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**Exploring the Interaction of Environmental and Genetic
Factors on the Development of Depressive Symptomatology
in an Animal Model**

Anne T.M. Konkle

A thesis submitted to the Faculty of Graduate and Postdoctoral Studies of the
University of Ottawa as partial fulfillment of the regulations for the degree of
Doctor of Philosophy

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*When you get in a tight place and everything goes against you
till it seems as though you could not hold on a minute longer,
never give up then,
for that is just the time and the place the tide will turn*

Harriet Beecher Stowe, in C.F. Kleinknecht,
Poor Richard's Anthology of Thoughts on Success (1947)

**One is never alone in accomplishing such an endeavor.
For this reason I would like to dedicate this dissertation to
my parents and Kate**

Mom and Dad, your continued love, support, and encouragement have helped me through many rough spots. Thank you for taking an active interest in my life and for respecting my choices. The thorough care with which you have read every one of my manuscripts has not gone unnoticed - there will be plenty more reading in the future. In sum, thank you for loving me so. Your loving daughter Anne Konkle!

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Abstract

Major depressive disorder is a devastating disease characterized by intense and prolonged sadness and feelings of hopelessness and worthlessness. Despite its incidence and severity, the exact etiology underlying this psychopathology remains uncertain, most likely involving interactions between an individual's neurobiological makeup and environment influences. While developments in drug therapy have provided some insight into the mechanisms underlying depression, investigations employing animal models have provided another strategy for studying the neurobiology of depression. The first generation of these models evolved to evaluate the therapeutic potential of novel drugs, and included the reserpine reversal test, forced swimming test, and the brain-stimulation reward (BSR) procedure. The rationale in the case of the latter was that since one of the core symptoms of depression is a decreased capacity to experience pleasure, interventions that relieve these symptoms in humans will enhance reward transmission; in animals, this was interpreted from decreases in thresholds for BSR. In the first experiment, we evaluated the effects of paroxetine, an antidepressant of the selective serotonin reuptake inhibitor class, on BSR thresholds and observed only modest decreases in thresholds. These results mimicked the minimal mood altering effects that antidepressants are reported to have in "normal" individuals. Next, as a means of inducing a depressive state in rats, the chronic mild stress (CMS) procedure, developed to model anhedonia, a core symptom of depression, was employed in the subsequent experiments. Its consequences were measured on several indices of depressive behaviours, sucrose consumption (1 and 24h intake and preference), BSR thresholds, and the forced swim test. Genetic variation was considered by comparing male and female rats of

two outbred strains. While CMS produced little change in BSR thresholds, its effects on sucrose consumption were varied; in male rats, we observed an initial reduction in 1h sucrose intake in Long Evans exclusively, while a CMS-induced reduction on the 24h intake measure was evident in both strains of female rats, with a greater effect in the Sprague-Dawley group. In evaluating the effects of CMS on forced swimming behaviour, we saw no group or strain difference in the female rats and a notable strain difference in male rats. Long Evans animals with a history of stress significantly reduced the duration of the test engaged in escape-type behaviours on the second exposure to forced swim, as would be expected in animals experiencing learned helplessness, a behaviour characteristic of depressed individuals.

In order to confirm the effectiveness of the stressors using other endpoints, a series of physiological and biochemical measures were included in these studies. For example, we observed a reduced rate of weight gain in both male and female rats exposed to the chronic stressors as well as a disruption in estrous cycling, most evident in the Long Evans female rats. Furthermore, corticosterone levels were increased as a consequence of CMS. Taken together, these findings suggest that the CMS procedure is a useful animal model of depression in producing the expected physiological and biochemical disturbances. The behavioural measures, at least those intended to assess the development of anhedonia as a consequence of stress are less reliable, possibly because these measures are too robust and the stressors too subtle to produce detectable signs of anhedonia.

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GENERAL INTRODUCTION

I shall never learn what "caused" my depression, as no one will ever learn about their own.

To be able to do so will likely forever prove to be an impossibility, so complex are the intermingled factors of abnormal chemistry, behaviour, and genetics. Plainly, multiple components are involved - perhaps three or four, most probably more, in fathomless permutations.

To discover why some people plunge into the downward spiral of depression, one must search beyond the manifest crisis - and then still fail to come up with anything beyond wise conjecture.

This passage by William Styron (2002) appropriately reflects the fallacy of the concept of depression and the difficulties that have long plagued those investigating its antecedents.

I. A CLINICAL PERSPECTIVE OF MOOD DISORDERS

1. Subtypes of depressive disorders

The Diagnostic and Statistical Manual of Mental Disorders characterizes mood disorders as those having a disturbance in mood as the predominant feature. Four categories of mood disorders have been identified: (1) Depressive Disorders, (2) Bipolar Disorders, (3) Mood Disorder due to a general medical condition, and (4) Substance-induced Mood Disorder (American Psychiatric Association, 1994). Only the first category, depressive disorders, will be examined in this section. In order to try to predict the best treatment for each individual

diagnosis, some investigators have further classified the depressive disorders into subtypes including melancholic depression, atypical depression, hostile depression, anxious depression, and depression with comorbid personality disorders (Fava, Uebelacker, Alpert, Nierenberg, Pava, and Rosenbaum, 1997).

2. Diagnostic Criteria and Symptoms of Major Depressive Disorder

In order to facilitate the task of differentiating clinically depressed individuals from others deemed “normal” but experiencing periods of sadness, professionals have standardized a list of diagnostic criteria; in North America the criteria are listed in the Diagnostic and Statistical Manual (American Psychiatric Association, 1994) and in other countries they appear in the International Classification of Diseases manual. In brief, for an individual to be diagnosed as suffering from a major depressive disorder, a major depressive episode must first have been identified (criteria are presented in Table 1) and the individual must fulfill the additional criteria necessary for diagnosing major depressive disorder (shown in Table 2). Identifying the features that characterize depression is used to determine the best course of treatment. Major depressive disorder with melancholic feature (criteria listed in Table 3) is a prominent diagnosis and the one on which the model of depression that forms the basis of the present work rests on.

3. Demographics

There has been an increase in the diagnosis of major depression in the last few decades. World wide, it is estimated to be the fourth most important cause of the loss in disability-

adjusted life-years, a measure that takes into account a person's productivity and the cost to society when expected productivity declines due to illness. The expectation is that it will rise to become the second most important cause by the year 2020 (Murray and Lopez, 1996). Approximately 5% of the general population is estimated to suffer from depression, with a greater prevalence in women - roughly twice that reported in men.

A variety of psychosocial factors have been linked to this disorder, such as, life difficulties, social roles and expectations, and exploratory styles (Nolen-Hoeksema, 1987), to name a few. In addition, neurobiological anomalies have also been identified in depressed humans, findings which have led to the postulation of theories focusing on neurobiological dysfunctions as the underlying cause of depression. A selected few of these are described in the following section.

II. NEUROBIOLOGY OF DEPRESSION

There are a myriad of hypotheses that have been proposed to explain the pathophysiology of depression. However, due to space constraints, in this section I will only present those directly related to the empirical work that underlies this dissertation, that is, the monoamine theory and the implication of stress in depression, and provide relevant sources for the others.

The predominant theory has been related to a deficit in monoaminergic neurotransmission. The section describes its emergence, evolution, and present understanding. A discussion of antidepressants will accompany the development of the theoretical explanations due to the crucial role that they have played in the formulation of such theories. Table 1 lists a variety

of compounds shown to have antidepressant properties, either in animal work or clinical tests; included is their primary mode of function and typical adverse effects.

1. Monoamines and depression

The view that monoamines play a role in depression dates back to the 1950s, with the finding that patients treated with the hypotensive drug reserpine, an alkaloid derived from the roots of the plant *Rauwolfia serpentina*, experienced symptoms of depression (Harris, 1957). This compound had been shown to be related to a depletion of monoamines from the central nervous system by interfering with their intracellular storage (Carlsson, Rosengreen, Bertler, and Nilsson, 1957). At about the same time, it was discovered that tuberculosis patients receiving a treatment of either isoniazid or iproniazid reported a significant elevation in mood unrelated to the drug's principal effect on symptoms of tuberculosis. At the cellular level, these drugs had previously been shown to inhibit the action of one of the enzymes involved in the catabolism of monoamines - monoamine oxidase (Zeller, Barsky, and Berman, 1955); it was only after clinical trials with iproniazid that its mode of action as an antidepressant was discovered. Taken together, these and other such findings suggested that a depletion in monoamines was related to depression. A further piece of evidence came from the realization that tricyclic compounds were successful in relieving depressive symptoms (Kuhn, 1957). Their mode of action was characterized as inhibiting the reuptake of monoamines into the presynaptic cell, through their action at presynaptic transporter sites, therefore lengthening the availability of monoamines at the synapse (de Paulis, Kelder, Ross, and Stjernström, 1978).

