ELECTROPHYSIOLOGICAL MEASURE OF NMDA ACTIVATION IN KINDLING

AN ELECTROPHYSIOLOGICAL MEASURE OF NMDA ACTIVATION IN PERFORANT PATH KINDLING

By

PAMELA ANN NELLIS, B.Sc.

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AUTHOR: Pamela Ann Nellis, B.Sc. (University of Toronto)

SUPERVISOR: Professor R.J. Racine

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ABSTRACT

High frequency stimulation of the perforant pathway triggers a prolonged field potential in the dentate gyrus that far outlasts that obtained with single pulses. The late rising component of this field potential has recently been shown to be mediated by N-methyl-D-aspartate (NMDA). In the present thesis, rats were implanted with stimulating electrodes in the perforant pathway and recording electrodes in the dentate gyrus of the hippocampus. Baseline input/output functions of field potentials (or population EPSPs) were established for each rat. Ketamine, an NMDA receptor antagonist, was then administered to confirm its effects on the late component of the EPSP. The late component was measured by subtracting the pulse-evoked from the train-evoked response. Ketamine was shown to significantly attenuate the late component. Diazepam, a GABA agonist, had no significant effect on the late component. Having established an NMDA component in the field potential allows for the monitoring of the levels of NMDA activation over prolonged periods. Hence, the effect of kindling, an animal model of temporal lobe epilepsy, was also determined. Fully kindled rats--defined as those who had experienced four Stage 5 seizures--also had significantly attenuated late components. In contrast to decreased late components, kindled rats

displayed increased population spike amplitudes and EPSP slopes. Such a decrease in the late component suggests that the NMDA receptor plays a role in kindling. Subjects were also given ketamine and diazepam following kindling, whereby the effects were proportionately the same as those observed prior to kindling.

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INTRODUCTION

Epilepsy is a complex condition caused by a variety of pathological processes in the brain. Neuroscientists have used animal models of epilepsy, such as kindling, to investigate the mechanisms of the disease. Kindling involves the progressive development of epileptiform activity and associated behavioural convulsions in response to the repeated application of initially subconvulsant electrical stimulation. It has been suggested that excitatory neurotransmitters play a role in kindling and, hence, epilepsy. In particular, the excitatory amino acid, N-methyl-D-aspartate (NMDA), has been shown both to mediate the development of kindling and to play a role in the expression of previously kindled seizures. Many of these studies have focused on the hippocampus, or its related pathways, because of their involvement in human temporal lobe epilepsy and because of the relative ease of kindling these sites. A characteristic progression to the kindled state, for example, may be elicited by stimulating the perforant pathway. Connectivity in this pathway can be monitored by pulsing the perforant path and recording the potential evoked in the target site, the dentate gyrus. High frequency stimulation triggers a prolonged field potential which far outlasts that obtained with single pulses. Subtraction of the pulse-evoked from the train-evoked response yields a waveform that peaks at about 15 msec and lasts for

about 50-70 msec. This late component is also thought to be mediated by NMDA and as such, will be used as a means of monitoring the involvement of the NMDA receptor in kindling.

Kindling

Kindling is an animal model of temporal lobe epilepsy characterized by a progressive and permanent increase in epileptogenicity (Racine, 1978). Kindling is accomplished by delivering initially subconvulsive, relatively low intensity electrical stimulation to the brain until electrical seizure activity and associated behavioural convulsions develop. The phenomenon was first defined by Goddard (Goddard, 1967; Goddard et al., 1969) and is currently considered to be the best available model of secondary generalized temporal lobe epilepsy (Goddard, 1983; McNamara, 1986, Racine, 1978), primarily because the animal seizure mimics human seizure behaviour and has a similar EEG pattern. Moreover, the mechanisms underlying kindling serve as an excellent example of neuronal plasticity and may, in fact, effect changes in synaptic function similar to those observed in learning and memory (Goddard and Douglas, 1975; Morrell and Toledo-Morrell, 1986). Kindling may represent a pathological distortion of the mechanisms of synaptic plasticity involved in normal synaptic transmission. As such, an understanding of the mechanisms of kindling would elucidate both normal and

pathological brain function.

Kindling has been demonstrated in a variety of animal species including rat, frog, rabbit, dog, and baboon (Goddard, 1967; Morrell and Tsura, 1976; Tanaka, 1972; Wada and Osawa, 1976; Wauquier, Ashton, and Melis, 1979). It has also been demonstrated in a wide range of brain sites, including anterior neocortex, entorhinal cortex, striatum, globus pallidus, hippocampus and other limbic structures, preoptic area, pyriform cortex, and brain stem (Goddard, McIntyre, and Leech, 1969; Racine, 1978). The amygdala is the structure most commonly kindled, primarily because relatively few stimulations are required to induce kindling.

The kindling procedure involves the implantation of an electrode and repeated, spaced application of a train of biphasic square waves (eg. 1 sec train at 60 Hz). The stimulation parameters may vary, as may the time interval between stimulations, with the primary concern being the elicitation of an electrographic afterdischarge (AD). The intensity of current used for kindling is determined by the AD threshold, defined as the weakest current that will evoke an electrographic AD. Racine (1972a,b) has demonstrated that the spaced and repeated occurrence of AD is both necessary and sufficient for the development of kindled convulsions. Racine (1972a) has also shown that repeated electrical stimulation at intensities below AD threshold will result in a decrease of

the AD threshold. Thus, an initially subthreshold stimulus, if delivered repeatedly, can ultimately evoke an AD.

The resultant increase in neural responsivity during kindling is reflected in the animal's behaviour as well as in its electroencephalogram (EEG). The epileptiform AD evoked by the first epileptogenic stimulation is relatively brief (about 10 s). Moreover, the EEG "spikes" that characterize the AD occur at a low frequency (1-2 Hz). As kindling progresses, the AD duration increases to about 60-100 s while the AD spike frequency increases to 4-5 Hz. With repeated elicitation of Ads, spaced in time, there is a progressive increase in the morphological complexity, amplitude, duration, and propagation of AD to other brain sites (Racine, 1972b).

The progressive increase in electrographic seizure strength reflected in the AD is accompanied by a progressive increase in the associated behavioural convulsions. The development of motor seizures is typically rated according to Racine's (1972b) five point scale: (1) mouth and facial movements; (2) head clonus; (3) unilateral forelimb clonus; (4) rearing with bilateral forelimb clonus; (5) loss of postural control, and clonus of all four limbs. Each later stage also encompasses the behavioural responses of the preceding stages. In the present paper, a fully kindled animal is defined as one that has experienced three Stage 5 seizures.

Immediately following the AD and convulsion, a brief (1-60 sec) flattening of the EEG and an absence of the animal's movement are observed. This state is referred to as the post-ictal depression. Afterwards, the rat's EEG and behaviour are relatively normal, with the exception of interictal spikes seen in the EEG. These are single epileptic spikes which occur most frequently following a seizure and decrease in frequency as time progresses.

The enhanced sensitivity to electrical stimulation observed in kindling is long lasting and may, in fact, be permanent. Animals kindled to stage 5 and left unstimulated for as long as twelve months or more will respond with a stage 5 seizure to one of the first two electrical stimuli delivered (Corcoran, 1988; Goddard, McIntyre, and Leech, 1969). Furthermore, it is possible to continue evoking ADs in a variety of brain sites until animals show spontaneous seizures. However, numerous stimulations are required before rats begin to seize spontaneously (Pinel, 1981; Pinel & Rovner, 1978). It is thought that the permanent functional changes that accompany kindling may be related to a mechanism that operates at the synaptic level.

There have been three prominent hypotheses regarding the neurophysiological mechanisms of kindling: these have been summarized by Racine et al., (1986). First, given that field potentials increase in amplitude following kindling (Douglas

and Goddard, 1975; Racine, 1972; Racine et al., 1983), it has been proposed that kindling may be due to long term potentiation (LTP) of excitatory synapses (Goddard and Douglas, 1976). However, for a variety of reasons (see Corcoran, 1988), it is now evident that this mechanism cannot completely account for kindling. Second, kindling has been proposed to be due to disinhibition, via loss of local inhibitory influences. However, rather than decreased inhibition, an increase in local inhibition has generally been found following kindling (Racine et al., 1986; Tuff, Racine, and Adamec, 1983), suggesting that kindling is due neither to a failure of recurrent GABA-mediated inhibition nor to a failure of GABA-independent long-latency inhibition.

A third putative mechanism of kindling may be the development of spontaneous epileptiform bursting in neurons that drive epileptiform bursts in other brain sites (Racine et al., 1986). Some neurons can develop patterns of high-frequency burst discharge after activation, with bursting evident both as spontaneous interictal discharges and as burst-like discharges of action potentials in response to electrical stimulation. These epileptic neurons become burst generators that drive epileptiform responses in other circuits of neurons, which ultimately results in the triggering of behavioural and electrographic seizures. The amygdala, pyriform cortex, entorhinal cortex, and the ventral

hippocampus have been implicated as burst generating sites (Kairiss et al., 1984). Thus, high-frequency stimulation can alter neurons in these sites such that a stronger burst response can be evoked in kindled neurons relative to control neurons.

In addition to the electrophysiological effects, kindling also induces neurochemical changes. There is substantial evidence for the involvement of excitatory amino acids (EAAs) in kindling. The application of EAA agonists directly into the brain can evoke epileptiform activity and convulsions. Glutamate administration in rats results in both epileptiform EEG activity and convulsions (Stewart, Coursin, and Bhagavan, 1972).

The N-methyl-D-aspartate (NMDA) receptor has been shown to play a facilitatory role in kindling. The NMDA antagonist DL-2-amino-5-phosphonovaleric acid (APV) has been shown to significantly retard the rate of amygdala kindling in rats (Cain, Desborough, and McKitrick, 1988; Holmes and Goddard, 1986). APV also reduced the duration of AD and acted as an anticonvulsant in fully kindled rats. Similar effects have been reported for the NMDA channel blocker, MK-801 (Gilbert, 1988; McNamara et al., 1988). Thus, the NMDA receptor appears to play a role in both kindling development and the expression of fully-kindled seizures. If NMDA receptors are also involved in mediating the late component of the EPSP, then

examining the size of the late component before and after kindling will help determine the degree of involvement of the NMDA receptor in kindling. Hence, the effect of kindling on the NMDA mediated late component will be the primary question addressed in this thesis.

N-Methyl-D-Aspartate

The N-methyl-D-aspartate (NMDA) receptor is an excitatory amino acid (EAA) receptor subtype. EAAs, primarily L-glutamate and related derivatives, are the major excitatory neurotransmitters in the vertebrate CNS. At least four receptor subtypes can be differentiated. Of these, three can be defined by agonists for which they show a relatively high affinity: N-methyl-D-aspartate (NMDA), quisqualate (quis), and kainate (kain) receptors (Watkins and Evans, 1981), with quis and kain often grouped together as "non-NMDA" receptors.

The development of specific antagonists that distinguish NMDA receptors from the other EAA receptor subtypes has resulted in clarification of the role of NMDA receptors in a variety of neural systems. **Competitive** antagonists selective for the NMDA receptor include DL-2-amino-5-phosphonovaleric acid (APV) and (3-2-carboxypiperazine-4-yl)-propyl-1-phosphonic acid (CPP) (Davies et al., 1981, 1986). Competitive antagonists interact at the recognition site of receptors and have been shown to block NMDA components of

EPSPs, block the induction of LTP and kindling, protect against the toxic action of EAAs, and act as anticonvulsants in some types of experimental epilepsy (eg. Kemp et al., 1987). Most of these antagonists have difficulty in passing the blood brain barrier. Consequently, development of NMDA blockers for therapeutic usage has been slow (Meldrum, 1985).

Other agents such as MK-801 (Wong et al., 1986) and the dissociative anaesthetics, ketamine and phencyclidine (PCP) (Anis et al., 1983), act as **non-competitive** NMDA antagonists. Unlike the competitive antagonists, the target of the dissociative anaesthetics is the ion channel associated with the receptor, rather than the agonist recognition site itself. Non-competitive antagonists are voltage dependent: blockade of the NMDA response depends upon the membrane potential. Antagonists appear to interact with the ion channel associated with the NMDA receptor almost exclusively when it is in the open state and not when it is closed. The relative potency of blockers can be accounted for by their relative rates of escape from the open channels. However, activation of the NMDA channel is dependent upon both glutamate and depolarization: it is chemical-dependent as well as voltage-dependent.

The use of antagonists has further established the role of EAA receptors in synaptic transmission, as well as their involvement in certain pathological conditions such as

epilepsy and ischemic cell death (Meldrum, 1985). As well, the NMDA receptor is known to be involved in the induction of long term potentiation (LTP); a phenomenon that has been utilized as a model of learning and memory storage (Thompson, 1986). In fact, intraventricular administration of APV can both prevent LTP within the perforant path-dentate gyrus system **and** selectively impair learning of a spatial task (Morris et al., 1986). APV has no effect on normal synaptic transmission.

During normal synaptic activity, NMDA receptors appear to be obstructed by a voltage dependent, Mg^{2+}-mediated blockade (Mayer et al., 1984; Nowak et al., 1984). The depolarization and glutamate release produced by the high frequency stimulus might be sufficient to open NMDA receptor channels by removing the Mg^{2+} block (Collingridge et al., 1988). This additional depolarization would in turn activate more NMDA receptors, producing a cascade effect and somehow initiating the process of LTP and possibly kindling induced potentiation (KIP). The activity of the NMDA receptors is most likely coupled to a mechanism for maintaining the synaptic enhancement through the kainate/quisqualate receptors as NMDA receptors are thought only to be involved in the induction of LTP and not its maintenance (Collingridge, 1985).

