EFFECT OF LONG-TERM AMYGDALA KINDLING
ON DEFENSIVE BEHAVIOUR IN RATS:
A MODEL OF THE INTERICTAL EMOTIONALITY ASSOCIATED WITH
TEMPORAL LOBE EPILEPSY

by

LISA EMILY KALYNCHUK
B.Sc., University of Alberta, 1990
M.A., University of British Columbia, 1993

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR
THE DEGREE OF DOCTOR OF PHILOSOPHY
in
THE FACULTY OF GRADUATE STUDIES
(.Department of Psychology)

THE UNIVERSITY OF BRITISH COLUMBIA
December 1996

© Lisa Emily Kalynchuk 1996
In presenting this thesis in partial fulfilment of the requirements for an advanced degree at the University of British Columbia, I agree that the Library shall make it freely available for reference and study. I further agree that permission for extensive copying of this thesis for scholarly purposes may be granted by the head of my department or by his or her representatives. It is understood that copying or publication of this thesis for financial gain shall not be allowed without my written permission.

Department of Psychology

The University of British Columbia
Vancouver, Canada

Date December 19, 1996
ABSTRACT

Temporal lobe epileptics often experience interictal (i.e., between-seizure) emotional disturbances such as fear and anxiety. Despite the problem that these disturbances present, little progress has been made in characterizing their nature and etiology because they are not amenable to experimental analysis in clinical populations. Accordingly, the general purpose of the experiments in this thesis was to demonstrate the potential of long-term amygdala kindling in rats as a model of the interictal hyperemotionality of temporal lobe epileptics.

Seven experiments comprise this thesis. Experiments 1 and 2 established that long-term amygdala kindling (i.e., 100 stimulations) results in large and reliable increases in emotionality. In Experiment 1, the long-term amygdala-kindled rats displayed more resistance to capture from an open field and more open-arm activity on an elevated plus maze than did the sham-stimulated rats; in Experiment 2, the magnitude of this hyperemotionality was shown to be dependent on the number of amygdala stimulations that the rats received. Experiment 3 showed that kindling-induced hyperemotionality is enduring; the hyperemotionality present 1 day after the final stimulation did not decline significantly over the ensuing month although some amelioration of symptoms was observed. Experiment 4 established that kindling-induced hyperemotionality is not unique to amygdala stimulation. Although increases in emotionality were greatest in amygdala-kindled rats, hippocampal-kindled, but not caudate-kindled, rats also displayed significant increases. Experiments 5 and 6 showed that kindling-induced hyperemotionality is fundamentally defensive in nature. In Experiment 5, amygdala-kindled rats displayed high levels of emotionality in an unfamiliar, but not in a familiar, situation; in Experiment 6, amygdala-kindled rats displayed more defensive, but less aggressive behaviour, in their interactions with other rats. Finally, Experiment 7 showed that 8-OH-DPAT binding to serotonin 5HT1A receptors is increased in the dentate gyrus of amygdala-kindled rats, but not in the amygdala, periaqueductal grey, perirhinal cortex, or CA1 or CA3 hippocampal subfields.
Together, the results of these experiments establish the potential of long-term amygdala kindling as a useful animal model of interictal emotionality in temporal lobe epileptics.
TABLE OF CONTENTS

ABSTRACT II

TABLE OF CONTENTS IV

LIST OF FIGURES VIII

LIST OF TABLES X

ACKNOWLEDGMENTS XI

GENERAL INTRODUCTION 1

NATURE OF TEMPORAL LOBE EPILEPSY 2

BEHAVIOURAL DISTURBANCES IN TEMPORAL LOBE EPILEPTICS 5

THE KINDLING MODEL OF TEMPORAL LOBE EPILEPSY 12

DEFENSIVE BEHAVIOUR IN ANIMALS AS A MEASURE OF EMOTION 15

THE ROLE OF THE AMYGDALA IN EMOTIONAL BEHAVIOUR 20

BEHAVIOURAL EFFECTS OF AMYGDALA KINDLING 24

RATIONALE AND GENERAL PURPOSES 27

GENERAL METHOD 30

SUBJECTS 30

SURGERY 30

KINDLING PROTOCOL 31

APPARATUS 31

BEHAVIOURAL TESTING 32

HISTOLOGY 32

STATISTICAL ANALYSES 33

EXPERIMENT 1: THE EFFECT OF LONG-TERM AMYGDALA KINDLING ON EMOTIONAL BEHAVIOUR IN RATS 33

METHOD 34

Kindling Protocol 34
EXPERIMENT 2: EFFECT OF DIFFERENT NUMBERS OF AMYGDALA STIMULATIONS ON THE DEVELOPMENT OF INCREASED EMOTIONALITY

METHOD
Kindling Protocol
Behavioural Testing
Statistical Analyses

RESULTS
Histology
Open Field Exploration
Resistance to Capture
Elevated Plus Maze

DISCUSSION OF EXPERIMENT 2

EXPERIMENT 3: INCREASED EMOTIONALITY PRODUCED BY LONG-TERM AMYGDALA KINDLING IS ENDURING

METHOD
Kindling Protocol
Behavioural Testing
Statistical Analyses

RESULTS
Histology
Open Field Exploration
Resistance to Capture
Elevated Plus Maze

DISCUSSION OF EXPERIMENT 3

EXPERIMENT 4: BRAIN SITE SPECIFICITY IN KINDLING-INDUCED EMOTIONALITY

METHOD
Kindling Protocol and Behavioural Testing
Statistical Analyses

RESULTS
Histology
Convulsion Class 69
Open Field Exploration 74
Resistance to Capture 74
Elevated Plus Maze 77

DISCUSSION OF EXPERIMENT 4 77

EXPERIMENTS 1 TO 4 REVISITED: HOW ARE THE BEHAVIORAL MEASURES RELATED TO EACH OTHER? 80

EXPERIMENT 5: ROLE OF UNFAMILIARITY IN THE EXPRESSION OF KINDLING-INDUCED HYPEREMOTIONALITY 83

METHOD 84
Kindling Protocol and Behavioural Testing 84
Statistical Analyses 85

RESULTS 85
Histology 85
Resistance to Capture 85

DISCUSSION OF EXPERIMENT 5 88

EXPERIMENT 6: FUNDAMENTAL NATURE OF KINDLING-INDUCED HYPEREMOTIONALITY: AGGRESSION OR DEFENSE? 88

METHOD 89
Kindling Protocol and Behavioural Testing 89
Statistical Analyses 90

RESULTS 90

DISCUSSION OF EXPERIMENT 6 90

EXPERIMENT 7: CHANGES IN SEROTONIN RECEPTOR BINDING ASSOCIATED WITH LONG-TERM AMYGDALA KINDLING 93

METHOD 94
Receptor Autoradiography 94
Statistical Analyses 95

RESULTS 95

DISCUSSION OF EXPERIMENT 7 95

GENERAL DISCUSSION 100

FUNDAMENTAL NATURE OF KINDLING-INDUCED EMOTIONALITY 102

CRITICAL FACTORS INFLUENCING THE EXPRESSION OF KINDLING-INDUCED EMOTIONALITY 106
LIST OF FIGURES

Figure 1. Schematic drawing of inputs and outputs of amygdalar nuclei thought to be involved in mediating emotional behaviour.........................................................22

Figure 2. Histological results from the three groups of rats in Experiment 1. ..................38

Figure 3. The mean resistance to capture displayed by the rats in each group in Experiment 1.................................................................39

Figure 4. The percentage of open-arm activity on the elevated plus maze displayed by the rats in each group in Experiment 1.................................41

Figure 5. Histological results from the four groups of rats in Experiment 2..................47

Figure 6. The mean number of squares entered during the first 30 s of the open-field test by the rats in each group in Experiment 2.................................49

Figure 7. The mean resistance to capture displayed by the rats in each group in Experiment 2.......................................................................................50

Figure 8. The percentage of open-arm activity on the elevated plus maze displayed by the rats in each group in Experiment 2.................................52

Figure 9. The percentage of rats in each group in Experiment 2 jumping off the open arms of the elevated plus maze.........................................................53

Figure 10. Histological results from the four groups of rats in Experiment 3...............60

Figure 11. The mean number of squares crossed in the open field by the rats in each group in Experiment 3.................................................................61

Figure 12. The mean resistance to capture displayed by the rats in each group in Experiment 3.......................................................................................62

Figure 13. The percentage of open-arm activity on the elevated plus maze displayed by the rats in each group in Experiment 3.................................64

Figure 14. Histological results from the amygdala-kindled and amygdala-sham rats in Experiment 4.................................................................70

Figure 15. Histological results from the hippocampal-kindled and hippocampal-sham rats in Experiment 4.................................................................71

Figure 16. Histological results from the caudate-kindled and caudate-sham rats in Experiment 4.................................................................72

Figure 17. The mean class of convulsions produced by the final five stimulations in each group of kindled rats in Experiment 4.................................73

Figure 18. The mean number of squares crossed in the open field by the rats in each group in Experiment 4.................................................................75
Figure 19. The mean resistance to capture displayed by the rats in each group in Experiment 4. .................................................................76

Figure 20. The percentage of open-arm activity on the elevated plus maze displayed by the rats in each group in Experiment 4. .................................................................78

Figure 21. Histological results from the two groups of rats in Experiment 5. .....................86

Figure 22. The mean resistance-to-capture scores displayed by the two groups of rats tested in their home cage and after repeated daily testing in an initially unfamiliar open field in Experiment 5. .................................................................87

Figure 23. The percent of kindled and sham-stimulated rats that engaged in defensive and aggressive behaviors when tested as intruders in a resident-intruder paradigm in Experiment 6. .................................................................91

Figure 24. The mean optical density of 8-OH-DPAT binding in several brain regions from the two groups of rats in Experiment 7. .................................................................96
LIST OF TABLES

Table 1. Traits of the Bear-Fedio Inventory ................................................................. 8
Table 2. Behavioural Side Effects of Anticonvulsant Medication ................................. 13
Table 3. Categories of Aggressive and Defensive Behaviour in the Rat ....................... 17
Table 4. The Results From the Elevated-Plus Maze in Experiment 1 ........................... 42
Table 5. Correlations Among the Behavioral Measures from Experiments 1 to 4 .......... 82
I thank my supervisor, Dr. John Pinel, for his guidance, wisdom, and friendship over the past 6 years. I thank Dr. Dallas Treit for helping me initiate the line of research that is presented in this thesis. I thank Dr. Dimitri Papageorgis, Dr. Don Wilkie, Dr. Chris Shaw, Dr. Richard Tees, Dr. Juhn Wada, and Dr. Antoine Depaulis for their contributions to the final copy of this thesis. I also thank the many undergraduate students who assisted me during the conduct of these experiments.

Success in graduate school demands time more than anything else. Unfortunately, the hours and hours I spent in the lab usually took their toll on my closest personal relationships. For this reason, I thank the people who made the biggest sacrifices to help me achieve this goal: my parents, Orest and Emily Kalynchuk, for their support and encouragement; Rhonda Smyl, for her unwavering friendship; and Ray Spiteri, for his patience, love, and acceptance.
GENERAL INTRODUCTION

Epilepsy is a chronic disorder that is characterized by spontaneously recurring seizures. Of the many forms of epilepsy, temporal lobe epilepsy represents the biggest problem for modern medicine: It is the most prevalent form, comprising 55% of all cases in adults, and it is the most resistant to treatment. Furthermore, 30 to 40% of temporal lobe epileptics experience profound interictal (i.e., between seizure) disturbances in emotional behaviour. Despite the problem that these interictal emotional disturbances present for temporal lobe epileptics, little progress has been made in characterizing their fundamental nature and etiology, and like temporal lobe epilepsy itself, interictal emotionality has been resistant to treatment. The interictal emotionality associated with temporal lobe epilepsy is the focus of this thesis.

Because of the difficulties inherent in the study of clinical populations, the development of an animal model for studying the interictal emotionality associated with temporal lobe epilepsy is critical for furthering our understanding of this problem and developing effective treatments for it. The three general purposes of the experiments in this thesis were to demonstrate the potential of long-term amygdala kindling as a model of the interictal hyperemotionality associated with temporal lobe epilepsy, to provide some parametric data about the interictal hyperemotionality, and to use the model to clarify the nature of the interictal hyperemotionality. Accordingly, the first six sections of the General Introduction deal in sequence with the following topics: (1) the nature of temporal lobe epilepsy, (2) the interictal behavioural disturbances in temporal lobe epileptics, (3) the kindling model of temporal lobe epilepsy, (4) defensive behaviour in animals as a measure of emotion, (5) the role of the amygdala in emotional behaviour, and (6) the behavioural effects of amygdala kindling. The seventh, and final, section of the General Introduction describes the general rationale and purposes of the thesis.
The psychological symptoms of temporal lobe epilepsy have both fascinated and frustrated medical scientists for centuries: Of all the epilepsies, temporal lobe epilepsy remains the biggest enigma. The psychological complexity of temporal lobe epilepsy is illustrated by the following two descriptions of temporal lobe seizures:

His seizures occurred periodically, every few months or days. Each one started with an ecstatic feeling which he could not fully describe. "You people have no idea of the bliss which epileptics experience in the moments preceding their attacks," he wrote. "For several moments, I have a feeling of happiness which I never experienced in my normal state and which one cannot imagine. It is a complete harmony in myself and in the wide world and this feeling is so sweet, so strong, that I assure you, for a few seconds of this felicity one could give ten years of his life, indeed his entire life." The rest of the seizure was less pleasant. He felt anguish, dread, and guilt, as if he had committed some "monstrous crime." He saw a blinding flash of light and paused as if searching for a word. With the sense that his voice belonged not to him but to a nonhuman being that had climbed inside his body, he cried out and lost consciousness for a second or two. Sometimes the epileptic discharge generalized across his brain, producing a secondary grand mal attack. Afterward he could not recall events and conversations that had occurred during the seizure, and he often felt depressed, guilty, and irritable for days.

At the time of her visit to Harvard Medical School, she was still taking Dilantin and another anticonvulsant, and still having between ten and fifty seizures daily. Gloria's typical seizure begins with an unpleasant smell lasting a minute or two. "It's a horrible stink," Gloria declared, "of feces, urine, or burning kerosene. It's not Chanel Number Five." Next, she has an automatism, mechanically turning her head or smacking her lips. Her left arm and the left side of her face twitch. From this point on in a seizure, she is not fully aware of what she is doing, and she will not recall her actions later. When asked where she is or what day or month it is, she cannot say for sure. She stares blankly, sometimes for as long as fifteen minutes. She has bursts of intense emotion--anger, sadness, or fear. Sometimes she loses consciousness.... (adopted from LaPlante, 1993)

These descriptions underline several common features of temporal lobe seizures:

They are variable and unpredictable in their course; they are frequent; they are difficult to control; they are accompanied by a extreme emotions that can change within a single seizure or from seizure to seizure; and they sometimes leave patients with feelings of irritability long after the actual seizure has ceased.
Temporal lobe seizures are of the complex partial type. In this context, the word “partial” means that the seizure has a focal onset (in this case, usually in a temporal lobe structure) and does not spread through the entire brain. Partial seizures are called "simple" if they are primarily sensory or motor with no impairment of consciousness, and they are called "complex" if—as in the case of temporal lobe epilepsy—consciousness is altered or lost.

About 50% of all temporal lobe seizures begin with an aura (Feldman, 1983). Auras may take many forms (Commission on Classification and Terminology of the International League Against Epilepsy, 1981): They may take the form of epigastric discomfort, sensory-motor problems that typically involve only the face and extremities, illusions or hallucinations, dysphasia, dysmnesia (e.g., *deja vu, jamais vu*, or flashbacks), cognitive disturbances (e.g., forced thinking or dreamy states), or affective disturbances (e.g., euphoria, fear, or anger).

After the aura, there is a period of semiconsciousness, which is often accompanied by behavioural arrest. During a period of behavioural arrest, the patient simply stops what she or he is doing and stares blankly ahead, often engaging in simple repetitive behaviours called automatisms. Common automatisms include repeated lip smacking, licking, chewing, swallowing, mimicking, gesturing, repeating nonsense phrases, tugging at pieces of hair, and doing and undoing buttons (Wada & Seino, 1990). However, some temporal lobe seizures involve complex sequences of nearly normal behaviour. The following four examples of such sequences were reported by Lennox (1960):

A war veteran subject to many automatisms read in the newspaper about a man who had embraced a woman in a park, followed her into a women’s toilet, and then boarded a bus. From the description given, he realized he was the man.

One morning a doctor left home to answer an emergency call from the hospital and returned several hours later, a trifle confused, feeling as though he had experienced a bad dream. At the hospital he had performed a difficult ... [operation] with his usual competence, but later had done and said things deemed inappropriate.
A young man, a music teacher, when listening to a concert, walked down the aisle and onto the platform, circled the piano, jumped to the floor, did a hop, skip, and jump up the aisle, and regained his senses when part way home. He often found himself on a trolley [bus] far from his destination.

A man in an attack went to his employer and said, "I have to have more money or [I] quit." Later, to his surprise, he found that his salary had been raised.

Whether the motor components of a temporal lobe seizure are primarily simple or complex, the patient typically has little or no recollection of them once full consciousness has been regained.

Temporal lobe seizures are sometimes accompanied by secondarily generalized convulsions. In such cases, the focal seizure discharge spreads throughout the brain, and the partial motor seizure develops into a generalized convolution. Because these secondary generalized convulsions are of the grand mal variety, some temporal lobe epileptics are misdiagnosed as grand mal epileptics (LaPlante, 1993).

It has been suggested that the form of temporal lobe seizures depends on the location of their focus within the temporal lobe (Lothman, Bertram, & Stringer, 1991). Those with medial temporal lobe onset (i.e., in the hippocampal-amygdala region) frequently begin with a behavioural arrest, whereas seizures with lateral temporal lobe onset (i.e., in the neocortex) frequently begin with auditory or visual hallucinations. However, there is a strong tendency for seizures with lateral temporal lobe onset to spread to the hippocampus and amygdala, resulting in considerable overlap in the behavioural manifestations of medial and lateral temporal lobe seizures in their latter stages.

Temporal lobe seizures are particularly resistant to treatment (Engel, 1987); indeed, approximately 65% of temporal lobe epileptics report dissatisfaction with the level of seizure control provided by their medication (LaPlante, 1993). In patients who experience complex partial seizures that develop into secondary generalized seizures, anticonvulsant medication often controls the generalized motor seizure but not the complex partial motor seizure (Lothman et al., 1991).
The resistance of temporal lobe epilepsy to treatment may be related to its neuroanatomical basis (Lothman et al., 1991). Weiser (1983, 1988) used depth electrodes to locate the epileptic foci of temporal lobe epileptics and found that two major subcortical structures of the temporal lobe, the hippocampus and amygdala, play the major role in seizure initiation—25% of the seizures in these patients appeared to originate in the hippocampus, 10% appeared to originate in the amygdala, and 65% appeared to originate in the hippocampus and amygdala simultaneously. Because the hippocampus and amygdala have particularly low seizure thresholds, seizure discharges that involve these structures may be particularly resistant to antiepileptic drugs.

Temporal lobe epilepsy is often associated with a variety of interictal behavioural disturbances that often include pathological increases in fear, anxiety, and depression (e.g., Bear & Fedio, 1977; Blumer & Benson, 1982; Devinsky, 1991; Gloor, 1990; Waxman & Geschwind, 1975). Gaining an understanding of the nature and cause of these disturbances is important for at least two reasons. First, the interictal behavioural disturbances associated with temporal lobe epilepsy are often more incapacitating and more difficult to control than the seizures themselves: They can disable epileptic patients to the point where they cannot work, sustain normal relationships, or otherwise function normally in society. Second, the study of the interictal behavioural disturbances associated with temporal lobe epilepsy may provide a basis for inferring the role of temporal lobe structures in psychological function. Because interictal behavioural disturbances are the focus of this thesis, current knowledge about them is reviewed in the following section.

**Behavioural Disturbances in Temporal Lobe Epileptics**

Recognition of the association between epilepsy and abnormal interictal behaviour has a long history. In the middle ages, epileptics were rejected as menaces to society and were often incarcerated or executed. In the late 19th century, pioneers of modern
psychiatry—such as Esquirol, Griesinger, Maudsley, Kraepelin, and Bleuler—began to
discuss the role of epilepsy as an important cause of mental pathology (Devinsky, 1991). At
about the same time, anecdotal reports relating temporal lobe seizures in particular to
interictal psychopathology started to be published; for example, Hughlings Jackson (1875)
concluded that “The more imperfect and shorter the paroxysm, the more likely...elaborate
mental actions will follow.” Similar anecdotal reports have continued to be published
throughout the 20th century: Sjobring (1944) described his temporal lobe epileptic patients
as “torpid, sticky, and affectively tense” and observed that they were prone to outbursts of
rage or anxiety; and Gibbs, Gibbs, and Fuster (1948) reported that patients with temporal
lobe seizures had a much higher incidence of “psychopathology” than did other epileptics.

In 1975, based on their own clinical observations, Waxman and Geschwind
characterized the interictal behavioural syndrome that is associated with temporal lobe
seizures (i.e., the "epileptic personality"). This syndrome comprised deepened emotions,
circumstantiality, hyperreligiosity, hyposociality, and hypergraphia. Waxman and
Geschwind stressed that although the behaviour of temporal lobe epileptics is different, it is
not qualitatively abnormal; they believed that temporal lobe epileptics were merely prone to
overdo the behaviours in which most people engage.

Efforts to measure the behavioural changes associated with temporal lobe epilepsy
have taken two forms. By far the most frequent approach has been to assess behavioural
changes with conventional psychiatric tests such as the Minnesota Multiphasic Personality
Inventory (MMPI). Unfortunately, the findings of studies that have taken this approach have
been equivocal (see Dodrill & Batzel, 1986). One problem with this approach is that it is
based on the a priori assumption that altered emotions in temporal lobe epileptics fit
traditional psychiatric diagnostic categories. A second problem is that some of the test items
reflect the experience of the seizures per se, rather than interictal emotional changes. For
example, consider the following items from the MMPI:
I have periods in which I carried on activities without knowing later what
I had been doing.

I have had attacks in which I could not control my movements or speech
but in which I knew what was going on around me.

I have had blank spells in which my activities were interrupted and I did
not know what was going on around me.

Clearly, temporal lobe epileptics who answer “yes” to these items are not necessarily
experiencing interictal behavioural problems.

The second approach to measuring the behavioural changes associated with temporal
lobe epilepsy has used the so-called Bear-Fedio Inventory (see Table 1). Motivated by the
inappropriateness of the MMPI for the task, Bear and Fedio (1977) constructed their
inventory for the expressed purpose of characterizing interictal changes in behaviour. The
Bear-Fedio Inventory assesses 18 behavioural traits; each of the 18 was either included in
Waxman and Geschwind’s (1975) clinical description of the temporal lobe epileptic
personality or described in previous anecdotal reports. In their initial validation study, Bear
and Fedio (1977) administered their inventory to a group of temporal lobe epileptics, a
group of neuromuscular-disorder patients, and a group of normal control subjects. All of the
subjects were asked to rate themselves by responding “true” or “false” to five questions
related to each trait. The temporal lobe epileptics scored significantly higher on each of the
18 traits than did the subjects in both of the other groups; in contrast, the neuromuscular-
disorder patients scored significantly higher than the normal controls on only 3 of the 18
traits.

