NEOCORTICAL EVOKED POTENTIALS:
EFFECTS OF ENRICHMENT AND LTP

NEOCORTICAL EVOKED POTENTIALS:
EFFECTS OF ENVIRONMENTAL ENRICHMENT
AND ELECTRICAL STIMULATION

By

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Abstract

Alterations in neural tissues associated with environmental variables have been studied for many years. Anatomical changes in the neocortex of rats in response to exposure to complex environments were observed and replicated in a number of studies both within and across species. These changes are not dependent on the age of the animal or on the duration of exposure, and have been demonstrated in structures outside of the cortex. Due to the undisputed involvement of both the neocortex and the hippocampus in learning and memory, researchers applied a widely used model system of a synaptic mechanism for learning, long-term potentiation (LTP), to the environmental enrichment paradigm and demonstrated significant enhancements in hippocampal field potentials in enriched rats. The present study examines whether the neocortex also showed evidence of plasticity in synaptic transmission. No effects for environmental enrichment were observed on the maximum amplitude of neocortical field responses evoked from the corpus callosum. To assess the plasticity of the chronic preparation used in the study, the animals were exposed to trains of pulses previously shown to induce electrical LTP in the cortex, but revealed only a slight, although significant, depression of the evoked response amplitude. An alteration in the stimulation parameters did not result in an enhanced response. Cortical depth measures suggested that the enriched environment was indeed sufficient to produce plastic changes in anatomy, if not in the efficacy of synaptic transmission. The importance of these findings in the neocortex leads us to question the validity of the LTP model of learning and memory.

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Introduction

Demonstrations of the brain's capacity to change in response to environmental stimulation are both intriguing and crucial to our understanding of learning mechanisms. Environmental alterations during early development can have a dramatic impact on later behavioural performance and on neural anatomy and chemistry. Both structural and functional effects of differential rearing can be observed in cortical and non-cortical areas of the mammalian brain. Several recent experiments have renewed interest in environmental variables by demonstrating that environmental effects were not exclusive to manipulations of an animal's early development. Environmental complexity can remain as a potent variable throughout the life-span of rodents, and it is apparently capable of producing comparable effects in either weanling or aged animals. Links between enrichment effects and putative synaptic mechanisms of memory storage can be derived from studies employing hippocampal evoked response measures following differential environmental exposure. There is a remarkable similarity between the observed effects of enrichment on evoked synaptic responses in the hippocampus and those of the phenomenon of LTP, a popular model of information storage. This resemblance, combined with analogous structural alterations in the hippocampus and the neocortex resulting from exposure to enriched environments, suggest a possible common mediator - namely, that differential demand for processing of environmental information results in changes in synaptic size and efficacy. An experiment to examine the effects of environmental enrichment on evoked responses in the neocortex is proposed to identify whether effects on evoked responses, similar to those in the hippocampus, can be induced in the neocortex. The results of this experiment and a follow-up study on cortical LTP are discussed with regard to the validity of potentiation phenomena as models of information storage.

Donald Hebb began studying the effects of environmental enrichment in rats in the 1940's with an interest in intelligence and its manipulation. He raised several groups of rats as pets in his own home in an attempt to enrich their environment shortly after weaning. Aside from exhibiting less emotional reactivity to being handled and tested, performance on a problem solving task was markedly improved in the enriched rats over their relatively impoverished counterparts reared in a laboratory setting (Hebb, 1949). Furthermore, the effect seemed to be long-lasting, if not permanent, and handling of the isolated group did not bring them closer to the performance levels of the enriched group. Similar effects, such as improvements in maze performance and agility (Pappas et al., 1987; Riege, 1971), have been demonstrated and replicated by other researchers. The link with intelligence was thought to be that the enriched animal possessed a greater ability to profit from new experiences at maturity, one of the hallmarks of the 'intelligent human being' (Hebb, 1949). Although the interest in intelligence declined with regards to environmental complexity, research continued to progress. Other effects of enrichment were reported, including increases in voluntary ethanol intake (Rockman, Gibson, & Benarroch, 1989) and decreases in food consumption with parallel reductions in body weight (Fiala, Snow, & Greenough, 1977). There were also enhancements of hoarding behaviour but decreases in exploration and object contact (Manosevitz, Campenot & Swencionis, 1968; Manosevitz & Montemayor, 1973; Morgan, 1973; Einon & Morgan, 1976), increases in sleep time (Tagney, 1973), changes in liver enzymes and increases in adrenal weight (Geller, Yuwiler, & Zolman, 1965).

The composition of the enrichment environments is highly variable between stduies, but most provide the animal with more cage space than the isolates, companions and objects (e.g. ramps, swings, balls, and seesaws) with which to interact. Animals were generally given opportunities to exercise, and a more spatially complex habitat that included barriers, tunnels, and mazes. Rockman and associates (1989) exposed animals to music as part of their enrichment procedure. It is apparent that almost any manipulation could be used to enrich the experience of the rat since enrichment is a relative term, always as a comparison with 'normal' laboratory housing conditions. A common comparison group for enrichment studies is the socially-reared animal. Several animals are housed together in colony cages but otherwise are not given extra stimulation. A study by Einon and Morgan (1976) observed several behavioural correlates of social rearing as compared to isolation. These included a significant increase in the number of total object contacts in an open field test and the manner in which the animals contacted objects or moved through the environment (e.g. climbing, rearing, touching with the paws). Standard wire laboratory cages were the most common environment for the isolated condition, although translucent plastic tubs have also been used (Green, Greenough, & Schlumpf, 1983).

An excellent review of the key issues and the inherent difficulties associated with interpreting the results of early environmental manipulation was prepared by Martin Daly in 1973. He cautions that we must be especially careful in our discussion of enrichment and impoverishment effects since the terms, by their very nature, tend to be anthropocentric. We must temper our understanding of environmental influences on developing animals with a

sense of ecological validity. It may be of little value to repeatedly impose visual tasks, for example, on an organism that relies greatly on olfactory information. One should understand species-specific behaviour patterns before evaluating the presumed benefits of these manipulations, generally believed to be accelerated growth, reduced emotional reactivity, more adaptive stress responses,and improved learning performance (Daly, 1973). Most importantly, one must consider whether the observed behavioural alterations are in fact adaptive in view of the organism's normal habitat. Failing to do so may lead to tangential discussions with little relevance to situations outside of the laboratory setting.

Behavioural adaptations are not the only consequences of environmental manipulation. There are also a number of anatomical and biochemical correlates of enrichment and isolation than have been replicated both within and across species (Manosevitz & Montemayor, 1973; Walsh & Cummins, 1975; Bryan & Riesen, 1989). These demonstrations include increases in gross cortical dimensions (Diamond, Krech, & Rosenzweig, 1964; Diamond, Lindner, & Raymond, 1967; Diamond et al., 1972), increased brain weight (Geller et al., 1965) altered neuronal structure (Globus et al., 1973; Rutledge, Wright, & Duncan, 1974), and alterations in enzymatic and RNA content of the brain (Krech, Rosenzweig, & Bennett, 1962; Ferchmin, Eterovic, & Caputto, 1970). The most common procedure to produce these varied effects is to place rats, just after weaning, into rooms with a multitude of objects with which they can interact. The animals can be housed either isolated or in groups, although group rearing may have some facilitating effects on object

manipulation (Einon & Morgan, 1976; Walsh & Cummins, 1975) and therefore on the quality of the enrichment experience itself.

Apparently, many of the effects are not dependent on the age at which enrichment is begun, nor are they greatly sensitive to the duration or the actual length of exposure each day. Similar enrichment effects have been produced in year-old (Riege, 1971), middle-aged (Green et al., 1983) and elderly rats (Cummins et al., 1973), and in weanlings, with as little as 4 days of exposure (Ferchmin et al., 1970; Diamond et al., 1976). Rosenzweig, Love, and Bennett (1968) observed changes in brain chemistry and anatomy with as little as 2 hr of exposure each day for 30 days. Riege (1971) observed that the brains of year-old rats were as susceptible to environmental manipulation as were weanlings, although longer exposure was required in the older animals to provide equivalent changes in maze learning and agility, brain weight, and acetylcholinesterase activity. These data suggest that, although enrichment effects are not entirely dependent on the age of exposure, some of the effects may be limited by chronological age.

With properties such as a rapid induction and an apparent capacity for life-long plastic change, environmental enrichment effects in the rodent brain sparked the curiosity of researchers interested in memory and information processing. The search for the possible mechanisms underlying these effects took many forms, but focused on changes in neurochemical and anatomical properties of the cortex thought to be involved in memory. Tissue concentrations of the neurotransmitters dopamine and serotonin are not altered by environmental manipulations of this type (Bennett et al., 1964; Geller et al., 1965), but norepinephrine has been shown to be significantly

involved in the behavioural, biochemical, and neuroanatomical manifestations of enrichment (O'Shea et al., 1983; Pappas et al., 1987). Using tritiated thymidine, Altman and Das (1964) found 59% more glial cells in the neocortex of enriched rats compared to their isolated counterparts, but found no significant changes in non-cortical areas. They injected the radio-labelled DNA precursor into adult rats and cats to determine if the rate of glial cell multiplication was altered in the enriched animals. As glial cells are capable of modifying both chemical and electrical properties of neighbouring neurons, these changes in neuroglia resulting from exposure to a spatially-enhanced habitat suggest that the cortex of enriched rats may exhibit alterations in its electrical properties as well.