It was at this point that tricyclic compounds and monoamine oxidase inhibitors (MAOI) were introduced as antidepressants. The classical MAOIs included phenelzine, tranylcypromine, and isocarboxazid and differed from the compounds that led to the original monoamine theories of depression in that they did not possess the unwanted antituberculosis properties. As previously mentioned, their antidepressant properties appear to be related to the inhibition in the deamination of monoamine neurotransmitters (serotonin, norepinephrine, and dopamine). Despite the benefit of these drugs as therapeutic agents, the discovery of dangerous and potentially lethal adverse effects of MAOIs eventually prompted their temporary discontinuation. The use of MAOIs as antidepressants were explored anew following advances in the molecular characterization of the two isoenzymes, MAO-A and MAO-B (Cawthon, Pinter, Haseltine, Breakefield, 1981; Johnston, 1968). It is believed that the antidepressant action of MAOIs stemmed from their inhibition of MAO-A, the isozyme more closely related to the deamination of serotonin, norepinephrine, epinephrine, and dopamine; monoamine oxidase B is more specific to the deamination of dopamine and to dietary source of amines, which are thought to be associated with some of the adverse effects of these drugs (Johnston, 1968). A further characterization of the mode of action of the classical MAOIs led to the discovery that these compounds bound to the enzyme in an irreversible manner. Thus, there were attempts to develop similar compounds with reversible capabilities. Monoamine oxidase inhibitors are still in use today but are not the predominant treatment for depression. However, patients who respond poorly to the preferred treatments of either tricyclic or second generation antidepressants (these will be discussed in the following sections) are often successfully treated with the newer MAOIs such as

moclobemide, a reversible and MAO-A specific inhibitor, or used in combination with traditional treatments (Konig and Wolfersdorf, 1997; Nolen, Hoencamp, Bouvy, and Haffmans, 1993).

The second class of drugs that were introduced for relieving symptoms of depression were the tricyclic antidepressants (TCA). The best known and classical prototype of this class is the compound imipramine. The predominant mode of action of the TCAs is in blocking the reuptake of serotonin and norepinephrine, and to a lesser extent dopamine (Horn, 1976; Waldmeier, Greengrass, Baumann, and Maitre, 1976). The drug binds to a site in close proximity to the reuptake transporter on the presynaptic nerve terminal and allosterically prevents binding of the neurotransmitter to its appropriate transporter, thus preventing reuptake of the neurotransmitter. While an increase in serotonergic and noradrenergic availability at the level of the synapse is related to the therapeutic action of TCAs, these compounds also block histamine receptors (H_1) and muscarinic cholinergic receptors, thus leading to substantial side effects that have been associated with lowered patient compliance (Lader, 1983).

Due to the therapeutic action of TCAs appearing to predominantly involve serotonin and norepinephrine transmission, different theories of depression specific to one or the other neurotransmitter were born. In the United States, Schildkraut (1965) was a fervent proponent of the catecholamine hypothesis, explained as an association between depression and absolute or relative deficiencies in norepinephrine at functionally relevant cerebral loci. These ideas helped to fuel interest in the role played by norepinephrine in the pathophysiology of this disorder.

Characterization of TCAs revealed a predominant inhibition of norepinephrine reuptake by nortriptyline, protriptyline, and desipramine whereas clomipramine, imipramine, and amitriptyline additionally inhibited serotonin reuptake. However, of particular interest was the finding that the metabolites of the compounds in the latter group, having a mixed action, were effective at noradrenergic sites. Further support for the catecholamine hypothesis came from the observation that norepinephrine metabolites were altered in cerebrospinal fluid and urine of many depressed patients (Muscettola, Potter, Pickar, and Goodwin, 1984; Schatzberg, Samson, Bloomingdale, Orsulak, Gerson, Kizuka, Cole, and Schildkraut, 1989). For recent reviews of these and other lines of evidence related to the catecholamine theory of depression, see Nutt (1997), Redmond and Leonard (1997), Ressler and Nemeroff (1999), and Anand and Charney (2000).

At about the same time that the catecholamine hypothesis came to light in America, in Europe Coppen (1967) focused on studying the role played by serotonin as an underlying agent in the neurobiology of depression. The theory relating serotonin to depression gained momentum and moved to the forefront in the 1970s with the introduction of the first “designer” antidepressant, fluoxetine (Fuller, Perry, and Molloy, 1974). The drug’s principle mode of action as a selective inhibitor of serotonin reuptake (SSRI), with increased tolerability as compared to the TCAs, provided further support for the importance of serotonin in depressive disorders. The introduction of new drugs of this class with an even greater selectivity for serotonin reuptake blockade, for instance sertraline and paroxetine, served to heighten the interest in mechanisms involving serotonin. Although the dangerous side effects typically associated with TCAs were eliminated, new problems were soon

reported. Agitation, akathisia (Gerber and Lynd, 1998; Leo, 1996), sexual dysfunction (Lane, 1997; Montejo-Gonzalez et al., 1997), weight gain (Fava, 2000; Harvey and Bouwer, 2000), and gastrointestinal bleeding (Aranth and Lindberg, 1992; Ottervanger, Stricker, Huls, and Weeda, 1994) are examples of some of the side effects associated with their long term administration.

However, overall the SSRIs appear to be as effective as other classes of antidepressants and possess fewer lethal effects, nonetheless, there have been reports of serious adverse effects in some patients and therapeutic failure in many severely affected individuals. This has prompted the search for novel compounds with equal or greater therapeutic value and reduced side-effects. Even though the serotonergic system had become the focus in the search for the mechanisms underlying depression, the role played by other neuromodulators in this disorder has gained momentum over the years. Research of this kind has led to the introduction of antidepressants with similar effectiveness and fewer of the undesirable side effects. The classes of compounds include serotonin noradrenergic reuptake inhibitors (SnaRIs) that lack the affinity for muscarinic and histaminergic receptors for example, the selective noradrenaline reuptake inhibitors (NaRIs), dopamine reuptake inhibitors, and herbal remedies. The reader is referred to Table 4 for a summary of antidepressant agents.

While the first line of action differs in the drugs mentioned above, they show a high rate of success in relieving symptoms of depression. Another commonality is the fact that while their activity at receptors, transporters, or enzymes is relatively immediate, they all show a delay in their therapeutic effect (6-21 days). Consequently, it has been suggested that symptom relief is not a direct result of activity at monoaminergic sites. In fact some

investigators have shown a down-regulation of β -adrenergic receptors following the chronic, but not acute, administration of different antidepressant treatments (Beer, Hacker, Poat, and Stahl, 1987; Heal, Bristow, Hurst, Elliott, and Bucket, 1989; Hytel, 1994), leading to further speculation as to a common antidepressant mechanism of action, assuming that there is one.

Given the diversity of compounds shown to be effective antidepressants (Table 1), for example rolipram - a phosphodiesterase E inhibitor, NMDA receptor antagonists, and neurotrophic factors such as brain-derived neurotrophic factor, elaborate theories regarding their interaction with monoamine systems have been formulated. Although discussion of these is beyond the scope of this dissertation, see Altar, 1999; Blows, 2000; Duman, Heninger, and Nestler, 1997; Malberg, Eisch, Nestler, and Duman, 2000; Manji, Drevets, and Charney, 2001; Skolnick, 1999, for comprehensive reviews of these theories.

2. Stress and depression

Given the variety of cerebral and visceral symptoms that are associated with depression, it is vital that the source of these be understood. One area of interest is the role played by stressful life events in the development of this disorder. While severe acute aversive events such as the loss of a loved one have been related to the induction of depression in some individuals, this situation is typically resolved over time and does not require long term medication for relief of symptoms. However, most individuals who suffer chronically from major depressive disorder are not necessarily reacting to such an event. This finding has led to the proposal that chronic daily life hassles, rather than a single disturbance, may provoke depressive symptomatology (Kendler, Karkowski, and Prescott, 1999; Mundt, Reck,

Backenstrass, Kronmüller, and Friedler, 2000). This idea has been further refined to highlight the importance of understanding how events become perceived as stressful in some individuals (Bedi, 1999).

There is biochemical evidence to support the view that stress underlies the etiology of depression. Before describing this literature, a brief overview of the stress response involving the hypothalamic-pituitary-adrenal (HPA) axis is presented. In the face of a stressful event, the following reactions have been shown to occur. Neurons of the periventricular nucleus of the hypothalamus amplify the production of corticotropin releasing factor, a peptide released at the level of the median eminence that acts at the anterior pituitary gland to stimulate the production of adrenocorticotrophic hormone. This endocrine factor causes the adrenal gland to induce the release of glucocorticoids (mainly cortisol in humans and corticosterone in rats) that in turn act at varied sites to appropriately alter autonomic nervous system function. A negative feedback loop of cortisol to the pituitary gland and hypothalamus acts as an inhibitor of hormone production, thus preventing its excessive manufacture.

