ABSENCE OF LONG-TERM POTENTIATION IN THE RETINOTECTAL SYNAPTIC REGION OF THE ADULT RAT SUPERIOR COLLICULUS

By

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ABSTRACT

To answer whether the mammalian retinotectal pathway is modifiable in the adult, an attempt was made to induce long-term potentiation (LTP) in retinal synapes in the superior colliculus (SC) of the adult rat, *in vivo*. Extracellular field potentials were recorded in the primary retinotectal afferent zone of the rat superior colliculus while electrically stimulating the optic chiasm. Induction of LTP in this primary visual pathway was attempted using a wide range of stimulus parameters. However, LTP was not observed. Iontophoretic application of bicuculline methiodide, before and during trains of stimuli, did not facilitate LTP in the rat SC.

The broad spectrum glutamatergic antagonist, kynurenic acid, greatly reduced the size of the field potentials. This supports suggestions that retinotectal neurotransmission may be mediated by excitatory amino acids.

An N-methyl-D-aspartate (NMDA) glutamate receptor mediated contribution to synaptic transmission in the evoked field potential was not evident. Iontophoretic application of the NMDA receptor selective antagonist 2-amino-5-phosphonovaleric acid (APV) had no effect on the field potentials. Even in the presence of bicuculline, there was no evidence for an NMDA component in the field potential response.

The non-NMDA glutamate receptor antagonist, 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX), did not affect the evoked potentials.

These data suggest that LTP was not observed in the retinotectal pathway due to several factors that may include: a loss of visual plasticity in the adult rat following the critical period, absence of necessary modulation factors and insufficient NMDA receptor mediated synaptic transmission.

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I am a part of all that I have seen

Ulysses

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Introduction

Activity-dependent, long-term modification of synaptic transmission may be the basis for information storage in the brain and serve as a substrate for learning and memory (Teyler and DiScenna, 1987). One of the most striking cellular examples of synaptic plasticity in the mammalian brain is the phenomena of long-term potentiation (LTP). This type of neural plasticity has been studied extensively in the hippocampus (Bliss and Lømo, 1970; Bliss and Gardner-Medwin, 1971; Bliss and Lømo, 1973b; Douglas and Goddard, 1975; Alger and Teyler, 1976; Lømo, 1971). These authors demonstrated that brief tetanic stimulation of the perforant path increased the amplitude of population responses of granule cells in the hippocampal dentate gyrus and that these changes lasted for hours or days, thereafter.

LTP has been produced in many other hippocampal preparations and pathways including: the perforant path (Bliss and Gardner-Medwin, 1973a; McNaughton et al, 1978), the mossy fibres (Alger and Teyler, 1976), the Schaffer collaterals (Schwartzkroin and Wester, 1975) and commissural projections from CA3 cells projecting to contralateral areas CA1 (Buzsaki, 1980) and CA3 (Bliss et al, 1983)

LTP has also be induced in other parts of the limbic system including the amygdala, septum, subiculum and entorhinal cortex (Racine et al, 1983), as well as, in rat cerebral cortex (Artola and Singer, 1987; Bindman et al, 1987; Lee, 1982; Kimura et al, 1988), cat cerebral cortex (Sakamoto et al, 1986; Komatsu et al, 1981), rat pyriform cortex (Stripling and Patneau, 1985) and the medial geniculate nucleus of the cat (Gerren and Weinberger, 1983)

In all of these central pathways, which are capable of LTP, the neurotransmitter involved has been suggested to be an excitatory amino acid (EAA). This may imply that all EAA pathways are capable of potentiation. To examine this possibility LTP was studied in the rat retinotectal tract, which

relays visual information from the retina to the superior colliculus (SC) or tectum. While the primary retinotectal neurotransmitter has not been fully established in the rat, evidence (reviewed below) suggests it may be glutamate, aspartate or an associated analog (Aizenman et al, 1988; Anderson et al, 1987; Tsai et al, 1990). Another reason for studying LTP in this pathway is that there is evidence to suggest that LTP-like phenomena play a role in developing and regenerating organizational and functional features in the non-mammalian tectum (Cline et al, 1987; Eisele and Schmidt, 1988; Meyer, 1983; Schmidt, 1990).

Superior Colliculus

The mammalian superior colliculus is a laminar structure located on the dorsal surface of the midbrain. This structure is a multi-modal sensory center whose superficial layers receive visual information from the retina and visual cortex, while deeper layers receive auditory and somatosensory input (Dean et al, 1989; Huerta and Harting, 1984; Moschovakis et al, 1988; Sparks, 1986). The signals from various sensory modalities converge within the superior colliculus onto premotor neurons which are involved in the generation of eye and head movements (Moschovakis et al, 1988). Therefore, the SC is a prominant subcortical sensorimotor structure that plays a role in guiding orienting responses of the head and eyes toward visual, auditory and somatosensory stimuli (Sparks, 1986).

In cross section, the superior colliculus is divided into seven anatomically recognizable cellular and fibrous layers (Huerta and Harting, 1984; Sparks, 1986). These include the stratum zonale (SZ), stratum griseum superficiale (SGS), stratum opticum (SO), stratum griseum intermediale (SGI), stratum album intermediale (SAI) and the stratum profunda (SP) which is often divided into the stratum griseum profundum (SGP) and the stratum album profundum (SAP). The fibrous layers include SZ, SO, SAI and SAP. The SZ, SGS and SO comprise the superficial layers; SGI and SAI the

intermediate layers and SP the deep layers.

Electrophysiological recordings from superficial layer collicular neurons show these cells exhibit a greater response to the appearance, disappearance or movement of a visual stimulus than to details of its form (Dean et al, 1989). Cells located within the intermediate and deep layers of the SC carry motor signals related to orienting movements of the eyes, head and trunk (Dean et al, 1989; Sparks, 1986; Moschovakis et al, 1988).

The superficial gray layers (SGL) have relatively few afferent and efferent connections. The afferent input to the upper layers originates primarily in the retina and visual cortex (Moschovakis et al, 1988; Sparks, 1986). These inputs terminate in a continuous horizontal sheet distribution within sublayers of the SGL (Huerta and Harting, 1984). In the rat, subcortical input from the locus coeruleus has been revealed (Sparks, 1986).

The terminal distribution of afferent pathways produce topographical maps which are an important organizational feature of the mammalian and non-mammalian superior colliculus. In particular, visual space is mapped in retinotopic fashion onto the superficial layers (Huerta and Harting, 1984; Sparks, 1986). This map of the contralateral hemifield, contains cells responsive to a restricted region of the visual field. The location of this receptive field is related to the cell's location within the superior colliculus. Medially located cells possess receptive fields in the dorsal visual space (overhead) while laterally positioned cells have fields in the ventral visual space (Cynader and Berman, 1982). Likewise, position in the nasal-temporal plane is topographically represented in the anterior-posterior direction.

Within the deeper layers, the visual world also remains topographically mapped and there are additional auditory and somatosensory topographic representations (Huerta and Harting, 1984; Sparks, 1986). The intermediate and deep layers also contain motor command maps, which in primates are concerned with saccadic eye movements (Moschovakis et al, 1988). Normally, all the sensory and motor maps are in spatial register.

How are these topographical maps initially organized and how is the alignment of different maps maintained? While these maps are laid down in

early development, recent evidence suggests that the fine-tuning of maps is activity-dependent (Eisele and Schmidt, 1988; Schmidt, 1990). In particular, this sharpening process may involve LTP-like phenomena in the retinotectal pathway.

Retinotectal Pathway

A major afferent pathway to the superficial layers, the retinotectal pathway, originates from the retina. This pathway consists of axons of the W- and Y-retinal ganglion cells.

The Y-retinal ganglion cells respond, in a transient manner, to large objects moving in the visual field, produce initial analysis of crude form and detect abrupt changes in diffuse illumination. Y-cells have large somas (20-30 and up to 40 μm) a large axon and the fastest conduction velocities of all retinal ganglion cells. The Y-cell component of the retinotectal pathway terminates in the deepest part of the SGS, SO and SGI (Hoffman, 1973).

The W-cells form a heterogeneous class which vary considerably in axonal conduction velocities, receptive field properties, somatodendritic morphology, retinal origin, optic chiasm decussation patterns and central target structure terminations (Cleland and Levick, 1974; Fukuda and Stone, 1974; Kirk et al, 1975; Leventhal et al, 1985; Rowe and Stone, 1977; Stanford, 1987; Stone and Fukuda, 1974). The W-component of the retinocollicular pathway terminates almost exclusively in the upper 50μm of the superficial gray layers (Berson, 1987; Freeman and Singer, 1983).

In the cat, retinal W-cells have been divided into two subclasses: W1 and W2 cells (Rowe and Stone, 1977; Stanford, 1987). W1 cells are characterized by sustained responses to light stimuli, small to medium sized somas, moderately slow conduction velocities and project uncrossed from the temporal retina. These cells are thought to contribute to the retinotectal pathway because there are tonic responses to visual stimuli in the SC (Fukuda and Stone, 1974) and the cell bodies of axons that do project to the

SC are medium sized (Stanford, 1987; Stone and Fukuda, 1974; Wassle and Illing, 1980) with moderately slow axonal conduction velocities (Freeman and Singer, 1983).

W2 cells respond transiently to light stimuli, have very small somas, extremely slow conduction velocities and almost always cross from the temporal retina. W2 cells probably comprise the majority of the retinotectal pathway as most retinal ganglion cells innervating the colliculus project contralaterally (Behan, 1981; Behan, 1982; Harting and Guillery, 1976; Sterling, 1973), are extremely small (Leventhal et al, 1985; Stone and Fukuda, 1974; Wassle and Illing, 1980) and have slow axonal conduction velocities (Freeman and Singer, 1983; Mc Ilwain, 1978).

A second major afferent pathway, the corticotectal tract, carries visual information from the visual cortex. The cortical cells are driven by Y-cell input from the lateral geniculate nucleus and their influence on the superficial layers arrives before the slow W-cell input (Berson, 1988).

When recording extracellular field potentials in the superficial layers during electrical stimulation of the optic nerve, the difference in conduction velocities and termination depth is evident. Specifically, various components of afferent input separate in different parts of the evoked field potential waveform. In addition, the polarity of these components changes at different depths as the electrode passes through various layers. These differences allow components of the retinotectal pathway to be studied separately and the active synaptic zones to be localized.

Activity and Map Formation

It has been known for sometime that, following optic nerve crush in frogs and fish, the retinotopic map regenerates and reestablishes on the tectum (Gaze and Jacobson, 1963). Initially, this projection is roughly retinotopic but later sharpens and becomes as precise as the original normal map (Rankin and Cook, 1986; Stuermer and Easter, 1984). This sharpening

or segregation can be blocked by either synchronizing all of the input activity (strobe illumination) or by blocking activity of all afferents by tetrodotoxin (Cook and Rankin, 1986; Schmidt and Eisele, 1985). The establishment of these specific connections may require the coincident activation of afferents from neighboring cells in the retina, so as to stabilize their inputs onto common postsynaptic cells. The requirement for coincident activation of converging afferents is quite similar to that of associative learning or LTP (discussed below).

A model system in which to study the fine-tuning of tectal retinotopic maps is provided by surgically producing three eyed tadpoles. In these preparations, retinal ganglion cells from the normal and supernumerary eyes project to the same optic tectum and produce segregated stereotyped ocular dominance stripes (Cline et al, 1987; Constantine-Paton and Law, 1978). Studies demonstrate afferent activity is required for both eye-specific segregation and retinotopic refinement (Meyer, 1983; Cline et al, 1987; Stryker and Harris, 1986). In addition, activation of the NMDA receptor complex, which is involved in synaptic plasticity, is essential in this process (Cline et al, 1987).