There is evidence that enrichment and isolation effects are not only limited to the cortex, but also are found in the hippocampus, the subcortical telencephalon, and the caudal diencephalon (Greer et al., 1982). Walsh, Budtz-Olsen, Penney, and Cummins (1969), reported a significant increase in hippocampal depth associated with environmental enrichment, but this difference disappeared when other researchers increased the sample size (Diamond et al., 1976). However, Fiala, Joyce, and Greenough (1978) reported that dendritic branching of hippocampal granule cells were significantly altered by a complex environment in young but not adult animals.

The analogies between these effects of differential environmental stimulation and the theoretical notions of long-term storage of information in the nervous system are obvious. The phenomenon of hippocampal LTP provides another model phenomenon for the mnemonic functions of the brain (e.g. Racine & Kairiss, 1987; Brown et al., 1988). Hippocampal LTP is a

lasting enhancement of synaptic transmission following high-frequency stimulation of fibres that terminate in the hippocampus (Bliss & Lomo, 1973; Bliss & Gardner-Medwin, 1973). Synaptic potentiation phenomena similar to LTP have also been induced in the rat visual neocortex (Artola & Singer, 1987; Berry, Teyler, & Han, 1989; Teyler, Perkins, & Harris, 1989), pyriform cortex (Stripling, Patneau, & Gramlich, 1988), and the frontal cortex (Sutor & Hablitz, 1989). Although there are obvious similarities between LTP in the hippocampus and the neocortex, including a comparable magnitude when cell densities are accounted for (Teyler, 1989), or a common dependence on the NMDA receptor (Artola & Singer, 1987; Sutor & Hablitz, 1989), there are also important differences. These sites differ in the stimulation parameters required for LTP induction, in the time-course of expression (Teyler, 1989), and of the form of the potentiated response itself (Stripling, Patneau & Gramlich, 1988). The neocortex and hippocampus also have a different time-course for structural development (Altman and Das, 1965), and a corresponding difference in the development of LTP (Wilson & Racine, 1983). Perkins and Teyler (1988) also describe a critical period for cortical LTP, but indicate that not all layers of the cortex demonstrate this property. They suggest that perhaps only the infragranular layers may remain plastic into adulthood. Although neocortical LTP has been observed in a slice preparation from both neonatal and adult rats, and in intact anaesthetized adults, it is not known whether it is supported in the awake and freely-moving animal. Evidence for a kindling-induced potentiation effect in the neocortex of freely-moving rats (Racine, Tuff, & Zaide, 1975) suggests that plastic phenomena like LTP may be possible in a chronic preparation. A kindling-like procedure in awake and

unrestrained cats by Rutledge, Wright, and Duncan (1974) produce morphological changes in neocortical neurons of remarkable similarity to those due to environmental enrichment. These results suggest that LTP and enrichment may be producing comparable effects through a shared mechanism.

Enrichment procedures themselves can lead to enhanced synaptic transmission in the hippocampus. Sharp, McNaughton, and Barnes (1985) observed an LTP-like effect in the hippocampus of rats induced by exposure to an enriched environment that consisted of a large room filled with boxes, ramps, and various surfaces for the animals to run on. Field recordings of evoked hippocampal responses showed an increase in the amplitude of both the population excitatory postsynaptic potentials (EPSPs - a measure of the strength of synaptic response) and the population spikes (a measure of cell discharge), although the relative increase in the population spike amplitude was more prominent. Sharp, Barnes, and McNaughton (1987) replicated and extended their findings to demonstrate that the phenomena also occurred in older animals. The decay rate of the environmentally produced increases in evoked response was greater for the older animals, with the decay time constants of 30.3 days for the younger group and 11.1 days for the older rats. It is notable that the decay constant for the young animals is equivalent to the value obtained for electrically induced potentiation in animals of the same age (Sharp et al., 1985).

As comparable structural adaptations result from environmental manipulations in both the neocortex and the hippocampus, and both structures support LTP, the report of Sharp and associates (1985) that enrichment could

induce potentiation-like effects in the hippocampus suggests that an analogous effect may be observable in the neocortex. Considering the involvement of both the neocortex and the hippocampus in the processing of complex environmental information, the neocortex is clearly a logical structure in which to look for such an effect. An experiment was designed to test whether environmental enrichment could induce an enhancement of evoked neocortical responses. A monosynaptic system between neocortical sites, running via the corpus callosum, was chosen for the simplicity and consistency of its response. This preparation provided a convenient system in which to examine the functional changes presumed to accompany the morphological plasticity resulting from environmental enrichment. If researchers could identify both a structural and a functional alteration resulting from explicit environmental stimulation, it would greatly facilitate the continuing search for a mechanism of permanent information storage in the brain.

EXPERIMENT 1

Methods

Sixteen male hooded rats, aged approximately 100 days and weighing 350-450 g, were obtained from the McMaster Psychology Department colony. Each animal was anesthetized with Somnotol (sodium pentobarbital, 60 mg/kg, i.p.) and given 0.20 mL i.p. atropine sulfate. The animal was then placed in a standard Kopf stereotaxic instrument with its head level. A topical anaesthetic (lidocaine hydrochloride with epinephrine, 0.20 mL s.c.) was applied to the scalp after which the skull was exposed. Five small holes were made in the skull with a hand-held drill. Stainless-steel jeweler's screws were inserted into four of these holes to serve as anchors, with one also serving as the ground or indifferent electrode. Dura was carefully sliced with a needle to allow for implantation of two bipolar stimulating/recording electrodes. Both electrodes consisted of two strands of teflon-coated stainless-steel wire (0.008") wound tightly together with a vertical tip separation of approximately 0.5 mm.

The recording electrode was carefully implanted so that its tip was placed in a somatosensory area of the frontoparietal neocortex (coordinates AP=+0.7, ML=+4.0, DV=-2.5 mm from Bregma at the skull surface; Paxinos & Watson, 1982). The stimulating electrode was lowered into the corpus callosum (approximately AP=+0.7, ML=+2.0, DV=-3.0) under physiological control to obtain the greatest amplitude evoked response from single test stimuli. During this implantation procedure, all animals were stimulated with 0.1 msec duration square wave biphasic test pulses at an intensity of 800 μA, once every 10 seconds.

Following the correct placement of the electrodes, gold-plated amphenol connectors attached to the electrode wires were inserted into a 9-pin plastic headcap assembly (Molino & McIntyre, 1972) and anchored to the skull with dental cement. As a precaution against infection, 0.30 mL of penicillin (Derapen-C) was injected intramuscularly, followed by the application of a topical antibacterial ointment (Furasone).

Following surgery, all animals were returned to their home cages and allowed to recover for at least 10 days before testing. The animals were housed individually in standard wire-mesh cages with solid sides and backs (preventing any social interaction - i.e. the "isolated condition") on a 12:12 hr light cycle. Food and water were available ad libitum.

Several days prior to the start of the experiment, all rats underwent screening for evoked responses. They were removed from their home cage, placed individually in an animal carrier, and brought to a testing room. They were placed in a wooden testing cage, measuring 50 cm x 50 cm x 60 cm, with a plexiglass front panel and a wire mesh bottom. Following this, each rat was attached via the headcap assembly to a set of electrical leads, connected to a Grass Model 7 Polygraph with Grass Model 7P5A preamplifiers, for the recording of electrical activity in the neocortex. Stimulating current was applied through the leads to the corpus callosum electrode by a Grass Model S88 stimulator. Constant current pulses were delivered via Photoelectric Stimulus Isolation Units. The rats were stimulated every ten seconds to determine the threshold and asymptotic current levels of the evoked response, and to identify an amplifier gain that produced a neocortical response meeting an arbitrary amplitude criterion. This gain was used subsequently to match

animals on the basis of response size and provided a means to assign the subjects to the experimental and control groups. Animals in each group were placed in random order for testing.

Standard procedure for each testing period consisted of removing the animals from their respective environment, transporting them individually in a small carrier, and then attaching them to the electrical leads in the testing cage. An automated input/output (I/O) procedure was begun, lasting 20 minutes, at which time the animal was returned to the appropriate environment.

The I/O procedure itself consisted of a series of 120 square wave pulses (0.1 msec in duration per half-cycle) delivered to the callosal electrode in twelve logarithmically-scaled ascending stimulus intensities, from 40 to 1200 μA. Pulse delivery was under the control of a Tandy 3000 Personal Computer running the Asyst Scientific System (Asyst Software Technologies, Rochester, N.Y.). Pulses were applied every eight seconds while a 50 msec sweep of each evoked response was recorded and digitized for later analyses.

Testing occurred every second day. The first two sessions were considered pre-baseline and allowed the animals to become accustomed to the testing procedure. Eight baseline sessions were run, followed by eight experimental sessions. Experimental animals were placed in an enriched environment 24 hours prior to the first experimental testing session and remained there for 17 days. Control animals remained in the isolated housing. All testing occurred at approximately the same time of day for each individual animal.

The environment for the "enriched condition" consisted of three large chambers in a separate room with popular music playing continuously and with the same light schedule as in the isolated condition. The first two chambers measured 100 cm x 110 cm x 50 cm while the third was 40 cm x 150 cm x 50 with an additional maze segment incorporating several small rooms and variable position doors attached via a tunnel to one end. The enrichment chambers were filled with wood chips as bedding and numerous objects that presented the animals with opportunities to interact with a variety of textures and odours. The components in each environment were changed randomly every day to provide constantly changing stimulation. Food cups and water bottles were placed in different locations in the environments each day. A typical environment included a large running wheel for exercise, metal, plastic, or cardboard tubes bent in different configurations, small boxes and 'houses' with doors and holes in them. In addition, there were pieces of wood, carpet, brick, a wire mesh structure for climbing, ramps and "teeter-totters", rolling balls, and jars with different odours (colognes and perfumes on cotton) inside.

