

†Weak activation (see Fig. 6) was obtained in 6 cells, others were unresponsiveness.

§Mean of data from 7 cells where activation was obtained (all in cerveau isolé animals), other cells were unresponsiveness.

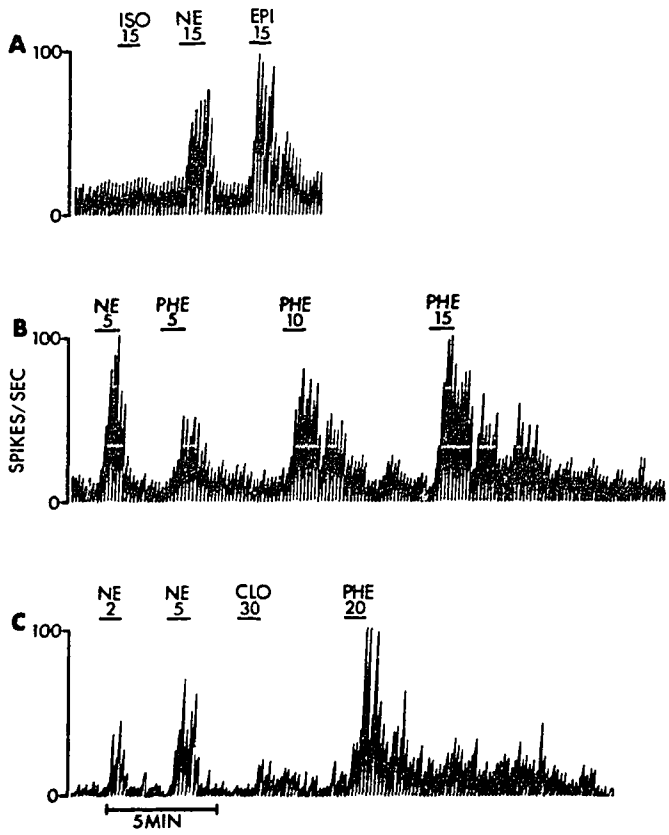


Fig. 6: Response of LGNd neurons to adrenergic agonists. A, Comparison of equal iontophoretic currents of isoproterenol (ISO), norepinephrine (NE) and epinephrine (EPI). B, Dose-dependent activation produced by phenylephrine (PHE). PHE has equal efficacy but less potency than NE. Note the prolonged nature of the response to PHE. C, Comparison of the weak activation produced by high currents of clonidine (CLO) with the response to NE and PHE.

4. Effects of Adrenoceptor Antagonists

Phentolamine, piperoxane and WB-4101 reliably blocked the response of LGNd neurons to NE (Fig. 7). With each drug, the effect was dose related and reversible. In addition, the blockade was selective in that low currents of the antagonists failed to attenuate glutamate excitations. The benzodioxane WB-4101 was the most potent of the antagonists studied. In 16 cells, WB-4101 applied with currents of 0-5 nA for 2 min or more produced a 79-100% reduction in the response to NE. ("0 nA" refers to the removal of the retaining current only without application of an ejecting current.) In most cases the activation was completely blocked but in one-half of the cells tested with currents of WB-4101 within the 2-5 nA range, there was some reduction in baseline firing rate.

Phentolamine was tested on 5 cells with currents of 5-10 nA. The response to NE was reduced 73-100% with ejections of 2 min or more. There was no decrease in baseline firing rate in any of the cells tested.

Piperoxane produced a 63-91% reduction in NE activations when currents of 5-10 nA were applied for 2 min or more (8 cells). Piperoxane also had a tendency to reduce the baseline firing rate of 50% of the cells.

Sotalol had variable effects on 15 cells with 5-25 nA ejections. Currents within the 5-10 nA range occasionally attenuated the response to NE but the effects were not consistent for repeated ejections on the same cell. In most cases, only a weak blockade was produced by these currents of sotalol and in 6 cells there was a potentiation of the response to NE. At higher currents (20-25 nA), sotalol did produce a consistent blockade of the response to NE (3 cells). In most cases sotalol did not alter the baseline firing rate significantly although 3 cells showed a slight

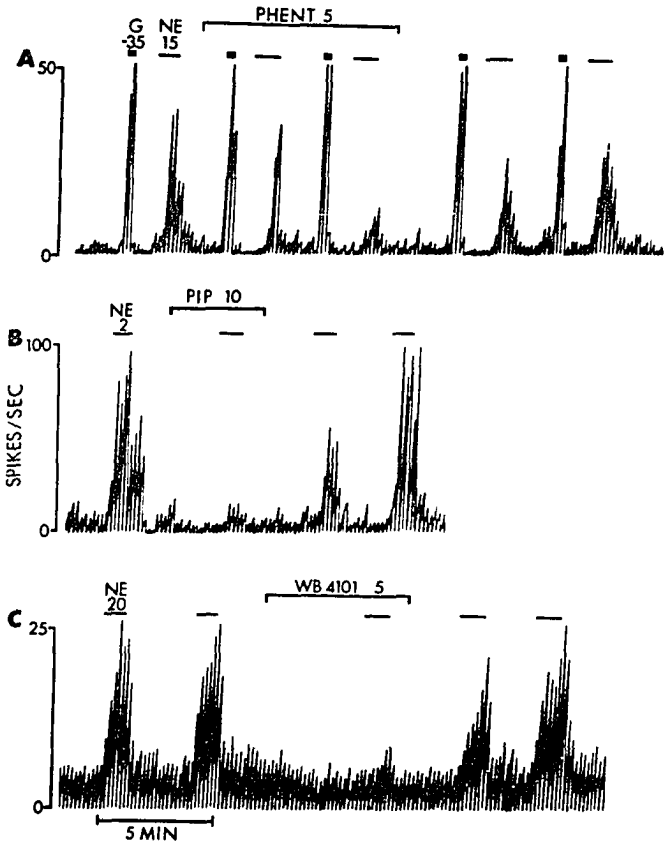


Fig. 7: Antagonism of norepinephrine (NE) induced activation of LGNd neurons by the α-adrenolytic drugs phentolamine (A), piperoxane (B) and WB-4101 (C). In A, the ability of these antagonists to selectively block the response to NE but not to glutamate (G) is illustrated.

increase in spontaneous activity.

The potencies of the antagonists were compared by determining the degree of inhibition of the response to NE attained at various times following the initiation of a continuous 5 nA ejection of the antagonist. These data are expressed graphically in Fig. 8. The rank ordering of potencies of the antagonists as determined by this procedure is WB-4101 > phentolamine $\geq$ piperoxane > sotalol.

5. Effects of Systemically Administered WB-4101

To confirm that the ability of α-adrenoceptor antagonists to attenuate the response to NE is not due to an artifact of the iontophoretic technique, in some experiments, WB-4101 was administered systemically. Doses within the 1-5 mg/kg range generally produced a selective blockade of the response to NE. In some cells, these doses of WB-4101 caused a depression of the baseline rate whereas in others no change in spontaneous firing was observed. An example of the selective blockade is given in Fig. 9B. Note that the effect of NE is markedly diminished after repeated intravenous injections of WB-4101 but that the responses to glutamate and acetylcholine are for the most part preserved. Fig. 9A demonstrates the effect of an intraperitoneal injection of a higher dose of WB-4101.

6. Effects of Clonidine

At low ejection currents, iontophoretic clonidine did not alter the spontaneous firing rate of LGNd neurons, whereas higher currents (20-35 nA) tended to produce a slight activation of firing (8 of 12 cells) (Fig. 6). The activations with clonidine usually occurred after the cessation of current ejection and were prolonged, lasting 4-12 min. The response to test doses of NE was occasionally attenuated following clonidine.

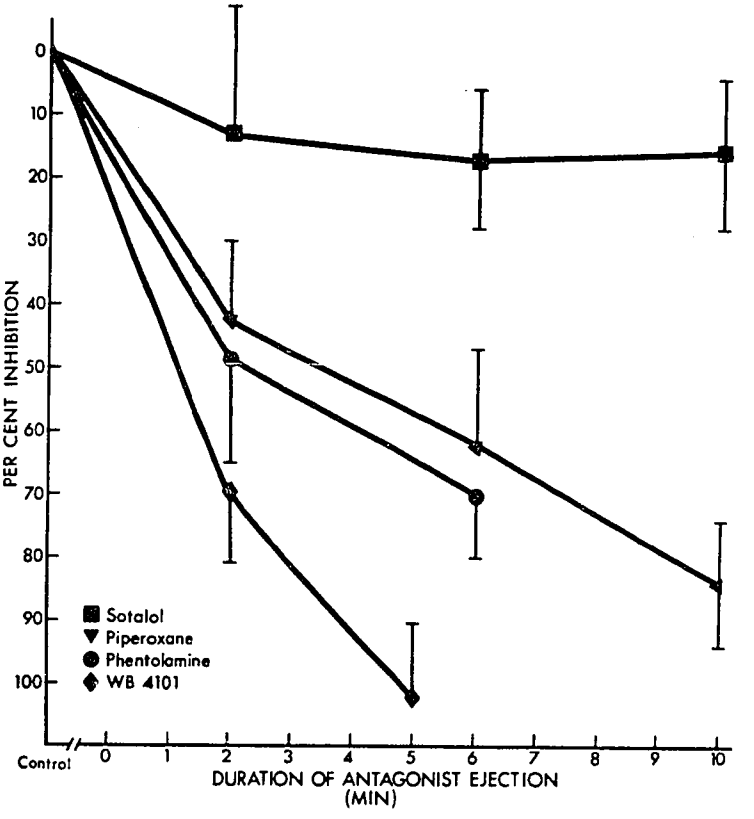
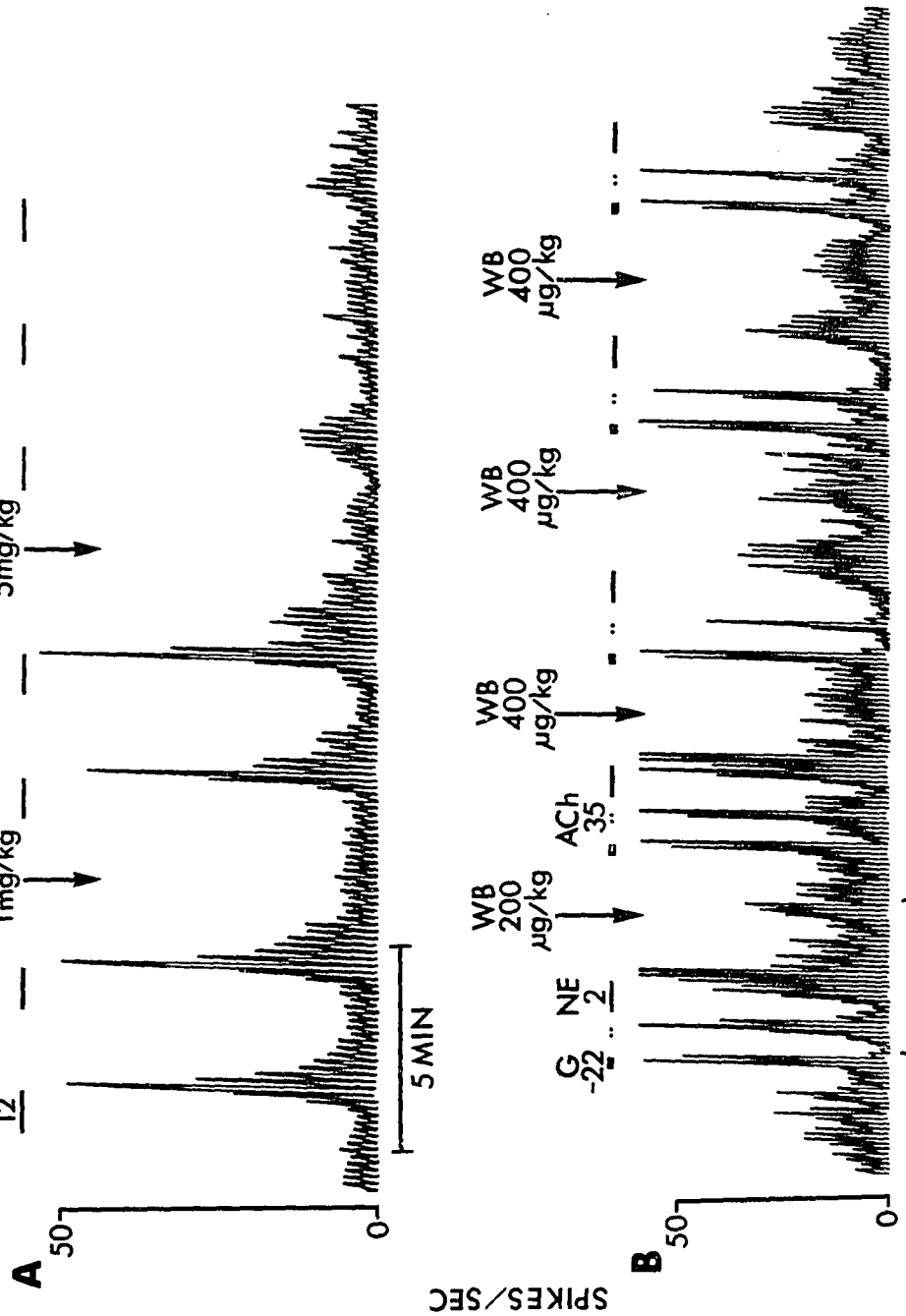


Fig. 8: Comparison of the effects of various adrenergic antagonists on the activation of LGNd neurons by NE. Each cell was tested with one min pulses of NE before (control) and at various times after the onset of a continuous ejection of the antagonist at 5 nA. The percent inhibition of the response to NE (ordinate) was calculated according to the formula $[(A_o - A_t) \div (A_o - S)] \times 100$ where A_o is the maximum rate per 10 sec epoch produced by the control NE pulse, A_t is the maximum rate produced by a pulse of NE initiated at time t during the antagonist ejection and S is the baseline spontaneous rate of the cell. The abcissa refers to the time during the antagonist ejection at which the NE test pulse began. Each point represents the mean ± S.E.M. of data from 5-8 cells.

Fig. 9: Antagonism of iontophoretically applied norepinephrine (NE) by intraperitoneal (A) or intravenous (B) WB-4101 (WB). In B, the response to glutamate (G) and acetylcholine (ACh) is relatively less affected than is the response to NE.



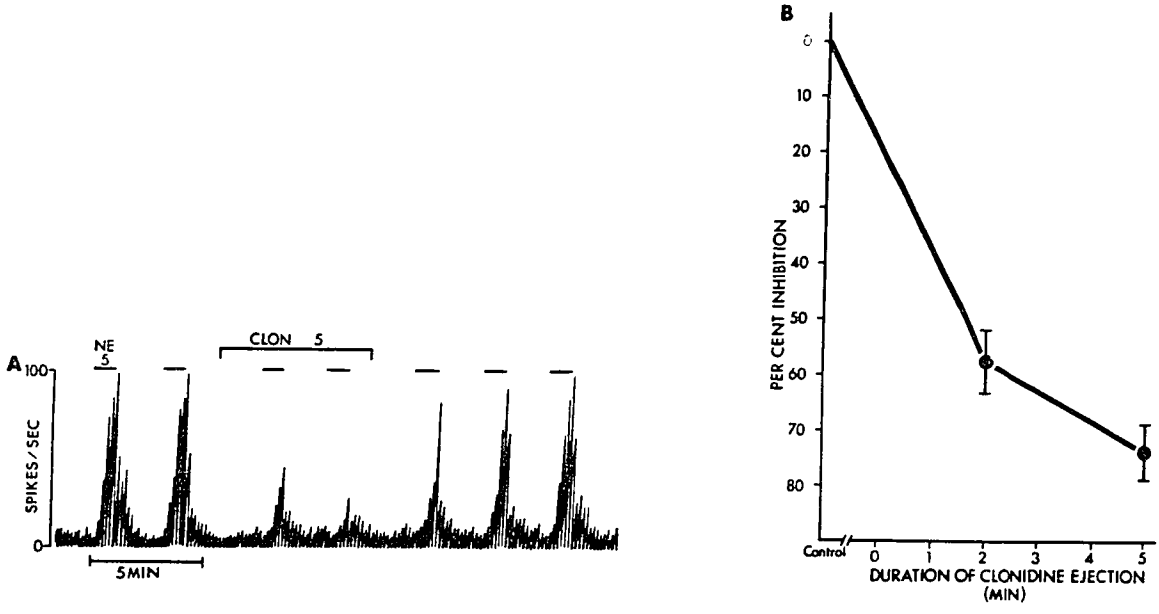


Fig. 10: Antagonism of norepinephrine (NE) induced activation of LGNd neurons by low iontophoretic currents of clonidine. A, Typical effect of clonidine. B, Pooled data from 6 cells expressed as described in the caption of Fig. 8. Clonidine was applied at currents of 5-10 nA.

