ENHANCEMENT OF ENTORHINAL-DENTATE EVOKED POTENTIALS FOLLOWING REPEATED ELECTROCONVULSIVE SHOCK SEIZURES IN THE RAT: NEUROPROTECTION STUDIES

by

Zoltan Gombos

A thesis submitted in conformity with the requirements for the degree of Master of Science
Graduate Department of Pharmacology
University of Toronto

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ABSTRACT

Burnham and colleagues have recently shown that repeated ECS seizures cause a long-lasting and perhaps permanent enhancement in entorhinal-dentate evoked potentials (EPs) in rats. These studies, however, involved "unmodified" ECS, whereas, in clinical practice, ECT is now given in its "modified" form. Experiment 1, therefore, repeated the study of Burnham et al. (1995) using "modified" ECS. Despite the use of the "modified" procedure, a significant and long-lasting (up to 3 months) enhancement in population spike amplitude was seen in the ECS group.

Stewart and Reid (1994) have reported that ketamine protects against EP enhancement following "unmodified" ECS. Experiment 2 was designed to confirm and extend the findings of Stewart and Reid (1994). Despite the use of ketamine, a significant enhancement in population spike amplitude was seen in the ECS groups. The "therapeutic" effect of ECS, however, as indexed by the Porsolt test, was diminished in animals receiving either ketamine or PB.
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LIST OF ABBREVIATIONS

AD     afterdischarge
AMPA   \( \alpha \)-amino-3-hydroxy-5-methyl-4-isoxazolepropionate
cm     centimetre
ECS    electroconvulsive shock
ECT    electroconvulsive therapy
EEG    electroencephalogram
EP     evoked potential
EPSP   excitatory postsynaptic potential
g     gram
hr     hour
Hz     Hertz
I/O    input/output
i.p.   intraperitoneal
i.v.   intravenous
kg     kilogram
LTP    long-term potentiation
mA     milliAmpere
\( \mu \)a  microAmpere
mg     milligram
min    minute
ml     millilitre
mm     millimetre
\( \mu \)m  micrometre
ms     millisecond
$\mu s$  microsecond

NMDA  N-methyl-D-aspartate

PB  pentobarbital

s  second

s.c.  subcutaneous

SEM  standard error of mean

v  volume

V  Volts
1. INTRODUCTION

1.1. DEPRESSION

1.1.1. DEFINITIONS/SYMPTOMS

Depression is defined as "a psychiatric syndrome consisting of dejected mood, psychomotor retardation, insomnia and weight loss, sometimes associated with guilt feelings and somatic preoccupations, often of delusional proportions" (Friel, 1974). Additional symptoms include tearfulness, loss of interest and motivation, lack of energy, poor concentration, and impaired memory. About 11% of depressed individuals commit suicide (Winokur and Tsuang, 1975).

Depressive illness may be characterized as "mild", "moderate" or "severe."

1.1.2. ETIOLOGY

The underlying causes of depression are unknown. The etiology usually involves an interaction between biological and psychosocial factors. In major ("endogenous") depression, biological factors appear prominent (Sylvälähti, 1994). In less severe ("reactive") depression, psychosocial factors, such as interpersonal loss or financial problems, play a major role (for a recent review, see Judd and Norman, 1990).
"Secondary" depression occurs in patients suffering from mental illnesses, such as schizophrenia, dementia, or anxiety (Judd and Norman, 1990). Other illnesses and certain drugs may also cause secondary depression.

To establish that a patient suffers from "primary" depression, a clinician must ensure that the condition is not due to (1) normal response to stress, (2) an underlying psychological illness, or (3) drugs or a physical illness (Judd and Norman, 1990). When patients are suffering from "secondary" depression, the root cause should be treated rather than the depressive state.

1.1.3. EPIDEMIOLOGY

The lifetime prevalence of clinical depression is 8.6% (Spaner et al., 1994). The mean age of onset is 25 years (Spaner et al., 1994). About twice as many women as men are affected. The occurrence of depression is related not only to gender and age, but also to residence, social class, and marital status (Lehtinen and Joukamaa, 1994).

1.1.4. THERAPY

Patients suffering from mild to moderate depression are usually treated with antidepressive drugs. About 30% of these patients, however, fail to respond to drug therapy (Dinan, 1995). These individuals are then usually treated with electroconvulsive
therapy.

In severely depressed individuals, ECT is considered the treatment of choice, especially when there is a significant risk of suicide (APA Task Force, 1990).

1.2. ELECTROCONVULSIVE THERAPY

1.2.1. DEFINITION

Electroconvulsive therapy (ECT) involves the use of electrical current to induce a series of generalized convulsive seizures (Abrams, 1992).

1.2.2. INDICATIONS

Convulsive therapy was originally used in the treatment of schizophrenic patients (von Meduna, 1937; von Meduna 1938). Verstraeten (1937), however, soon reported its usefulness in the treatment of "manic depression," and, in recent years, it has been used primarily in the treatment of major depression or depression that fails to respond to antidepressant drugs (e.g., Abrams, 1992). Between 3-5% of psychiatric patients in Europe and North America receive ECT (Kendell, 1981).
1.2.3. EFFICACY

ECT is effective in about 80-90% of patients with major depression (Weiner and Coffey, 1991a). Recovery occurs in a few days, as opposed to several weeks for drug therapy (Abrams, 1992).

1.2.4. MECHANISM OF ACTION

The mechanism of action of ECT is currently unknown. Many hypotheses have been advanced, often involving changes in monoaminergic transmission. For reviews, see: Deakin et al., 1981; Sackeim et al., 1983; Sackeim et al., 1986; Pearlman, 1991.

It is known, however, that seizures are essential to the treatment process (Ulett et al., 1956; Ulett and Johnson, 1957; Ottoson, 1960). According to Fink (1979), it is the seizure that is the therapeutic factor; the stimulus only acts as a trigger to elicit the convulsion.

1.2.5. "UNMODIFIED" ECT - THE HISTORICAL PROCEDURE

Convulsive therapy was originally administered to conscious, unanaesthetized patients. This is now called "unmodified" convulsive therapy. Originally, chemical convulsants, such as camphor and pentylenetetrazole (Metrazol®), were used to trigger the seizures (von Meduna, 1937; von Meduna, 1938). Later, Cerletti and Bini (1938) modified the procedure by using electric current
instead of chemical convulsants. With the electrical stimulus, the onset and offset of the seizures are more rapid, and easier to control (Abrams, 1992).

Even with the electrical stimulus, however, there were significant problems with "unmodified" ECT (Abrams, 1992):

1. Breaks and dislocations of various bones were major complications in the era of "unmodified" ECT. Fractures and dislocations (mainly jaw) were seen in up to 30% of patients (Kendell, 1981).

2. High-threshold patients, who failed to seize, found the procedure extremely unpleasant. Kalinowsky and Hoch (1946) argue that most patients undergoing "unmodified" ECT showed a fear of the procedure.

3. Apnea occurs during the convulsions, and it was feared it might lead to brain damage.

4. The use of high-intensity sine-wave currents led to a high level of memory loss.

These problems led to the gradual evolution of the modern procedure of "modified" ECT.
1.2.6. "MODIFIED" ECT - THE MODERN PROCEDURE

Modern "modified" ECT is administered under anaesthesia, with an anticholinergic, muscle relaxant and oxygenation. Low-intensity square-wave current is used to trigger the seizures (Swartz, 1993). This procedure is believed to eliminate most of the negative effects associated with "unmodified" ECT (Abrams, 1992):

1. The use of a muscle relaxant prevents breaks and dislocations of the bones. Initially, curare was used (Bennett, 1940; Bennett, 1941), but currently, succinylcholine, a short-acting neuromuscular blocker is the drug of choice (Avramov et al., 1995).

2. The use of a short-acting barbiturate, such as methohexital, achieves a brief period of unconsciousness. This avoids the recall of muscle paralysis and stimulation in case of a missed seizure (Selvin, 1987). It eliminates much of the fear associated with ECT.

3. The use of ventilation and oxygen throughout the procedure prevents hypoxia and any risk of brain damage (Haines, 1944). Many practitioners also use an anticholinergic premedication, such as atropine (Kramer, 1993), which decreases salivation and the risk of aspiration.
4. The use of a square-wave, brief-pulse stimulus, pioneered by Liberson (1944), decreases the cognitive side effects of ECT (Weiner et al., 1986a; Weiner et al., 1986b). The square-wave current is as effective as sine wave current in treating depression, and it requires less energy to elicit the seizures (Liberson, 1948; Goldman, 1949; Harris-Brandts and Martin, 1983). Currently, intensity is often set at "average threshold plus 20%" (Abrams, 1992; see below).

Thus, the modern, "modified" procedure is believed to eliminate all of the discomfort and other side-effects associated with "unmodified" ECT.

1.2.7. TECHNICAL PARAMETERS - STIMULUS INTENSITY

There has been much discussion of stimulus intensity in ECT (Abrams, 1992). A moderately suprathreshold stimulus is significantly more effective than a near-threshold stimulus (Bean et al., 1991; Sackeim et al., 1993). It is the extent to which the stimulus exceeds seizure threshold that is important and not the absolute stimulus intensity itself (Sackeim et al., 1987a). With near-threshold stimulation, mean energy is usually about 13-29 Joules (Sackeim, 1987a; Sackeim et al., 1987b; Sackeim, et al., 1987c). With suprathreshold stimulation, values can be as high as 63 Joules (Bean et al., 1991).
Unfortunately, suprathreshold stimuli also cause more side effects (Ottoson, 1960). In particular, more memory impairment is seen (Sackeim et al., 1987b). Currently, "average threshold plus 20%" is frequently used (Abrams, 1992).