Animals were placed in a random order in the three chambers (2 or 3 per chamber) each day to reduce aggression between the social group members. The rats were observed to thoroughly explore all aspects of the environment and were found to exercise and play frequently.

Statistical analyses were accomplished by a repeated measures analysis of variance with p=.05 as the criterion level of significance.

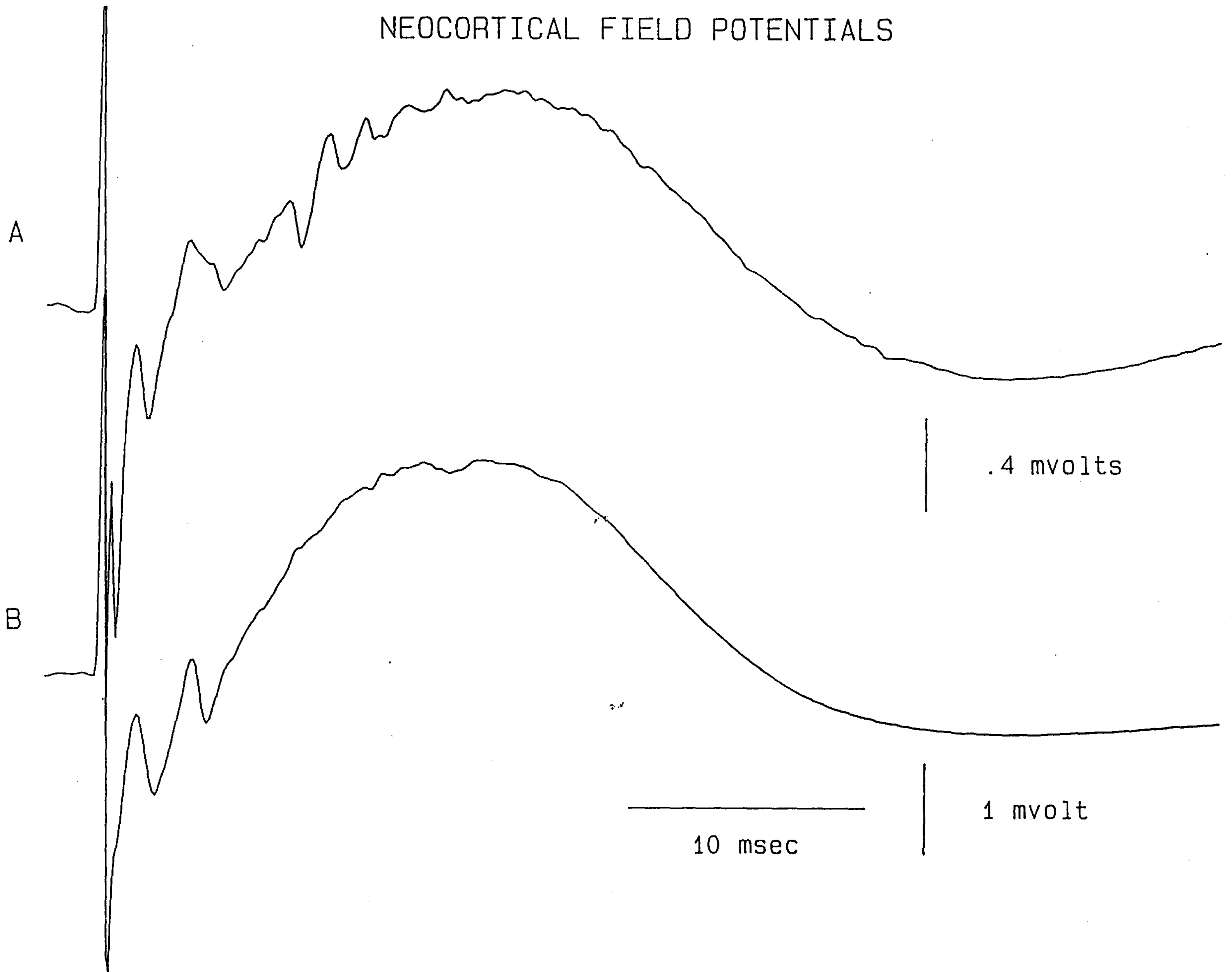
Results

The callosal evoked neocortical responses were of a stable morphology with two main components. The first component was a rapid negative-positive wave with a latency to onset of approximately 2 msec and a latency to peak of 2.4 msec. The second component had a latency to peak of approximately 24 msec, but due to the first component's location in the waveform, it was difficult to determine its latency to onset (see Figure 1, A and B).

The first component of the response was interpreted as reflecting an antidromic activation while the second, and larger, component was believed to be a population EPSP. Some of the responses had additional, but smaller, 'spikes' superimposed on the rising phase of the EPSP ('A' in Figure 1), but they were not seen clearly in all animals ('B' in Figure 1). To characterize these components further, and to verify that they resulted from monosynaptic activation, several animals were tested for the ability of the evoked responses to remain intact with rapidly-repeating stimulus pulses. This frequency-following test confirmed that the callosal-neocortical preparation used in this study was indeed a monosynaptic system.

Chang (1953) and Vogt and Gorman (1982) reported response morphologies similar to ours, with components representing an antidromic spike, EPSP, and a long hyperpolarization. These data, however, come from either anaesthetized or sliced cortical preparations that significantly differ from the present experiment which applied an intact and awake animal. Bremer (1955) discussed an early component, or spike, from callosal evoked responses in the striate area of the cat that preceded the positive-negative spike complex discussed by Chang. He interpreted these as afferent fibre

Figure 1: Neocortical Field Potentials. Representative examples of neocortical evoked responses from two different animals (A and B) showing morphology, amplitude, and duration.


NEOCORTICAL FIELD POTENTIALS
A
B
.4 mvolts
1 mvolt
10 msec

potentials. This component may be masked by the stimulation artifact in our responses.

Spontaneous spindle activity at a frequency of 6-7 Hz was noted, but appeared to occur less frequently during movement. Spindles evoked by the single pulses used for I/O testing had a similar frequency yet had an unusually long duration of up to 14 seconds. If the animal moved during an evoked spindle, however, the duration would be attenuated to approximately 2 seconds.

The threshold for evoked responses was between 60 and 100 μA, with a mean of 75 μA. Asymptotic amplitudes were reached between 800 and 1000 μA. The evoked responses were exceptionally stable throughout the experiment, with only minor differences in the waveform from Day 1 to the final recording session 44 days later (see Figure 2). Many of the 'spikes' were stereotypical, in that they remained with the same latency throughout and were still apparent with little distortion in averages of 10 or more single responses. The longer latency spikes, particularly those following the peak of the EPSP, were more variable in latency and amplitude.

The results showed no main effect due to the environmental manipulation for maximum EPSP amplitude and no Group x Manipulation interaction. There was, however, a significant group effect, $F(1, 14) = 9.00$, $p<.01$. This presumably reflected a small sampling bias. Correspondingly, there was a significant amount of drift in the repeated measures over time, $F(7, 98) = 2.51$, $p<.05$, but this was regarded as consistent between groups as there were no significant interactions for Group x Time nor for Manipulation x Time. Figure 3 is a three-dimensional representation of the amplitude of averaged evoked

Figure 2: Neocortical Field Potentials. Representative examples of neocortical evoked responses indicating stability over time (44 days).