Long-Term Potentiation

The characteristics and requirements for LTP have been demonstrated electrophysiologically in the hippocampus where synchronous synaptic activity evokes large field potentials (Bliss et al, 1983; Bliss and Lømo, 1970; Bliss and Gardner-Medwin, 1971; Douglas and Goddard, 1975; Alger and Teyler, 1976; Andersen et al, 1971; Buzsaki, 1980; Harris and Teyler, 1984; Schwartzkroin and Wester, 1975; Wigström and Gustafsson, 1983). Due to the highly laminated nature of the hippocampus, the small currents generated by a large population of cells sum and generate waveforms which can be attributable to certain cellular events.

A population EPSP is produced by the extracellular current generated

by a population of synchronously activated synapses (Lømo, 1971). The population spike reflects the synchronous discharge of a large number of postsynaptic neurons (Andersen et al, 1971). In LTP, the size of the field EPSP and the amplitude of the population spike both increase.

There are several requirements for LTP. Among them is cooperativity between afferents. This is evident in LTP of perforant path synapses which require coactivation of a considerable number of fibers (McNaughton et al, 1978). These inputs are thought to display cooperativity by producing sufficient postsynaptic depolarization (through spatial summation of EPSPs) to activate NMDA receptors which then trigger potentiation (Artola and Singer, 1990; Ascher and Nowak, 1986; Bliss and Lynch, 1988; Harris and Teyler, 1984). In practice, this cooperative process requires a minimum threshold stimulus intensity during high frequency stimulation (McNaughton et al, 1978). In this study, in order to satisfy this requirement, stimulation of the retinotectal tract was carried out at intensities that produced a near maximum response.

Another prerequisite for LTP induction is the removal of strong inhibition. In the hippocampus, stimulation of the commissural projection to the dentate area, a known inhibitory pathway (Douglas, 1983), during or immediately prior to a tetanus to the perforant path suppresses LTP induction (Douglas, 1978; Douglas et al, 1982). Conversely, application of the gabaergic antagonists bicuculline or picrotoxin in the slice preparation facilitates induction of LTP (Wigström and Gustafsson, 1983). In several cases of this study, to facilitate potentiation, bicuculline methiodide was iontophoresed, in the presence of stimulation trains, in an attempt to reduce any possible gabaergic inhibition.

Other non-gabaergic substances can profoundly affect LTP induction. Wigström and Gustafsson (1983) demonstrated the mode of action of excitatory amino acid antagonists on hippocampal LTP. Specifically, they studied the effects of the N-methyl-D-aspartate (NMDA) receptor antagonists 2-amino-5-phosphonovalerate (APV) and γ-D-glutamylglycine on the induction of LTP in guinea pig hippocampal slices. Experiments were

performed in the presence of picrotoxin to eliminate gabaergic inhibition. In this paradigm, both NMDA antagonists prevented the induction of LTP which implicated NMDA receptor subtype involvement.

Glutamate as a Neurotransmitter

The CNS contains many different neuronal populations that produce excitatory synaptic potentials in their postsynaptic target cells. In many of these populations, the neurotransmitters which mediate synaptic transmission have not yet been characterized. However, for many synapses there is evidence that glutamate, aspartate or a structural analog of these amino acids such as N-acetylaspartylglutamate (NAAG) functions as an excitatory neurotransmitter (Curtis and Johnston, 1974; Johnson, 1972; Krnjevic, 1970; Tsai et al, 1990). However, since glutamate occupies a central role in brain metabolism, it has been difficult to prove conclusively that neuronal populations release it as a neurotransmitter (Lund Karlsen and Fonnum, 1978).

Pharmacological studies of synaptic transmission in central nervous system (CNS) tissue slices (Foster and Fagg, 1984) and cell cultures (O'Brien and Fischbach, 1986; Rothman and Samaie, 1985) have provided the best evidence that glutamate or a closely related compound functions as an excitatory transmitter in certain neuronal populations or pathways. These studies indicate that the transmitter is exerting its effects through excitatory amino acid (EAA) receptors but the chemical identity of the endogenous transmitter remains uncertain.

There are 3 well known classes of glutamate receptors which have been named N-methyl-D-aspartate (NMDA), kainate and quisqualate (Fagg, 1985; Watkins and Evans, 1981). Recently, the quisqualate receptor has been referred to as the AMPA receptor (Keinanen et al, 1990) because of its high affinity for alpha-amino-2,3-dihydro-5-methyl-3-oxo-4-isoxazolepropanoic acid hydrobromide (AMPA). In the present study, this receptor will be referred to

as the AMPA receptor. In addition to these subtypes, there is at least one other putative glutamate receptor class, the L-2-amino-4-phosphonobutyrate (APB) receptor subtype (Bridges et al, 1986).

The NMDA subtype is particularly important in LTP. In the hippocampus, where the proposed neurotransmitters of excitatory pathways are glutamate and aspartate (Cotman and Nadler, 1981; Fonnum, 1984; Storm-Mathisen, 1981), the NMDA receptor subtype is known to be involved in inducing, but not in maintaining or expressing, LTP (Bliss and Lynch, 1988; Collingridge et al, 1983; Collingridge and Bliss, 1987; Harris and Teyler, 1984). Given that LTP is readily inducable within the hippocampus, it is interesting to note that this structure contains the highest number of NMDA binding sites in the brain (Monaghan and Cotman, 1985).

A possible mechanism for the role of the NMDA receptor complex in LTP formation has been proposed (Collingridge and Bliss, 1987). Under physiological conditions, the voltage-dependent Ca^{2+} channel (Ascher and Nowak, 1986; Mayer and Westbrook, 1985) associated with the NMDA receptor is blocked by magnesium. To open the channel, binding of the endogenous ligand to the NMDA receptor (Collingridge et al, 1983) and sufficient postsynaptic membrane depolarization (Malinow and Miller, 1986) must occur. The depolarization produced by glutamate binding to non-NMDA receptors removes the magnesium block of the voltage dependent Ca^{2+} channel (Hvaldy et al, 1986; Mayer et al, 1984).

In this study, three well characterized antagonists of excitatory amino acid receptors were employed in an attempt to determine if a glutamate mediated component was present in the evoked field potential. The drugs utilized were kynurenic acid, 2-amino-5-phosphonovaleric acid (APV) and 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX).

Kynurenic acid is considered to be a broad spectrum glutamatergic antagonist (Ganong et al, 1983) while APV is a selective antagonist of the NMDA class of glutamate receptor (Collingridge et al, 1983; Davies et al, 1981). CNQX blocks non-NMDA glutamate receptors but is predominantly selective for AMPA receptors (Honore et al, 1988).

The main drug utilized in this study was kynurenic acid. This substance is known to block the NMDA current when it is expressed in the absence of APV or Mg^{2+} (Ganong et al, 1983; Huettner and Baughman, 1986; Perouansky and Grantyn, 1989). In cultured tectal and retinal neurons (Coleman et al, 1986; Perouansky and Grantyn, 1989) kynurenic acid has demonstrated high selectivity for kainate receptors. However, high concentrations of kynurenic acid exhibit little selectivity over different excitatory amino acid receptor classes (Perkins and Teyler, 1982).

Excitatory Neurotransmission in the Superficial Gray Layers

As previously noted, the primary retinotectal transmitter has not been identified in the rat. Evidence supporting glutamate, aspartate or an associated analog as an excitatory neurotransmitter in the superficial gray layers is accumulating (Aizenman et al, 1988; Langdon and Freeman, 1986). Cultured neuron preparations from the rat superior colliculus show that the density of binding sites for L-glutamate is elevated in the superficial gray layer. In fact, the highest concentration of glutamate binding sites in the brainstem occurs in the superior colliculus superficial layers (Greenamyre et al, 1984; Halpain et al, 1984; Monaghan and Cotman, 1985). In addition, electrophysiological evidence indicates that cultured superficial gray tectal neurons respond to glutamate and glutamate agonists (Grantyn et al, 1987). Of the four agonists: NMDA, quisqualate, kainate and APB; only APB failed to elicit a response in cultured rat tectal neurons. The APB receptor subtype may be expressed in tectal neurons but may have a higher affinity for kainate as a ligand (Perouansky and Grantyn, 1989).

There is additional evidence supporting glutamate involvement in collicular neurotransmission (Dean et al, 1988; Golden et al, 1989). For example, amino acid profiles in Long-Evans rat superior colliculus indicate high levels of glutamate, gaba, ß-alanine, glutamine, taurine, aspartate and glycine (Golden et al, 1989). Furthermore, Dean et al (1988) have shown

microinjection of glutamate, into the superior colliculus of rats, produces responses resembling defensive behavior. This indicates the SC contains neurons that are glutamate sensitive. In another study, Langdon and Freeman (1986) examined retinotectal neurotransmission in isolated sections of goldfish tectum, by applying antagonists of excitatory amino acids, while recording extracellular field potentials. Kynurenic acid, γ-D-glutamylglycine and cis-2,3-piperidine dicarboxylic acid reduced postsynaptic components of the evoked potentials by over 90%. APV was without conspicuous effect. Therefore, Langdon and Freeman propose retinotectal neurotransmission in the goldfish to be glutamatergic. These examples illustrate that glutamate or a structural analog may function as the candidate neurotransmitter of the retinotectal pathway.

Recently, evidence in favor of the glutamate analog, N-acetylaspartylglutamate (NAAG), as a putative retinotectal neurotransmitter has been presented. Through *in vivo* microdialysis, NAAG has been demonstrated to be released from the rat retinotectal tract in the superficial layers (Tsai et al, 1990). However, the physiological differences, if any, between NAAG and glutamate have not yet been determined.

Long-Term Potentiation in the Superior Colliculus

There is already some evidence that LTP may be induced in the SC. Miyamoto and Okada (1988) demonstrated LTP formation in guinea pig superior colliculus slices *in vitro*. They recorded field potentials in the superficial gray layer, during stimulation of the optic layer. After an initial train of 50Hz for 20 seconds the postsynaptic potential (PSP) increased to 190%. Following this, a second train delivered 25 minutes after the first tetanus produced an increase in the PSP to 270%. LTP was observed when slices were stimulated for 1, 10 and 20 sec at 100Hz and 1, 10, 20, and 30 sec duration at 50 Hz. In addition, these authors demonstrated the addition of APV to the slice bath inhibited the induction of LTP as further evidence

implicating NMDA receptor involvement.

Lewis and Teyler (1986) produced long term potentiation of the synaptic response in an *in vitro* goldfish optic tectum preparation. Extracellular field potentials were recorded in the primary retinotectal (stratum fibrosum et griseum superficiale) synaptic area. The LTP observed had a slow time course and restricted low frequency dependence (optic nerve stimulated at 1 and 5Hz for 100 and 20 seconds, respectively).

LTP was also observed in regenerating or developing systems. Schmidt (1990) found that the regenerating retinotectal projection of the goldfish had an increased capacity for LTP. A train of 20 stimuli at 0.1Hz delivered to this projection, was capable of inducing potentiation. He suggested that the increased LTP sensitivity was related specifically to the activity-dependent sharpening of retinotopic maps.

Cline et al (1987) tested whether activity-driven NMDA activation could play a role in eye-specific segregation in three eyed tadpoles. They found APV produced eye-specific desegregation while chronic NMDA application produced enhanced segregation of ocular dominance stripes. These observations are consistent with an LTP-like process. Thus, correlated spatiotemporal patterns of pre- and postsynaptic activity produce long-term gains in synaptic efficacy through the activation of postsynaptic NMDA receptor complexes (Bliss and Gardner-Medwin, 1973a; Malinow and Miller, 1986). This work suggests that a single process such as LTP could be the mechanism for both sharpening of topographical maps in the developing CNS and plasticity in the mature brain.