The Role of EAAs in Hippocampal Synaptic Transmission

Throughout the central nervous system, the highest density of specific binding sites for NMDA can be found in the dendritic field of CA1 pyramidal neurons, where the Schaffer collateral-commissural fibres terminate (Monaghan et al., 1984; Olverman et al., 1984). Selective NMDA receptor antagonists do not affect normal, single-pulse elicited synaptic potentials in this system. However, NMDA receptors have been shown to participate in high-frequency synaptic transmission in the hippocampus, their involvement during low-frequency transmission being greatly suppressed by endogenous Mg^{2+} (Herron et al., 1986). Non-NMDA receptors (especially kainate receptors) have been shown to mediate voltage-independent fast-acting conductances in the hippocampal tri-synaptic circuit (Collingridge et al., 1983; Lambert et al., 1989), while NMDA receptors mediate voltage-dependent slower acting responses (Nowak et al., 1984; Mayer and Westbrook, 1987).

The voltage-dependence of the NMDA receptor-activated currents is due to a block by extracellular Mg^{2+}. The blocking action of Mg^{2+} has been proposed to occur by the binding of Mg^{2+} to a site within the channel complex that is sensitive to the membrane potential. Both the affinity of the binding site for Mg^{2+} and the Mg^{2+} block of current flow through the channel increase with hyperpolarization (Mayer and Westbrook, 1987).

Current flow through the NMDA receptor-associated channel is conditional upon sufficient depolarization of the membrane to remove the Mg^{2+} block of the channel. Thus, at the level of the synapse, NMDA receptor-induced responses depend upon concurrent presynaptic release of transmitter and postsynaptic depolarization.

Activation of NMDA receptors is capable of transiently elevating intracellular concentrations of Ca^{2+} (Mayer et al., 1987) and a portion of this increase comes from a flux of Ca^{2+} directly through NMDA channels (Ascher and Nowak, 1988). Intracellular Ca^{2+} could then lead to subsequent biochemical and structural processes necessary for long-term changes in synaptic strength (Lynch and Baudry, 1984; Miller and Kennedy, 1986). This increase in permeability to Ca^{2+} also appears to be involved in the modulation of intracellular second messenger systems involved in trans-synaptic signalling and in a number of pathological conditions such as epilepsy.

Synaptic potentials which can be blocked by NMDA receptor antagonists are usually only revealed by experimental manipulations that facilitate unblocking of the NMDA-operated ionophore. One such manipulation is the removal of extracellular Mg^{2+} ions (Coan and Collingridge, 1985; Collingridge et al., 1988a; Mody et al., 1987). When the hippocampal slice perfusate was changed from one containing 1mM Mg^{2+} to one with no added Mg^{2+}, Coan and Collingridge

(1987a, 1987b) observed a pronounced increase in population spike amplitude, the appearance of secondary population spikes, and in some slices, the development of spontaneous epileptiform discharges. The latter two events were abolished by the selective NMDA antagonist APV, suggesting that these spikes and discharges are NMDA mediated. As well, ketamine and PCP selectively blocked the secondary component of the synaptic response (Coan and Collingridge, 1987b). This effect is unlikely to involve cholinergic or adrenergic systems as agonists and antagonists of these systems neither blocked nor mimicked the effects of PCP. Coan and colleagues (1987) also found MK-801 to selectively block the putative NMDA receptor-mediated component.

Another manipulation used to study the late component involves blockage of GABA-ergic feed-back and feed-forward inhibition with picrotoxin or bicuculline (Hablitz and Langmoen, 1986; Wigstrom et al., 1985). This inhibition causes the membrane to return rapidly to the resting potential following an EPSP. As well, depolarization to levels where the Mg^{2+} blockade of the channel is partially alleviated can be achieved by repetitive stimulation at a frequency which allows temporal summation of the EPSPs (Collingridge et al., 1988b; Herron et al., 1986). During the induction of LTP, delivery of high frequency bursts is required in order to depolarize postsynaptic neurons to a degree that is sufficient

to remove the Mg2+ blockade and thus allow Ca^{2+} to enter the postsynaptic spines (Herron et al., 1986; Larson and Lynch, 1988). In the present thesis, high frequency trains of pulses will be delivered to the perforant path. The field potential thus elicited in the dentate gyrus will include a prolonged waveform referred to as the late component. This component is positive at the soma layer and most likely results from removal of the Mg^{2+} blockade by the trains, as described above.

The Role of NMDA Receptors in Kindling

There is now considerable evidence that the NMDA receptor is important in the genesis of seizures. EAA transmitters participate in normal synaptic transmission throughout the CNS, so it is not surprising that such excitatory pathways are involved in the initiation and propagation of seizures. Electrophysiological studies indicate that seizure activity is associated with increased participation of NMDA receptors in excitatory synaptic transmission. Epileptiform discharge is characterized by large depolarizations which may represent a strong activation of the NMDA receptor. In the dentate gyrus, NMDA receptors become actively involved in synaptic transmission following long-lasting neuronal changes induced by kindling of the amygdala or hippocampus. Biochemical studies suggest that a change intrinsic to the NMDA receptor-channel complex may contribute to the increase in NMDA

receptor-mediated synaptic transmission.

The non-competitive NMDA receptor antagonists ketamine and MK-801 have been shown to retard the kindling process in a dose-dependent fashion, as evidenced by both an increased latency to stage 4 or 5 seizures and shorter AD duration (Gilbert, 1988; Morimoto, 1989; Trommer and Pasternak, 1990). Several selective, competitive NMDA receptor antagonists have also been shown to have an inhibitory effect on kindling. The competitive antagonist 2-amino-7-phosphonoheptanoic acid (AP7) has been shown to inhibit both fully kindled seizures and the development of the kindling process itself (Holmes et al., 1990; Watkins and Olverman, 1987).

Similarly, APV has been shown to retard seizure development dose-dependently (Holmes and Goddard, 1986; Holmes et al., 1990). As well, CPP has been shown to dose-dependently retard focal and generalization stages in rats kindled in the amygdala (Davies et al., 1986; Harris et al., 1986; Holmes et al., 1990). Finally, glutamate-induced kindling of the amygdala is inhibited by the NMDA receptor antagonists AP7 and CPP (Croucher and Bradford, 1989).

The expression of seizures in previously kindled adult rats has also been found to be inhibited by NMDA receptor antagonists, as evidenced by reductions in seizure stage and AD duration. Ketamine (Trommer and Pasternak, 1990) and MK-801 (Mintz, Rose, and Herberg, 1990; Trommer and Pasternak,

1990) both show significant anticonvulsant activity when administered to fully kindled rats. Taken together, the evidence indicates that the blockade of the NMDA receptor complex delays both kindling seizure development and the expression of seizures in previously kindled rats.

Mody and his colleagues (Mody, Stanton, and Heinemann, 1988) found an increase in the amplitude and duration of granule cell EPSPs in kindled rats. Perfusion of the NMDA receptor antagonist APV into the slice chamber reduced the amplitude of EPSPs in kindled animals only. Furthermore, a large, late-rising component of the kindled granule cell EPSP was also seen, especially at membrane potentials 10-20 mV depolarized from rest. Since this component was blocked by APV, it is most likely mediated by NMDA receptors. Interestingly, this NMDA-mediated component was only seen in EPSPs recorded from kindled tissue and not at all in control responses.

Removal of Mg^{2+} from the perfusate resulted in a significant increase in EPSP amplitude of kindled granule cells which could not be attributed to the hyperpolarizing effect of low Mg^{2+} concentration (Mody et al., 1988). Thus, kindled granule cells were found to have an NMDA component in their EPSPs that was further enhanced by the removal of the voltage-dependent Mg^{2+} blockade.

Possible Mechanisms

Kindling trains produce a collapse of GABA-ergic inhibition (Kapur et al., 1989) which allows NMDA receptors to become active. This permits Ca^{2+} entry into the cell: such entry is likely to be massive during most seizures and may, therefore, damage the neuron transiently or permanently. Ca^{2+} is also a crucial factor in the activation of second messenger cascades, processes that converts short-lived electrophysiological processes occurring at the membrane into much longer lasting intracellular processes. These may include plastic changes at the synaptic and receptor level and may account for kindling (Gloor, 1989). Thus, kindling may result in more intense activation of the NMDA receptor complex (Anderson, Swartzwelder, and Wilson, 1987; Mody, Stanton, and Heinemann, 1988; Slater, Stelzer, and Galvan, 1985) and/or changes of intracellular second messenger systems mediated by NMDA receptors (Akiyama, Yamada, and Sato, 1987; Yamada, Akiyama, and Otsuki, 1989).

Most of the above research involved stimulation of the hippocampus and its pathways. The hippocampus is part of the limbic system and has been widely implicated in temporal lobe epilepsy. As well, the late component has only been observed in hippocampal field potentials. Since both kindling and the late component appear to be mediated to some extent by NMDA, the presence of an NMDA component in the field potential

will enable the degree of NMDA activation due to kindling to be monitored. To this extent, animals in the present thesis have electrodes chronically implanted in (i) the dentate gyrus of the hippocampus and (ii) the perforant pathway, the latter connecting the entorhinal cortex to the hippocampus.

Hippocampus: Anatomy

The hippocampus and dentate gyrus are widely used in the study of neurobiological phenomena, primarily because of their simple and regular structure. The transverse connectivity within the hippocampus--the so-called trisynaptic circuit--has been investigated in detail using both in vitro and in vivo preparations.

In the rat, the hippocampus curves from the septum along the medial wall of the inferior horn of the lateral ventricle to the caudal part of the amygdala, resembling the shape of a cashew. The hippocampus consists of Ammon's horn (cornu Ammonis or CA), the dentate gyrus, and the subiculum. In the rat, as in all mammals, the hippocampus consists of two C-shaped, interlocking principal cell layers: the granular cell layer of the dentate gyrus (DG) and the pyramidal cell layer of Ammon's horn. Ammon's horn is typically divided into four subfields: CA1, CA2, CA3, and CA4 (see Figure 1).

The basic architecture of the hippocampal subfields is very similar in that they all consist of one single lamina of

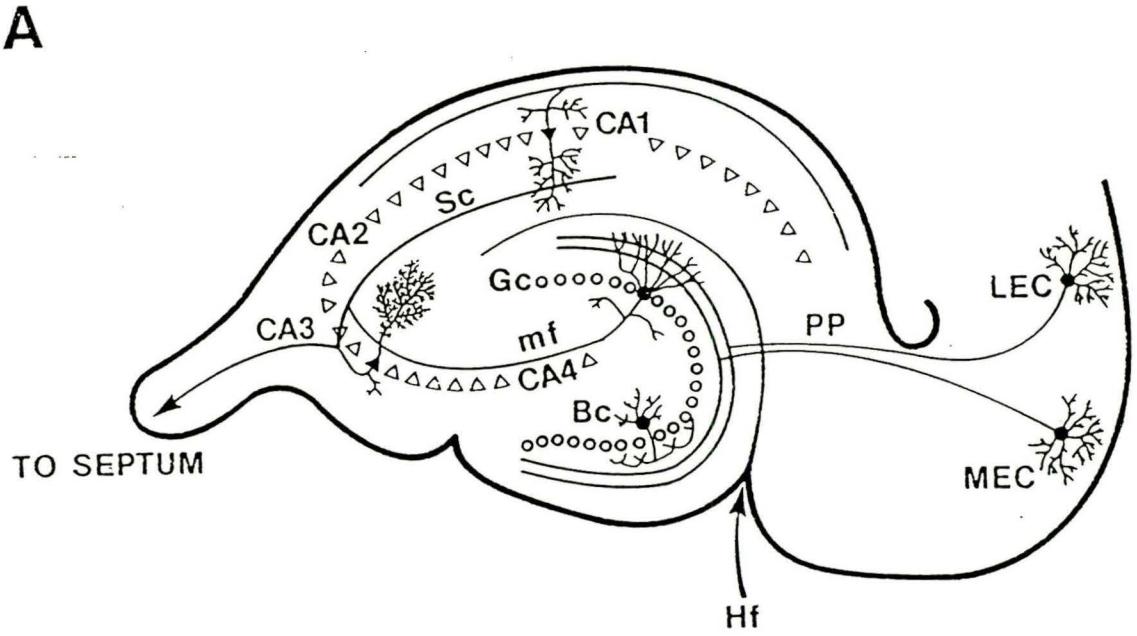
neurons of which the apical dendrites extend into the stratum moleculare in the DG and the subiculum, as well as the stratum lacunosum-moleculare and stratum radiatum in Ammon's horn. The DG consists of a densely packed single cell layer, with granule cells as the major cell type. The area between the upper and lower granule cell body layers is known as the "hilus", which also includes the proximal part of the pyramidal cell layer of CA3.