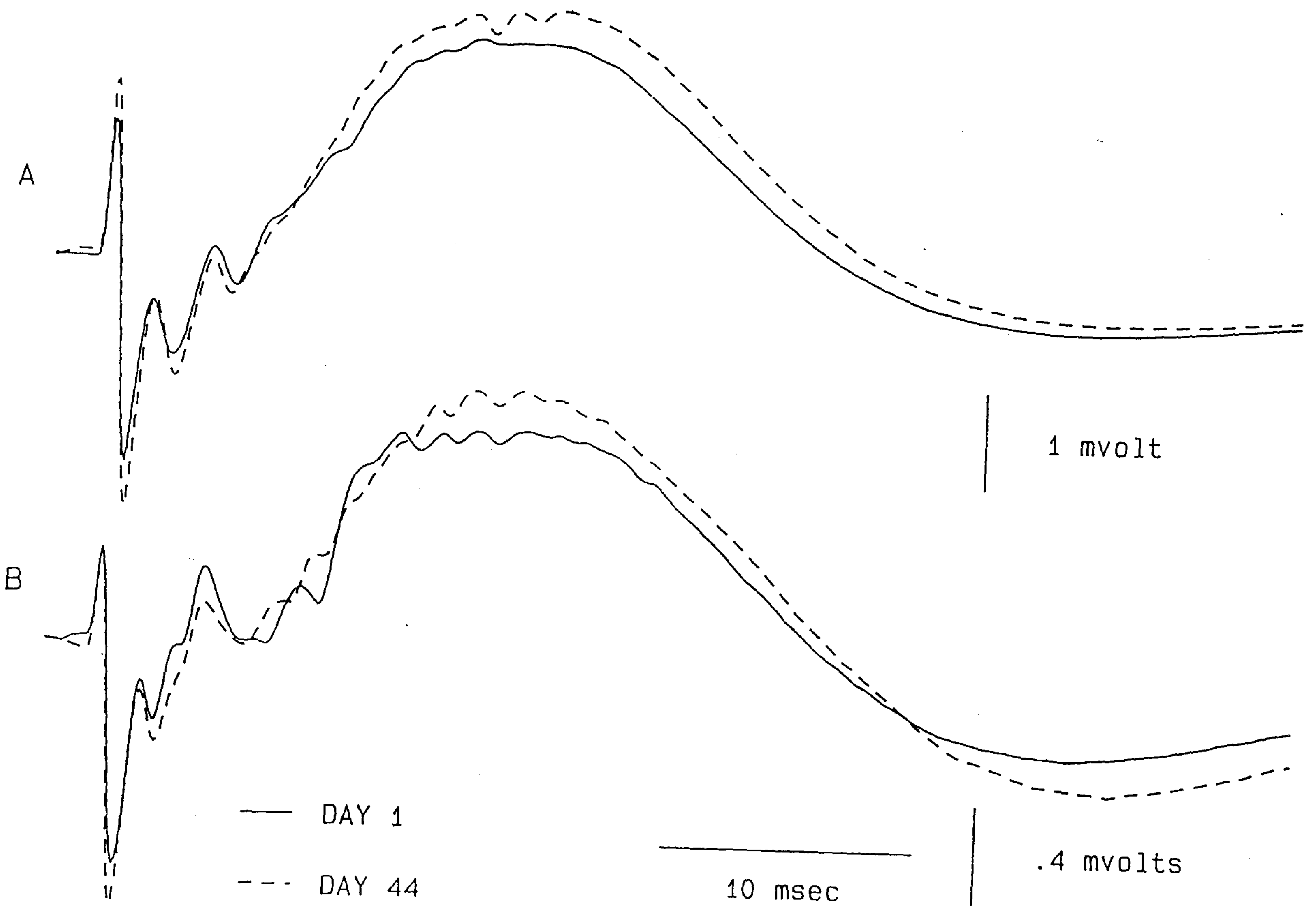
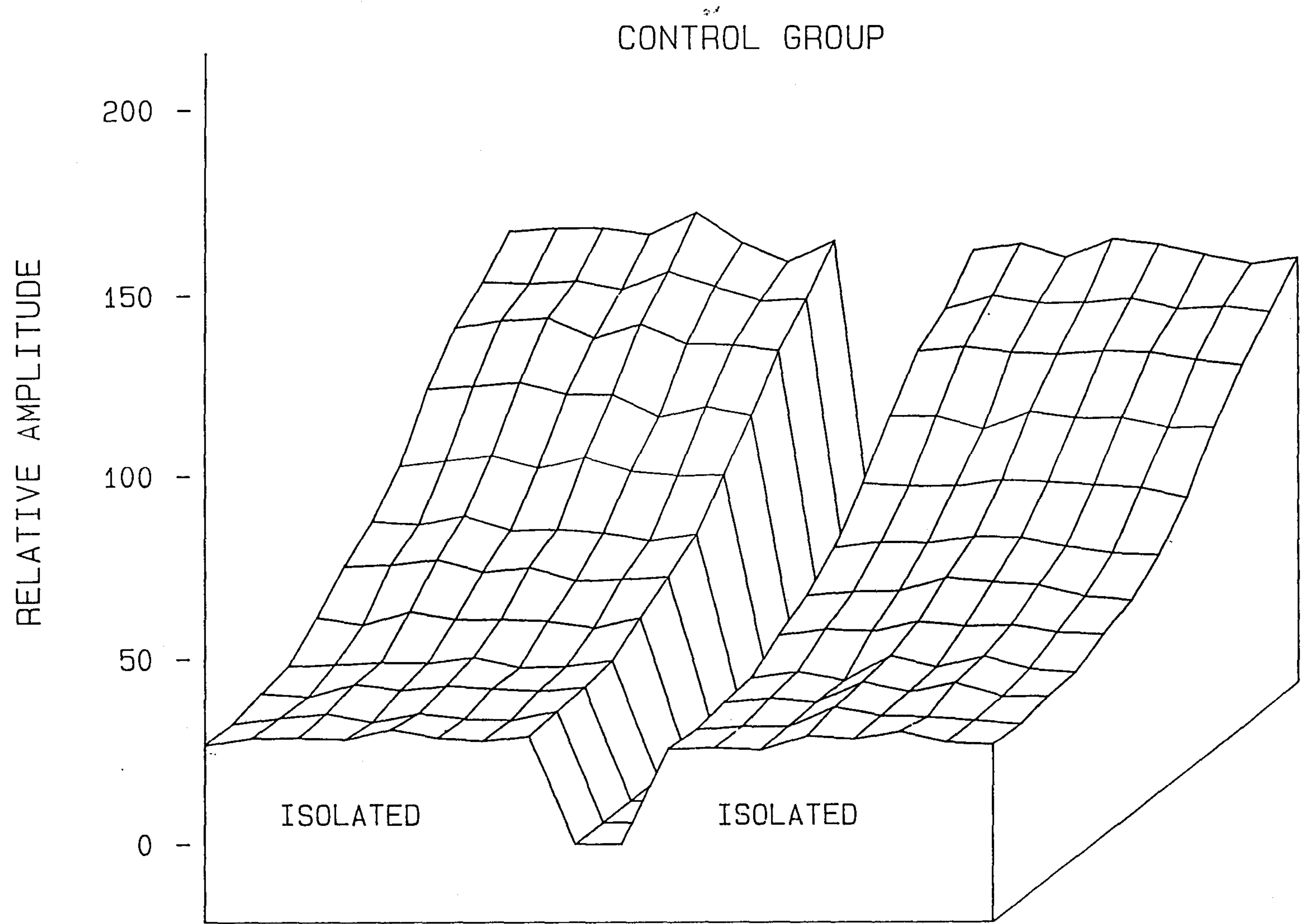
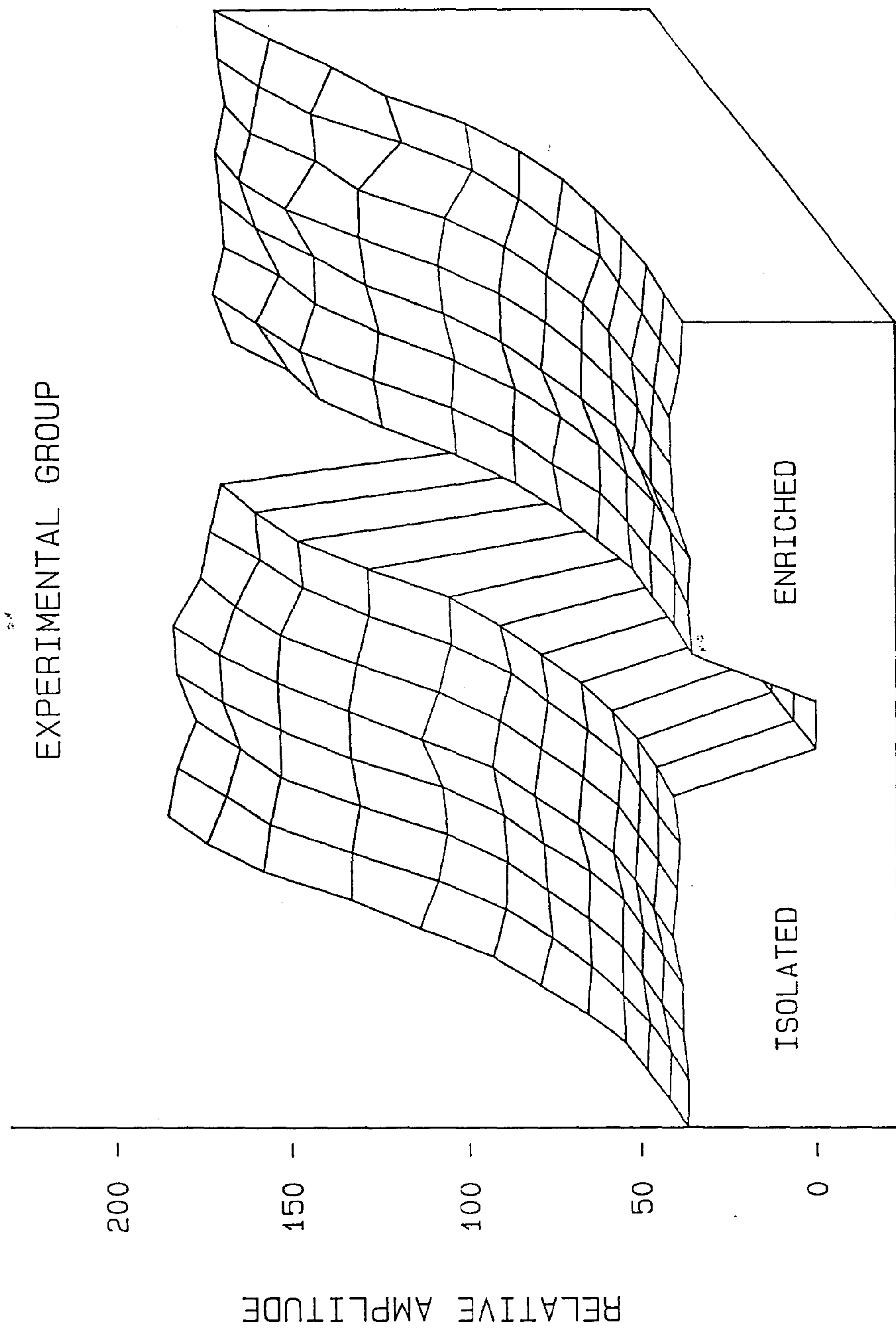

NEOCORTICAL FIELD POTENTIALS
A
B
1 mvolt
.4 mvolts
10 msec
DAY 1
DAY 44

Figure 3: Control Group. 3-Dimensional plot of control group for the environmental enrichment experiment showing response amplitude, time period for tests during the control and experimental environmental manipulation phases, and intensity of stimulation (in depth).