LTP in Regeneration of Central Mammalian Pathways

The possibility of inducing LTP in the tectum is interesting because potentiation may play a role in regeneration of this and other central nervous system (CNS) pathways. Interruption of axons in the peripheral nervous system (PNS) of vertebrates and certain CNS tracts in fish and amphibia

leads to extensive regrowth and restoration of anatomical and functional connections (Aguayo et al, 1987). In contrast, severed axons in the CNS of adult mammals fail to regrow substantially and are restricted to short range changes in neuronal connectivity and synapse organization (Raisman, 1985). Optic nerve transection in mammals leads to abortive axonal growth and retrograde degeneration of many retinal ganglion cells (Misantone et al, 1984; Richardson et al, 1982). However, there is hope that functional regeneration of central pathways can occur in mammals (Vidal-Sanz et al, 1987).

The regenerative capacity of adult CNS neurons has been demonstrated in the rat retinotectal system. Transplanted segments of peripheral nerve, which joined the severed axons to the SC, were used as "bridges" to provide injured neurons with critical non-neuronal component interactions for regrowth (Aguayo, 1985). In the presence of these PNS transplants, many retinal ganglion neurons produced lengthy regrowth of their interrupted axons (Aguayo, 1985). This author concluded that functional regeneration of the retinotectal tract requires the promotion and guidance of regenerating axons, and regenerated terminals must reform specific terminal synapses.

Although this pathway is capable of regrowth and synapse formation, only a limited number of retinal ganglion cell axons actually penetrate the superior colliculus (Vidal-Sanz et al, 1987). It remains unclear if these penetrating axons form appropriate, sustained or functional synapses with SC neurons (Aguayo et al, 1987).

Once the techniques for promoting large numbers of these axons to regrow into the tectum have been achieved, then the formation of functional synapses and retinotopic maps may occur. Knowledge about LTP in the SC may be beneficial in promoting this critical stage in regeneration. As described earlier, Schmidt (1990) found that the retinotectal projection in the goldfish displayed an increased capacity for LTP during the time regenerating axons reached the tectum and formed synapses. This indicates that the initial step in stabilizing appropriate branches may be through long-term increase in synaptic gain.

Thesis Objective

The primary purpose of this thesis work was to determine if long-term potentiation could be elicited in the retinal synapes in the adult rat superior colliculus. The plasticity of this selective connection may contribute to our knowledge and enhance superficial layer function with regards to retinotectal mapmaking and inherent regenerative capabilities.

In an attempt to induce LTP in the tectum an attempt was made to optimize the conditions for obtaining LTP. An extremely wide range of stimulation parameters were employed. In order to achieve cooperativity, stimulation of the retinotectal tract was carried out at intensities that produced a near maximal response. Bicuculline methiodide was iontophoresed prior to and during stimulation trains so as to reduce gabaergic inhibition and facilitate LTP.

In addition, the EAA receptor antagonists kynurenic acid, APV and CNQX were utilized in an attempt to demonstrate an NMDA and a non NMDA receptor mediated component in the evoked tectal field potential.

Materials and Methods

Preparation

Experiments were carried out on 89 Long-Evans rats of both sexes weighing from 200 to 450 g. All animals were anesthetized with urethane (1.5-2.0 g/kg ip). The animals were held in a stereotaxic apparatus with the dorsal surface of the head level. An incision was made lengthwise on the head and the skull was subsequently exposed. A craniotomy was performed at stereotaxic coordinates "0" Bregma and 7mm posterior, 1mm lateral (right hemisphere) from Bregma "0" to provide access to the optic chiasm and right superior colliculus respectively. Rectal temperature was maintained at 36-38°C using a servo controlled heating pad (Frederick Haer and Co.). At the conclusion of the experiment, animals were given a lethal dose of urethane.

Stimulation and Recording Electrodes

For the activation of the retinotectal pathway, a concentric bipolar stimulating electrode (Rhodes Medical) was placed in the optic chiasm. Two stimulus isolation units (Neurolog-Medical Systems Corp.) were utilized to deliver constant-current, diphasic square-wave pulses between the core and sleeve of the stimulating electrode. Single test pulses varied according to stimulating electrode position in each animal and ranged from 700 to 2500 μA. All pulses were of 100μs duration.

Collicular field potentials were recorded extracellularly using filament (7 micron carbon fibre) containing multibarrel glass micropipettes (2 or 3 barrel glass, 1.2 mm x 0.6mm, 4" - A.M. Systems Inc.). Micropipettes were pulled using a vertical puller (Narishige). The filament containing the recording barrel was filled with O.9% NaCl. The remaining barrels were filled with the fol-

lowing test solutions: kynurenic acid, 100mM in 1ml ethanol and 4ml, 150mM NaCl soln., pH 8.5 (Perkins and Stone, 1982); CNQX, 50mM in 200mM NaCl soln., pH 5.5 (Honore et al, 1988); APV, 50mM in 150mM NaCl soln., pH 4 (Davies et al, 1981; Perkins et al, 1981); bicuculline methiodide, 10mM in 0.9% NaCl soln., pH 3.5. Typical ejection currents and time of application were: kynurenic acid, 45-150nA for 1-3 minutes; CNQX, 50-80nA for 3-6 minutes; APV, 100nA for 7 minutes; bicuculline methiodide, 40nA for 5-10 minutes. Saline was iontophoresed as a current control. All drugs were obtained from Research Biochemicals Inc..

Stimulation Train Parameters

To test for LTP, high and low frequency trains were delivered to the optic chiasm. Various combinations of frequencies and numbers of pulses were tested: 0.1,1, 2, 5,10, 20, 30 40 50, 60, 100, 200, 400, and 500Hz; 20, 100, 140, 160, 200, 300, 400, 440, 800, 900 and 1000 pulses.

In an attempt to remove gabaergic inhibition and facilitate LTP induction, bicuculline methiodide was iontophoresed in conjunction with stimulus trains. The following trains were delivered to the optic chiasm while bicuculline methiodide was iontophoresed: 10 Hz: 200 pulses; 20 Hz: 400 and1000 pulses; 30 Hz: 100 and 200 pulses; 60 Hz: 140, 200, and 400 pulses; 500 Hz: 800 pulses.

Bicuculline was also co-iontophoresed with APV in an attempt to reveal the NMDA receptor mediated component of the field potential. 2 Hz trains with 440 pulses and 5Hz trains of 200 and 300 pulses were given while APV was applied.

In order to test for late onset LTP, synaptic efficacy was monitored immediately after a tetanus was administered until up to 4 hours later.

Test stimuli, both pre- and post-tetanus, were usually delivered at 0.1Hz.

Electrophysiology

Field potentials were amplified with an A.M. Systems differential AC amplifier. Waveforms were monitored and stored using the Macintosh VAST software program (Douglas, 1990). This unique electrophysiology program provides detailed and convenient analysis of recorded field potentials.

The location of the recording electrode in the superficial gray layer W or Y synaptic layers was determined by the characteristics of field potentials evoked by optic tract stimulation. Thus, the recording electrode was lowered approximately 2500μm where a maximal field response was observed corresponding to the Y-synaptic terminal zone of the retinotectal tract.

Results

Field Potential Characteristics

In this study, the evoked field potentials were similar to those observed in other studies (Berson, 1987; Berson, 1988; Hoffman, 1966). Figure 1 shows two field potentials recorded at different depths in the superficial layers in response to optic chiasm stimulation. The three components of the waveform are labeled in all figures as "P" for a presynaptic fiber response; "Y", the response to the Y-cell input and "W", the component of the waveform evoked by W-cell input. On the rising slope of the waveform between the Y- and W-component, often there were spikes that may correspond to indirect Y-input. However, this component was variable and difficult to measure.

Amplitude measurements were taken of the large negative postsynaptic Y-component measuring from the peak positivity after the initial short latency presynaptic fiber response, to the initial peak negativity. For most of the experiments descibed below, the focus was on the Y-component because this early part of the waveform was not contaminated by prior activity. Also, this component of the waveform was most easily quantified and reproducible from rat to rat.

The initial presynaptic component may serve as one control for changes within the field potential response. That is, if the presynaptic component remains unchanged throughout the course of the experiment, then the observed field response changes are probably due to proper test manipulations.

Increasing stimulus intensity to the optic chiasm elicited correspondingly larger pre- and postsynaptic field responses, up to a maximum response (Fig. 2). In general, the shape of the recorded waveform increases in size in response to increasing stimulus intensity with only small decreases in the latencies of the various components. There was no emergence of additional components with increasing stimulation intensity.

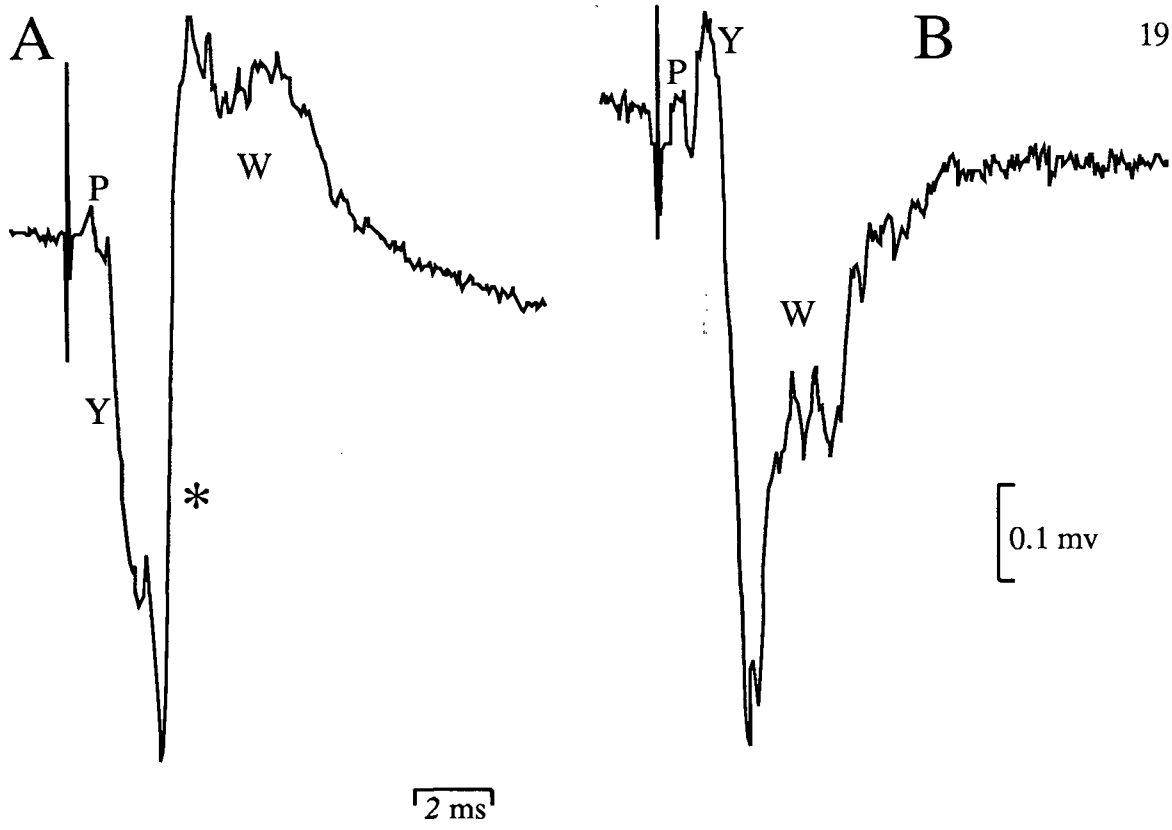

Fig. 1. Field potential recordings in the superficial layers of the rat superior colliculus *in vivo*. A: Field potential response recorded in the deeper superficial layers to a 2500μA stimulus delivered to the optic chiasm. P: the initial short latency components reflect the presynaptic retinotectal afferent volley. Y: the large negative potential is the postsynaptic response to the Y-cell input. W: The long latency positive waveform is the postsynaptic response to the slow conducting W-cell afferent input (Hoffman, 1966; Berson, 1987; Berson, 1988). B: Field potential response recorded in the very superficial layers. Note the P, W and Y components are reversed in the two traces. * denotes where the indirect Y is often evident.