Furthermore, continuous low current ejections of clonidine (5-10 nA) effectively blocked the activation produced by pulses of NE (Figs. 10A,B).

7. Responses in Unanesthetized Animals

In order to determine if general anesthesia alters the pattern of pharmacological sensitivity of LGNd neurons experiments were carried out in 5 unanesthetized, cerveau isolé animals. Twenty-five of 27 cells tested in the transected rats showed at least a two-fold increase in rate with 1-10 nA, one min pulses of NE. The response pattern was qualitatively identical to that observed in anesthetized animals, although the cells appeared to be somewhat more sensitive to NE.

As in chloral hydrate anesthetized animals, many cells in the unanesthetized preparation were not activated by any current of isoproterenol which could be passed. However, some cells which were particularly sensitive to NE, did show a weak response to high currents of isoproterenol (7 cells). In no case was it possible to achieve full activation with isoproterenol; in fact, increasing the current beyond that giving maximal activation tended to reduce the magnitude of the response.

Phentolamine, when applied at currents of 5-10 nA for 2 min or more, was as effective an antagonist of the response to NE in unanesthetized animals (6 cells) as it was in the anesthetized preparation.

8. Discussion

Microiontophoresis of NE and other sympathomimetic amines caused an increase in the firing rate of most of the spontaneously active LGNd neurons which were examined. This response was reproducibly elicited only when low iontophoretic currents were applied for relatively prolonged periods. Increasing the ejection current strength within a limited range

usually shortened the latency and enhanced the magnitude and duration of the responses. However, currents in excess of this range often produced biphasic effects in which the facilitation was followed by normal or diminished activity. The loss of responsiveness observed with high currents could be due to overdepolarization, to a direct or indirect action of the drug on inhibitory receptors or to tachyphylaxis. Since glutamate had an excitatory action during this depression in the cases where it was tested, the effect cannot be attributed to overdepolarization in all instances. Nevertheless, some cells did show signs of overdepolarization (widening and decrease in amplitude of the extracellular action potential) when tested with supramaximal agonist currents.

As outlined in Part I, previous investigators have reported variable effects of iontophoretic NE in the LGNd. I attribute the discrepancy between the present findings and these earlier studies to the fact that iontophoretic dose was not systematically controlled in the previous investigations. Rather than characterizing the response of a particular cell on the basis of a single ejection current, in the present study most cells were tested with a range of currents. In this way it was possible to adjust for variations in electrode properties and differences in the local environment of the electrode tip. It was possible to produce depressant responses to NE quite easily by using supramaximal ejection currents, suggesting that use of excessive doses was responsible for the variable effects observed in previous studies.

Since most cells demonstrated a uniform activation with adrenergic agonists under the present experimental conditions, it was possible to systematically determine the pharmacological characteristics of the response. In peripheral tissues, two procedures are used to classify

responses mediated by adrenergic receptors. In the first procedure, the relative potencies of a series of adrenergic agonists are compared; in the second, a determination is made of the potencies of antagonists for inhibiting the response to an agonist.

According to the definition proposed by Furchgott (1972), a response mediated by an adrenergic receptor of the α-type is pharmacologically characterized by: (1) a relative potency series in which epinephrine > NE > phenylephrine > isoproterenol and (2) a susceptibility to specific blockade by low doses of phentolamine and other α-adrenergic antagonists. The activation of LGNd neurons by sympathomimetic amines satisfys both of these criteria. The rank ordering of the potency ratios of the agonists fits the appropriate sequence. Furthermore, phentolamine and other α-blockers were potent and specific antagonists of the response to NE. β-adrenergic drugs exhibited weak or no activity. A similar pattern of pharmacological sensitivity has been observed in other brain areas where NE has apparent excitatory actions, although systematic comparisons between agonists and antagonists have not been carried out in most cases (Bevan et al., 1977; Boakes et al., 1971; Bradley et al., 1966; Kirsten and Sharma, 1976; Yamamoto, 1967; also see Part I).

The pharmacological profile of the activation of LGNd neurons corresponds closely with that of adrenergic responses of the α-type present in peripheral tissues. Thus, for example, sympathomimetic agents causing constriction of smooth muscle in the aorta (Besse and Furchgott, 1976; Sheys and Green, 1972), pulmonary artery (Starke et al., 1975), spleen (Sheys and Green, 1972), vas deferens or intestine (Van Rossum, 1965) show a relative potency series: epinephrine > NE > phenylephrine > α-methylnorepinephrine > dopamine. Phentolamine is a potent antagonist

of these responses and in the vas deferens, as in the LGNd, the potencies of antagonists have the following rank ordering: WB-4101 > phentolamine > piperoxane (Mottram and Kapur, 1975; Van Rossum, 1965). α-receptors demonstrating this pattern of sensitivity have been termed "postsynaptic" or α_1-adrenergic receptors to distinguish them from the receptors regulating the release of NE from sympathetic neurons which, although strictly of the α-type, do show certain pharmacological differences from classical α-receptors. These neuronal receptors have been referred to as "presynaptic" or α_2-adrenergic receptors (Langer, 1974; Berthelsen and Pettinger, 1977).

The α_1-adrenoceptors in the LGNd exhibit certain striking pharmacological differences from other adrenergic receptors in the central nervous system. For example, catecholamine receptors mediating depression of noradrenergic neurons in the locus coeruleus have been classified as α_2 based upon their equal sensitivity to NE, epinephrine and dopamine, slightly greater sensitivity to α-methylnorepinephrine, and marked sensitivity to clonidine (Cedarbaum and Aghajanian, 1977). Receptors on neurons in other brain regions such as the cerebellum (Hoffer et al., 1971) and hippocampus (Segal, 1974) which show a predominantly depressant response to NE have characteristics resembling those of peripheral β-adrenoceptors (see Part I).

Using the radioligand ^{3}H-WB-4101, it has recently become possible to specifically label a population of receptors in brain and peripheral tissues which show drug specificities characteristic of α_1-adrenoceptors (U'Prichard and Snyder, 1979). With one major exception, there is an excellent correspondence ($r=0.997$) between the affinities of agonists for brain α_1-receptors determined by their potencies as displacers of ^{3}H-WB-4101 binding and their ability to activate LGNd neurons (Fig. 11). However,

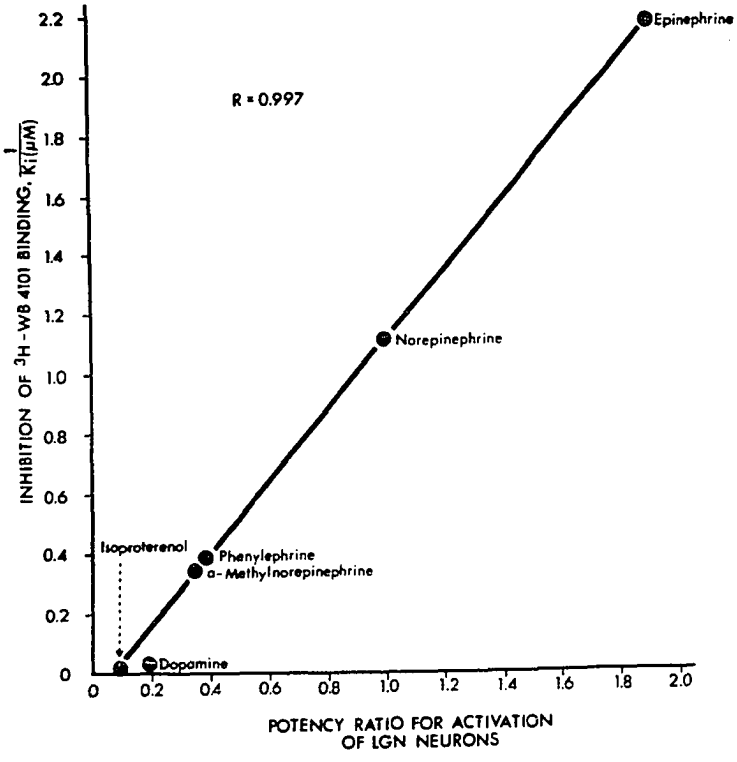


Fig. 11: Comparison between affinities of sympathomimetic amines for brain α-adrenoceptors and ability of drugs to activate LGNd neurons. Data concerning the potencies of drugs in displacing specific ^{3}H-WB-4101 binding to rat brain membranes are taken from U'Prichard and Snyder (1979) and U'Prichard et al. (1977). Affinities (ordinate) are expressed as the reciprocol of the K_i. Iontophoretic potency rations (abcissa) are from Table I.

clonidine, a ligand with extremely high affinity for brain α_1-receptors, is only weakly active as an agonist in the LGNd. This discrepancy may be explained by noting that, unlike the other α-agonists, clonidine did not appear to exhibit full efficacy even at high ejection currents, indicating that it may be a partial agonist with weak intrinsic activity. Clonidine was also able to block the response to NE at currents at which it produced no activation. These observations suggest that clonidine acts as a mixed agonist-antagonist at central postsynaptic α-receptor sites. This conclusion is consistent with other physiological (Boissier et al., 1968; Hodge and Robinson, 1972; Schüman and Endoh, 1976; Stone and Taylor, 1978) and biochemical (Davis and Maury, 1978; Skolnick and Daly, 1975; Vetulani et al., 1977) evidence indicating that clonidine can act as an adrenergic antagonist. However, it would appear that the dose of clonidine required to block LGNd α_1-receptors is significantly higher than that necessary to depress locus coeruleus neurons (Svensson, 1975). Therefore, low doses of clonidine would be expected to act primarily at pre- rather than postsynaptic α-adrenergic sites.

B. EFFECTS OF NOREPINEPHRINE ON EVOKED ACTIVITY; COMPARISON WITH SEROTONIN

1. Identification of Geniculocortical Relay Neurons

In subsequent studies, most LGNd units were identified using orthodromic activation according to the criteria of Burke and Sefton (1966) and Sumitomo and Iwama (1977). Only "P-type" units were studied. As indicated earlier, these units first respond to electrical stimulation of the afferent visual pathway with a single short latency spike and then, following a silent period of variable duration (typically 70-230 msec),

discharge groups of up to 4 spikes at regular intervals. No attempt was made to further classify the P-type units.

In some cases, antidromic activation of the visual cortex was used to confirm that the cell exhibiting the P-type response was indeed a geniculocortical relay neuron. The criteria used for identifying antidromically-evoked action potentials were: (1) constant latency of the initial response, (2) ability to follow paired shocks at a high frequency and (3) collision with spontaneous action potentials occurring immediately prior to the stimulus (Figs. 12B-D).

2. Comparison of the Response of Spontaneously Active LGNd Neurons to Norepinephrine and Serotonin

In all subsequent experiments, 6-barrel electrodes were used which consisted of a fine single glass micropipette glued to a conventional 5-barrel array (see Methods and Materials). These pipettes allowed superior unit discrimination because extracellular action potentials tended to be of greater amplitude. Initially, I examined the response of spontaneously firing relay neurons to NE delivered with 6-barrel electrodes and compared the effect with that produced by 5-HT. Units were selected which demonstrated a stable rate of firing under conditions of constant low level illumination. As was found with conventional 5-barrel pipettes, these cells responded to low microiontophoretic currents of NE (1-20 nA) with a delayed increase in firing frequency. The latency to the onset of the response was typically 30-120 sec. (The delay was, in general, greater in the present series of experiments presumably because of the separation between the recording and iontophoretic barrels.)

In contrast to NE, 5-HT, at similar iontophoretic currents, depressed the firing of all of the LGNd units tested (Figs. 12A; 13A,B).

Fig. 12: Response of an antidromically identified LGNd relay neuron to iontophoretically applied norepinephrine (NE) and serotonin (5-HT). A, The spontaneous firing rate of the cell was facilitated by NE and depressed by 5-HT. B, Antidromic activation of the cell by stimulation in the region of the visual cortex (VC). Stimuli (arrowhead) produced an all-or-none response of constant latency. Five superimposed sweeps. C, Ability of cell to follow high frequency paired shocks. At the interstimulus interval shown (2 msec), the cell followed the second shock without failure. Slightly shorter intervals resulted in failure of the second spike. D, Collision between spontaneous and antidromic action potentials. The spontaneous spike (dot) triggered the oscilliscope sweep and, at a predetermined delay, a VC stimulus. When the VC stimulus followed the spontaneous spike by 2.4 msec (upper trace) no collision occurred and an antidromic spike (asterisk) is observed. With a 1.7 msec delay (lower trace), collision occurred as indicated by failure of the antidromic spike. Stimulation parameters: 0.2 msec, 1.25 mA, biphasic. Filtering: low frequency cutoff, 100 Hz; high frequency cutoff, 2 kHz.

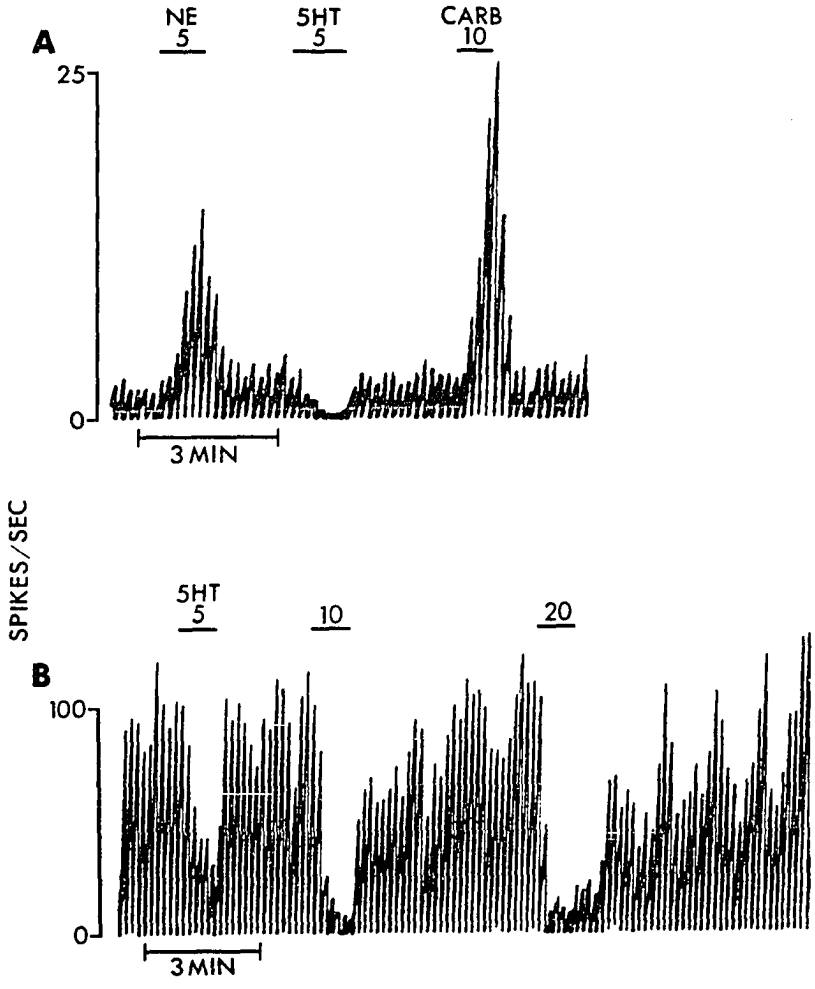


Fig. 13: A, Comparison between the effects of iontophoretically applied norepinephrine (NE), serotonin (5-HT) and carbachol (CARB) on a spontaneously active LGNd neuron. B, Dose dependent depression of another cell by 5-HT.