CONTROL GROUP
RELATIVE AMPLITUDE
200
150
100
50
0
ISOLATED
ISOLATED
I/O TESTS (48 HR INTER-TEST INTERVALS)

responses for each intensity on each day of the experiment. An examination of the surface produced by the interaction of these three variables easily confirms that the control group did not differ between the control and the experimental phases. An artificial separation of one time period was inserted between the two phases for clarity only, and does not reflect the actual time-course of the experiment. Figure 4 depicts the experimental group in the same format. A slightly greater degree of variability is apparent at both the high and the low ends of the intensity dimension, and, in combination with the significant repeated measures drift, it may in part account for a significant 4-way interaction of Group x Manipulation x Time x Intensity, $F(35, 490) = 1.45$, $p<.05$. It should be emphasized that these significant differences reflect very small changes in an otherwise highly stable response, and that the manipulation of concern, enrichment, had no significant effect (Figure 3 and Figure 4).

Figure 4: Experimental Group. 3-Dimensional plot of experimental group for the environmental enrichment experiment showing response amplitude, time period for tests before and during enrichment, and intensity of stimulation (in depth).


EXPERIMENTAL GROUP
ISOLATED
ENRICHED
I/O TESTS (48 HR INTER-TEST INTERVALS)
RELATIVE AMPLITUDE
200
150
100
50
0

Discussion

Leah, Allardyce, and Cummins (1985) evoked potentials from the surface of the somatosensory cortex with a direct somatic (forepaw) stimulus. They observed a significant habituation to repeated external stimulation in both urethane anaesthetized and enriched animals. Isolated animals were unable to habituate to the repetitive stimulation unless given an inter-trial interval between sets of stimuli of at least one hour. They interpreted their results as indicating that sensory isolation impairs the development of control over sensory input. Correspondingly, enrichment should enhance the animal's management of sensory information, and may alter aspects of the registration and long-term storage of that information.

Several alternative explanations arise from the failure to produce a significant effect for environmental enrichment on neocortical evoked responses. Three obvious possibilities are: 1) that enrichment effects occurred but the preparation was either inappropriate or the measures were not sensitive to the changes, 2) that enrichment effects were not sufficiently robust in our paradigm to produce changes in field potentials, or, 3) that environmental enrichment cannot alter neocortical evoked responses. Each of these alternatives will be dealt with in turn.

It is possible that the callosal-neocortical system used in the present study underwent significant plastic changes, but, for some reason, the measures used to index those changes were not sufficient for that purpose. Evoked response amplitude may have been an inappropriate choice for the dependent variable. However, the same measures were effective for the demonstration

of enrichment effects on hippocampal responses (Sharp et al., 1985). The demonstration of a kindling-induced potentiation of a transcallosal response by Racine, Tuff, and Zaide (1975) also employed evoked response measures. They used a monosynaptic callosal system that differed from the present study mainly in the location of the stimulating electrode, which was positioned in the contralateral neocortex. As the corpus callosum consists primarily of axons connecting homologous structures from each hemisphere (Chang, 1953), it is unlikely that a significantly different population of neurons was accessed in these related experiments. Callosal axons arborize and terminate mainly in the supragranular layers (where the recording electrode was placed), with smaller numbers of terminations in the infragranular regions (Jacobson, 1970). Unlike the regular laminar arrangement of the hippocampus, the neocortex allows more opportunity for complex interactions between the direct synaptic responses and indirect collateral and recurrent stimulation. It is expected that, in the neocortex, in order for a field potential to be large enough for detection, there must be a relatively synchronous activation of cells within the region of the recording electrode. In view of this, it is doubtful that an indirect activation of the target site could effectively mask a plastic modification of the magnitude expected. The choice of evoked response measures does not appear to be a likely alternative explanation for the failure to reveal an enrichment effect.

The choice and age of animal may have influenced the results in a number of ways. Our animals were approximately 100 days old at the onset of the experiment. Although animals of the same age were capable of showing alterations in neocortical and hippocampal anatomy (Diamond et al., 1972; Walsh et al., 1969) and in hippocampal function (Sharp et al., 1987) as a result

of enrichment, there is evidence that suggests that the neocortex may not respond with a functional plasticity over the entire life-span of the rat. Perkins and Teyler (1988) suggest that only the infragranular layers of the cortex remain plastic into adulthood for the induction of LTP. This may have accounted for the lack of enrichment effect, although LTP has been demonstrated in supragranular regions in adult rats (e.g. Artola & Singer, 1987; Stripling et al., 1988). Another alternative is that the effect was lost to biological variability. The present study randomly assigned animals from several litters to each group instead of applying the common littermate pairing procedure that is used to minimize variability from extraneous sources (Rosenzweig, 1966). In view of the magnitude of the effects from other studies, it is doubtful whether the procedural differences in subject selection could account for the data. Other studies have successfully used non-littermate pairs randomized in their assignment to conditions (Green et al., 1983; Pappas et al., 1987) and have shown significant anatomical and biochemical differences between the enriched and isolated groups. An additional criticism of the experimental design is that enhanced synaptic transmission was present in the neocortex following enrichment, but it had either decayed by the time the response was measured or perhaps the testing procedure had somehow masked a facilitated response. There are no data available to adequately address this criticism at this time.

The lack of enrichment effect could be due to a poorly conceived enrichment environment or a poor selection of cortical test site. Diamond, Lindner, and Raymond (1967) took cortical depth measures and found no significant changes in the slices from the "somesthetic area". They did,

however, find significant decreases in some areas (medial rostral) and increases in others, especially the visual cortex. It is reasonable to expect that the specific pattern of cortical change resulted from the unique characteristics of the environment used for enrichment. Bennett and associates (1964) suggest that modifying the amount of experience in one modality can affect rather specifically the brain regions serving that modality. Considering that Diamond and coworkers (1967) used an environment that emphasized spatial and visual abilities, it would not be particularly surprising for that system to show preferential effects. Sur, Pallas, and Roe (1990) suggest that modality-specific surgical manipulations in early development can induce a subsequent 're-wiring' of the sensory neocortex in ferrets, producing plastic changes that alter the functional properties of specific cortical regions. It is reasonable to assume that plastic phenomena in the adult neocortex are also sensitive to specific types of sensory input. The present study emphasized stimuli that were assumed to be preferentially processed in somatosensory areas of the cortex, providing various textures, shapes, and numerous opportunities for exercise. Anatomical measures on these animals may yield important information to further assess the efficacy of the enrichment environment in producing alterations within the somatosensory cortical systems.

Finally, assuming that all the conditions were optimized for demonstrating a functional plasticity, it is possible that the adult callosal-neocortical system applied in this study is just not plastic, at least in response to the environmental stimuli used in our study. We know that the morphology of the neurons in much of the neocortex have been shown to be plastic to enrichment effects, if not in this specific area of the cortex. Also, long-term

potentiation has been induced within a similar region. Before we can conclude that the system is not as plastic as was originally believed, we must first confirm that LTP is possible in the same animals that underwent environmental manipulation, and second, whether there are any demonstrable anatomical effects that could verify the adequacy of the enrichment experience. Experiment 2 is designed to address these questions.

EXPERIMENT 2

Methods

Half of the animals from each environmental group of Experiment 1 were randomly chosen to receive trains of pulses in an attempt to induce electrical LTP. For each rat there was a corresponding control, balanced for response size. All animals were housed in standard (isolated) laboratory cages.

Induction of LTP consisted of a sequence of 5 sets of trains. Each set of trains consisted of a pair of smaller 4-pulse trains, both at 400 Hz, applied to the corpus callosum electrode. The duration of the pulses was 0.1 msec per half-cycle with 2.5 msec between each pulse and 200 msec between the trains in the pairs. There was a 10 s delay between each of the train pairs. This sequence of trains was repeated at each of the 10 highest stimulus intensities used in the I/O tests of Experiment 1 and was interspersed with 30 single test pulses (900 μA at 10 s intervals) from a separate Grass Model S88 stimulator. Both stimulators were under the control of the computer that recorded the responses in the neocortex evoked by the train and test pulses.

The experimental protocol involved the initial exposure to the potentiation trains (approximately 1 hour in duration), I/O tests at 20 min and 24 hr after the trains, and then one test every second day thereafter for a total of 6 I/O measures. Baseline recordings were taken from the final 8 I/O tests of Experiment 1.

Several days after the final day of testing, all animals were euthanized with an overdose of chloral hydrate, perfused intracardially with 10% formalin in saline, and had their brains removed. The tissues were stored in the formol-saline solution until 24 hr prior to sectioning, at which time they were

transferred to a formol-sucrose (30%) solution. The brains were then frozen and sectioned at 40 µm thickness in a cryostat (Reichert-Jung Cryocut-E). The sections were mounted on slides and stained with buffered thionin to verify electrode placement and to allow for the measurement of cortical depth.