Electrophysiological (Andersen et al., 1971) and to a lesser extent anatomical data (Blackstad et al, 1970; Hjorth-Simonsen and Jeune, 1972) have indicated that the hippocampus is organized in a lamellar fashion. Each lamella contains a sequence of almost completely unidirectional connections from the DG to the subiculum, via CA3 and CA1. The DG represents the major target structure for input into the hippocampus, with prominent cortical afferents arriving from the entorhinal cortex. The subiculum gives rise to most of the hippocampal efferents to widespread subcortical and cortical areas, including the entorhinal cortex, although CA1 and CA3 also contribute to many of these efferent systems (Swanson et al., 1987).

Axons from dentate gyrus granule cells, the mossy fibres, synapse onto CA3 pyramidal cells. From these CA3 neurons, the Schaffer collaterals travel to the apical dendrites of CA1 pyramidal cells. Both the granule and pyramidal cells have

Figure 1: (a) Schematic diagram of hippocampal formation and adjacent entorhinal cortex (lateral = LEC; medial = MEC). Bc = basket cell; CA = cornu ammonis; Gc = granule cell; Hf = hippocampal formation; mf = mossy fibre; PP = perforant path; Sc = Schaffer collateral.

(b) Dentate gyrus circuit diagram indicating excitatory (+) and inhibitory (-) connections that affect granule cell firing. ac = axon collateral.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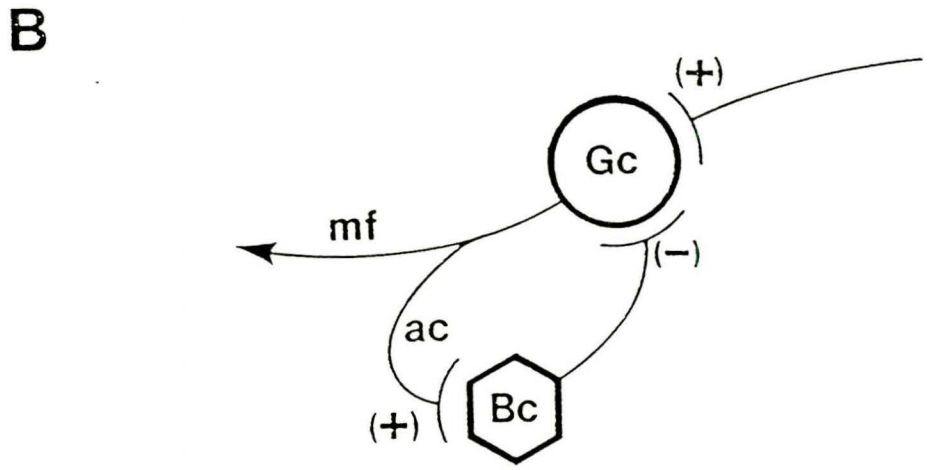
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collateral axons that innervate inhibitory basket cells (Andersen et al., 1966).

The entorhinal cortex gives rise to the largest projection to the hippocampus--the perforant pathway. Within the entorhinal cortex, six cortical layers are distinguished that constitute superficial (layers I-III) and deep (IV-VI) layers. Projections to the DG and CA3 arise predominantly from neurons in layer II, whereas projections to CA1 arise from cells in layer III (Steward and Scoville, 1976). Minor contributions may originate in the deeper layers of the entorhinal cortex (Kohler, 1985a).

The perforant path consists of both a lateral and medial component (Hjorth-Simonsen and Jeune, 1972). Medial perforant path fibres leave layers II and III of the dorsomedial entorhinal cortex and enter the underlying white matter and angular bundle. From here they traverse the pyramidal cell layer of the subiculum proper (with some fibres crossing the presubiculum or the distal part of CA1) and synapse on the middle third of the DG's molecular layer. The lateral perforant path originates from cells in the ventrolateral entorhinal cortex and terminates in the outer third of the molecular layer.

Hippocampus: Electrophysiology

The major pathways of the hippocampus have also been

explored by functional analysis. Electrical stimulation of the PP, in the form of brief afferent volleys, produces reliable waveforms or evoked potentials (EPs), whose shape is largely dependent upon the recording location within the hippocampus (Andersen, Bliss, and Skrede, 1971; Andersen, Holmqvist, and Voorhoweve, 1966; Lomo, 1971). This response is largest when the recording electrode is positioned in the hilus of the dentate gyrus.

The first component of the DG evoked response (Figure 2) represents the stimulus artifact (Fig. 2a) and is largely caused by inductive and capacitive coupling and by current flowing directly from the stimulating electrode to the recording electrode via low resistance cytoplasm. The second element consists of a positive change in potential caused by the depolarization of the granule cell dendrites. The slope of this component is proportional to the amplitude of the cellular excitatory post-synaptic potential (EPSP) and is often called the population EPSP (Fig. 2b) (Lomo, 1971; Bliss, 1979). If the intensity of stimulation is great enough, a third component can be elicited. It consists of a negative inflexion, the population spike (Fig. 2c), superimposed on the population EPSP and reflects the near synchronous discharge of granule cells (Lomo, 1971; Andersen et al., 1971). The population spike height is proportional to the number of granule cells firing.

Figure 2: Population excitatory postsynaptic potential (EPSP) evoked in the dentate gyrus by perforant path stimulation. The solid line represents the response evoked by a single pulse while the dashed line represents the response evoked by a train of pulses.

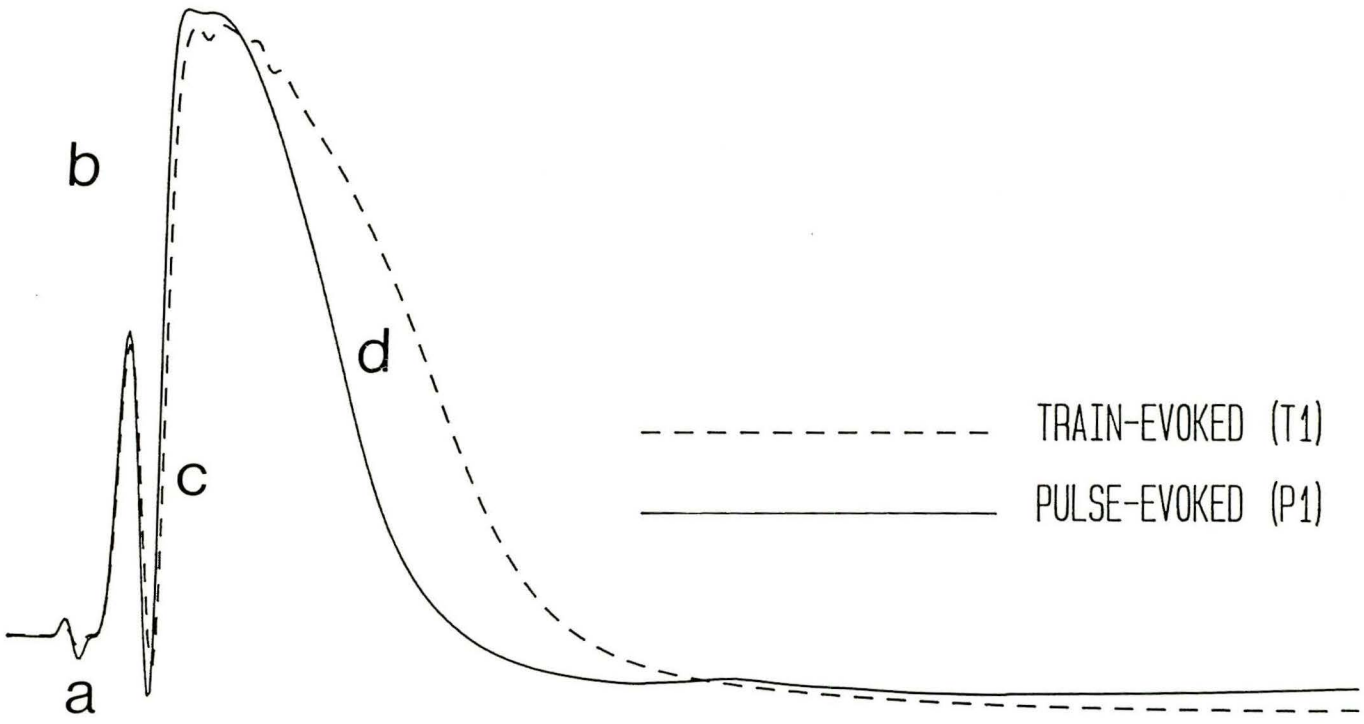
(A) Stimulus artifact.

(B) Population EPSP.

(C) Population spike

(D) Late component.

PULSE-EVOKED AND TRAIN-EVOKED RESPONSES



When a high frequency train of pulses, rather than a single pulse, is used to evoke a response in the dentate gyrus, the descending phase of the resultant EPSP is increased in amplitude and area. This component is not seen during normal (low-frequency) synaptic transmission and will be referred to throughout the thesis as the "late component" (Fig. 2d). The stimulation trains used to elicit this late component evoke population EPSPs and population spikes that are almost identical to those evoked by single pulses. Recent studies have implicated the NMDA receptor as mediating a late-rising component of EPSPs evoked in hippocampal slices (Mody, Stanton, and Heinemann, 1988; Muller and Lynch, 1990). The late component observed in these in vitro preparations resembles that obtained in our laboratory with in vivo preparations. Racine and co-workers have shown that the late component of the PP-DG EPSP is attenuated by administration of either MK-801 or ketamine (Racine, Moore, and Wicks, 1991). Thus, the train-evoked response in the hippocampus can be used as a measure of the degree of NMDA activation in chronic preparations.

General Introduction

NMDA receptors most likely participate in the initiation and/or propagation of epileptiform discharge, since NMDA antagonists have been shown to have potent anticonvulsant

effects in a variety of seizure models (eg. Croucher et al., 1982; Davies et al., 1986; Trommer and Pasternak, 1990). Furthermore, several investigators have reported that kindling produces an increase in the activity of the NMDA receptor (Mody et al., 1988; Morrisett et al., 1989; Yeh et al., 1989). Using a hippocampal slice preparation, Mody and co-workers reported a large, late-rising component in the granule cell (GC) EPSP following kindling (Mody et al., 1988). They suggested that kindled GC EPSPs resemble those of certain cortical neurons--in which NMDA receptors contribute to normal synaptic transmission (Thomson, West, and Lodge, 1985)--and concluded that the NMDA component was strengthened in the kindled animal.

If kindling does enhance NMDA receptor sensitivity, this increased activation of the NMDA system may yield a larger late component in the dentate gyrus response. However, preliminary investigations in our laboratory intimated that, similar to ketamine and MK-801, kindling of the perforant path tends to attenuate the late component of the dentate gyrus granule cell EPSP, although these data failed to reach significance (Racine et al., 1991). On one hand, Mody suggests that kindling strengthens the late component, while on the other hand, Racine suggests that kindling weakens it. Hence, the present thesis will attempt to resolve the apparent contradiction concerning the effects of kindling on the late

wave, a component thought to be mediated by NMDA receptors.

The contribution of NMDA receptors to the generation of EPSPs and in particular, the late component, will first be confirmed. It has previously been shown **in vitro** that ketamine attenuates the secondary component of field potentials evoked in the Schaffer collateral-commissural/CA1 system (Coan and Collingridge, 1987; Davies et al., 1988). A recent study from our laboratory found both ketamine and MK-801 to attenuate the late component of the train-evoked PP-DG field potential in vivo (Racine et al., 1991). Before drawing any conclusions regarding the effect of kindling on the late component, with subsequent implications for the role of NMDA in kindling, the NMDA-dependency of the late component will first be verified. Ketamine is the NMDA receptor antagonist of choice in this study as it is one of the few available ligands that crosses the blood brain barrier. Hence, ketamine can be injected systemically, eliminating the need for incannulation or slice work.

The NMDA component appears to coincide with the period of recurrent inhibition following stimulus induced potentials. Any treatment that increases background inhibition could be expected to attenuate the NMDA component. In the PP-DG system, recurrent inhibition is mediated by gamma-amino-butyric-acid (GABA) (Adamec et al., 1981). The GABA agonist sodium pentobarbitol has been shown to block or reduce NMDA

responses in hippocampal slice preparations (Miljkovic and MacDonald, 1986; Sawada and Yamamoto, 1985). As well, Riveros and Orrego (1986) reported that GABA was effective in blocking NMDA-induced Ca^{2+} flux. Treatment with the GABA agonist diazepam will determine the contribution of GABA receptors, if any, to the late wave. Furthermore, given that kindling causes a reduction of GABAergic inhibition, the effect of diazepam on the late component of kindled animals will also be investigated.

If both ketamine and kindling are found to attenuate the late component, this would suggest that the kindling process involves antagonism of the NMDA receptor complex. Mody and colleagues found that APV consistently reduced the amplitude and duration of EPSPs following kindling (Mody et al., 1988). Moreover, they also reported a distinct NMDA receptor-mediated component of EPSPs, recorded in the presence of APV, in kindled slices only. If kindling causes an attenuation of the late component, administration of ketamine to kindled animals should aid in establishing the degree of involvement of the NMDA system in kindling.

METHODS

Animals and Surgical Procedures

Subjects were 18 male Long Evans hooded rats, bred at McMaster University and weighing between 350 and 450 grams. Following surgery, animals were housed individually in wire mesh cages and allowed food and water ad libitum. Although 30 rats were implanted with electrodes, only 18 of the animals were deemed to have classical dentate gyrus field potential morphology. Those animals who did not exhibit classical responses were discarded from the study.