Is there a single underlying psychological change that is the basis of all 18 of the
behavioural changes documented by the Bear-Fedio Inventory? In their paper, Bear and
Fedio (1977) suggested that all 18 behavioural traits associated with temporal lobe epilepsy
are the product of a single underlying psychological change: enhanced affective association
to previously neutral stimuli, events, or concepts. Thus, in Bear and Fedio’s view, for
temporal lobe epileptics objects and events augmented with affective coloration may
<table>
<thead>
<tr>
<th>Trait</th>
<th>Clinical Observations</th>
<th>Studies</th>
</tr>
</thead>
<tbody>
<tr>
<td>Emotionality</td>
<td>Deepening of all emotions; sustained intense affect</td>
<td>Davidson &amp; Bagley, 1969; Glaser, 1964; Hill, 1953; Slater, 1969; Slater &amp; Beard, 1963; Waxman &amp; Geschwind, 1974</td>
</tr>
<tr>
<td>Elation, Euphoria</td>
<td>Grandiosity, exhilarated mood; manic-depressive symptoms</td>
<td>Flor-Henry, 1969; Slater &amp; Beard, 1963</td>
</tr>
<tr>
<td>Sadness</td>
<td>Discouragement, tearfulness, self-depreciation; diagnosis of depression, suicide attempts</td>
<td>Glaser, 1964; Slater &amp; Moran, 1969; Williams, 1944</td>
</tr>
<tr>
<td>Anger</td>
<td>Increased temper, irritability</td>
<td>Falconer, 1973; McIntyre et al., 1976; Sweet et al., 1969; Taylor, 1969; Trefert, 1964</td>
</tr>
<tr>
<td>Aggression</td>
<td>Overt hostility, rage attacks, Violent crimes</td>
<td>Davidson, 1947; Mark &amp; Ervin, 1970; Mark et al., 1968; Serafetinides, 1965</td>
</tr>
<tr>
<td>Guilt</td>
<td>Tendency to self-scrutiny and self-recrimination</td>
<td>Blumer, 1975; Dominian et al., 1963; Waxman &amp; Geschwind, 1974</td>
</tr>
<tr>
<td>Hypermoralism</td>
<td>Attention to rules with inability to distinguish significant from minor infraction, desire to punish offenders</td>
<td>Blumer, 1975; Mark &amp; Ervin, 1970; Waxman &amp; Geschwind, 1974</td>
</tr>
<tr>
<td>Obsessionalism</td>
<td>Ritualism; orderliness; compulsive attention to detail</td>
<td>Blumer, 1975; Bruens, 1969; Waxman &amp; Geschwind, 1974</td>
</tr>
<tr>
<td>Circumstantiality</td>
<td>Loquaciousness, pedantic; overly detailed, peripheral</td>
<td>Slater &amp; Beard, 1963; Waxman &amp; Geschwind, 1974</td>
</tr>
<tr>
<td>Trait</td>
<td>Clinical Observations</td>
<td>Studies</td>
</tr>
<tr>
<td>-------------------------------</td>
<td>----------------------------------------------------------------------------------------</td>
<td>----------------------------------------</td>
</tr>
<tr>
<td>Sense of personal destiny</td>
<td>Events given highly charged, personalized significance; divine guidance ascribed to many features of patient's life</td>
<td>Glaser, 1964; Slater &amp; Beard, 1963; Waxman &amp; Geschwind, 1974</td>
</tr>
<tr>
<td>Hypergraphia</td>
<td>Keeping extensive diaries, detailed notes; writing autobiography or novel</td>
<td>Blumer, 1975; Waxman &amp; Geschwind, 1974</td>
</tr>
<tr>
<td>Religiosity</td>
<td>Holding deep religious beliefs, often idiosyncratic; multiple conversions, mystical states</td>
<td>Dewhurst &amp; Beard, 1970; Irvin, 1967; Hill, 1969; Slater, 1969; Slater &amp; Beard, 1963</td>
</tr>
<tr>
<td>Philosophical interest</td>
<td>Nascent metaphysical or moral speculations, cosmological theories</td>
<td>Slater &amp; Beard, 1963; Waxman &amp; Geschwind, 1974</td>
</tr>
<tr>
<td>Dependence, passivity</td>
<td>Cosmic helplessness, &quot;at hands of fate&quot;, protestations of helplessness</td>
<td>Blumer, 1975; Slater &amp; Beard, 1963</td>
</tr>
<tr>
<td>Viscosity</td>
<td>Stickiness, tendency to repetition</td>
<td>Blumer, 1975; Glaser, 1964</td>
</tr>
<tr>
<td>Humorlessness, sobriety</td>
<td>Overgeneralized ponderous concern; humor lacking or idiosyncratic</td>
<td>Ferguson et al., 1969; Waxman &amp; Geschwind, 1974</td>
</tr>
<tr>
<td>Paranoia</td>
<td>Suspicious, overinterpretative of motives and events; diagnosis of paranoid schizophrenia</td>
<td>Bruens, 1969; Hill, 1969; Pond, 1959; Slater &amp; Beard, 1963</td>
</tr>
</tbody>
</table>

from Bear & Fedio, 1977

engender a mystically religious world view or an inflated sense of personal destiny. A felt significance behind events that others dismiss constitutes a seed for paranoia or may confirm the feeling that the patient is a passive pawn in the hands of powerful forces that structure the world. Sensing emotional importance in even the smallest acts leads patients to perform them ritualistically and repetitively.
Bear (1979) later suggested that affective associations to previously neutral stimuli could be the result of repeated seizure activity in limbic structures such as the amygdala, which links sensory association cortices with drive-controlling centres within the hypothalamus. According to Bear, the epileptic process could promote sensory-limbic associations through hyperconnection, just as lesions produce sensory-limbic dissociations through disconnection. The sensory-limbic hyperconnection produced by epilepsy could lead to a coloration of experience with emotion in temporal lobe epileptics. This hypothesis is consistent with the large research literature implicating the amygdala both in the mediation of emotional behaviour (see Davis, Rainnie, & Cassel, 1994) and in the processing of complex stimuli (e.g., LeDoux, 1992).

Efforts to replicate the 18 trait differences between temporal lobe epileptics and normal controls that were originally reported by Bear and Fedio (1977) have been only marginally successful. Rodin and Smaltz (1984) did find that a mixed group of epileptics (i.e., temporal lobe and grand mal seizures) scored significantly higher on all 18 traits than normal controls; however, Brant, Seidman, and Kohl (1985) found that a similar mixed epilepsy group scored higher than normal controls on only 5 of the 18 traits (i.e., circumstantiality, humorlessness, viscosity, sadness, and dependence). Similarly, Seidman (1980) found that temporal lobe epileptics rated themselves as significantly higher than normal controls on only 8 of the 18 traits (i.e., viscosity, obsessiveness, emotionality, circumstantiality, paranoid, depression, anger, dependence, and guilt).

Comparisons between temporal lobe epileptics and other patients using the Bear-Fedio Inventory have also been equivocal. Rodin and Smaltz (1984) found that a group of psychiatric inpatients scored higher on all 18 traits than did a mixed group of epileptics and that within the mixed epilepsy group itself, the temporal lobe epileptics had a significantly higher score than the grand mal epileptics on only 1 trait (i.e., hypergraphia). Bear, Levin, Blumer, Chethan, and Ryder (1982) found that temporal lobe epileptics scored significantly higher on 7 traits (i.e., viscosity, circumstantiality, religiosity, philosophical interest,
humorlessness, paranoia, and hypermoralism) compared to psychiatric patients, but Mungas (1982) found no significant differences on any of the traits between a group of temporal lobe epileptics with a diagnosed psychiatric illness and a group of nonepileptic psychiatric controls. Finally, Hermann and Reil (1981) compared temporal lobe epileptics to grand mal epileptics and found significantly higher scores in the temporal lobe epileptics on only 4 of the 18 traits (i.e., sense of personal destiny, dependence, paranoia, and philosophical interest).

The Bear-Fedio Inventory has been criticized, largely because it does not reliably differentiate between temporal lobe epileptics and other neurological or psychiatric patients (e.g., Adamec, 1990; Devinsky, 1991; Dodrill & Batzel, 1986) and because the original Bear-Fedio validation study (1977) involved both small samples (i.e., \( n_s = 12 \)) and a sample of temporal lobe epileptics who were particularly prone to psychiatric problems. However, despite these problems, studies using the Bear-Fedio Inventory have confirmed that temporal lobe epileptics do differ from healthy control subjects in their behavioural traits and that the difference is primarily emotional—even though there is no general consensus on the exact nature of these emotional differences. Particularly problematic is the widely-held belief that increased emotionality in temporal lobe epileptics makes them prone to outbursts of aggression (see Blumer & Benson, 1982; Fenwick, 1991). This belief persists despite a paucity of empirical support (Gloor, 1992) and its adverse social repercussions for temporal lobe epileptics.

In summary, there has been little progress in the study of the interictal emotional disturbances associated with temporal lobe epilepsy: The fundamental nature of the disturbances, the factors that influence their development and expression, and their neural basis are still unknown. This lack of progress is largely attributable to problems inherent in the study of epileptic patients. First, because temporal lobe epilepsy is difficult to diagnose, many temporal lobe epileptics are initially diagnosed as grand mal epileptics (LaPlante, 1993). This may lead to confusion in studies comparing interictal behavioral changes in
temporal lobe epileptics and grand mal epileptics. Second, many patients diagnosed with temporal lobe epilepsy also experience other types of seizures, making it difficult to select a homogeneous group of “pure” temporal lobe epileptics for study (Dodrill & Batzel, 1986). Third, most studies of interictal disturbances in temporal lobe epileptics are fraught with methodological problems: For example, in many cases, the control subjects are grand mal epileptics whose seizures likely invade limbic regions, and in almost all cases, behaviour is examined at only one moment in time instead of during the entire course of epileptogenesis (see Devinsky, 1991). Fourth, the interictal emotional disturbances associated with temporal lobe epilepsy per se are often clouded by the side effects of anticonvulsant medication (see Table 2), by the experience of suffering from a disorder that is both traumatic and unpredictable, and from the emotional impact of the social stigma that is attached to it (Engel, 1989). And finally, the diffuseness and variability of the structural and functional brain pathology in temporal lobe epileptics (Armstrong, 1993; de Lanerolle, Kim, Robbins, & Spenser, 1989; Gloor, 1992) makes it difficult to link the emotional changes associated with temporal lobe epilepsy to changes in particular cerebral structures. Consequently, the availability of a useful animal model of interictal changes in emotional behaviour would greatly facilitate the study of the interictal emotional disturbances associated with temporal lobe epilepsy. To that end, the kindling model of temporal lobe epilepsy was used in the experiments in this thesis—it is described in the next section.

THE KINDLING MODEL OF TEMPORAL LOBE EPILEPSY

Periodic administration of initially subconvulsive stimulations to certain brain structures results in the development and progressive intensification of elicited motor seizures—this phenomenon has been termed kindling (Goddard, McIntyre, & Leech, 1969). Kindling is the most widely studied model of temporal lobe epilepsy. It can be induced by a variety of different convulsive agents, delivered either diffusely or focally to particular brain loci. For example, it has been induced by electrical or chemical stimulation of the pyriform
### Table 2. Behavioural Side Effects of Anticonvulsant Medication

<table>
<thead>
<tr>
<th>Drug</th>
<th>Side Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetylureas</td>
<td>Confusional states, depression, personality change, psychoses with thought disorder</td>
</tr>
<tr>
<td>Barbiturates</td>
<td>Drowsiness, dysarthria, excitation, hyperactivity</td>
</tr>
<tr>
<td>Phenobarbital</td>
<td></td>
</tr>
<tr>
<td>Methylphenobarbital</td>
<td></td>
</tr>
<tr>
<td>Benzodiazepines</td>
<td>Aggression, anorexia, confusion, depression, drowsiness, dysarthria, hallucinations, irritability</td>
</tr>
<tr>
<td>Clonazepam</td>
<td></td>
</tr>
<tr>
<td>Clorazepate</td>
<td></td>
</tr>
<tr>
<td>Lorazepam</td>
<td></td>
</tr>
<tr>
<td>Diazepam</td>
<td></td>
</tr>
<tr>
<td>Carbamazepine</td>
<td>Anorexia, depression, dizziness, drowsiness, psychosis</td>
</tr>
<tr>
<td>Hydantoins</td>
<td>Anorexia, dementia, dizziness, drowsiness, dysarthria, hyperreactivity, organic brain syndrome, personality change, progressive encephalopathy</td>
</tr>
<tr>
<td>Phenytoin</td>
<td></td>
</tr>
<tr>
<td>Ethotoin</td>
<td></td>
</tr>
<tr>
<td>Oxazolidinediones</td>
<td>Anorexia, dizziness, drowsiness, irritability, personality change</td>
</tr>
<tr>
<td>Paramethadione</td>
<td></td>
</tr>
<tr>
<td>Trimethadione</td>
<td></td>
</tr>
<tr>
<td>Succinimides</td>
<td>Anorexia, dizziness, drowsiness, insomnia, irritability, personality change, psychosis</td>
</tr>
<tr>
<td>Valproate</td>
<td>Aggression, depression, hyperactivity, psychosis</td>
</tr>
</tbody>
</table>

cortex, amygdala, entorhinal cortex, ventral hippocampus, olfactory bulb, septum, caudate, and anterior cortex in such species as frogs, mice, gerbils, rats, rabbits, cats, dogs, rhesus monkeys, and baboons (McNamara, Byrne, Dasheiff, & Fitz, 1980; Racine, 1978). However, despite the variety of agents, structures, and species that can be kindled, it has been most frequently studied in rats subjected to daily electrical stimulations of the amygdala.

At first, electrical stimulation of the amygdala at an intensity sufficient to evoke afterdischarges (i.e., sufficient to evoke epileptic spiking in the EEG record that outlasts the stimulation) elicits little or no behavioural response. Then, with each subsequent
stimulation, the afterdischarges at the site of stimulation last longer and generalize further from the site of stimulation, and motor seizures begin to accompany them. After about 15 periodic stimulations, rats respond reliably to each stimulation with a generalized clonic convulsion, which is characterized in sequence by jaw clonus, head clonus, forelimb clonus, rearing, and falling (McNamara, 1988; Racine, 1972) and has a duration of about 40 s (Kalynchuk, Kippin, Pinel, & McIntyre, 1994; Pinel, Mana, & Kim, 1986; Pinel & Rovner, 1978a).

There are three particularly interesting features of kindling. The first is that the neural changes underlying kindling are enduring, if not permanent. Once an animal has been kindled (i.e., once it displays three consecutive generalized convulsive seizures), it will continue to respond to each stimulation with a generalized convulsion even after a stimulation-free period lasting several months in rats (Goddard et al., 1969). The second is that kindling is produced by distributed, as opposed to massed, stimulations. The rate of kindling (i.e., the number of stimulations required to induce a generalized motor seizure) declines considerably if the interval between stimulations is less than an hour, and no kindling at all may occur if the stimulations are administered every few minutes (Racine, Burnham, Gartner, & Levitan, 1973). The third interesting feature of kindling is that kindling one site in the brain will usually facilitate kindling of another site in the same brain (Cain, 1986; Racine, 1972). This “transfer” effect suggests that the permanent neural changes underlying kindling include synaptic changes that occur far from the originally-stimulated site. This idea is supported by lesion studies: Racine (1972) showed that kindling transfers even after the original kindling site has been lesioned.

Several lines of evidence support the view that rats kindled by amygdala stimulation are valid models of human temporal lobe epilepsy. First, drug effects on amygdala-kindled convulsions in rats are predictive of drug effects on complex partial seizures in humans (Racine & Burnham, 1984; Löscher, Jackal, & Czuczwar, 1986). Second, kindled rats display patterns of cell loss (Cavazos, Das, & Sutula, 1994; Scharfman & Schwartzkroin,
1990; Sloviter, 1987) and mossy fibre sprouting (Sutula, 1990; Wasterlain & Shirasaka, 1994) similar to those observed in the brains of human temporal lobe epileptics. And third, extensive kindling (e.g., 250 stimulations in rats) ultimately results in the recurrence of spontaneous motor seizures, which is the defining feature of clinical epilepsy (Pinel, 1981; Wada & Osawa, 1976). Kindling is particularly useful for studying epileptogenesis because animals can be studied at particular stages in the kindling process, up to and including the emergence of spontaneous motor seizures. Accordingly, several investigators interested in the interictal emotional disturbances associated with temporal lobe epilepsy have studied the changes in interictal emotional behaviour that accompany amygdala kindling (see Adamec, 1990; Depaulis, Helfer, Deransart, & Marescaux, in press).

**DEFENSIVE BEHAVIOUR IN ANIMALS AS A MEASURE OF EMOTION**

Increases in fear and anxiety are the behavioural sequelae of temporal lobe epilepsy that are most often modelled in laboratory animals using the kindling model of temporal lobe epilepsy. There are three primary reasons for this: Hyperemotionality is thought to underlie the majority of behavioural problems experienced by temporal lobe epileptics (Bear, 1979); fear and anxiety are the most prominent emotional problems reported in temporal lobe epileptics (Dodrill & Batzel, 1986); and there are numerous behavioural paradigms available for modeling human fear and anxiety in rats. The purposes of this section of the General Introduction are the following: to explain some of the early difficulties that were encountered in modeling human fear and anxiety in rats, to explain how these difficulties have been largely resolved, and to introduce some of the measures that were employed in the present experiments.

Most efforts to model human fear and anxiety in laboratory animals stem from Charles Darwin's (1872) conclusion that the human emotions of fear and anxiety are manifested in the form of defensive behaviour. Consequently, most efforts to infer heightened levels of fear or anxiety in laboratory animals have involved demonstrations of
elevated levels of defensive behaviour. For example, increases in fear and anxiety have been inferred from such defensive behaviours as increased burying of the shock source in the defensive burying paradigm (Pinel & Treit, 1978; Pinel, Symons, Christensen, & Tees, 1989; Pinel, Mumby, Dastur, & Pinel, 1994), decreased explorations in the hole-board test (File & Wardill, 1975), decreased contact with conspecifics in the social-interaction test (File & Hyde, 1978), decreased open-arm exploration in the elevated-plus-maze test (Hogg, 1996; Pellow, Chopin, File, & Briley, 1985; Rodgers & Cole, 1994; Treit, 1985), increased reactivity in the acoustic startle and fear-potentiated startle tests (Davis, Hitchcock, & Rosen, 1987; Davis, 1992), and decreased exploration in the open-field test (Kalynchuk, Pinel, Treit, & Kippin, in press; Takahashi, Kalin, Vanden Burgt, & Sherman, 1989; Walsh & Cummins, 1976). These tests all measure an animal's behavioural response to a threatening stimulus, whether it be the source of an electric shock, an unfamiliar conspecific, or a novel situation.

Attempts to model the increases in fear and anxiety associated with temporal lobe epilepsy in laboratory animals have until recently been complicated by the lack of a general consensus about whether particular agonistic behaviours of laboratory animals are fundamentally aggressive or defensive. Indeed, until recently, this issue was largely ignored. However, over the last decade, it has become apparent that there are important differences between aggressive and defensive behaviours and that each comes in many different forms (see Table 3). It is critical to distinguish among the various types of defensive and aggressive behaviours because each serves a different function, has a different topography, occurs in different situations, and is mediated by different neural circuits (e.g., Blanchard & Blanchard, 1988; Albert, Walsh, & Jonik, 1993).

From a general functional perspective, the difference between aggressive and defensive behaviours is clear: The primary function of aggressive behaviour is to threaten or harm other animals, whereas the primary function of defensive behaviour is protection from threat or harm. Although this difference is clear at a conceptual level, it is in practice not
Table 3. Categories of Aggressive and Defensive Behaviour in the Rat

<table>
<thead>
<tr>
<th>Aggressive Behaviour</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Predatory Aggression</td>
<td>The stalking and killing of members of other species for the purpose of eating them. Rats kill prey, such as mice and frogs, by delivering bites to the back of the neck.</td>
</tr>
<tr>
<td>Social Aggression</td>
<td>Unprovoked aggressive behaviour that is directed at a conspecific for the purpose of establishing, altering, or maintaining a social hierarchy. In mammals, social aggression occurs primarily among males. In rats, it is characterized by piloerection, lateral attack, and bites directed at the defender's back.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Defensive Behaviour</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intraspecific Defense</td>
<td>Defense against social aggression. In rats, it is characterized by freezing and flight and by various behaviors, such as boxing, that are specifically designed to protect the back from bites.</td>
</tr>
<tr>
<td>Defensive Attacks</td>
<td>Attacks that are launched by animals when they are cornered by threatening conspecifics or members of other species. In rats, they include lunging, shrieking, and biting attacks that are usually directed at the face of the intruder.</td>
</tr>
<tr>
<td>Freezing and Flight</td>
<td>Responses that many animals use to avoid attack. For example, if a human approaches a wild rat, it will often freeze until the human penetrates its safety zone, whereupon it will explode into flight.</td>
</tr>
<tr>
<td>Maternal Defensive Behaviors</td>
<td>The behaviors by which mothers protect their young. Despite their defensive function, they are similar to male social aggression in appearance.</td>
</tr>
<tr>
<td>Risk Assessment</td>
<td>Behaviors that are performed by animals in order to obtain specific information that helps them defend themselves more effectively. For example, rats that have been chased by a cat into their burrow do not emerge until they have spent considerable time at the entrance scanning the surrounding environment.</td>
</tr>
<tr>
<td>Defensive Burying</td>
<td>Rats and other rodents spray sand and dirt ahead with their forepaws to bury dangerous objects in their environment, to drive off predators, and to construct barriers in their burrows.</td>
</tr>
</tbody>
</table>

always easy to categorize agonistic behaviour as aggressive or defensive on this basis. For example, consider the behaviour of rats in the shock-elicited aggression paradigm (i.e., Powell & Creer, 1969). When two rats are placed in a box with a grid floor and given noncontingent shocks, they rear up on their hindlegs facing one another and push at one another with their forepaws—a behaviour often referred to as “boxing.” The initial assumption was that this boxing behaviour is aggressive, and the behaviour was termed “shock-elicited aggression”; however, there is now compelling evidence that boxing behaviour is fundamentally defensive.