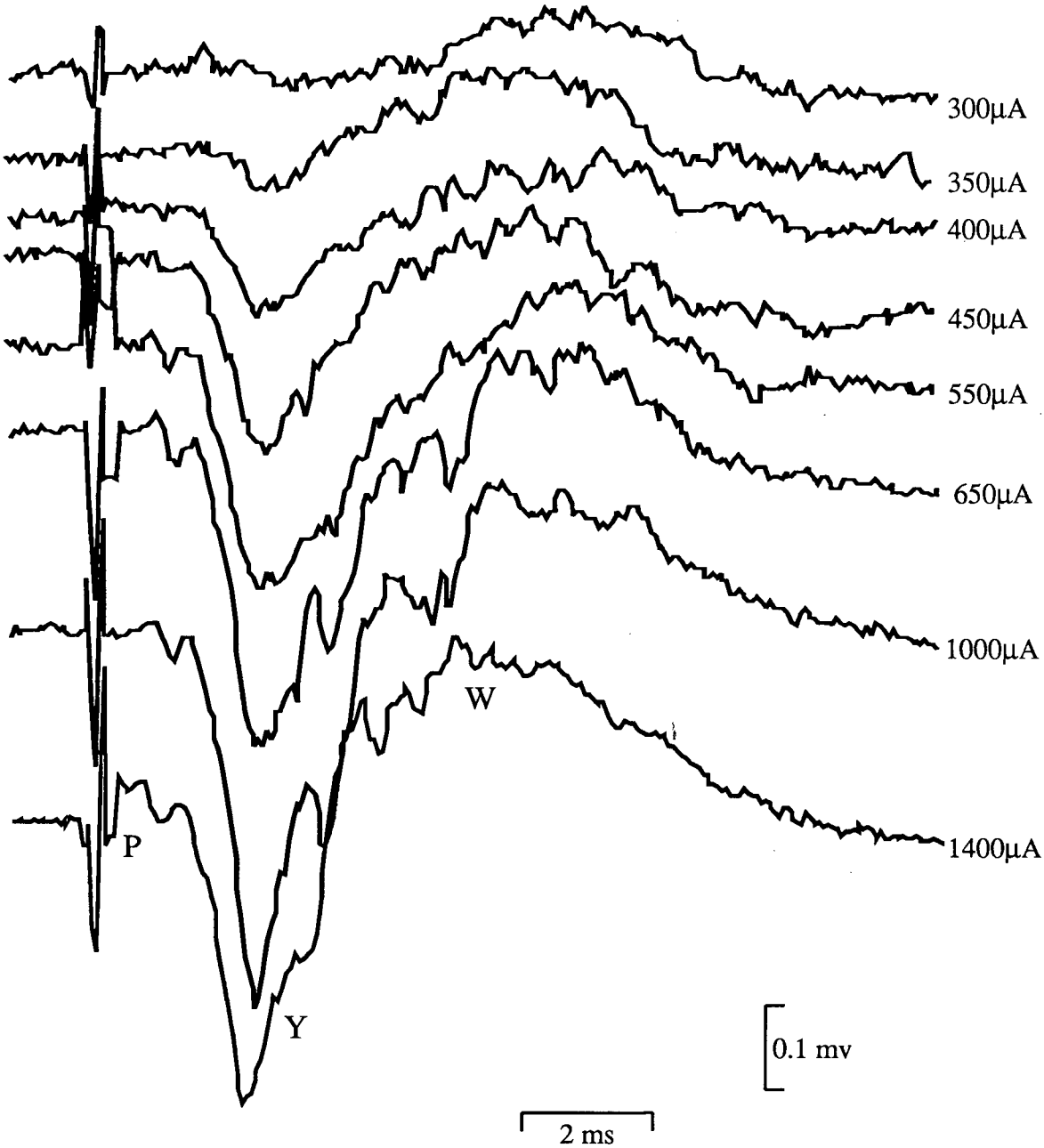

Fig. 2. Field potential responses in the rat superficial gray layers to increasing stimulus intensities (300μA-1400μA, top to bottom) delivered to the optic chiasm. Stimulus intensity is identified for each trace. P: the short latency components increase with stimulus intensity and comprise the presynaptic input. Y: the large negative component also increases with stimulation intensity and is the Y-cell induced postsynaptic field response. W: the large long latency positive potential also increases with stimulus intensity. Recordings were taken in a region responding maximally to optic chiasm stimulation, approximately 2500μm below the dorsal surface of the brain.

This simplified data analysis.

The minimum stimulation intensity needed to produce the maximum synaptic response amplitude was used for most of the test stimuli and trains in the LTP experiments. However, to determine that failure to potentiate was not due to the response itself being saturated, several experiments involved submaximal stimulation.

Accurate placement of the recording electrode was particularly critical for iontophoresis, as the drugs must be applied within the active synaptic zones. In the example depth profile (Fig. 3) the initial recordings were taken above the superior colliculus at approximately 1700μm below the dorsal surface of the brain. The electrode was subsequently lowered in small steps. In this example, at a depth of 2375μm (within the upper layers of the SC) the W-component became maximally negative. Within the next 100μm (between 2375 and 2475μm), the potential rapidly reversed. Below this reversal potential, the Y-component became prominant and reached a peak negativity. Both the Y-and W-components reversed at a similar depth. In this study, these characteristics and general depths were common for all depth profiles of the collicular superficial layers. Other studies (Berson, 1987; Berson, 1988; Hoffman, 1966) have observed the W- and Y-terminal fields with a reversal potential close to their maximum negativity.

Induction of LTP

There are several reports of LTP in tectal slice preparations of goldfish and guinea pig (Lewis and Teyler, 1986; Miyamoto and Okada, 1988). In the first series of experiments, the same stimulation parameters were used in the rat *in vivo*.

Miyamoto and Okada (1988) observed LTP in superior colliculus slices from the guinea pig using a stimulation train of 50 Hz for 20 seconds in duration. However, when similar stimulation trains were delivered in three

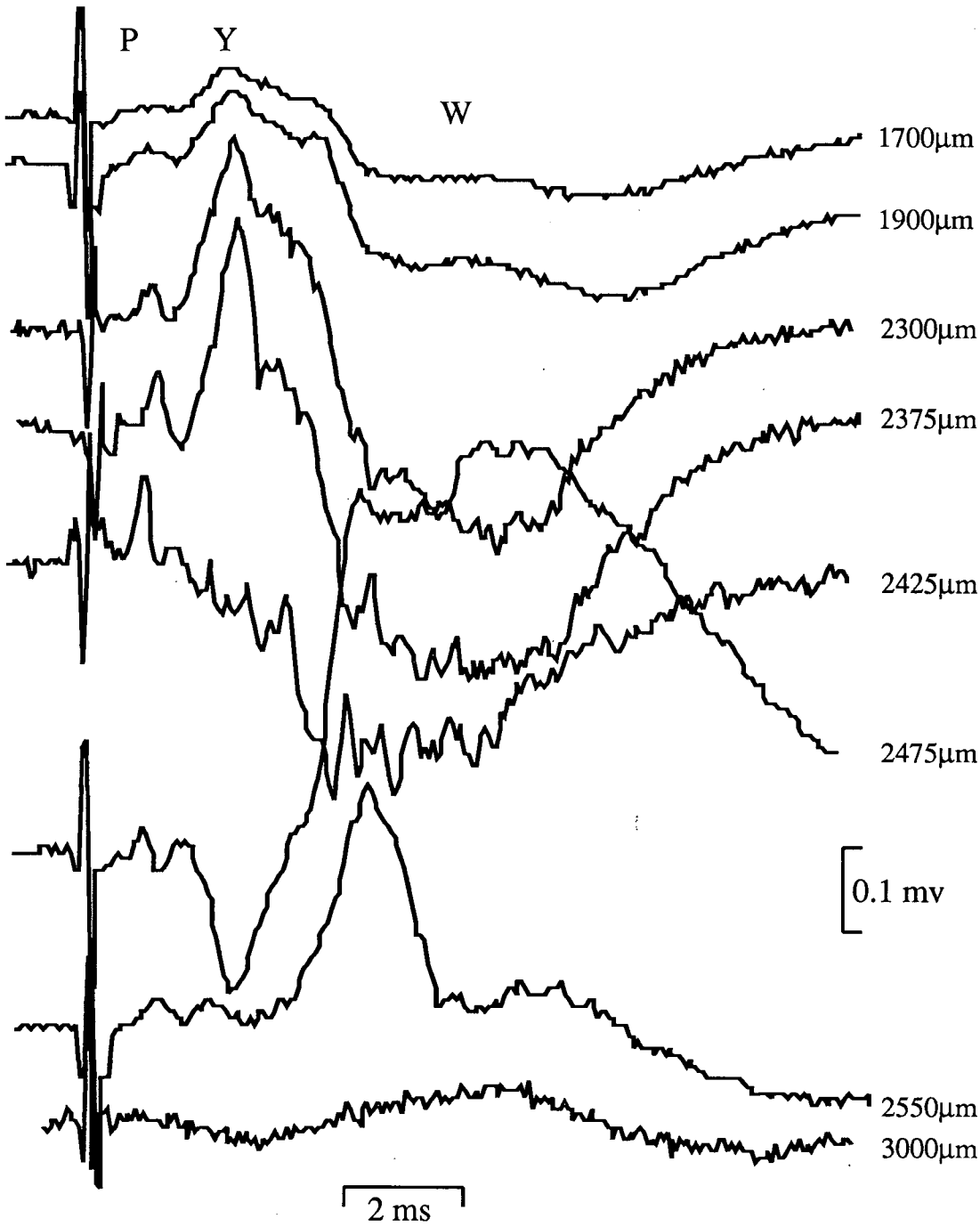

Fig. 3. A depth profile of field potentials in the superficial gray layers of the rat superior colliculus evoked by optic chiasm stimulation. For each trace the recording depth below the dorsal surface of the brain is indicated on the right. Note the change in polarity of the potential between 2375µm and 2475µm.

rats in this study, there was no evidence of LTP in either the Y- or W-component of the field potential. Other 50Hz trains of 2 and 18 second durations were tested in three other rats but again no potentiation was observed.

In another 13 animals, similar stimulation parameters to those employed by Lewis and Teyler (1986) in the goldfish optic tectum slice preparation were used. In these cases, stimulus intensity was first adjusted to produce half maximal field potential response, trains of stimuli at 1 (n=3) and 5 Hz (n=10) for 100 and 20 seconds respectively, were presented (Fig. 4 and Fig. 5). The 1 and 5Hz trains of stimuli did not produce LTP.

Schmidt (1990) produced LTP in the regenerating retinotectal projection of the goldfish using a train of 20 stimuli at 0.1Hz. Similar stimulation trains to those used by Schmidt failed to induce LTP in the superficial layers of 3 rats tested (test stimuli were delivered at 0.01Hz). In fact, throughout the course of most of the other experiments in this study, test stimuli were delivered at this frequency (0.1Hz) and did not appear to induce potentiation.