Rats were anaesthetized with sodium pentobarbitol (Somnotol, MTC Pharmaceuticals) at 60 mg/kg I.P. and treated with atropine (0.2 ml I.P.) to prevent respiratory distress. Animals were secured in a stereotaxic apparatus (David Kopf Instruments) with bregma and lambda in the same horizontal plane. Bipolar stimulating and recording electrodes were constructed from two teflon coated stainless steel wires (125 um diameter) twisted together, with a vertical tip separation of 0.5 mm. Electrodes were implanted according to the atlas of Paxinos and Watson (1982).

Recording electrodes were implanted into the hilus of the dentate gyrus (3.8 mm posterior and 2.2 mm lateral, relative to bregma). Stimulating electrodes were positioned in the

ipsilateral perforant path (7.6 mm posterior and 4.1 mm lateral to bregma). To ensure optimal placements, the evoked response to electrical stimulation of the perforant path was monitored while slowly lowering recording and stimulating electrodes during surgery.

A ground screw/electrode and three additional support screws were placed in the skull. The connecting pins of each electrode were inserted into a plastic headcap and the entire assembly was fixed in place with dental acrylic. At least two weeks were allowed for recovery from surgery prior to testing.

Apparatus

All testing was conducted in a plywood recording chamber with an acrylic front and a mirror on the back wall. Electrical stimulation to the perforant path was delivered by means of a Grass S88 stimulator. Constant current output was provided by Grass PS106 photoelectric stimulus isolation units. The evoked response measured in the dentate gyrus was passed to a Grass model 7P5A wide-band A.C. EEG pre-amplifier. A Grass polygraph driver amplifier (model 7DAE) was used when recording a paper record of the EEG. Cutoff frequencies were set at 1 Hz and 3 kHz. Evoked responses were monitored on a Tektronix D-13 dual beam storage oscilloscope and channelled into a Zenith 386 computer via an A/D converter. The responses were digitized at 10 points per msec, stored on

computer disk, and analyzed using programs written in ASYST (Keithley/ASYST Software Company).

Stimulation Parameters

Evoked Responses

Excitatory post-synaptic potentials (EPSPs) were recorded from the dentate gyrus in response to perforant path stimulation. Stimulation was delivered as biphasic pairs of pulses, with an interpulse interval of 200 ms. Such an interval typically results in inhibition of both the EPSP and population spike amplitudes, but maximizes the train-evoked late component of interest in this study. Pulse pairs were separated by 12 s.

In order to determine input/output (I/O) relationships, 120 pulse pairs were delivered to the perforant path at a rate of 0.1 Hz. The duration of each sampled sweep was 50 msec, to allow sufficient time for the complete response to be recorded for each animal. Twelve pulse intensities were used in all sessions. For half of the animals the intensities were: 40, 60, 80, 100, 150, 200, 250, 350, 500, 700, 900, and 1200 μA. This particular range of intensities was selected as it yields a roughly logarithmic I/O function. For the second half of the animals, a programmable stimulator replaced the manual system formerly used in the laboratory. The latter set of intensities, which yields a roughly logarithmic scale, was as

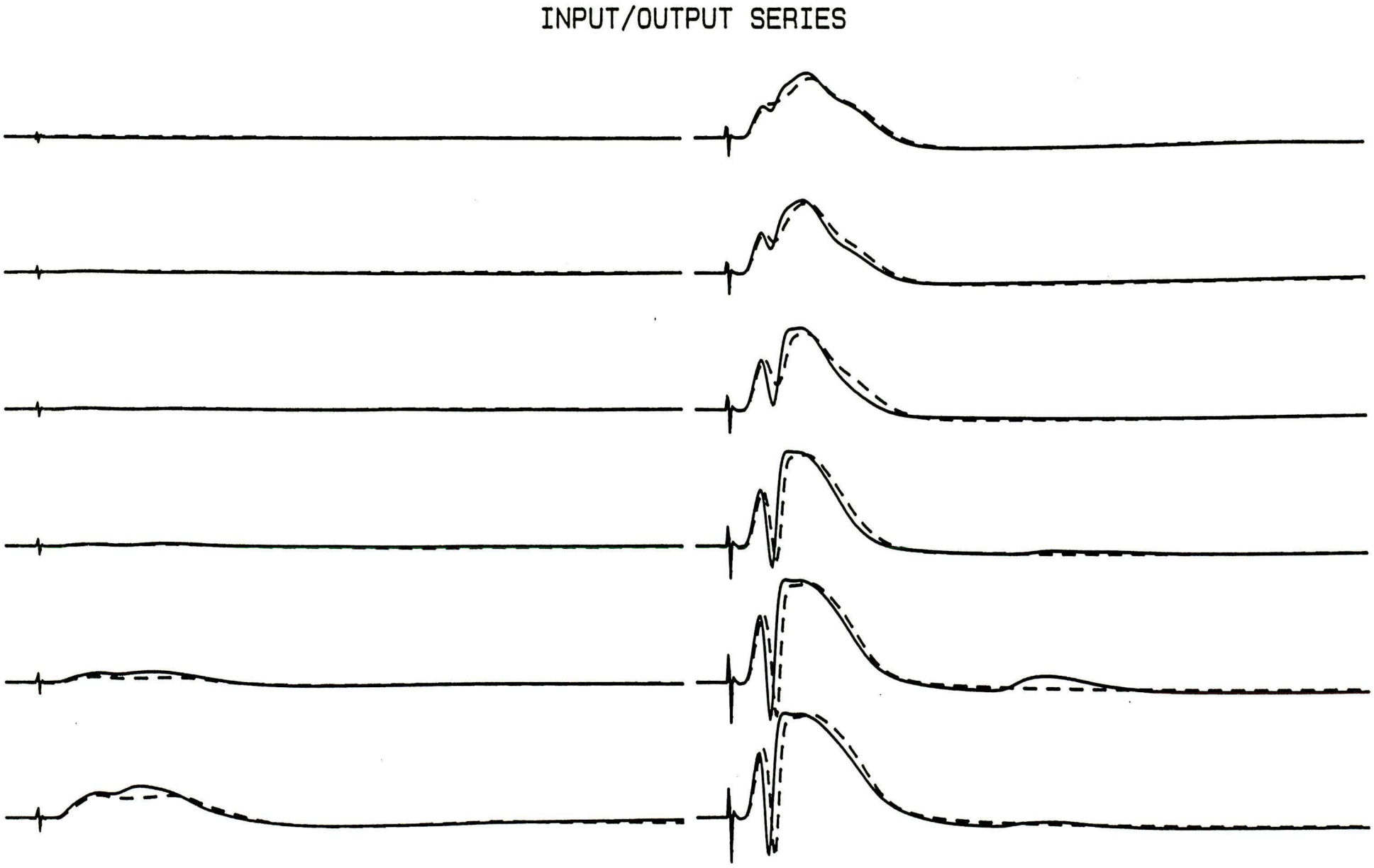
follows: 45, 64, 91, 128, 215, 256, 362, 512, 724, 861, and 1218. Ten pulse pairs were delivered at each of the 12 intensities and the responses were then averaged. An intensity of 40 or 45 μA was usually below the EPSP threshold. The responses then increased in magnitude until they reached asymptotic levels, around 900 - 1200 μA (see Figure 3). When discussing the results, stimulation intensities will be referred to as ordinal numbers from 1 to 12 to facilitate comparison across the two sets of stimulation parameters.

Paired trains were also delivered, with an intertrain interval of 200 ms. Each train consisted of four biphasic pulses delivered at a frequency of 400 Hz with a 9 ms duration and a 100 μsec interval between positive and negative pulses. Intensities were varied as described above and I/O relationships were determined for the train stimuli.

Kindling

Each kindling train consisted of a 1 s train of 1 ms biphasic pulses, at a frequency of 60 Hz. The stimulation was delivered to the perforant path. The minimum intensity required to elicit an electrographic afterdischarge (AD) was used as the kindling intensity. These ranged from 400 μA to 800 μA. On the first day of kindling, only one AD was triggered. Subsequently, three ADs were triggered each day, five hours apart, until three Stage 5 seizures were observed.

Figure 3: Typical input/output curve. The solid line represents the response to the first pulse while the dashed line represents the response to the second pulse. The evoked potentials increase in magnitude as the stimulation intensity increased from 40 μA (top left) to 1200 μA (bottom right). This animal's EPSP threshold is approximately 150 μA.


INPUT/OUTPUT SERIES
RESPONSE TO 1ST PULSE
RESPONSE TO 2ND PULSE
10 MSEC

A Stage 5 generalized convulsion consists of tonic/clonic rearing with a loss of postural control (Racine, 1972b). Control animals did not receive kindling stimulation.

Drugs

All animals were administered both ketamine and diazepam. Ketamine hydrochloride was delivered I.M. as Rogarsetic (rogar/STB Inc.) at 22 mg/kg. Diazepam was administered I.P. as Valium (Hoffmann-LaRoche Ltd.) at 2 mg/kg. Each electrophysiological testing session began as soon as behavioural effects of the drug were observed (five to ten minutes after drug administration). The combined paired pulse and train sessions lasted for 50 minutes.

Experimental Procedures

Following two weeks recovery from surgery, animals were randomly assigned into either an Experimental (kindled, n = 10) or a Control (n = 8) group. All animals received two baseline I/O sessions (sessions 1 and 2), with each session consisting of a paired-pulse run and a paired-train run as described above. Two days later, animals received a third baseline session (session 3), followed 6 h later by administration of ketamine and another I/O test (session 4). Animals were tested again 48 h after administration of the drug (session 5). Forty-eight hours later, animals received

a control trial (session 6), followed 6 h later by a session (session 7) with diazepam. Subjects were also tested 48 h post-drug (session 8).

Rats in the Experimental group were then kindled, with each animal's stimulation intensity set at individual threshold level. Subjects in the Control group received no electrical stimulation during this period. Each rat required a different number of ADs to reach the criterion of three Stage 5 seizures. Consequently, a fourth Stage 5 seizure was elicited from each kindled rat prior to the post-kindling I/O tests. EEG was recorded from both the dentate gyrus and perforant path electrodes.

Following kindling, I/O sessions 2 to 8 above were repeated for all animals in order to determine (1) the effect of kindling on the late component and (2) the effect of ketamine and diazepam on the late component of kindled rats.

Data Analysis

The slope of the population EPSP and the peak amplitude of the population spike were measured from each averaged field potentials. In addition, the area of the late component was determined by subtracting the paired pulse response from the paired train response. This process was facilitated by the collection and storage of data on computer. The EPSPs were matched according to stimulation intensity: an input/output

curve for the late component area was generated across the 12 intensities.

Multivariate analyses of variance (MANOVA) were performed on the data using SPSS, a statistics software package. A mixed ANOVA was used, with one between subject variable (group) and two within subject variables (stimulation intensity and treatment).

Histology

Upon completion of the experiment, rats were perfused intracardially with a 10% formalin solution. The brains were removed and coronal sections of 40 μm thickness were taken using a freezing microtome. Representative sections of the areas of implantation were mounted on glass slides and stained with thionin. The placements of the electrodes were then determined. All electrodes were found to be in the target sites.

RESULTS

Pulse and train-evoked sweeps are shown in Figures 4 and 5. Figure 4a depicts two pulse-evoked responses for each of five kindled animals: one response prior to kindling and one following kindling (stimulation intensity = 1200 μA). Figure 4b illustrates the pulse-evoked responses for five control animals. Although the control rats did not receive kindling stimulation, their responses for the same time period are shown.

Figures 5a and 5b illustrate the train-evoked responses before and after kindling for the same animals (kindled and control, respectively). Notice here that the late component is attenuated following kindling (i.e. the late component is smaller after kindling, as compared to before). This will be illustrated in greater detail by subtracting the pulse-evoked response from the train-evoked response.

Figure 6a depicts the pulse-evoked responses for five animals prior to and following ketamine administration. Figure 6b shows the train-evoked responses for the same sessions. Both the pulse-evoked and train-evoked responses were attenuated by ketamine treatment. The peak attenuation was at about 10 to 12 msec for pulse-evoked responses and 15 msec for train-evoked responses. These results are consistent

Figure 4: Pulse-evoked responses at maximum stimulation intensity for 5 typical experimental (kindled) and 5 typical control animals. The field potentials were evoked in the dentate gyrus via stimulation applied to the perforant path. The solid lines represent the response before kindling and the dashed lines represent the response after kindling (or, for the control animals, after the same period of time).