1.2.8. TECHNICAL PARAMETERS - ELECTRODE PLACEMENT

Electrode placements for ECT may be unilateral (both electrodes on one side of the head) or bilateral (one electrode on each side of the head). In either case, they are often placed over the temporal region of the brain (Daniel and Crovitz, 1983). Unilateral electrode placements are associated with more missed seizures than bilateral placements (d'Elia and Raotma, 1975; Gregory et al., 1985; Pettinati and Nilsen, 1985), and - although right unilateral placements cause less memory loss - they may also be less effective therapeutically (Sackeim et al., 1987a). At the Toronto Hospital, bilateral placements are currently in use (Carmichael, personal communication).

1.2.9. TECHNICAL PARAMETERS - SEIZURE DURATION

The goal of ECT is to induce a generalized tonic-clonic seizure lasting 20-30 s (Sackeim et al., 1987b; APA Task Force, 1990; Sackeim and Devanand, 1991; Sackeim et al., 1991). A seizure lasting less than 20 s does not provide therapeutic benefit to the patient (Maletzky, 1978; d'Elia et al., 1983).
Usually, the electrographic seizure continues after the conclusion of the motor seizure (Weiner and Krystal, 1994). Therefore, electroencephalographic (EEG) monitoring is essential to observe the true duration of the seizure. EEG monitoring is routinely done in modern ECT (Abrams, 1992).

1.2.10. TECHNICAL PARAMETERS - NUMBER OF TRIALS

For an effective treatment, a minimum of 4 ECT trials is necessary. Most clinicians administer 5-10 trials in total, with 8 treatments as the average (Galletly et al., 1991). Seizures are generally administered 3/week, since more frequent administration is associated with pronounced side-effects (e.g., Weiner and Krystal, 1994).

While initial therapy may involve only 8 trials, some authorities argue that, to avoid relapse, "maintenance" ECT is necessary (Avramov et al., 1995). Maintenance ECT is given first at weekly intervals and later at monthly intervals (Avramov et al., 1995). Patients receiving maintenance ECT may receive far more than 8 ECTs. Some patients have been documented as having as many as 2400 ECTs during the course of their therapy (Kramer, 1990).
1.2.11. RISKS OF ECT - CARDIOVASCULAR MORBIDITY

From the beginning, there have been questions about the safety of ECT (Abrams, 1992). These have mainly centred around 3 topics: (1) cardiovascular morbidity, (2) structural brain changes, and (3) memory problems/confusion.

Today, the primary risk of ECT is considered to be cardiovascular morbidity (Fink, 1979; Husum et al., 1983). Arrhythmias and changes in arterial blood pressure during ECT can lead to myocardial infarction, congestive heart failure, cardiac arrest and other problems. This is thought to occur because of mixed sympathetic and parasympathetic activation during ECT (Weinger et al., 1991). These autonomic changes lead to the release of circulating catecholamines from central and peripheral stores (Jones and Knight, 1981; Foster and Ries, 1988). Gravenstein et al. (1965) showed a 3-15 fold increase in noradrenaline and adrenaline serum levels following ECT.

The cause of these abnormalities is thought to be the current passed and not the subsequent convulsion (Partridge et al., 1991). According to Abrams (1992), cardiovascular mortality among patients receiving ECT is about 0.03%.
1.2.12. RISKS OF ECT - STRUCTURAL BRAIN CHANGES

In the early literature, there were several reports of structural brain abnormalities following ECT. These studies were done in schizophrenic patients (e.g., Weinberger et al., 1979) and elderly depressed patients (Calloway et al., 1981).

More recently, investigators have criticized these early studies for their "unscientific" testing methods (Coffey et al., 1991). Later investigators have found no evidence of structural brain damage (Weiner, 1984; Coffey et al., 1988; Coffey et al., 1991). It is now generally accepted that there are no irreversible metabolic or gross structural brain changes following ECT (Weiner, 1984; Coffey et al., 1991).

1.2.13. RISKS OF ECT - MEMORY DISTURBANCES/CONFUSION

There is clear evidence of both retro- and anterograde amnesia in the period immediately after ECT (Daniel and Crovitz, 1983; Calev et al., 1991). Ottoson (1962) conducted the first experiments in this area. He concluded that it was the current rather than the seizure that caused amnesia.

Most authorities agree that the amnesic effects of ECT disappear with time. Psychological tests conducted 6 months after ECT, show no measurable memory or learning deficits (Squire and Chace, 1975) - except for the period immediately preceding the ECT stimulus (Squire et al., 1981). Even with large numbers of ECT
trials (>100), patients have not been shown to suffer any measurable memory impairments 6 months after ECT (Squire et al., 1979; Devanand et al., 1991).

It is worth noting, however, that patients sometimes complain of subjective feelings of memory dysfunction after ECT (Squire et al., 1979; Squire and Slater, 1983). These complaints go on for at least 10 years (Weeks et al., 1980). Thus, although there is no objective evidence of long-term memory impairment following ECT, patients feel that their memories have been impaired.

Another problem described immediately after ECT is confusion. Patients are disoriented, and may become agitated and require restraint (Abrams, 1992). This problem appears to be progressive in nature. Patients undergoing repeated ECT require more time to regain consciousness and suffer increased confusion, which occasionally progresses to delirium (Fink, 1993; Fredman et al., 1994).

1.2.14. RISKS OF ECT - "KINDLING"

"Kindling" is an animal model of epilepsy, in which repeated seizures lead to long-lasting changes in brain and behaviour (Goddard et al., 1969; Racine, 1972a; Racine, 1972b). These changes occur in the absence of structural brain damage. Eventually, after repeated stimulation, kindled seizures become spontaneous (Pinel, 1981).
There have been suggestions that ECT may have "kindling-like" effects, resulting in the development of spontaneous seizures (Pacella and Barrera, 1945; Abrams, 1992). However, this question has been hotly debated. Proponents of ECT argue that ECT acts as an anticonvulsant, since seizure threshold is elevated after ECT trials (Blackwood et al., 1980; Small et al., 1981). This threshold rise is believed to suppress any "kindling-like" phenomenon (Abrams, 1992).

1.3. ELECTROCONVULSIVE SHOCK - THE ANIMAL MODEL OF ECT

Electroconvulsive shock (ECS), the animal analog of ECT, is an experimental model of generalized tonic-clonic seizures. It was developed by Toman et al. (1946), and has been widely used for antiepileptic drug screening (Burley and Ferrendelli, 1984; Löscher and Schmidt, 1988; Löscher et al., 1988).

In ECS, as in ECT, current is passed through the brain to cause a generalized convolution. Corneal electrodes are generally used to administer the stimulus (Browning and Nelson, 1985), although transauricular stimulation through ear-clip electrodes is also used (e.g., Quattrone et al., 1978; Mason and Corcoran, 1979).
1.3.1. "THERAPEUTIC" EFFECTS OF ECS - THE PORSOLT TEST

It is not clear that true depression can exist in lower animals. There are animal preparations, however, which can "model" depression and which show the therapeutic effects of ECS. The best known of these is the Porsolt test (Porsolt et al., 1977).

In Porsolt's "behavioural despair" or "forced swimming" test, rats are forced to swim in an upright cylinder. After an initial period of vigorous movements (attempts to escape), the animal stops moving and floats passively ("immobile posture"), except for occasional activity that enables it to keep its nose above the water. The experimenter measures the duration of the immobile posture. Antidepressant treatments reduce the period of immobility (Porsolt et al., 1977).

The Porsolt test has been widely used in the development of new drugs with antidepressive properties (Baldessarini, 1996). Porsolt and colleagues (1978) have also showed that non-pharmacological treatments, such as ECS, can reduce the period of immobility. For a review of this test and its ability to detect antidepressive properties see Porsolt (1981).

Porsolt argues that the immobility seen in his test suggests a state of despair and a lowered mood (Porsolt et al., 1977). This may or may not be true. The test, nevertheless, remains a good empirical predictor of the effects of antidepressant therapies.
1.3.2. "RISKS" OF ECS - STRUCTURAL BRAIN CHANGES

Devanand et al. (1994) review 22 separate animal studies, and conclude that there is "no evidence of structural brain damage" following "unmodified" ECS. The sole study to employ partially "modified" ECS, also found no evidence of ECS-induced structural brain changes (Brennan et al., 1972). ECS, like ECT, does not appear to induce structural lesions in the brain.

1.3.3. "RISKS" OF ECS - MEMORY CHANGES

ECS, like ECT, causes both anterograde (McGaugh and Madsen, 1964) and retrograde amnesia (Leonard and Zavala, 1964). Although there is much controversy regarding the mechanism and the type of memory affected, there is little doubt that ECS is a powerful amnesic agent (e.g., Shavalia et al., 1981).

1.3.4. "RISKS" OF ECS - KINDLING-LIKE CHANGES

In kindled animals, several long-lasting changes have been reported, including gliosis, sprouting, "aggressive" behaviour, and modified gene expression (Pinel et al., 1977; Dragunow and Robertson, 1987; Geinesmann et al., 1988; Racine et al., 1989).

Burnham and colleagues have recently begun to search for long-lasting changes after repeated ECS, using treatments and tests that
have shown long-lasting changes after kindling. Their work is based on the hypothesis that long-lasting and functionally significant brain changes can occur in the absence of gross structural pathology.

Following repeated ECS seizures, Burnham and colleagues have now found significant changes in brain metabolism, gliosis, sprouting, monaminergic receptor binding, "emotional" behaviour, evoked potentials and gene expression (Nobrega et al., 1993; Burnham et al., 1995; Cottrell et al., in preparation; Gombos et al., in preparation; Mingo et al., in preparation). These findings confirm and extend the findings of several other groups, including monaminergic receptor binding and gliosis (Lerer, 1984; Pazos et al., 1985; Barkai et al., 1990; Orzi et al., 1990; Kragh et al., 1993).

These data suggest that repeated generalized seizures "kindle" the brain just as repeated focal seizures do. Actually, Pinel and others showed "kindling-like" effects of repeated ECS over fifteen years ago (Ramer and Pinel, 1976; Pinel and van Oot, 1976; Sangdee et al., 1982).