Using an ethoexperimental approach, Blanchard and Blanchard (e.g., 1988; 1990) have illustrated the variety of rat aggressive and defensive behaviours by describing the interactions of a large male rat and a small male rat placed in a colony cage in which the large male has already established itself as the alpha male (i.e., the dominant male of the colony). The premise underlying this resident-intruder paradigm is that the behaviour of the large resident will be primarily aggressive and the behaviour of the small intruder will be primarily defensive. The interaction between the two rats typically begins with the resident sniffing the perianal area of the intruder and chasing it around the cage. The intruder, unable to escape from the apparatus, eventually turns to face the resident and rears up on its haunches in a “boxing” posture. Then, the resident approaches the intruder sideways (lateral approach) so that it is in a position to make a darting attack around the intruder to deliver a bite to its back, which is the target site of all aggressive social attacks in rats. The intruder typically defends itself by pivoting on its hindlegs and fending off the resident with its forepaws (i.e., by boxing); however, if sufficiently harassed, it will launch a shrieking, biting, defensive jump attack directly at the face of the resident, which is somewhat protected from the attack by its own sideways attack posture. Accordingly, during agonistic social interactions between male rats, sideways approach and back bites are aggressive; whereas rearing, boxing, jump attacks, and face bites are defensive. In contrast, predatory bites of rats are always directed at the back of the neck of the prey (Blanchard, Brain,
Blanchard, & Parmigiani, 1989). The resident-intruder paradigm was used in Experiment 6 of this thesis to characterize the fundamental nature of kindling-induced changes in emotional behaviour.

A second line of recent research that has illustrated the variety of defensive responses in the rat involves placing them alone in a threatening environment and carefully observing their behaviour—the threat is typically generated by exposing the rat to a cat in the same environment before the test (Blanchard, Blanchard, & Rodgers, 1990), by shocking the rat in the environment (Pinel & Treit, 1978), or by testing the rat in an environment that is both large and unfamiliar (Kalynchuk et al., in press). Rats placed directly into such environments usually flee if they have the opportunity; if fleeing is not possible, they usually freeze.

A rat that has initially fled will eventually return to the testing environment, and a rat that has initially frozen will eventually begin to move about in the test environment. In both instances, the rats engage in a variety of approach behaviours whose function appears to be risk assessment (Blanchard & Blanchard, 1988)—the function of risk assessment behaviours is to identify the nature of the threat so that the rat can select the most appropriate defensive alternatives. The rat begins by slowly exploring the apparatus in a “stretched approach” posture (e.g., Pinel, Mana, & Ward, 1989), staying as close to the walls as possible (i.e. thigmotaxis, see Treit & Fundytus, 1988). If a source of potential risk is located, the rat will direct a series of “approach-avoidance” sequences (Blanchard & Blanchard, 1988) at it. If no direct threat to the rat occurs, the risk assessment behaviours subside and the rat’s behaviour returns to normal. The discovery that risk assessment behaviours constitute an important component of the rat’s defensive repertoire was important because it disproved the widely-held assumption that approach behaviours are always indicative of positive affect (Blanchard & Blanchard, 1988).

In conclusion, although the study of aggressive and defensive behaviour has been characterized by a great deal of confusion, recent studies--only a few of which have been
described here—have greatly increased our understanding of differences between aggressive and defensive behaviours and among their various forms. Consequently, the use of animal defensive behaviours as components of animal models of the hyperemotionality associated with temporal lobe epilepsy now stands on much firmer ground than it did a decade or two ago.

THE ROLE OF THE AMYGDALA IN EMOTIONAL BEHAVIOUR

With the recent characterization of the differences between defensive and aggressive behaviour has come a greatly enhanced understanding of the neural circuits involved in fear and anxiety. This purpose of this section of the General Introduction is to briefly present some evidence implicating the amygdala in the mediation of emotional behaviour and to discuss how the amygdala might accomplish this task in conjunction with other brain structures.

The importance of the amygdala in the mediation of emotional behaviour was first recognized in the mid 20th century, when in 1956, Weiskrantz reported that the dramatic changes in emotional behaviour seen in the Kluver-Bucy syndrome (Kluver & Bucy, 1937) were largely attributable to amygdala damage. The Kluver-Bucy syndrome is characterized by tameness, indifference, hypersexual activity, and “psychic blindness” (i.e., visual agnosia) in monkeys. Although Kluver believed that the Kluver-Bucy syndrome was caused by damage to the entire temporal lobe, Weiskrantz (1956) showed that amygdalar damage was responsible for most of the emotional changes associated with the syndrome, a finding that has been repeatedly confirmed (e.g., Downer, 1961; Horel, Keating, & Misantone, 1975). Weiskrantz’s discovery had a major impact because neither of the two theories of the neural basis of emotion that were influential at the time included a role for the amygdala: Cannon and Bard had proposed that emotion was mediated by the hypothalamus and cerebral cortex (Bard, 1929; Cannon, 1931) and Papez (1937) had proposed the existence of
a neural circuit of emotion involving the hypothalamus, anterior thalamus, cingulate cortex, and hippocampus (i.e., the Papez Circuit).

Since the initial studies of the contribution of amygdala damage to the Kluver-Bucy syndrome, the major role of the amygdala in emotional behaviour has been confirmed many times (e.g., Davis, 1992; Graeff, 1993; LeDoux, 1992). For example, electrical stimulation of the amygdala in humans has been found to elicit feelings of fear or anxiety with little or no anger (Halgren, 1981); and in several species of laboratory animals, it has been found to produce behavioural changes that are similar to those produced by stressful or fearful stimuli (Davis et al., 1994). In addition, bilateral lesions of the amygdala in rats have been found to abolish conditioned fear (e.g., Coover, Murison, & Jellestad, 1992; Hitchcock & Davis, 1986; Kopchia, Altman, & Commissaris, 1992; Miczek, Kelsey, & Grossman, 1972), increase exploratory behaviour in novel situations (Grijalva, Levin, Morgan, Roland, & Martin, 1990), decrease plasma corticosterone levels after restraint stress (Beaulieu, DiPaolo, & Barden, 1986), and attenuate fear-induced increases in heart rate (Kapp, Frysinger, Gallagher, & Haselton, 1979). Moreover, bilateral infusions of benzodiazepine anxiolytic drugs into the rat amygdala have been found to reduce fear-related behaviour on the elevated plus maze (Pesold & Treit, 1995) and in a punished-responding test (Katoaka, Shibata, Yamashita, & Ueki, 1987). Thus, the current and most popular model of fear implicates the amygdala, but models implicating other neural structures do exist (e.g., the septo-hippocampal system, see Gray, 1982).

How does the amygdala mediate emotional behaviour? The current hypothesis is that the amygdala integrates complex sensory input and directs other brain structures to respond with an appropriate amount of emotional arousal (see Figure 1). Sensory input enters the basolateral and lateral nuclei of the amygdala (i.e., the basolateral complex) via the entorhinal, perirhinal, and secondary sensory cortices, the thalamus (LeDoux, Cicchetti, Xagoraris, & Romaski, 1991), and the subicular region of the hippocampus (Phillips & LeDoux, 1991). The basolateral complex contains neurons with a high density of dendritic
Figure 1. Schematic drawing of inputs and outputs of amygdalar nuclei thought to be involved in mediating emotional behaviour. (Abbreviations: La, lateral amygdaloid nucleus; BLA, basolateral amygdaloid nucleus; Ce A, central amygdaloid nucleus; PAG, periaqueductal gray).
spines, which are capable of integrating a wide array of synaptic input (Davis et al., 1994). Once the afferent input has been integrated, the basolateral complex directs the output via a reciprocal activation of cortical regions, and unidirectional activations of the caudate nucleus, nucleus accumbens, and central nucleus of the amygdala. The output to various cortical regions is thought to be involved in the conscious perception of fear or anxiety, whereas the output to the caudate and nucleus accumbens is thought to relay motivationally significant information to motor areas to initiate motor responses (Davis et al., 1994). The spine-sparse neurons of the central nucleus project to the lateral hypothalamus and several brain stem regions capable of initiating autonomic and somatic components of the fear reaction. The central nucleus also sends output to the paraventricular nucleus of the hypothalamus (PVN) and to the periaqueductal gray (PAG) (Siegel, 1991). The output to the PVN organizes neuroendocrine responses to fearful or stressful stimuli (Schulkin, McEwen, & Gold, 1994), whereas the output to the PAG organizes defensive responses to unfamiliar stimuli (Graeff, Silveira, Nogueira, Audi, & Oliveira, 1993; LeDoux, Iwata, Cicchetti, & Reis, 1988).

The identification of a defense pathway between the amygdala and the PAG has supported the hypothesis that the neurotransmitter serotonin (5-HT) plays a key role in mediating emotional behaviour such as fear and defense (e.g., Graeff, 1993; Graeff, Guimaraes, Andrade, & Deakin, 1996; Handley, McBlane, Critchley, & Njung’e, 1993). Both the amygdala and the PAG are innervated by 5-HT-containing fibres originating from the dorsal raphé nucleus. The axons projecting to the amygdala follow the dorsal raphé-forebrain tract, whereas those projecting to the PAG and medial hypothalamus run through the dorsal raphé-paraventricular tract (Azmitia, 1978). Lesions that reduce 5-HT output in these areas have anxiolytic effects in a punished-responding paradigm (Tye, Everitt, & Iversen, 1977). Similarly, administration of the 5-HT1A receptor agonist 8-OH-DPAT into the dorsal PAG, which decreases 5-HT output, produces anxiolytic effects in a social interaction test (Hogg, Andrews, & File, 1994) and in footshock-induced ultrasonic
vocalizations (Schreiber, & Devry, 1993). Thus, there is anatomical and pharmacological evidence for the participation of 5-HT in the mediation of fear and defensive behaviour by the amygdala and PAG. In Experiment 7 of this thesis, the hypothesis that 5-HT plays a role in kindling-induced changes in emotional behaviour was investigated.

In summary, there is now clear evidence that the amygdala plays an important role in mediating emotional behaviour. The amygdala also plays a key role in the initiation and maintenance of temporal lobe seizures (Gloor, 1992; Weiser, 1983). Thus, excitation of the amygdala is a potential mechanism involved in the increased emotionality observed in temporal lobe epileptics.

**BEHAVIOURAL EFFECTS OF AMYGDALA KINDLING**

Given the involvement of the amygdala in both temporal lobe seizures and emotional behaviour, amygdala kindling is an obvious means of modelling the interictal hyperemotionality associated with temporal lobe epilepsy, and consequently there have been several studies of the effects of amygdala kindling on emotional behaviour in animals.

Adamec was the first to document the effects of kindling on interictal emotional behaviour (Adamec, 1976). Adamec found that partial kindling (i.e., kindling that produces afterdischarges but no convulsions) of the amygdala or ventral hippocampus in cats results in behavioural changes that appear to be independent of convulsions or interictal spiking for their maintenance (Adamec, 1990). After partial kindling, cats displayed increased defensive responses when they were exposed to rats, mice, and conspecific threat vocalizations (Adamec, 1976) or when they received electrical stimulations of the ventromedial hypothalamus (Siegel, 1984). These behavioural changes were reversible, lasting from several days to several weeks (Adamec, 1990), and could be blocked by low doses of flumazenil, a benzodiazepine receptor antagonist (Adamec, 1993). Interestingly, flumazenil normally has no anxiolytic effect on cat behaviour; its anxiolytic effects appear only after partial kindling.
More recently, two groups have begun to study the effects of partial amygdala kindling on emotional behaviour in rats. Helfer, Deransart, Marescaux, and Depaulis (in press) found that partial amygdala kindling decreased the percentage of open-arm activity in an elevated plus maze, and Rosen, Hamerman, Sitcoske, Glowa, and Schulkin (1996) found that partial amygdala kindling exaggerated conditioned fear-potentiated startle.

Taken together, the studies of partial kindling in cats and rats have provided important data regarding the effects of repeated seizures on defensive behaviour; the advantage of such studies of partial kindling is that they assess behavioural changes at a stage in kindling before afterdischarges have become generalized. However, because the animals in these studies were only partially kindled, it is unclear how directly their findings relate to temporal lobe epilepsy.

Pinel, Treit, and Rovner (1977) were the first to document amygdala-kindling-induced increases in interictal emotional behaviour in rats. In their experiment, rats received 99 stimulations of the amygdala, hippocampus, or caudate and their defensive response to a pencil tap on the back and their resistance to capture were assessed. The amygdala- and hippocampal-kindled rats displayed a significantly greater response to tail tap and greater resistance to capture than the sham-stimulated control rats, but the caudate-kindled rats did not.

Since the seminal experiments of Adamec and of Pinel and his colleagues, most investigators interested in the interictal emotional disturbances associated with temporal lobe epilepsy have studied the changes in emotional behaviour that accompany short-term amygdala kindling (see Adamec, 1990). Short-term kindling refers to a protocol in which animals receive enough stimulations to induce three consecutive generalized convulsions, usually between 15 and 25 in the case of amygdalar stimulations in rats. The subjects, typically rats, are tested at least 24 hr after the final kindling stimulation, in order to be sure that any apparent behavioural differences are interictal and not due to postictal electrical activity. Using this protocol, amygdala kindling in squirrel monkeys has been shown to
cause increases in defensiveness and social withdrawal (Lloyd, Kling, & Ricci, 1989); in cats, it has been shown to decrease the threshold to electrically elicit defensive hissing and growling (Hiyoshi, Matsuda, & Wada, 1990) and in rats, it has been shown to decrease exploratory behaviour (Adamec & Morgan, 1994; Helfer et al., in press; Nieminen, Sirvio, Teittinen, Pitkanen, Airaksinen, & Riekkinen, 1992), decrease the latency to muricide in spontaneous mouse-killers (McIntyre, 1978), increase corticotrophin releasing-factor-induced defensive fighting (Weiss, Post, Gold, Chrousos, Sullivan, Walker, & Pert, 1986), and increase stress-induced stomach ulcers (Henke & Sullivan, 1985).

Kindling-induced alterations in interictal emotional behaviour appear to be specifically related to changes in fear or anxiety. No differences have been found between short-term amygdala-kindled rats and control rats on tests of depression, such as the sucrose-preference or the forced swimming tests (Helfer et al., in press), or on tests of memory, such as the Morris water maze (Nieminen et al., 1992; Holmes, Chronopoulos, Stafstrom, Mikati, Thurber, Hyde, & Thompson, 1992) or radial arm maze (Letty, Lerner-Natoli, & Rondouin, 1995). Furthermore, the changes in emotional behaviour observed in kindled rats do not appear to be due to either a general increase or decrease in motoric activity (Adamec & Morgan, 1994; Helfer et al., in press).

Although the results of studies of amygdala-kindling-induced changes in interictal behaviour have consistently shown increases in emotional behaviour, they have not been consistent with respect to the precise nature of the emotional changes produced by kindling. For example, the effects of short-term kindling in rats have been inconsistent with respect to behaviour on the elevated plus maze--left basolateral-amygdala kindling has been associated with both anxiolytic (Adamec & Morgan, 1994) and anxiogenic (Nieminen et al., 1992) effects. Neither of these authors has offered an explanation for this discrepancy.

The neural mechanisms underlying kindling-induced interictal emotionality remain largely unknown. The emotionality does not appear to be related to interictal spiking or any other aberrant interictal electrical activity (Adamec, 1990; Helfer et al., in press). However,
there are many other speculative ideas: lasting hyperexcitability via long-term potentiation of amygdala neurons (Adamec, in press); activation of a defense pathway between the central nucleus of the amygdala and the periaqueductal gray (Adamec, 1993; Depaulis et al., in press; Helfer et al., in press); functional modification of the benzodiazepine binding site on the GABA<sub>A</sub> complex (Adamec, 1993); over-production of the stress neuropeptide corticotrophin releasing-factor (Weiss et al., 1986); and withdrawal from the effects of opioid peptides that are released during generalized seizures (Engel & Rocha, 1986). However, no direct evidence linking kindling-induced emotionality to any of these changes has been provided in support of these hypotheses.

**RATIONALE AND GENERAL PURPOSES**

The three general purposes of this thesis were to demonstrate the potential of long-term amygdala kindling as a model of the interictal hyperemotionality associated with temporal lobe epilepsy, to provide some parametric data about the interictal hyperemotionality, and to use the model to clarify the nature of the interictal hyperemotionality. Although amygdala kindling itself is widely regarded as a model of temporal lobe epilepsy, the ability of long-term amygdala-kindling-induced changes in emotional behaviour to model the interictal hyperemotionality of temporal lobe epilepsy is the focus of this thesis.

The experiments comprising this thesis are studies of the effects of long-term (i.e., 100 stimulations) amygdala kindling on emotional behaviour in rats. Why would I choose to study long-term, as opposed to partial or short-term, kindling in rats? The reason is that kindling is a progressive disorder that is far from complete once three consecutive generalized convulsions have been elicited: Although rats who display three consecutive generalized convulsions in response to stimulation are often said to be "fully kindled,” if the programme of stimulations is continued, the severity of their motor seizures increases (i.e., multiple fits of rearing and falling, running fits, and tonic seizures develop), interictal
epileptic spikes begin to punctuate the EEG records, and after about 250 stimulations, motor seizures begin to recur spontaneously (e.g., Pinel, 1981). This suggested to me that long-term kindling might produce some changes in interictal emotional behaviour that are not apparent after short-term kindling and some changes that are larger and more reliable than those that are apparent after short-term kindling. I selected 100 stimulations as the standard treatment because my experience with long-term kindling suggested that 100 stimulations would be enough to guarantee that all subjects would be well kindled, but not so well kindled that they would be displaying spontaneous convulsions (i.e., motor seizures), which would confound the behavioural testing. In short, the studies comprising this thesis are based on the premise that long-term amygdala kindling is likely to be superior to short-term amygdala kindling as a model of the interictal hyperemotionality associated with temporal lobe epilepsy.

What constitutes a good animal model of a human disorder? There are three types of animal models of human disorders: homologous, isomorphic, and predictive (Kornetsky, 1977). Homologous animal models duplicate human disorders in every respect; the etiology, symptoms, and prognosis of a homologous model resemble those of the human disorder. They serve as a basis for studying all aspects of a disorder, including its causes. Isomorphic animal models resemble human disorders, but are artificially produced in the laboratory in a way that does not reflect normal etiology. They permit predictions and the study of underlying neural mechanisms. Finally, predictive animal models do not resemble the human disorder, but they are of value in predicting some aspects of the disorder, such as the potential of drug therapies to reduce the disorder. Most animal models of behavioral disorders are predictive with a few key isomorphic components: In general, unless a model is at least partially isomorphic, it cannot be used to study the mechanisms of the disorder.

Developing an animal model is like exploring a section of an unknown maze. One enters an unfamiliar section with little more than a hope that its exploration will prove fruitful, and it is only after each of its arms has been carefully explored that it is possible to
know whether the decision to enter the section was wise. In the same way, it is not possible to evaluate the potential of an animal model until it has been thoroughly explored. Each of the seven experiments in this thesis is relevant to each of its three purposes. In developing an animal model of a human behavioural disorder that is not well understood, each time that one demonstrates a similarity between the model and what is currently believed about the disorder, one increases the validity of the model as well as providing a clearer picture of the disorder. However, as this thesis progresses, the experiments become less and less concerned with establishing the validity of the model and more and more concerned with using the model to clarify the nature of the disorder. Accordingly, Experiment 1 was designed to establish that long-term amygdala kindling results in large and reliable changes in emotionality; Experiment 2 was designed to determine the effect of stimulation number on the development of these changes; Experiment 3 was designed to assess the persistence of these changes after the cessation of kindling; Experiment 4 was designed to determine the effect on emotionality of kindling different brain sites; Experiments 5 and 6 were designed to characterize the fundamental nature of the changes in emotional behavior; and Experiment 7 was designed to investigate changes in binding to serotonin 1A receptors in the brains of long-term amygdala-kindled rats.
GENERAL METHOD

This section describes the methods common to all seven experiments. Any variations in this general methodology are described in the Method sections of the relevant experiments.

SUBJECTS

The subjects in all seven experiments were male Long-Evans rats (Charles River, Canada) weighing between 250 and 350 g at the time of surgery. They were individually housed in steel hanging cages in a colony room with an ambient temperature of about 21 degrees C and a 12:12-hr light:dark cycle (lights on at 8:00 a.m.). Purina rat chow and water were available continuously. All experimental manipulations occurred during the light phase of the light-dark cycle.

SURGERY

A single bipolar stimulating electrode (Plastic Products Company, MS-303-2) was implanted in the appropriate brain region of each rat under sodium pentobarbital anesthesia (65 mg/kg, i.p.). Amygdalar electrodes were aimed at a site 2.8 mm posterior, 5.0 mm left, and 8.5 mm ventral to the skull surface at bregma; hippocampal electrodes were aimed at a site 5.7 mm posterior, 5.0 mm left, and 5.5 mm ventral to the skull surface at bregma; and caudate electrodes were aimed at a site 0.2 mm anterior, 3.2 mm left, and 5.7 mm ventral to the skull surface at bregma. All coordinates were taken from the Paxinos and Watson (1986) stereotaxic atlas. Each electrode was secured to the skull with four stainless steel screws and dental acrylic. Powdered tetracycline was sprinkled on the incision before suturing to reduce the risk of infection.
**Kindling Protocol**

Following a postsurgical recovery period of at least 12 days, each rat was stimulated three times per day, 5 days per week. The total number of stimulations varied in each experiment. Some of the rats received convulsive stimulations (1 sec, 60 Hz, 400 μA) and some received an equal number of sham stimulations. A few seconds prior to each convulsive stimulation, each rat was placed in a plastic box (58 x 58 x 25 cm) containing a thin layer of commercial bedding material, and the stimulation lead was attached. Once all convulsive activity had ceased, each rat was returned to its home cage. Rats receiving sham stimulations were treated in exactly the same way except that no current was delivered. There was a minimum of 2 hr between consecutive stimulations.

As is usual (see Pinel & Rovner, 1978a), the initial convulsive stimulations produced no behavioural response other than a momentary behavioural arrest; but after 10 days, almost every stimulation elicited a clonic convulsion characterized by facial clonus, forelimb clonus, rearing, and loss of equilibrium. The measure of convulsion severity was the convulsion class elicited by each stimulation. Convulsion class was scored according to Pinel and Rovner's (1978a) extension of Racine's (1972) widely used 5-class scale (class 1: head nodding only; class 2: head nodding and jaw clonus; class 3: head nodding, jaw clonus, and forelimb clonus; class 4: head nodding, jaw clonus, forelimb clonus, and rearing; class 5: head nodding, jaw clonus, forelimb clonus, rearing, and falling once; class 6: a class 5 with multiple rearing and falling episodes; class 7: a class 6 with running fits; class 8: any of the preceding symptoms with periods of tonus).

**Apparatus**

Most of the behavioural testing was conducted in one of two pieces of apparatus: an open field or an elevated plus maze.

**Open Field.** The open field was a 60 x 60 x 60 cm wooden box with no top. It was located in a small brightly lit testing room. For Experiment 1, it had a 2-cm layer of commercial
bedding on the floor; this bedding was removed for the other experiments and 36 identically-sized squares were marked on the floor with bright yellow tape. A video camera was mounted 2 m above the floor to record each rat’s open-field activity.

**Elevated Plus Maze.** The elevated plus maze was constructed of wood and painted black. It consisted of four 50 x 10 cm arms, two of which were open and two of which were enclosed on both the sides and the end by a 50-cm-high wall. The maze was mounted 50 cm above the floor in a testing room that was lit by a shaded 40 W light reflected off the back wall.