Given the biochemical reactions of the HPA axis incurred during a stressful event, abnormalities at many levels of the axis in depressed individuals suggest an association between stress and depression. One finding, first shown by Board et al. (1956), that has been consistently replicated is hyperactivity of the HPA axis as shown by raised levels of urinary, plasma, and cerebrospinal fluid cortisol levels in individuals suffering from major depressive disorder. Alterations related to other steps of the HPA axis in response to stress have also been reported in depressed individuals. These include increased corticotropin releasing

factor levels in the cerebrospinal fluid of unmedicated patients (Banki, Bissette, Arató, O'Conner, and Nemeroff, 1987; Nemeroff, Widerlöv, Bissette, Walleus, Karlsson, Eklund, Kilts, Loosen, and Vale, 1984) and its normalization with successful antidepressant treatment (De Bellis, Gold, Geraciotti, Listwak, and Kling, 1993). Elevated corticotropin releasing factor concentrations (Raadsheer, Hoogendijk, Stam, Tilders, and Swaab, 1994) and corticotropin releasing factor mRNA expression (Raadsheer, Van Heerikhuize, Lucassen, Hoogendijk, Tilders, and Swaab, 1995) in the periventricular nucleus have been detected in postmortem tissue of depressed individuals. Another finding that supports the role of stress in depression is that when depressed patients are administered corticotropin releasing factor, they exhibit a blunted adrenocorticotrophic hormone, but normal cortisol, response (Plotsky, Owens, and Nemeroff, 1998). In line with previous work that has shown the importance of various neuromodulatory systems in depression based on the mode of action of antidepressants, administration of glucocorticoid synthesis inhibitors (e.g. Metyrapone) has been shown to have therapeutic effects in depressed individuals (rev. in Murphy, 1997; Wolkowitz and Reus, 1999). These previous findings appear to be specific to patients suffering from major depressive disorder; other clinical populations, such as individuals inflicted with bipolar disorder or schizophrenia, do not show the same pattern of HPA axis alterations (Banki et al., 1987; Nemeroff et al., 1984).

In animal studies, changes in HPA axis activity have been shown to be induced by manipulations believed to give rise to a "depressive" state. For example, exposure to stress early in life (an animal model of depression that will be discussed in the following section) has been shown to elevate corticotropin releasing factor concentrations in the cerebrospinal

fluid of adult primates (Coplan, Andrews, Rosenblum, Owens, Friedman, Gorman, and Nemeroff, 1996) and in the hypothalamus of adult rats (Plotsky and Meaney, 1993). The application of chronic mild stress in rodents (another model discussed below) has been shown to significantly increase corticotropin releasing factor concentration in the bed nucleus of the stria terminalis (Stout, Mortas, Owens, Nemeroff, and Moreau, 2000), a region with many corticotropin releasing factor immunoreactive cells that receives corticotropin releasing factor projections from the amygdaloid nuclei (Sakanaka, Shibasaki, and Lederis, 1986).

While there is support for the view that stress is linked to depression, it is not a foregone conclusion that stress causes depression. Some investigators believe that adversity in early life will predispose an individual to develop depressive symptomatology when faced with perceived stress as an adult (Heim, Owens, Plotsky, and Nemeroff, 1997). However, many depressed individuals do not report a history of severe distress at an early age. In light of the fact that depression is a heterogeneous disorder, there may be multiple mechanisms underlying its occurrence. One of these is that in certain individuals there is a genetic predisposition to stress susceptibility rendering them more likely to develop depressive symptoms. Even though a role for stress in depression has generally been well accepted, a genetic component also appears to be of great importance given the high rate of depression, or other mood disorder, in monozygotic twins (Kendler, Neale, Kessler, Heath, & Eaves, 1992; McGuffin, Katz, and Rutherford, 1991).

In trying to understand the role played by stress in the monoamine theory of depression, which is to date the most investigated theory pertaining to this disorder, it is important to note that there is increasing evidence relating the monoaminergic system with HPA axis

regulation. For example, there is CRF innervation of major sources of adrenergic cell bodies (Heit, Owens, Plotsky, and Nemeroff, 1997) suggesting an interaction between these neuromodulatory systems, although their functional relationship is unclear. Another is the interplay between serotonin and HPA axis function, as shown by the stimulatory effect of serotonin on the secretion of HPA axis-related endocrine and neuromodulators (reviewed in Dinan, 1996). The connection between stress and new factors (trophic factors), sites (NMDA receptors), and events (site specific neuronal death), thought to play a part in depression, continue to be investigated. For a review of these interactions please consult the following review articles (Duman, 1998; Duman et al., 1997; Jacobs, van Praag, and Gage, 2000; Manji et al., 2001).

3. Gonadal hormones and depression

Only a brief mention of gonadal hormones and their possible implication in depression will be presented here. As mentioned earlier, the incidence of depression is greater in women than in men, and a variety of psychosocial, cognitive, and personality factors may explain part of this gender difference. It is also clear that gonadal hormones contribute significantly to this difference. In fact, this distinction with respect to depression is apparent from puberty on, and is less pronounced again after menopause, corresponding to periods of cyclical but constant flux in gonadal hormones. Other evidence suggesting a role for gonadal hormones in gender differences related to depression is the finding that some disturbances of mood are closely linked to fluctuations in absolute or relative levels of these hormones, that occur for example in premenstrual dysphoric disorder, depression with postpartum onset, and

depression in perimenopause (American Psychiatric Association, 1994). However, given that mood disorders affect both men and women, there may be a common etiological basis with alterations in gonadal hormones potentially interacting with this underlying mechanism to render women more susceptible. The greater susceptibility in women may be related to the effects of gonadal hormones on neurotransmitter systems or interactions with the HPA axis; the latter is discussed in the fourth section of this introduction.

The causes of major depressive disorder are probably multiple, depending on genetic, environmental, neuroanatomical, and biochemical factors. A few of the biochemical components have been discussed. The next section examines studies that explore the importance of environment, using animal models, in order to understand its contribution to the underlying causes of depression.

III. ANIMAL MODELS OF DEPRESSION

Depression is one psychopathology that is increasingly studied in animal models, principally for two reasons - to screen and develop new therapeutic drugs and to simulate human depression in order to investigate its neurobiological basis. These aims are not mutually exclusive since increasingly, antidepressant drug trials are being carried out in models that simulate depression, in an attempt to better qualify the time frame of the therapeutic effect.

One problem with modeling depression in animals is the subjectivity associated with its diagnosis in humans. Consequently, investigators have focused instead on modeling the

behavioural symptoms and known neurochemical changes that occur in depressed individuals. Due to the limitations of this approach, the usefulness of a model is typically determined by evaluating the common features it shares with the clinical disorder through a process of validation (adapted from McKinney and Bunney, 1969; Willner, 1984). The criteria involve 1) predictive validity defined as the observation of similar responses in humans and animals to analogous therapeutic treatments, 2) construct validity defined as homologous responses between humans and the model species, and 3) face validity defined as the use of similar but ecologically relevant conditions. Most animal models of depression do not satisfy all of these criteria. Nevertheless, investigators make use of these models all the while noting their limitations.

The following is a description of different animal models of depression; note that this is not meant to be an exhaustive list but rather a look at the types of models that have received the most attention with respect to this disorder. The reader is referred to Table 5 for a comprehensive list of models and relevant sources for each. Although the focus of the dissertation is on the chronic mild stress (CMS) model, an overview of other representative ones is included in order to demonstrate the broadness of this application and their influence on the development of the CMS paradigm.

1. Pharmacological Induction Models

Following the finding that reserpine treatment disrupts monoamine transmission, the first animal model of depression was developed, which involved the study of therapeutic agents that reverse reserpine-induced syndrome in animals (Costa, Garattini, and Valzelli, 1960).

Through the years, the model has proven to have predictive validity, although many of the antidepressants introduced following TCAs have been ineffective in this model. Since then, given our better understanding of the neurobiological causes of depression, more appropriate models have been developed.

Another pharmacological model that is exploited to induce a “depressive” state in adult rats is the chronic administration of the serotonin reuptake inhibitor clomipramine (Anafranil®) in neonates. In young pups, administration of clomipramine from postnatal days 8-21 has been shown to disrupt rapid eye movement sleep (Mirmiran, van de Poll, Corner, van Oyen, and Boer, 1981). Treatment with clomipramine at this developmental stage coincides with the maturation of the monoaminergic system, thus potentially interfering with this process (Vogel, Neill, Hagler, and Kors, 1990). When these animals are studied in adulthood, they display behavioural alterations resembling symptoms of depression in humans. For example, impaired male sexual activity (Neill, Vogel, Hagler, Kors, and Hennessey, 1990), altered circadian rhythmicity (Vogel, Neill, Kors, and Hagler, 1990), a reduced aggressiveness (Vogel, Hartley, Neill, Hagler, and Kors, 1988), increased hyperactivity and exploration in an open field paradigm (Hartley, Neill, Hagler, Kors, and Vogel, 1990), as well as diminished pleasure-seeking behaviour as revealed by an increase in current thresholds for brain-stimulation reward are observed (Vogel, Neill, Hagler, Kors, and Hartley, 1990). The neurochemical changes in adulthood associated with this model have only recently been investigated. These include a reduction in hypothalamic serotonin concentration (Feenstra, van Galen, Te Riele, Botterblom, and Mirmiran, 1996), and decreased serotonin and norepinephrine levels in frontal cortex, hippocampus, brain stem, and septum (Vijayakumar and

Meti, 1999). Furthermore, similar to many depressed patients, animals neonatally treated with clomipramine exhibit elevated corticosterone levels in adulthood as well as a decreased capacity to suppress a corticosterone response following a dexamethasone test (Prathiba, Kumar, and Karanth, 1998). Although this model has helped us understand the impact of early life events on adult behaviour, it is criticized as lacking in face validity; it is difficult to find a parallel between the early life events reported in humans and the drug-induced neonatal disturbances associated with this model.