1.4. ECS ENHANCEMENT OF ENTORHINAL-DENTATE EVOKED POTENTIALS

The best documented of the long-term effects of ECS is the long-term enhancement seen in entorhinal-dentate evoked potentials (EPs). The present section will discuss: (1) anatomical projections from the entorhinal cortex to the hippocampus; (2)
entorhinal-hippocampal EPs; (3) long-term potentiation, a temporary enhancement of the potentials; (4) the permanent enhancement seen after kindling; and (5) the permanent enhancement seen after ECS.

1.4.1. ANATOMICAL PROJECTIONS FROM THE ENTORHINAL CORTEX TO THE HIPPOCAMPUS

A major input to the hippocampus comes from the entorhinal cortex through the perforant path, which can be divided into dorsomedial and ventrolateral components (McNaughton and Barnes, 1977). Both pathways synapse on the granule cells of the dentate gyrus. The granule cells of the dentate gyrus project to the pyramidal cells of CA3 through the mossy fiber pathway. The pyramidal cells of CA3 synapse on the pyramidal cells of CA1, through the Schaffer collateral (CA1) pathway (Fig. 1-1) and also send projections out to the septal area and hypothalamus (Anderson et al., 1971; Amaral, 1993). Damage to any of these pathways produces some form of memory disturbance (Zola-Morgan et al., 1986).

1.4.2. ENTORHINAL-DENTATE EP'S

Stimulation of the perforant path by electrodes placed in the entorhinal cortex results in the monosynaptic activation of dentate granule cells (Andersen et al., 1966; Lømo, 1971a; Lømo, 1971b). This is seen in gross-electrode recordings as the population
excitatory postsynaptic potential (EPSP). With the stimulation of sufficient number of perforant-path fibers, the granule cells discharge synchronously. This results in the appearance of a "population spike" superimposed on the population EPSP.
FIGURE 1-1. Diagram of the 3 major pathways in the hippocampus. The perforant path from the entorhinal cortex synapses on the granule cells of the dentate gyrus. These granule cells project to the CA3 region through the mossy fiber pathway. The pyramidal cells of CA3 synapse on the pyramidal cells of CA1.
Schaffer collateral pathway

CA1

CA3

Dentate region

Mossy fiber pathway

Perforant pathway
The population spike is partially or completely inhibited if the fibers are restimulated within 100 ms (McNaughton and Barnes, 1977). The spike can also be potentiated by LTP, kindling or repeated ECS seizures.

1.4.3. LONG-TERM POTENTIATION

In 1973, Bliss and Lømo showed that hippocampal neurons have plastic capabilities, and suggested that this could relate to memory storage. Tetanic stimulation of any of the hippocampal pathways produces a transient increase in synaptic strength of the pathway (Bliss and Lømo, 1973; Alger and Teyler, 1976; Schwartzkroin and Wester, 1975). This increase - termed "long-term potentiation" (LTP) - lasts for hours in anaesthetized animals and for days or weeks in freely moving animals (Bliss and Lømo, 1973; Bliss and Gardner-Medwin, 1973). LTP is believed to play a possible role in learning and memory (Bliss and Lømo, 1973).

LTP appears to depend on glutamatergic neurotransmission. It has been shown, ex vivo, that all of the major hippocampal pathways use glutamate as their neurotransmitter (e.g., Nicoll and Malenka, 1995). Glutamate, an amino acid, has a potent neuroexcitatory property when applied to neurons (Curtis et al., 1960; Curtis and Watkins, 1960; Curtis and Watkins, 1963), including hippocampal neurons (Biscoe and Straughan, 1966; Steiner, 1969; Dudar, 1974). There is controversy about the pre- and post-synaptic nature of LTP. Many believe, however, that in the perforant path, LTP is
initiated by the postsynaptic cell (Malenka, et al., 1988; Malenka and Nicoll, 1993), however, there is much controversy regarding this issue. Nevertheless, it is accepted that the influx of calcium ions (Ca\(^{2+}\)) is necessary for LTP induction (Lynch et al., 1983). It is believed that this influx of Ca\(^{2+}\) is mediated by the glutamatergic N-methyl-D-aspartate (NMDA) channel (Bliss and Collingridge, 1993). LTP is blocked by NMDA receptor antagonists (e.g., Morris et al., 1986) or by a mutation in one of the NMDA receptor subunits (e.g., Sakimura et al., 1995).

1.4.4. PERMANENT ENHANCEMENT SEEN AFTER KINDLING

Racine and colleagues have reported a long-lasting enhancement of EPs in several neural pathways, including the entorhinal-dentate pathway. In the perforant path, both population spike amplitude and population EPSP slope are elevated following kindled seizures. Although the enhancement of the population EPSP slope was potentiated throughout their tests (10 EP tests during the course of 10 kindling stimulations), the population spike amplitude was elevated only until the eighth stimulation, which was followed by a decline to near pre-kindling levels. The reasons for this decline is not clearly understood (Racine et al., 1973).
1.4.5. PERMANENT ENHANCEMENT SEEN AFTER ECS

In 1993, Stewart and Reid reported another sort of long-lasting enhancement in entorhinal-dentate EPs. This occurred following repeated "unmodified" ECS seizures. Later, they showed that this enhancement reaches a maximum after 5 convulsions (Stewart et al., 1994).

Burnham et al. (1995) subsequently confirmed the findings of Stewart and Reid, and showed that enhancement lasts for at least 3 months following the last ECS seizure. They hypothesized that the change might be permanent in nature.

Stewart and Reid (1993) also investigated the effects of permanent (ECS) potentiation on LTP. They found that LTP-induction in the perforant path was significantly lessened after repeated ECS. Stewart and Reid (1993) hypothesize that ECS maximally potentiates the pathway, so that it is already "saturated" at the time of LTP testing. They believe that a similar phenomenon may produce the memory impairment seen in psychiatric patients undergoing ECT. If the hippocampal pathways are "saturated" by ECT, LTP will not take place and memories will not be formed (Stewart et al., 1994).
1.4.6. KETAMINE ANTAGONISM OF PERMANENT POTENTIATION

As mentioned above, NMDA antagonists block LTP. Recently, Stewart and Reid (1994) have reported that ketamine, a non-competitive NMDA antagonist, prevents the ECS-induced enhancement of EPs.

Ketamine, a dissociative anaesthetic, is believed to act by binding within the ionophore portion of the NMDA receptor-channel complex (MacDonald et al., 1987; MacDonald et al., 1991). Since ketamine has a high affinity for the NMDA receptor, it antagonizes NMDA-mediated responses at low concentrations (ffrench-Mullen and Rogawski, 1989).

Stewart and Reid (1994) preadministered ketamine to subjects before each ECS trial. They reported that it completely antagonized the ECS-induced enhancement of EPs. They also found that seizure length was reduced in animals receiving ketamine. They do not believe, however, that the reduction in seizure length was related to the blockade of enhancement, or that it was sufficient to change the "therapeutic" effects of ECS (Stewart and Reid, 1994).
1.5. OBJECTIVES

The objectives of the present work were: (1) to investigate whether the use of "modified" ECS would eliminate the ECS-induced enhancement of entorhinal-dentate EPs; and (2) to confirm the finding of Stewart and Reid (1994) that pre-treatment with ketamine eliminates the ECS-induced enhancement of entorhinal-dentate EPs.

These goals were pursued in the following experiments:

1. In Experiment 1, a new model was developed - "modified" ECS - which closely parallels modern "modified" ECT. In "modified" ECS animals are anaesthetized, given an anticholinergic and a muscle relaxant, oxygenated, and stimulated using low-doses of square-wave current. The effect of "modified" ECS was investigated on the entorhinal-dentate EPs.

2. In Experiment 2, "unmodified" ECS was used to elicit seizures in animals pre-injected with saline, ketamine, or PB. PB was used to shorten seizure duration without blocking the NMDA channel.

The long-term goal of these experiments was to see whether the negative effects of ECT could be eliminated without losing its therapeutic effects.
2. EXPERIMENT 1

Long-Term Enhancement of Entorhinal-Dentate EPs Following "Modified" ECS in the Rat

2.1. RATIONALE

Studies by Stewart et al. (1994) have shown that repeated ECS seizures significantly enhance entorhinal-dentate EPs in the rat. Burnham et al. (1995) have confirmed this finding and have shown that the changes are long-lasting (>3 months) and perhaps permanent. These studies, however, have all involved "unmodified" ECS, whereas in modern clinical practice ECT is usually given in its "modified" form. Experiment 1, therefore, was designed to determine whether enhancement in entorhinal-dentate EPs would be found following "modified" ECS.

2.2. METHODS

2.2.1. SUBJECTS

Male, Long-Evans rats (275-300 g; Charles River, St. Constant, PQ) were individually housed in hanging cages in a temperature-controlled vivarium (21 °C) with a 12-hr light-dark cycle (lights on at 8:00 a.m.). Ad lib food and water were available at all times.
2.2.2. IMPLANTATION OF ELECTRODES

One week (minimum) following arrival from the breeding farm, bipolar electrodes were implanted in the left fascia dentata and entorhinal cortex of each subject, using PB anaesthesia (Somnolut\textsuperscript{R}, 65 mg/kg, i.p.; MTC Pharmaceuticals, Cambridge, ON) and standard stereotaxic techniques (Skinner, 1971). Electrodes were constructed from 2 teflon-coated, stainless steel wires (0.011 in.; A-M Systems Inc., Everett, WA) which were twisted together. One end of each wire was attached to a male Amphenol\textsuperscript{R} insert (Electrosonic, Willowdale, ON).