To determine if the enrichment environment in this experiment was in fact sufficient to produce the structural changes noted by other researchers (e.g. Greer et al., 1981), cortical depth measures were taken in a procedure similar to Diamond, Lindner, and Raymond (1967). Four measures of cortical depth were taken with a microscope and an ocular micrometer at 40x from two different brain slices from each animal in the experimental and control groups. Measurements were recorded 0.7 mm anterior to Bregma in the hemisphere opposite to that of the stimulating and recording electrodes. Depth was interpreted as the distance from pia to a point immediately lateral to the elevation of the corpus callosum (Diamond, Lindner, & Raymond, 1967). A t-test was used to analyze the results to determine if the enriched and isolated groups had different cortical thicknesses in the hemisphere opposite to the structures studied electrophysiologically.

Results

There was a small and transient, but significant, depression in evoked response amplitude due to the exposure to potentiating trains evidenced by the interaction of Group x Manipulation x Intensity, $F(5, 70) = 2.34$, $p<0.05$. A three-dimensional plot, similar to those of Experiment 1, shows the relatively stable control group (consisting of equal numbers of enriched and isolated

animals from the Experiment 1) in the time periods corresponding to the baseline and post-train periods for the experimental group (Figure 5). There was a tendency for a small upward drift in the amplitude of the response.

Similarly, Figure 6 demonstrates the responses of the experimental group both prior to and following the trains. It is apparent that the post-train responses are slightly depressed, especially at the higher stimulation intensities during the third post-LTP session. One must, however, remember that the time intervals for the post-train period are not equal. The figure does not indicate that the first period is only 20 minutes after trains, the second test at 24 hr post-train, and the remaining four tests at 48 hr intervals. Caution must be exercised when considering the significance of the depression caused by the potentiation trains since the responses of the control and experimental groups are obviously diverging on at least the third day after the trains (Figure 7). The marginal statistical significance of the 3-way interaction and the divergence in response between groups suggests that there was little, if any, effect of potentiation trains in this preparation.

Electrode placements were verified histologically and are shown in Figure 8. There were no obvious correlations between the few animals that showed variable or unusual responses and the position of the stimulating or electrode tips.

The mean cortical depth of the enriched animals (1.787 mm, $\underline{s}^2$=.0385) was 8.9% greater than that of the isolated group (1.699 mm, $\underline{s}^2$=.0306). Although this difference was not significant ($\underline{t}(62) = 1.888$, $\underline{p}$=.06), the trend suggests that the cortex increased in thickness in response to the

Figure 5: Control Group. 3-Dimensional plot of control group for the neocortical LTP experiment showing response amplitude, time period for tests during the pre- and post-train potentiation phases, and intensity of stimulation (in depth).

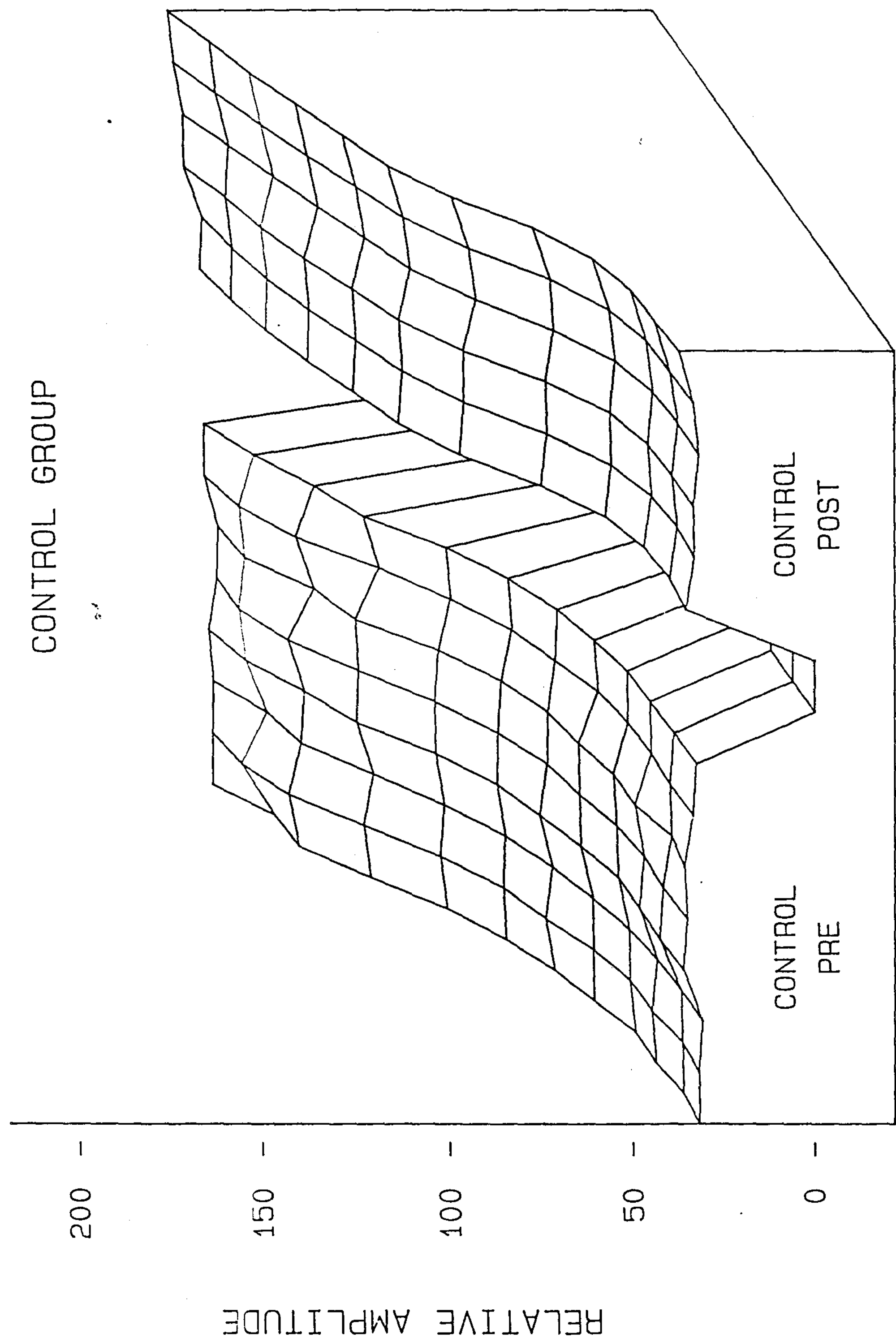

CONTROL GROUP
CONTROL PRE
CONTROL POST
I/O TESTS (48 HR INTER-TEST INTERVALS)
RELATIVE AMPLITUDE
200
150
100
50
0

Figure 6: Experimental Group. 3-Dimensional plot of experimental group for the neocortical LTP experiment showing response amplitude, time period for tests before and after potentiation trains, and intensity of stimulation (in depth)..