The onset of the response to 5-HT, typically 10-20 sec, was somewhat more rapid than the onset of the response to NE. In paired comparisons on 25 cells, an opposite effect of the two amines on the spontaneous firing rate was invariably observed (Figs. 12A & 13A). For these cells, NE (2.5-20 nA, mean 8.4 nA) produced a 272 ± 175% increase in rate whereas 5-HT (5-20 nA, mean 10.2 nA) caused a 93 ± 10% fall in activity. In 10 cells, identical currents of NE and 5-HT produced a greater than 100% increase or a 90-100% fall in rate, respectively. Two cells showed rebound activations following cessation of the 5-HT ejections. Five cells were positively identified as geniculocortical relay (P-type) neurons by satisfying the criteria for antidromic activation given above (Figs. 12B-D). Most of the other cells were catagorized as P-type on the basis of their response to optic chiasm (OX) stimulation.

3. Effects of Acetylcholine and Carbachol

Relay neurons were also activated by acetylcholine (ACh) (Fig. 9B) and by the nonhydrolyzable cholinergic agonist carbamylcholine (carbachol) (Fig. 13A). The effect with ACh was variable and occasionally even high iontophoretic currents were ineffective. However, uniform responses were obtained with carbachol.

4. Comparison of the Effects of Norepinephrine and Serotonin on Electrically Evoked Activity

To examine the interaction of NE and 5-HT with the major synaptic input to the LGNd, relay neurons were orthodromically driven by stimulation of the afferent visual pathway at the level of the OX. Poststimulus time histograms (PSTHs) (100 sweeps) were generated during the application of constant current, rectangular pulses (150 μsec duration) to the OX at

1 Hz. The histograms were prepared immediately before and during the iontophoresis of NE and 5-HT. Since responses to the amines tended to be delayed, collection of the histograms was begun up to 120 sec following the onset of the ejections.

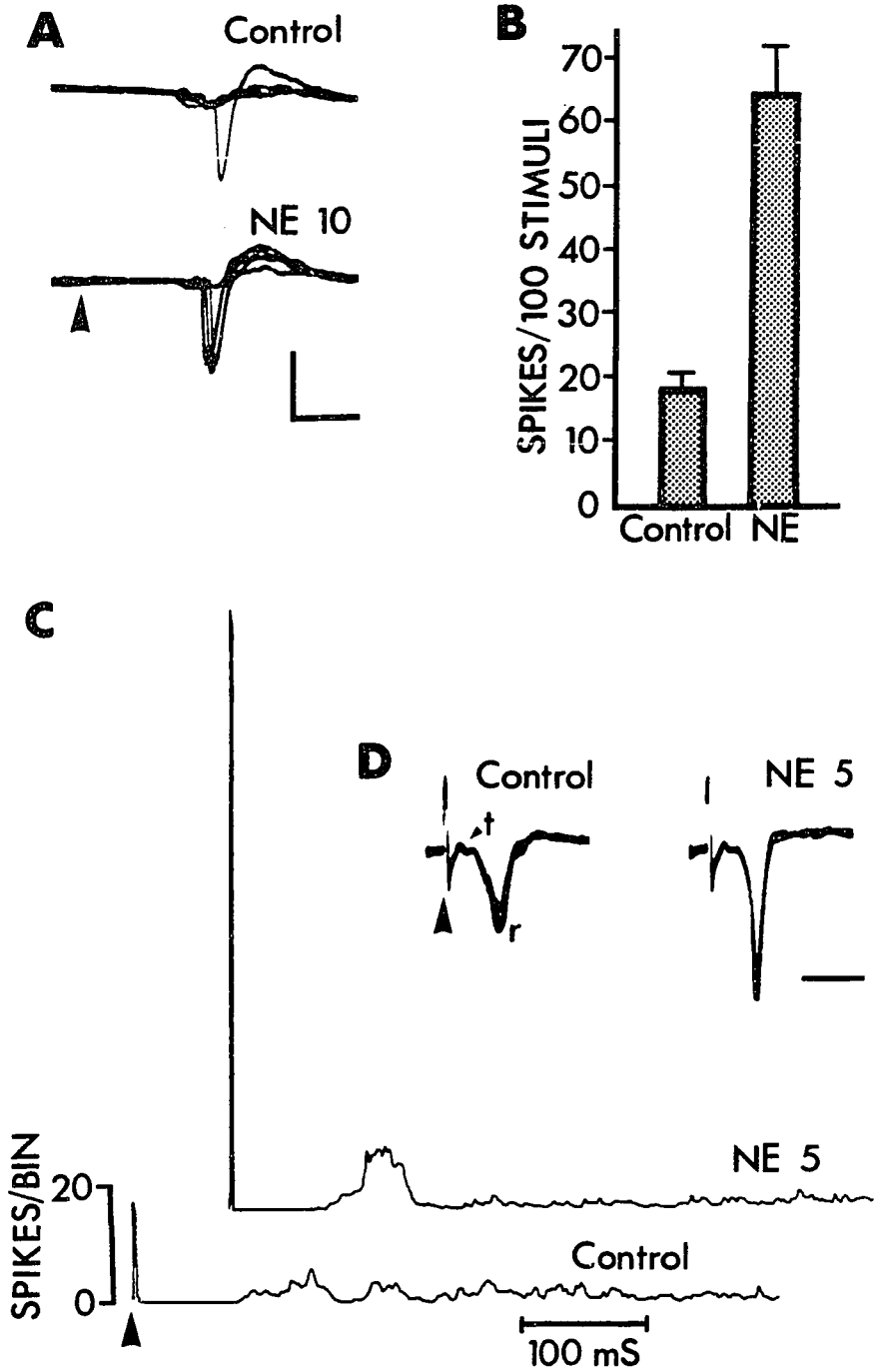
Iontophoresis of NE (5-10 nA) caused a marked facilitation of the early response to subthreshold OX stimulation. This was manifested by an increased probability of spike generation during the short latency response (11 cells) (Fig. 14). Facilitation of this component was apparent whether the stimuli produced a low (Fig. 15A) or moderate (Fig. 15B) percentage of short latency spikes under control conditions. In addition, there was a tendency for more spikes to occur immediately following the inhibitory period in what is referred to as the "post-inhibitory-pause rebound" in activity.

Occasionally, it was possible to adjust the stimulus intensity so that approximately 20% of the stimuli produced initial spikes. Under these conditions, iontophoresis of NE resulted in a 255% increase in the frequency of spike generation (Figs. 14A,B).

NE also enhanced the amplitude of the postsynaptic (r) component of the field response to optic chiasm stimulation which is believed to represent the mass activity of LGNd principal cells (see Part I) (Fig. 14D). During NE iontophoresis (5-30 nA), there was a 72 ± 19% (mean ± S.E.M.; 5 animals) increase in the peak-to-peak amplitude of this component.

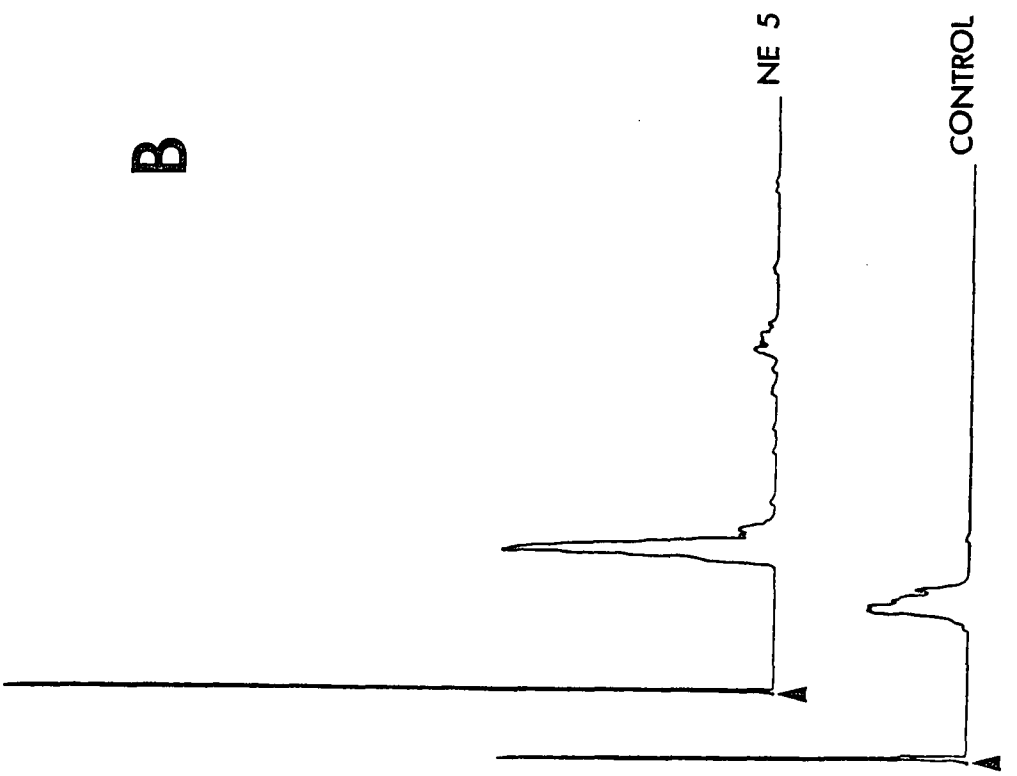
5-HT (5-15 nA) had depressant and essentially opposite effects from NE on the PSTH (7 cells) (Fig. 16A,B). The short latency component of the response appeared to be especially sensitive to 5-HT but later activity was also depressed. In many cases cellular activity was completely

Fig. 14: Norepinephrine (NE) facilitation of unitary action potentials (A,B,C) and field response (D) evoked by optic chiasm stimulation. In A, subthreshold shocks were applied to the optic chiasm at a frequency of 1 Hz. The stimulus strength was adjusted so that approximately 1 out of 5 stimuli produced a short latency spike discharge (upper trace). For the cell shown, 0.5 mA, 0.1 msec duration biphasic rectangular pulses satisfied this condition. The position of the stimulus artifact is indicated on the storage oscilliscope record by an arrowhead. Shock artifacts were electronically blanked in these traces. During NE iontophoresis (10 nA, 60 sec), 4 out of 5 stimuli now produce spikes under identical stimulation conditions (lower trace). Five superimposed sweeps. Calibration: 2 msec, 1 mV. Positivity is upward. B, Summary of data from 5 cells in 4 animals. The experimental procedure was as in A except that each unit was tested with 100 shocks before and during the iontophoresis of NE at 5-20 nA for 2-5 min and this was repeated 2-8 time per cell. Data are the mean ± S.E.M. of averages for each cell. C, Poststimulus time histogram demonstrating the effect of NE on the early and late responses to optic chiasm stimulation. In addition to a facilitation of the initial spike response (vertical line at left of each histogram), NE (5 nA, 50 sec) causes a shift in the late activity to a peak of high probability firing immediately following the inhibitory pause (upper histogram). Each histogram represents the summed responses to 100 stimuli delivered at 1 Hz. Stimulation para-



meters: 1 mA, 0.15 msec. Bin width: 2 msec. D, Field response before (left) and during (right) NE iontophoresis (5 nA). Stimulation parameters: 1 Hz, 1 mA, 0.25 msec. Five superimposed sweeps. Time Calibration: 2 msec.

Fig. 15: Effect of norepinephrine (NE) on the response of two LGNd neurons to optic chiasm stimulation. In A, under control conditions (lower histogram), the stimulus intensity is sub-threshold for production of an initial spike response but late activity does occur. In the presence of NE (upper histogram), short latency spikes are now frequent and a clear late peak ("post-inhibitory-pause rebound") is visible. Collection of the upper histograms was initiated 120 sec following the onset of the NE ejection. There was little change in the overall firing rate of the cell during NE. In this and subsequent figures, each PSTH represents the summated responses to 100 stimuli delivered at 1 Hz. The bin width is 2 msec. Numbers to the right of drug abbreviations refer to the iontophoretic currents in nanoamperes. Stimulation intensity: 1.5 mA. In B, under control conditions (lower histogram), one-half of the stimuli produced short latency spikes. A small late peak is also seen. NE (upper histogram) causes an enhancement of the initial and late peaks. Collection of this histogram was begun 60 sec after the onset of the NE ejection. There was a 180% increase in the total activity of the cell during NE iontophoresis. Stimulation intensity: 0.18 mA.



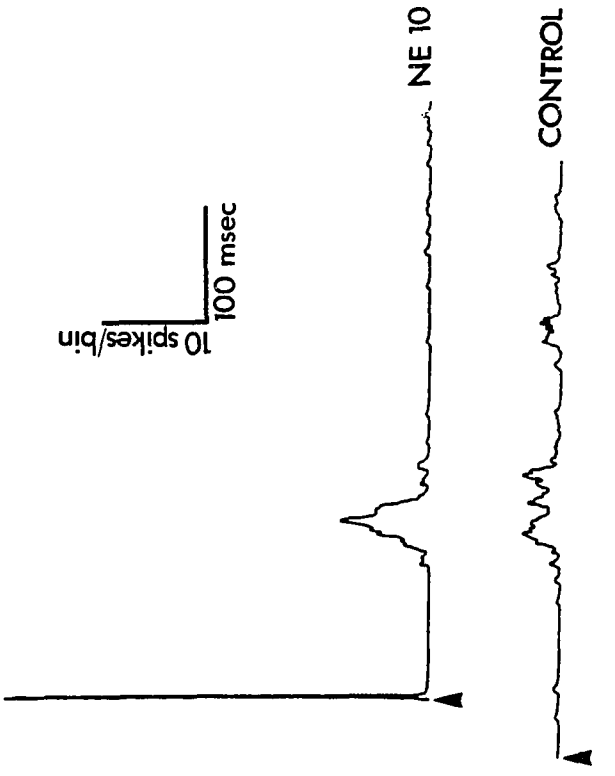
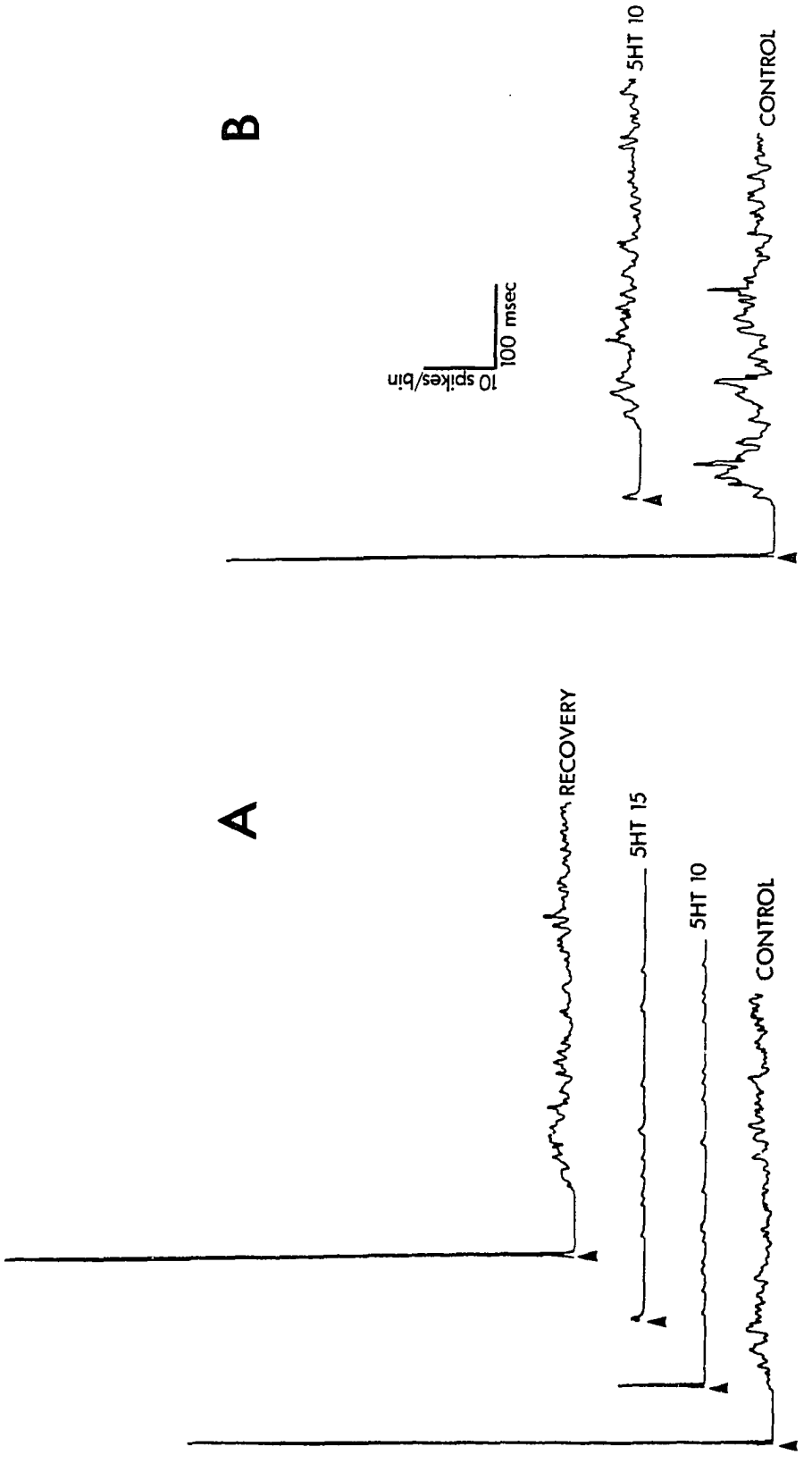


Fig. 16: Effect of serotonin (5-HT) on the response of two LGNd neurons to optic chiasm stimulation. A, Dose-dependent depression of poststimulus activity. Both early and late firing is reduced. Histograms were begun 72 sec following initiation of the 5-HT ejection. Recovery histogram was begun 85 sec after cessation of 5-HT ejection. Stimulation intensity: 3.75 mA. B, Response of second cell showing preferential sensitivity of early response to 5-HT. Begun 48 sec following 5-HT ejection. Stimulation intensity: 4.0 mA.



suppressed by 5-HT. In paired comparisons (12 cells), identical iontophoretic currents of NE and 5-HT applied to the same cell could be shown to have reciprocal effects on the response to OX stimulation (Fig. 17).