2. Stress Induction Models

A second category of animal models involves induction of depressive symptomatology following or during exposure to a variety of stressors. Similar to the pharmacological developmental model, stress in the neonate, in the form of maternal separation, has been argued to lead to depressive symptomatology (Checkley, 1996; Meaney, Dorio, Francis, Widowson, LaPlante, Caldji, Sharma, Seckl, and Plotsky, 1996; Stanton, Gutierrez, and Levine, 1988). It is important to begin by differentiating between the early handling and maternal separation procedures. Although both involve a period of separation, the behavioural and neuroendocrine profiles of these animals differ substantially. Such factors as duration of separation and prolonged human contact appear to play a role in distinguishing the behavioural effects associated with these. The model of most interest is that of maternal separation *per se*, administered between postnatal days 4 and 14 in rats. Endocrine consequences of this deprivation can be seen at different developmental stages. Some investigators have demonstrated elevated basal corticosterone levels (Avishai-Eliner, Yi,

Newth, and Baram, 1995; Kuhn, Pauk, and Schanberg, 1990; Lehmann, Russig, Feldon, and Pryce, 2002; Levine, Huchton, Wiener, and Rosenfeld, 1992) in these animals while others have reported HPA axis hyperresponsiveness as indexed by increased corticosterone and ACTH levels following a subsequent stressor, if measured soon after the separation procedure (Stanton et al., 1988; Suchecki, Mozaffarian, Gross, Rosenfeld, and Levine, 1993). The effects of previous maternal separation on the adult rat are less consistent. Some report similarly elevated basal corticosterone levels in adulthood (Rots, De Jong, Workel, Levine, Cools, and De Kloet, 1996) while others do not (Suchecki and Tufik, 1997). Conflicting results are also found in the degree of HPA axis responsiveness in adult animals following exposure to an acute stressor; one group has observed a faster recovery (Suchecki and Tufik, 1997) while another (Lehmann and Feldon, 2000) reports the opposite trend. Behavioural indices associated with stress and depression are equally affected by maternal separation but generally only observed before weaning; these include increased grooming and defecation, and prolonged high levels of activity in response to novelty. In adults, few minor behavioural deficits are reported (Kaneko, Riley, and Ehlers, 1994; Lehmann, Pryce, Bettschen, and Feldon, 1999). Even though maternal separation models the later effects of this experience in humans, the lack of procedural uniformity between laboratories may be one explanation for inconsistent behavioural and endocrine results that have been reported; the duration, frequency, and onset of the separation are factors that must be considered (rev. in Lehmann and Feldon, 2000). Even with this caution, the strength of the maternal deprivation procedure appears to be in modeling HPA axis dysfunction.

One paradigm with high predictive validity (Willner, 1984) is the forced swimming test

(FST) developed by Porsolt et al. (1977). The procedure involves placing a rat or mouse in a swimming chamber for 15 min on the first day, administering antidepressant treatment the next day followed by a 5 min swim test. Behavioural monitoring reveals that animals first exposed to the water show escape-related activities that are eventually replaced by immobility (Porsolt, Lepichon, and Jalfre, 1977), interpreted as behavioural despair - a characteristic symptom of depressed individuals. Successful therapeutic treatments are reported to lengthen the period of mobility and escape, thus preventing the development of despair. Tests have successfully been conducted with TCAs, SSRIs, atypical compounds such as mianserin as well as electroconvulsive shock and REM sleep deprivation; in contrast, anxiolytics have been typically ineffective in this paradigm (for a review see Bourin, Fiocco, and Clenet, 2001). Its predictive validity comes into question by the finding that psychomotor stimulants are equally effective as antidepressants in this paradigm, presumably due to their ability to increase motoric activity in the animals (Porsolt et al., 1977; see rev. Borsini and Meli, 1988). This dilemma has been overcome by conducting tests of locomotor activity prior to FST in order to detect doses that produce stimulant effects. Finally this paradigm has been criticized for its lack of a chronic induction phase and the fact that in contrast to the delayed onset of therapeutic effect in the clinical population, immediate antidepressant effects are observed in the FST.

Another model that has been negatively viewed due to its acute nature is the learned helplessness paradigm. In spite of its shortcomings, this model has served as the basis for other stress models and deserves particular attention. Learned helplessness is produced by exposing animals to a series of uncontrollable, inescapable electric shocks that produce

deficits in escape behaviour in other aversive situations that are escapable. Shock administration also decreases food and water intake (Weiss, 1968) as well as aggressiveness and social dominance (Maier, Anderson, and Lieberman, 1972; Rapaport and Maier, 1978). Increases in neophobia and decreases in social interaction are also evident (Job and Barnes, 1995; Short and Maier, 1993), suggesting increased anxiety. However, these behavioural changes are not observed when animals are instead presented with escapable (controllable) shock. Another consequence of shock exposure is a prolonged decrease in response rate for BSR from the ventral tegmental area and nucleus accumbens (Kamata, Yoshida, and Kameyama, 1986; Zacharko, Bowers, Kelley, and Anisman, 1984), a performance decrement that resembles the reported deficits in motor activity (Anisman, DeCatanzaro, and Remington, 1978; Weiss, Goodman, Losito, Corrigan, Charry, and Bailey, 1981). Modest motivational deficits are also observed as interpreted by threshold increases for brain-stimulation reward following uncontrollable footshock (Zacharko, Kasian, MacNeil, and Anisman, 1990). Although the perception of stressor control in this paradigm parallels some of the theories pertaining to human depression, the relatively short-lasting effect of stressor application has proven problematic. One redeeming quality of this model is the fact that re-exposure to the stimulus context (not the uncontrollable stress itself) is sufficient to prolong the behavioural depression (Maier, 2001). Thus, it appears that awakening memories of the stressor may mimic the rumination process reported in depressed humans.

In keeping with the basic tenet that exposure to stressors induces depression, the chronic unpredictable stress model improves upon the learned helplessness one by varying the stressors and presenting them over a prolonged period in order to track the development of

behavioural and other reactions as well as to prevent habituation from re-exposure. An example of a chronic schedule is the following: over a 21 day period, the stressors might include three 60-min sessions of unpredictable foot shock, two 40-h sessions of food deprivation and two separate sessions of water deprivation, repeated exposure to heat stress, shaker stress, and reversed light/dark cycle, presented intermittently. A great deal of this work was originally carried out by Katz's group. They reported lowered activity in the open field test induced by chronic stress, interpreted as an indicator of the animal's emotional state (Katz, Roth, and Carroll, 1981). Further support for an hedonic deficit following the stress procedure stemmed from the finding of a reduction in the consumption of a mildly sweet fluid void of caloric content, which was then reversed by chronic antidepressant administration (Katz, 1982). The chronic unpredictable stress procedure was also shown to elicit an increase in plasma corticosterone levels (Katz, Roth, and Carroll, 1981). This model was argued to demonstrate construct validity in that it gave rise to the homologous symptomatology (decreased hedonic capacity and neurochemical alterations) reported in the clinical literature. Further tests were conducted to verify the model's predictive validity. The atypical antidepressants, bupropion and mianserin, drugs of the tricyclic class (imipramine and iprindole), and the MAOI tranylcypromine were shown to reverse the behavioural deficits induced by the chronic stress procedure in the open field test (Katz, Roth, and Schmaltz, 1981; Katz and Sibel, 1982; Roth and Katz, 1981) whereas other (non-antidepressant) drugs did not (Katz and Sibel, 1982). Most of the therapeutic compounds were also associated with a return to basal plasma corticosterone levels. Although the model was refined to distinguish between the effects of chronic emotional versus physical stress

(Rodríguez Echandía, Gonzalez, Cabrera, and Fracchia, 1988), a major drawback remained the severity of the stressors employed. In order to possess face validity, the stressors must be analogous to those endured by humans and be ecologically relevant to the model species. Granted, some clinically depressed individuals do suffer from a reactive-type of depression following exposure to severe exogenous stress; however they typically recover without medical intervention. The severity of the stressors and eventual recovery not necessitating any intervention is more akin to stress exposure in the learned helplessness paradigm in rodents.