**BEHAVIOURAL TESTING**

The behavioural testing in the experiments in this thesis employed several tests of emotional behaviour—open-field exploration, resistance to capture, open-arm activity on the elevated plus maze, and resident-intruder interactions. These tests were chosen for several reasons: They are conventional and well-characterized tests of emotional behaviour in rats, they avoid the use of shock to induce a response, and with the exception of resident-intruder interactions, they have been used to assess emotional behaviour in previous studies of short-term kindling. Not all tests were used in each experiment. The specific tests that were used are described in the Method sections of the individual experiments.

**HISTOLOGY**

At the conclusion of each experiment, all subjects were sacrificed with CO$_2$ according to the Canada Council on Animal Care guidelines. Then, their brains were removed and preserved in formalin for at least 1 month. The brains were subsequently frozen and sliced along the coronal plane through the structure in which the electrode had been implanted: the amygdala, the hippocampus, or the caudate. Each slice was 30 μm thick, and every fifth slice was mounted on a slide and stained with cresyl violet. The position of each electrode tip was estimated from the stained slices using the Paxinos and Watson (1986) atlas.
In Experiment 7, the use of the receptor autoradiographic technique precluded a histological analysis of electrode position. Instead, the brains from the rats in this experiment were rapidly removed, frozen in dry ice and isopentane, and stored at -80 degrees Celsius until the receptor autoradiographical analysis was performed.

**STATISTICAL ANALYSES**

The statistical significance of the results in the experiments of this thesis was evaluated using both parametric and nonparametric techniques (Siegel & Castellan, 1988). The use of nonparametric analyses was necessary in some cases because some of the data were measured on an ordinal scale and because there was extreme differences in variability among the conditions—there was no variability whatsoever in some conditions. The specific tests used are outlined in the statistics section of each experiment. The level of significance was $p < .05$ for all comparisons. Only the data of those rats completing each experiment were subjected to statistical analyses.

**EXPERIMENT 1: THE EFFECT OF LONG-TERM AMYGDALA KINDLING ON EMOTIONAL BEHAVIOUR IN RATS**

In the only published study of long-term kindling and emotionality, Pinel et al. (1977) found that 99 amygdala stimulations greatly increased the resistance of rats to capture. The purpose of Experiment 1 was to replicate this finding and extend it in two ways. First, in addition to the single measure of emotionality that was recorded in the Pinel et al. experiment, the rats' behaviour on an elevated plus maze was also assessed—both resistance to capture (Albert & Richmond, 1975) and open-arm exploration of the elevated plus maze (Pellow et al., 1985; Treit, 1985) are widely recognized measures of emotionality in rats. Second, because the site of stimulation within the amygdala has been reported to

---

1 The results of Experiments 1 and 2 have been accepted for publication: Kalynchuk, L.E., Pinel, J.P.J., Treit, D., & Kippin, T.E. (in press). Changes in emotional behavior produced by long-term amygdala kindling in rats. Biological Psychiatry.
influence the emotional behaviour of rats subjected to short-term kindling (Adamec & Morgan, 1994), the effect of the site of stimulation (i.e., basolateral amygdala vs. central amygdala) on the emotional behaviour produced in rats by long-term kindling was assessed.

**METHOD**

**Kindling Protocol**

A bipolar electrode was implanted in the left amygdala of each of 48 rats. Following a postsurgical recovery period, some of the rats received a convulsive stimulation 3 times per day, 5 days per week, for 33 days \( n = 37 \), and some received an equal number (i.e., 99) of sham stimulations \( n = 11 \).

**Behavioural Testing**

**Resistance to Capture.** One day after the final kindling stimulation, each rat was placed by itself in the centre of the open field for 2 min while an experimenter who was naive to the experimental history of each rat and had not previously handled the rats sat quietly in the room out of sight of the rat. After the 2 min, the rat was picked up forcefully from above by the experimenter, who was wearing a leather glove that was unfamiliar to the rat. The rat's resistance to capture by the unfamiliar experimenter was scored according to the following 7-point scale adapted from Albert and Richmond (1975): 0 = easy to pick up, 1 = vocalizes or shies away from hand, 2 = shies away from hand and vocalizes, 3 = runs away from hand, 4 = runs away and vocalizes, 5 = bites or attempts to bite, 6 = launches a jump attack.

**Elevated Plus Maze.** One day after the resistance-to-capture test, each rat was tested on the elevated plus maze for 5 min. The rat was placed on the centre of the maze and its behaviour was recorded by an experimenter naive to the history of each rat. The experimenter sat quietly approximately 1 m from the maze. If a rat jumped or fell off one of the open arms, it was quickly picked up and returned to the place on the maze that it last occupied. The amount of time spent in each of the arms as well as the number of entries made into each of
the arms was recorded—an arm entry was defined as all four paws moving into an arm. From these measures, the two main indices of elevated-plus-maze activity were calculated: time spent on open arms as a percentage of time spent on all arms (i.e., percentage of open-arm time) and number of open-arm entries as a percentage of all arm entries (i.e., percentage of open-arm entries) (Pellow et al., 1985). In addition to these two main indices of elevated-plus-maze activity—percentage of open-arm entries and percentage of time on the open arms—the following measures of elevated-plus-maze activity were subjected to analysis: total number of arm entries, number of closed-arm entries, number of open-arm entries, time spent on the closed arms, and time spent on the open arms.

Statistical Analyses

The statistical significance of differences among the groups in their resistance to capture and in their elevated-plus-maze activity was evaluated nonparametrically using a Kruskal-Wallis one-way ANOVA by ranks test, followed by post hoc multiple comparisons (see Siegel & Castellan, 1988). The Kruskal-Wallis test was used because the resistance to capture data were measured on an ordinal scale and because there were large differences in variability among the conditions in elevated-plus-maze activity.

RESULTS

In this experiment, the amygdala-kindled rats displayed substantially more resistance to capture from the novel open field and a greater percentage of open-arm activity on the elevated plus maze than did the sham-stimulated rats.

Histology

Figure 2 illustrates the location of the electrode tip in each subject. Of the rats that received convulsive stimulations, 24 had electrodes terminating in the basolateral complex (i.e., the lateral and basolateral amygdalar nuclei) of the amygdala, 9 had electrodes
terminating in the central amygdala, and 4 had their electrodes terminating outside the amygdala. The data of these latter 4 rats were not included in the analysis. All 11 control rats had electrodes terminating within the basolateral complex. Accordingly, the behavioural analyses were based on 24 basolateral-amygdala-stimulated rats (basolateral-kindled rats), 9 central-amygdala-stimulated rats (central-kindled rats), and 11 sham-stimulated rats (sham-stim rats).

**Resistance to Capture**

Figure 3 illustrates the mean resistance to capture from the open field for the three groups. It is apparent from the figure that both the basolateral-kindled and central-kindled rats were substantially more resistant to capture than were the sham-stim rats. Analysis of these differences revealed a significant group effect, $H (2) = 19.166, p < .0001$, and post hoc comparisons revealed a significant difference between the basolateral-kindled and the sham-stim rats, $|R_1 - R_2| = 19.47, p < .001$, and between the central-kindled and the sham-stim rats, $|R_2 - R_3| = 19.85, p < .001$, but not between the basolateral-kindled and the central-kindled rats, $|R_1 - R_2| = .375, p > .05$.

**Elevated-Plus Maze**

The open-arm activity of the three groups on the elevated plus maze is summarized in Figure 4: The mean percentage of open-arm entries is illustrated in panel 4A, and the mean percentage of time spent on the open arms is illustrated in panel 4B. It is apparent from Figure 4 that both the basolateral-kindled and central-kindled rats made a greater percentage of entries into the open arms and spent a greater percentage of time on the open arms than did the sham-stim rats. Analysis of the differences in percentage of open-arm entries revealed a significant group effect, $H (2) = 20.49, p < .0001$, and post hoc comparisons revealed that both the basolateral-kindled, $|R_1 - R_2| = 20.96, p < .001$, and central-kindled, $|R_2 - R_3| = 17.44, p < .001$, rats displayed a significantly greater percentage of open-arm entries than the sham-stim rats. Similarly, analysis of the differences in
Figure 2. Histological results from the three groups of rats in Experiment 1. The stimulated rats were divided into two groups according to the location of their electrodes: The electrode placements of the basolateral-kindled rats are illustrated in Panel A; those of the central-kindled rats are illustrated in Panel B. The electrode placements of the sham-stim rats are illustrated in Panel C. Abbreviations: LaDL, lateral amygdalar nuclei; BLA, BLP, BMP, basolateral amygdalar nuclei; CeM, CeL, central amygdalar nuclei.
Figure 3. The mean resistance to capture displayed by each group in Experiment 1. The basolateral-kindled and central-kindled rats were significantly more resistant to capture from the open field than were the sham-stim rats.
percentage of time on the open arms revealed a significant group effect, $H(2) = 20.011, p < .0001$, and post hoc comparisons revealed that both the basolateral-kindled, $|R_1 - R_3| = 20.146, p < .001$, and central-kindled, $|R_2 - R_3| = 19.611, p < .001$, rats spent a significantly greater percentage of time on the open arms than did the sham-stim rats.

Differences in the absolute number of entries into open and closed arms and in the absolute durations of time spent on open and closed arms confirmed the selective tendency for the kindled rats to display more open-arm entries and to spend more time on the open arms (see Table 4). Analysis of the differences in total arm entries revealed a significant group effect, $H(2) = 7.059, p < .03$, and post hoc comparisons revealed that the basolateral-kindled rats entered significantly more arms than did the sham-stim rats, $|R_1 - R_3| = 12.22, p < .025$. However, there was no significant group effect of total time spent on the four arms, $H(2) = 2.889, p > .23$. Next, separate analyses of group differences in open-arm and closed-arm entries and time revealed selective increases in open-arm activity in the basolateral-kindled and central-kindled groups. Analyses of open-arm entries and time spent on open arms revealed that the basolateral-kindled and the central-kindled rats made significantly more open-arm entries, $H(2) = 21.595, p < .0001; |R_1 - R_3| = 21.73, p < .025, |R_2 - R_3| = 15.389, p < .025$, and spent significantly more time on the open arms, $H(2) = 20.052, p < .0001; |R_1 - R_3| = 20.312, p < .025, |R_2 - R_3| = 19.167, p < .025$, than did the sham-stim rats. However, analyses of closed-arm entries and time in the closed arms revealed that there were no significant differences among the groups in closed-arm entries, $H(2) = 1.028, p > .59$, and that the basolateral-kindled and central-kindled rats spent significantly less time on the closed arms, $H(2) = 16.593, p = .0002; |R_1 - R_3| = 17.549, p < .025, |R_2 - R_3| = 19.647, p < .025$, than did the sham-stim rats.

Interestingly, several of the basolateral-kindled and central-kindled rats, but none of the sham-stim rats, jumped off the open arms of the elevated plus maze. These jumps were made in a purposive manner: The rats peered over the edge of the maze; then they gathered themselves and leapt. Although rats sometimes fall from the open arms of the elevated plus
Figure 4. The percentage of open-arm activity on the elevated plus maze displayed by the sham and kindled rats. Panel A illustrates the mean percentage of time spent on the open arms. Both groups of kindled rats made a significantly greater percentage of open-arm entries and spent a significantly greater percentage of time on the open arms than did the sham-saline rats.

Panel B illustrates the mean percentage of open-arm entries. Both groups of kindled rats made more open-arm entries than the sham-saline rats.
Table 4. The Results From the Elevated-Plus Maze in Experiment 1

<table>
<thead>
<tr>
<th></th>
<th>Basolateral Kindled</th>
<th>Central Kindled</th>
<th>Sham Stim</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total Arm Entries</strong></td>
<td>11.29 ± .96*</td>
<td>9.56 ± 2.15</td>
<td>6.27 ± 1.17</td>
</tr>
<tr>
<td><strong>Total Time On Arms</strong></td>
<td>183.2 ± 6.8</td>
<td>167.8 ± 14.9</td>
<td>206.5 ± 12.0</td>
</tr>
<tr>
<td><strong>Open-Arm Entries</strong></td>
<td>3.33 ± .5*</td>
<td>2.44 ± 1.13*</td>
<td>0 ± 0</td>
</tr>
<tr>
<td><strong>Open-Arm Time (s)</strong></td>
<td>55.5 ± 8.58*</td>
<td>55.5 ± 15.36*</td>
<td>0 ± 0</td>
</tr>
<tr>
<td><strong>Closed-Arm Entries</strong></td>
<td>7.96 ± .91</td>
<td>7.11 ± 1.64</td>
<td>6.27 ± 1.17</td>
</tr>
<tr>
<td><strong>Closed-Arm Time (s)</strong></td>
<td>126.2 ± 9.76*</td>
<td>112.3 ± 18.4*</td>
<td>206.5 ± 11.99</td>
</tr>
</tbody>
</table>

Values are means ± s.e. For each measure, significant differences between the two kindled groups and the sham-stim group are indicated with an asterisk (*).

DISCUSSION OF EXPERIMENT 1

In Experiment 1, long-term amygdala kindling increased the resistance of rats to capture by an unfamiliar experimenter from an unfamiliar open field, and it increased their percentage of open-arm entries and their percentage of time spent on the open arms of the elevated plus maze. Neither the increase in percentage of open-arm entries or the increase in the percentage of time on the open arms of the elevated plus maze appeared to reflect nonspecific motor changes because both effects were specific to the open arms--there were no significant differences among the groups in the number of closed-arm entries. File (1991) has suggested that the number of closed-arm entries is the best measure of locomotor
activity in the elevated plus maze. Based on this suggestion, the number of closed-arm entries were used in the remaining experiments as a single measure of motoric effects on the elevated plus maze.

The site of stimulation within the left amygdala had no effect on the development of emotionality in Experiment 1: Both the basolateral-kindled and the central-kindled rats displayed similar changes in emotional behaviour. In addition, there was no effect of varying the stimulation site along the anterior-posterior plane of the amygdala (data not presented), which Adamec and Morgan (1994) found to influence the development of kindling-induced increases in open-arm activity on the elevated plus maze. The absence of differences in the effects of basolateral and central nucleus kindling appears to be at odds with evidence that the central and the basolateral amygdala mediate different types of emotional behaviour (Davis 1992) and respond differently to the anxiolytic effects of benzodiazepines (Pesold & Treit, 1995). However, after the first few stimulations, the afterdischarges triggered by each kindling stimulation generalize throughout the forebrain, and it is thus unlikely that slight variations in electrode placements would influence the consequences of long-term kindling--although it may be a factor in short-term kindling.

The results of Experiment 1 are noteworthy in two respects. First, the effect of kindling on emotional behaviour was particularly large and reliable; unlike the results of most previous studies of kindling and emotional behaviour, there was virtually no overlap between the kindled and sham-stimulated subjects in terms of their reactivity to handling, their percentage of open-arm entries, or their percentage of time on the open arms. I attribute the magnitude of these effects to the fact that the basolateral-kindled and central-kindled rats were subjected to long-term, as opposed to short-term, kindling. Second, the effects of kindling on resistance to capture and on elevated-plus-maze activity were seemingly inconsistent. Increased resistance to capture is a widely used indicator of anxiogenesis (see Albert & Richmond, 1975), whereas increased percentage of open-arm entries and time
spent on the open arms are widely used indicators of anxiolysis (see Pellow et al., 1985; Treit, 1985). One purpose of Experiment 2 was to resolve this apparent inconsistency.

**EXPERIMENT 2: EFFECT OF DIFFERENT NUMBERS OF AMYGDALA STIMULATIONS ON THE DEVELOPMENT OF INCREASED EMOTIONALITY**

The primary purpose of Experiment 2 was to determine the developmental course of the effects observed in Experiment 1 by comparing the behaviour of kindled rats that had received different numbers of stimulations. The effect of number of stimulations on kindling-related changes in emotionality had not been previously assessed. It is possible that number of stimulations is the factor underlying the difference between the results of Experiment 1 and the results of Nieminen et al. (1992), who reported a decrease, rather than an increase, in the open-arm activity of rats on the elevated plus maze after 17 amygdalar stimulations.

Another purpose of Experiment 2 was to resolve the apparent inconsistency between the two major findings of Experiment 1: between the kindling-produced increase in resistance to capture from an unfamiliar open field and the kindling-produced increase in the percentage of open-arm activity on the elevated plus maze. In an attempt to resolve this apparent inconsistency, two additional measures of anxiety were recorded: amount of open-field activity and the number of jumps from the open arms of the elevated plus maze.

**METHOD**

**Kindling Protocol**

A bipolar electrode was implanted into the left amygdala of each of 59 rats. After a 2-week postsurgical recovery period, the rats were divided into six groups: Three kindled groups received 20 (20-stim rats, \( n = 14 \)), 60 (60-stim rats, \( n = 13 \)), or 100 (100-stim rats, \( n = 13 \)) stimulations. The remaining three unkindled groups served as controls. The kindling protocol consisted of 100 stimulations at a stimulus intensity of 10 mA for 1 second, delivered at 10 Hz.
= 14) convulsive stimulations; and three control groups received either 20 \((n = 6)\), 60 \((n = 6)\), or 100 \((n = 6)\) sham stimulations.

**Behavioural Testing**

To serve as a basis for recording open-field activity, 36 identical squares were marked by tape on the floor of the open field that was used in Experiment 1. One day after the final kindling stimulation or sham stimulation, each rat was placed in the centre of the open field and left there for 5 min. The rat’s activity was recorded by a video camera mounted 1.5 meters above the open field. Later, the numbers of squares entered by each rat during each 30-s segment of the test were scored from a television monitor—a rat received credit for 1 square entry when the centre of its back crossed over from one square and into an adjacent square. Activity was recorded in 30-s segments because I expected fear-related reductions in exploration between stimulated and sham-stimulated rats to be greatest during the first 30 s of the session, when the open field was most unfamiliar.

After the open-field test, each rat was picked up forcefully from above, as in Experiment 1, by an unfamiliar experimenter wearing an unfamiliar leather glove, and the rat's resistance to capture was scored according to the 7-point scale used in Experiment 1. One day later, each rat was tested individually on the elevated plus maze for 5 min in the same manner as in Experiment 1, except that the experimenter also recorded each instance of jumping off the maze. Jumping off the maze and falling off the maze were easily distinguishable: Jumping off always occurred after a rat peered over the edge of an open arm, and it always proceeded head first, whereas falling occurred when a rat was turning on an open arm, lost its balance, and left the arm rear first. If a rat jumped or fell off one of the open arms, it was quickly picked up and returned to the place on the maze that it last occupied.
Statistical Analyses

The three groups of rats that received sham stimulations were combined for the purposes of statistical analysis (i.e., sham-stim rats). Differences among the three groups of stimulated rats and the combined group of sham-stim control rats in resistance to capture and elevated-plus-maze behaviour were analyzed as in Experiment 1. Differences among the groups in open-field activity were analyzed by a one-way ANOVA of the total number of squares entered during the 5-min session and 10 separate one-way ANOVA's of the total number of squares entered during each 30-s segment of the session. When appropriate, these tests were followed by post hoc analyses of mean differences using Newman-Keuls tests. Finally, differences among the groups in the proportion of rats that jumped off the open arms of the elevated plus maze were analyzed using a Chi-square test.

RESULTS

In this experiment, the kindled rats displayed less open-field exploration in the first 30 s of the session, greater resistance to capture, more open-arm activity on the elevated plus maze, and more purposive jumping from the elevated plus maze than did the sham-stim rats. The magnitude of each of these effects increased as a function of the number of stimulations.

Histology

Figure 5 illustrates the location of the electrode tip in each rat from Experiment 2. Because Experiment 1 did not reveal a significant effect of electrode placement within the amygdala, kindled rats with electrodes terminating in various parts of the amygdala were not divided into separate groups for the purposes of analysis. Two sham-stim rats and one 20-stim rat had electrodes that terminated outside the amygdala, and their data were not subjected to analysis. The analyses were therefore based on 13, 13, 14, and 16 rats in the 20-stim, 60-stim, 100-stim, and sham-stim groups, respectively.
Figure 5. Histological results from the four groups of rats in Experiment 2. Because Experiment 1 showed that the placement of the electrode within the amygdala had no significant effect on the results, kindled rats with electrodes terminating in various parts of the amygdala were not divided into separate groups for purposes of analysis. Abbreviations: LaDL, lateral amygdalar nuclei; BLA, BLP, BMP, basolateral amygdalar nuclei; CeM, CeL, central amygdalar nuclei.
**Open Field Exploration**

Figure 6 illustrates the number of squares entered during the first 30-s segment of the open-field test for the four groups. At the beginning of the session, both the 60-stim and 100-stim rats crossed fewer squares than the 20-stim and sham-stim rats did. Analysis of the differences in number of entries during the first 30 s revealed a significant group effect, $F(3, 52) = 16.89, p < .001$. Post hoc comparisons revealed that both the 60-stim and the 100-stim rats entered significantly fewer squares than did the 20-stim or sham-stim rats, $p < .01$. This effect continued through the second 30-s segment of the session for the 60-stim rats, $F(3, 52) = 4.41, p < .008$; Newman-Keuls, $p < .01$. As the session progressed, there were a few 30-s segments in which either the 100-stim rats, 90-120 s: $F(3, 52) = 3.916, p < .02$, Newman-Keuls, $p < .05$; 180-210 s: $F(3, 52) = 5.019, p < .004$, Newman-Keuls, $p < .05$, or the 60-stim rats, 240-270 s: $F(3, 52) = 6.21, p < .002$, Newman-Keuls, $p < .01$, entered significantly more squares than did the sham-stim rats. However, a one-way ANOVA of total square crosses made during the entire 5-min session revealed no significant differences among the groups, $F(3, 52) = 1.587, p > .20$.

**Resistance to Capture**

Figure 7 illustrates the mean resistance to capture from the open field for the four groups. Increases in resistance to capture above control levels were greatest in the groups that received the most stimulations. Analysis of the differences in resistance to capture revealed a significant group effect, $H(3,56) = 28.305, p > .0001$, and post hoc comparisons revealed a significant difference between the 60-stim and the sham-stim rats, $|R_3 - R_4| = 22.9, p < .01$, between the 100-stim and the sham-stim rats, $R_3 - R_4 = 28.77, p < .01$, and between the 100-stim and the 20-stim rats, $|R_3 - R_1| = 19.79, p < .01$. 
Figure 6. The mean number of squares entered during the first 30 s of the open-field test by the rats in each group in Experiment 2. Both the 60-stim and the 100-stim rats entered significantly fewer squares during the first 30 s of the test than did the sham-stim rats.
Figure 7. The mean resistance to capture displayed by the rats in each group in Experiment 2. The 100-stim rats were significantly more resistant to capture than both the 20-stim and the sham-stim rats; the 60-stim rats were significantly more resistant to capture than the sham-stim rats.
Elevated Plus Maze

The open-arm activity of the rats in each of the four groups on the elevated plus maze is summarized in Figure 8: Mean percentage of open-arm entries is illustrated in panel 8A, and mean percentage of time spent on the open arms is illustrated in panel 8B. Both the 60-stim and 100-stim rats engaged in more open-arm activity than did the 20-stim or sham-stim rats. Analysis of the differences in percentage of open-arm entries revealed a significant group difference, $H(3) = 13.531, p < .004$, and post hoc comparisons revealed a significant difference between the 60-stim and the sham-stim rats, $|R_2 - R_4| = 19.66, p < .025$, and between the 100-stim and the sham-stim rats, $|R_3 - R_4| = 16.52, p < .05$. Similarly, analysis of the differences in percentage of time spent on the open arms revealed a significant group difference, $H(3) = 13.598, p < .004$, and post hoc comparisons revealed a significant difference between the 60-stim and the sham-stim rats, $|R_2 - R_4| = 19.62, p < .025$, and between the 100-stim and the sham-stim rats, $|R_3 - R_4| = 16.63, p < .025$.