In a few cells, the effect of 5-HT on glutamate activated firing was examined. In confirmation of previous observation in the cat (Curtis and Davis, 1962; Tebecis and DiMaria, 1972), excitation by the amino acid was less sensitive to 5-HT than was spontaneous or synaptically evoked activity. Iontophoretic currents of 5-HT between 40 and 60 nA were generally required to produce clear depression of the response to glutamate.

5. Effects of Phentolamine and Methysergide

Since α-adrenoceptor antagonists were able to selectively antagonize the activation of spontaneous firing produced by NE, it was of interest to examine whether the facilitatory action of NE on evoked activity could be blocked in a similar fashion. PSTHs to OX stimulation were generated before and during the application of NE at 5 or 10 nA. The OX stimuli were applied with a current intensity just above threshold for the short-latency response. Recording of the histograms was begun 36 to 114 sec after the onset of the NE ejection. The sequence of two histograms (control and during NE) was then repeated during the continuous application of phentolamine at 10 nA. Results from a typical cell are shown in Figs. 18A-D. Note that phentolamine markedly diminishes the NE-induced facilitation of the short latency response, although the enhancement of the delayed response is also reduced. Six units were held sufficiently long to collect all histograms. On the average, NE caused a 1754% increase in the initial spike response of these units. In the presence of phentolamine, this increase was reduced to 424%, a 76% diminution in the effect of NE. By itself, phentolamine had no consistent effect on the PSTH: in two cells

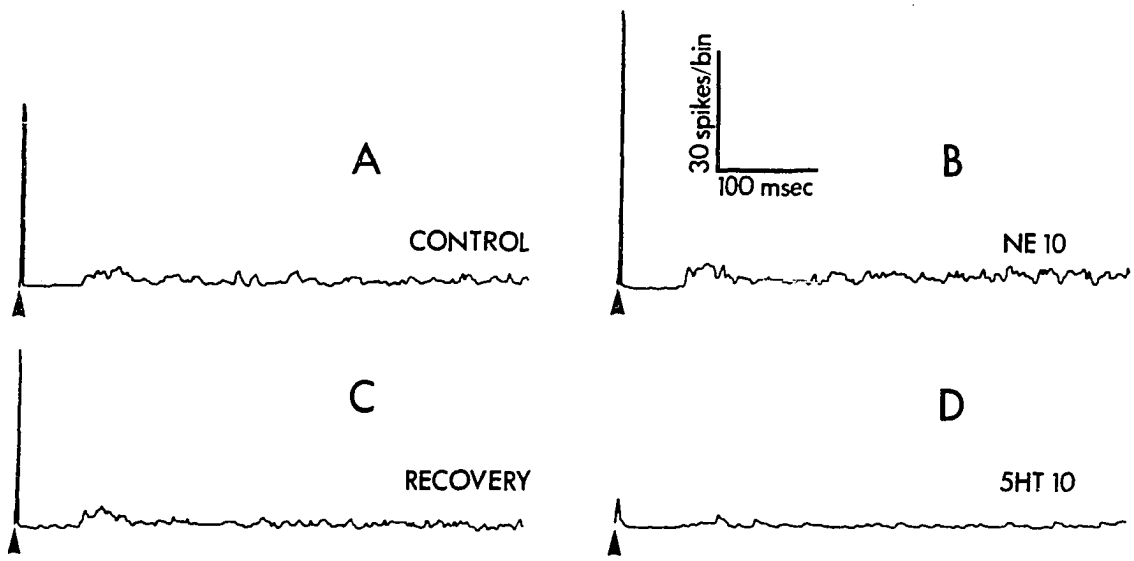
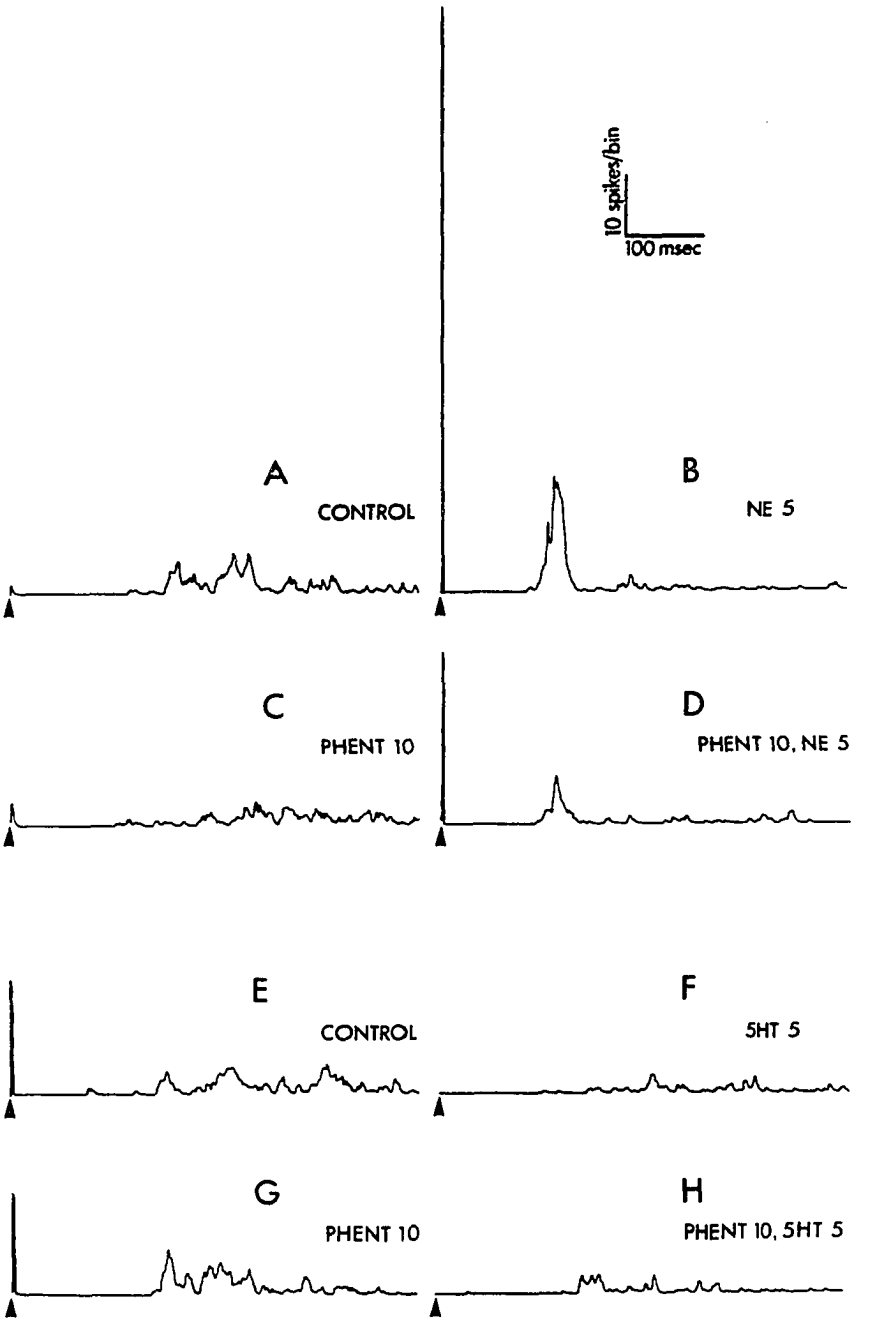


Fig. 17: Comparison of the effects of norepinephrine (NE) and serotonin (5-HT) on the response to optic chiasm stimulation. A, Short latency spikes were produced by 46% of stimuli. B, NE causes a 52% increase in the number of spikes in the early peak. C, The control response pattern in regained following cessation of the NE ejection. D, 5-HT causes a 83% reduction in the number of spikes in the early peak. Histograms were begun 30 sec following initiation of the drug ejections. Stimulation intensity: 0.25 mA.

Fig. 18: Effect of phentolamine (PHENT) on the modification of post-stimulus responses by norepinephrine (NE) and serotonin (5-HT). A, Under control conditions, the stimulus intensity is just subthreshold for production of the short latency spike response. B, NE increases the frequency of initial spike generation from 3% to 95%; a prominent late peak is also seen. C, PHENT alone produces a slight depression of the poststimulus response. In comparison with A, the total number of spikes is reduced by 20%. (PHENT had no consistent effect on the PSTH; see text.) D, In the presence of PHENT, the effect of NE is markedly attenuated. The total number of spikes in the initial and late peaks are reduced by 71% and 66%, respectively. E, At slightly higher stimulation intensities an initial spike response is now present under control conditions. F, 5-HT depresses the early and late activity. G, PHENT has little effect on the control activity and, H, does not alter the response to 5-HT. Collection of the histograms was begun 60 sec after the onset of the NE and 5-HT ejections. PHENT was ejected for at least 3 min prior to recording of histograms. Stimulation intensity: 2 mA.



there was a slight decrease in activity, in three cells a slight increase and in one cell there was no change. On average, there was a 13% increase in the initial peak during phentolamine. In three cells, the response to 5-HT was also tested before and during phentolamine. In none of these cells did phentolamine antagonize the depressant effect of 5-HT (Figs. 18E-H).

The peripheral 5-HT antagonist methysergide has recently been demonstrated to block the facilitation of motoneuron excitation by 5-HT (McCall and Aghajanian, 1979). Therefore, I tested its ability to antagonize the depression of evoked activity produced by 5-HT. In three of four cells, methysergide (10-20 nA) did not block the depression of the initial spike produced by 5-HT. In fact there was a tendency for the response to 5-HT to be prolonged during methysergide. In a fourth cell, there appeared to be a partial blockade of the response to 5-HT but no recovery occurred following cessation of the methysergide ejection.

6. Comparison of Norepinephrine and Serotonin Effects on Light Evoked Activity

As a complement to studies on electrically evoked, I also examined the effect of NE and 5-HT on LGNd cells which were activated by bright flashes of light. In general, relay neurons respond to such stimuli with a burst of firing (up to 10-30 msec in duration) beginning 30-80 msec following the flash. There is then a period of reduced activity, typically 30-150 msec, followed by one or more peaks of enhanced firing.

In 12 cells, iontophoretic application of NE (2-10 nA) produced an increase in both the initial and the first post-inhibitory pause rebound peak (Fig. 19). The mean increase in the total number of spikes in the initial peak was 43 ± 54%. 5-HT (5-10 nA) uniformly depressed light

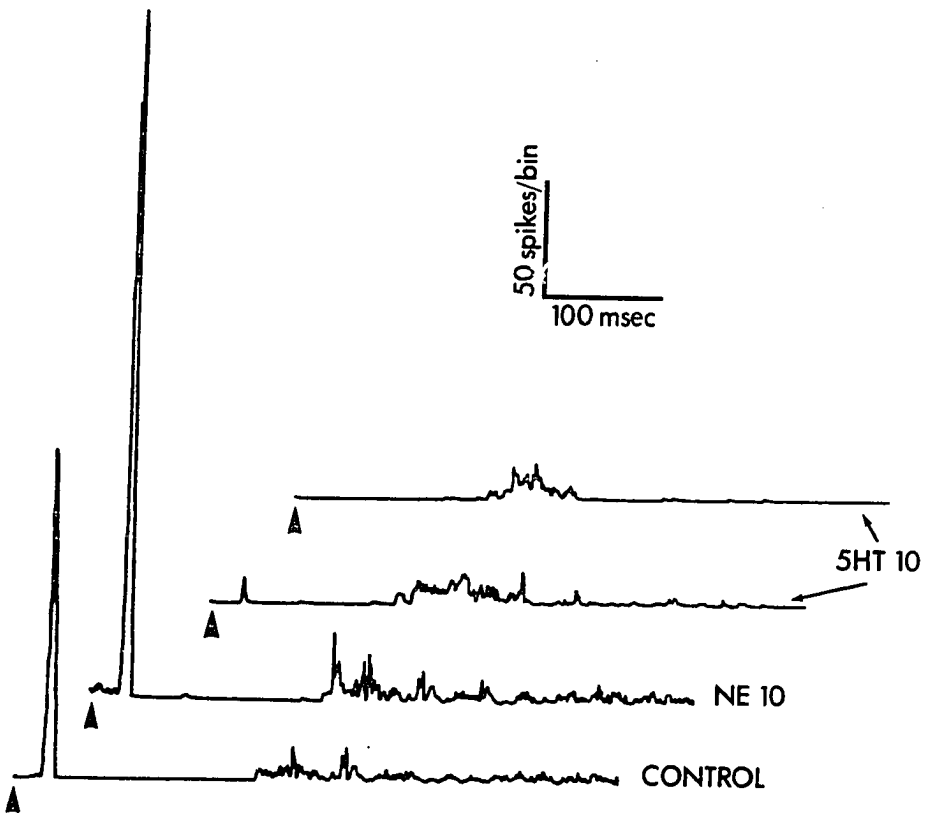


Fig. 19: Comparison of the effects of norepinephrine (NE) and serotonin (5-HT) on the response to visual stimulation. Light flashes were delivered to the animal's eyes at 1 Hz (arrowheads). NE iontophoresis produces a marked facilitation of the early peak in the PSTH and a moderate increase in the late response. The histogram was begun 25 sec following the onset of the NE ejection. 5-HT depresses the early peak; late activity is relatively less affected. 5-HT histograms were begun 25 and 135 sec following onset of the ejection. Each histogram represents the response to 100 flashes. Bin width: 1 msec.

activated firing and at appropriate currents completely suppressed cellular activity (3 cells).

7. Discussion

In the present section, it is demonstrated that NE causes a facilitation of relay neuron spontaneous firing when applied with 6-barrel microelectrodes, confirming the observations obtained with 5-barrel pipettes described in the previous section. In contrast to NE, 5-HT is a powerful depressant of the spontaneous activity of these cells.