Other obstacles in achieving face validity have been the chronicity of the induction phase and the use of a single behavioural assessment. While employing a chronic schedule is an improvement over the application of a single acute stressor, it remains that hedonic evaluations are typically only carried out once, at the end of the stress schedule, so that the development of anhedonia is unknown as is its relationship to the time course of drug therapeutic action. These limitations were addressed by Willner et al. (1987) and their modifications to this paradigm are presented in the next section.

In addition to the behavioural deficits found in the open field test, Katz (1982) reported altered consumption of mildly sweetened water arising from exposure to the chronic unpredictable stressors. These findings suggested a change in the hedonic quality of the solution, that is a decrease in the perceived rewarding property of the sweetened water, a state reminiscent of anhedonia in depressed individuals. In order to increase the paradigm's construct validity in such a way as to better model the everyday life strains endured by humans that may induce depression, Willner and colleagues (1987) selected less severe

stressors than those employed by Katz. Additionally, they monitored hedonic status during the stress phase in order to better discern the time course of the effect.

The CMS methodology involves the application of a series of relatively mild stressors such as overnight food and/ or water deprivation, cage tilting, paired or grouped housing in soiled bedding, periods of reversal of the light/ dark cycle, and overnight illumination, just to name a few, applied over a seven day schedule and repeated for a minimum of three weeks. This model has been extensively tested in order to verify its pharmacological validity as well as to evaluate its behavioural, neurochemical, and physiological consequences.

A variety of behavioural measures have been employed to assess the effectiveness of novel antidepressants on this model, of which three address specifically the induction of anhedonia following CMS; they are conditioned place preference, consumption of a mildly sweet solution, and brain-stimulation reward (BSR); a thorough description of these will be presented in a later section of the Introduction.

Chronic mild stress has been shown to decrease place preference conditioning associated with drugs, amphetamine and morphine, and feeding-related reinforcers (food and sweet solutions)(Benelli, Filafarro, Bertolini, and Genedani, 1999; Cheeta, Broekkamp, and Willner, 1994; D'Aquila, Monleon, Borsini, Brain, and Willner, 1997; Muscat, Papp, and Willner, 1992; Papp, Willner, and Muscat, 1991; Papp, Willner, and Muscat, 1993; Valverde, Smadja, Roques, and Maldonado, 1997; Willner, Lappas, Cheeta, and Muscat, 1994) while no effects have been found for aversive place conditioning (Papp, Lappas, Muscat, and Willner, 1992). When changes in BSR thresholds are instead evaluated following the application of these chronic stressors, the results are not as consistent; some investigators

report a gradual (up to 30%) increase in frequency thresholds (Moreau, Borgulya, Jenck, and Martin, 1994; Moreau, Bös, Jenck, Martin, Mortas, and Wichmann, 1996; Moreau, Bourson, Jenck, Martin, and Mortas, 1994; Moreau, Jenck, Martin, Mortas, and Haefely, 1992; Moreau, Scherschlicht, Jenck, and Martin, 1995; Stout et al.), while others find no effect of the stressors (Nielsen, Arnt, and Sanchez, 2000) or only a modest decrease in thresholds (Lin, Bruijnzeel, Schmidt, and Markou, 2002). The data pertaining to the consumption of a sweet solution as a reward are plentiful and no less confusing. While many report a decrease in the intake of 0.7, 1 or 2% sucrose solutions (Cheeta et al., 1994; Dziejzicka-Wasylewska, Willner, and Papp, 1997; Grippo, Moffitt, and Johnson, 2002) others fail to replicate these findings (Matthews, Forbes, and Reid, 1995; Nielsen et al., 2000). Evaluating sucrose preference (ratio of sucrose intake to total fluid intake in a two-bottle test) instead does not provide a much more reliable picture (Benelli et al., 1999; Kioukia, Bekris, Antoniou, Papadopoloulou-Daifoti, and Christofidis, 2000; Matthews et al., 1995; Willner et al., 1987). Some have argued that the rewarding property of sucrose is confounded by its caloric content and the food deprivation that is typically employed prior to the sucrose test, so trials with saccharin were conducted, a substitute without calories. The results of these studies reveal no particular difference from that obtained using low concentration sucrose solutions (Harris, Zhou, Youngblood, Smagin, and Ryan, 1998; Hatcher, Bell, Reed, and Hagan, 1997; Pucilowski, Overstreet, Rezvani, and Janowsky, 1993; Willner et al., 1987). Even though there is controversy as to the use of these tests as behavioural measures of CMS-induced anhedonia, pharmacological validation of the model remains robust.

Pharmacological agents that have successfully reversed the stressed-induced anhedonia as

monitored with at least one of the three behavioural paradigms introduced above include monoamine oxidase inhibitors (Moreau, Jenck, Martin, Mortas, and Haefely, 1993), tricyclic antidepressants (Benelli et al., 1999; Dziedzick-Wasylewska et al., 1997; Gittos and Papp, 2001; Kioukia et al., 2000; Kubera, Basta-Kaim, and Papp, 1995; Moreau et al., 1992; Papp, Nalepa, and Vetulani, 1994; Willner et al., 1987), SSRIs (D'Aquila, Monleon et al., 1997, Dziedzick-Wasylewska et al., 1997; Luo and Tan, 2001; Muscat et al., 1992), as well as the atypical antidepressants maprotiline (Muscat, Papp, and Willner, 1992), mianserin (Cheeta, Broekkamp, and Willner, 1994; Moreau, Bourson, Jenck, Martin, and Mortas, 1994), and buspirone (Papp, Moryl, and Willner, 1996). Drugs that have proven unsuccessful in reversing this effect are neuroleptics (Papp, Moryl, and Willner, 1996), the anxiolytic, chlordiazepoxide (Muscat, Papp, and Willner, 1992), and the psychostimulant, amphetamine (Papp, Moryl, and Willner, 1996). The effectiveness of novel compounds has also been assessed; for example the methyl donor S-adenosyl-L-methionine (SAME) (Benelli et al., 1999; Genedani, Saltini, Benelli, Filaferro, and Bertolini, 2001), the tryptophan hydroxylase activation inhibitor AGN 2979 (Gittos and Papp, 2001), and the sigma ligand Lu 28-179 (Sánchez and Papp, 2000), have all been shown to reverse the CMS-induced reduction in sucrose consumption. Although important for the development of new antidepressants, these tests have additionally helped us glean some insight into the mechanisms that may be implicated in the pathophysiology of depression.

Responsiveness to rewards has also been monitored by evaluating sexual behaviour although no pharmacological tests have yet been conducted. An overall decrease in sexual activity is apparent in male rats exposed to chronic stressors; reported are reductions in

mounting behaviour (Brotto, Gorzalka, and LaMARre, 2001; D'Aquila, Brain, and Willner, 1994), lowered ejaculation frequencies, and a lengthened post-ejaculatory interval (Brotto et al., 2001), similar to the sexually related difficulties experienced in the clinical population.

Other behaviours, not necessarily associated with hedonia, have also been assessed following the application of this stress regime. In the open field apparatus, animals in one study showed a reduction in rearing frequency (Harro, Häidkind, Harro, Modiri, Gillberg, Pähkla, Matto, and Oreland, 1999) while others reported an increase in this behaviour (Benelli et al., 1999; Harris et al., 1998). Conflicting results are also found when evaluating motility in the open field; compared to control (unstressed) animals, those exposed to CMS have shown either a decrease (D'Aquila, Peana, Carboni, and Serra, 2000b) or no change (Benelli et al., 1999) in this behaviour. To date, only one study has examined the effects of the mild stressors on forced swimming behaviour (FST); no effect of CMS was evident. However, when animals were first treated with the noradrenergic denervation agent DSP-4, CMS decreased their FST immobility time compared to their control counterparts (Harro et al.,) suggesting an antidepressant role for CMS under these conditions.

Other behavioural alterations associated with CMS include a reduced latency to enter rapid eye movement sleep and sustain it (Cheeta, Ruijt, van Proosdij, and Willner, 1997; Moreau et al., 1995). Results of employing measures of anxiety reveal that CMS animals typically decrease the number of attacks versus control animals in a resident-intruder test, interpreted as a reduction in aggression or an increase in submissiveness. On a test of social interaction, stressed animals spend less time in active social contact with a same-sex partner (D'Aquila et al., 1994), thus displaying an anxiogenic profile on this behavioural test.

Aside from the behavioural assessments, evaluation of the physiological, biochemical, and neurochemical alterations induced by the CMS paradigm have also been conducted. A weight loss or a decrease in the rate of weight gain has typically been associated with application of these mild stressors (Barr and Phillips, 1998; Brotto et al., 2001; D'Aquila, Monleon et al., 1997; D'Aquila, Peana, Carboni, and Serra, 2000b; Dunčko, Kiss, Škultétyová, Rusnák, and Jezová, 2001; Dziedzicka-Wasylewska et al., 1997; Harris et al., 1998; Harro et al., 1999; Hatcher et al., 1997; Matthews et al., 1995; Muscat, Towell, and Willner, 1988; Sánchez and Papp, 2000; Willner et al., 1994). Other physiological changes include adrenal gland hypertrophy (Muscat and Willner, 1992), a decrease in thymus weight (Kubera, Basta-Kaim, Holan, Simbirtsev, Roman, Pigareva, Prokopieva, and Sham, 1998), and a reduction in the amount of epididymal fat (Harris et al., 1998).