In order to verify that test stimuli were not potentiating the SGL responses in the rat tectum, the field potentials evoked by the initial 65 test stimuli were recorded (Fig. 6). This was accomplished by stereotaxically positioning the stimulating and recording electrodes in the optic chiasm and superficial layers respectively, without using any stimulation. Then the very first evoked potentials were recorded but there was no progressive change in the size of the responses. Therefore, the failure of higher frequency trains to potentiate was not a result of initial test stimuli inducing LTP.

Low and medium frequency trains did not induce LTP in the rat superficial layers. Therefore, high frequency trains were administered. Frequencies of over 100Hz are often used to obtain LTP in the hippocampus (Racine et al, 1983; Teyler and DiScenna, 1987). Figure 7 shows an example in which trains of 800 pulses at 200 and 500Hz were used. A variety of high frequencies and durations were used in 8 rats. Table I is a summary of the different stimulation conditions which were utilized. In all cases LTP was not observed.

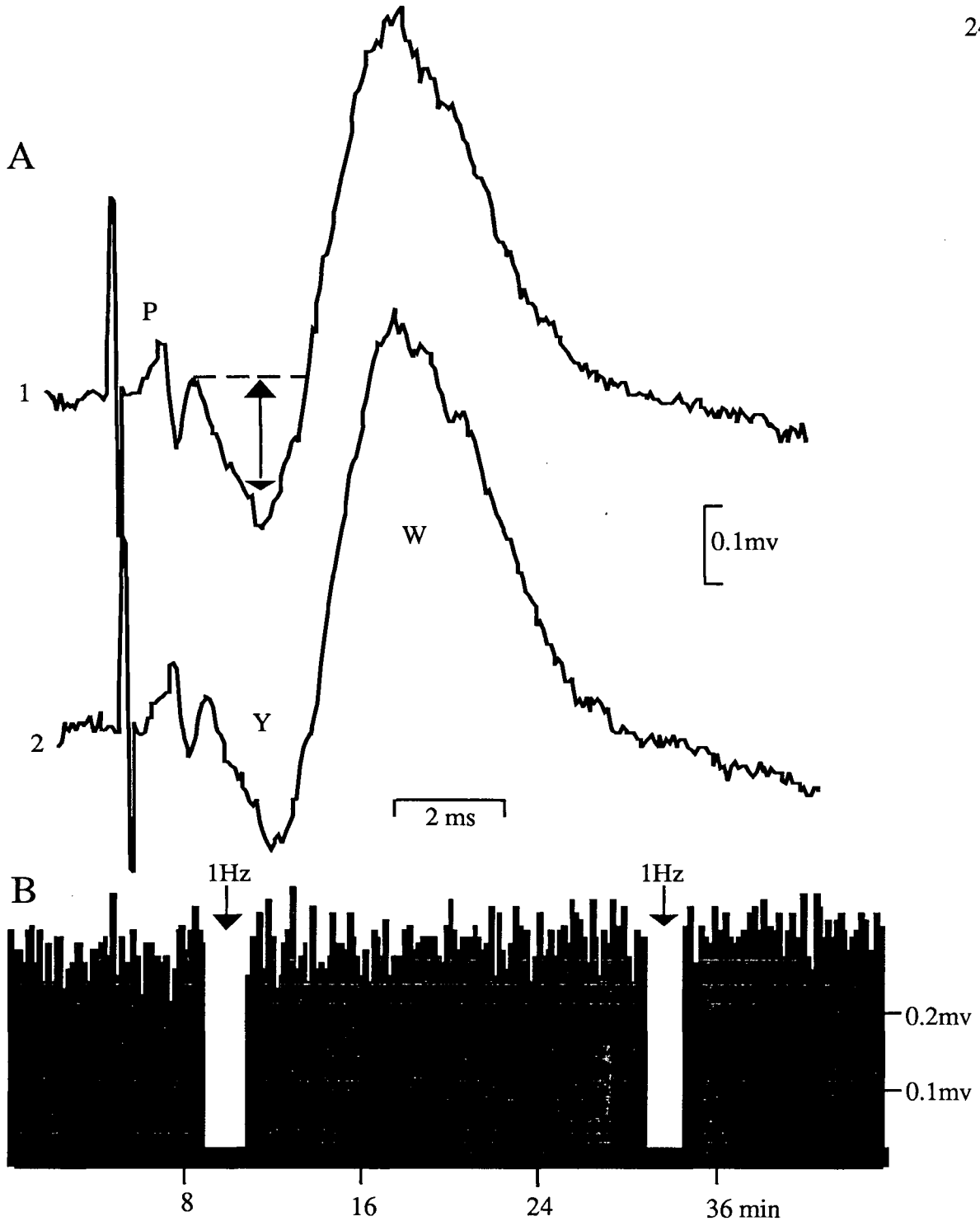

Fig. 4. Field potential responses recorded in the Y-synaptic layer of the superficial layers. A: representative waveforms recorded in the Y-synaptic superficial layer (1) before and after (2) the trains of stimuli (100 pulses at 1Hz). The arrow indicates where the Y-component was measured. B: Size of Y-component over the course of the experiment. Test stimuli were delivered at 0.1Hz.The two breaks in the histogram represent individual trains of stimuli (100 pulses at 1Hz) delivered to the optic chiasm.

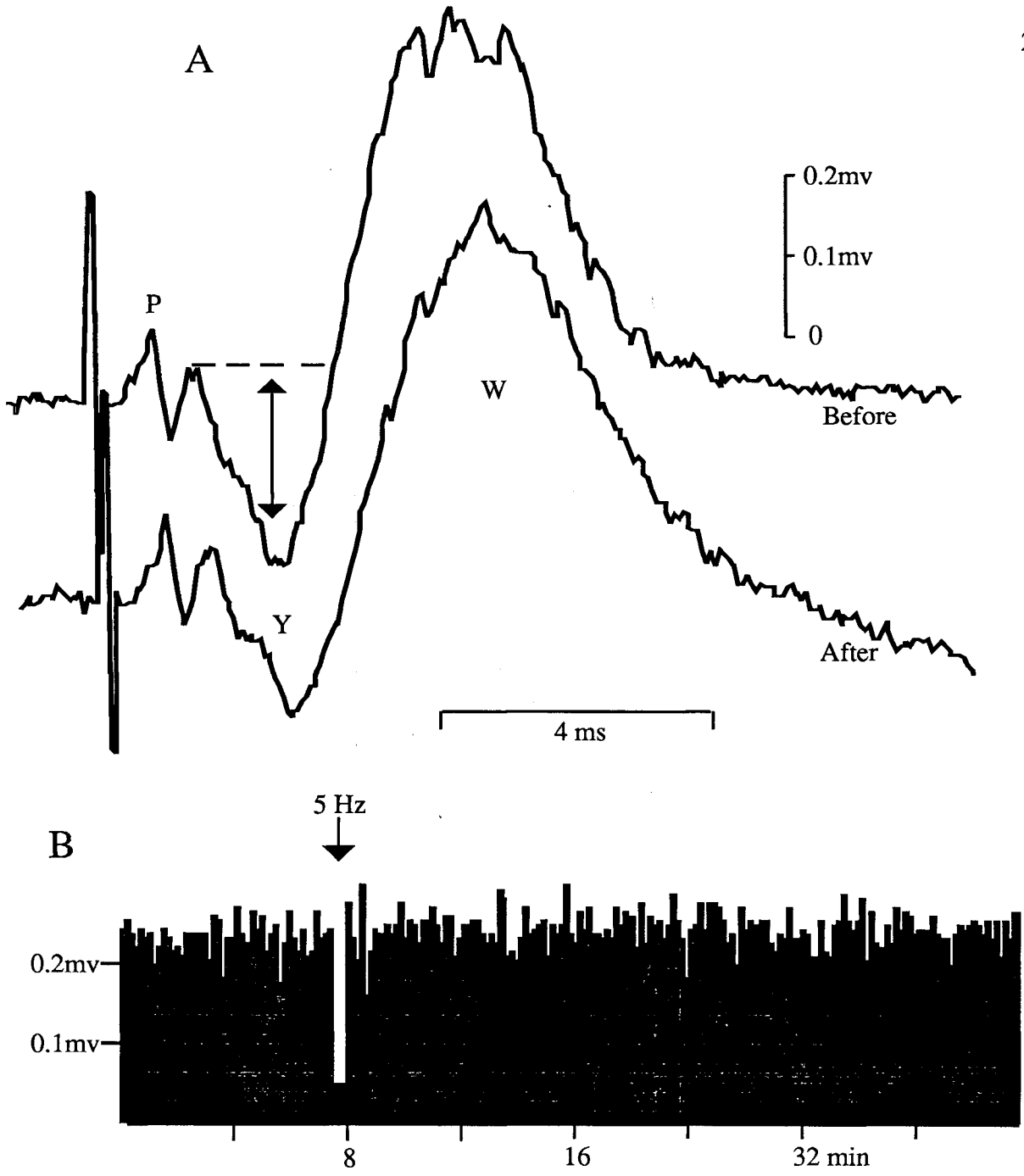

Fig. 5. A: Effect of a 5Hz, 100 pulse train on the field response in the Y-synaptic superficial layer. B: Size of the Y-component measured as indicated in "A" by the arrow.Test stimuli were delivered at 0.1Hz.The break in the histogram denotes the point at which a train of stimuli (100 pulses at 5Hz) was delivered to the optic chiasm. Note there were no changes observed.

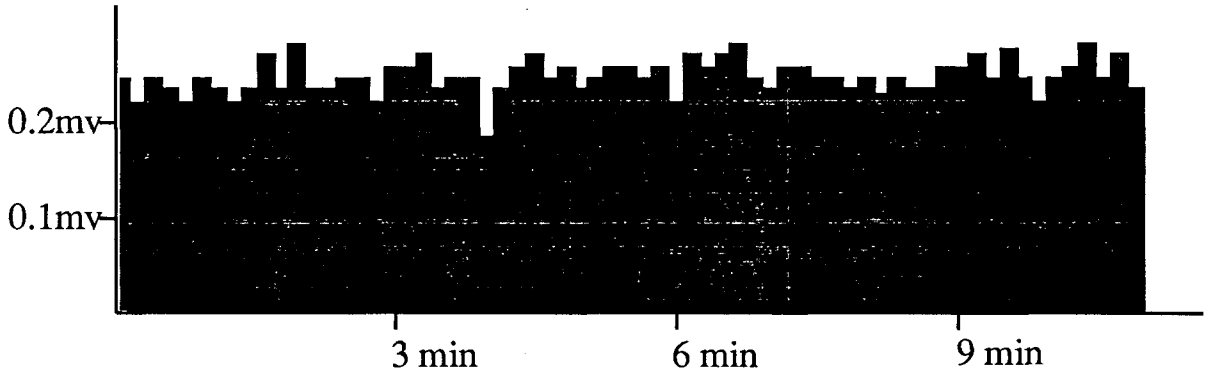

Fig. 6. Size of the amplitude of the Y-synaptic component in the very first field potential responses evoked in the rat optic chiasm. Stimulation was presented at 0.1Hz which is the frequency most commonly used as test stimuli in other LTP experiments.There was no potentiation observed.