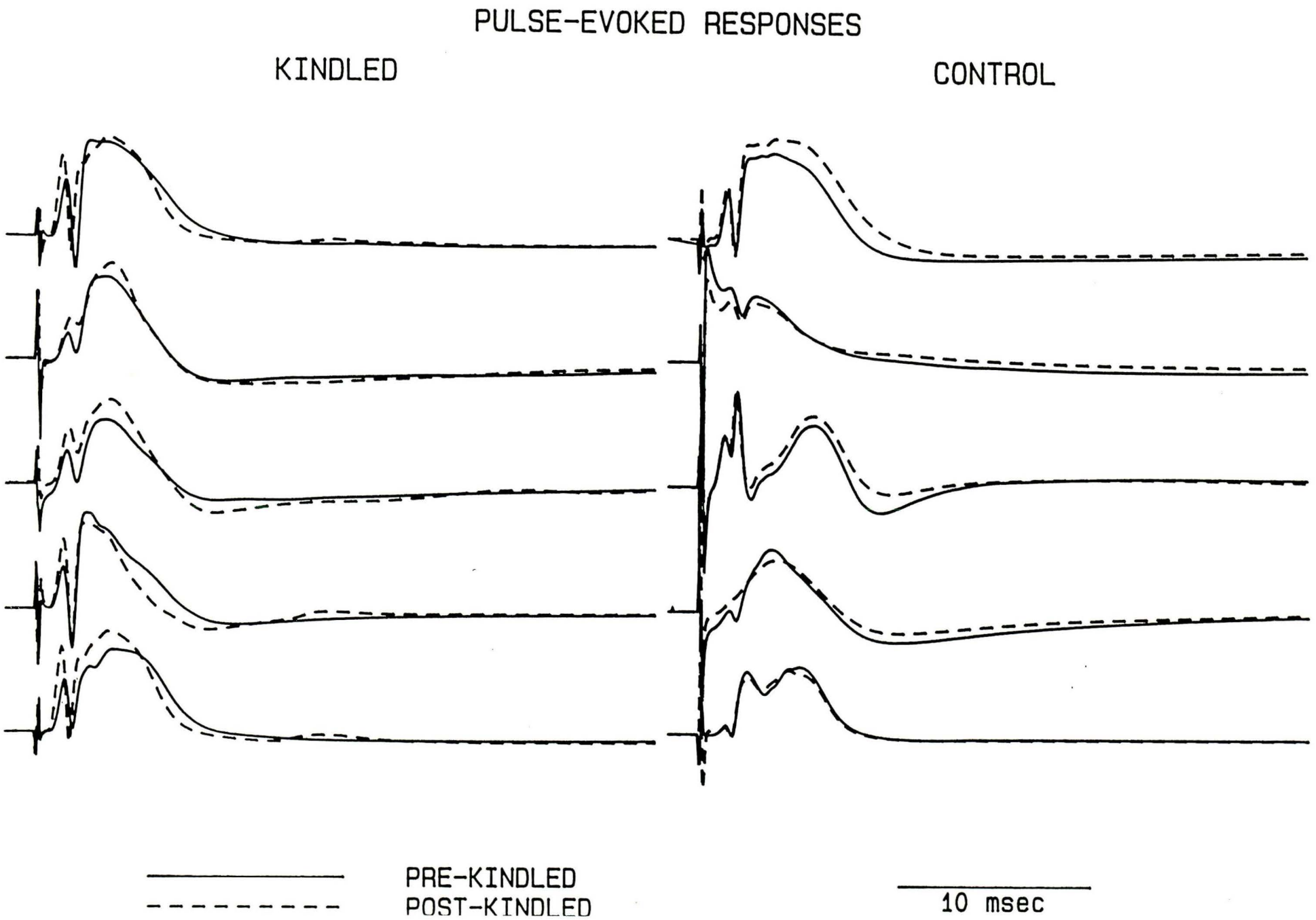

PULSE-EVOKED RESPONSES
KINDLED
CONTROL
PRE-KINDLED
POST-KINDLED
10 msec

Figure 5: Train-evoked responses at maximum stimulation intensity for 5 typical kindled and 5 typical control animals. The trains were delivered in pairs with an inter-train interval of 200 msec. The solid lines represent the response before kindling and the dashed lines represent the response after kindling. The spiking seen in the first part of the EPSP represents the stimulus artifacts for the train of pulses delivered.

The late component is depressed for each of the kindled rats. Conversely, it appears to be elevated for 4 of the 5 control rats shown here.

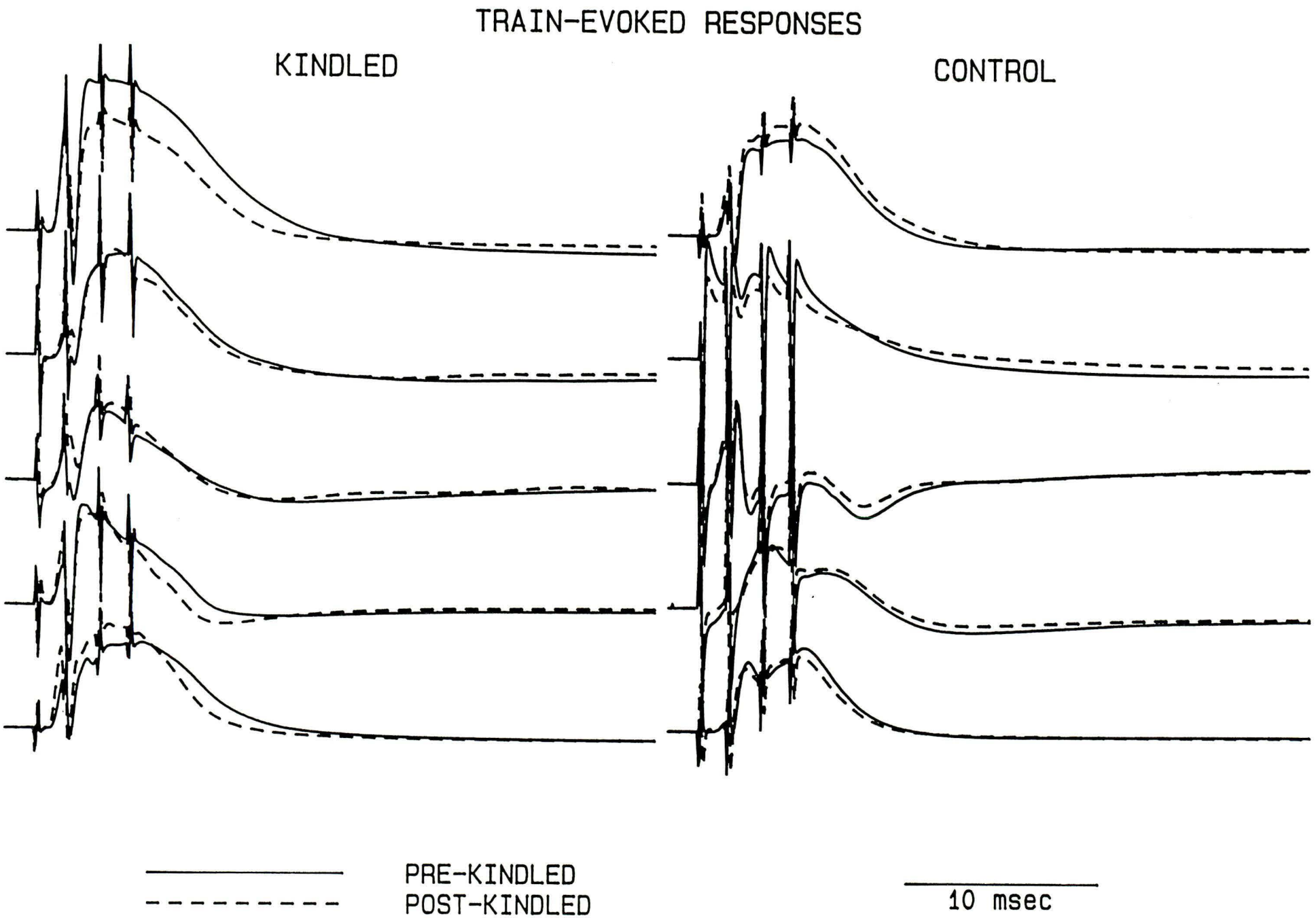
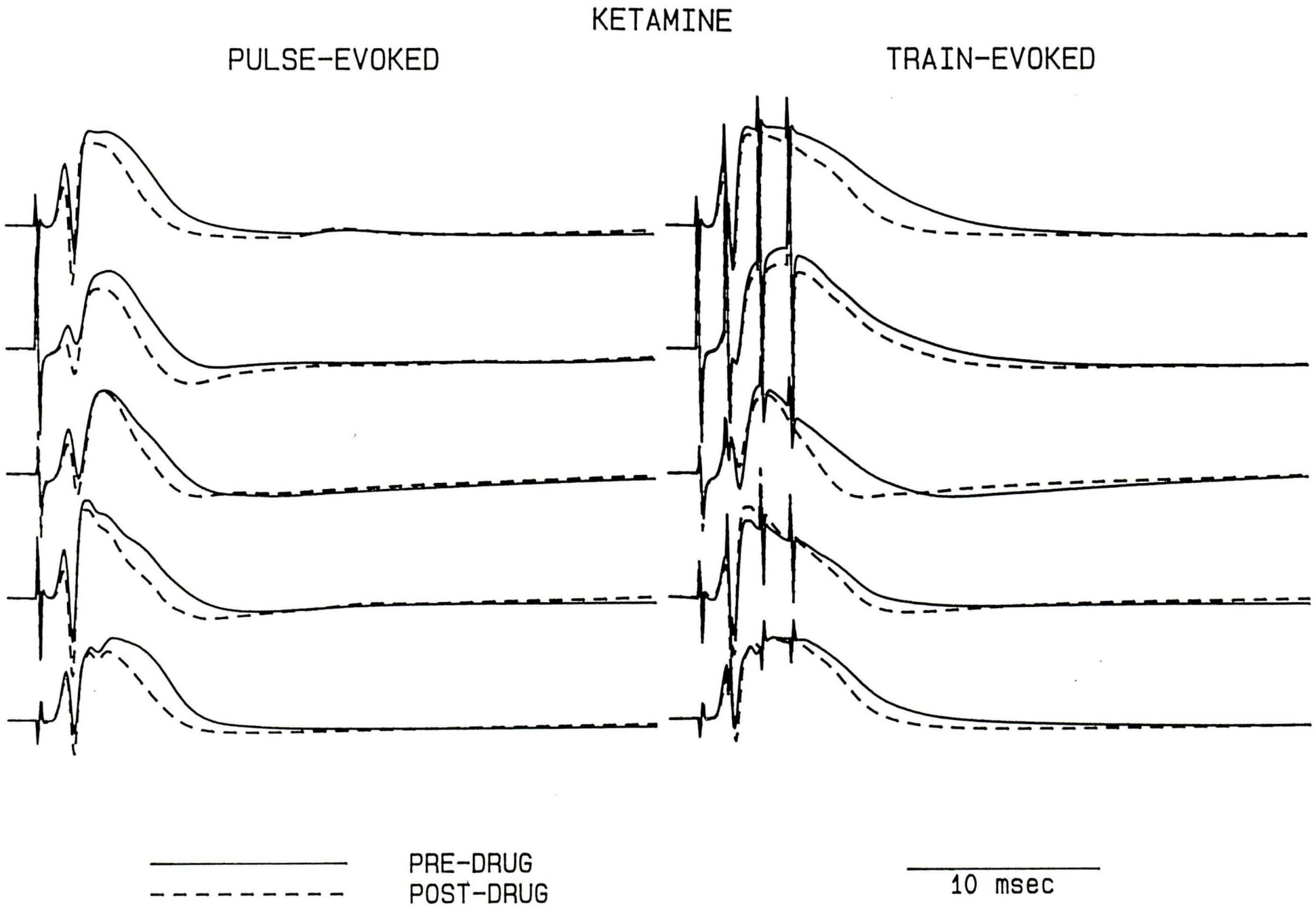

TRAIN-EVOKED RESPONSES
KINDLED
CONTROL
PRE-KINDLED
POST-KINDLED
10 msec

Figure 6: Pulse-evoked and train-evoked responses at maximum stimulation intensity for 5 animals treated with ketamine (22 mg/kg). The solid lines represent the response before ketamine and the dashed lines represent the response evoked immediately after ketamine was administered. Note that ketamine produced a substantial depression of the late component, observable in both the pulse- and train-evoked responses.


KETAMINE
PULSE-EVOKED
TRAIN-EVOKED
PRE-DRUG
POST-DRUG
10 msec

with those reported by Racine et al., (1991) indicating the presence of an NMDA component in the pulse-evoked as well as the train-evoked response. Figure 7a illustrates the pulse-evoked responses for the same five animals prior to and following diazepam administration. Figure 7b shows the train-evoked responses for the same sessions. Neither train-evoked nor pulse-evoked responses are significantly altered by diazepam treatment.

The late component was determined by subtracting the pulse-evoked response from the train-evoked response. Given the evidence for some NMDA involvement in the pulse-evoked response, this procedure presumably provided an underestimate of the NMDA component in the train-evoked response. The upper panel of Figure 8 shows the pulse- and train-evoked responses superimposed to facilitate comparison. The resulting area under the curve was measured and referred to as the late component area (Figure 8, lower panel). This waveform peaked at roughly 15 msec and lasted approximately 50-70 msec.

Administration of ketamine was shown to attenuate the area of the late component when compared to non-drugged responses for the same animals ($F = 5.90$, $p < .026$; ANOVA). Figure 9a illustrates this effect, averaged over 18 animals. The solid line represents the average of two I/Os taken prior to ketamine administration while the dashed line represents

Figure 7: Pulse-evoked and train-evoked responses at maximum stimulation intensity for 5 animals treated with diazepam (2 mg/kg). The solid lines represent the response before diazepam and the dashed lines represent the response immediately following diazepam. While it appears as though the trend is towards an attenuation of the late component following diazepam, when considering all 18 animals over 12 intensities, the trend is one of an elevation (see Figure 9b).