Experiments have also been carried out to investigate any stress related biochemical and neurochemical changes. The results of a few studies report an increase in corticosterone levels in animals exposed to the chronic stress regime (Ayensu, Pucilowski, Mason, Overstreet, Rezvani, and Janowsky, 1995; Harris et al., 1998; Lanfumey, Pardon, Laaris, Joubert, Hanoun, Hamon, and Cohen-Salmon, 1999) while others failed to observe any such changes (Azpiroz, Fano, Garmendia, Arregi, Cacho, Beitia, and Brain, 1999; Dunčko, Kiss et al., 2001; Stout et al., 2000; Willner et al., 1987). Given the importance of serotonin in hypotheses related to the etiological basis of depression and the action of antidepressant drugs, Lanfumey et al. (1999) evaluated brain levels of serotonin in mice exposed to eight weeks of CMS. They report a reduction of the 5-HIAA/ 5-HT ratio in the brain stem, hippocampus, and striatum, suggesting a decrease in serotonin neurotransmission (Lanfumey

et al., 1999).

Alterations in immune system function are commonly associated with stress (Irwin, Daniels, Risch, Bloom, and Weiner, 1988; Kiecolt-Glaser, Dura, Speicher, Trask, and Glaser, 1991) and such changes have also been reported in depressed individuals (Irwin, Lacher, and Caldwell, 1992; Kronfol and House, 1989; Maes, Bosmans, Suy, Minner, and Raus, 1989). Thus, it follows that immune system alterations would be investigated in animals models of depression, particularly those employing stress as inducing factors. Results of these studies have shown a reduction in thymus weight following CMS (Harris et al., 1998; Kubera et al., 1998) as well as an increase in the proliferative response of mice and rat splenic cells to the mitogen Concanavalin A (Azpiroz et al., 1999; Kubera, Symbirtsev, Basta-Kaim, Borycz, Roman, Papp, and Claesson, 1996). Further evidence of immune anomalies associated with this paradigm include an increase in the production of interleukin-1 and 2 in splenocytes (Kubera et al., 1996) as well as a decrease in the hemolytic complement activity (Ayensu et al., 1995).

The CMS paradigm appears to be one of the most extensively studied models of depression. The results of a variety of drug tests lend to its predictive validity, while many of the CMS related physiological and biochemical changes are similar to those reported in depressed humans, conferring upon it construct validity. Face validity is achieved by employing a variety of unpredictable mild stressors in a chronic fashion, and importantly, relevant to the species studied. Although the validity of this model appears firm, problems arise with respect to its reliability; while many investigators have observed the expected behavioural patterns, many have not. The lack of agreement across studies is probably

attributed to methodological differences such as animal species, strain, and gender employed, as well as variations in the combination of stressors that are used and differences in housing conditions, just to name a few. These issues will be addressed in detail as the individual experiments are presented.

3. Genetic Models

In addition to drug and stress-induced models of depression, at least two different rat strains have been used as genetic models of this disorder; these include the Wistar-Kyoto and Flinders Sensitive lines of rats. The Wistar-Kyoto rat is mainly exploited for its hyper-responsiveness to stress, as expressed by an increase in ulcer formation when exposed to stressors. This heightened stress response may also potentially mimic the particular susceptibility of some humans to experience stressors more intensely and subsequently develop depressive symptomatology. This inbred rat strain exhibits a typical (Allen-Rowlands, Allen, Greer, and Wilson, 1980) diurnal rhythm in corticosterone and adrenocorticotrophic hormone (Solberg, Losee Olson, Turek, and Redei, 2001); however the peaks of both of these endocrine factors are prolonged when compared to the outbred Wistar strain (Solberg, Losee Olson, Turek, and Redei, 2001). Furthermore, they also show an exaggerated response in these measures following 15 min of swim stress and no suppression of adrenocorticotrophic hormone when administered dexamethasone (Rittenhouse, López-Rubalcava, Stanwood, and Lucki, 2002). These findings are suggestive of a dysfunctional HPA axis. Behaviourally, the Wistar-Kyoto rats, compared to either Wistar or Sprague-Dawley rats, show greater immobility in the FST (Paré, 1994; Rittenhouse et al., 2002) and

reduced exploration in the open field paradigm (Paré, 1994). Following tail shock stress, males display less approach to a novel female (Paré, 2000).

The Flinders Sensitive lines and their counterparts the Flinders Resistant line of rats were bred for their responsiveness to the anticholinergic agent diisopropyl fluorophosphate (Overstreet, Russell, Helps, and Messenger, 1979; Russell, Overstreet, Messenger, and Helps, 1982). Sensitivity to this agent was shown in the Flinders Sensitive line by a decrease in body temperature and weight, as well as a reduction in fluid intake. In addition to the supersensitivity to cholinergic agents experienced by Flinders Sensitive line of rats and many depressed humans, there are physiological and behavioural similarities between the two species. For instance, lower body weight and feeding levels have been found in Flinders Sensitive line of rats compared to their progenitor Sprague-Dawley rats (Overstreet, 1993), similar to the reduction found in the clinical population relative to “normal” individuals.

Biochemical assessments have revealed that corticosterone levels are not elevated in Flinders Sensitive line compared to the Flinders Resistant line, both in a resting state (Overstreet, Booth, Dana, Risch, and Janowsky, 1986) and following CMS (Ayensu et al., 1995). However, results from behavioural tests do support the claim that the Flinders Sensitive line of rats be considered an animal model for studying depression. These rats have been reported to exhibit decreased activity in the open field paradigm (Overstreet, 1986), exaggerated immobility in the FST (Overstreet, 1986), and a reduction in saccharin preference following CMS (Pucilowski et al., 1993). The behavioural responses in these genetic lines thought to model depression are reminiscent of the anhedonia experienced in depressed individuals. Other similarities with the clinical population include altered rapid

eye movement sleep (Benca, Overstreet, Gilliland, Russell, Bergmann, and Obermeyer, 1996) and altered immune response to stress as shown by reduced killer T cell activity (Friedman, Irwin, and Overstreet, 1996).

Thus, both the Flinders Sensitive line and Wistar-Kyoto rats display physiological and behavioural alterations similar to those associated with clinical depression. Even more striking in these lines is their hyper-responsiveness and sensitivity to stress.

The models presented in the previous sections provide a brief overview of the approaches developed in animal paradigms to screen potential antidepressants and understand the mechanisms underlying depression. Even though drug-induced and major depression are considered separate categories according to the Diagnostic and Statistical Manual of Mental Disorders (American Psychiatric Association, 1994), the means by which these drugs alter hedonic state have helped shed some light on the substrate underlying this disorder. Animal models implicating stress as a precipitating or co-existing factor with depression have also been presented and the findings from this work tend to parallel the clinical literature. Finally, specific rat strains have been bred that model the hyper-responsiveness to stress believed to play an important role in differentiating between individuals who will develop symptoms of depression subsequent to daily life strains and those who will remain resistant. The implication of genetic factors in the etiology of depression will be explored in greater detail in the following section.

IV. GENETIC ISSUES

In the previous section were presented some environmental factors that appear to be

important in the development or progression of depression, the most important being stress; however, little information is available concerning the role of genetic factors in this disorder. There are as yet no criteria that would help in distinguishing those individuals who may have a genetic predisposition to this disorder. Biological predispositions undoubtedly play a role in depression, potentially interacting with environmental factors such as stress, which itself is known to be related to the onset of depression. In order to better understand the underlying neurobiology and treatment for this disorder, it would be important to be able to identify susceptible individuals. Note that while all individuals endure stressful experiences, only relatively few suffer from depression. With this goal in mind, geneticists have studied whether polymorphisms of genes for the serotonin transporter and for the enzyme catechol-O-methyltransferase, both important in the degradation of serotonin or other monoamines, could be detected in depressed individuals; to date, the results of these investigations have not been fruitful and the findings inconsistent between different laboratories (Bellivier, Henry, Szöke, Schürhoff, Nosten-Bertrand, Feingold, Launay, Leboyer, and Laplanche, 1998; Henderson, Korten, Jorm, Jacomb, Christensen, Rodgers, Tan, and Easteal, 2000; Hoche, Wendel, Grunewald, Chiaroni, Levy, Morris-Rosendahl, Macher, Sander, and Crocq, 1998; Ogilvie, Battersby, Bubb, Fink, Hammar, Goodwin, and Smith, 1996). This section will further explore how genetic differences can be exploited in research with animal models of human disorders. Instead of focusing on detailed and limited genome differences that have been investigated in humans, a broader genetic difference, that of strain and gender, will be examined.