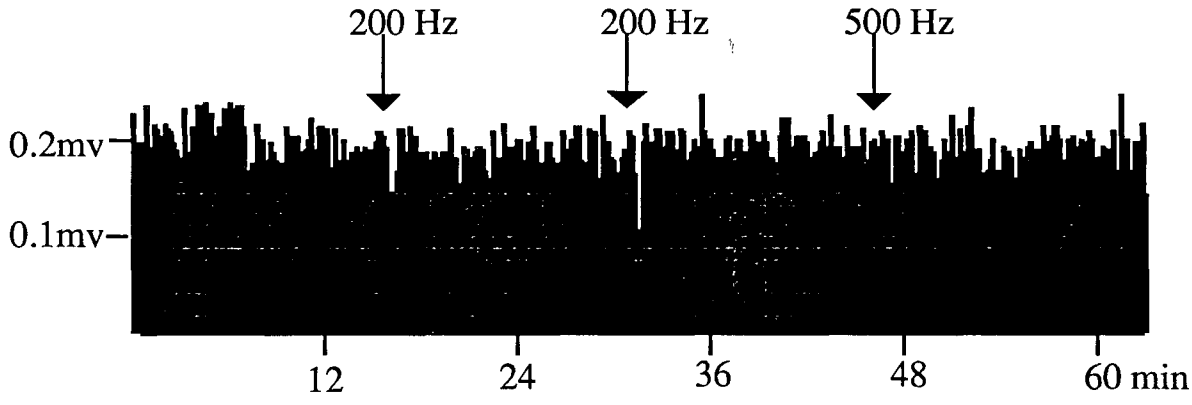

Fig. 7. Size of the amplitude of the Y-component in the rat superficial Y-synaptic layer in response to high frequency trains of 800 pulses at 200Hz (2) and 500Hz delivered to the optic chiasm.Test stimuli were delivered at 0.1Hz.

Table I. Stimulation train parameters utilized in an attempt to induce long-term potentiation in the superficial gray layer synaptic region of the adult rat superior colliculus *in vivo*.

Frequency Hz	Number of Pulses	n
0.1	20	3
1.0	100	3
2	140, 440	2
5	100, 200, 300	17
10	160, 200	3
20	100, 400, 1000	16
30	100, 200, 400	6
40	400	1
50	100, 900, 1000	6
60	100,140,200,400	24
100	800	3
200	800	3
500	800	2

It is important to note that throughout the course of most experiments the field potentials were monitored for delayed onset of LTP. In many cases, several trains were given and recording lasted for several hours. Not only was there no evidence of LTP immediately after trains but there was no evidence for delayed onset of LTP.

Finally, in several experiments, to facilitate cooperativity, the visual cortex was electrically stimulated simultaneously with the optic chiasm. However, the convergent input failed to potentiate the SGL synaptic region. This may be due to the non-overlapping terminal fields of the corticotectal and retinotectal pathways (Huerta and Harting, 1984; Lund, 1966).

Effects of Bicuculline

Since gabaergic inhibition is known to block LTP (Douglas et al, 1983; Douglas et al, 1982; Wigström and Gustafsson, 1983), bicuculline methiodide (10mM, pH 3.5, ejection current of 40nA), a $GABA_A$ antagonist, was iontophoresed within either the W- or Y-synaptic layers. In twenty rats tested, bicuculline was iontophoresed at least 3 minutes before each train. Table II contains the stimulation parameters used in the presence of bicuculline. No LTP was observed in any of these animals.

Bicuculline did have transient effects on the field potentials within the upper layers. There was a change in the long latency components of the field potential response during bicuculline iontophoresis (Fig. 8). The Y- and W-components were unaffected.

Table II. Stimulation train parameters utilized in conjunction with bicuculline methiodide iontophoresis in the rat SC.

Frequency Hz	Number of Pulses	n
10	200	1
20	100, 400, 1000	6
30	100, 200, 400	2
60	100,140,200,400	10
500	800	1

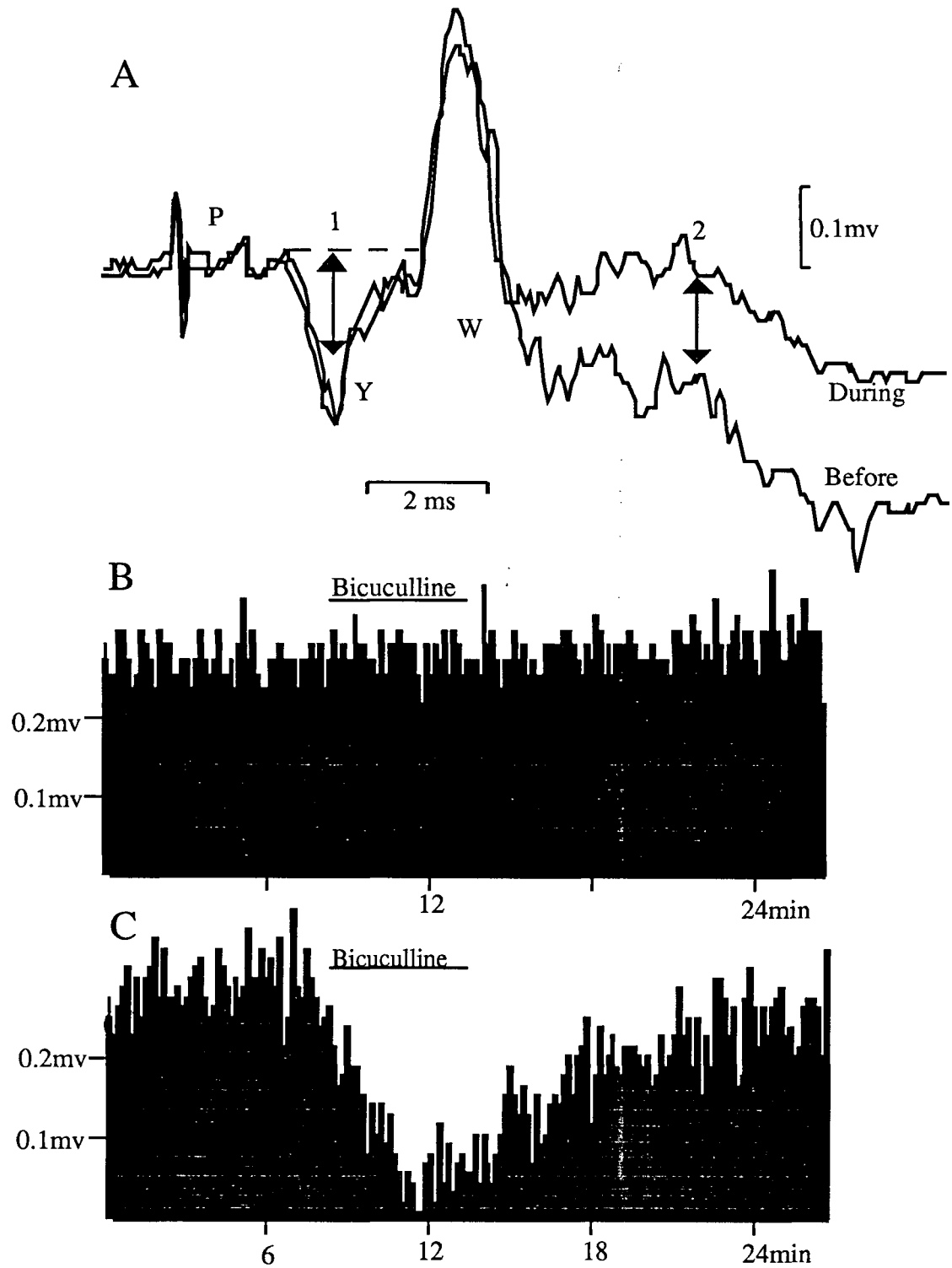

Fig. 8. Action of iontophoretically applied bicuculline methiodide (10mM, pH 3.5; ejection current of 40nA) on the field potential responses evoked in the superficial layers of the rat superior colliculus. A: comparison of representative waveforms before and during bicuculline application. B: size of Y-component as indicated by arrow (1) in "A" to iontophoretically applied bicuculline for 5 minutes. C: size of long latency components as indicated by arrow (2) in "A". Bicuculline was applied for 5 minutes. Test stimuli were delivered at 0.1Hz.

Retinotectal Neurotransmission and Glutamate Antagonists

When applied with cathodal currents of 45-150 nA for 1-3 min, kynurenic acid (100mM, pH 8.5) had a strong effect on the field potentials recorded in the superficial gray layers. This broad spectrum excitatory amino acid antagonist reduced different components of the postsynaptic field response (Fig. 9). Specifically, while stimulating the optic chiasm and recording in the Y-synaptic layer of the superficial layers, kynurenic acid application reduced the Y-component of the postsynaptic response. The W-component was unaffected. The opposite occurred when recording in the W-synaptic layer. There, kynurenic acid reduced the W-synaptic component while leaving the Y-synaptic component unaffected (Fig. 9).

The second drug iontophoresed, CNQX, a non-NMDA glutamatergic antagonist, failed to reduce the field potential response of the superficial layers. Various concentrations, pH's and vehicles were used for iontophoresis in an attempt to reduce or abolish the field response. However, in all cases there were no effects observed (Fig. 10). To determine the effectiveness of the iontophoretic solution, CNQX was applied to the dentate gyrus of the rat hippocampus. While stimulating the perforant path, CNQX significantly reduced the field potential response in this structure (Fig. 10).

Finally, the NMDA receptor antagonist APV, was iontophoresed within the superficial layers. When APV was iontophoresed at 20mM, pH 4, 40-100 μA for 3-5 min, there were no observed changes in the postsynaptic field response (Fig. 11). Furthermore, APV had no effects on long latency components even when bicuculline was coiontophoresed (Fig. 12). This suggests that a significant contribution to the evoked field potential response was not NMDA mediated.