Electrodes were aimed to the perforant path. Coordinates for implantation were derived from the atlas of Paxinos and Watson (1986), which sets the incisor bar at 3 mm below the interaural midline. Electrodes were implanted under physiological control in the sites that gave the largest EPs. For the fascia dentata, the coordinates were as follows: 3.5 mm posterior to bregma, 2.2 mm lateral to midline, and 4-5 mm down from the skull surface. For the entorhinal cortex, the coordinates were: 7.5 mm posterior to bregma, 4.1 mm lateral to the midline, and 3.5-5 mm down from the skull surface. Three screws (3.2 mm stainless steel; Plastics One, Roanoke, VA) were set into the skull to anchor the headcap. These were also used for EEG recording from the cortex. Two of the screws were implanted 3.0 mm anterior to bregma and 2.0 mm lateral on each side of the midline. The third was implanted on the midline, 2.0 mm posterior to lambda. Each screw was soldered to
the end of a teflon-coated stainless steel wire. The other end of the wire was attached to a male Amphenol® pin. Following implantation of the skull screws, the EP and EEG electrodes were cemented in place using cranioplastic powder (Plastics One, Roanoke, VA), liquified by repair material liquid (Dentsply Trubyte, York, PA). The Amphenol® pins were directed into an ABS plug (Carleton University, Ottawa, ON), which was fixed on the animal's head using the cranioplastic cement. The skin was sutured closed around the headcap using surgical thread (Dexon II 4-0; Davis+Geck, Wayne, NJ).

2.2.3. FEMORAL CANNULATION

Ten days (minimum) after the implantation of electrodes, a permanent indwelling cannula (below) was inserted in the femoral vein of each subject to simplify subsequent drug administration. Under halothane anaesthesia (Halocarbon Laboratories, River Edge, NJ), a 2-cm incision was made in the right femoral region, and the right femoral vein was isolated using blunt dissection techniques. Two pieces of surgical thread were placed underneath the vein. The vein was then ligated by knotting the caudal thread. An incision was made rostral to the ligation and the Silastic® portion of the cannula (below) was inserted to a length of 2 cm and tied in place with the second surgical thread. Following cannulation of the femoral vein, a midline incision (2 cm) was made in the dorsal aspect of the animal’s neck. A subcutaneous tunnel from the
femoral vein to the dorsum of the neck was made with a blunt needle driver, and the free end of the cannula was pulled through it. The injection port was then sutured to the neck, allowing a 1 cm piece to protrude outward.

The cannulas were manufactured out of 5 cm lengths of polyethylene tubing (PE-50; Clay-Adams, Parsippany, NJ). The end of the tubing that was to be inserted into the lumen of the vein was tipped with 1 cm of Silastic tubing (0.025" ID, 0.047" OD; Dow Corning, Midland, MI) - which is soft and flexible - to prevent luminal damage. Chloroform (Fisher Scientific Co., Fair Lawn, NJ) was used to ensure adhesion of the component parts. The other end of the polyethylene tubing was affixed to 1 cm of white heat-shrink tubing to form the injection port. A 2-cm piece of polyethylene tubing (PE-205; Clay-Adams, Parsippany, NJ), occluded at one end, formed the safety cap for the injection port (Fig. 2-1).

2.2.4. PROCEDURE FOR EP MEASUREMENTS

Two days (minimum) after cannulation, entorhinal-dentate EPs were measured. The test stimulus consisted of a biphasic pair (positive and negative going) of 100 μs rectangular pulses, separated by an interval of 100 μs. The stimulus, which was provided by a Grass S-88 stimulator (Grass Instruments, Quincy, MA), was applied once every 10 s. Ten responses were evoked and averaged at each of 16 different intensities - using a program written in Asyst - to allow determination of an input/output (I/O)
function. Intensities ranged from 30 to 3200 μA (peak-to-peak).
FIGURE 2-1. Schematic representation of the cannula. The cannulas were manufactured out of 5 cm lengths of polyethylene tubing. The end of the tubing that was to be inserted into the lumen of the vein was tipped with 1 cm of Silastic\textsuperscript{R} tubing. The other end of the polyethylene tubing was affixed to 1 cm of white heat-shrink tubing to form the injection port. A 2-cm piece of polyethylene tubing, occluded at one end, formed the safety cap for the injection port.
Safety cap

Heat-shrink tubing (protruding from animal)

Polyethylene tubing

Silastic® tubing (inserted into vein)
2.2.5. PROCEDURE FOR "MODIFIED" ECS

"Modified" ECS or sham "modified" ECS trials were initiated 2 days (minimum) after the first EP test. The "Modified" ECS Group received 8 ECS trials, administered on a 48-hr schedule (3/week). This schedule matches the number and time course of ECT trials commonly given in clinical practice (Abrams, 1992). The "Modified" Control Group received 8 "sham ECS" trials, during which drugs (below) and handling were administered, but no current was passed through the electrodes applied to the head.

"Modified" ECS was administered using the following procedure: Fifteen minutes before the administration of the ECS stimulus, animals were injected s.c. with atropine (0.05 mg/kg; Ormond Veterinary Supply, Ancaster, ON). Thirteen minutes later (2 min before the stimulus), sodium methohexital (Brietal® Sodium, 10 mg/kg; Eli Lilly, Toronto, ON) was injected via the cannula. After the animal was anaesthetized (i.e., had lost its righting reflex), succinylcholine chloride (Quelicin® Chloride, 0.15 mg/kg; Abbott Laboratories, Mississauga, ON) was injected via the cannula. The cannula was flushed with normal saline. (These doses and procedures had been worked out in a set of preliminary experiments that are described in Appendix 1. All injections were given in a volume of 1 ml/kg). The region between the ears and the eyes was then cleaned with 95% ethanol. The animal was gently restrained with a purpose-made Spandex cover to prevent movement during the administration of the stimulus. Oxygenation (100% oxygen; Boc
Gases, Mississauga, ON) was initiated by placing the rat's nose and mouth into a 10-ml syringe from which the plunger had been removed.

ECS was administered within 1 min after oxygenation was started. Electrodes were placed on both sides of the animal's head at the midpoint between the external auditory meatus and the eye's outer canthus. This corresponds to the "temporal" region of the human head. The ECS stimulus was then administered. The "modified" ECS stimulus consisted of a 1 s train of biphasic equidistant 1 ms rectangular pulses (Fig. 2-2). Pulses were delivered by a Grass S-88 stimulator, at a rate of 70 Hz, through 9 mm cup-shaped gold-plated electrodes (Grass Instruments, Quincy, MA) to which conductive gel (Redux Gel; Medical Products Group, Waltham, MA) had been applied. Stimulus intensity was set at "average threshold plus 20%," average threshold having been determined in a separate group of animals (Appendix 5-2). "Threshold plus 20%" was 85 V.

During the seizures, EEGs were recorded using a Grass Model 6 Electroencephalograph (Grass Instruments, Quincy, MA). Each stimulation resulted in an electrographic discharge lasting 5 s or more. If a 5+ s AD failed to occur, the stimulation was repeated, at 100 V (base-to-peak), after a 1 min interval. Motor seizures, which were almost completely suppressed by the muscle relaxant, consisted of mild to moderate forelimb tremor (spasmodic muscle contractions) and mild head clonus.
FIGURE 2-2. The square wave pulse pattern used in "modified" ECS. The pattern consisted of a 1 s train of biphasic equidistant 1 ms rectangular pulses, delivered at 70 Hz. Every second pulse was positive going. The distance between positive and negative pulses was 6.1 ms.
2.2.6. PROCEDURE FOR EP RE-MEASUREMENTS

Following "modified" ECS or sham "modified" ECS, EPs were re-measured 24 hrs, 28 days and 3 months after the eighth ECS seizure using procedures identical to those described above (Section 2.2.4.).

2.2.7. HISTOLOGY

Following the completion of all testing, subjects were anaesthetized with PB (100 mg/kg; Somnotol®; MTC Pharmaceuticals, Cambridge, ON) and perfused through the heart with a 0.9% saline solution, followed by a 4% (v/v) formaldehyde solution (in saline; Fisher Scientific Co., Fair Lawn, NJ). The brains were removed and placed in 4% (v/v) formaldehyde solution (in saline) for a minimum of 4 days. Frozen coronal sections (30 μm) were then cut on a Leica JUNG CM3000 cryostat (Nussloch, Germany), and stained with thionine (Skinner, 1971). Placement of electrodes was confirmed via light microscopy. All electrodes were in the expected brain structures.
2.2.8. DATA SCORING AND STATISTICAL ANALYSIS

Population spike amplitude was measured as the vertical distance (in mV) from the peak of the spike to a line tangent to both the spike onset and spike offset. Population EPSP slope was evaluated as the slope of the middle 0.5 ms of the rising phase of the population EPSP (Fig. 2-3). To simplify the statistical analysis, the responses evoked by the 5 highest voltage levels in each I/O test were averaged together, providing a single measure for each test day. Population spike amplitudes and population EPSP slope measures were calculated as a percent increase from the baseline (pretreatment) measures. Differences between the "Modified" ECS Group and the "Modified" Control Group were analyzed using a two-way analysis of variance with test day as the repeated measures factor and group membership (ECS or sham) as the between subjects factor.
FIGURE 2-3. A diagram of an entorhinal-dentate EP. The population spike amplitude (a) and the population EPSP slope (b) are designated.
2.3. RESULTS

2.3.1. EEG OBSERVATIONS DURING "MODIFIED" ECS

Figure 2-4 shows a typical EEG trace from a single animal receiving "modified" ECS. A primary AD lasting for 11 s is seen. It is followed by a secondary AD lasting for 21 s.

Figure 2-5 shows the mean duration of the primary AD, the mean latency of the secondary AD, and the mean duration of the secondary AD for all subjects during each of the 8 ECS seizures. The data reveal a steady increase in the duration of the primary AD (Fig. 2-5A). A one-way repeated measures analysis of variance showed this effect to be significant ($F_{7,87}=2.6069$, $p=0.0189$). The number of subjects displaying a secondary AD varied from 5/11 to 10/11 on different trials (Fig. 2.5). The latency of the secondary AD did not change significantly over time (one-way repeated measures analysis of variance: $F_{7,58}=1.0211$, $p=0.4311$; Fig. 2-5B), nor did its duration (one-way repeated measures analysis of variance: $F_{7,58}=1.6963$, $p=0.1369$; Fig. 2-5A).
FIGURE 2-4.  A typical EEG trace from an animal receiving "modified" ECS.  A primary AD is followed by a secondary AD.
2.3.2. PERFORANT PATH EP'S FOLLOWING "MODIFIED" ECS

Figure 2-6 presents typical I/O data from single subjects in the "Modified" ECS and the "Modified" Control groups. The traces were taken before the first seizure (solid line) and 24 hrs after the last seizure (dotted line). As indicated, "modified" ECS appeared to increase both the amplitude of the population spike and the slope of the population EPSP. In control subjects, no consistent change was seen.