1. Strain differences in animal research

Rat strains have been found to differ in emotionality, as assessed on tests of passive-avoidance, forced swimming, and the open field paradigm, as well as others. In fact, strains have been developed for such differences, for example the Roman high-and low-avoidance lines (Bignami, 1965). A differential response to stress has also been reported in these lines (Gentsch, Lichtsteiner, Driscoll, and Feer, 1982). Extensive differentiation in reactivity to stress has been carried out on two inbred and related rat strains, the Fischer 344 and the Lewis rats. The Lewis line, reportedly hyporeactive to stress (Sternberg, Glowa, Smith, Calegero, Listwak, Aksentijevich, Chrousos, Wilder, and Gold, 1992) show an increase in risk assessment behaviour in the elevated-plus maze as defined by greater exploration of the external perimeters of the structure (Stöhr, Szuran, Welzl, Pliska, Feldon, and Pryce, 2000). This same group has also reported a stronger startle response in the Lewis rats compared to the Fischer 344 line (Stöhr et al., 2000). Furthermore, in the FST, Lewis rats show signs of immobility much sooner than the Fischer 344 ones (Sternberg et al., 1992). A comparison of plasma corticosterone levels following a brief period of restraint stress has yielded data that support the behavioural differences reported in these strains; Fischer 344 rats exhibit a greater corticosterone response than their Lewis counterparts (Dhabar, Miller, McEwen, and Spencer, 1995; Stöhr et al., 2000). Further examination of these biochemical differences at the molecular level has revealed no difference between the strains in the number of mineralocorticoid and glucocorticoid adrenal steroid receptors found in neural tissue (Dhabar, McEwen, and Spencer, 1993); however Fischer 344 rats show a greater magnitude of glucocorticoid receptor activation in brain tissue in response to stress (Dhabar et al., 1995).

Recall that these steroid receptors are involved in the HPA-axis response to stress (Spencer, Kim, Kalman, and Cole, 1998).

Characterization of differences in stressor reactivity has also been examined in other strains - the Fawn-hooded, Brown Norway, spontaneously hypertensive, and the previously mentioned Wistar-Kyoto rats. However, these inbred rats represent somewhat extreme phenotypes of stress sensitivity. Therefore, even though they are useful in understanding dysfunctional stress responses, they may not properly represent the genotypic variations observed in individuals suffering from depression. Given the very specific neurochemical anomalies found in these inbred rat strains, they may instead typify a very small sub-population of individuals who suffer from depression.

Our limited knowledge of the neurobiological basis of depression makes it difficult to discern if the neurochemical disturbances reported in some depressed individuals result from depression or are causative factors of the disease. Thus, animal models of depression have better generalizability when the research is not limited to animals with specific genotypes and phenotypic traits. Outbred rats are more representative of this condition, particularly when they are not bred for any specific attributes. Even then, we still observe strain differences in behavioural tests. Sprague-Dawley rats exhibit increased activity and exploration in the open field and elevated plus maze compared to PVG rats (Schmitt and Hiemke, 1998 a,b) whereas a similar stress-induced decrease in open field activity is reported for Sprague-Dawley and Long Evans rats (Faraday, 2002). Furthermore, when tested in the acoustic startle paradigm, Sprague-Dawley rats exhibit a greater startle response following stress versus their Long Evans counterparts (Acri, Brown, Saah, and Grunberg, 1995; Faraday, 2002). Behavioural

sensitivity to specific drugs have also been characterized in these strains; for example, cocaethylene-induced behaviours are reportedly less prominent in Long Evans versus Sprague-Dawley animals (Baumann, Horowitz, Kristal, & Torres, 1998; Horowitz, Kristal, and Torres, 1997).

Physiological distinctions between these strains have also been described. Long Evans males tend to show a reduced rate of weight gain during exposure to a chronic regime of restraint stress compared to unstressed animals; in Sprague-Dawley animals, this reduction is much less pronounced, occurring only after 19 days of stress administration (Faraday, 2002).

These data demonstrate some of the behavioural differences that exist between outbred rat strains. While much information has been gained regarding the biochemical and physiological characteristics that may account for distinctions between inbred strains, little is known about the mechanisms that differentiate outbred rat strains. Some of the inbred rat strains appear to be appropriate for the study of particular pathologies, for example, the spontaneously hypertensive rat line that models conditions related to hypertension and heart disease. However, given that the anomalies that characterize inbred strains are not always representative of dysfunctions found in human disorders, the use of outbred rat strains may be more appropriate to their study. Characterization of the distinctions between some of these strains may provide insight as to the neurobiological underpinnings of certain human conditions.

2. Gender differences in animal research

While strain differences represent one source of genetic variation in animal research,

gender differences are equally important. This section will deal with such differences and the importance of including female responses in animals studies.

Gender differences begin in utero. In rats, differentiation of the sexes begins at day 18 of gestation, until which point the animals have indifferent gonads. At this time, chromosomal differences begin to exert their effects; the presence, on the male -Y chromosome, of the gene responsible for sex determination will lead to development of the testes. Ultimately, production of androgens from the male gonads results in masculinization of the central nervous system. A critical step in masculinization is estradiol-stimulation of estrogen receptors in neuronal cells, a process achieved in "males" by aromatization of testosterone to estradiol. Although the gonads of female fetuses produce only low levels of estrogen, females are unable to escape estrogenic exposure due to the elevated levels produced by the mother and the capacity for estrogen to cross the placental barrier. However, fetuses of both sexes produce α -feto protein, a blood-borne protein that binds estrogen and limits its entry to the fetal brain; small amounts appear to be important in the feminization process. By producing testosterone, males by-pass the action of this protein; testosterone enters neuronal cells where it is aromatized to estrogen, beyond the reach of α -feto proteins. Estrogen can then bind to the nuclear estrogen receptors and initiate the events necessary to prevent nervous system feminization. Therefore, the processes of feminization and masculinization of the genitalia coincide with that of the nervous system and as a consequence, behaviour. However, the critical period for each differs. For example, prenatal exposure of female rats to testosterone will induce the development of testes, whereas neonatal androgen treatment will instead defeminize the developing rat such that ovulation and lordosis - typical female

sex-related behaviours - are prevented from occurring in adulthood (Booth, 1977; McDonald and Doughty, 1974).

Differentiation of the nervous system and behaviours related to reproduction have been extensively investigated as have gender differences linked to nonreproductive behaviours. In fact, sexual dimorphisms in aggression, as well as in the gender specific behaviours expressed in a variety of paradigms including the open-field, plus-maze, and passive-avoidance learning paradigm, are commonly studied. In order to better understand sexually dimorphic behaviours, it is important to determine whether the differences arise from developmental organization or transitory activational processes. If defeminization or demasculinization of the behaviour, in female and male animals respectively, is achieved by altering developmental exposure to gender appropriate gonadal steroids, then it is said to be under organizational control; however, if changes in endocrine (e.g. by gonadectomy) exposure as an adult induce said behavioural alterations, then it is believed that these are under transient activational control. Further investigation can help in discerning the involvement of more specific neuroanatomical or neurochemical processes.

While sexual dimorphism of the HPA axis response has been reported, these have been shown to be under the control of transient activational processes. Whereas diurnal patterns of basal plasma corticosterone levels are similar between male and female rats, their absolute values are greater in females depending on the phase of their estrous cycle; peak plasma corticosterone levels are greatest in females in proestrus versus males or females in any other estrous phase (Atkinson and Waddell, 1997). For the most part, basal functioning of the HPA axis appears to be similar in males and females. However, differences arise when the

organism is challenged. For example, exposure to physical or neurogenic stressors such as forced swimming, immobilization, or footshock for a duration of either 5, 15, or 60 min, induces similar corticosterone levels in female rats, irrespective of the length of exposure to the stressor while in males, at least 15 min of exposure is required to significantly alter corticosterone levels from basal counts (Kant, Lenox, Bunnell, Mougey, Pennington, and Meyerhoff, 1983). When animals are instead repeatedly exposed to a stress regime, the direction of the gender difference is less consistent. While one group reports that females are more resistant than males on behavioural tests when faced with chronic unpredictable physical stress (Rodríguez Echandía et al., 1988), another finds that although females do not greatly react to acute restraint stress, they failed to adapt to its repeated exposure, a finding in direct contrast with results in male rats (Kennett, Chaouloff, Marcou, and Curzon, 1986). These findings suggest that the type of stressor employed may be important in evaluating gender differences related to the stress-induced HPA axis response.

A further consideration that affects stress responses is housing condition. Investigators have shown that crowding, defined as an elevated spatial density of rats rather than simply group housing, is stressful in male rats while isolation is a stressor in females (Brown and Grunberg, 1995). A recent study has further qualified these housing conditions; the results suggest that aggression and defeat ensuing from crowded housing is the important factor in group housing males while social instability is the crucial qualifier in housing female rats (Haller, Fuchs, Halász, and Makara, 1998). These findings suggest that gender comparisons in responding during or following administration of a variety of stressors, must be interpreted with caution as basal stress levels appear to be dependent on housing conditions. This

exemplifies potential differences in gender susceptibility to stress.