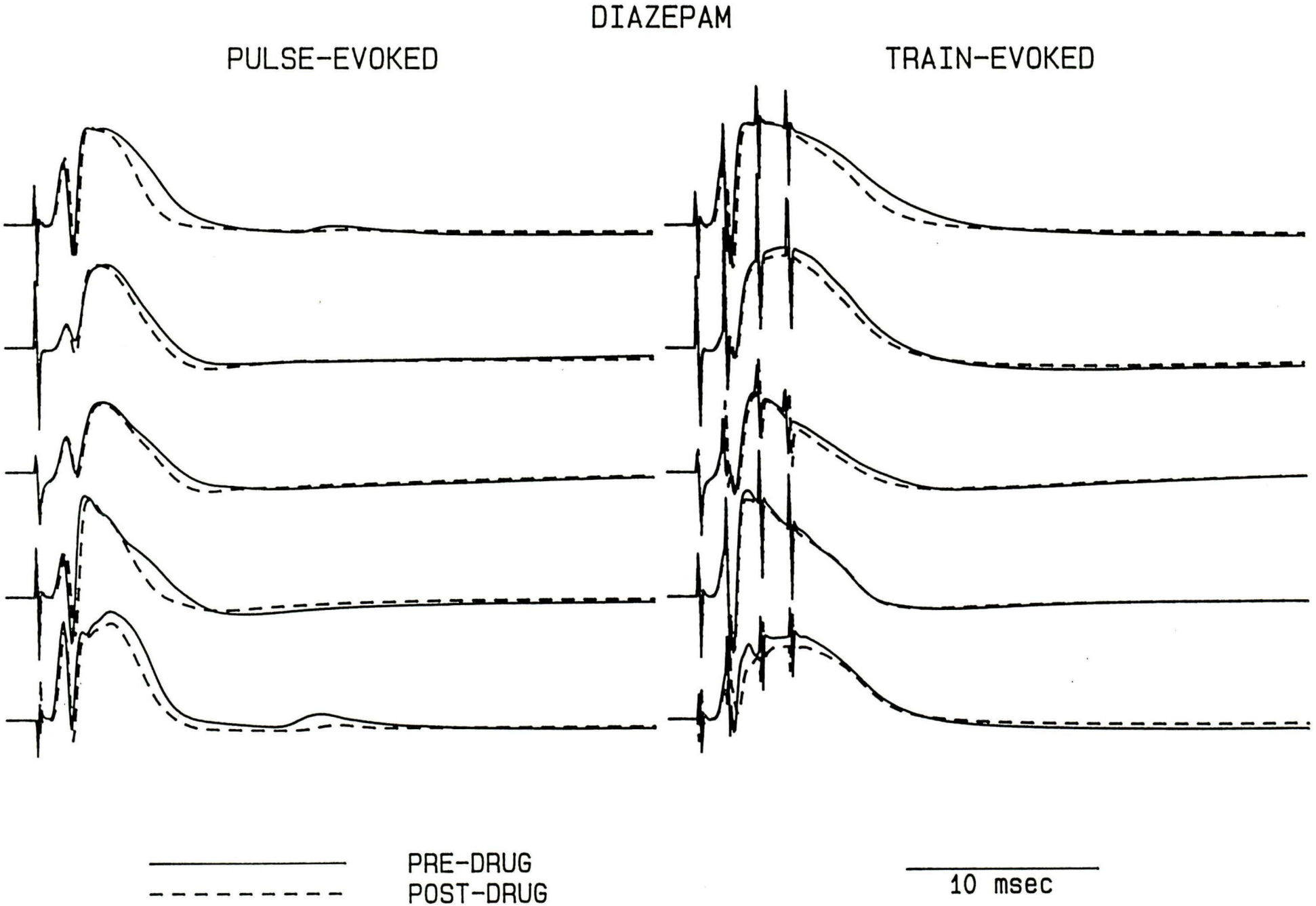

DIAZEPAM
PULSE-EVOKED
TRAIN-EVOKED
PRE-DRUG
POST-DRUG
10 msec

Figure 8: The upper panel illustrates typical field potentials evoked in the dentate gyrus by either pulse (solid line) or train (dashed line) stimulation applied to the perforant path. The stimulation intensity for these responses was 1024 μA. The population EPSP and population spike are very similar for both types of stimulation. The trains, however, triggered a positive long-lasting component. The lower panel illustrates the results of subtracting the pulse-evoked response from the train-evoked response.

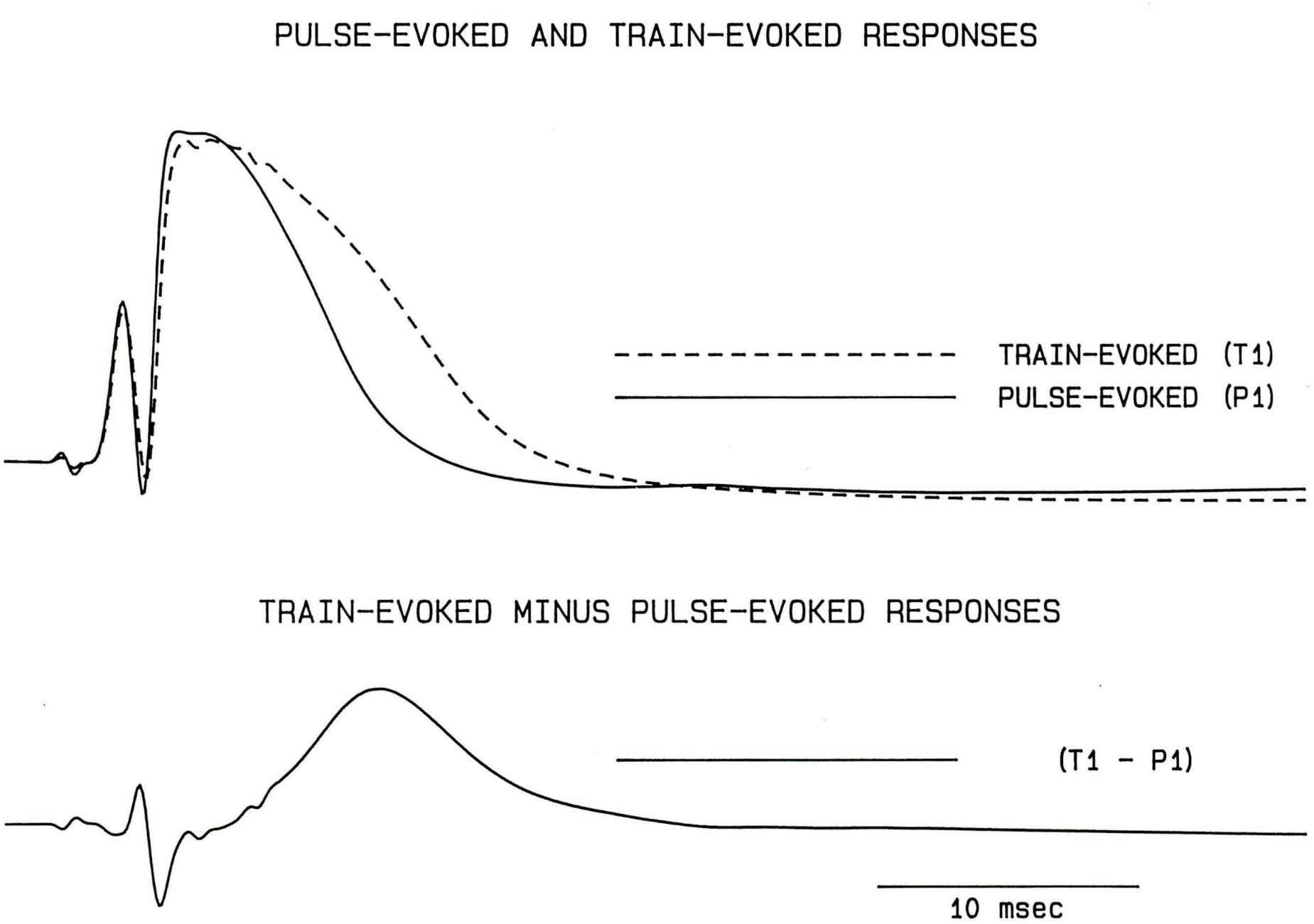
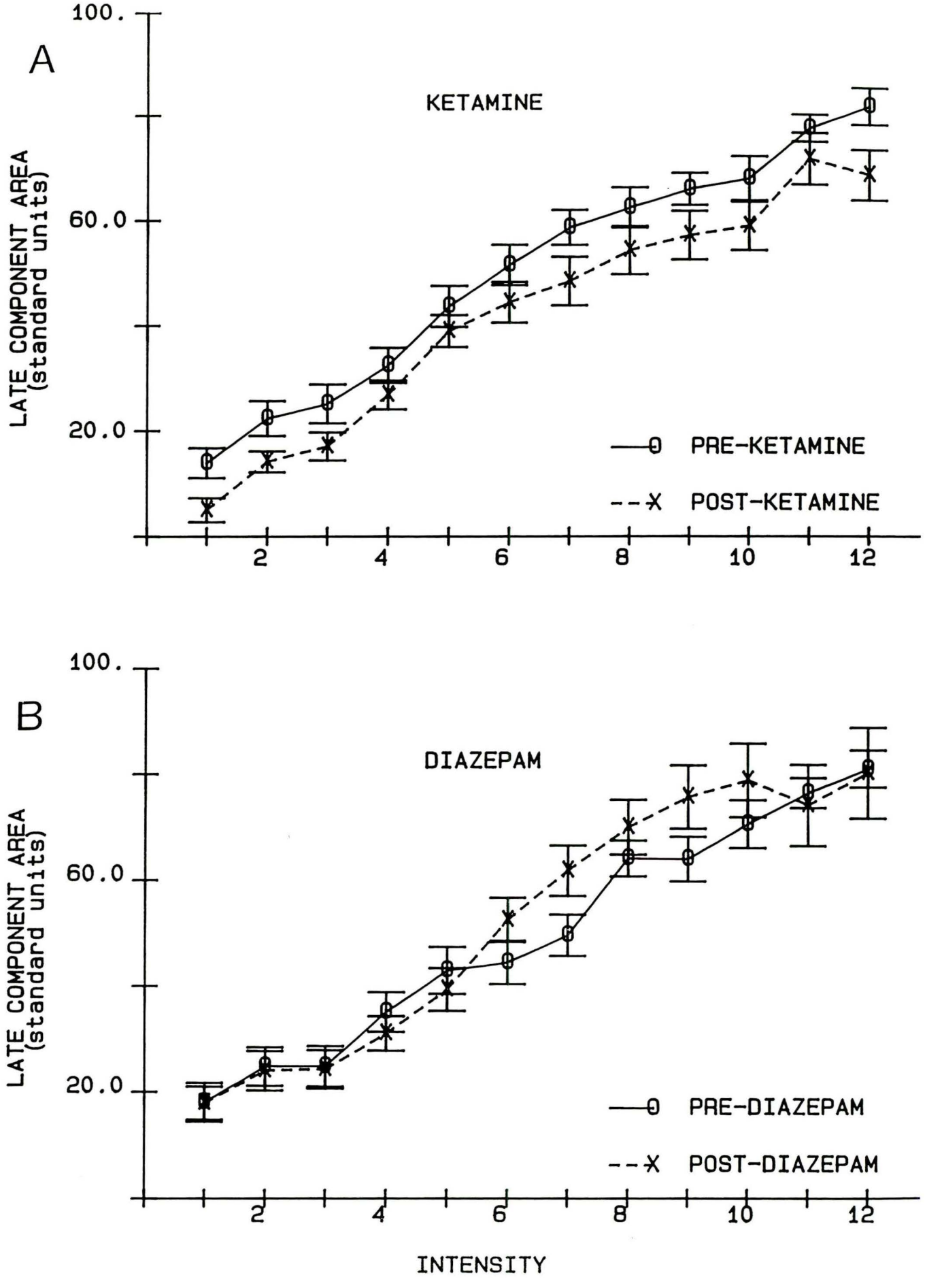

PULSE-EVOKED AND TRAIN-EVOKED RESPONSES
TRAIN-EVOKED (T1)
PULSE-EVOKED (P1)
TRAIN-EVOKED MINUS PULSE-EVOKED RESPONSES
(T1 - P1)
10 msec

Figure 9: (a) Input/output (I/O) curves averaged for 18 animals pre- and post-ketamine treatment. Both late component area and stimulation intensity are presented in relative standard units. There is a significant attenuation of the late component area across every intensity following administration of ketamine (dashed line).

(b) I/O curves averaged for 18 animals pre- and post-diazepam treatment. No significant effect was found. If anything, diazepam appears to slightly enhance the late component.


A
KETAMINE
100.
60.0
20.0
LATE COMPONENT AREA
(standard units)
PRE-KETAMINE
POST-KETAMINE
2
4
6
8
10
12
B
DIAZEPAM
100.
60.0
20.0
LATE COMPONENT AREA
(standard units)
PRE-DIAZEPAM
POST-DIAZEPAM
2
4
6
8
10
12
INTENSITY

the average of two I/Os under the influence of ketamine.

Diazepam had no significant effect on the area of the late component ($F = 0.93$, $p < .348$). In Figure 9b, the solid line represents two I/Os taken prior to diazepam administration and the dashed line represents two I/Os taken under the influence of diazepam.

Kindling

The average number of kindling stimulations required for rats to experience three Stage 5 seizures was 41.8. After the last animal reached criterion, all of the kindled rats were given a fourth Stage 5 seizure. Figure 10 illustrates baseline EEG (Fig. 10a) and EEG recorded during a Stage 5 seizure (Fig. 10b). Because the second phase of I/O testing was begun at the same time for all animals (i.e. after the slowest kindled animal reached criterion), approximately three weeks elapsed between the pre-kindling I/Os and the post-kindling I/Os.