A number of anatomical regions in the central nervous system are innervated by both noradrenergic and serotonergic fibers as is the LGNd. In those areas where the response of identified neurons to the two amines have been studied, NE and 5-HT usually have similar net effects on neuronal activity although the responses tend to be mediated by pharmacologically distinct receptors. Thus, hippocampal pyramidal cells (Biscoe and Straughn, 1966; Segal, 1975; Segal and Bloom, 1974), olfactory bulb mitral cells (Bloom et al., 1974) and neurosecretory cells in the hypothalamic supraoptic nucleus (Barker et al., 1971) are uniformly depressed by both NE and 5-HT. The two monoamines also have similar effects on motoneurons in the brainstem (McCall and Aghajanian, 1979) or spinal cord (White and Neuman, 1980) except in these areas facilitation of excitation rather than depression is seen. LGNd relay neurons are therefore unique in that they invariably respond in an opposite fashion to NE and 5-HT.

The two amines modified evoked activity in a manner consistent with their effects on spontaneous firing. Thus, NE markedly facilitated the early (monosynaptic) spike response to OX stimulation. In addition, a complex alteration in the pattern of late poststimulus activity was

noted. In PSTHs prepared during NE iontophoresis, there was a selective enhancement of peaks of high probability firing relative to between-peak activity (Figs. 15A,B). One can speculate that this represents a gain in the "signal-to-noise" ratio for the transmission of specific signals through the LGNd.

The facilitation of poststimulus firing by NE was antagonized by phentolamine at doses which did not alter the control response pattern. This indicates that NE acts to facilitate evoked activity via a receptor with at least some pharmacological characteristics in common with that mediating the effects of NE on spontaneous firing.

5-HT, at low doses, consistently depressed the response to OX stimulation, as would be predicted on the basis of its action on spontaneously firing cells. The depressant response to 5-HT was neither antagonized by phentolamine, nor by the "peripheral" 5-HT antagonist methysergide. Under similar conditions, methysergide is highly effective as an antagonist of 5-HT-induced facilitation of firing in the reticular formation (Boakes et al., 1970; Haigler and Aghajanian, 1974b), cerebral cortex (Roberts and Straughn, 1967) or facial nucleus (McCall and Aghajanian, 1979; 1980), suggesting that there are significant pharmacological differences between the receptors mediating the depressant and facilitatory actions of 5-HT. Because of the lack, at present, of an effective antagonist for the depressant response to 5-HT it is not possible to show pharmacological identity between the effects of 5-HT on spontaneous and evoked activity, as has been done for NE.

Since glutamate activated firing is relatively insensitive to 5-HT, it appears that 5-HT does not reduce excitability in the same fashion as conventional inhibitory agents such as γ-aminobutyric acid (GABA). In

an earlier study, Tebecis and DiMaria (1972) demonstrated that GABA is more effective than 5-HT as a depressant of amino acid-induced excitation, whereas 5-HT is better against spontaneous or synaptically-evoked activity. A number of mechanisms could account for this finding. First, low currents of 5-HT could have a predominantly presynaptic site of action, i.e., block the release of transmitter from retinal terminals (Curtis and Davis, 1962; Tebecis and DiMaria, 1972). Second, 5-HT could selectively reduce the responsiveness of relay neurons to the transmitter of the retinal ganglion cells, which, as yet, has not been identified. Third, 5-HT's effectiveness could be dependent upon the localization of the excitatory input, so that strong excitation produced at the soma by locally applied amino acids would be relatively less affected than synaptic excitation arising at more dispersed loci. Finally, 5-HT could be acting as a neuromodulator which selectively facilitates synaptic inhibition. On the basis of the presently available data, it is not possible to distinguish among these possibilities.

C. FACILITATORY EFFECTS OF NOREPINEPHRINE UNDER CONDITIONS OF SUPPRESSED SPONTANEOUS ACTIVITY

1. Facilitation of Glutamate Excitation

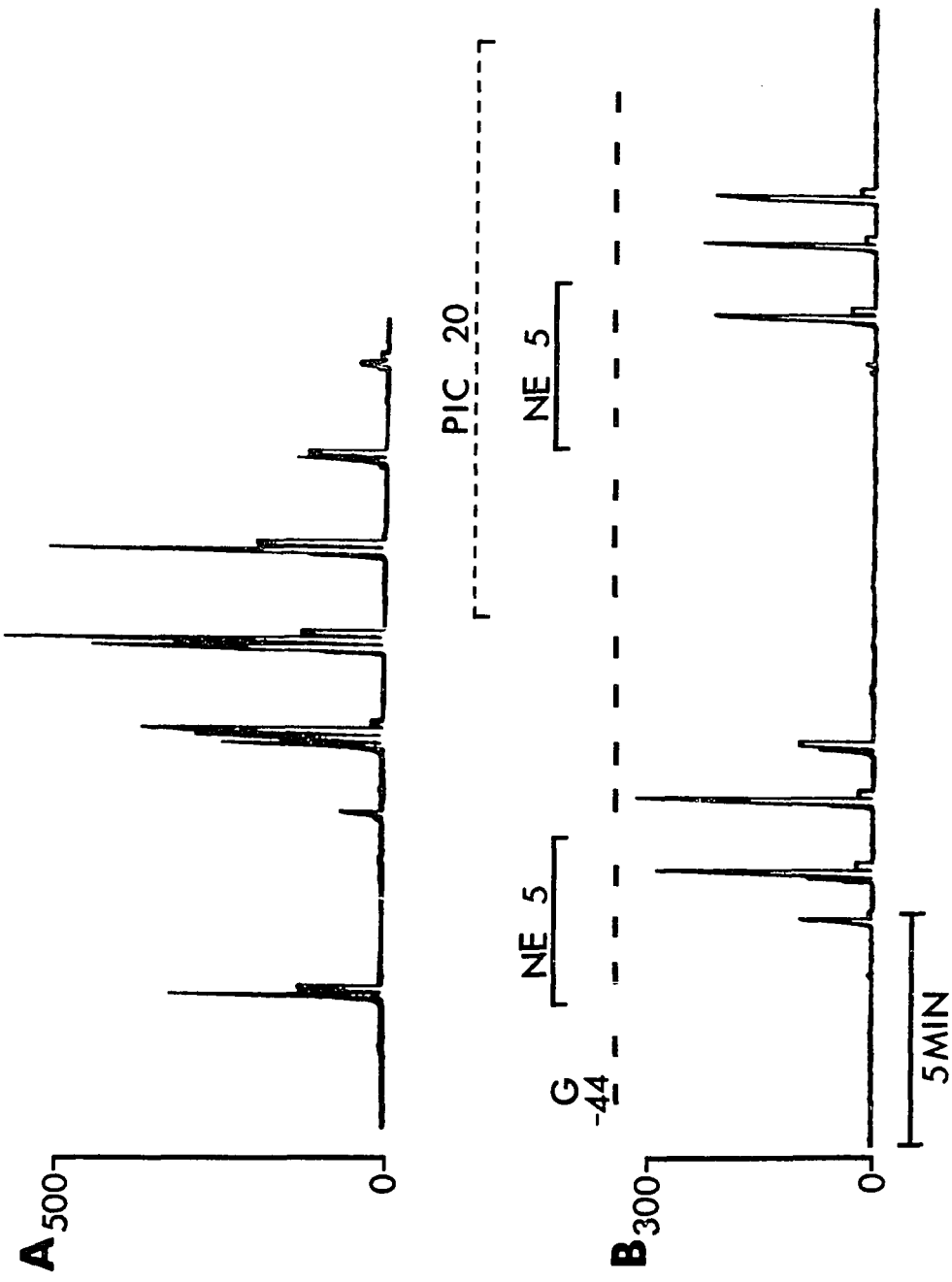
The results presented in the previous section indicate that NE can facilitate the responsiveness of LGNd relay neurons to retinal ganglion cell excitation and, thus, presumably enhance signal transfer from retina to visual cortex. The question examined in the present section is whether this effect is due to a direct excitatory action of NE or to an interaction of the amine with afferent synaptic activation in a manner more appropriately termed "neuromodulatory."

One approach to answering this question is to suppress the ongoing tonic excitation of relay neurons so that the effects of NE can be examined in isolation. Relay neurons receive tonic synaptic excitation under conditions of either light or darkness. This excitation is believed to arise primarily from the retina and is the cause of the "spontaneous" activity exhibited by relay neurons (Freund, 1973). Acute enucleation of the eyes temporarily eliminates the spontaneous activity of 63% of relay neurons (Bishop et al., 1962a), presumably by removing the major source of the ongoing tonic excitation. Thus, in the enucleated preparation it is possible to examine the effects of NE on relay neurons without the complicating presence of significant extrinsic excitatory drive.

In enucleated animals, NE failed to produce an excitation of silent LGNd units although cells could be activated easily by iontophoretic application of the excitatory amino acid glutamate. Thus, NE, in contrast to glutamate, did not appear to be a direct excitant. However, low currents of NE (2-15 nA) were able to dramatically facilitate the excitatory action of the amino acid. Iontophoresis of glutamate at currents which produced little or no excitation prior to the application of NE, resulted, with concurrent ejection of NE, in marked activation of firing (22 cells in 14 rats) (Fig. 20A). The effect of NE required 30 to 90 sec to develop and lasted up to 6 min following cessation of the ejection.

Iontophoretic ejection of Mg^{2+} (30-90 nA) was found to produce a rapid suppression of the spontaneous firing of most LGNd neurons. The exact mechanism of this effect is unclear, but it could be due to any of the following known actions of Mg^{2+}: (1) inhibition of excitatory transmitter release from presynaptic terminals (del Castillo and Engback, 1954; Potashner, 1978), (2) blockade of the postsynaptic receptor for the

Fig. 20: Norepinephrine (NE) facilitation of glutamate (G)-induced excitation under conditions of suppressed spontaneous activity. A, Constant current, 30-sec pulses of G were applied to a silent neuron in an acutely enucleated rat. NE causes a reversible potentiation of the response to subthreshold G. In this and subsequent examples, silent neurons were identified by optic chiasm stimulation or iontophoresis of G. B, Response of another cell demonstrating that NE but not picrotoxin (PIC) facilitates the action of G (20-sec pulses). Note that the response to NE is slightly diminished during the PIC ejection. Ordinate represents spikes per 10 sec epoch.



excitatory transmitter (Evans et al., 1977; Davies and Watkins, 1977) or (3) increase in the threshold for excitation (Kato and Somjen, 1969; Kelly et al., 1969).

Whatever the mechanism, Mg^{2+} was used as an alternate to acute enucleation for reducing the spontaneous activity of relay neurons. During the continuous iontophoretic application of Mg^{2+}, most silent cells failed to respond to NE. In a few cases, even silent cells were excited by NE but this could be prevented by increasing the Mg^{2+} ejection current. As was the case in acutely enucleated animals, however, low currents of NE were able to markedly facilitate the excitatory action of glutamate on cells which were silenced by Mg^{2+} (16 cells in 10 rats) (Fig. 21). These observations suggest that the receptivity or excitability of the cells had not been markedly impaired by Mg^{2+}. (It did appear, however, that glutamate was antagonized to a certain extent since higher ejection currents were required to excite cells in the presence of Mg^{2+}.) These results indicate that enucleation is not the only maneuver which allows the demonstration of the ability of NE to facilitate relay neuron excitability in the absence of any direct excitatory effect of its own. In fact, silent cells which were occasionally encountered in normal animals (especially under conditions of deep chloral hydrate anesthesia) responded in a similar fashion.

2. Effects of Picrotoxin

The ability of NE to produce an enhanced responsiveness to glutamate suggested that NE could facilitate the general excitability of relay neurons, presumably through a postsynaptic mechanism. This mechanism may account for the ability of NE to mimic the facilitation of sub-threshold excitation and the potentiation of the field response to optic

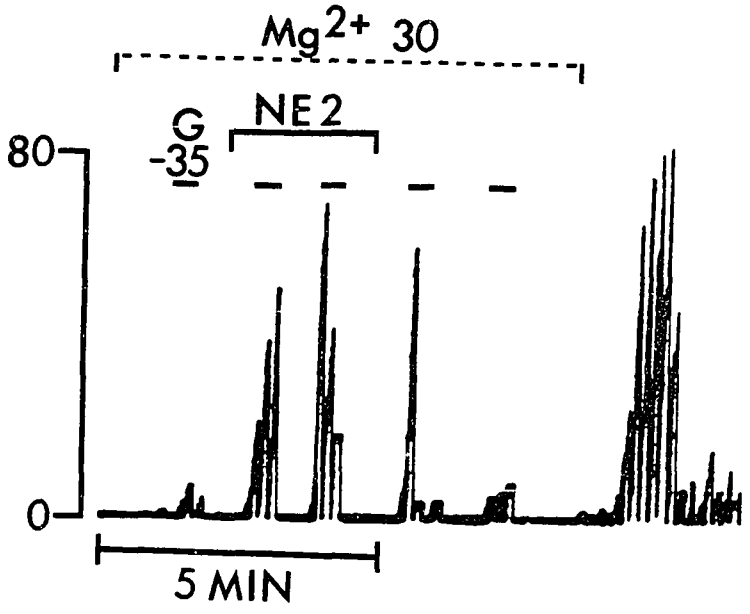


Fig. 21: Norepinephrine (NE) facilitation of glutamate (G) excitation with spontaneous activity suppressed by Mg^{2+}. Upon cessation of Mg^{2+} ejection there is rebound excitation followed by a return to normal spontaneous firing. Ordinate represents spikes per 10 sec epoch.

chiasm stimulation which Nakai and Takaori (1974) observed following conditioning stimulation of the LC (see Part I). However, on the basis of the apparent depressant effects of LC stimulation on certain supposed I-cells, the latter authors speculated that NE may facilitate relay neurons indirectly by suppressing inhibitory interneurons.

As discussed in Part I, two populations of local inhibitory elements are believed to synapse upon P-cells (relay neurons). The first group, originally considered to be intrinsic interneurons by Burke and Sefton (1966), are now recognized as residing in the thalamic reticular nucleus which is adjacent to the LGNd, but separate from it. These neurons, known as perigeniculate reticular (PGR) cells, probably mediate the post-excitation inhibition of relay cells (Sumitomo et al., 1976). Three considerations argue against the participation of PGR neurons in the facilitatory action of iontophoretic NE. First, NE generally produces only a small decrease in the postexcitation inhibitory period yet the responsiveness, especially of the short latency spike, is markedly facilitated. Second, PGR neurons are silent in enucleated animals (Waring, 1979), although NE can readily facilitate glutamate under these conditions. Finally, the activity of NE is independent of the proximity of the iontophoretic pipette to the reticular nucleus which is situated rostral to the geniculate, as much as 1.5 mm anterior to the caudal extent of the LGNd (Sumitomo et al., 1976).

I-cells intrinsic to the LGNd proper, the second class of neurons providing inhibition to relay cells, have been described only recently (Sumitomo and Iwama, 1977). Although sparse in number (approximately 6% of the total neuronal population in the rat LGNd; see Part I), these true interneurons could conceivably mediate the action of NE. Recent evidence

indicates that GABA is the neurotransmitter utilized by these cells (Sterling and Davis, 1980). Therefore, if the facilitatory action of NE on relay neurons were mediated by depression of these interneurons or suppression of GABA release from presynaptic dendrites, blockade of GABA-mediated transmission should produce similar effects. This hypothesis was tested with the GABA antagonist picrotoxin. In preliminary experiments, I found that picrotoxin was able to block GABA-induced depression of spontaneously active LGNd neurons when applied with currents of 15-20 nA for 1-5 min. (Doses of GABA producing 90-100% suppression of spontaneous activity were arbitrarily used in these trials.) Picrotoxin was then applied to silent neurons in enucleated animals. Even with prolonged administration (up to 25 min), the GABA antagonist (15-50 nA) did not produce a facilitation of the response to glutamate (5 cells). In fact, during the iontophoresis of picrotoxin, the ability of NE to facilitate the action of glutamate was unaffected or occasionally slightly deptessed (Fig. 20B), rather than enhanced as would be predicted if GABAergic I-cells provided tonic inhibition to relay neurons. These observations suggest that the facilitatory action of NE is not mediated transynaptically via inhibition of adjacent interneurons or blockade of GABA release from presynaptic dendrites, although an interaction between NE and either class of inhibitory neurons could play a role in the overall physiological action of the coeruleogeniculate pathway. It is of interest to note that the facilitatory action of iontophoretic NE on LGNd neurons is similar in many respects to that described in a previous study of motoneuron excitability in the rat facial nucleus where intrinsic neurons are absent (McCall and Aghajanian, 1979).