As in Experiment 1, these effects were not due to a nonspecific motor effect—there were no significant differences in the number of closed-arm entries made by the rats in each group (sham-stim $M = 8.25$; 20-stim $M = 11.1$; 60-stim $M = 11.3$; 100-stim $M = 9.93$, $H(3) = 3.977, p > .26$).

Figure 9 illustrates the percentage of rats in each group that jumped from the open arms of the elevated-plus maze. A chi-square analysis of this jumping behaviour revealed a significant group difference, $X^2 (3, N = 56) = 14.179, p < .003$. Post hoc comparisons revealed that a significantly greater percentage of the 100-stim rats jumped from the open arms than did the 60-stim rats, $p < .05$, the 20-stim rats, $p < .001$, or the sham-stim rats, $p < .001$.

Finally, I also observed extreme piloerection in rats who jumped from the open arms of the elevated plus maze. However, because these observations were not systematically recorded, they were not subjected to formal statistical analyses.
Figure 8. The percentage of open-arm activity on the elevated plus maze displayed by the rats in Experiment 2. Panel A illustrates the mean percentage of open-arm entries and Panel B illustrates the mean percentage of time spent on the open arms. The 100-stim and the 60-stim rats made a significantly greater percentage of open-arm entries and spent significantly more time on the open arms than did the sham-stim rats.
Figure 9. The percentage of rats in each group in Experiment 2 jumping off the open arms of the elevated plus maze. A significantly greater percentage of the 100-stim rats jumped off than did the 60-stim, 20-stim, or sham-stim rats.
DISCUSSION OF EXPERIMENT 2

In Experiment 2, amygdala-kindling decreased open-field activity during the first 30 s of the test, increased resistance to capture, increased both open-arm entries and time spent on the open arms of the elevated plus maze, and increased purposive jumping from the maze. The magnitude of each of these effects was directly related to the number of stimulations that the rats received: Relative to sham-stim controls, rats that received 20 stimulations displayed no statistically significant changes in emotional behaviour; rats that received 60 stimulations displayed significant decreases in open-field activity during the first 30 s of the test, increases in resistance to capture, and increases in open-arm activity on the elevated plus maze; and rats that received 100 stimulations displayed all of the significant changes displayed by the 60-stim rats plus significant increases in purposive jumping from the elevated plus maze. The rats that received 100 stimulations also displayed significantly greater resistance to capture than did the 20-stim rats.

The mean resistance to capture of the 100-stim rats in Experiment 2 ($M = 4.7$) was higher than that for the rats that received 99 stimulations in Experiment 1 ($M = 3.1$). This may have been due to the fact that the rats in Experiment 2 spent 5 min in the open field before their resistance-to-capture test, whereas the rats in Experiment 1 spent only 2 min. Albert and Richmond (1975) found that lengthening the amount of time rats are left in an unfamiliar open field increases their subsequent reactivity to a variety of tests, including resistance to capture.

The results of this experiment, like the results of Experiment 1, are not attributable to a nonspecific motoric effect. There were no significant differences among the 20-stim, 60-stim, and 100-stim rats in terms of number of closed-arm entries in the elevated plus maze or total square crosses during the 5-min open-field test, yet there were considerable differences among these rats in terms of their emotion-related behaviour. In addition, the rats that displayed the greatest increases in emotionality (the 60-stim and 100-stim rats) were more active than the sham-stim control rats on the elevated plus maze but less active...
during the first 30 s in the open field. Thus, neither a general increase nor a general decrease in motor activity is sufficient to explain the pattern of behavioural changes observed in the kindled rats.

In view of the fact that the percentage of open-arm entries and the percentage of time spent on the open arms are commonly used behavioural measures of anxiolytic drug effects (e.g., Treit, 1985), the observed increases in these two measures are seemingly at odds with the other observed effects of long-term amygdala kindling, which all suggest anxiogenesis. The observation that many rats in the 100-stim group jumped from the elevated plus maze while displaying extreme piloerection suggests a possible reconciliation of these ostensibly incompatible findings. It suggests that there are two reasons why rats might venture onto the open arms of an elevated plus maze. One is that their level of fear may have declined to the point where it is no longer sufficient to inhibit open-arm exploration (i.e., anxiolysis); the other is that their level of fear may have increased to the point that it motivates a search for escape routes from the apparatus (i.e., anxiogenesis). I speculate that in conventional studies of anxiolytic drug action, rats typically enter the open arms for the former reason; whereas in the present studies, the kindled rats entered the open arms for the latter reason. Supporting this interpretation are the jumping, extreme piloerection, and increased number of arm entries of the 100-stim rats and the increases in other measures of emotionality in the same rats.

Brandao and his colleagues (1994) have suggested that flight is at the high end of a continuum of defensive behaviour, with passive avoidance being near the low end. Based on this conception, it is possible that kindling-induced anxiogenesis progresses through the following three stages of elevated-plus-maze behaviour as the rats become more thoroughly kindled. First, if control levels of open-arm activity are sufficiently high, there may be an initial reduction in open-arm activity after the first few stimulations as the level of maze-induced anxiety becomes high enough to motivate passive avoidance (i.e., remaining in the closed arms). Second, as additional stimulations increase the levels of anxiety, rats may be
motivated by fear of the apparatus to venture onto the open arms in search of an escape route and to engage in risk assessment (see Blanchard & Blanchard, 1988; Pinel et al., 1989) but still be insufficiently motivated to jump. Third, after long-term kindling, rats may become sufficiently motivated by fear of the apparatus to venture onto the open arms of the maze, to engage in risk assessment, and to escape from the maze by jumping.

This three-stage interpretation of kindling-induced changes in elevated-plus-maze activity sheds light on two previous inconsistent findings from studies of short-term kindling—left basolateral amygdala kindling has been reported to produce both anxiolytic (Adamec & Morgan, 1994) and anxiogenic (Nieminen et al., 1992) effects. First, Adamec and his colleagues (Adamec & Morgan, 1994; Adamec & McKay, 1993) reported that short-term amygdala kindling in rats results in increases in open-arm activity on the elevated-plus maze. This finding was confirmed by the 20-stim rats in Experiment 2. Consistent with the conventional view of increases in open-arm activity, Adamec et al. attributed their observation to an anxiolytic effect; however, given the leaping behaviour and piloerection of long-term kindled rats in this experiment, it is more parsimonious to conclude that the kindling-induced increases in open-arm activity following short-term kindling are a consequence of anxiogenesis. This interpretation is consistent with Adamec's own research on the anxiogenic effects of partial amygdala kindling in cats (Adamec & Stark-Adamec, 1983; Adamec, 1990) and with the literature on the emotionality of temporal lobe epileptics (e.g., Gloor, 1992). Second, Niemenen et al. (1992) found that short-term kindling decreased, rather than increased, open-arm activity on the elevated plus maze. I attribute this finding to the fact that Niemenen et al. did not test their rats until 2 weeks after the last amygdala stimulation—a point at which the moderate levels of anxiety induced by short-term kindling could have declined to the point where the levels of anxiety could have motivated passive avoidance (i.e., stage 1 of the three-stage interpretation of elevated-plus-maze behavior). One purpose of Experiment 3 was to test this idea.
EXPERIMENT 3: INCREASED EMOTIONALITY PRODUCED BY LONG-TERM AMYGDALA KINDLING IS ENDURING

The results of the preceding two experiments clearly demonstrate that amygdala kindling produces significant changes in interictal emotional behaviour in rats and that the magnitude of these effects increases monotonically during the course of long-term kindling. The purpose of Experiment 3 was to assess the persistence of these changes following the discontinuation of kindling stimulations.

Whether or not kindling-induced hyperemotionality persists after the kindling stimulations have been discontinued is an important issue because it has implications for understanding the mechanisms that underlie this hyperemotionality. If the hyperemotionality is enduring, it would suggest that the mechanisms underlying it are the same as those underlying the kindled state, which itself is enduring if not permanent. Alternatively, if the hyperemotionality dissipates quickly, it would suggest that the mechanisms underlying it are related to some less enduring aftereffect of the stimulations. The permanence of kindling-induced interictal hyperemotionality also has important implications for the development of strategies for the treatment of interictal emotional disturbances in human epileptics. For example, if the hyperemotionality were permanent, then anticonvulsant medication after the diagnosis of epilepsy would be unlikely to have any prophylactic effect; however, if the hyperemotionality were dependent on the continued occurrence of seizures, then using anticonvulsants to control the seizures may offer some relief from emotional disturbances. It was surprising, therefore, that the persistence of kindling-induced changes in emotional behaviour has never been systematically assessed (however, see Adamec, 1991).

In Experiment 3, all subjects were subjected to long-term amygdala kindling. Then, their emotional behaviour was assessed either 1 day, 1 week, or 1 month after the final stimulation.
METHOD

Kindling Protocol

A bipolar electrode was implanted in the left amygdala of each of 53 rats. After a postsurgical recovery period, some of the rats received 99 kindling stimulations and others received an equal number of sham stimulations. After the kindling phase, the rats were divided into six groups for behavioural testing: Three kindled groups were tested either 1 day (1-day rats, \( n = 12 \)), 1 week (1-week rats, \( n = 14 \)), or 1 month (1-month rats, \( n = 13 \)) after their last stimulation; and three sham-stimulation control groups were tested after the same time intervals (sham-stim rats, \( n = 14 \)).

Behavioural Testing

The behavioural tests were the same as in Experiment 2: open-field activity, resistance to capture, and open-arm activity on the elevated plus maze. However, unlike Experiment 2, open-field activity was scored for only the first 30 s of the 5-min session because Experiment 2 had shown that kindling induces the greatest changes in open-field exploration in the first 30 s of the session, when the open field is the most unfamiliar.

Statistical Analyses

The three sham-stimulation groups were combined for the purposes of the statistical analyses. The statistical significance of differences among the groups on all the measures was assessed using the same tests as in Experiment 2.

RESULTS

In this experiment, long-term kindling produced substantial decreases in open-field exploration, increases in resistance to capture, and increases in open-arm activity on the elevated plus maze in the 1-day rats; in general, these effects were persistent but they did decline somewhat in the 1-week and 1-month rats.
Histology

Figure 10 illustrates the location of the electrode tip in each rat in Experiment 3. It is apparent from the figure that each electrode terminated in one of the amygdalar nuclei.

Open Field Exploration

Figure 11 illustrates the number of squares crossed during the first 30 s of the open-field test for each group. The kindling-induced decrease in the number of squares crossed dissipated somewhat once the stimulations were discontinued, but this effect was not significant. Analysis of the differences in the number of squares crossed during the 30-s test revealed a significant group effect, $F (3, 49) = 3.776, p < .02$. Post hoc comparisons revealed that both the 1-day and 1-week rats crossed significantly fewer squares than did the sham-stim rats, $p < .05$, but not the 1-month rats, $p > .09$. However, the 1-month and the sham-stim rats did not differ significantly, $p > .10$.

Resistance to Capture

Figure 12 illustrates the mean resistance to capture from the open field for the four groups. It is apparent from the figure that the kindling-induced resistance to capture dissipated somewhat once the stimulations were discontinued; however, the decline was not statistically significant. Analysis of the differences in resistance to capture revealed a significant group effect, $H (3) = 14.677, p > .002$. Post hoc comparisons revealed a significant difference between the 1-day and the sham-stim rats, $|R_1 - R_4| = 20.57, p < .03$, and between the 1-week and the sham-stim rats, $|R_2 - R_4| = 17.61, p < .03$, but not between the 1-day and the 1-month rats, $|R_1 - R_3| = 12.37, p > .05$. Although the resistance to capture of the 1-day and the 1-month rats did not differ significantly, the 1-month rats did not differ significantly in this respect from the sham-stim rats, $p > .20$. 
Figure 10. Histological results from the four groups of rats in Experiment 3. All rats had electrode placements within the amygdala. Abbreviations: LaDL, lateral amygdalar nuclei; BLA, BLP, BMP, basolateral amygdalar nuclei; CeM, CeL, central amygdalar nuclei.
Figure 11. The mean number of squares crossed in the open field by the rats in each group in Experiment 3. Both the 1-day and the 1-week rats entered significantly fewer squares during the 30 s test than did the sham-stim rats. There were no significant differences between the 1-day and the 1-month rats, or between the 1-month and the sham-stim rats.
Figure 12. The mean resistance to capture displayed by each group in Experiment 3. The 1-day and 1-week rats were significantly more resistant to capture from the open field than the sham-stim rats. There were no significant differences between the 1-day and 1-month rats, or between the 1-month and sham-stim rats.
Elevated Plus Maze

Figure 13 illustrates the performance of the four groups on the elevated plus maze. Mean percentage of open-arm entries is illustrated in panel 13A, and mean percentage of time spent on the open arms is illustrated in panel 13B. The 1-day rats engaged in the greatest percentage of open-arm activity, and the 1-month rats engaged in the smallest percentage of open-arm activity. Analysis of the percentage of open-arm entries revealed a significant group effect, $H (3) = 13.815, p < .003$. Post hoc comparisons of this effect revealed that the 1-day rats engaged in a significantly greater percentage of open-arm entries than did the 1-week rats, $|R_1 - R_2| = 16.58, p < .05$, and the 1-month rats, $|R_1 - R_2| = 21.448, p < .025$, but not the sham-stim rats, $|R_1 - R_4| = 9.226, p > .05$. Similarly, analysis of the percentage of time spent on the open arms revealed a significant group effect, $H (3) = 15.037, p < .002$, and post hoc comparisons revealed that the 1-day rats spent a significantly greater percentage of time on the open arms than did the 1-week rats, $|R_1 - R_2| = 17.27, p < .05$, and the 1-month rats, $|R_1 - R_2| = 22.69, p < .025$, but not the sham-stim rats, $|R_1 - R_4| = 10.56, p > .09$. Interestingly, the percentage of open-arm entries and percentage of time spent on the open arms was substantially less in the 1-week and 1-month rats than in the sham-stim rats; however, these differences did not quite reach statistical significance.

The number of closed-arm entries made by the rats in each group were similar (1-day, $M = 10.92$; 1-week, $M = 8.64$; 1-month, $M = 8.77$; sham-stim, $M = 9.21$). Analysis of the number of arm entries in the elevated plus maze revealed no significant differences, $H (3) = 4.004, p > .26$. 
Figure 13. The percentage of open-arm activity of each group on the elevated plus maze in Experiment 3. Panel A illustrates the mean percentage of open-arm entries, and Panel B illustrates the mean percentage of time spent on the open arms. The 1-day rats engaged in a significantly greater percentage of open-arm entries and percentage of time spent on the open arms than the 1-week rats and 1-month rats.
DISCUSSION OF EXPERIMENT 3

As in Experiment 2, the amygdala-kindled rats tested 1 day after the last stimulation in Experiment 3 displayed a substantial decrease in activity in an unfamiliar open field and an increase in resistance to capture compared to the sham-stim rats. Experiment 3 established that these effects are enduring; they endured over a stimulation free 1-month period although they did decline substantially in magnitude. This intermediate performance of the 1-month rats on the open-field activity and resistance-to-capture tests was indicated by the fact that they did not differ significantly on either of these two measures from the 1-day rats or the sham-stim rats. In contrast to the results for the open-field and resistance-to-capture tests, the high percentage of open-arm activity in the elevated plus maze observed in the 1-day rats did decline significantly in the 1 month following the final stimulation. In fact, the percentage of open-arm activity in the 1-month rats decreased to well below control levels. Thus, it appears that there may still be lingering effects of amygdala kindling on elevated-plus-maze open-arm activity 1 month after the cessation of stimulations but the effect is opposite to the effect observed 1 day after.

The decrease in open-arm activity in the 1-week and 1-month rats in this experiment is consistent with the three-stage interpretation of kindling-induced anxiogenesis in the elevated plus maze that I proposed in the Discussion section of Experiment 2, assuming that kindling-induced fear dissipates over time once the stimulations cease. Compared to control rats, kindled rats tested shortly after the last stimulation should prefer an active avoidance response, engaging in more open-arm activity. Kindled rats tested 1 week after the last stimulation should prefer a more passive response, engaging in less open-arm activity. Finally, kindled rats tested 1 month after the last stimulation should prefer a completely passive response, engaging in little or no open-arm activity. There is some support for this interpretation: Nieminen et al. (1992) found that a group of short-term amygdala-kindled rats tested 2 weeks after their final stimulation engaged in less open-arm activity on the elevated plus maze than did a group of sham-stimulated rats.
Of direct relevance to the results of Experiment 3 are the results of a recent unpublished experiment (Kalynchuk, Pinel, McEachern, Treit, & Kippin, in preparation). In this experiment, we found that the long-term amygdala-kindling-induced increase in resistance to capture was still significantly above control levels 2 months after the final stimulation, although it did dissipate significantly. Interestingly, the kindled rats responded to the first or second stimulation after the 2-month stimulation-free period with a generalized convulsion, but 30 additional stimulations was required to significantly increase the resistance to capture again.

What do the findings from Experiment 3 imply about the mechanisms underlying kindling-induced interictal hyperemotionality? In designing this experiment, I thought that the results would show one of two things: Either the kindling-induced emotionality would dissipate entirely after the 1-month stimulation-free period, implying that the mechanism underlying it was some short-term aftereffect of the seizures, or the emotionality would not decline at all over the 1-month stimulation-free period, implying that the mechanism underlying it was the same as the mechanism underlying the kindled state. However, the results of this experiment are intermediate: The kindled rats tested 1 month after the final stimulation did not differ significantly in their resistance to capture or open-field exploration from either the kindled rats tested 1 day after the last stimulation or the sham-stimulated rats. Thus, although Experiment 3 (and our more recent unpublished observations) have clearly established that the increases in emotionality produced by long-term amygdala kindling are enduring, the time course of the decline provides no clear indication of the nature of the mechanism that underlies it. One possibility is that some short-term aftereffect of the seizures and some kindling-related mechanism both contribute to the effect.

The present intermediate finding that kindling-induced hyperemotionality declines monotonically after the most recent seizure but still endures for at least a month or two may account for some of the confusion in the literature. Some studies have indicated that the recent occurrence of seizures is critical for the expression of seizure-induced interictal
emotionality: Post, Kennedy, Shinohara, Squillace, Miyaoka, Suda, Ingvar, and Sokoloff (1984) found that lidocaine-treated rats displayed biting and extreme resistance to capture only when the lidocaine was administered in a dose high enough to induce seizures; and Griffith, Engel, and Bandler (1987) found that cats treated with kainic acid displayed interictal defensive behaviour only during periods in which they experienced seizures. However, Engel, Bandler, Griffith, and Caldecott-Hazard (1991) found that blocking temporal lobe seizures by pharmacological or surgical means does not reliably suppress the expression of interictal hyperemotionality, implying that the recent occurrence of seizures is not critical for the expression of hyperemotionality.

**EXPERIMENT 4: BRAIN SITE SPECIFICITY IN KINDLING-INDUCED EMOTIONALITY**

In the preceding three experiments, long-term amygdala kindling produced robust increases in emotionality. Are kindling-induced increases in emotional behaviour specific to amygdala kindling? There are two major reasons for hypothesizing that kindling of the amygdala, rather than some other structure, might produce particularly great increases in emotionality: the amygdala's putative role in emotional behaviour (e.g., Davis, 1992; LeDoux, 1994) and the relation between emotional pathology and temporal lobe epilepsy (Gloor, Olivier, Quesney, Andermann, & Horowitz, 1982; Gloor, 1990). However, there are also two major reasons for hypothesizing that kindling-induced increases in emotionality are not any greater after amygdala kindling than after kindling some other structure: Pinel et al. (1977) reported that long-term kindling of either the amygdala or hippocampus in rats increased resistance to capture and reactivity to a pencil tap and Adamec (1993) reported that partial kindling of either the amygdala or hippocampus in cats increased defensive responding to rats.
The purpose of this experiment was to compare the development of emotionality in rats that received either long-term amygdala, hippocampal, or caudate kindling using the battery of tests developed in the previous experiments in this thesis.

**METHOD**

**Kindling Protocol and Behavioural Testing**

A bipolar electrode was implanted in the left basolateral amygdala, dorsal hippocampus, or caudate nucleus of 79 rats. After a 2-week postsurgical recovery period, the rats were divided into six groups: amygdala kindled (n = 17), amygdala sham (n = 10), hippocampal kindled (n = 14), hippocampal sham (n = 12), caudate kindled (n = 14), and caudate sham (n = 12). All rats received 99 kindling or sham stimulations. The rats were then subjected to the same behavioural tests as in Experiment 3.

**Statistical Analyses**

Differences among the four groups in open-field exploration, resistance to capture and elevated-plus-maze behaviour were analyzed as in Experiment 2. In addition, differences among the groups in the mean convulsion class elicited by the final five stimulations were analyzed nonparametrically using a Kruskal-Wallis ANOVA, followed by post hoc multiple comparisons.

**RESULTS**

Long-term amygdala and hippocampal kindling produced a higher class of convulsions, and greater changes in open-field exploration, resistance to capture, and open-arm activity in the elevated plus maze compared to long-term caudate kindling.
Histology

Figures 14, 15, and 16 illustrate the location of the electrode tips in the amygdala, hippocampal, and caudate rats respectively. Each rat’s electrode terminated within the target structure; therefore, the data of all the rats were included in the statistical analyses.