Figure 2-7 presents mean population spike amplitudes (expressed as a percent of baseline) for all ECS and control subjects, before and after "modified" ECS or sham "modified" ECS. The amplitude of the population spike was increased in ECS subjects at all intervals following treatment. A two-way repeated measures analysis of variance on the 24 hr and 28 day data showed a significant effect of treatment \( F_{1,19}=12.5037, p=0.0035 \), but no significant effect of time \( F_{1,19}=0.0775, p=0.7887 \). This suggests that ECS enhanced the amplitude of the population spike, and that the enhancement was stable. The population EPSP slopes did not differ significantly, perhaps due to the large variability of these data. (Data not shown.)

EP enhancement was still observed in the ECS subjects at 3 months, but statistical analysis was not possible, due to a loss of control subjects to sickness and electrode displacement.
FIGURE 2-5. Mean duration of the primary and secondary ADs (A) and the mean latency of the secondary AD (B) during each of the 8 ECS seizures. **A.** There is a steady increase in the duration of the primary AD (filled circles), however, there is no significant change in the duration of the secondary AD (open circles). **B.** The latency of the secondary AD did not change significantly over time.
$2^\circ$ AD LATENCY MEAN±SEM (s)

AD DURATION MEAN±SEM (s)

NUMBER OF SEIZURES

$N=9$

$N=8$

$N=11$

$N=10$

$N=11$

$N=6$

$N=9$

$N=5$

$N=6$

$N=9$

$N=8$

$N=5$

$N=6$

$N=11$

$N=6$

$N=9$

$N=8$

$N=5$

$N=6$

$N=11$

$N=6$

$N=9$

$N=8$
FIGURE 2-6. Representative I/O traces of subjects in the "Modified" ECS and the "Modified" Control groups. The traces were taken before the first seizure (solid line) and 24 hrs after the last seizure (dotted line). "Modified" ECS appeared to increase both the amplitude of the population spike and the slope of the population EPSP. In control subjects, no consistent change was seen.
FIGURE 2-6. Mean population spike amplitude expressed as a percent of baseline, for ECS (△) and control (○) subjects. The amplitude of the population spike was increased in ECS subjects at all time points following treatment.
POPULATION SPIKE HEIGHT
(% change from baseline mean±SEM)
3. EXPERIMENT 2

The Effect of Ketamine on ECS-Induced Brain Changes

3.1. RATIONALE

The results of Experiment 1 indicate that entorhinal-dentate EPs are enhanced following repeated "modified" ECS seizures. The "modified" procedure does not protect against these long-lasting brain changes. Similar changes may be occurring in the brains of psychiatric patients undergoing "modified" ECT.

Since these changes are very long lasting, it seems unlikely that they contribute to the (transitory) therapeutic effects of ECS. Stewart and Reid (1993) have suggested, in fact, that they relate to the unwanted amnesic effects of ECT. If so, it would be desirable to prevent them, if one could so without losing the therapeutic effects of the treatment.

Recently, Stewart and Reid (1994) have reported that ketamine, a dissociative anaesthetic and non-competitive NMDA antagonist, can block the enhancement of entorhinal-dentate EPs caused by repeated "unmodified" ECS. They argue that ketamine exerts this effect without significantly altering the seizures, although there is actually a decrease in the duration of the behavioural seizures (from 18.4 s to 13.9 s).

If Stewart and Reid are correct, ketamine might be substituted for methohexital when ECTs are given, and some of the unwanted effects of ECT might disappear. Ketamine has been used in the past
for ECT administration (Weiner and Coffey, 1991b; Weiner and Krystal, 1994), and Reid is now using it in his own practice (Reid, personal communication).

Experiment 2 was designed to confirm Stewart and Reid's report of the neuroprotective effects of ketamine against EP enhancement. A PB control group was added in the present study to test the possibility that ketamine's effects were due to the shortening of the seizures rather than to its neuroprotective effects. (PB - which is not an NMDA antagonist - was expected to shorten the seizures without providing neuroprotection.) In addition, the Porsolt test - an index of the "therapeutic" effects of ECS - was used to determine whether the "therapeutic" efficacy of the ECS procedure was diminished by ketamine.

3.2. METHODS

3.2.1. SUBJECTS AND ELECTRODE IMPLANTATION

Male Long-Evans rats were implanted with bipolar electrodes as in Experiment 1. The subjects and the procedure for electrode implantation were similar to those described above (Section 2.2.1. and Section 2.2.2.), except that the skull screws were not attached to EEG electrodes. (EEG recording was not done in Experiment 2.)
3.2.2. PROCEDURE FOR EP MEASUREMENTS

Ten days (minimum) after electrode implantation, EPs were measured, using procedures identical to those described above (Section 2.2.4.).

3.2.3. PROCEDURE FOR "UNMODIFIED" ECS

ECS or "sham ECS" trials were initiated 2-5 days after the first EP test. The ECS subjects received 8 "unmodified" ECS trials, administered on a 48-hr schedule (3/week). The number and time course of ECS trials were chosen to match the procedure commonly used in clinical practice (Abrams, 1992). The "Control Group" received "matched handling" trials. Electrodes were applied to the eyes, but no current was passed.

The stimulus for "unmodified" ECS consisted of 0.2 s of 150 mA, 60 Hz sine-wave pulses. This is the stimulus traditionally used to produce "maximal" ECS seizures (Swinyard, 1972). Pulses were delivered by a purpose-made stimulator via corneal electrodes. Each stimulation resulted in a motor seizure (see Section 3.3.1.).
3.2.4. DRUGS AND DRUG ADMINISTRATION

Fifteen minutes before the administration of each ECS or sham ECS trial, subjects received an injection of drug or saline. Animals in the control group were injected i.p. with 1 ml/kg normal saline. Animals in the ECS groups received i.p. injections of: (1) 1 ml/kg normal saline (ECS-SAL); (2) 100 mg/kg ketamine (Vetalar®; Vetrepharm, London, ON) (ECS-KET); or (3) 20 mg/kg of PB (BDH, Toronto, ON) (ECS-PB).

The dose of ketamine that was used matches that used previously by Stewart and Reid (1994). The dose of PB was worked out in preliminary studies involving a different group of animals. It was designed to produce the diminution in behavioural seizures seen in the presence of ketamine. (See Appendix 5-3.)

Ketamine was obtained in pre-mixed injectable form, with a concentration of 100 mg/ml ketamine hydrochloride in saline with negligible amount of preservative. PB was obtained as a powder and dissolved in normal saline. It was mixed fresh daily at a concentration of 20 mg/ml.

3.2.5. PROCEDURE FOR THE PORSOLT TEST

Six days after the eighth ECS seizure or matched handling trial, Porsolt testing was begun. Animals were placed individually in vertical plexiglass cylinders (36 cm in height, 18 cm in diameter) containing 24 cm of water maintained at 25 °C. The depth
of the water forced the animals to swim or float, since their feet did not reach the bottom of the cylinder. After 15 min in the water, animals were removed and allowed to dry for 15 min before being returned to their cages. Twenty-four hours later, the animals were again subjected to forced swimming. At 5 s intervals the mobility or immobility of each animal was recorded for a 10 min period. The rat was considered "immobile" whenever it remained floating passively in a hunched position, its head just above the water.

3.2.6. PROEDURE FOR EP RE-MEASUREMENT

Twenty-four hours after the last ECS seizure, EPs were re-measured using procedures identical to those described above (Section 2.2.4.).

3.2.7. HISTOLOGY

Following the completion of all testing, subjects were anaesthetized with PB (100 mg/kg; Somnotol®; MTC Pharmaceuticals, Cambridge, ON) and histology was done as described in Experiment 1 (Section 2.2.7.). The placements of electrodes were confirmed via light microscopy and found to lie within the expected brain structures.
3.2.8. STATISTICAL ANALYSIS

In the EP tests, population spike amplitudes and population EPSP slopes were measured as in Experiment 1 (Section 2.2.8.). They were expressed as percent increase from the baseline (pretreatment) levels. To simplify the analysis, a single evoked response was used to provide a measurement for each test day. The EP used was the potential produced by the intensity which had produced a population spike amplitude 50% of the maximal amplitude in the pre-ECS tests. This procedure was similar to that of Stewart and Reid (1994). Differences between the ECS-SAL, ECS-KET, ECS-PB and Control groups were analyzed using a one-way analysis of variance, followed by Student-Newman-Keuls multiple comparison analysis.

In the Porsolt test, differences in immobility between the ECS-SAL, ECS-KET, ECS-PB and Control groups were analyzed using a one-way analysis of variance, followed by Student-Newman-Keuls multiple comparison analysis.

3.3. RESULTS

3.3.1. PERFORANT PATH EP'S FOLLOWING "UNMODIFIED" ECS

Figure 3-1 shows typical I/O data from single subjects in the ECS-SAL, ECS-KET, ECS-PB and Control groups. Measurements were taken before (solid line) and 24 hrs after the last seizure (dotted
As indicated, "unmodified" ECS appeared to increase both the amplitude of the population spike and the slope of the population EPSP in all of the ECS groups, regardless of drug treatment. In control subjects, no consistent changes were seen.

Figure 3-2A presents mean population spike amplitudes (expressed as a percent of baseline) for all ECS-SAL, ECS-KET, ECS-PB and Control subjects 24 hrs after ECS or sham ECS. The amplitude of the population spike was increased in all ECS subjects, irrespective of the drug administered. A slight decrease in population spike amplitude was noted in the control subjects. A one-way analysis of variance showed a significant overall treatment effect \( (F_{3,28}=3.5261, \ p=0.0294) \). Student-Newman-Keuls post-hoc t-tests showed that the ECS Groups were all significantly different from the Control Group \( (p<0.05 \ in \ each \ case) \), but were not significantly different from one another.