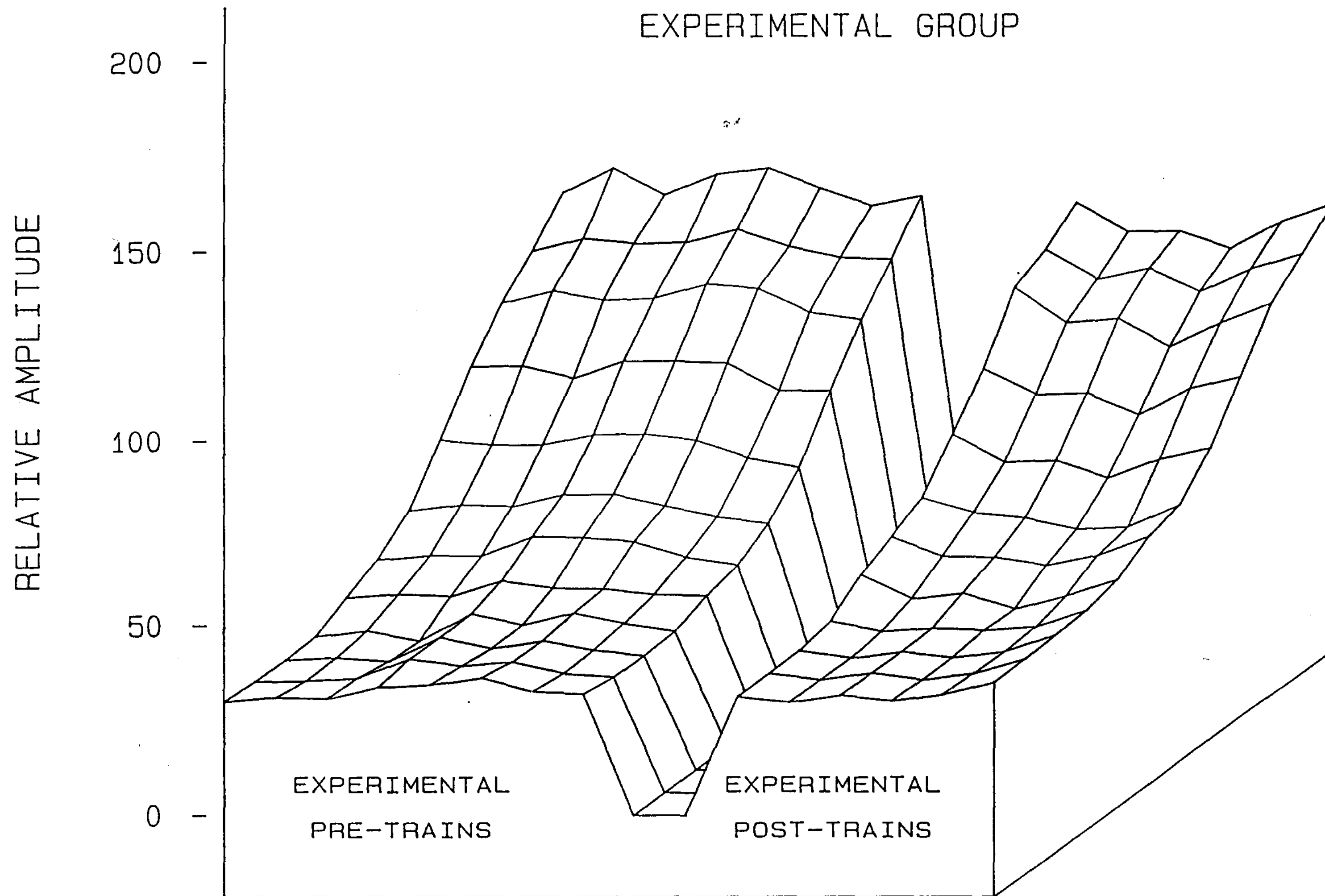

EXPERIMENTAL GROUP
RELATIVE AMPLITUDE
200
150
100
50
0
EXPERIMENTAL
PRE-TRAINS
EXPERIMENTAL
POST-TRAINS
I/O TESTS (48 HR INTER-TEST INTERVALS)

Figure 7: I/O Curves. Input/Output relations for the neocortical LTP experiment comparing the final day of the baseline phase and the third time period of the post-train phase.

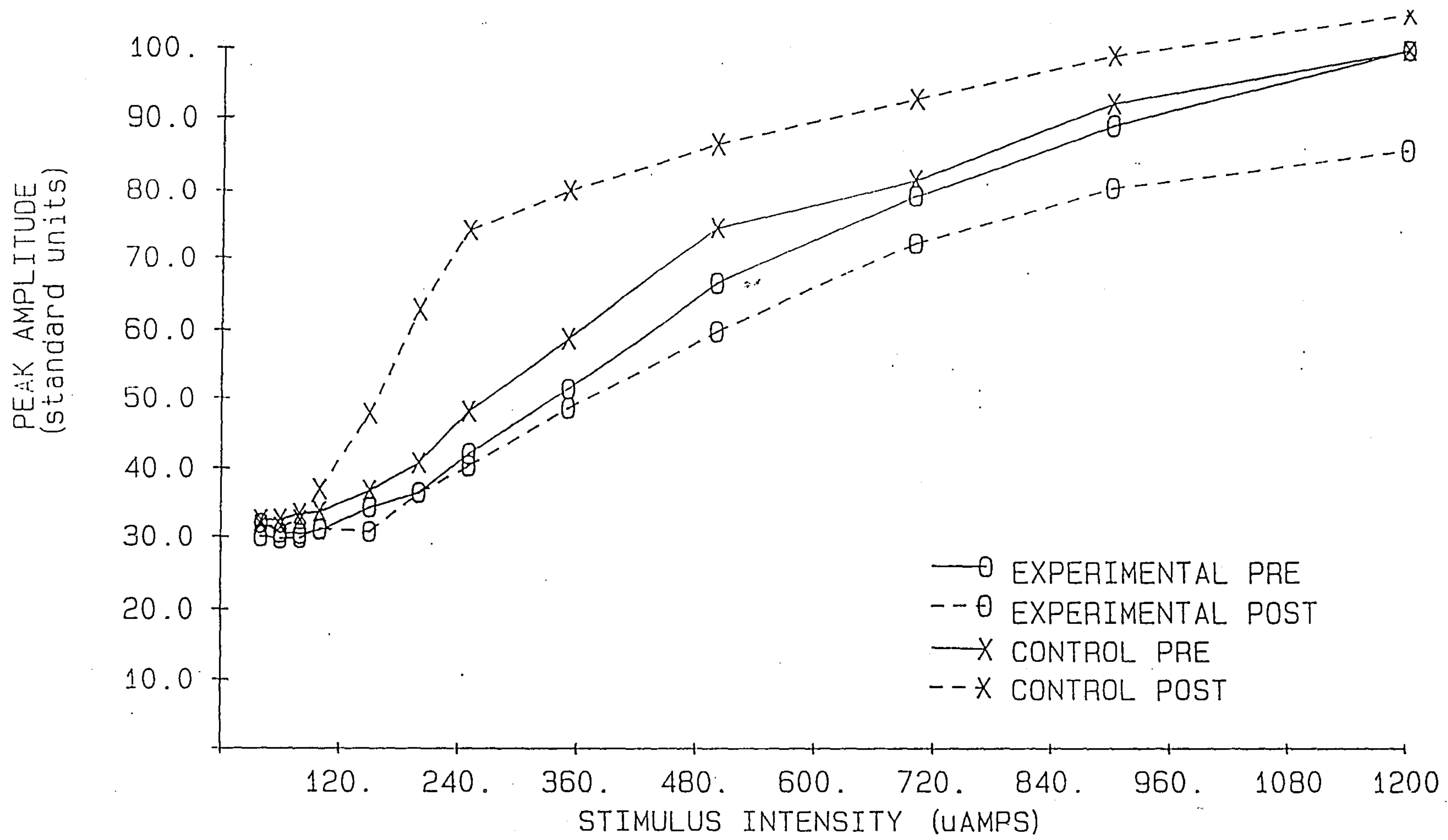
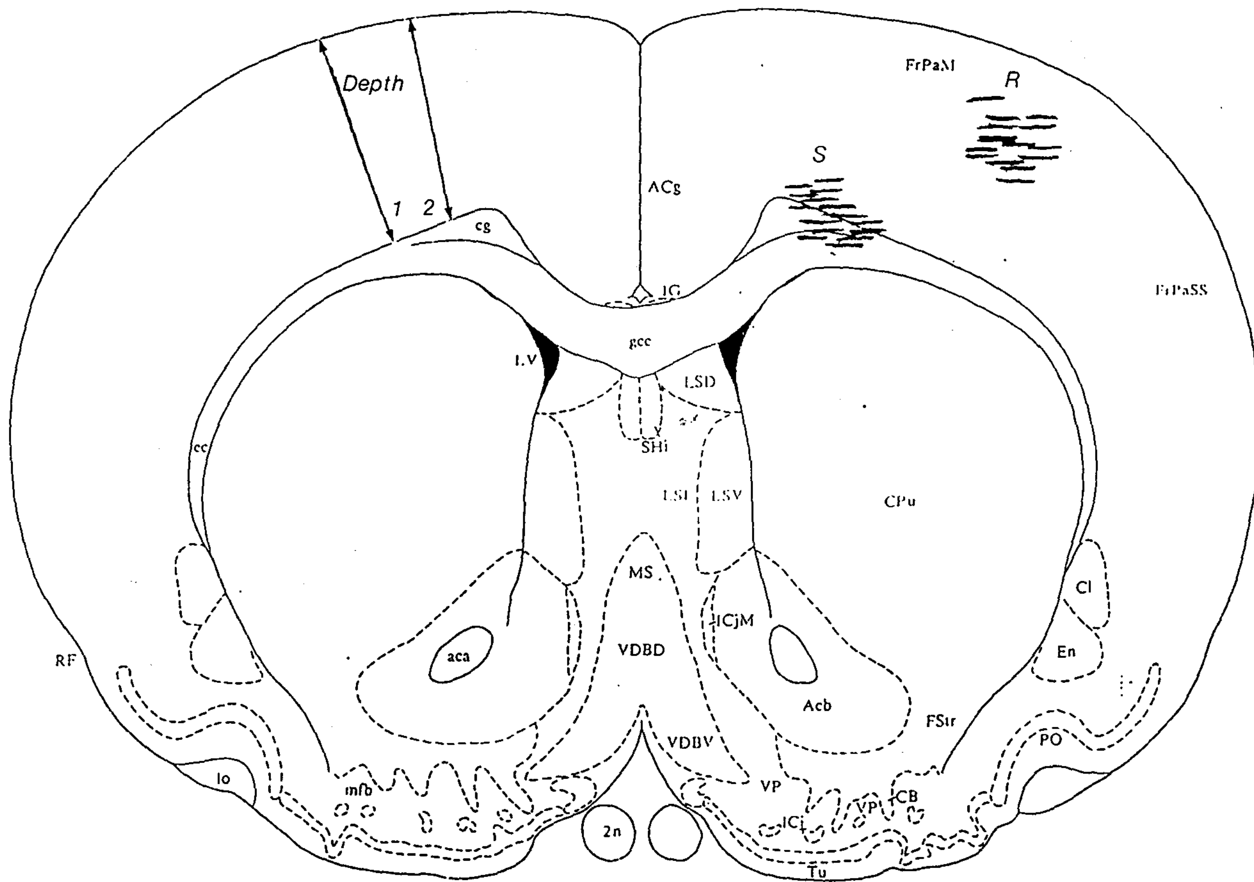

PEAK AMPLITUDE
(standard units)
100.
90.0
80.0
70.0
60.0
50.0
40.0
30.0
20.0
10.0
120.
240.
360.
480.
600.
720.
840.
960.
1080
1200
STIMULUS INTENSITY (uAMPS)
EXPERIMENTAL PRE
EXPERIMENTAL POST
CONTROL PRE
CONTROL POST

Figure 8: Histology. Histological verification of electrode placements showing the position of the recording (R) and stimulating (S) electrode tips and indicating the position for cortical depth measurements (from Paxinos & Watson, 1982).