3. Discussion

The major findings presented in this section are that NE can facilitate the excitability of relay neurons to glutamate without having a direct excitatory effect itself. To demonstrate this phenomenon, it was necessary to eliminate the ongoing tonic excitation these cells normally receive from the retina. In addition, it was probably advantageous that the experiments were carried out in chloral hydrate anesthesized rats so that other excitatory inputs to the nucleus (i.e., cerebral cortex) were suppressed. On the basis of the observations presented in this section, it can be concluded that NE satisfys the definition of a "neuromodulator" outlined in Part I. Moreover, the longlasting character of the response to NE is consistent with the general characteristics of monoamine (and also non-monoamine, i.e., peptide) modulators in other systems.

It is, of course, possible that the effects of glutamate and NE are simply additive and that NE is, in fact, acting as a conventional excitatory transmitter. Although this possibility cannot be excluded with certainty using extracellular recording techniques, it would appear unlikely since even with high currents NE generally did not excite silent cells in enucleated animals.

The fact that NE acts as a modulator suggests that the facilitation of spontaneous activity it produces under control conditions is a manifestation of its capacity to enhance the excitability of relay neurons to ongoing synaptic activation rather than to excite these cells directly. This presumably accounts for the prolonged nature of the activating effect which is strikingly different from the time course of the response to, for example, glutamate.

Finally, the observation that NE can act as a neuromodulator might explain the unique patterns of responsiveness to NE observed in brain regions other than the LGNd where the local circuitry and the configuration of synaptic inputs (or endogenous pacemaker activities) are different. Thus, much of the inconsistency observed in iontophoretic studies may be a reflection of the variability among brain regions with respect to synaptic architecture rather than intrinsic responsiveness to NE.

Variations in experimental technique, especially with respect to anesthesia, may also be important. For example, experiments conducted under deep barbituate anesthesia often give different results from those carried out under light or no anesthesia. This is readily explainable if the substance being tested is a neuromodulator since under conditions of deep anesthesia the excitatory drive to the target cell might be suppressed. Since the action of a neuromodulator is dependent upon this excitation, variations in responsiveness with anesthesia level would be predicted.

D. LOCUS COERULEUS STIMULATION

1. Effects on Evoked and Spontaneous Activity

To provide additional evidence that NE is the transmitter substance in the coeruleogeniculate pathway, experiments were conducted in which trains of electrical stimuli were applied directly to the LC. The response of LGNd neurons to LC activation was then compared with the effects of iontophoretic NE on the same neurons.

In these experiments, concentric bipolar (coaxial) stimulating electrodes were implanted in or near the ipsilateral LC. Single shocks

applied to the LC did not drive LGNd neurons nor was a field potential ever observed in the LGNd even at high stimulation intensities. In order to obtain effects in the LGNd, it was necessary to apply trains of stimuli to the LC, as has been the case in previous studies in other target areas (Hoffer et al., 1973; Segal and Bloom, 1974b; Sasa et al., 1974; Phillis and Kostopoulos, 1977; Dillier et al., 1978).

Two stimulation protocols were used. In the first, a conditioning train was applied to the LC at predetermined intervals prior to an OX shock. In this way the interaction between LC activation and the response to an afferent volley in the optic pathway could be examined. Generally, the stimulus train consisted of four 1 msec duration rectangular pulses at a frequency of 200 Hz.

In the second stimulation protocol, continuous trains were applied to the LC for periods of 10-60 sec. During the stimulus, average rate activity was recorded in a conventional manner except that counting was electronically blanked for a few milliseconds on either side of each pulse. In this way an accurate representation of the rate was obtained even during periods of stimulation. The trains consisted of 1 msec duration pulses at 10 Hz.

Fig. 22 demonstrates the effect of a conditioning train applied to the LC on the response to a subthreshold OX shock. The OX stimulus was adjusted so that under control conditions approximately 20% of the shocks produced short latency spikes. In the presence of a train to the LC, the probability of spike generation in the early component of the response is markedly facilitated. In addition, the slow sweep storage oscilliscope records and the PSTHs demonstrate that there is a facilitation of late firing. Integration of the PSTH for the experiment shown indicated that

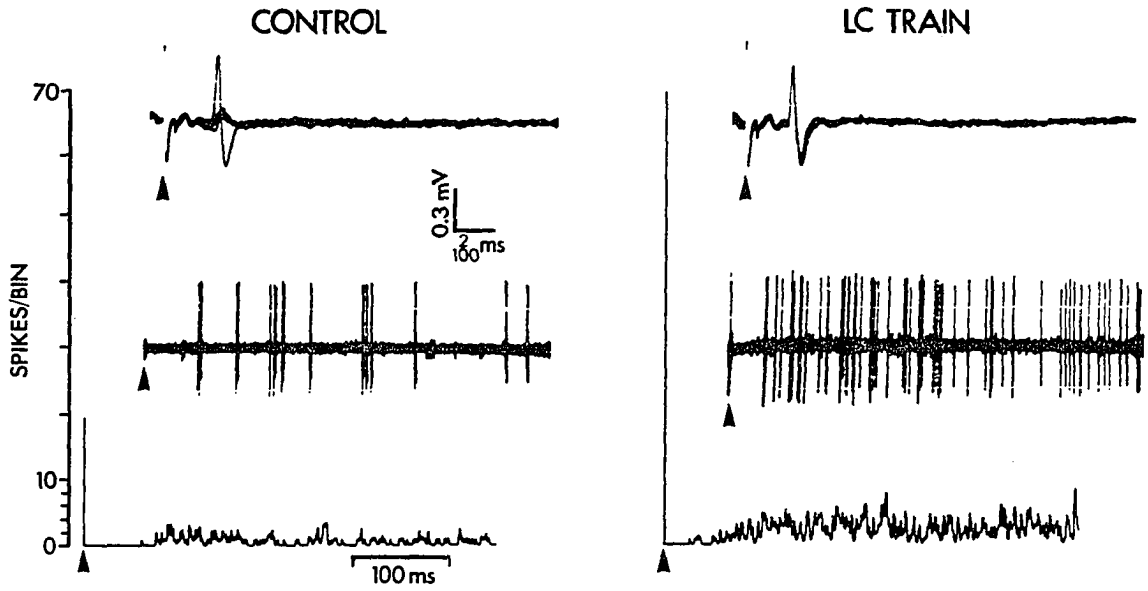


Fig. 22: Effect of conditioning locus coeruleus (LC) stimulation on the response of a LGNd neuron to optic chiasm (OX) stimulation. The intensity of the OX stimulus was adjusted so that under control conditions approximately 1 out of 5 stimuli gave short latency spikes. The records on the left show the response to OX stimulation alone; the records on the right are after a train of four 1 msec shocks to the LC at 200 Hz (4 mA). The interval between the LC and OX stimuli was 50 msec. Stimulation parameters: 0.15 msec, 1 mA.

there was a 241% increase in the total number of spikes with conditioning stimulation of the LC. Note that the pattern of late activity seen with LC stimulation is somewhat different from that produced by iontophoretic NE (see Figs. 14C & 15). With LC stimulation, the duration of the post-excitation pause is shortened and a large post-inhibitory-pause rebound peak is not usually observed. This may be due to the fact that LC stimulation suppresses the activity of PGR cells (Nakai and Takaori, 1974) which are believed to mediate the post-excitation inhibitory period (Sumitomo et al., 1976). As spikes move to earlier times due to the facilitatory effects of LC stimulation, suppression of the PGR cells allows them to fall within the inhibitory period and prevents a peak of activity from occurring at the end of the inhibitory pause. Intervals of 40 or 50 msec between the conditioning LC train and the OX shock produced optimal effects on the short latency spike. However, intervals between 20 and 100 msec were generally effective.

In subsequent experiments, continuous trains at 10 HZ were applied to the LC. Spontaneously active cells in normal animals invariably responded to these trains with a dramatic increase in firing rate. Stimulation intensities of 0.3 to 0.8 mA were used; the threshold current required appeared to depend upon the electrode placement. The degree of activation was roughly correlated with the stimulation intensity (Fig. 23) although in some cases it appeared as if the effect was "all-or-none." As with iontophoretically applied NE, the effect of LC stimulation was delayed in onset (requiring 1-30 sec to attain a peak response) and sustained beyond the duration of the stimulus train (up to 20 sec).

2. Placement of Stimulating Electrodes

Optimal effects of LC stimulation were obtained when the electrode

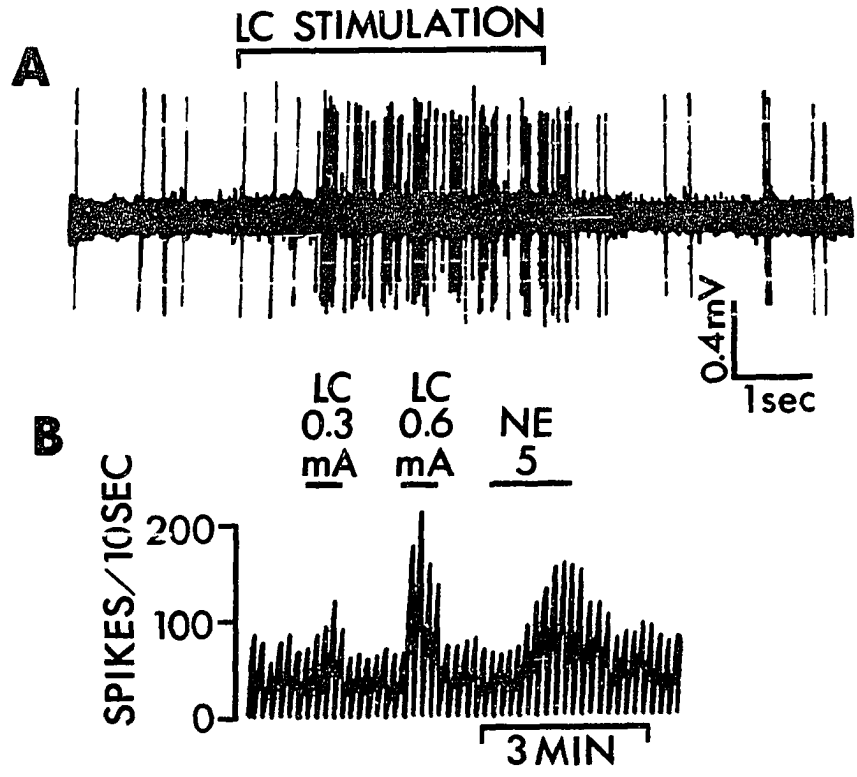


Fig. 23: A, Storage oscilliscope record demonstrating the effects of locus coeruleus (LC) stimulation on the spontaneous firing of a LGNd neuron. Stimulation parameters: 10 Hz, 1 msec, 0.3 mA. B, Rate histogram of another cell comparing LC stimulation with iontophoretically applied norepinephrine (NE).

tip was either within the ipsilateral LC or just anterior to the LC, presumably in the dorsal NE axon bundle. Uniform activating effects were also obtained with the electrode just alongside the LC in the mesencephalic nucleus of the trigeminal nerve. Fig. 24 illustrates the electrode placements in 12 rats. Since coaxial bipolar electrodes were used, the actual current path is from the points shown on the diagram (representing the position of the electrode tip) to a location approximately 1 mm dorsal. Most of the electrode placements produced uniform activating effects in the LGNd; an example of such a placement is shown in Fig. 25. However, in two cases, the electrode was positioned somewhat below the LC. In these animals, variable effects of stimulation were obtained: some cells were activated, others were depressed and many were unaffected. This is consistent with previous studies on the effects of reticular formation stimulation on cellular activity in the LGNd (Phillis et al., 1967b; Matsuoka and Domino, 1972; Foote et al., 1974). In one animal, the electrode was placed in the contralateral LC. Many cells were activated by stimulation at this site, but the responses were weak even with high stimulation intensities.

3. Comparison with Response to Iontophoretic Norepinephrine

Paired comparisons were carried out between iontophoretic NE and LC stimulation on 23 cells in 10 animals. In all cases, when the stimulating electrode was verified to be in or near the LC, both treatments gave a comparable facilitation of the spontaneous firing rate (Fig. 23B).

WB-4101 was tested for its ability to antagonize the response to

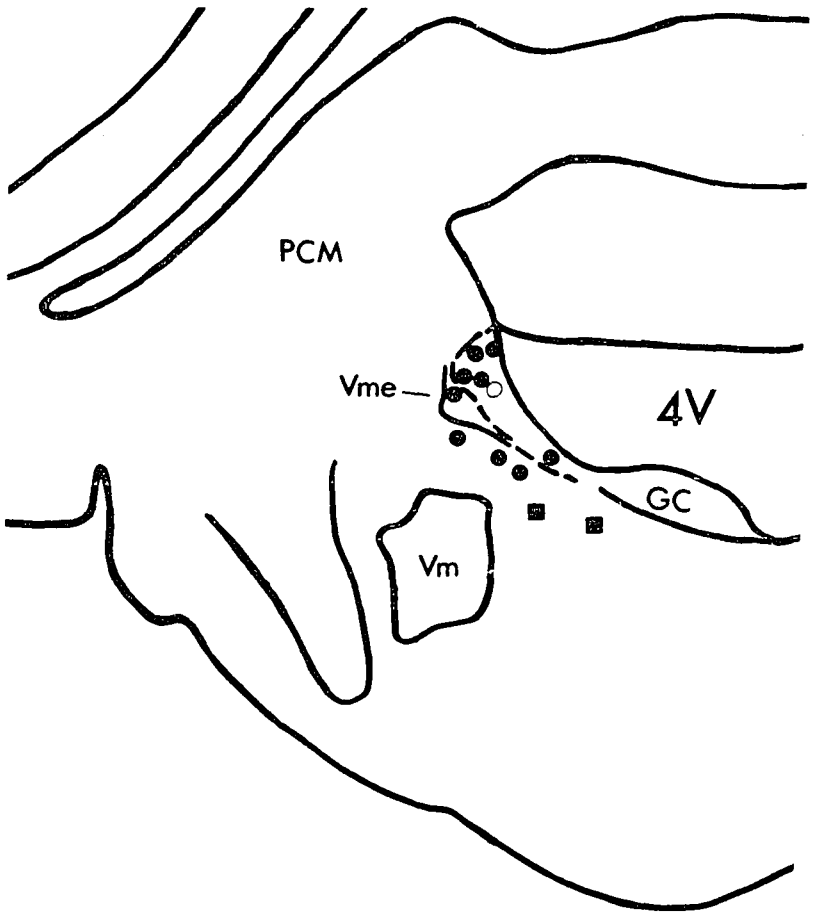


Fig. 24: Schematic representation of LC stimulating electrode placements. Electrode position is marked by producing a lesion at the tip. Sites from all frontal plane levels are mapped onto this representative section through the anterior LC. Most of the actual electrode sites were close to this frontal plane level. Two electrodes were placed anterior to the LC and are not marked. ● = uniform activation of spontaneously active cells; ■ = variable effects; ○ = contralateral side.