Figure 3-2B shows the slope of the population EPSP's (expressed as a percent of baseline) in ECS-SAL, ECS-KET, ECS-PB and Control subjects 24 hrs after the last ECS seizure. The slope was increased in all ECS subjects, irrespective of the drug given. A slight decrease in the slope of population EPSP's was observed in control subjects. A one-way repeated measures analysis of variance, however, showed that this effect was not statistically significant \( (F_{3,28}=1.2724, \ p=0.3053) \).
FIGURE 3-1. Representative EPs taken during I/O tests in a control subject (A), an ECS-SAL subject (B), an ECS-KET subject (C), and an ECS-PB subject (D). For each subject, the solid lines illustrate the potential seen during the initial baseline test and the dashed lines illustrate the potential seen 24 hrs after the last ECS trial. A. Control subjects show no change in EPs 24 hrs after sham treatment. B. Both the population EPSP slope and the population spike were potentiated in the ECS-SAL subject 24 hrs after the last ECS seizure. C. Both the population EPSP slope and the population spike were potentiated in the ECS-KET subject, 24 hrs after the last ECS seizure. D. Both the population EPSP slope and the population spike were potentiated in the ECS-PB subject 24 hrs after the last ECS seizure.
FIGURE 3-2. Mean population spike amplitude (A) and mean population EPSP slope (B) in animals subjected to ECS or sham treatment. A. In all ECS groups, population spike amplitude was significantly elevated 24 hrs after the last ECS seizure. B. In all ECS groups, population EPSP slope was elevated 24 hrs after the last ECS seizure, however, this effect did not reach statistical significance.
POPULATION EPSP SLOPE
(% change from baseline mean±SEM)

POPULATION SPIKE HEIGHT
(% change from baseline mean±SEM)

Legend:
P<0.05
3.3.2. PORSOLT TEST

Figure 3-3 shows the mean duration of immobility in ECS-SAL, ECS-KET, ECS-PB and Control subjects during the 10-min Porsolt test. Measurements were taken 1 week after the last ECS seizure. As indicated, the ECS-SAL Group shows a greatly decreased duration of immobility as compared to the Control Group. The duration of immobility was also decreased in the ECS-KET and ECS-PB groups, but the decreases were much smaller. A one-way analysis of variance showed a significant overall treatment effect ($F_{3,35}=4.7259$, $p=0.0077$). Student-Newman-Keuls post-hoc tests revealed that there was a significant difference between the ECS-SAL Group and all of the other groups ($p>0.05$). There were no significant differences among the other groups.
FIGURE 3-3. Mean duration of immobility in the Porsolt test in animals subjected to ECS or sham treatment. Only the ECS-SAL Group shows a significant reduction in immobility.
4. DISCUSSION

4.1. EXPERIMENT 1

The present work was designed to determine whether the long-term effects of ECS - an animal model of ECT - could be prevented by either the "modified" procedure or by pre-administration of ketamine. Two large experiments were done: (1) Experiment 1, which tested the effects of the "modified" procedure; and (2) Experiment 2, which tested the effects of the neuroprotective drug, ketamine.

Experiment 1 utilized a new animal model - "modified" ECS - which parallels the "modified" ECT used clinically. It was designed to determine whether the "modified" procedure would prevent the enhancement of entorhinal-dentate EPs seen with "unmodified" ECS. Previously, Burnham et al. (1995) had shown EP enhancement lasting for a minimum of 3 months after repeated "unmodified" ECS.

The results of Experiment 1 indicate that the adoption of a "modified" procedure does not prevent the ECS-induced enhancement of evoked potentials. Evoked potential enhancement was found at all time points following "modified" ECS (Fig. 2-7).

Three major points will be discussed below: (1) the new "modified" ECS model; (2) the growth of EEGs during modified ECS; and (3) the clinical significance of enhanced EPs after "modified" ECS.
4.1.1. THE "MODIFIED" ECS MODEL

Previous experimenters have duplicated part, but not all, of the clinical ECT procedure.

British investigators, for instance, routinely use anaesthesia - usually halothane - before the administration of the ECS current. This partially duplicates the anaesthetics used in "modified" ECT. Modern "modified" ECT, however, is administered under methohexital anaesthesia, with atropine, succinylcholine, and oxygenation, using low-doses of square-wave current (e.g., Abrams, 1992).

Shaw (1985) studied EPs in rats curarized and oxygenated during a single ECS seizure. They found enhanced somatosensory EPs lasting for at least 20 min after ECS, an effect also seen in patients receiving ECT. Sine-wave stimulation was used in this study. There was no anesthesia.

Brennan and colleagues (1972) looked for structural changes following a single "modified" ECS seizure in rats. Their model more closely approached the clinical situation, since subjects were pre-treated with atropine, anaesthetized with ether, ventilated with 100% oxygen and injected i.m. with succinylcholine. A single seizure caused no structural brain changes. Sine-wave stimulation was used in this study.

Shavalia and colleagues (1981) used a "partially modified" ECS model to investigate the amnesic properties of ECS seizures. Their model - which was the closest to the current clinical practice - included pre-administration of atropine, curarization and
ventilation with air. They used a square-wave stimulus (with intensities up to 480 V) administered through electrodes - made of needles - which were implanted subdermally over the temporalis muscle. No anesthesia was used in this study.

Thus, although several past ECS studies have partially duplicated the procedures used in "modified" ECT, none of them has followed the full clinical paradigm. The model used in Experiment 1 is the first model to fully duplicate modern ECT procedures.

Clinicians have argued that "modified" ECT does not cause any long-lasting brain changes. To test this assertion, it was necessary to fully replicate the procedure used clinically.

4.1.2. EEG'S DURING "MODIFIED" ECS

In current ECT, EEGs are recorded during each seizure (e.g., Abrams, 1992). This is necessary to assess seizure duration, since patients receive succinylcholine, which partially suppresses the behavioural convulsions. Furthermore, the behavioral seizures often end before the EEG seizures do (Liston et al., 1988).

EEGs were also recorded during our study of "modified" ECS. These yielded additional important information. It was found, for instance, that animals subjected to repeated "modified" ECS showed a significant increase in the duration of the primary AD. The later primary ADs were almost twice as long as the early ones. Although AD growth has been reported in kindled subjects (Racine, 1972a), it has not been previously described in ECS subjects.
Two-thirds of the animals also showed secondary ADs. These are seen in kindled animals (Racine, 1972a), but they have not been previously described in ECS animals. No change in the latency or the duration of the secondary AD was seen as seizures were repeated.

There have been 2 previous reports of EEGs in rats during ECS seizures. Urca and Frenk (1982) studied EEG during "unmodified" ECS seizures. In that study there is no mention of a secondary AD, and no mention of an increase in the duration of the primary AD (Urca and Frenk, 1982). Shavalia et al. (1981) published 1 EEG trace taken during a "partially modified" ECS seizure. Their trace resembles those seen in Experiment 1, although the primary AD is longer than most primary ADs seen in the present study. This may be because they used a stimulus intensity of 480 V, about 6 times higher than the stimulus used in Experiment 1. A secondary AD is seen in their trace, but the authors do not comment on it.

As noted above, a previous study by Pinel and van Oot (1976) reported an increase in the strength of behavioural convulsions following repeated ECS. The present study also showed an increased incidence of "maximal" seizures in the later ECS trials (see Appendix 5-3). Pinel and van Oot (1976) suggested that ECS had a "kindling-like" effect. The present data - showing a growth in ADs over time - complement Pinel's work since they also suggest a "kindling-like" effect of ECS.
Writers in the ECT field have argued that repeated ECT seizures do not cause kindling (Abrams, 1992). The classic data of Pinel and van Oot (1976) cast doubt on this assertion. The present study - done with "modified" ECS, which closely parallels the modern clinical procedure - showed progressive growth of ADs. This also suggests that ECS causes "kindling-like" changes.

Further support for this view came from our study of EP enhancement after "modified" ECS.

4.1.3. EP'S AFTER "MODIFIED" ECS

Following a series of 8 "modified" ECS seizures, entorhinal-dentate EPs were enhanced for at least 3 months. These findings confirm and extend past reports of evoked potential enhancement following repeated ECS (Stewart and Reid, 1994; Burnham et al., 1995). They also are in general agreement with reports of EP enhancement following repeated kindled seizures (Racine et al., 1972; Racine et al., 1975; Racine et al., 1983; Racine et al., 1991). It appears that repeated seizures produce EP enhancement in at least 2 different paradigms.

Clinicians have argued that the use of a "modified" procedure protects against any long-term effects of electroconvulsive seizures (Abrams, 1992). The results of Experiment 1 make it clear that this is not true, at least in rats. Since the "modified" procedure used in the present study closely parallels the "modified" ECT procedure used in clinical practice, it may not be
true in human patients either.

The mechanism of the ECS-induced EP enhancement is presently unknown. The phenomenon was first reported only three years ago, and no studies of mechanism have, as yet, been done.

One might hope that studies of LTP enhancement might provide some clues. A great deal is known about the mechanism of LTP. It appears, however, that the LTP and ECS-induced enhancement have distinct mechanisms. In the perforant path, LTP is NMDA mediated (Nicoll and Malenka, 1995). As noted above, NMDA antagonists block LTP. In the present study, however, ketamine, an NMDA antagonist, had no effect on ECS-induced enhancement.

It is possible that the kindling literature will provide some useful clues as to the mechanism of ECS-induced enhancement. EPs are enhanced by seizure activity in both models, and similar mechanisms may be involved. Unfortunately, the mechanisms involved in kindling are still unknown. Burnham and Cottrell (1990) list three possible basic mechanisms for kindling - neuron growth, neuron death and permanent changes in neuron function - and note that there is some evidence for each of these. Further studies will be necessary to elucidate the mechanism of kindling and, if it is similar, of ECS-induced potentiation.