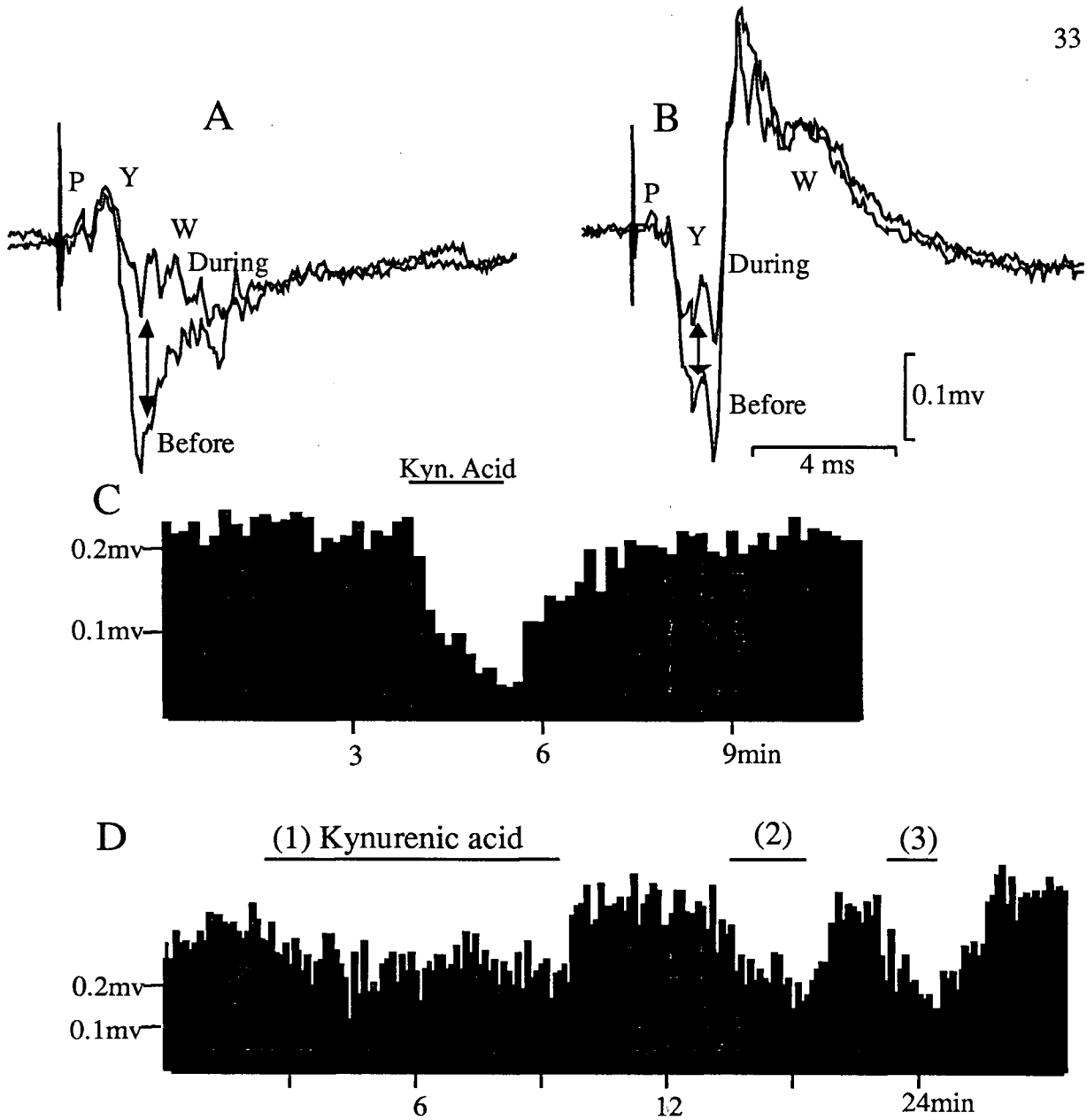

Fig. 9. Action of kynurenic acid iontophoresis in the superficial layers of the rat superior colliculus. A: two sample waveforms evoked in the W-synaptic layer in response to kynurenic acid iontophoresis (100mM, pH 8.5). Waveforms were recorded before and during iontophoresis. B: two sample waveforms evoked in the Y-synaptic layer before and during kynurenic acid application. C: size of the amplitude of the W-component as indicated by the arrow in "A". Kynurenic acid was iontophoresed for 1.5 min. with a cathode ejection current of 80nA. D: size of the amplitude of the Y-component as indicated by the arrow in "B". Kynurenic acid was applied for (1) 7 minutes with a 45nA cathode ejection current; (2) 2 minutes with a 60nA cathode ejection current; (3) 1.5 minutes with a 85nA cathode ejection current. Test stimuli were delivered to the optic chiasm at 0.1Hz.

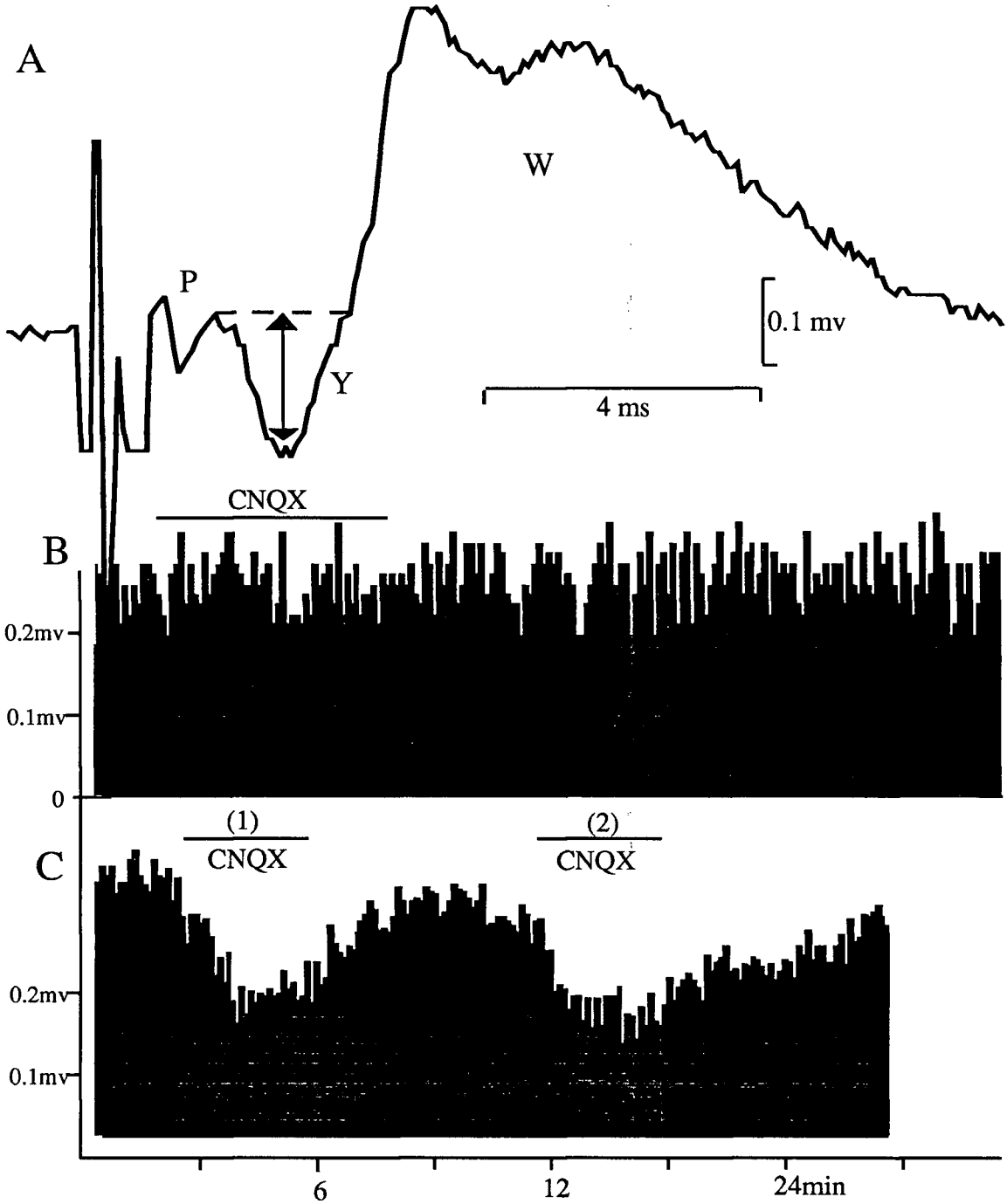

Fig. 10. Effects of CNQX in the hippocampus and superficial layers of the rat superior colliculus. A: sample waveform indicating the Y-component was measured as indicated by the arrow. B: size of field potential responses evoked in the Y-synaptic layer of the collicular superficial layers. CNQX (50mM, pH 5.5) was applied for 5.9 minutes as indicated with a cathode ejection current of 80nA. C: size of the field responses recorded in fascia dentata of the adult rat hippocampus (amplitude of population EPSP measured) to iontophoretically applied CNQX *in vivo*. (1) CNQX applied for 3 min. with a cathode ejection current of 50nA. (2) CNQX applied for 3 min. with a cathode ejection current of 60nA.

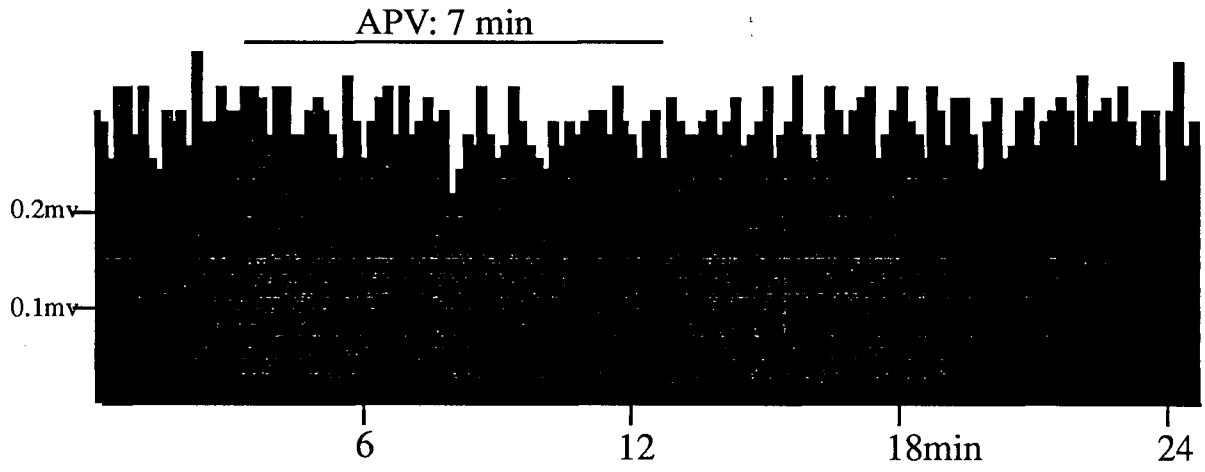

Fig. 11. Effect of APV on the size of the Y-component of the field potential responses.These responses were evoked in the Y-synaptic layer of the rat superior colliculus. APV (50mM soln. in 150mM NaCl, pH 4) was applied with an ejection current of 100nA for seven minutes.Test stimuli were delivered to the optic chiasm at a frequency of 0.1 Hz.

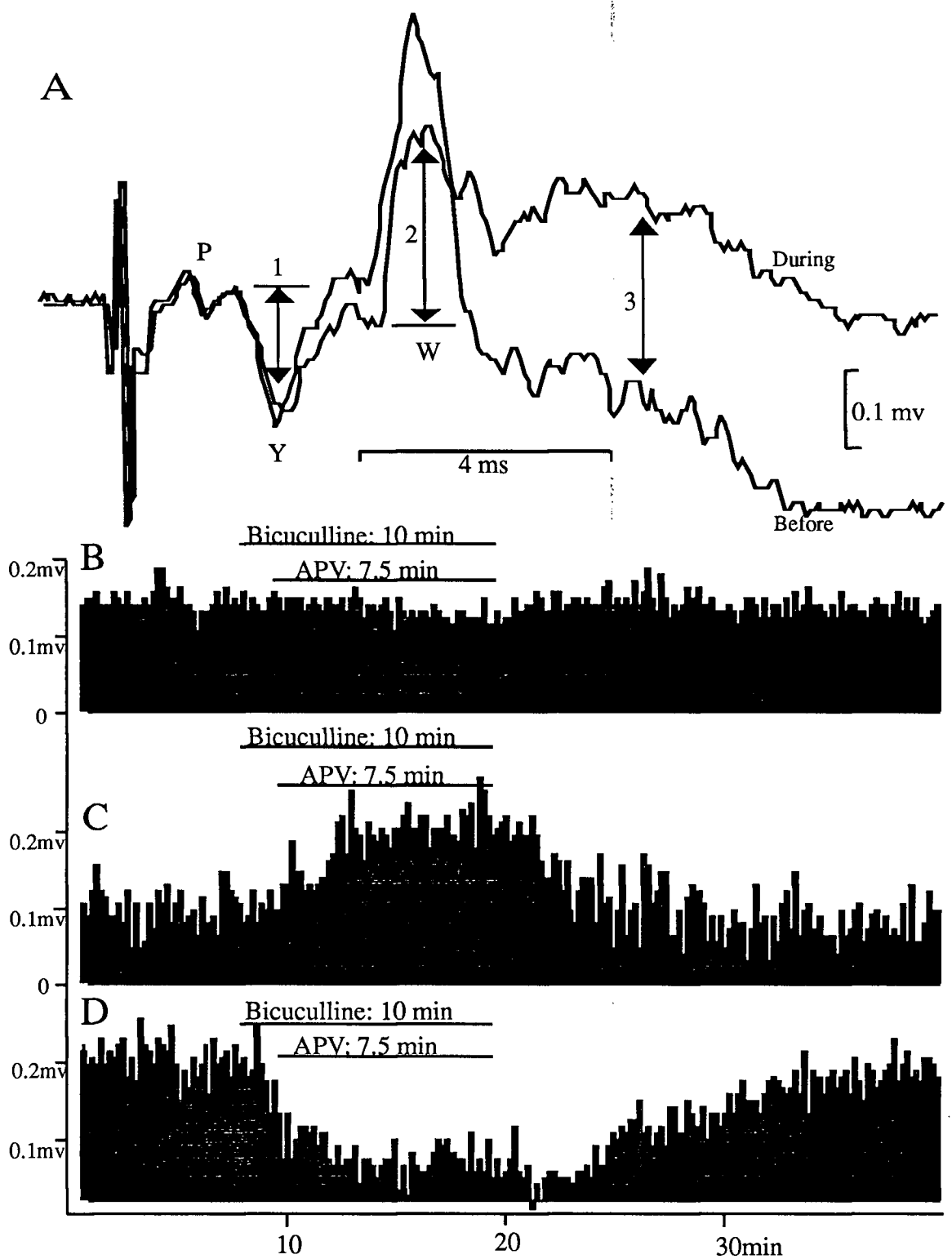

Fig. 12. Action of cointophoretical application of bicuculline methiodide and APV. A: comparison of individual waveforms evoked in Y-synaptic layer before and during bicuculline (10mM, pH3.5: ejection current of 40nA) and APV (50mM soln. in 150mM NaCl, pH 4; ejection current of 100nA) application. The drugs were applied at the times as indicated. B: size of Y-component as indicated by arrow 1 in "A". C: size of W-component as indicated by arrow 2 in "A". D: size of long latency component as indicated by arrow 3 in "A" (note the similarity of bicuculline iontophoresis in Figure 8).Test stimuli were delivered at 0.1Hz.