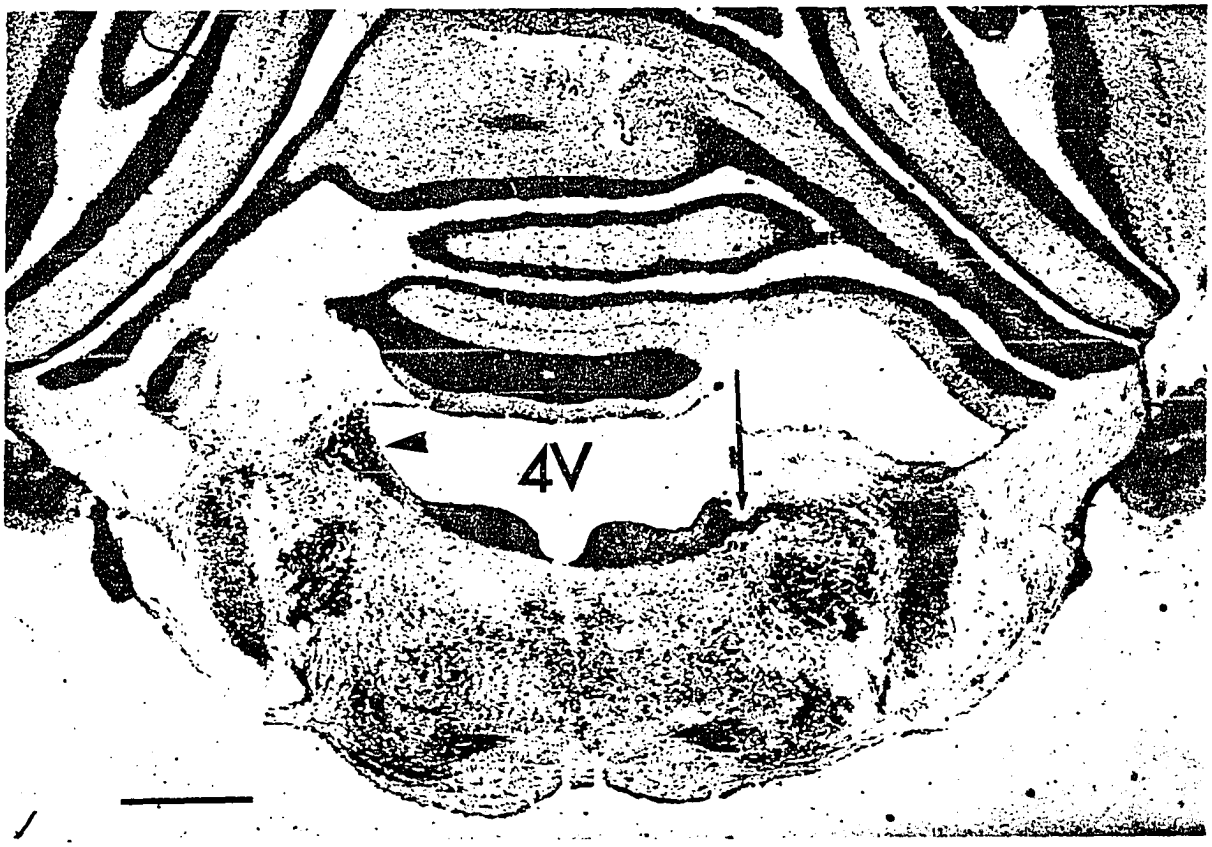


Fig. 25: Electrolytic lesion produced at tip of stimulating electrode indicating typical placement in LC. Cresyl Violet stained, 50-μm thick coronal section of formalin fixed tissue. Position of electrode tract is marked with an arrow. The lesion was produced by passing a current of 20 μA for 20 sec. The arrowhead points to the contralateral LC. 4V = fourth ventricle. Calibration bar = 1 mm.

LC stimulation. Intravenous administration of WB-4101 (100 μg/kg) caused a transient suppression of the activating effect in 3 animals (Fig. 26). At the low doses used, no depression of the baseline firing rate was observed, although with higher intravenous doses the baseline rate is reduced. Iontophoretic WB-4101 (15-30 nA) also blocked the response to LC stimulation as demonstrated in Fig. 27. Again the baseline rate is not significantly affected by the antagonists at the doses used. (Somewhat higher ejection currents of WB-4101 are required with 6-barrel electrodes to obtain blockade of the response to NE. However, with these pipettes less depression of the baseline rate is observed even at higher ejection currents than with 5-barrel pipettes. This may be due to the ability of 6-barrel pipettes to disperse the antagonist throughout a larger area of the cell's dendritic field without obtaining high concentrations at the soma which could conceivably cause non-specific depressant effects.)

In a few cells it was possible to demonstrate parallel blockade of the response to iontophoretic NE and LC stimulation (Fig. 28). In general, the effect of iontophoretic NE was more easily antagonized by WB-4101 than was the response to LC stimulation. In the example shown in Fig. 28, WB-4101 completely eliminated the response to NE while the effect of LC stimulation was markedly reduced but not entirely suppressed.

4. Effects in Enucleated Animals

In contrast to the effects on spontaneously active cells, silent cells in enucleated animals were generally not activated by LC stimulation. However, as with iontophoretic NE, LC stimulation markedly enhanced the excitatory action of glutamate (5 cells) (Fig. 29).

To provide further evidence that LC stimulation and iontophoretic

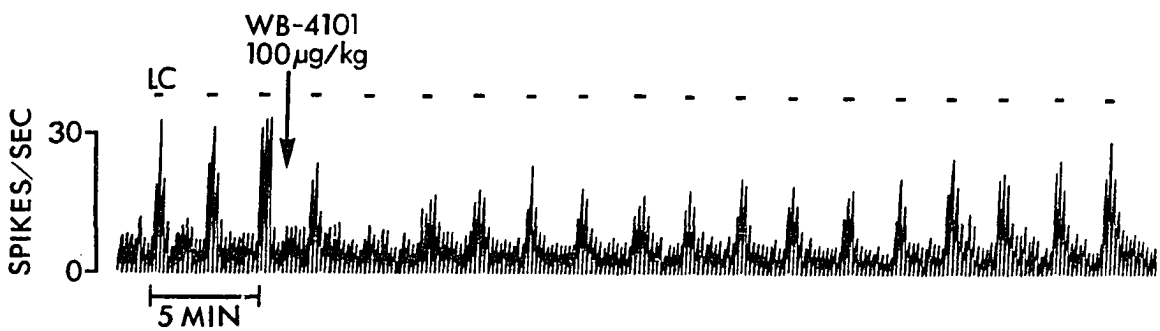


Fig. 26: Blockade by intravenous WB-4101 of the activation of a LGNd neuron by locus coeruleus (LC) stimulation. Stimulation parameters: 10 Hz, 1 msec, 0.6 mA.

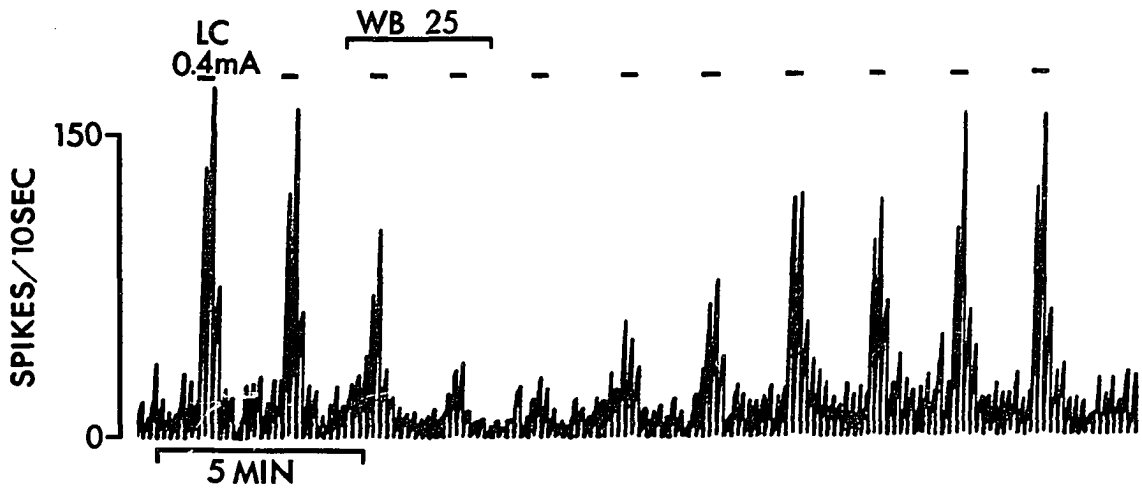


Fig. 27: Blockade by iontophoretic WB-4101 (WB) of the activation of a LGNd neuron by locus coeruleus (LC) stimulation. Stimulation parameters: 10 Hz, 1 msec, 0.4 mA.

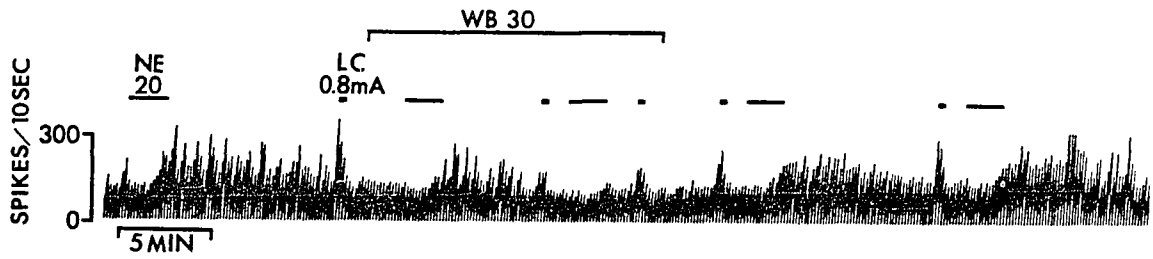


Fig. 28: Blockade by iontophoretic WB-4101 (WB) of the activation of a LGNd neuron by norepinephrine (NE) and by locus coeruleus (LC) stimulation. Stimulation parameters: 10 Hz, 1 msec, 0.8 mA.

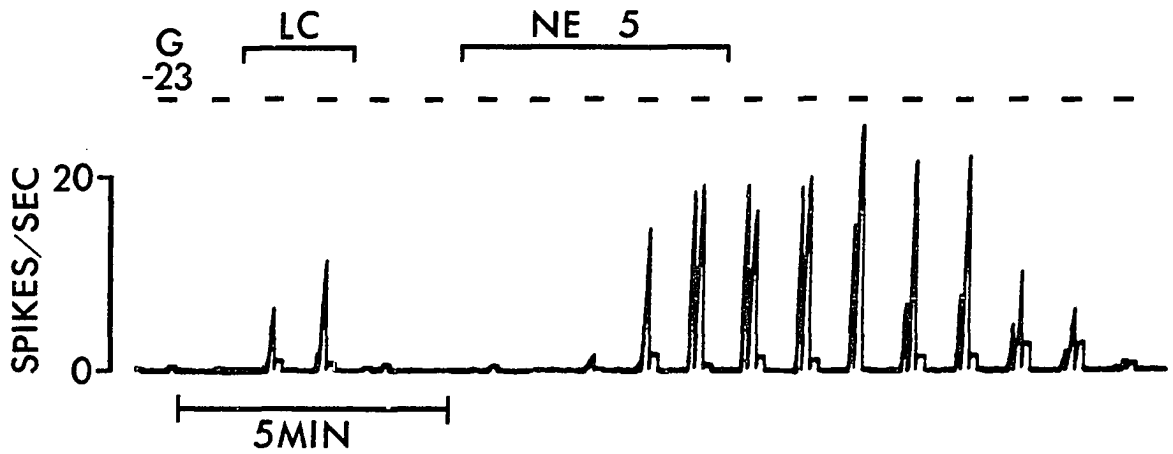


Fig. 29: Comparison between the effects of locus coeruleus (LC) stimulation and iontophoretic norepinephrine (NE) under conditions of suppressed spontaneous activity due to bilateral enucleation. Both LC stimulation and iontophoretic NE facilitate the excitatory activity of glutamate (G) but neither directly excites the cell. Stimulation parameters: 10 Hz, 1 msec, 1.0 mA.

NE evoke identical postsynaptic actions, I examined whether the enhanced excitability each produced was sensitive to WB-4101. In 4 cells, WB-4101 at currents of 15-30 nA produced a blockade of the facilitatory action of NE (5 nA) (Figs. 30A,B). Spike amplitude was unaffected by WB-4101 (Fig. 30A) suggesting that local anesthetic effects do not occur at the doses used. Similar iontophoretic currents also markedly attenuated the facilitatory action of LC stimulation (3 cells) (Fig. 30C).

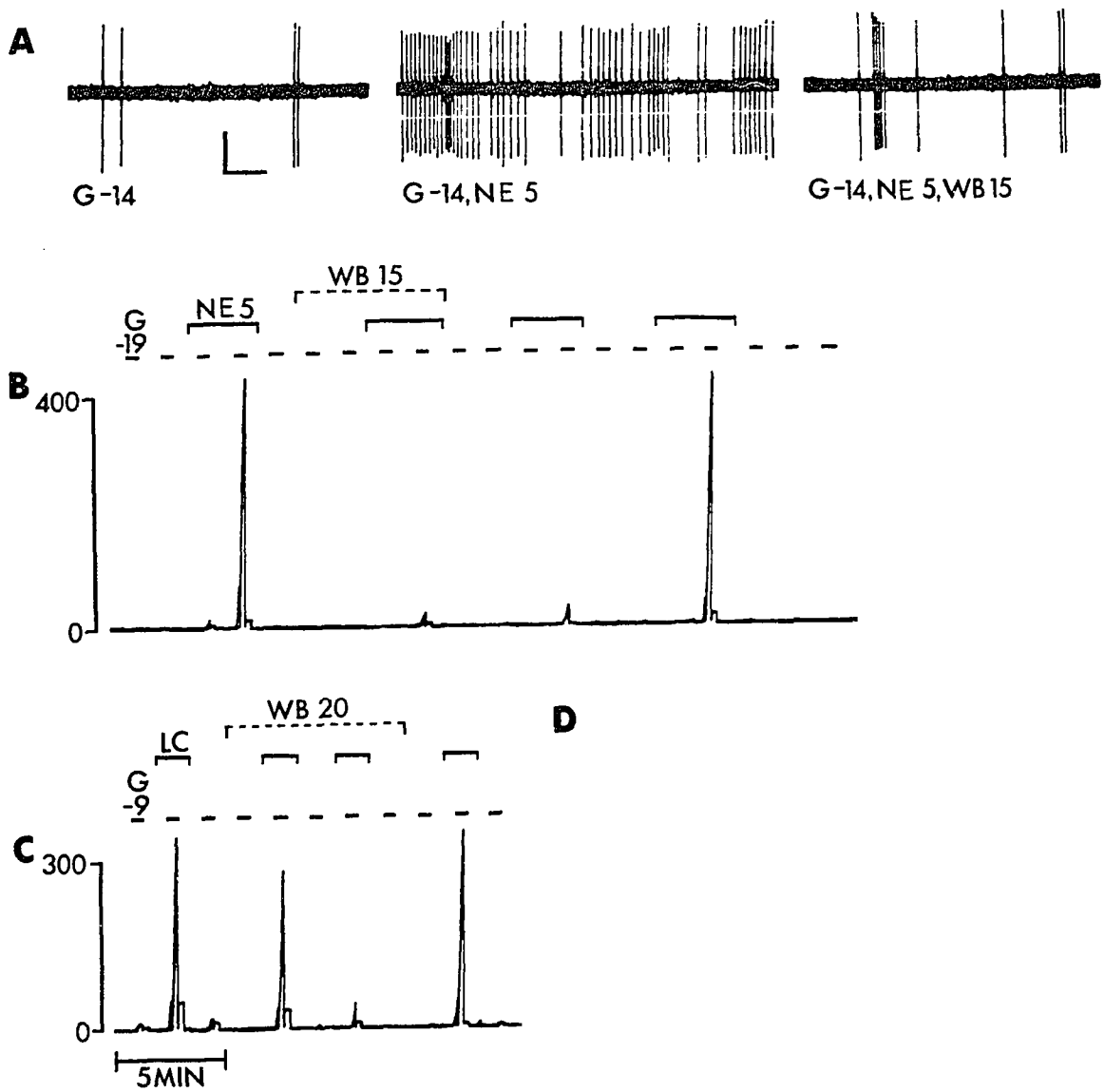
5. Discussion

Conditioning stimulation of the LC facilitated the initial spike response to an afferent volley along the optic pathway. This finding confirms the observations of Nakai and Takaori (1974) in the cat. In the present study, the effect of LC stimulation on late activity was also examined. In general, there appeared to be a facilitation of longer latency activity as well as of the early response.