Rats that had been kindled displayed a significantly smaller late component area on I/O measures collected after kindling. ($F = 6.41$, $p < .022$). Control animals who were not stimulated during the kindling period failed to show any such depression. In fact, the late component tended to increase over time, in the absence of any stimulation. Figure 11a illustrates the late component area over 12 intensities for

Figure 10: (a) Baseline EEG from dentate gyrus, recorded prior to kindling.

(b) EEG recorded from dentate gyrus during Stage 5 seizure.

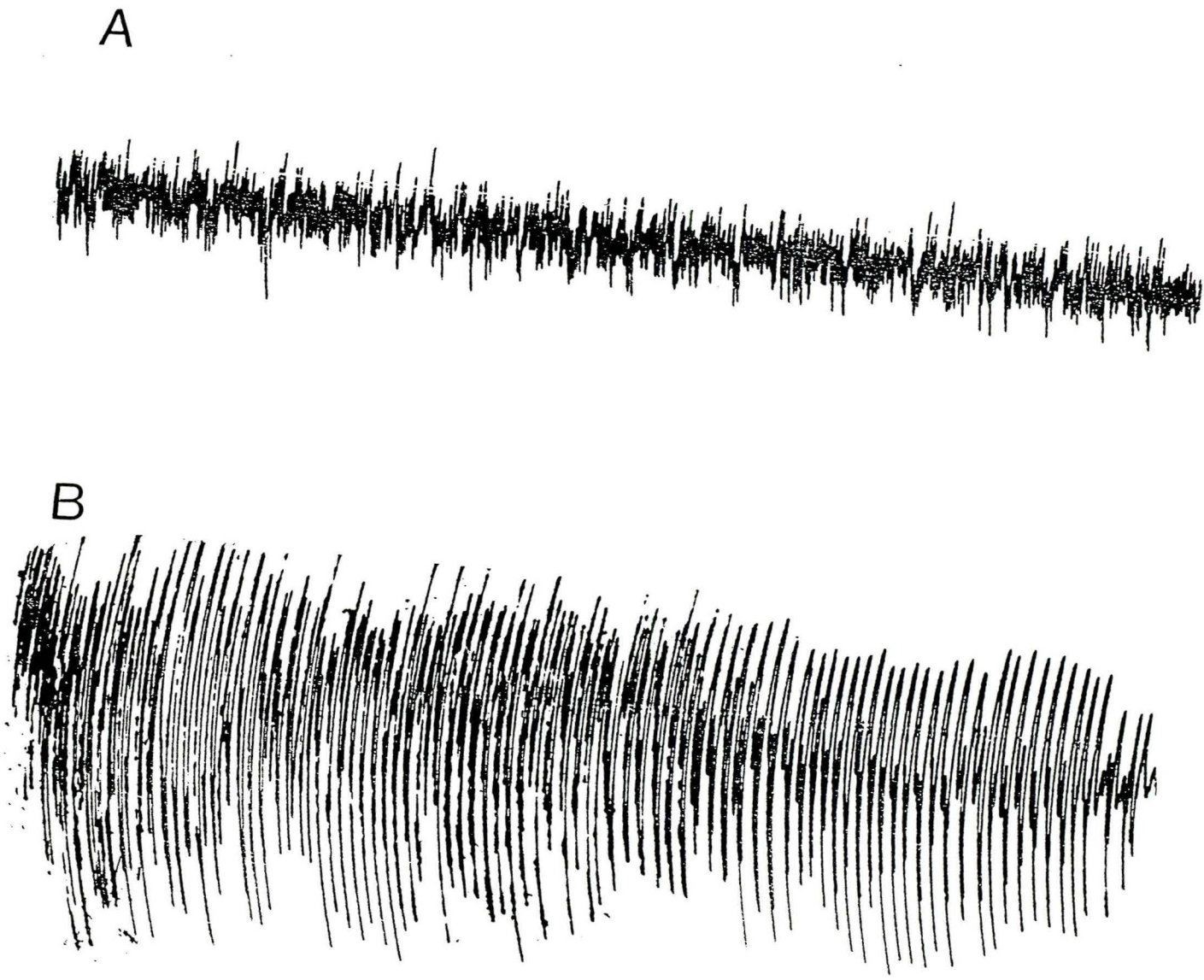
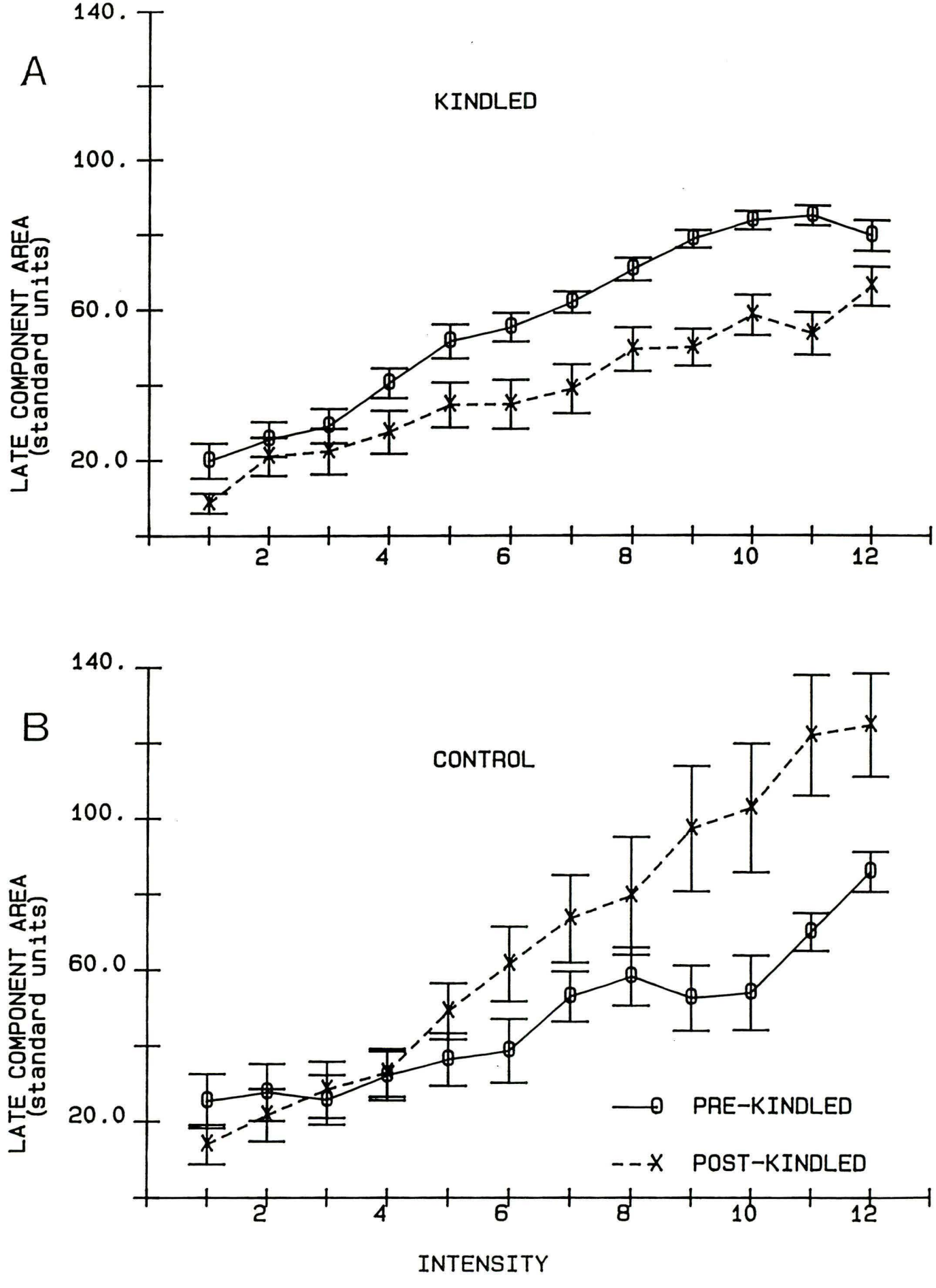

A
B

Figure 11: (a) I/O curves for the 10 kindled animals, before and after kindling. Fully kindled animals experienced a total of four Stage 5 seizures each. There is a significant attenuation of the late component following kindling (dashed line), seen over all intensities.

(b) I/O curves for the 8 control animals, for the same time period as shown in Figure 8(a). Control animals received no kindling stimulation. These untreated animals showed an increase in the area of the late component approximately three weeks after the "pre-kindled" I/O curves were elicited.


A
KINDLED
140.
100.
60.0
20.0
LATE COMPONENT AREA
(standard units)
2
4
6
8
10
12
B
CONTROL
140.
100.
60.0
20.0
LATE COMPONENT AREA
(standard units)
PRE-KINDLED
POST-KINDLED
2
4
6
8
10
12
INTENSITY

the 10 kindled rats averaged together. The solid line is pre-kindling and the dashed line is post-kindling. Figure 11b shows the late component for the 8 control (non-kindled) animals averaged together. Note the significantly attenuated area post-kindling, especially at higher intensities.

In contrast, kindling produced a potentiation of both the population EPSP slope ($p < .005$) and the population spike ($p < .028$). Figure 12a illustrates the kindling induced potentiation (KIP) of the EPSP slope. Figure 13a illustrates KIP of the population spike, a less robust effect. Notice that kindling potentiates part of the EPSP (slope and spike), yet depresses another (late component).

Control rats remained unstimulated for the entire period of time during which the experimental animals were kindled. Over the course of time, the area of the late component tended to drift upward (in the absence of treatment). Figure 11b depicts the late component area for the 8 control rats averaged together. The solid line corresponds to the pre-kindling period while the dashed line corresponds to I/Os taken after the experimental rats were kindled. The area of the late component is significantly greater three weeks following the "pre-kindling" measure.

The administration of ketamine to kindled rats did not yield any differences in the effect on the late component compared to the administration of ketamine prior to kindling

or to the non-kindled rats following kindling ($F = .51$, $p < .480$). Similarly, the effect of diazepam was not altered following kindling ($F = .50$, $p < .490$).

Figure 12: (a) In contrast to a depression of the late component (Figure 8a), kindling produced a significant enhancement of the population EPSP slope. This is known as kindling-induced potentiation (KIP). Shown are I/O curves for slope taken before (solid line) and after (dashed line) kindling.

(b) I/O curves representing EPSP slope for the control (non-kindled) subjects. There were no significant effects of time on the slope.