Discussion

This study provides evidence that *in vivo* retinotectal synapses in the adult rat do not potentiate. This is in direct opposition to positive findings in *in vitro* preparations (Kimura et al, 1988; Miyamoto and Okada, 1988; Perkins and Teyler, 1988), lower vertebrates studies (Lewis and Teyler, 1986; Schmidt, 1990) and evidence that many other glutamatergic synapses potentiate (Bliss and Lømo, 1970; Bliss and Gardner-Medwin, 1971; Bliss and Lømo, 1973b; Douglas and Goddard, 1975; Racine et al, 1983; Stripling and Patneau, 1985). There are several possibilities why LTP was not observed.

Stimulation Frequency

Tetanus frequency and duration play a key role in triggering LTP (Komatsu et al, 1981; Lee, 1982). In this study, a wide range of high and low frequencies (from 0.1Hz to 500Hz with durations from 2 to 60 seconds) were employed. Many of these stimulus parameters have been used successfully in other structures such as the hippocampus and cortex (Kimura et al, 1988; Racine et al, 1983; Teyler and DiScenna, 1987). Other stimulation trains utilized were similar to those which had induced LTP in superior colliculus slice preparations (Lewis and Teyler, 1986; Miyamoto and Okada, 1988). However, regardless of the stimulation protocol, LTP was not observed.

In addition, short-term potentiation was not induced in any of the experiments. In the hippocampus, short and long-term potentiation can be easily demonstrated (McNaughton, 1982). Short-term potentiation may facilitate the induction of LTP as excitation builds faster than inhibition. Therefore, it appears that the synapses of the tectum and hippocampus differ with respect to short-term synaptic modification. This difference may contribute to the absence of LTP in the tectal superficial layers.

Furthermore, providing the necessary conditions for LTP in the superior colliculus through electrical stimulation may not be possible. This type of stimulation excites large numbers of axons in a synchronous manner and depolarizes many postsynaptic cells simultaneously. Any inhibitory circuits present that could block LTP, may also be concurrently activated.

Cooperativity

In the hippocampus, synapse modification requires the coactivation of a considerable number of fibers (McNaughton et al, 1978), presumably to depolarize the postsynaptic cells to the NMDA threshold (Collingridge et al, 1983; Harris and Teyler, 1984). In an attempt to satisfy the cooperativity requirement, relatively high intensity pulses were employed in this study to produce maximum field potential responses. Since the retinotectal pathway is the major afferent input, this requirement should have been achieved. However, extracellular recordings can not confirm this. The absolute size of the field potential varies enormously with recording location and the geometry of the cells. It is unclear whether stimulation, which produces a maximum field response, is sufficient to meet the cooperativity requirement. In fact, the observations are consistent with the possibility that the cooperativity threshold was not reached, since field potentials within the SGL were smaller than in the hippocampus and there were no obvious population spikes.

Inhibitory Influences

In the hippocampus, the high cooperative threshold is due, in part, to the level of background inhibition (Douglas and Vetter, 1988; Wigström and Gustafsson, 1983). Therefore, to lower the cooperativity threshold and facilitate LTP induction in the SGL, gabaergic inhibition was reduced through

iontophoretic application of bicuculline methiodide. It was observed that the later components of the tectal field potential became broader and larger. This indicates that gabaergic inhibition was present and may have been shunting the late components.

Stimulation trains administered, while gabaergic inhibition was suppressed, did not produce LTP. Even in the presence of bicuculline, there were no obvious population spikes. Therefore, it would appear that gabaergic inhibition does not contribute to the evoked field potential response. However, it is possible that there was a large non-$Gaba_A$ inhibitory component present that was blocking LTP.

APV and NMDA Receptors

Cooperativity and the removal of strong inhibition are insufficient to induce LTP (Bliss and Gardner-Medwin, 1973a). The presence of NMDA glutamate receptors is also required (Collingridge and Bliss, 1987). Therefore, the number of NMDA receptors in retinotectal synapses is an important determinant for LTP capability in this synaptic region. Binding studies show that the number of L-glutamate binding sites in the brainstem is highest in the superficial layers of the SC (Greenamyre et al, 1984; Monaghan and Cotman, 1985). The NMDA-sensitive tritiated glutamate (100μM) binding sites within the superficial layers is reported to be .231 +/- .018 pmol/mg of protein and .131 +/- .026 pmol/mg protein in the deep layers (Monaghan and Cotman, 1985). This is 5 times less than in the hippocampus (Monaghan and Cotman, 1985). Therefore, even though the cooperativity requirement may have been achieved, there may be insufficient NMDA receptors present to trigger the next stage of LTP. There is also no evidence that these receptors are localized in retinotectal synapses.

In this study, the application of APV demonstrated an insignificant NMDA receptor contribution to the field potential response in the superficial layers. This is consistent with observations in the hippocampus and cortex

where APV has little effect on the amplitude and initial time course of monosynaptic EPSPs (Collingridge et al, 1983; Crunelli et al, 1983; Davies and Watkins, 1983; Honore et al, 1988). During high frequency trains an APV-sensitive contribution may be observed in the slow time course of large EPSPs. It is possible that retinotectal synaptic NMDA receptor numbers are so few, that even when the conditions are met for their channels to open, the response to trains of stimulation is insufficient to overcome a critical threshold for LTP induction. If the absence of LTP in the superficial layers is due to the relative efficiency of NMDA receptor mediated synaptic transmission, then NMDA receptor numbers play a critical role.

Retinotectal Neurotransmission and Non-NMDA Glutamate Receptors

Since glutamate and the NMDA subtype of glutamate receptor are candidate prerequisites of LTP, the nature of retinotectal neurotransmission is an important issue in this study. Though the putative neurotransmitter of this pathway remains uncertain, increasing evidence favors an excitatory amino acid or a structural analog such as N-acetylaspartylglutamate (NAAG) as the endogenous neuroactive substance(s) (Tsai et al, 1988; Tsai et al, 1990; Westbrook et al, 1986).

The present study demonstrates that kynurenic acid (100 mM) consistently inhibits both the Y- and W-components of the field potential. These data indicate that excitatory retinotectal transmission is mediated, at least in part, by excitatory amino acid neurotransmitters. However, kynurenic acid is not useful in identifying the receptor subtype, due to the lack of receptor specificity. It has been suggested that kynurenic acid effects are predominantly mediated through non-NMDA receptors (Ganong et al, 1983; Perouansky and Grantyn, 1989).

It is intriguing that the non-NMDA glutamate receptor antagonist, CNQX, had no observable effects on the retinotectal field potentials. This was

not due to technical problems with iontophoresis or diffusion, since CNQX reduced the field potential in the dentate gyrus of the hippocampus.

There are two common non-NMDA receptors: AMPA and kainate receptors. Comparing different binding studies (Greenamyre et al, 1984; Insel et al, 1990; Monaghan et al, 1984; Monaghan and Cotman, 1985), it would appear that there are fewer AMPA receptors than kainate receptors. It has been shown that CNQX has a higher affinity for AMPA receptors than for kainate receptors (about 1/5 as effective at kainate receptors) (Honore et al, 1988). Thus, CNQX may not be expected to have profound effects on a largely kainate receptor mediated field response.

There is another possible explanation for the lack of a CNQX response in the evoked field potentials of the superficial layers. Recently the AMPA receptor has been shown to consist of at least four different subtypes (Keinanen et al, 1990). If in addition there are kainate subtypes present, it is possible that the inability of CNQX to elicit a response is due to the presence of AMPA and kainate receptor subtypes which are CNQX insensitive.

Age

In mammals, the development of the visual system depends on visual experience (Frégnac and Imbert, 1984). During a definitive period of post-natal development, called the critical period, the visual system is susceptible to various modifications by environmental influences (Kimura et al, 1988). There may be a critical period for plasticity in some components of the visual cortex (Komatsu et al, 1981; Perkins and Teyler, 1988). In rats, the critical period for some aspects of visual function extends up to about 40 days (Stafford, 1984). During this time, glutamate binding sites in the brain peak at postnatal day 15 which coincides with eye opening. Thereafter, the number of glutamate binding sites drastically reduces to adult levels by day 25 (Schliebs et al, 1985).

These conditions are similar to those Schmidt (1990) found during the

regeneration of the retinotectal pathway of the goldfish, when it is particularly sensitive to LTP induction and NMDA receptor blockers. Therefore, LTP may occur at an early age when the number of NMDA receptors have peaked and activity-dependent processes are involved in development.

Miyamoto and Okada (1988) were able to induce LTP in tectal slices from 30 day old guinea pigs. These animals may still have been within their critical period. In cat (Komatsu et al, 1988) and rat (Perkins and Teyler, 1988) visual cortex there is strong evidence for a critical period, but LTP has been observed in the adult rat visual cortex (Artola and Singer, 1987; Artola and Singer, 1990).

There are several factors that may contribute to the reduction in LTP susceptibility following the critical period. For example, a decrease in NMDA receptor number and changes or increases in patterns of inhibition. Furthermore, some modulation factor which is present during development may become absent or be reduced in the adult.

There may be other reasons why LTP has been observed in the slice preparations of young animals. First of all, the level of tonic activity would be quite different in the slice protocol. For example, there would be no spontaneous retinal presynaptic activity in the *in vitro* paradigm. Also, there would be no spontaneous activity on any modulatory inputs or there may be a complete loss of inhibitory modulation. Miyamoto and Okada (1988) also stimulated the optic layer and thus, may have activated many non-retinotectal synapses. It is possible that these non-retinotectal synapses can potentiate, or that their "activation"may be needed to potentiate the retinal input.

Summary

In conclusion, this thesis provides evidence that long-term potentiation can not be induced within the retinotectal synapses of the adult rat *in vivo*. The most likely hypothesis for this observation is that the number of NMDA receptors may be too low for LTP induction. Future studies should involve the use of a complete developmental series with particular emphasis on 15 day old animals when NMDA receptor numbers have peaked and activity-dependent processes, like LTP, may contribute to different aspects of development.

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