One recent study may offer some clues. Recently, Naylor and colleagues (1996) found that, following repeated ECS, the expression of GluR1, an α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA) receptor, was elevated in the hippocampus. The elevation was seen at 24 hrs, the longest
interval studied. NMDA expression was unchanged. If this elevation is a long-lasting one, it should result in a long-lasting increase in transmission in the glutamatergic pathways. This would result in enhanced EPs.

4.2. EXPERIMENT 2

The results of Experiment 1 indicated that the use of a "modified" procedure did not protect against EP enhancement. Experiment 2 was designed to determine whether enhancement of EPs could be prevented by a neuroprotective drug. Recently, Stewart and Reid (1994) had reported that ketamine, a dissociative anaesthetic and non-competitive NMDA antagonist, could block the enhancement of entorhinal-dentate EPs caused by repeated "unmodified" ECS. Experiment 2, therefore, was designed to confirm and extend their findings.

The results of Experiment 2 indicate that ketamine did not, in our hands, prevent the ECS-induced enhancement of EPs. EP enhancement was found in all ECS groups following the last ECS trial (Fig. 3-2). Thus, we have failed to replicate the findings of Stewart and Reid (1994).

There were several differences in experimental procedure that may explain the conflict between these 2 studies. The following points will be discussed below: (1) differences in rat strain used; (2) differences in drug administration procedure; (3) differences in mode and intensity of ECS administration; and (4) differences
in data analysis. Following that, the Porsolt data will be discussed.

4.2.1. DIFFERENCES IN RAT STRAINS

Stewart and Reid (1994) used Lister rats in their experiments. These are "hooded" rats that are predominantly used in Britain. Experiment 2 used Long-Evans rats, another strain of hooded rat. This strain is predominantly used in North America.

Although there are no experiments that specifically compare seizure thresholds or other parameters in these 2 strains, it is possible that inter-strain differences exist.

Intrastrain differences have been shown to affect seizure-related parameters in other studies. Animals from different rat strains have been shown to kindle at different rates, for instance (Racine et al., 1973).

4.2.2. DIFFERENCES IN DRUG ADMINISTRATION PROCEDURES

Stewart and Reid (1994) do not report the exact time of ketamine injection. Their injection took place "about" 10-15 min before each ECS administration (Stewart, personal communication), but the exact injection-test interval was not monitored. In Experiment 2, on the other hand, all drugs were injected exactly 15 min before the administration of the ECS stimulus. It is possible, therefore, that we injected the drug at a slightly
earlier time than Stewart and Reid (1994).

It is possible that administering ketamine at a earlier time before seizure onset (e.g., 5-10 min) might affect its neuroprotective actions. Following administration, ketamine is rapidly hydrolysed to a metabolite, norketamine. There has been much controversy regarding this metabolite and whether it is active or inactive following its formation (e.g., Reich and Silvay, 1989). The plasma half-life of ketamine itself is estimated to be less than 3 min (Cohen et al., 1973).

A few minutes' difference in injection time, therefore, might significantly affect blood levels at the time of seizure onset. The negative results in Experiment 2, therefore, may have been due to low blood levels produced by a slightly earlier time of drug-injection.

4.2.3. DIFFERENCES IN MODE AND INTENSITY OF ECS ADMINISTRATION

Stewart and Reid (1994) used the following stimulation parameters in their experiment: 200V, 50 mA, 2 s, with transcranial administration. They administered 10 ECS seizures on a 48-hr interval. Experiment 2, on the other hand, used the following stimulation parameters: 150 mA, 0.2 s, with corneal administration. Eight seizures were administered on a 48-hr schedule (3/week). These parameters matched the parameters used in the previous experiments of Burnham et al. (1995). Therefore, Experiment 2 used stimulation parameters that were somewhat different from those used
by Stewart and Reid (1994).

Browning and Nelson (1985) have reported that ECS seizures elicited by transauricular stimulation are different from those elicited by corneal stimulation. Tonic seizures are more easily triggered by transauricular stimulation, suggesting that the 2 types of stimulations engage slightly different brain areas.

The neuroprotective property of ketamine reported by Stewart and Reid (1994), therefore, might depend on part on electrode placement. Further investigation will be necessary to determine the effect of ketamine with the 2 different ECS procedures.

4.2.4. DIFFERENCES IN EP DATA ANALYSIS

Stewart and Reid (1994) used a single stimulation intensity (700 μA) to elicit EPs. They fail to report, however, whether these intensities reflect base-to-peak or peak-to-peak values. In Experiment 2, sixteen different stimulation intensities were used. The intensities used were 30-3200 μA (peak-to-peak).

The use of different current intensities is important in electrophysiological experiments involving whole animal preparations. "Ceiling" effects can occur, and the ceiling may differ from animal to animal. As seen in Fig. 3-2, I/O sweeps recorded with stimulation intensities greater than 900 μA (peak-to-peak) did not show further increase in amplitude; they appear to have reached a maximum size. Interpretation of these I/O sweeps, therefore, would yield incorrect scientific conclusions.
Consequently, it is important to produce I/O functions at several stimulation intensities.

Stewart and Reid (1994) worked at a single intensity. Conceivably, it was too high, and they were at "ceiling" in some subjects. In that case, no potentiation would be seen. If more subjects at "ceiling" happened to be in the experimental group, the lack of potentiation would be falsely attributed to ketamine.

It is worth noting, however, that Stewart and Reid (1994) had proper controls and have shown an enhancement of EPs following ECS. The failure to replicate their work, therefore, requires further investigation.

4.2.5. THE "THERAPEUTIC" EFFECTS OF "UNMODIFIED" ECS

Stewart and Reid (1994) did not attempt to assess the therapeutic efficacy of ECS given in the presence of ketamine. They simply assumed that therapeutic effects would be unchanged.

It seemed possible to us, however, that the reduction in seizure "intensity" might lead to a change in the antidepressive efficacy of ECS. The Porsolt test was used to investigate this question.

The Porsolt test showed that in animals injected with ketamine, ECS did not produce a significant "therapeutic" effect. Animals injected with PB, which produced a similar reduction in seizure intensity, also failed to show a significant "therapeutic" effect. These data suggest that treatments which reduce seizure
intensity also reduce therapeutic efficacy. They also show a further dissociation between EP enhancement and the "therapeutic" effects of ECS. Drug treatment suppressed the "therapeutic" effects of ECS - as modelled by the Porsolt test - but not the enhancement of EPs.

The Porsolt test is only an animal model. It may not accurately model the effects of drugs and/or ECT on humans. Assuming some validity, however, the present data are not encouraging. They suggest that even if ketamine can be shown to antagonize EP enhancement - at other doses or treatment intervals (below) - it may do so at the expense of ECS/ECT therapeutic effects.

Data from the Porsolt test also provide further evidence that enhancement of EPs following ECS are not associated with the "therapeutic" effects of the ECS. Ketamine and PB did not prevent the enhancement of EPs, however, both drugs eliminated the "therapeutic" efficacy of ECS as measured by the Porsolt test. This effect, therefore, requires further investigation.
4.3. FUTURE EXPERIMENTS

4.3.1. FURTHER EXPERIMENTS WITH "MODIFIED" ECS

As mentioned above, Burnham and colleagues have now found several long-lasting changes after ECS. Presently, these changes - except for EP enhancement - have all been studied only in experiments involving "unmodified" ECS. It is possible that the "modified" procedure will eliminate some of these other changes. Studies designed to test this possibility are currently in progress.

4.3.2. FURTHER EXPERIMENTS WITH KETAMINE

Further investigations regarding the neuroprotective properties of ketamine are essential. A slight deviation from the experimental procedure used by Stewart and Reid (1994) may have eliminated the neuroprotective properties previously observed. Therefore, the following three experiments are suggested: (1) An exact replication of Stewart and Reid (1994); (2) A dose-response study with different doses of ketamine using our own model; and (3) a study, using our model, where the times of drug injection are varied.
4.3.2.1. EXACT REPLICATION OF STEWART AND REID (1994)

Experiment 2 did not replicate the findings of Stewart and Reid (1994). This might be due to any of several differences between their model and ours. Therefore, an exact replication of their study is necessary. This study would involve the same species of rat used by Stewart and Reid (1994), plus the same stimulation parameters, injection-test interval, and so forth.

4.3.2.2. DOSE-RESPONSE STUDY

Running a dose-response study is important, since different doses of ketamine might exert different properties. Furthermore, the different strains of rat used in the 2 experiments - Lister and Long-Evans - might have different sensitivities to ketamine. Therefore, Experiment 2 should be repeated using a variety of ketamine doses in Long-Evans rats. The doses should cover the whole range from ineffective to toxic. The effects of these doses on the "therapeutic" effects of ECS should also be evaluated using the Porsolt test.
4.3.2.3. TIME-RESPONSE STUDY

The dose-response study alone might not provide sufficient evidence regarding the neuroprotective effect of ketamine. It is possible that the time of injection is more important than the dose used. Therefore, Experiment 2 should be repeated with different injection-test intervals. These should range from 15 min to 5 min before ECS administration.

If a ketamine effect is found, administration of ketamine after ECS administration might also be tried. If the mechanism of action for the neuroprotective effects is NMDA-mediated - as hypothesized by Stewart and Reid (1994) - injection of an antagonist before or after seizure onset should be equally effective (e.g., Wasterlain et al., 1993). Injection after ECS administration, moreover, would not interfere with the "intensity" of the seizure. The Porsolt test should also be run in this experiment, to establish that injection of ketamine after seizure onset does not interfere with the "therapeutic" effect of ECS.
5. APPENDICES

5.1. APPENDIX 1 - DETERMINATION OF DRUG REGIMENS FOR EXPERIMENT 1

Figure 5-1A shows a dose-response curve for the anaesthetic effect of methohexital alone. Anaesthesia was measured by the tail-pincht test. Animals receiving 10 mg/kg of methohexital i.v. became ataxic but still responded to tail-pincht; therefore, these animals were not deeply anaesthetized. With increased doses, the animals became progressively more anaesthetized. At the dose of 22.5 mg/kg, they became apneic. A dose of 17.5 mg/kg - which produced 2-3 min of anaesthesia - was initially chosen. Less than 5 min of anaesthesia are induced in human ECT patients (McCleave and Blakemore, 1975).