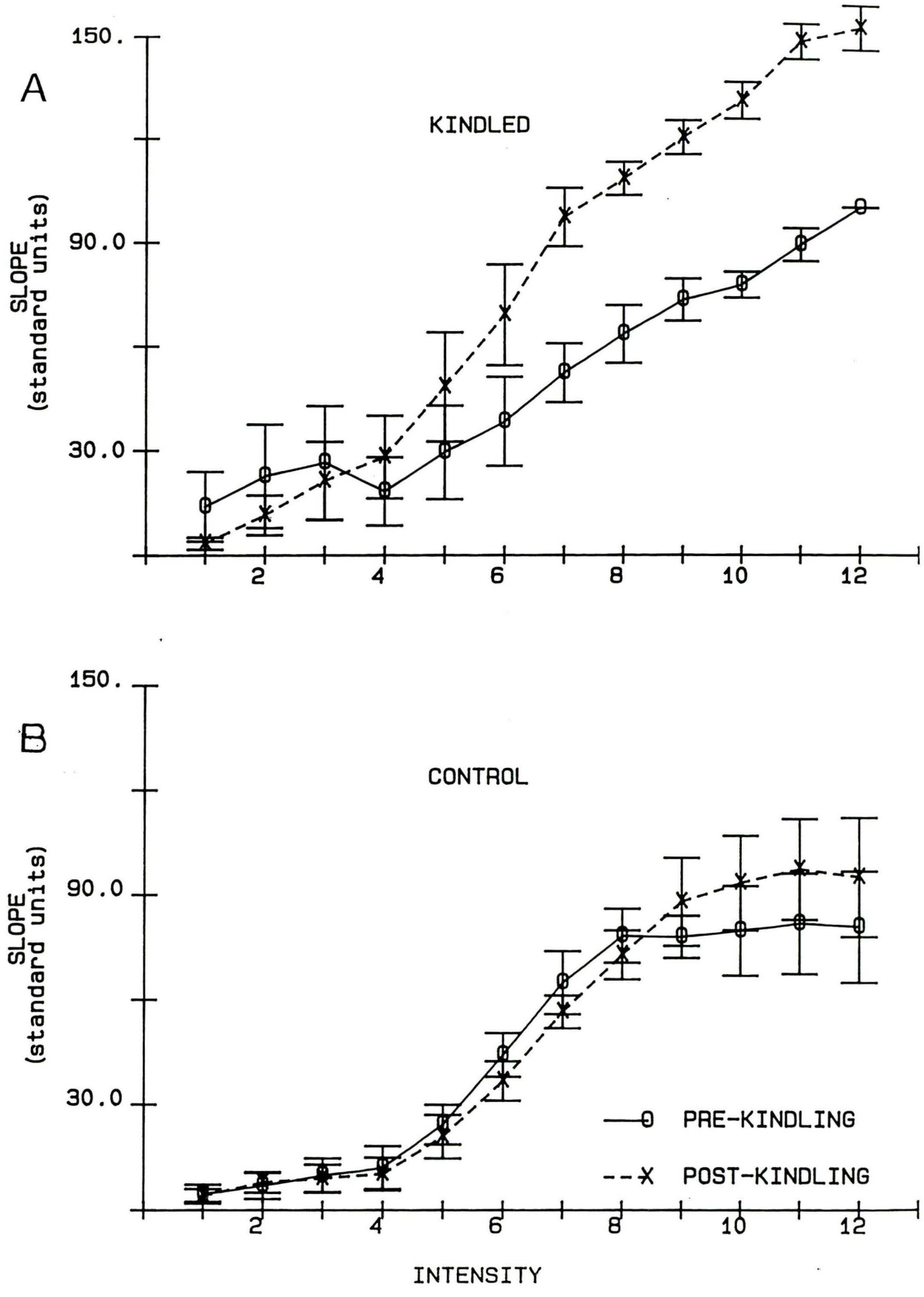
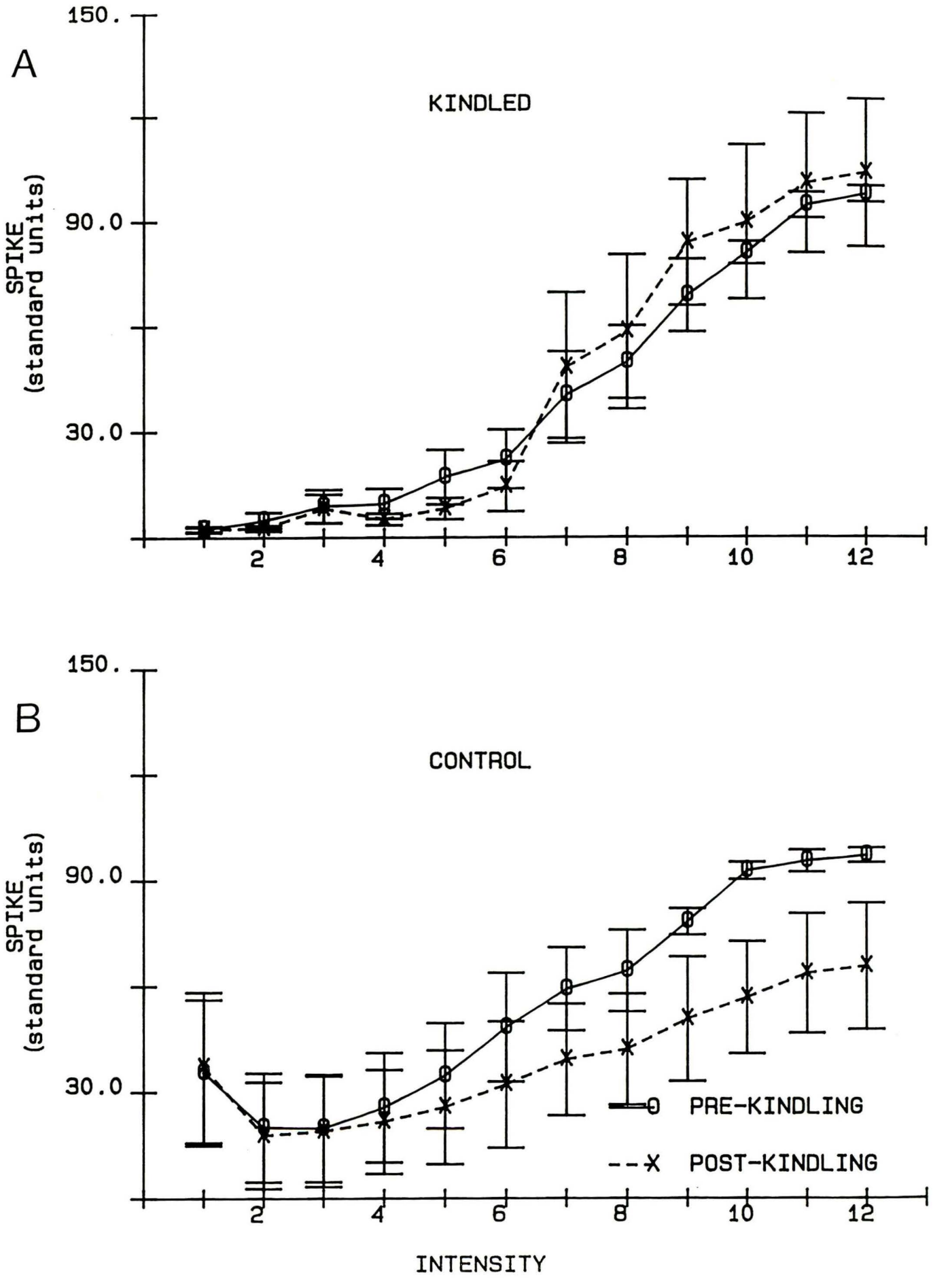

A
KINDLED
150.
90.0
30.0
SLOPE
(standard units)
2
4
6
8
10
12
B
CONTROL
150.
90.0
30.0
SLOPE
(standard units)
PRE-KINDLING
POST-KINDLING
2
4
6
8
10
12
INTENSITY

Figure 13: (a) As with EPSP slope, kindling appeared to cause an increase in population spike amplitude when compared with the downward drift seen in control animals. Shown are I/O curves for population spike taken before (solid line) and after (dashed lines) kindling.

(b) Population spike I/O curves for control (non-kindled) animals. The passage of time resulted in some downward drift in the pop spike amplitude.


A
KINDLED
150.
90.0
30.0
SPIKE
(standard units)
2
4
6
8
10
12
B
CONTROL
150.
90.0
30.0
SPIKE
(standard units)
PRE-KINDLING
POST-KINDLING
2
4
6
8
10
12
INTENSITY

DISCUSSION

Systemic administration of ketamine attenuated the area of the late component by 27 percent. This is consistent with the results reported by Racine et al. (1991) and supports the conclusion that the late component of the EPSP is partially mediated by NMDA receptors. The fact that diazepam failed to significantly effect the late component suggests that GABA-mediated inhibition does not play a major role, if any, in the currents contributing to the late component of the train-evoked response. However, the late component is not likely to be entirely NMDA-mediated as administration of ketamine failed to abolish it completely. Although full dose-response curves were not generated, a previous study showed that non-anaesthetic doses of ketamine produced an apparently maximum attenuation of around 35% (Racine et al., 1991). The dose of ketamine used in both Racine's and the present study was half the recommended maximum anaesthetic dose. It would be of theoretical value to administer higher concentrations of ketamine in order to determine how much of the late component can be blocked by antagonism of the NMDA receptor.

The membrane currents driving the late component are most likely related to ionic flux through NMDA receptor channels. As well, a similar train-evoked late component has been

reported in the hippocampal slice preparation, and this component has also been attributed to activation of NMDA receptors (Wigstrom, Gustafsson, and Huang, 1985). While NMDA receptors have not been found to play a role in normal synaptic transmission, they have been shown to contribute to events during high frequency transmission (Herron et al., 1986). During high-frequency stimulation, a neuron may become depolarized long enough to reduce the Mg^{2+} block of NMDA channels (Collingridge et al., 1988). Removal of extracellular Mg^{2+} alone, in the hippocampal slice preparation, results in an increased population spike amplitude, the appearance of secondary population spikes, and, occasionally, the development of spontaneous epileptiform discharges (Coan and Collingridge, 1987b). A similar mechanism is most likely activated during the high-frequency stimulation used in kindling. Each kindling train causes sustained depolarization resulting in a removal of the Mg^{2+} blockade which, in turn, causes activation of the NMDA channel.

The kindling-induced change observed in the NMDA mediated response goes counter to that reported in the literature. Mody, Stanton, and Heinemann (1988) found that perfusion of the NMDA receptor antagonist APV had no effect on the EPSPs of non-kindled granule cells, but that it consistently reduced the amplitude and duration of EPSPs in kindled cells. They thus reported the existence of an NMDA mediated component in

kindled slices that was not present in control slices. Similarly, Morrisett and his colleagues found that hippocampal slices from kindled rats exhibited a long-lasting enhanced sensitivity to NMDA (Morrissett et al., 1989). However, in both of these studies, the hippocampal slice preparation was used. Inherent in such a technique are several sources of potential error that may lead to misinterpretation of results. Specifically, in preparing a slice, it is necessary to sever cortical afferents that may contribute to the late component and/or other aspects of synaptic transmission. As well, the bath medium may not exactly mimic cerebral spinal fluid (CSF). There may be trace elements present in CSF that alter the sensitivity of the NMDA receptor. In the chronic preparation, the physiological and chemical environment most closely resemble that found in the untouched brain. The discrepancy between Mody's and the present work is most likely one of methodology, with the more probable situation being a decreased sensitivity of the NMDA receptor.

Furthermore, the diminished activation of the NMDA component following kindling is consistent with reductions in NMDA receptor binding after kindling. Using a quantitative radiohisto-chemical method, Okazaki and co-workers found NMDA receptor binding to be decreased in CA1, motor cortex, and in layers I-IV of somatosensory cortex, in animals tested 28 days following their last kindled seizure (Okazaki, McNamara, and

Nadler, 1988). Hence, kindling lead to a down-regulation of NMDA receptor binding in selected brain regions.

Ketamine and kindling treatments caused an attenuation of the late component that was almost equal in magnitude. The effects of this antagonism are not transient, since I/O measures collected up to two weeks following the last Stage 5 seizure still revealed an attenuated late component. The administration of ketamine to fully kindled animals yielded an attenuation of the late component that was similar to that obtained when ketamine was administered to naive animals. This suggests that kindling rendered a certain proportion of the NMDA receptors inaccessible to activation. The drug effects would thus be expected to remain proportionally similar to those found before kindling. Since the effects of ketamine were indeed the same both prior to and following kindling, it is possible that many of the NMDA receptors were incapacitated as a result of kindling and could no longer contribute to the late component. If this is the case, then similar results should be obtained with specific receptor antagonists, such as APV.

Given that (1) the attenuating effects of ketamine on the late component remained proportionately constant before and after kindling and (2) kindling also served to attenuate the late component, significant implications can be proposed regarding the neurochemical nature of the late component.

The results of the present study and others strongly indicate that the late component is mediated by the NMDA receptor (Mody et al., 1988; Morrisett et al., 1989; Wigstrom et al., 1985). However, given that NMDA receptor antagonists do not completely abolish the response, there is clearly another transmitter contributing to the late wave. If decreased activation of this other chemical were responsible for the attenuated late component seen following kindling, then that portion of the late wave remaining would consist almost entirely of an NMDA-mediated component. If that were the case, ketamine would be expected to have an even greater attenuating effect than that obtained prior to kindling. Since the effects of ketamine were proportionately similar both before and after kindling, it can be claimed that kindling did, indeed, decrease the sensitivity of the NMDA receptor.

Since kindling has been shown to result in a number of permanent cellular and functional changes, it is feasible that the late component is slightly attenuated with each kindling stimulation and that the combined effect of these attenuations is a permanent, measurable decrease in the magnitude of the late component. It would, thus, have been of value to collect late component measures at regular intervals throughout the kindling procedure in order to substantiate this.

Systemic administration of kainic acid, an EAA agonist,

causes status epilepticus with subsequent tissue damage in pyriform cortex and other brain regions. However, if rats are first kindled, the intensity and duration of status epilepticus as well as the extent of neuropathology are all diminished (McIntyre, personal communication). Hence, kindling serves to protect cells from the neurotoxic effects of kainic acid--an EAA agonist. If kindling causes a decreased sensitivity to NMDA, and if NMDA activation mediates the seizure discharge triggered by EAAs, then the effects of any EAA agonist should be less intense following kindling. Similarly, the effects of EAA antagonists such as ketamine may also be less intense following kindling. This was seen when ketamine administered after kindling affected the late component in the same manner as ketamine administered before kindling, even though the baseline late component after kindling was significantly attenuated with respect to that obtained prior to kindling.

Although kindling produced a significant depression of the late component, it substantially **increased** the mean population spike amplitude and EPSP slope. Kindling-induced potentiation (KIP) of the population spike and the EPSP slope are well-documented phenomena (deJonge and Racine, 1987). That the late component was not potentiated following kindling suggests that KIP is not due to an increase in neurotransmitter release. All components of the field

potential that were measured in this study are due, in part, to the release of glutamate from perforant path terminals. If there was an increase in transmitter release as a result of kindling, then all components should have been enhanced.

The principal result of the thesis is a decrease in the amplitude of a late component in the train-evoked field potential following kindling. Evidence is presented that this component is partially NMDA-dependent. Although this challenges some previous findings, the evidence provided herein is consistent with previous results from our laboratory. As this component has been shown to be mediated, in part, by NMDA receptors, attenuation of the late component may reflect a decreased contribution of NMDA receptors to synaptic transmission. This decreased participation of NMDA receptors is consistent with a reported reduction of NMDA receptor binding following kindling (Okazaki et al., 1988). Furthermore, a decreased seizure response to EAA agonists, such as kainic acid, is also seen following kindling, providing further evidence for a decreased sensitivity to EAAs and possibly NMDA following kindling. The decreased sensitivity to NMDA may reflect either a degenerative or a compensatory mechanism and may be a critical event underlying the kindling process.

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