Convulsion Class

Figure 17 illustrates the mean class of convulsions produced by the final five stimulations in each group of kindled rats. It is important to note that there were some differences in the convulsions elicited by caudate as opposed to amygdalar and hippocampal stimulations. At the beginning of the kindling phase, each amygdalar and hippocampal stimulation produced no obvious motor response other than a behavioural arrest, whereas each caudate stimulation elicited a convolution characterized by rapid falling over and rolling to one side and a brief period of forelimb clonus. This behavioural sequence of falling over and rolling immediately after a stimulation was never observed in the amygdala-kindled or hippocampal-kindled rats and as the kindling phase progressed, it appeared less frequently in the caudate-kindled rats. By the end of the kindling phase, each of the amygdala-kindled, hippocampal-kindled, and caudate-kindled rats responded consistently to each convulsive stimulation with a generalized motor seizure; however, the final five stimulations produced a significantly higher mean convolution class in both the amygdala-kindled and the hippocampal-kindled rats than in the caudate-kindled rats, $H (3) = 18.008, p < .001$. Post hoc comparisons revealed a significant difference between the amygdala-kindled rats and the caudate-kindled rats, $|R_1 - R_5| = 17.651, p < .025$, and between the hippocampal-kindled rats and the caudate-kindled rats, $|R_3 - R_5| = 18.286, p < .025$. 
Figure 14. Histological results from the amygdala-kindled and amygdala-sham rats in Experiment 4. All the electrode tips were located in the amygdala. Abbreviations: LaDL, lateral amygdalar nuclei; BLA, BLP, BMP, basolateral amygdalar nuclei; CeM, CeL, central amygdalar nuclei.
Figure 15. Histological results from the hippocampal-kindled and hippocampal-sham rats in Experiment 4. All the electrode tips were located in the hippocampus.
Figure 16. Histological results from the caudate-kindled and caudate-sham rats in Experiment 4. All the electrodes were located in the caudate nucleus.
Figure 17. The mean class of convulsions produced by the final five stimulations in each group of kindled rats in Experiment 4. Although all the rats were “kindled” by the end of the stimulation phase of the experiment, both the amygdala- and hippocampal-kindled rats displayed a significantly higher mean convulsion class than the caudate-kindled rats did. Convulsion classes: class 1: head nodding only; class 2: head nodding and jaw clonus; class 3: head nodding, jaw clonus, and forelimb clonus; class 4: head nodding, jaw clonus, forelimb clonus, and rearing; class 5: head nodding, jaw clonus, forelimb clonus, rearing, and falling once. See the General Method section for a more complete description.
Open Field Exploration

Figure 18 illustrates the number of squares crossed during the first 30 s of the open-field test. The amygdala-kindled and hippocampal-kindled rats crossed fewer squares during the open-field test than did their respective sham rats. In contrast, the caudate-kindled rats crossed more squares than their respective sham rats did. These differences were statistically significant—a one-way ANOVA revealed a significant group main effect, $F(5, 78) = 12.671$, $p < .0001$, and Newman-Keuls post hoc tests revealed a significant difference between the amygdala-kindled rats and the amygdala-sham rats, $p < .01$, between the hippocampal-kindled and the hippocampal-sham rats, $p < .05$, between the amygdala-kindled and the caudate-kindled rats, $p < .01$, and between the hippocampal-kindled and the caudate-kindled rats, $p < .05$.

Resistance to Capture

Figure 19 illustrates the mean resistance to capture displayed by each group in Experiment 4. The amygdala-kindled rats displayed the greatest resistance to capture from the open field, followed by the hippocampal-kindled rats, the caudate-kindled rats, and the rats in the three sham groups, which all displayed virtually the same low level of resistance to capture. Analysis of the differences in resistance to capture among the groups revealed a significant group effect, $H(5) = 37.089$, $p < .0001$. Post hoc comparisons revealed a significant difference between the amygdala-kindled and amygdala-sham rats, $|R_1 - R_2| = 38.015$, $p < .025$, between the hippocampal-kindled and the hippocampal-sham rats, $|R_3 - R_4| = 28.654$, $p < .025$, and between the amygdala-kindled and the caudate-kindled rats, $|R_1 - R_5| = 38.015$, $p < .025$. 
Figure 18. The mean number of squares crossed in the open field by the rats in each group in Experiment 4. The amygdala-kindled rats crossed significantly fewer squares during the first 30 s than did the amygdala-sham rats and the caudate-kindled rats. Similarly, the hippocampal-kindled rats crossed significantly fewer squares than did the hippocampal-sham rats and the caudate-kindled rats.
Figure 19. The mean resistance to capture displayed by the rats in each group in Experiment 4. Both the amygdala-kindled and the hippocampal-kindled rats were significantly more resistant to capture from the open field than their respective sham rats. In addition, the amygdala-kindled rats were significantly more resistant to capture than the caudate-kindled rats.
Elevated Plus Maze

The performance of the six groups on the elevated plus maze is summarized in Figure 20: Mean percentage of open-arm entries is illustrated in panel 20A, and the mean percentage of time spent on the open arms is illustrated in panel 20B. It is apparent from this figure that the amygdala-kindled rats engaged in the highest percentage of open-arm entries and spent the greatest percentage of time on the open arms, followed in both cases by the hippocampal-kindled rats and the caudate-kindled rats. Analysis of the differences among the groups in the percentage of open-arm entries revealed a significant group effect, $H(5) = 32.273, p < .0001$, and post hoc comparisons revealed a significant difference between the amygdala-kindled and the amygdala-sham rats, $|R_1 - R_2| = 37.64, p < .025$, and between the hippocampal-kindled and the hippocampal-sham rats, $|R_3 - R_4| = 31.25, p < .025$. Similarly, analysis of the differences among the groups in the percentage of time spent on the open arms revealed a significant group effect, $H(5) = 34.718, p < .0001$, and post hoc comparisons revealed a significant difference between the amygdala-kindled and the amygdala-sham rats, $|R_1 - R_2| = 38.729, p < .025$, and between the hippocampal-kindled and the hippocampal-sham rats, $|R_3 - R_4| = 30.536, p < .025$.

In addition, although the amygdala-kindled, hippocampal-kindled, and caudate-kindled rats all entered more closed arms than did their respective sham rats (amygdala kindled, $M = 12.18$; amygdala sham, $M = 10.1$; hippocampal kindled, $M = 12.43$; hippocampal sham, $M = 10.4$; caudate kindled, $M = 13.1$; caudate sham, $M = 9.5$), none of these differences was statistically significant, $H(5) = 9.45, p > .09$.

**DISCUSSION OF EXPERIMENT 4**

In Experiment 4, long-term amygdala and hippocampal kindling produced a significantly greater degree of emotionality than did long-term caudate kindling. Compared to the caudate-kindled rats, the amygdala-kindled and hippocampal-kindled rats explored less in the open-field test, were more resistant to capture from the open field, and engaged in
Figure 20. The percentage of open-arm activity of each group on the elevated plus maze in Experiment 4. Panel A illustrates the mean percentage of open-arm entries, and Panel B illustrates the mean percentage of time spent on the open arms. Both the amygdala-kindled and the hippocampal-kindled rats engaged in a significantly greater percentage of open-arm entries and spent a significantly greater percentage of time on the open arms than did their respective sham-stim rats.
a greater percentage of open-arm activity on the elevated plus maze. They also displayed a higher mean class of convulsions elicited by the last five stimulations than the caudate-kindled rats did. Although the magnitude of emotionality was greatest in the amygdala-kindled rats, the results of Experiment 4 demonstrate that repeated stimulation of the hippocampus can also produce a significant degree of emotionality. Thus, long-term kindling-induced increases in emotionality are not limited to amygdala kindling.

The findings from Experiment 4 are consistent with those of others. The significantly higher mean convulsion class displayed by the amygdala- and hippocampal-kindled rats replicates a previously published report of Pinel and Rovner (1978b). They found that after 96 convulsive stimulations, amygdala and hippocampal-kindled rats displayed a significantly higher mean convulsion class than caudate-kindled rats. Interestingly, this effect disappeared after their rats had received 192 stimulations. In addition, the finding that long-term amygdala and hippocampal kindling produces a greater degree of resistance to capture replicates Pinel et al.’s (1977) previously published report that amygdala and hippocampal kindling produces more resistance to capture and reactivity to a pencil tap on the base of the tail than does long-term caudate kindling. Finally, the fact that kindling of both the amygdala and hippocampus can produce increased emotionality is consistent with Adamec’s findings that partial kindling of both the amygdala and hippocampus in cats can increase defensive behaviour (see Adamec, 1990).

The class of convulsions elicited by the final few stimulations may be important for the expression of hyperemotionality. The hippocampal-kindled and amygdala-kindled rats displayed both a higher mean class of convulsions elicited by the final five stimulations and a greater degree of emotionality than the caudate-kindled rats did. This may be because the electrographic brain activity accompanying more severe convulsions facilitates the activation of neural pathways involved in the development of hyperemotionality after long-term kindling.
The fact that the class of convulsions elicited by the final few stimulations may be important for the development of emotionality also suggests that caudate kindling could eventually produce a similar amount of emotionality to that observed after amygdala and hippocampal kindling. Pinel and Rovner (1978b) found that after 192 stimulations, each stimulation elicited a similar class of convulsion in amygdala-, hippocampal-, and caudate-kindled rats. This suggests that more caudate stimulations than the 99 administered in Experiment 4 might produce a level of emotionality comparable to that in the amygdala- and hippocampal-kindled subjects.

**EXPERIMENTS 1 TO 4 REVISITED: HOW ARE THE BEHAVIORAL MEASURES RELATED TO EACH OTHER?**

In Experiments 1 to 4, there were four main measures of emotionality: open-field activity, resistance to capture, percentage of open-arm entries on the elevated plus maze, and percentage of time spent on the open arms of the elevated plus maze. The long-term kindled rats in each experiment were significantly different from the sham-stimulation control rats on each of these measures. This return visit to the data of Experiments 1 to 4 has two purposes, one general and one specific. The general purpose was to assess the strength of the relation between the measures of emotionality used in Experiments 1 to 4. The specific purpose was to test my three-stage interpretation of kindling-induced anxiogenesis in the elevated plus maze. If the increased open-arm activity observed in the long-term kindled rats in Experiments 1 to 4 is an anxiogenic effect, then it should be positively correlated to resistance to capture; if it is an anxiolytic effect, as conventional wisdom suggests, then it should be negatively correlated to resistance to capture.

In order to assess the degree of association among the measures of emotionality used in the first four experiments of this thesis, a series of correlations among the data from the amygdala-kindled rats that received 99 or 100 stimulations in each experiment were calculated. The statistical significance of the correlation between resistance to capture and
each of the other measures (i.e., resistance to capture versus open-field exploration, resistance to capture versus the percentage of open-arm entries, and resistance to capture versus the percentage of time spent on the open arms) was assessed using the nonparametric Spearman Rank Order test because the resistance-to-capture data were measured on an ordinal scale. The more sensitive Pearson product-moment correlational analysis, which requires that data be measured on an interval or ratio scale, was applied to the remaining relations.

Table 5 shows the results of these correlational analyses. It is evident from Table 5 that the resistance to capture of the long-term amygdala-kindled rats is significantly correlated to the other three measures of emotionality in each experiment except Experiment 3. In addition, open-field exploration, the percentage of open-arm entries, and the percentage of time spent on the open arms are significantly correlated in each experiment except in Experiment 4, in which the correlation between open-field exploration and the percentage of open-arm entries failed to reach statistical significance. Accordingly, in general, the rats that displayed the highest levels of resistance to capture also displayed the least open-field exploration and the greatest percentage of open-arm activity in the elevated plus maze.

These results are important for two reasons. First, the correlations among resistance to capture, open-field exploration, and the percentage of open-arm activity in the elevated plus maze (rs = .3 to .6) are large enough to suggest that each individual measure may be assessing a common effect produced by kindling (i.e., increased fearfulness), but they are small enough to suggest that the use of the test battery is important in interpreting the nature of that common effect. Second, the positive correlation between resistance to capture and the percentage of open-arm activity in the elevated plus maze supports my three-stage interpretation of kindling-induced anxiogenesis in the elevated plus maze.

The data from Experiment 3 are problematic because due to unavoidable circumstances, the resistance to capture data were collected by a student assistant, whereas I collected all the other data in Experiment 3 and all the data in the other experiments in this
Table 5. Correlations Among the Behavioral Measures from Experiments 1 to 4

<table>
<thead>
<tr>
<th>Relation</th>
<th>Expt 1</th>
<th>Expt 2</th>
<th>Expt 3</th>
<th>Expt 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resistance to capture vs. Open-field exploration</td>
<td>no data</td>
<td><em>r = -.58</em></td>
<td><em>r = -.21</em></td>
<td><em>r = -.45</em></td>
</tr>
<tr>
<td></td>
<td>*p &lt; .002</td>
<td>*p &gt; .12</td>
<td>*p &lt; .0001</td>
<td></td>
</tr>
<tr>
<td>Resistance to capture vs. % Open-arm entries</td>
<td><em>r = .60</em></td>
<td><em>r = .29</em></td>
<td><em>r = .11</em></td>
<td><em>r = .31</em></td>
</tr>
<tr>
<td></td>
<td>*p &lt; .0001</td>
<td>*p &lt; .03</td>
<td>*p &gt; .44</td>
<td>*p &lt; .007</td>
</tr>
<tr>
<td>Resistance to capture vs. % Time on open arms</td>
<td><em>r = .56</em></td>
<td><em>r = .31</em></td>
<td><em>r = .08</em></td>
<td><em>r = .36</em></td>
</tr>
<tr>
<td></td>
<td>*p &lt; .0002</td>
<td>*p &lt; .02</td>
<td>*p &gt; .54</td>
<td>*p &lt; .001</td>
</tr>
<tr>
<td>Open-field exploration vs. % Open-arm entries</td>
<td>no data</td>
<td><em>r = -.45</em></td>
<td><em>r = -.26</em></td>
<td><em>r = .15</em></td>
</tr>
<tr>
<td></td>
<td>*p &lt; .001</td>
<td>*p &lt; .05</td>
<td>*p &gt; .10</td>
<td></td>
</tr>
<tr>
<td>Open-field exploration vs. % Time on open arms</td>
<td>no data</td>
<td><em>r = -.45</em></td>
<td><em>r = -.22</em></td>
<td><em>r = .23</em></td>
</tr>
<tr>
<td></td>
<td>*p &lt; .001</td>
<td>*p &lt; .05</td>
<td>*p &lt; .03</td>
<td></td>
</tr>
<tr>
<td>% Open-arm entries vs. % Time on open arms</td>
<td><em>r = .90</em></td>
<td><em>r = .97</em></td>
<td><em>r = .97</em></td>
<td><em>r = .96</em></td>
</tr>
<tr>
<td></td>
<td>*p &lt; .0001</td>
<td>*p &lt; .0001</td>
<td>*p &lt; .0001</td>
<td>*p &lt; .0001</td>
</tr>
</tbody>
</table>

Significant differences are denoted with an asterisk (*).

thesis. My student assistant may have been less forceful in picking up the rats in the resistance-to-capture test in Experiment 3. This may account for the fact that the correlations between resistance to capture and the other three measures in this experiment were not significant—the resistance-to-capture scores in Experiment 3 are much lower than those that I typically observe when I am collecting these data.

Although the statistically significant correlations among open-field exploration, resistance to capture, and the percentage of open-arm activity in the elevated plus maze suggest that these tests all measure a common effect produced by kindling, it is important to note that they do not by themselves indicate that there is only one underlying influence governing the rats' behaviour in these tests. A factor analysis of these variables could be
useful to further illuminate the relation among them. However, in this case, factor analysis was not possible for two reasons: First, because the data were measured on different numerical scales (e.g., resistance to capture and percentage of open-arm activity), it is not possible to factor analyze them (Cattrell, 1978, pp., 500); and second, there were an insufficient number of variables to conduct a meaningful factor analysis (see Cliff, 1987, pp. 339). In this case, there would be only three variables—the percentage of open-arm entries and the percentage of time spent on the open arms cannot be used as two separate variables because they are nonlinear functions of each other. Cliff (1987) recommends the inclusion of at least 12 variables in any factor analysis and at least three to five times as many variables as components.

EXPERIMENT 5: ROLE OF UNFAMILIARITY IN THE EXPRESSION OF KINDLING-INDUCED HYPEREMOTIONALITY

The first four experiments in this thesis demonstrated that long-term kindling increases a variety of related interictal emotional behaviors. I have hypothesized that these behaviors are fundamentally defensive rather than aggressive in nature. This distinction has great clinical and theoretical significance. There has long been a belief that temporal lobe epileptics are prone to outbursts of aggression (Fenwick, 1991), despite the fact that little empirical evidence exists to support this claim. This label of “aggressive” has caused considerable psychosocial problems for epileptic patients. Alternatively, it has been suggested that the emotional outbursts are fundamentally defensive in nature (Gloor, 1992). If interictal hyperemotionality is fundamentally defensive, it is important that corroborative evidence be gathered so that it can serve as a basis for reducing the psychosocial problems associated with labelling epileptic patients as aggressive. Such evidence would also be critical in guiding the research on the neural mechanisms underlying interictal emotionality: The neural substrates of aggression have been shown to be different from the neural substrates of defense (Blanchard & Blanchard, 1988).
One way to test the hypothesis that kindling-induced emotional behaviors are fundamentally defensive in nature is to assess the effect on them of unfamiliar situations—unfamiliarity is known to potentiate fear (e.g., Hendrie, Weiss, & Eilam, 1996; Lister, 1991) and to result in an increased release of stress hormones (e.g., Romero, Levine, & Sapolsky, 1995) in rats. It also decreases aggression and increases defense—in a threatening situation, such as exposure to an unfamiliar object or situation, the primary responses of a rat are defensive (Blanchard & Blanchard, 1988). In each of the previous experiments, the behavioural testing was done in an environment unfamiliar to the rats and by an experimenter unfamiliar to the rats; however, the impact of the unfamiliarity was unclear because no rats were tested in familiar conditions.

The purpose of Experiment 5 was to test the hypothesis that kindling-induced increases in emotional behaviour are fundamentally defensive by investigating the role of unfamiliarity in kindling-induced emotionality in two ways: by comparing the resistance-to-capture scores of kindled rats in their home cages as opposed to an unfamiliar open field and by assessing the effects of repeated testing of kindled rats in the open field.

METHOD

Kindling Protocol and Behavioural Testing

A bipolar electrode was implanted into the left amygdala of each of 26 rats. After a postsurgical recovery period of 10 days, one group of rats received 99 convulsive stimulations (kindled, \( n = 13 \)) and another group of rats received an equal number of sham stimulations (sham stim, \( n = 13 \)).

Two days after the final stimulation, each rat's resistance to capture from its home cage was assessed using the same 7-point scale that was used in the first four experiments. The next day, the open-field testing began. For 5 consecutive days, each rat was placed in the open field for 5 min, and then its resistance to capture was assessed.
**Statistical Analyses**

The significance of the differences in resistance to capture between the kindled and the sham-stim rats were analyzed nonparametrically in two ways. First, the differences in resistance to capture on the first day of exposure to the open field were assessed using a Mann-Whitney U test. Second, the between-day differences in resistance to capture in the kindled rats were assessed using a Wilcoxon-Signed Ranks test.

**RESULTS**

Kindling induced high levels of resistance to capture from an unfamiliar open field but not from the rats' home cage. However, the high levels of resistance to capture from the open field declined with repeated exposure to the open field.

**Histology**

Figure 21 illustrates the location of the electrode tip in each rat from Experiment 5. Each electrode terminated in the amygdala, thus the data of all 26 subjects were included in the statistical analyses.

**Resistance to Capture**

Figure 22 illustrates the mean resistance to capture displayed by the kindled and sham-stim rats tested in their home cages and after repeated daily exposure to an initially unfamiliar open field. The kindled and sham-stim rats did not differ significantly in their resistance to capture from the home cage, $U = 63.5, p > .28$. However, during the first exposure to the open field, the kindled rats were significantly more resistant to capture than the sham-stim rats, $U = 169 \ p < .0001$. The resistance to capture displayed by the kindled rats during their first exposure to the open field declined significantly by day 5, $z = -3.18, p < .0015$. 
Figure 21. Histological results from the two groups of rats in Experiment 5. All the electrodes terminated within the amygdala. Abbreviations: LaDL, lateral amygdalar nuclei; BLA, BLP, BMP, basolateral amygdalar nuclei; CeM, CeL, central amygdalar nuclei.
Figure 22. The mean resistance-to-capture scores displayed in Experiment 5 by the kindled and sham-stim rats tested in their home cage and after repeated daily testing in an initially unfamiliar open field.
DISCUSSION OF EXPERIMENT 5

In Experiment 5, neither the kindled nor the sham-stim rats were resistant to capture from their home cages, and the sham-stim rats were not resistant to capture from the open field. However, the kindled rats were extremely resistant to capture on their first day of exposure to the open field; then their resistance to capture declined monotonically as the open field became more familiar. Thus, the intensity of the emotional behaviour produced by long-term amygdala kindling is greatest when the subjects are tested in an unfamiliar situation.

The fact that kindled rats react more emotionally in unfamiliar situations explains why some investigators who have handled kindled rats for years during their daily stimulations have not noticed major increases in emotional behaviour (e.g., Cain, 1992). The present results suggest that as long as kindled rats are handled in situations that are familiar to them, few handling-related emotional problems should be elicited.

The findings from this experiment are the first to provide conclusive evidence that unfamiliarity plays a key role in the expression of kindling-induced changes in emotional behaviour. No previous studies of kindling-induced emotionality have compared animals tested in both familiar and unfamiliar situations. Because rodents are neophobic, exposure to unfamiliarity should increase defensive but not aggressive behaviors (Blanchard, Kleinschmidt, Flannelly, & Blanchard, 1984). Thus, the fact that the emergence of kindling-induced emotionality is contingent on the degree of unfamiliarity associated with the testing situation supports the hypothesis that kindling increases the rats' defensive, rather than aggressive, behaviour.

EXPERIMENT 6: FUNDAMENTAL NATURE OF KINDLING-INDUCED HYPEREMOTIONALITY: AGGRESSION OR DEFENSE?

The results of Experiment 5 provided one piece of evidence in favor of the hypothesis that kindling-induced increases in interictal emotionality are fundamentally
defensive in nature: High levels of resistance to capture were dependent on the unfamiliarity of the situation in which the rats were tested. The purpose of Experiment 6 was to test this hypothesis in another way—by comparing the behaviour of kindled and sham-stimulated control rats as intruders in the resident-intruder paradigm. The resident-intruder paradigm is particularly useful for assessing aggressive and defensive behaviour because sequences of the aggressive and defensive behaviours that commonly occur in this situation are readily discriminable (see Blanchard et al., 1989). The resident rat is generally dominant, and the intruder rat submissive. Accordingly, the resident rat first sniffs the perianal area of the intruder and chases it around the cage. The intruder, unable to escape from the apparatus, eventually turns to face the resident and rears up on its haunches in a “boxing” posture. Then, the resident approaches the intruder sideways (lateral approach) so that it is in a position to make a darting attack around the intruder to deliver a bite to its back, which is the target site of all aggressive social attacks by rats. The intruder defends itself by pivoting on its hindlegs and fending off the resident with its forepaws; however, if sufficiently pressed, it will launch a defensive biting jump attack directly at the face of the intruder. If kindling-induced interictal emotionality is fundamentally defensive in nature, then the kindled rats in this paradigm should react more defensively than the sham-stimulated rats when approached by the dominant resident rat.

**METHOD**

**Kindling Protocol and Behavioural Testing**

The rats used in this experiment were the same as those used in Experiment 5. As described in the Method section of Experiment 5, one group of rats received 99 stimulations (kindled, \(n = 13\)) and another group of rats received an equal number of sham stimulations (sham stim, \(n = 13\)). One day after the final stimulation, half the kindled and half the sham-stim rats were tested in the resident-intruder paradigm. Each rat was placed into the cage of a different weight- and age-matched resident rat for 10 min, and the interaction of the two
rats was videotaped for later analysis. The next day, the other half of the kindled and sham-stim rats were tested with the same resident rats.