Continuous stimulation of the LC activated spontaneously active LGNd neurons in a manner similar to that produced by iontophoretically applied NE. The onset of the response was delayed and the effect persisted after the cessation of the stimulus train. The time course of the effect was similar to that observed in other LC target sites such as the cerebellar cortex (Hoffer et al., 1973) and hippocampus (Segal and Bloom, 1974b). In these areas, the depression of spontaneous activity produced by LC stimulation may outlast the period of stimulation by up to 2 min.

It is unlikely that peripheral effects of LC stimulation are responsible for the activation of LGNd neurons since stimulation of the contralateral LC produced weak responses. Moreover, Hoffer et al. (1973) demonstrated that arterial blood pressure does not change under stimulation

Fig. 30: Blockade by WB-4101 (WB) of the facilitatory action of norepinephrine (NE) (A,B) and locus coeruleus (LC) stimulation (C). A, Storage oscilliscope tracings of the extracellular spike records of a "silent" cell in an acutely enucleated rat. The unit was activated by glutamate (G) (left panel) and this response was enhanced by NE (middle panel). WB applied for 16 min antagonized the effect of NE (right panel). Note that the spike amplitude is unaltered during prolonged application of WB. Calibration: 0.5 sec, 0.5 mV. B, Rate histogram demonstrating the effect of WB on another cell. C, Blockade by WB of LC-stimulation-induced facilitation of G in a third cell. LC stimulation does not activate the cell in the absence of G. Stimulation parameters: 10 Hz, 1 msec, 0.5 mA.



conditions similar to those used in the present study.

WB-4101 was an effective antagonist of both the responses to iontophoretic NE and to LC stimulation. Certain discrepancies were observed, however, with respect to dose. On the one hand, the response to iontophoretic NE was antagonized by somewhat lower iontophoretic currents of WB-4101 than was the stimulation effect. Similar discrepancies have been observed in other systems and it has been suggested that this may be due to differences in the locus of action of synaptically and iontophoretically released transmitter agents (see Tebecis, 1973). With stimulation, release of transmitter presumably occurs over a large area of the dendritic field of the target neuron whereas with iontophoretic application the effect is limited to the vicinity of the pipette. The iontophoretically ejected antagonist has access to approximately the same local region of membrane as the iontophoretically released agonist and therefore it is reasonable that iontophoretically applied NE is more easily antagonized than is activation of the pathway.

On the other hand, it was found that LC stimulation was highly sensitive to systemically administered WB-4101 while iontophoretic NE was less strongly affected. Again the discrepancy may be related to geometric considerations. Therefore, although the present findings do not prove that physiologically released NE mediates the effects of LC stimulation, the evidence is highly suggestive. Moreover, it appears that both responses are mediated by an α-adrenoceptor.

The depressant response to LC stimulation reported by previous investigators appears to have a different pharmacological specificity from the activating effect. A number of studies have found that the depressant effects of LC stimulation are antagonized by β-antagonists

such as sotalol (Hoffer et al., 1973; Segal and Bloom, 1974; Phillis and Kostopoulos, 1977; Dillier et al., 1978). In the spinal trigeminal nucleus, intraventricular administration of the β-blockers sotalol and propranolol selectively reduced the inhibition produced by LC stimulation while the α-blockers phenoxybenzamine and phentolamine were inactive (Sasa et al., 1976). Thus, the facilitatory effect of LC stimulation appears to be mediated by an α-receptor; whereas the depressant response more closely resembles a β-mediated effect.

In addition to the more readily apparent similarities between the effects of LC stimulation and iontophoretic NE (in particular, the time course of action), it was found that both treatments are inactive in the setting of reduced afferent excitation. In a manner similar to that produced by locally applied NE, LC stimulation was able to facilitate the excitatory action of glutamate under these conditions and this effect was antagonized by WB-4101. Thus, receptor mediated modulatory effects of pathway stimulation can be demonstrated under appropriate circumstances. A similar (modulatory) facilitation of excitability appears to occur with mesencephalic reticular formation stimulation due to activation of the cholinergic pathway to the LGNd (Phillis et al., 1967b).

PART IV: SUMMARY AND CONCLUSIONS

The studies presented in this dissertation provide a physiological characterization of the pharmacological actions of norepinephrine in a model postsynaptic area, the dorsal lateral geniculate nucleus. The aim of these experiments is to provide evidence for a transmitter role of NE in the coeruelogeniculate pathway and to further the understanding of central noradrenergic systems.

A number of traditional criteria must be satisfied before a substance can be accepted as a chemical transmitter agent in a specific neuronal pathway. Although several authors have proposed criteria, those given by Paton (1961) and Phillis (1966) are reasonable and concise. These criteria are summarized in Table II. In the following discussion, I indicate the degree to which these criteria have been met for NE in the coeruleogeniculate pathway and point out areas for further investigation.

Criteria 1.- Biochemical and histofluorescence evidence indicate that NE is present within axons which innervate the LGNd (Kromer and Moore, 1980; Fuxe, 1965; Lindvall et al., 1974). The critical enzymes in the biosynthesis of NE, tyrosine hydroxylase and dopamine-β-hydroxylase, have also been localized to these fibers by immunocytochemical methods (Hökfelt et al., 1978; Swanson and Hartman, 1975).

Criteria 2.- With presently available methods, it is not possible to detect the release of NE from a single nerve terminal, either in the peripheral or in the central nervous system. It is feasible, however,

TABLE II

Criteria for Transmitter Identification

1. The presynaptic neuron should contain the suspected transmitter substance and be able to synthesize it.

2. The substance should be released on stimulation of the presynaptic neuron.

3. Application of the substance to the postsynaptic cell should reproduce the effects of the synaptically released transmitter.

4. The action of the substance on the postsynaptic cell should be affected by blocking agents in the same way that synaptic transmission is ("identity of action").

5. There may be a system for the inactivation of the transmitter.

After Paton (1961) and Phillis (1966).

to stimulate a noradrenergic cell group and measure the change in NE turnover in its target nuclei. Although this has not yet been carried out specifically for the coeruleogeniculate pathway, data is available for other LC projection areas (Korf et al., 1973; Arbuthnott et al., 1970; Walter and Eccleston, 1973). In general, stimulation of the LC (in a manner similar to that used in the physiological studies reported here) produces a decrease in the concentration of NE and a large increase in the levels of 3-methoxy-4-hydroxyphenylglycol sulfate, the major metabolite of NE in the brain. This indicates that the turnover of NE is accelerated by LC stimulation and suggests that NE is released upon stimulation, although the exact site of this release is not known.

Criteria 3.- The present study (see Part III, Section D) demonstrates that iontophoretic application of NE and stimulation of the LC have similar effects on the activity of LGNd neurons. However, experiments utilizing intracellular recording techniques will be required to confirm that both treatments produce identical changes in membrane electrical properties.

Criteria 4.- The α-adrenoceptor antagonist WB-4101 blocks the actions of both locally applied NE and LC stimulation providing evidence for "identity of action." Further studies with additional adrenergic antagonists could be carried out to strengthen this point since no receptor blocker is entirely specific. Nevertheless, WB-4101 at the doses used was selective for NE when compared with glutamate or acetylcholine, the only two substances currently considered as possible excitatory transmitters in the LGNd.

Criteria 5.- It is widely believed that the synaptic actions of NE are terminated via a specific high-affinity uptake mechanism. Bio-

chemical studies have demonstrated this uptake activity in the diencephalon. Furthermore, lesions of the LC significantly reduce its activity in this brain region, suggesting that the uptake activity is, at least in part, localized to LC neurons (Kuhar, 1973). Although this criteria is not absolutely required (a transmitter system could have no inactivation system other than diffusion), it does appear to be satisfied for NE in the diencephalon and therefore presumably in the LGNd.

Taken together, the evidence cited above strongly suggests that NE is a chemical transmitter in the coeruleogeniculate pathway. An understanding of the precise mechanism whereby NE influences the activity of LGNd neurons must await studies utilizing intracellular recording techniques. Moreover, until such studies are carried out, the possibility remains open that NE exerts membrane actions which alter relay neuron excitability in a more complex fashion than simple monitering of extracellular spike activity can reveal.

Realizing the limitations of the experimental methods, the present study has demonstrated that NE, acting through an α_1-adrenoceptor, can facilitate the excitability of geniculocortical relay neurons. This appears to take place via a neuromodulatory mechanism in which the cellular responsiveness to synaptic excitation is enhanced. In the absence of synaptic inputs, this excitability change is not sufficient by itself to cause action potentials to be generated. Thus, the activity of NE is significantly different from that of conventional transmitters such as excitatory amino acids (in the central nervous system) or acetylcholine (at the neuromuscular junction). It remains to be determined whether NE can facilitate synaptic inhibition as well as excitation.

It can be speculated that the physiological correlates at the

membrane level of this modulatory action are similar to those observed with NE or 5-HT on facial motoneurons (VanderMaelen and Aghajanian, personal communication) or with 5-HT on tonic-type myenteric plexus neurons (Wood and Mayer, 1979). In both cases (see Part I) the facilitation of excitability is associated with a small, long lasting depolarization and an increase in membrane input resistance. The exact biochemical and biophysical mechanisms mediating the excitability change are presently unknown but will certainly be a fruitful area for future investigation.

On the basis of previous studies beginning to explore this area, it can be speculated that monoamine-induced increases in an intracellular messenger such as cyclic adenosine monophosphate (cAMP) or Ca^{2+} might mediate the excitability change possibly through a subsequent alteration of the voltage-sensitive ion channels which mediate the conductances involved in action potential generation. Thus, Weiss et al. (1979) have suggested that 5-HT-induced enhancement of buccal muscle contractility in Aplysia occurs via an increase in cAMP production, however, subsequent steps in the modulatory process in these cells have not yet been elucidated. On the other hand, Wood et al. (1979) have provided preliminary evidence in myenteric neurons that 5-HT reduces the magnitude of an inward Ca^{2+} current which, in turn, suppresses Ca^{2+}-dependent K^+ conductances. The K^+ conductance change is postulated to directly regulate cellular excitability. The participation of cAMP or other second-messenger as the initial event in 5-HT's action on myenteric neurons has not been excluded.

Whatever the mechanism for the facilitation of excitability in the LGNd, it can be speculated that such an action would endow LC neurons

with the capacity to enhance the transmission of visual signals through the geniculate. In order to appreciate the significance of this phenomenon, it is necessary to consider the physiological activity of the LC neurons which provide the noradrenergic innervation of the LGNd.

In the awake, freely moving rat (Jones et al., 1979) or monkey (Foote et al., 1979) LC neurons respond to a wide range of sensory modalities. Auditory, visual, tactile and possibly other stimuli produce a burst of firing at a latency of 20 to 50 msec followed by a transient decrease in rate. With repeated presentations of the stimulus, this response rapidly habituates.

These observations, in conjunction with the present findings concerning the action of NE in the LGNd, suggest that the coeruleo-geniculate pathway may serve to facilitate the transmission of visual information when the organism is presented with a novel stimulus of the same or different sensory modality. When the stimulus loses its novel character (and therefore an awareness of it presumably becomes less important to survival), the LC system no longer produces its "significance enhancing" function.

It has been speculated that at higher levels of activity the LC may function as an "alarm system" in response to threatening stimuli and serve to prepare the organism for a "survival struggle" (Redmond and Huang, 1979). In such circumstances, it would be adaptive to provide maximum visual awareness as would be the case if the activity of the LC was increased.

In addition to the phasic facilitation of sensory activity in response to novel (and possibly threatening) stimuli, the LC system may act, in part, to set the overall responsiveness of the LGNd with respect to the level of consciousness. For example, during behaviorial alertness

(characterized by desynchronization of the electroencephalogram) the activity of LC neurons tends to be highest. On the other hand, during drowsiness and slow-wave sleep, firing is sporadic (Foote et al., 1979) and when rapid eye movement (REM) sleep commences the cells become silent (Jones et al., 1979). Thus during REM sleep the transmission of visual information through the geniculate would be suppressed possibly allowing an internal dream generator to override any external visual stimuli.

The LC system is characterized by projections to many brainstem sensory nuclei and cortical sensory areas as well as to thalamic sensory relay nuclei such as the LGNd (Levitt and Moore, 1979; Moore and Bloom, 1979). The LC is thus anatomically situated to induce a state of attention or awareness with respect to sensory modalities other than vision, if its physiological action in some or all of these sensory nuclei were similar to its action in the LGNd.

Recent studies in the monkey auditory cortex (Foote et al., 1975) and rat somatosensory cortex (Waterhouse and Woodward, 1980) have indicated that NE can enhance the responsiveness to sound and tactile stimulation in these areas. Interestingly, preliminary evidence suggests that in the somatosensory cortex this facilitatory effect is mediated via an α-adrenoceptor (Waterhouse et al., 1979).

It thus appears that the LC system can have modulatory effects on sensory transmission and processing in many central nervous system areas and that these modulatory actions are mediated, in at least some cases, by α-adrenoceptors. Drugs with α-adrenergic activity which gain access to the brain would therefore be predicted to produce alterations in sensory perception.

Amphetamine, a drug which is believed to increase the synaptic availability of catecholamines by inducing release from presynaptic terminals and by blocking reuptake (Schildkraut and Kety, 1967), does in fact have such effects. In human subjects, characteristic behavioral features of amphetamine use and abuse are hightened awareness to sensory stimuli and an acute sense of novelty and curiosity (Ellinwood, 1967). With chronic use, there may be auditory or visual hallucinations (cf. Bell, 1973) and an overwhelming sense of fear or terror (Ellinwood, 1967). Generally subjects taking amphetamine are free from disorders of thought in spite of these profound psychological effects. The toxic effects of amphetamine are counteracted by chloropromazine (Espelin and Done, 1968), a neuroleptic with potent α-blocking activity (Peroutka et al., 1977).

The psychotomimetic activity of drugs such as mescaline or LSD may also, in part, depend upon an interaction with brain noradrenergic systems which project to sensory areas. Both of these drugs have been found to increase the reactivity of LC neurons to peripheral stimuli (Aghajanian, 1980). In man, the net effect of this might be to increase awareness (a commonly described attribute of the drug experience), produce hallucinations (particularly visual as experienced with these drugs), and possibly create a state of fright or terror (which occasionally occurs in some users).

Under extraordinary circumstances in the absence of drugs, high levels of activity in the LC system may also be perceived as a state of anxiety, fear or terror (Redmond and Huang, 1979). The therapeutic activity of drugs with antianxiety effects such as the benzodiazepines (S. Grant and D.E. Redmond, personal communication) or opiates (see Redmond and Huang, 1979) might be mediated by an action at the LC. It

would therefore be of interest to examine specific postsynaptic α-adrenoceptor-active drugs for behavioral activity and potential therapeutic usefulness in man.

APPENDIX

Portions of the work presented in this dissertation have been published or submitted for publication in the following papers and abstracts:

1. M.A. Rogawski and G.K. Aghajanian (1979): Norepinephrine activates lateral geniculate neurons and facilitates retinal inputs via an α-adrenergic receptor, Soc. Neurosci. Abstr. 5: 350.
2. M.A. Rogawski and G.K. Aghajanian (1980): Activation of lateral geniculate neurons by norepinephrine: mediation by an α-adrenergic receptor, Brain Res. 182: 345-359.
3. M.A. Rogawski and G.K. Aghajanian (1980): Norepinephrine and serotonin have opposite effects on the excitability of lateral geniculate neurons, Abstracts, Ninth Annual Meeting, New England Pharmacologists.
4. M.A. Rogawski and G.K. Aghajanian (1980): Norepinephrine and serotonin: opposite effects on the activity of lateral geniculate neurons evoked by optic pathway stimulation, Exp. Neurol., in press.
5. M.A. Rogawski and G.K. Aghajanian (1980): Modulation of lateral geniculate neuron excitability by noradrenaline microiontophoresis or locus coeruleus stimulation, Nature, in press.
6. M.A. Rogawski and G.K. Aghajanian (1980): Locus coeruleus modulates lateral geniculate neuron excitability via an α-adrenoceptor, Soc. Neurosci. Abstr., in press.

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