Figure 5-1B presents a dose-response curve for the combined effect of 17.5 mg/kg methohexital, injected i.v., and various doses of i.v. injected succinylcholine. As demonstrated, succinylcholine greatly extended the duration of methohexital-induced anaesthesia. In the presence of 0.125 mg/kg succinylcholine - which produced an acceptable level of muscle relaxation - 17.5 mg/kg methohexital produced over 7 min of anaesthesia. This duration is longer than the duration of anaesthesia used for clinical ECT. The dose-response curve was therefore repeated with an i.v. injection of 10 mg/kg methohexital, followed by varying succinylcholine doses injected i.v. An anaesthetic effect lasting for 3-4 min was observed with a combination of 10 mg/kg methohexital and 0.15 mg/kg
succinylcholine was used. This combination of doses was used in all subsequent experiments.
FIGURE 5-1. Dose-response curve for anaesthetic effects of (A) methohexital and (B) combinations of methohexital and succinylcholine. A. Methohexital-induced anaesthetic duration increases dose-dependently. At the 10 mg/kg dose, there is no anaesthesia as measured by the tail-pincher test. At the 22.5 mg/kg dose, both animals became apneic. (Error bars are not seen because SEMs were so small at most points that they fell within the symbols). B. Succinylcholine extended the duration of methohexital-induced anaesthesia. The 17.5 mg/kg methohexital (MTH) dose (open circles) combined with various concentrations of succinylcholine produced anaesthesia with >8 min durations. The 10 mg/kg methohexital dose (full circles), combined with 0.15 mg/kg succinylcholine, produced an anaesthetic effect lasting 3-4 min.
5.2. APPENDIX 2 - DETERMINATION OF AVERAGE SEIZURE THRESHOLD

In clinical ECT, threshold is not measured in individual patients. "Average" thresholds - derived from studies of other subjects - are used. A similar procedure was adopted in our ECS studies.

In a separate group of animals, 3 skull screws were implanted as described in Section 2.2.2. One week after the implantation of electrodes, a permanent indwelling cannula was inserted in the femoral vein of each subject to facilitate subsequent drug administration (see Section 2.2.3.).

Two days later, the threshold testing procedure was initiated. Animals received "modified" ECS. The technique described in Section 2.2.5. was used, except that stimulus intensity was varied. The initial ECS stimulus was set at 30 V. Animals were restimulated at 1-min intervals with 10 V increments until a 5-s AD was recorded. The threshold testing procedure was repeated 48 hrs later, to check the stability of the threshold readings.

Table 5-1 shows the mean seizure threshold of animals receiving "modified" ECS. Seizure threshold was defined as the voltage that elicited a 5-s (minimum) AD. It was found that the mean threshold was 70 V (base-to-peak). This value remained stable when the threshold test was repeated 48 hrs later.

In current ECT practice, a stimulus intensity of "seizure threshold plus 20%" is recommended for eliciting seizures (Abrams, 1992). Therefore, the intensity of 85 V (base-to-peak) was used
in all the subsequent studies.
TABLE 5-1. SEIZURE THRESHOLD OF ANIMALS RECEIVING "MODIFIED" ECS

<table>
<thead>
<tr>
<th>ANIMAL</th>
<th>DAY 1 THRESHOLD (V; base-to-peak)</th>
<th>DAY 3 THRESHOLD (V; base-to-peak)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1</td>
<td>90</td>
<td>70</td>
</tr>
<tr>
<td>A2</td>
<td>100</td>
<td>80</td>
</tr>
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<td>A3</td>
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<td>40</td>
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<td>A5</td>
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<td>50</td>
</tr>
<tr>
<td>A6</td>
<td>40</td>
<td>-</td>
</tr>
<tr>
<td>MEAN±SEM</td>
<td>70±12.1</td>
<td>70±12.2</td>
</tr>
</tbody>
</table>

* Subject died of respiratory complications following the induction of anaesthesia.
5.3. APPENDIX 3 - DETERMINATION OF THE PB DOSE FOR EXPERIMENT 2

Stewart and Reid used 100 mg/kg of ketamine in their experiments (Stewart and Reid, personal communication). Although that dose did not completely suppress the seizures, it shortened them. In the present study, a new control group was added - a group of subjects in which PB was used to partially suppress the seizures (ECS-PB Group). This group was designed to determine whether the anti-enhancement effects of ketamine related to seizure shortening rather than to neuroprotection.

The PB dose that matched the behavioural seizure produced by 100 mg/kg ketamine was established using the following procedure:

One week following arrival from the breeding farm, a group of animals (separate from those used in Experiment 2) was implanted with 3 skull-screws, plus attached Amphenol pins to allow for EEG recording. Procedures were identical to those described in Section 2.2.2.

"Unmodified" ECS (see Section 3.2.3.) was then initiated on a 48-hr schedule (3/week). Before each ECS trial, the animals were injected i.p. with either: 1) saline; 2) 100 mg/kg of ketamine; or 3) varying doses of PB (10-30 mg/kg). Each animal was tested 5 times, using the schedule and doses shown in Table 5-2. Drugs were obtained and mixed as described in Experiment 2. Injections were given 15 min before each ECS trial to match the procedure of Stewart and Reid (Stewart and Reid, personal communication). PB is known to be effective at this time interval (Krall et al., 1978).
EEGs were recorded on a Grass Model 6 Electroencephalograph (Grass Instruments, Quincy, MA). Motor seizure "intensity" was rated according to the following scale: 0 - no motor seizure; 1 - fore-limb flexion; 2 - brief fore-limb extension (<2 s); 3 - longer fore-limb extension (>2 s); 4 - hind-limb extension.

Figure 5-2A shows the duration of ADs recorded under the different experimental conditions. AD durations during the 3 saline tests were very similar, indicating that there was no shift in the baseline over time. The duration of AD in the ketamine group was very similar to the saline durations, indicating that ketamine had little effect on ADs. AD durations under PB, however, were considerably shorter than under saline. PB reduced the AD duration at all 3 doses. A one-way repeated measures analysis of variance for these data showed a significant effect of treatment ($F_{6,68}=4.4858$, $p=0.0011$). Student-Newman-Keuls post-hoc testing, however, showed that only the 10 mg/kg PB dose reduced the AD duration significantly, as compared to the second saline group ($p>0.05$).

Figure 5-2B shows the motor seizure "intensities" observed in the different groups. The animals receiving saline showed classic electroshock convulsions. The convolution was predominantly tonic, with the head flexed and fore-limbs in rigid extension. Many subjects also showed tonic hind-limb extension (7/14 first test day; 12/14 by fifth test day), which is the hallmark of "maximal" electroshock seizures. The median rank was 4.
Animals receiving ketamine exhibited milder, "submaximal" convulsions, which consisted of tonic fore-limb flexion (with a duration of about 1 s), followed by fore-limb clonus (with a duration of about 4 s). A few of the animals also showed fore-limb extensions of brief (<2 s; 2/7) or longer (>2 s; 1/7) duration. None showed hind-limb extension. The median rank was 1.

Animals that received the low dose of 10 mg/kg PB also showed "submaximal" seizures. These predominantly consisted of fore-limb extension (>2 s; 6/7). A single animal showed hind-limb extension. The median rank was 3, which was higher than the median rank of ketamine subjects.

Animals that received 20 or 30 mg/kg PB also showed "submaximal" seizures. These consisted of fore-limb flexion (6/7 and 4/7, respectively), with a few subjects showing brief (<2 s; 1/7 at 30 mg/kg) or longer (1/7 at 20 mg/kg; 2/7 at 30 mg/kg) fore-limb extensions. The median rank for both doses was 1, which was identical to the rank of the ketamine subjects.

A Kruskal-Wallis nonparametric one-way analysis of variance showed a significant effect of treatment ($H_\text{e}=45.4438$, $p=3.82\times10^{-8}$). Dunn's post-hoc test revealed significant differences between the saline groups and the ketamine, 20 mg/kg PB, and 30 mg/kg PB groups ($p<0.05$). There was no significant difference among the saline groups themselves or between the saline groups and the 10 mg/kg PB group.
### TABLE 5-2. SCHEDULE AND DRUG DOSES TO DETERMINE EEG AD DURATION AND BEHAVIOURAL INTENSITY IN "UNMODIFIED" ECS

<table>
<thead>
<tr>
<th>TEST WEEK</th>
<th>TEST DAY</th>
<th>GROUP 1</th>
<th>GROUP 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>SALINE (1 ml/kg)</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>3</td>
<td>PB (20 mg/kg)</td>
<td>KETAMINE (100 mg/kg)</td>
</tr>
<tr>
<td>1</td>
<td>5</td>
<td>SALINE (1 ml/kg)</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>8</td>
<td>PB (10 mg/kg)</td>
<td>PB (30 mg/kg)</td>
</tr>
<tr>
<td>2</td>
<td>10</td>
<td>SALINE (1 ml/kg)</td>
<td></td>
</tr>
</tbody>
</table>
FIGURE 5-2. AD duration (A) and seizure "intensity" (B) during "unmodified" ECS. A. The AD durations in the 3 saline tests and in the ketamine group are very similar. PB reduced the AD duration at all 3 doses. B. Motor seizure "intensity" was rated according to the following scale: 0 - no motor seizure; 1 - fore-limb flexion; 2 - brief fore-limb extension (<2 s); 3 - longer fore-limb extension (>2 s); 4 - hind-limb extension. Animals receiving saline primarily showed maximal seizures (median rank = 4), while those that received ketamine or PB showed submaximal seizures (median rank = 3 or less).
The 20 mg/kg dosage of pentobarbital was selected for use in Experiment 2, since this was the lowest dose that produced motor seizures similar to those observed in animals receiving 100 mg/kg ketamine.
6. REFERENCES