Two measures of aggressive behaviour and one measure of defensive behaviour were scored from the videotapes of resident-intruder interactions. The two measures of aggressive behaviour were the percentage of rats engaging in lateral attacks and the percentage of rats biting the back of the resident, and the one measure of defensive behaviour was the percentage of rats launching jump attacks at the resident.

**Statistical Analyses**

The significance of the differences in the percentage of rats from each group displaying lateral attacks, back bites, and jump attacks was assessed nonparametrically using a Chi-square test.

**RESULTS**

Figure 23 illustrates the percentage of rats from each group engaging in lateral attacks, back bites, and jump attacks. A substantially greater percentage of kindled rats launched defensive jump attacks and a smaller percentage displayed lateral attacks or back bites compared to the sham-stim rats. A Chi-square analysis revealed that these differences were statistically significant for jump attacks \( X^2 (1, N = 26) = 3.846, p < .05 \) and lateral attacks \( X^2 (1, N = 26) = 5.85, p < .02 \) but not back bites \( X^2 (1, N = 26) = 3.391, p > .06 \).

**DISCUSSION OF EXPERIMENT 6**

The results of Experiment 6 are as follows: Intruder rats that had been subjected to long-term kindling were much more likely to launch defensive attacks than were the sham-stim rats and they were also much less likely to display aggressive lateral attacks and back bites. Accordingly, by these measures, rats that have been subjected to long-term amygdala kindling are more defensive and less aggressive than sham-stimulated controls.
Figure 23. The percent of kindled and sham-stim rats that engaged in defensive and aggressive behaviors when tested as intruders in a resident-intruder paradigm in Experiment 6.
These findings are consistent with the findings of others. Racine and Bawden (1979) found that amygdala-kindled rats did not differ from controls in the latency to predatory attack of mice or in the amount of aggression directed at an unfamiliar conspecific in a social interaction test. In addition, Mellanby, Strawbridge, Collingbridge, George, Rands, Stroud, and Thompson (1981) found that rats made epileptic by an injection of tetanus toxin displayed substantially more defensive behaviour toward an intruder rat introduced into their home cages--7 of 11 epileptic rats displayed defensive behaviour when confronted by the intruder rat compared to only 1 of 12 control rats. I have also tested kindled rats as resident rats in a resident-intruder paradigm and found that they are uncharacteristically passive and defensive toward naive intruder rats (Kalynchuk & Pinel, unpublished observations). This was a particularly compelling finding because resident rats normally act very aggressively toward intruder rats (Blanchard & Blanchard, 1988).

The combined results of Experiment 5 and Experiment 6 provide clear evidence that kindling-induced changes in interictal behaviour are defensive in nature. The fact that long-term amygdala kindling produces increased defensive behaviour is at odds with the widely held notion that temporal lobe epileptics are highly aggressive (Fenwick, 1991). They suggest instead that temporal lobe epileptics tend to become excessively fearful and display heightened levels of defensive behavior when threatened. These results provide support for the results of recent retrospective studies of human temporal lobe epileptics: although epileptics tend to respond excessively to threatening situations, few engage in acts of premeditated aggression (Gloor, 1992).
EXPERIMENT 7: CHANGES IN SEROTONIN RECEPTOR BINDING ASSOCIATED WITH LONG-TERM AMYGDALA KINDLING

The preceding six studies of long-term kindled rats clearly establish that long-term kindling produces large and reliable increases in emotional behaviour. However, the neural mechanisms that underlie these increases remain unknown.

The purpose of Experiment 7 was to determine whether serotonin (5-HT) could be involved in the development of kindling-induced hyperemotionality by assessing the amount of agonist binding to 5-HT$_{1A}$ receptors in six different brain regions--the CA1, CA3, and dentate gyrus regions of the hippocampus, the amygdala, the perirhinal cortex, and the periaqueductal grey (PAG)--of long-term amygdala-kindled rats. These structures were chosen for analysis for two reasons: They contain a significant number of 5-HT$_{1A}$ receptors (Wright, Seroogy, Lundgren, Davis, & Jennes, 1995), and they have been implicated either in the mediation of seizures, emotional behaviour, or both (Graeff, 1993; Lothman et al., 1991). Serotonin receptors were chosen for analysis because excess amounts of brain serotonin have been shown to produce anxiogenic effects in several animal models of anxiety (Graeff, 1993).

Serotonin is released from the raphé nuclei, and projections from these nuclei innervate a number of structures that have been implicated in the mediation of various types of emotional behaviour (Graeff et al., 1993; Guimaraes, Del Bel, Padovan, Netto, & de Almeida, 1993). Of the 13 subtypes of 5-HT receptors that have been identified in the mammalian brain (Lucki, 1996), 5-HT$_{1A}$ receptors appear to play a particularly important role in anxiety and defensive behaviour. These receptors are located both on the cell bodies of raphé nuclei, where they act as autoreceptors, and at various postsynaptic raphé axon innervation sites. Agonists of 5-HT$_{1A}$ receptors are inhibitory: They reduce 5-HT neuronal function by reducing raphé neuron firing (Handley et al., 1993) and thus decreasing 5-HT release at innervation sites such as the hippocampus (Huston, Sarna, O'Connell, & Curzon, 1989). They may also produce inhibitory effects through their synaptic connections with
GABAergic interneurons in the hippocampus (Freund, 1992). Indeed, the 5-HT$_{1A}$ agonist buspirone is a clinically effective anxiolytic drug, and other 5-HT$_{1A}$ agonists have anxiolytic effects in animal models of anxiety (File & Gonzalez, 1996; Higgins, Bradbury, Jones, & Oakley, 1988; Treit, Robinson, Rotzinger, & Pesold, 1993). Thus, in Experiment 7, the effects of long-term amygdala kindling on the binding of 8-OH-DPAT, a 5-HT$_{1A}$ receptor agonist, was assessed.

**METHOD**

A bipolar electrode was implanted into the left amygdala of 15 rats. After a postsurgical recovery period, one group of rats received 99 convulsive stimulations (kindled, $n = 10$), and another group of rats received an equal number sham stimulations (sham-stim, $n = 5$).

**Receptor Autoradiography**

Each rat was sacrificed 36 hr after the final stimulation, and its brain was rapidly removed and frozen in isopentane and dry ice. The brains were stored in a -80°C freezer for approximately 1 month. Then, frozen 20 μm coronal sections were cut through the hippocampus and periaqueductal grey, thaw-mounted on gel-albumin-coated slides, and stored at -30°C until assay. The slides were then pre-incubated in 4 °C buffer, incubated in radiolabelled [³H]-8-OH-DPAT, rinsed in a cold buffer, and dried under a stream of cool air. They were then apposed to ³H-Hyperfilm along with a set of ³H standards (Amersham Microscales, activity 3.03-109.08 nCi) for 8 weeks, after which the film was developed. The regional receptor binding in six different brain regions—the CA3, CA1, and dentate gyrus regions of the hippocampus, as well as the amygdala, PAG, and perirhinal cortex—was quantified using optical density measurements provided by an Imaging Research Inc. image analysis system calibrated with the ³H standards.
Statistical Analyses

The statistical significance of the differences between the kindled and sham-stim rats in 8-OH-DPAT binding in each brain region was evaluated parametrically using 2-tailed t-tests.

RESULTS

Figure 24 illustrates the mean optical density of 8-OH-DPAT binding in specific regions of the brains of the kindled and sham-stim rats. A higher optical density indicates a greater amount of binding. Thus, it is evident from Figure 24 that the amount of 8-OH-DPAT binding was relatively low in all brain regions except the CA1 and dentate gyrus regions of the hippocampus. Furthermore, the amount of binding in the two groups was virtually identical in every region except the dentate gyrus, where the kindled rats displayed a much greater degree of 8-OH-DPAT binding than did the sham-stim rats. This difference was statistically significant, \( t(9) = 2.35, p < .05 \).

DISCUSSION OF EXPERIMENT 7

In Experiment 7, 8-OH-DPAT binding to 5-HT\(_{1A}\) receptors was significantly greater in the dentate gyrus of long-term amygdala-kindled rats. The increased binding observed in the dentate gyrus may reflect either an increase in the affinity of 8-OH-DPAT for 5-HT\(_{1A}\) receptors or an increase in the number of these receptors. Interestingly, no statistically significant effects were detected in the amygdala or periaqueductal grey, two brain regions thought to be important in the mediation of emotional behavior. In fact, the amount of 8-OH-DPAT binding in these areas was relatively low.

The results of this experiment are consistent with the results of the one previous study to investigate 5-HT\(_{1A}\) receptor binding in the brains of kindled rats: Clark, Weiss, and Post (1993) found that short-term amygdala kindling induced a persistent increase in 8-OH-DPAT binding in the dentate gyrus but not in the cortex. They are also consistent with the
Figure 24. The mean optical density of 8-OH-DPAT binding in several brain regions from the two groups of rats in Experiment 7. A higher optical density indicates more binding. Only the difference in the amount of binding in the dentate gyrus was statistically significant.
results of a study using a different animal model: Hayakawa and his colleagues (1994) found that repeated treatment with electroconvulsive shock in rats increased 8-OH-DPAT binding sites in the dentate gyrus but not in the CA1 and CA3 subfields of the hippocampus, the dorsal raphé nucleus, or the septal nucleus.

What might be the significance of increased 5-HT\textsubscript{1A} binding in the dentate gyrus for kindling-induced emotionality? The increased 5-HT\textsubscript{1A} binding suggests that kindling produces a decrease in serotonin levels in the dentate gyrus. On the surface, this is inconsistent with the popular view that an increase in serotonin levels precipitates anxiety (e.g., Graeff, 1993). However, after a review of the literature, Panksepp (1991) recently concluded that it is unclear whether serotonin serves to increase or decrease anxiety. One possibility is that anxiety arises when serotonin is increased in some brain regions and decreased in others. For example, Deakin and Graeff (1991) have suggested that activation of the 5-HT\textsubscript{2} pathway between the dorsal raphé and the amygdala increases fear, whereas deactivation of the 5-HT\textsubscript{1A} pathway between the median raphé and the hippocampus impairs adaptive responses to stress. Based on this suggestion, increased serotonin in the amygdala and decreased serotonin in the hippocampus may mediate the emotionality of long-term amygdala-kindled rats.

The speculative idea that low levels of serotonin in the hippocampus may mediate some of the emotionality produced by long-term amygdala kindling is consistent with the evidence suggesting that antidepressant medication has anxiolytic effects in patients suffering from generalized anxiety disorder. Antidepressant drugs act to increase the availability of serotonin in the hippocampus. It would be interesting to determine whether antidepressant drugs would attenuate kindling-induced increases in emotionality.

The dentate gyrus seems to be a key region of convergence for several forms of plasticity—it is considered to be the seizure initiation area of the brain (Houser, 1992; Lothman, Stringer, & Bertram, 1992; Sloviter, 1994); it has been implicated in the mediation of neophobia in rats (Belzung, 1992; Maren, Tocco, Chavanne, Baudry,
Thompson, & Mitchell, 1994); it has an abundance of 5-HT and GABA receptors, which are both thought to be important in the expression of abnormal levels of emotional behaviour (File, 1991); and it is the site of stress-induced changes, some of which are regulated by 5-HT binding in the hippocampus (Mendelson & McEwen, 1992; Mitchell, Iny, & Meaney, 1990). Furthermore, there are interesting interactions among each of these forms of plasticity. Particularly noteworthy is the fact that the serotonergic axons from the raphé nucleus to the dentate gyrus synapse predominantly on GABAergic interneurons (Freund, 1992). Accordingly, the possibility exists that serotonin may work in concert with GABA in the dentate gyrus to affect kindling-induced changes in emotional behaviour. Although this hypothesis is highly speculative, support for it comes from the recent finding that $^3$H-SR95531 binding to GABA receptors and $^3$H-flunitrazepam binding to benzodiazepine receptors are selectively increased in the dentate gyrus, but not the in the amygdala or cortex, of long-term amygdala-kindled rats (Kalynchuk, McEachern, Barr, Pinel, & Shaw, 1995).

The relevance of the results of Experiment 7 for understanding of the mechanisms involved in kindling-induced hyperemotionality are currently unclear. Experiment 7 was a preliminary study and further research is needed to explore the possible role of serotonin in mediating kindling-induced changes in emotional behaviour. Currently, there is no evidence whatsoever that the observed changes in 8-OH-DPAT binding in the dentate gyrus are related to the increased emotionality observed in long-term amygdala-kindled rats--correlational analysis of the results of the battery of tests of emotional behaviour used in the previous experiments in this thesis and 8-OH-DPAT binding in the dentate gyrus is an important next step in establishing evidence for such a relation. Furthermore, if the existence of such a correlation is established, it will still be necessary to establish that it is a causal one. Experiment 7 was also limited by the fact that only one type of serotonin receptor was analyzed. There are currently 13 subtypes of serotonin receptors that have been identified (Lucki, 1996), and several of them may play a role in the development of abnormal
emotionality (Dubovsky & Thomas, 1995). Moreover, there is reason to believe that serotonin may interact with other neurotransmitters or neuropeptides to produce effects on emotional behaviour (Graeff, Brandao, Audi, & Schutz, 1986; Shephard, 1986; Suranyi-Cadotte, Bodnoff, & Welner, 1990). Thus, although Experiment 7 was a positive step forward, many more studies are needed to determine if and how serotonin may mediate the effects of long-term kindling on emotional behaviour.
GENERAL DISCUSSION

The three general purposes of this thesis were to demonstrate the potential of long-term amygdala kindling as a model of the interictal hyperemotionality associated with temporal lobe epilepsy, to provide some parametric data about the interictal hyperemotionality associated with temporal lobe epilepsy, and to use the model to clarify the nature of the interictal hyperemotionality associated with temporal lobe epilepsy. The results of the experiments in this thesis fulfilled these purposes.

Experiments 1 and 2 demonstrated that long-term amygdala kindling can induce in rats a syndrome of increased emotionality that is both reliable and large. This syndrome included decreased exploration in an unfamiliar open field, increased resistance to capture from the open field, increased percentage of entries on the open arms of an elevated plus maze, increased percentage of time spent on the open arms of the elevated plus maze, and increased jumping from the elevated plus maze.

The observation in Experiment 2 that the number and magnitude of these effects tends to be greater following long-term kindling than short-term kindling confirms the rationale on which my decision to study the effects of long-term kindling was initially based. That rationale was based on the fact that kindling is a progressive disorder: as the number of stimulations increases, rats begin to experience seizures of greater severity; and eventually, spontaneously recurring seizures develop. Thus, I hypothesized that long-term kindling would produce changes in interictal emotional behaviour that are larger and more reliable than those typically produced by short term kindling, as well as some changes in interictal emotional behaviour that are not produced by short-term kindling. Indeed, in Experiment 2, some changes in emotional behaviour that were present in kindled rats after short-term kindling (i.e., after 20 stimulations) were far greater after long-term kindling (i.e., after 100 stimulations)—for example, decreased activity in the open field and increased resistance to capture from the open field. In addition, one change in emotional behaviour
that was not present at all in rats after short-term kindling was readily apparent after 100 stimulations: jumping from the elevated plus maze.

Experiments 3, 4, 5, and 6 provided some parametric data about the interictal hyperemotionality of temporal lobe epilepsy and clarified its nature. Experiment 3 showed that the substantial increases in emotionality produced by long-term amygdala kindling declined after the final stimulation but were still quite enduring. The level of emotionality observed in the rats tested 1 month after the final stimulation was not statistically significant from either the high levels observed in the rats tested 1 day after the final stimulation or the baseline levels observed in the sham-stimulated rats. Experiment 4 showed that long-term-kindling-induced increases in emotionality are not specific to amygdala stimulation: Compared to sham-stimulated and caudate-kindled rats, amygdala-kindled and hippocampal-kindled rats displayed significantly less open-field exploration, significantly more resistance to capture from the open field, and a significantly greater percentage of open-arm activity on the elevated plus maze. Although the amygdala-kindled rats did not differ significantly from the hippocampal-kindled rats, they were more emotional on every measure. Experiment 5 showed that unfamiliarity plays a critical role in determining the intensity of the emotional responses produced by long-term amygdala kindling—the kindled rats displayed very little resistance to capture from their home cages but extreme resistance to capture from a novel open field. This extreme resistance to capture dissipated monotonically with repeated exposures to the open field. Experiment 6 showed that kindled rats display more defensive jump attacks and fewer aggressive lateral displays and back bites than sham-stimulated rats when tested as intruders in a resident-intruder paradigm. These findings are critical because they suggest that the fundamental interictal behavioural problem of temporal lobe epilepsy is hyperdefensiveness, not hyperaggressiveness.

Finally, Experiment 7 showed that 8-OH-DPAT binding to 5-HT1A receptors is increased in the dentate gyrus, but not the amygdala, the periaqueductal grey, the CA1 and CA3 subfields of the hippocampus, or the perirhinal cortex of long-term amygdala-kindled
rats. Although preliminary, this finding suggests that serotonin levels in the dentate gyrus could play a role in the mediation of kindling-induced emotional behaviour, either on its own, or in conjunction with changes in GABA and glucocorticoid receptors, which are also prevalent in the dentate gyrus.

Taken together, the results of these experiments confirm that studies of the emotional changes induced by long-term kindling have the potential for making unique contributions to the study of epilepsy-related emotionality. The relevance and implications of the results from these experiments are discussed in the following five sections of the General Discussion: (1) the fundamental nature of kindling-induced emotionality, (2) the critical factors influencing the development of kindling-induced emotionality, (3) a hypothesis concerning the neural mechanisms underlying kindling-induced emotionality, (4) the relevance to human temporal lobe epilepsy, and finally (5) the conclusions and future directions of this work.

FUNDAMENTAL NATURE OF KINDLING-INDUCED EMOTIONALITY

What does the pattern of behavioural change observed in the experiments in this thesis suggest about the fundamental nature of the emotional consequences of long-term amygdala kindling? With the exception of the observed changes in open-arm behaviour on the elevated plus maze, all of the observed behavioural changes strongly suggest that long-term amygdala kindling increases levels of fearfulness. This interpretation is supported by the fact that the changes in behaviour induced by long-term amygdala kindling are all defensive in nature and are most likely to manifest themselves in fear-inducing situations. For example, the kindled rats displayed great resistance to capture from an unfamiliar open field by an unfamiliar experimenter despite the fact that they were not at all resistant to capture from their home cages, and this extreme resistance to capture declined monotonically as the rats became more familiar with the open field. Finally, when exposed to a resident conspecific, the kindled rats displayed more defensive jump attacks and fewer
aggressive lateral displays than did the sham-stimulated rats. Together, these findings provide strong support for the hypothesis that kindling produces defensive responses to fear-provoking stimuli.

How can the results of the elevated-plus-maze test be reconciled with the conclusion that long-term kindling induces extreme increases in fearfulness and defensiveness in rats? In Experiments 1 to 4, the long-term kindled rats engaged in a much greater percentage of open-arm entries on the elevated plus maze and spent a much greater percentage of time on the open arms of the elevated plus maze than did the sham-stimulated rats. An increase in percentage of open-arm activity on the elevated plus maze is traditionally interpreted as an anxiolytic effect because it is produced in rats by the administration of benzodiazepine-anxiolytic drugs (Pellow & File, 1986; Rodgers & Cole, 1994). Indeed, Adamec (in press) has concluded that his observations of increased open-arm activity in the elevated plus maze in short-term amygdala-kindled rats are indicative of an anxiolytic effect. However, in the Discussion section of Experiment 2, I proposed a three-stage interpretation of kindling-induced anxiogenesis on the elevated plus maze to account for the apparent paradox between the results of the elevated plus maze and the results of the other behavioural measures used in the experiments in this thesis. I suggested that the administration of a few stimulations should produce a level of maze-induced anxiety that motivates passive avoidance (i.e., remaining in the closed arms); additional stimulations should increase the level of maze-induced anxiety to motivate rats to venture out on the open arms of the elevated plus maze, engage in risk assessment, and search for an avenue of escape; and a large number of stimulations should produce high levels of maze-induced anxiety that motivate open-arm exploration, risk assessment, and escape from the maze by jumping.

An alternative interpretation of kindling-induced anxiogenesis in the elevated plus maze is that short term and long-term kindling have opposite effects on elevated-plus maze behaviour; that is, that short-term kindling produces an anxiolytic effect, whereas long-term kindling produces an anxiogenic effect. However, three pieces of evidence in favor of the
three-stage interpretation have emerged from the experiments in this thesis. First, in Experiment 2, the resistance-to-capture measure of emotionality indicated that the development of kindling-induced emotionality is monotonic. Rats that received 20 stimulations showed some increases in resistance to capture, rats that received 60 stimulations showed more increases, and rats that received 100 stimulations showed the greatest increases relative to the sham-stimulated rats. If short-term kindling produced anxiolytic effects, then the rats that received 20 stimulations would be expected to show about the same amount or even less resistance to capture than did the rats that received the sham-stimulations. Second, in Experiment 3, the rats tested 1 month after the last stimulation engaged in almost no open-arm activity—substantially, although not significantly, less than did the sham-stimulated rats. This is consistent with the prediction from the three-stage interpretation of kindling-induced anxiogenesis that kindled rats tested shortly after the last stimulation should prefer an active avoidance response and engage in more open-arm activity; kindled rats tested a few days after the last stimulation should prefer a more passive response and engage in less open-arm activity; and kindled rats tested a few weeks after the last stimulation should prefer a completely passive response and engage in no open-arm activity. The third piece of evidence in favour of the three-stage interpretation is that the ‘revisit’ of the data from Experiments 1 to 4 revealed that the decrease in open-field exploration, increase in resistance to capture, and increase in the percentage of open-arm activity in the rats that received 100 stimulations are significantly correlated with each other. It is unlikely that these measures would be significantly correlated if open-field exploration and resistance to capture represented an anxiogenic effect and the percentage of open-arm activity on the elevated plus maze represented an anxiolytic effect.

Support for the three-stage interpretation of kindling-induced anxiogenesis is also provided by the findings of other studies that have documented the anxiogenic effects of short-term kindling. Amygdala kindling causes decreases in predatory aggression and
increases in defensiveness in cats (Adamec, 1990), increases in defensiveness and social
withdrawal in squirrel monkeys (Lloyd et al., 1989), increases in CRF-induced defensive
fighting in rats (Weiss et al., 1986), and increases in stress-induced ulcers in rats (Henke &
Sullivan, 1985). Kindling with lidocaine has been shown to increase defensive responses to
handling in rats (Post et al., 1984); kindling with RO5-4864 has been shown to increase
conspecific fighting in rats (Weiss, Post, Marangos, & Patel, 1986); and kindling with FG
7142 has been shown to increase footshock-produced freezing both in mice (Jeevanjee,
Little, Martin, Nicholass, & Nutt, 1985) and rats (Corda, Giogi, Gatta, & Biggio, 1985). It is
thus highly unlikely that short-term kindling would produce an anxiolytic effect in the
elevated plus maze.