Depth
1
2
FrPaM
R
S
ACg
cg
IG
gcc
LV
LSD
SHi
LSI
LSV
CPu
cc
FrPaSS
MS
ICjM
VDBD
aca
Acb
Cl
En
RF
FStr
VDBV
PO
lo
mfb
VP
ICj
CB
2n
Tu
AP= +0.7mm

environmental treatment. The relative positions at which the depth measures were recorded is indicated in Figure 8.

Discussion

The application of trains of pulses in a manner known to produce potentiation in the neocortex (Wilson & Racine, 1983) failed to enhance the evoked response of the monosynaptic corpus callosum-neocortex system, and in fact, produced a significant depression of the response. It is possible that the population of neurons stimulated in this experiment responds maximally to different stimulation parameters. Teyler (1989) described that the most effective parameters for neocortical potentiation in the slice were either low (2 Hz) or high frequencies (100 Hz), although Wilson and Racine (1983) were able to produce LTP in intact, yet anaesthetized, rats at 400 Hz. To gain more information on whether a lower stimulation frequency would be capable of inducing potentiation, a brief pilot experiment on two animals with comparable and stable responses was undertaken. These animals were exposed to 1 hour of pulses delivered at 2 Hz at 900 μA. Since Teyler (1989) indicated that neocortical potentiation effects were possible with this set of parameters but that it took up to 30 minutes to develop, the timing of the first I/O measure after the trains was altered from 20 minutes to 2 hours. Therefore, the pilot animals had a baseline I/O measure taken, they were exposed to trains, and then they remained in the apparatus for 2 hours. At this time another I/O measure was performed, with the next following 24 hours later. The results suggest that there may have been a slight depression of the response after the trains that was maintained for at least 24 hours. Although the sample size was admittedly low, there was no evidence of any potentiation in either animal.

Amplitude measures may have been insufficient to reveal complex changes in response morphology. Other researchers have been able to

demonstrate post-tetanic potentiation of both the EPSP and the antidromic response (Clare et al., 1961) in the cat. But it was the negative component of the waveform was more strongly enhanced than the positive component, and they suggest that it was an increase in the area more than the amplitude. The latency to peak of the negative component was also noted to be delayed. Our maximum (positive) amplitude measures undoubtedly would not be sufficient to reveal these types of response modifications. The application of slope and latency measures may have yielded more conclusive data on the post-LTP responses. Also, it would certainly be advantageous to attempt to examine later components (past 50 msec) of the evoked response in future experiments to more fully characterize the complex nature of neocortical responses. A careful examination of the plots of our waveforms, however, failed to reveal any clear changes in morphology.

The depression that was observed after potentiation trains may have been a real effect if one considers negative changes as a means of information storage. Rosenzweig and associates (1972) postulate that negative structural changes, in contrast to positive events, such as growth of new or more effective synapses, should be given more thought as a valid method for encoding information. There is also little reason to believe that negative functional alterations could not be a effective mnemonic process. There are, however, little data to support such a contention.

The fact that the increase in cortical thickness was not significant is not particularly surprising, since both the sample size and the number of measures per animal were low. Perhaps if the animals were sacrificed immediately after being removed from the environments, instead of 2 weeks

later after the intervening LTP experiment, the results may have reached significance.

Finally, it is possible that this particular cortical system is not plastic, at least in the adult animal. It is unknown whether the callosal-neocortical system of the awake and unrestrained rat behaves in a manner similar to the anaesthetized or slice preparation, but this procedural difference may account for the lack of potentiation effects with either high or low frequencies of stimulation. There may be some unique or complex property of the cortex that prevents these types of externally mediated effects while the tissue is fully operational. However, more study is required before such conclusions can be made.

General Discussion

The complexity of mammalian neocortex has prevented a clear understanding of its functions for centuries. Eccles (1981) describes the structure as composed of over 3 million repeating functional units, or modules, and that it has the capacity to store immense numbers of spatio-temporal patterns. The precise role of the neocortex in memory or in the processing of information, however, has never been identified. The concept of the 'engram' has been the leading guide to research into the mechanisms of learning and memory, with much of the attention focused on the cortex. The application of a model system, long-term potentiation, has been one of the most useful approaches to the problem and has only been recently applied to cortical phenomena (Teyler et al., 1989). The induction, expression, and duration of LTP seem to mimic many aspects of learning, and it is reasonable to speculate that LTP-like processes may be at work in the neocortex.

The 19th century doctrine that exercise may increase the size of the brain was applied to the study of memory by application of a convenient model (Bennett et al., 1964; Rosenzweig, 1966). This model, the environmental enrichment paradigm, became more widely studied when it was learned that the structural changes that it produced were not limited to the cortex (Walsh et al., 1969). Even greater interest was stimulated when it was demonstrated that an LTP-like enhancement of hippocampal synaptic transmission was associated with enriched experience (Sharp et al., 1985; 1987). The present study addressed whether similar effects could be produced in a simple, monosynaptic cortical preparation.

No environmental influences on evoked responses were observed in the corpus callosum-somatosensory neocortex of awake and freely-moving adult rats. Maximum evoked response amplitudes were unaltered after 2 weeks of exposure to an enriching environment, remaining stable in morphology and absolute size over at least 44 days. Subsequent to this, another study was undertaken to induce electrical LTP in the same animals, but it also failed to produce an enhanced response. A slight, but significant, depression occurred three days after exposure to the potentiation trains, although this effect may not have been due to the manipulation itself, since the responses of the control and experimental groups were found to be drifting in opposite directions on that day. It is unlikely that the choice of stimulation parameters had a significant influence on the results, as a brief pilot experiment applying a much lower frequency of stimulation suggested a similar depression of the callosal evoked response. Cortical depth measures indicated a trend towards previously reported increases in thickness that were associated with enrichment, although the differences did not reach significance in this study. The cortical depth data were interpreted as being highly influenced by the low sample size and to the fact that the histology was not completed immediately after the end of the environmental phase of the experiment. It is not known if the changes in cortical thickness are permanent or whether they can be eliminated or influenced by other variables. Also, the use of depth measurement as an indication of structural change throughout the cortex has been questioned, as there are significant regional differences in cortical depth (Bennett et al., 1964). Other factors, such as heredity or the choice of cortical test site, may have significantly biased the results. Henderson (1970) found

that brain weight differences in mice reared in differential environments was accentuated in hybrids as compared with highly inbred strains.

The neocortex as a whole is very important for the processing and recording of environmental information. Theoretically, the cortex should also have a relatively high degree of functional plasticity in order to fully accomplish this task, and should undoubtedly be affected in some way by the increased processing demand imposed by environmental enrichment procedures. As LTP is used as a model phenomenon for the mnemonic functions of the brain, it might be expected to be supported in the cortex. If LTP is to continue to be used further in this regard, it should at least demonstrate a rough parametric resemblance to memory in preparations that unequivocally display the encoding and storage of information, such as in the enriched environment paradigm. This, of course, is based on the assumption that LTP itself may be involved as a mechanism for the storage of information in the neocortex. Since both potentiated evoked responses and increases in thickness were noted following environmental enrichment in the hippocampus, it was thought that an analogous potentiation of electrically evoked synaptic responses might accompany the observed increase in thickness of the neocortex. No effects on evoked neocortical responses were found to be associated with enriched experience. Furthermore, since the application of trains of electrical pulses results in a change in synaptic response in the hippocampus but were not observed in a callosal-neocortical system, LTP again fails to live up to theoretical expectations.

The present experiments suggest that potentiation-like phenomena are not among the mechanisms involved in information storage in the neocortex, and

in turn, these data raise serious doubts about the validity of LTP as a model of memory. If LTP is to model mnemonic functions, at minimum it should be present, if not prevalent, in the structures most commonly associated with the processing of information. On the other hand, LTP may be involved in the encoding of information, but in a manner quite different from our original expectations. Synaptic potentiation may not be the mechanism through which an animal references environmental events and information, at least in the neocortex. Perhaps the environmentally produced synaptic responses in the hippocampus were recording some other specific aspect the animal's environment, possibly a spatial component. Information about the composition of the environment may still be recorded in the neocortex, possibly through a process that does not resemble LTP but which causes a structural ramification in a manner similar to that which occurred in the hippocampus. Since there is no reason to assume that the processes in the hippocampus are precisely the same as those in the neocortex for both the structural and functional changes observed after environmental enrichment, the cortex could very well support information storage.

More study of other cortical areas is warranted with the enriched environment paradigm to determine if the failure to show alterations in evoked responses is consistent throughout the cortex or whether it is a property unique to the sensory neocortex. It is possible that some of the areas of the cortex are 'hard-wired' and are not malleable to environmental experience. It is reasonable to expect this to be the case in some regions, especially in sensory association cortex, if consistent processing of environmental information is desired. The somatosensory area of the fronto-parietal

neocortex may unfortunately be one of those systems that does not show plastic changes readily, affording the animal a reliable and relatively stable representation of its sensory environment. Nevertheless, as a result of the present experiments, LTP becomes less likely as a candidate for the preeminent neocortical mechanism of learning, but it does, however, still have support as one of the many processes that may be involved in the encoding of information in the brain.

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