Unlike the amygdala-kindled rats in Experiment 2, those in Experiments 3 and 4 did
not jump from the elevated plus maze significantly more often than did the sham-stimulated
rats. However, there are some differences in the degree of emotionality produced in both
kindled and control animals from experiment to experiment. Furthermore, the rats in
Experiments 3 and 4 did engage in substantial risk assessment on the open arms—they
leaned over the edge of the maze and peered down at the floor, but they stopped just short of
jumping off. It is also worth noting that the statistically significant increase in jumping from
the elevated plus maze in the rats that received 100 stimulations in Experiment 2 has
recently been replicated (Kalynchuk, Pinel, & Meaney, in preparation).

What is it that makes the kindled rats so fearful and defensive on the tests in these
experiments? When placed in unfamiliar situations, the kindled rats appear to be constantly
on the brink of an explosive reaction in response to the slightest noise or change in
environment. Two different kinds of changes might account for this effect: The kindled rats
could have a perceptual problem that leads them to perceive danger in unfamiliar situations
in which it is not perceived by control rats, or they could perceive the degree of threat
presented by unfamiliar situations correctly but just overreact to it. Although this theoretical
distinction is an important one and has been repeatedly raised in the discussions of the
hyperemotionality of epileptic and anxiety-disorder patients (Gloor, 1992; Kelly, Mitchell-Heggs, & Sherman, 1971; Uhde, 1990), it has not proven amenable to experimental investigation.

CRITICAL FACTORS INFLUENCING THE EXPRESSION OF KINDLING-INDUCED EMOTIONALITY

There are three important points about the critical factors influencing the level of kindling-induced hyperemotionality made by the experiments in this thesis. Experiment 2 showed that the level of hyperemotionality depends on the number of stimulations that the rats have received; Experiment 3 showed that the level of hyperemotionality depends on the recency of seizures; and Experiment 4 showed that the level of hyperemotionality depends on the site of kindling. In this section of the General Discussion, each of these findings will be briefly discussed and integrated.

Experiment 2 was the first to provide experimental evidence that the degree of emotionality produced by amygdala kindling is dependent on the number of stimulations. All previous studies of kindling and behaviour had compared the behaviour of rats that had received the same number of stimulations. The finding that the number of stimulations influences the development of emotionality is consistent with the fact that kindling is progressive in nature—rats that receive more stimulations display more severe convulsions and eventually spontaneous convulsions (Pinel, 1981). Presumably, the greater the number of stimulations, the greater the activation of neural circuits involved in the hyperemotionality—Engel, Wolfson, and Brown (1978) found that 2-deoxyglucose uptake became greater and more widespread in limbic brain regions as rats experienced more convulsions. This suggests that the spontaneous seizure state produced by kindling should receive more experimental attention, not only for elucidating the mechanisms of epilepsy-induced emotionality, but also for enhancing our understanding of temporal lobe epilepsy as a whole.
Experiment 3 was the first to investigate the persistence of kindling-induced emotionality after the cessation of stimulations. The results of this study suggested that kindling-induced changes in emotionality are enduring but that they depend to some extent on the recent occurrence of seizures. Siegel (1984) has reported similar findings in cats that received massed electrical stimulations of the amygdala. The amygdala seizures reduced the electrical threshold to elicit hypothalamic defense reactions and decreased the latency to the occurrence of this defensive behavior. The magnitude of these changes declined over time, but in some cases, they lasted for 6 weeks after the last seizure. Note that these amygdala seizures did not result in kindling because the stimulations were timed very closely together; massed stimulations do not produce kindling (Racine, 1978).

Experiment 4 replicated the previous finding of Pinel, Treit, and Rovner (1978) that long-term amygdala and hippocampal kindling can both produce substantial increases in emotionality. The fact that long-term amygdala kindling consistently induced the greatest changes in the various measures of emotional behavior in Experiment 4 is consistent with the extensive research literature linking the amygdala to the expression of fear-motivated behaviour (e.g., Davis, 1992; Graeff et al., 1993; LeDoux, 1994; Treit, Pesold, & Rotzinger, 1993a, 1993b). Similarly, the increased emotionality observed after hippocampal kindling is consistent with two previous reports: Mellanby et al. (1981) reported that seizures induced by administration of tetanus toxin directly into the hippocampus produced defensive reactions to handling and passive responses toward an intruder rat in a resident-intruder paradigm; and Tanaka, Kailima, Daita, Ohgami, Yonemasu, and Riche (1982) reported that seizures induced by microinjections of kainic acid into the hippocampus made cats extremely difficult to handle.

Because both the amygdala and the hippocampus have been implicated in various types of emotional behaviour and because they have the lowest thresholds for the elicitation of limbic seizures, it is tempting to conclude that they are the critical regions for the neural changes underlying kindling-induced emotional behaviour. However, it is unclear from the
results of Experiment 4 whether the repeated activation of the amygdala and hippocampus
during the kindling stimulations was the critical factor in the development of
hyperemotionality, or whether the class of convulsions elicited by those stimulations was the
critical factor. For example, the caudate-kindled rats were much less emotional than the
amygdala or hippocampal-kindled rats and they displayed a much lower mean class of
convulsions elicited by the final 5 stimulations.

In summary, the results of Experiments 2, 3, and 4 make three points: They indicate
that the severity of kindling-induced increases in emotionality depends on the number of
stimulations, the region of the brain that is stimulated, and the time since the last seizure.
The clinical relevance of these points is discussed after the next section of the General
Discussion, in which I outline a hypothesis about the mechanisms of kindling-induced
hyperemotionality.

A HYPOTHESIS CONCERNING THE NEURAL MECHANISMS UNDERLYING KINDLING-
INDUCED HYPEREMOTIONALITY

The preliminary finding from Experiment 7 that 8-OH-DPAT binding to 5-HT\textsubscript{1A}
receptors is increased in the dentate gyrus but not the amygdala, the periaqueductal grey, the
perirhinal cortex, or the CA1 and CA3 subfields of the hippocampus is suggestive. It
implicates the dentate gyrus in both temporal lobe seizures and their effects on emotional
behaviour. In this section of the General Discussion, other evidence for the involvement of
the dentate gyrus in both temporal lobe seizures and their effects on emotional behaviour is
summarized. The section concludes with a hypothesis of how processes within the dentate
gyrus might mediate kindling-induced emotionality.

There is ample evidence for the idea that the dentate gyrus is important in the
mediation of temporal lobe seizures. For example, damage to dentate hilar neurons, mossy
fibre sprouting from dentate granule cells, and reorganization of the dentate granule cell
layer have been observed in the brains of temporal lobe epileptics (Houser, 1992;
Masukawa, O'Connor, Lynott, Burdette, Uruno, McGonigle, & O'Connor, 1995) and
amygdala-kindled rats (Cavazos et al., 1994; Sutula, 1991). Interestingly, these neural changes depend on the number of seizures experienced, as does the expression of kindling-induced hyperemotionality. In addition, kindled seizures produce the greatest amounts of c-fos activation in the pyriform cortex and dentate gyrus (Dragunow, Currie, Faull, Robertson, & Jansen, 1989), and administration of colchicine, which selectively destroys dentate granule cells while leaving neurons in the CA1-CA3 subfields of the hippocampus intact, retards the development of amygdala kindling (Dashieff & McNamara, 1989). Finally, a strong correlation has been found between the duration of maximal dentate activation and the severity of amygdala-kindled convulsions (Lothman et al., 1992). These findings are consistent with the hypothesis that the dentate gyrus acts as a ‘promoter’ or ‘amplifier’ of seizure activity.

There is indirect evidence that neural changes within the dentate gyrus play a role in the interictal hyperemotionality observed after temporal lobe seizures. The dentate gyrus contains serotonin, GABA\(_A\), and glucocorticoid receptors, all of which have been implicated in the mediation of emotional behaviour (Graeff et al., 1996). Furthermore, both GABA\(_A\) and glucocorticoid receptor systems have been associated with seizure-induced alterations in behaviour. GABA has been implicated by Adamec’s (1993) observation that interictal increases in feline defensiveness following partial hippocampal kindling are blocked by the administration of flumazenil, a benzodiazepine receptor antagonist, and by our (Kalynchuk et al., 1995) observations of selective increases in binding to GABA\(_A\) and benzodiazepine receptors in the dentate gyrus of long-term amygdala-kindled rats. Glucocorticoids have been implicated in interictal hyperemotionality by a series of reports. Amygdala-kindling has been found to produce high levels of circulating corticosterone (Szafreczyk, Caracchini, Rondouin, Ixart, & Malaval, & Assenmacher, 1986), which are associated with increased conditioned fear in rats (Corodimas, LeDoux, Gold, & Schulkin, 1994), an enhanced susceptibility of rats to pentylenetetrazol- and kainic acid-induced seizures (Roberts & Keith, 1994), dendritic atrophy in the CA3 region of the hippocampus (Wooley, Gould, &
McEwen, 1990), and decreased glucocorticoid receptor density in the dentate gyrus (de Kloet, 1991). In contrast, postnatally handled rats that are tested as adults display decreased fearfulness in an unfamiliar environment (Meaney, Diorio, Francis, Widdowson, LaPlante, Caldji, Sharma, Seckl, & Plotsky, 1996), decreased resistance to handling (Ader & Grota, 1965), decreased defensive behaviour in response to the presence of an intruder rat (Hilakivi-Clarke, Turkka, Lister, & Linnoila, 1991), and increased glucocorticoid receptor gene expression in the hippocampus, but not the septum, the amygdala, the hypothalamus or the pituitary.

Based on the converging evidence from studies of seizures and emotion, I speculate that morphological and neurochemical alterations observed in the dentate gyrus after repeated seizures could underlie the expression of interictal emotionality. This putative mechanism begins with the actions of the stress hormone, corticosterone. Normally, the presence of corticosterone in the brain after a stressful event triggers negative feedback that inhibits its further release from the adrenal glands (Dallman, Akana, Cascio, Darlington, Jacobson, & Levin, 1987); this feedback minimizes the increase in circulating corticosterone and thus prevents corticosterone-induced changes within the hippocampus. Long-term amygdala kindling may represent a form of repeated stress in which each kindling stimulation acts like an external stressor, initiating the release of corticosterone. Repeated kindling stimulations and corticosterone release may reduce the capacity of the negative feedback to maintain circulating corticosterone levels under control, and as a result, hippocampal damage, glucocorticoid receptor activation, and a compensatory downregulation of dentate glucocorticoid receptors would occur. Interestingly, amygdala stimulation increases corticotrophin releasing factor (CRF) in hippocampal neurons that normally do not produce CRF (Smith, Weiss, Abedin, Kim, Post, & Gold, 1991), further increasing circulating corticosterone levels, and these same sites project back from the hippocampus to the amygdala (Swanson & Simmons, 1989).
Several aspects of this speculation are consistent with the results of the experiments in this thesis. The idea that each kindling stimulation acts as a stressor to potentiate the presence of pathological amounts of corticosterone in the dentate gyrus is consistent with the findings of this thesis that the development of hyperemotionality depends on the number of stimulations that the rats receive and that its maintenance depends in part on the recent occurrence of seizures. Once the seizures cease, the repeated release of corticosterone would also cease, allowing the negative feedback loop to catch up and return the levels of circulating corticosterone to manageable levels. Moreover, the fact that extended exposure to pathological amounts of corticosterone produces dendritic atrophy and decreases in gene expression in hippocampal regions suggests that some remnants of the kindling-induced emotionality would be more lasting. This suggestion is consistent with the conclusion from Experiment 3 that some increases in emotionality remain persistent despite the fact that the stimulations have been discontinued. Finally, the premise that kindled convulsions release corticosterone is supported by the observation in Experiment 7 that binding to 5-HT1A receptors is increased in the dentate gyrus of long-term amygdala-kindled rats—5-HT1A agonists are known to cause the release of corticosterone (de Boer, Slangen, & van der Gugten, 1990).

In addition to its direct pathological effects, the excessive release of corticosterone may also alter the actions of GABA. Interestingly, despite the loss of dentate hilar cells observed in human temporal lobe epileptics and kindled rats, there is a preferential preservation of GABAergic neurons in this region (Babb, Pretorius, Kupfer, & Crandall, 1989). Orchinik, Weiland, and McEwen (1994) recently reported that glucocorticoids can modulate hippocampal excitability by altering the expression of specific GABA\(_A\) receptor subunits in the dentate gyrus. This suggests that corticosterone-induced changes in subunit expression might alter GABAergic synaptic inhibition by changing the density of GABA\(_A\) receptors or their subunit composition and affecting the pharmacological properties of the receptors. This hypothesis is supported by the fact that blockade of spontaneous seizures in
kainate treated cats by anticonvulsant drugs that affect GABA release exacerbated their interictal defensiveness (Griffith, Bandler, & Engel, 1987). Thus, an interaction between GABA and corticosterone within the hippocampus may mediate the hyperdefensiveness associated with long-term amygdala kindling.

**RELEVANCE TO HUMAN TEMPORAL LOBE EPILEPSY**

The symptoms and etiology of interictal changes in emotion associated with temporal lobe epilepsy are diverse. For example, many temporal lobe epileptics experience fear as a prominent ictal and interictal symptom (Hermann, Dikman, Schwartz, & Karnes, 1982; Gloor, 1990) and have significant atrophy of the amygdala (Cendes, Andermann, Gloor, Gambardella, Lopes-Cendes, Watson, Evans, Carpenter, & Olivier, 1994); however, many do not. In addition, many temporal lobe epileptics experience behavioural disturbances almost continuously, but some experience them intermittently (Devinsky, 1991).

The following are factors that may account for the diversity of the interictal behavioural pathology associated with temporal lobe epilepsy. First, ictal events may produce sudden, intense alterations in affect; however, these changes are not likely to be manifested unless emotion-inducing stimuli are concomitantly present. Second, the nature of interictal disturbances may depend on the cerebral hemisphere in which the epileptic focus is located (Adamec, in press; Adamec & Morgan, 1994; Devinsky, Ronsaville, Cox, Witt, Fedio, & Theodore, 1994). Third, patients with temporal lobe epilepsy may also have structural lesions (i.e., hippocampal sclerosis) that can alter their ability to process and attach affective tone to certain types of information. Fourth, in addition to the seizure disorder itself, patients may experience alterations in their emotional behaviour as a result of pharmacological treatment. These alterations may arise from the common side effects of the medication or from some uncommon effects that potentiate the interictal behavioral disturbances (Trimble, 1988). And finally, patients may experience the effects of various
psychological reactions and social-interpersonal variables related to the knowledge that they have a seizure disorder. Mittan and Locke (1982) report that 78% of their sample of 147 epileptic patients fear that their seizures will become life-threatening; 49% are concerned about losing their jobs because they are epileptic, and 55% withdraw socially as a means of coping with their fear about their illness. The tendency of epileptics to withdraw socially could contribute to family relationship disturbances; it could also prevent them from seeking out extra-familial social support networks.

Because of the diversity of interictal emotionality and the numerous factors that can affect its course, caution must be exercised in directly applying the results of the experiments in this thesis to the human epileptic condition. Nevertheless, there are several pertinent points worthy of discussion. For example, the findings from Experiment 2 suggest that seizure number may account for some of the diversity in interictal emotionality. Indeed, seizure number is a major predictor of emotional problems in epileptics who experience generalized tonic-clonic convulsions (Dodrill, 1986); personality disorders are found more frequently the earlier the seizures start and if the seizures are generalized (Rutter, Graham, & Yule, 1970; Taylor, 1972); behavioral alterations may be intensified in some patients during periods of increased seizure frequency (Hermann & Melyn, 1984); and greater degrees of neuropsychological impairment in human epileptics are associated with the onset of seizures at an early age, a large total number of seizures, and a long history of seizures (Dikman 1980). Unfortunately, to my knowledge, the relationship between seizure number and the onset of hyperemotionality has not been systematically studied in a selective population of temporal lobe epileptics. However, in a study of 114 epileptic patients suffering from complex partial seizures, Adamec (1990) found that the degree of change in affect varied directly with the reported frequency and intensity of aura experiences. The change in affect was manifested as an increase in intensity as well as lability of emotional responses. Because auras are thought to originate from focal epileptic limbic system discharges, Adamec’s data suggest that greater numbers of limbic seizures precipitate
greater changes in mood. These findings confirm the results of Experiment 2 and suggest that long-term kindling may be a particularly useful way to model the affective changes of temporal lobe epileptics.

The conclusion of Experiment 3 that the kindling-induced hyperemotionality results both from the transient aftereffects of recent seizures and from the changes underlying the epileptic state is consistent with reports from the clinical literature. Certain behavioural disturbances in epileptic patients disappear when seizures are treated successfully (Taylor, 1972). For example, Spiers, Schomer, Blume, and Hochanadel (1992) reported the case of a patient whose primary complaint was of constant dysphoric feelings that were refractory to psychiatric and antidepressant therapies. However, these symptoms disappeared once for nearly a week after he had attempted suicide by phenobarbital (i.e., an anticonvulsant drug) overdose. During a subsequent EEG recording session, constant periodic bursts of small spike and wave seizure activity from the right posterior temporal region were discovered, and when the patient was given a single dose of paraldehyde, a potent short-acting anticonvulsant, these bursts of seizure activity disappeared and the dysphoric mood lifted. Unfortunately, his affect returned once the paraldehyde wore off and the spike and wave activity returned. However, not all patients experience relief from interictal hyperemotionality once their seizures have been treated. Some patients complain of tension, irritability, depression, and poor attention span after prolonged seizure-free intervals (Devinsky, 1991). In some such patients, suppressing seizures actually exacerbates undesirable interictal behaviors.

It came as no surprise that kindling of both the amygdala and the hippocampus produced high levels of emotionality in rats in Experiment 4 for three reasons. First, depth recording studies in human epileptics suggest that the anxiety and fear are associated with amygdala and hippocampal discharges (Nickell & Uhde, 1991). Second, patients who received a series of periodic amygdala or hippocampal stimulations eventually displayed extreme stimulus-bound emotional behaviors, such as fear or rage; these emotions occurred
suddenly and without any relation to the motive state of the patient just prior to the stimulations (Spiers et al., 1992). And third, the Kluver-Bucy syndrome, which results largely from damage to the amygdala in monkeys (Weiskrantz, 1956), is characterized by changes in emotional behaviour that are opposite to those observed in temporal lobe epileptics. Thus, there is a convergence of evidence implicating both the amygdala and hippocampus in the generation of interictal hyperemotionality in human epileptics, to which the results of Experiment 4 can be added.

The finding of Experiments 5 and 6 that kindling-induced emotionality is defensive rather than aggressive in nature has important practical implications. For decades, the stigma of being labelled aggressive has rested on the shoulders of epileptics, despite the paucity of relevant empirical evidence. Indeed, the few adequately designed studies that have attempted to document a relation between temporal lobe epilepsy and aggression have been largely unsuccessful; for example, Riley and Neidermayer (1978) found that temporal lobe epileptics are not particularly prone to acts of violence, recurrent aggressive behaviour, or outbursts of anger that were serious enough to warrant neuropsychiatric evaluation. Although the prevalence of aggression is somewhat higher in people with temporal lobe epilepsy than in the general population, it is not higher than that found in people suffering from other forms of epilepsy or other chronic illnesses (Strauss, 1989). Thus, the finding of Experiments 5 and 6 that the emotional outbursts are fundamentally defensive may help chip away at the unwarranted assumption that temporal lobe epileptics are particularly aggressive.

**CONCLUSIONS AND FUTURE DIRECTIONS**

The results of the experiments in this thesis have fulfilled the three general purposes on which it was based. First, the present experiments established that long-term kindling produces components of hyperemotionality that are isomorphic to those observed in temporal lobe epileptics--long-term amygdala kindling induced large, reliable, and
systematic increases in emotional behaviour that are similar in major respects to those reported in temporal lobe epileptics. In addition, the potential of long-term kindling as an isomorphic model was strengthened by the parametric data identifying the number of seizures, the site of stimulation, the testing environment, and the time since the last seizure as factors influencing the expression of kindling-induced hyperemotionality. These factors have also been suggested by the observations of epileptic patients to play a role in the emergence of the interictal hyperemotionality of temporal lobe epilepsy. Finally, the nature of the hyperemotionality produced by long-term kindling was defensive, a finding that buttresses recent conclusions based on the study of temporal lobe epileptics that the fundamental interictal emotional change is an increase in defensiveness rather than an increase in aggression (Gloor, 1992).

Developing a useful and valid animal model of a human behavioural disorder is a long and difficult process. Most animal models of human behavioural disorders are heralded before they have been completely validated. The ultimate test of the value of a model is to determine its usefulness in predicting neural mechanisms underlying the disorder or therapies to reduce the severity of the disorder. The fact that long-term amygdala kindling encompasses components isomorphic of the interictal emotionality of temporal lobe epileptics suggests that it may be very useful in identifying both the neural mechanisms underlying the emotionality and the therapeutic strategies effective in eliminating it. Indeed, the finding from Experiment 7 that serotonin and the dentate gyrus may have some role to play in the neural changes underlying interictal emotional behaviour is a first step in that direction. However, the degree to which neural mechanisms and drug therapies that are implicated from future studies of long-term kindled rats are corroborated by studies of epileptics patients will constitute the ultimate test of the validity of long-term kindling as a model of the interictal hyperemotionality of temporal lobe epilepsy.
REFERENCES

Adamec, R.E. (1976). Behavioural and epileptic determinants of predatory attack

Psychiatry, 27, 249-279.

changes in limbic physiology which accompany changes in feline aggression and defense.
Physiology & Behavior, 49, 443-453.

benzodiazepine receptor. Physiology & Behavior, 54, 531-545.

Adamec, R.E. (in press). Amygdala kindling and rodent anxiety. In M. Corcoran (Ed.),


Adamec, R.E. & Morgan, H.D. (1994). The effect of kindling of different nuclei in the
left and right amygdala on anxiety in the rat. Physiology & Behavior, 55, 1-12.

cat: lasting after-effects of partial kindling of ventral-hippocampus. I. Behavioral changes.
Behavioral & Neural Biology, 38, 223-239.

Physiology & Behavior, 4, 303-305.

within and adjacent to the septum. Physiology & Behavior, 15, 339-347.

biological foundation? Neuroscience and Biobehavioural Reviews, 17, 405-425.

Neuropathology and Experimental Neurology, 52, 433-443.

Azmitia, E.C. (1978). The serotonin-producing neurons of the midbrain median and
dorsal raphé nuclei. In L.L. Iversen, S.D. Iversen, & S.H. Snyder, (Eds.), Handbook of

decarboxylase-immunoreactive neurons are preserved in human epileptic hippocampus.
Journal of Neuroscience, 9, 2562-2574.

Bard, P. (1929). The central representation of the sympathetic system: As indicated by
certain physiological observations. Archives of Neurology and Psychiatry, 22, 230-246.

hyperconnection. Cortex, 15, 357-384.


Hitchcock, J. & Davis, M. (1986). Lesions of the amygdala, but not of the cerebellum or red nucleus, block conditioned fear as measured with the potentiated startle system. *Behavioral Neuroscience, 100,* 11-22.