These genetic (strain and gender) differences must be taken into account in behavioural measures of anhedonia. The next section will define and explore the behavioural measures that are typically employed in the assessment of CMS-induced anhedonia.

V. MEASURES OF REWARD AND HEDONIA

Anhedonia, one of the two core symptoms of depression, is defined as a loss of interest or pleasure in daily activities (Fawcett, Clark, Scheftner, and Gibbons, 1983). There are three behavioural measures typically employed in assessing CMS-induced anhedonia in rats; these are place preference conditioning, consumption of a mildly sweetened solution, and BSR. A description of each measure and the effects of CMS on it is described next.

1. Place Preference Conditioning

This paradigm involves conditioning of an animal for preference of a place associated with administration of a reward; the preference must be sustained once the reward is no longer available. The animal is first taught the association between the reward and the location by placing it in a two compartment box and offering the reward when the animal is in a particular compartment, the idea being that the particular compartment will become associated with the reward. Often, the two compartments are distinguished by different sensory cues, for instance horizontal lines on the wall of one compartment and vertical lines on the other. Preference is interpreted if the duration in the compartment associated with a reward exceeds that of the other compartment. A variation of the place preference paradigm

is place aversion. The procedure is similar to that previously described except in this case, animals are conditioned to spend less time in the compartment associated with the aversive stimulus, either footshock or noxious drugs.

The main use of the place preference procedure is in assessing the rewarding properties of drugs. Drugs of abuse, such as cocaine, heroin, amphetamine, and morphine have been shown to induce place preference (reviewed in Bardo, Rowlett, and Harris, 1995).

Furthermore, if animals are pretreated with appropriate antagonists, place preference can not be conditioned, at least while the antagonist is active.

Place preference conditioning has also been used to assess changes in hedonia following CMS. Typically, animals are exposed to CMS for a period of three weeks and then trained in the place preference procedure with one of the two compartments associated with a reward of either drugs or food. Chronic mild stress has been shown to prevent the association of rewarding stimuli with a distinctive environment (Papp, Lappas, Muscat, and Willner, 1992; Papp et al., 1991) which is normalized with antidepressant treatment (Benelli et al., 1999; Cheeta et al., 1994; D'Aquila, Monleon et al., 1997; Muscat et al., 1992; Valverde et al., 1997; Willner et al., 1994). It is important to qualify that the effects of CMS are particular to rewarding stimuli and have not been extended to place conditioning of aversive stimuli (Papp et al., 1992). Taken together these results suggest that CMS induces anhedonia, assessed as a decreased sensitivity to reward in the place preference paradigm.

One major limitation to evaluating anhedonic changes following CMS using place preference conditioning is that the nature of the test does not allow repetitive assessment of CMS induced changes. Given that deficits in sucrose intake (Benelli et al., 1999; Grippo et

al., 2002; Willer et al., 1987) and BSR thresholds (Moreau et al., 1992) are typically gradual, using the results of a single time point may be misleading and precludes the evaluation of the development and progression of anhedonia and its potential reversal with antidepressants.

2. Consumption of a mildly sweetened solution

The consumption of any sweet food source is said to be rewarding for rats. In fact, animal behaviour is often trained through reinforcement with a sweet treat. Palatable solid foods have been employed in such procedures, ranging from high sucrose containing rat chow pellets to vanilla or “Graham” wafers. Others employ sweetened solutions that can be made up of sucrose or saccharin sweetened water or even sweetened condensed milk.

Although intake of most of these varieties of sweetened foods has been evaluated as an index of the effects of CMS, a low concentration sucrose solution is most commonly employed (Willner, 1997a). Determining the consumption of a sucrose solution, following the application of CMS, has served as an indication of hedonic status in rodents. It is believed that a CMS-induced gradual deficit in the consumption of the sweetened food, a stimulus with previously rewarding properties, is indicative of anhedonia (Willner et al., 1987). However, the concentration of the solution that is presented to the animals is important in this paradigm. The following patterns of sucrose consumption have been useful in determining the optimum concentration to be used. Animals concurrently presented with three bottles, each containing a different sucrose concentration tend to choose the highest level (34%) over 0.7 and 7% sucrose solutions. However, in a two bottle test, animals presented with the choice of water and one of three concentrations of sucrose exhibit a

monotonic rise in intake and concentration, with greatest preference found at 7% (Muscat, Kyprianou, Osman, Phillips, and Willner, 1991); solutions of greater concentration no longer show a monotonic relationship between concentration and rewarding value. Therefore, the optimum concentration needed to detect increases or decreases in the rewarding value of the stimulus is defined as the value transecting the middle, or the 50% point, of the ascending limb of the concentration-intake function; typically a 1% sucrose solution is employed with rats.

Early evaluations of CMS on sucrose consumption were conducted using a two bottle test, such that both sucrose intake and preference for sucrose over water could be assessed over a 1h period (Willner et al., 1987). Since then, many investigators employ a single bottle paradigm, choosing instead to monitor water intake at a separate time, although there are those who argue that changes in preference rather than intake is a better index of CMS-induced anhedonia (Matthews et al., 1995). This topic is further investigated in the third and fourth studies of the dissertation.

The use of sucrose consumption as an index of anhedonia has many advantages over other methods. For one, it is conducive to repeated testing, allowing investigators to track the time course of hedonic changes. Once the CMS-induced deficit in consumption is deemed appropriate, antidepressant trials can be conducted to determine if the drug reinstates hedonic value. Third, it is a relatively simple procedure to carry out. However, there are limitations to this measure that require some attention. For example, it has been shown that mice tend to respond better to a slightly more concentrated sucrose solution (Monleon, D'Aquila, Parra, Simon, Brain, and Willner, 1995), so that a concentration-intake function should be

determined for different species and strains.

A second limitation to the use of sucrose consumption as an index of anhedonia is the potential confound of food deprivation used as part of the CMS schedule. Typically, the 1h sucrose test is conducted immediately following a prolonged (12-24h) period of food deprivation, a procedure that undoubtedly induces a physiological state of hunger. It is therefore important that control animals also be food deprived as part of the procedure. This however gives rise to another problem in that alterations in food availability have been shown to be stressful in rats (Heiderstadt, McLaughlin, Wright, Walker, and Gomez-Sanchez, 1999; Leal and Moreira, 1997). A way around this is to conduct longer test sessions so that the deprivation is less tightly tied to sucrose consumption; for example, some investigators monitor overnight consumption of a mildly sweetened solution (Ayensu et al., 1995; Hatcher et al., 1997). A final and related concern has to do with the caloric value of the sucrose solution. Even though the concentration employed is generally thought to be of little caloric consequence, it is uncertain whether the nutritional or caloric value of the solution remains relatively neutral to stressed animals. One way to overcome this potential confound is to use a substitute with little caloric value, for example saccharin. While most investigators have shown a reduction in low concentration saccharin consumption following CMS (Ayensu et al., 1995; Pucilowski et al., 1993; Willner et al., 1987), some have also related this decrease to the food or water deprivation stressors (Harris et al., 1998; Hatcher et al., 1997). In order to circumvent any nutritional and feeding related confounds, BSR (presented in the next section) was introduced as a behavioural endpoint to evaluate CMS-induced changes in hedonia.

3. Brain-Stimulation Reward

Brain-stimulation reward, a term used to describe the behaviour of an animal self-administering electrical stimulation via a probe implanted in a select brain structure, was first reported by Olds and Milner (1954). Since their adventitious discovery, investigators have employed the paradigm to search for other reward-related loci. Sites shown to support BSR in rodents range from anterior structures such as the olfactory bulbs to posterior sites including the dorsal raphe and locus coeruleus (rev. Stellar and Stellar, 1985). Rewarding brain stimulation has also been demonstrated in other species such as the fish, cat, dog, monkey, and humans (Angyan, 1975; Bishop, Elder, and Heath, 1963; Boyd and Gardner, 1963; Rolls, Burton, and Mora, 1980; Routtenberg, Gardner, and Huang, 1971; Sadowsky, 1972) suggesting the existence of a “neural” reward system.

Brain-stimulation reward has been employed as a paradigm to assess changes in the rewarding property of a given stimulus. Early on, investigators indexed such changes by observing alterations in the response rates; however, it was soon acknowledged that factors unrelated to reward could alter this measure. Today, we evaluate changes in reward based on frequency or current thresholds, a rate-independent procedure adapted from psychophysical methods. It has been employed to measure the rewarding effects of different classes of drugs. For example, cocaine (Frank, Manderscheid, Panicker, Williams, and Kokoris, 1992; Gilliss, Malanga, Pieper, and Carlezon Jr., 2002), morphine (Carlezon and Wise, 1993), and nicotine (Bozarth, Pudiak, and Kuo Lee, 1998; Huston-Lyons and Kornetsky, 1992) have been shown to produce reward-facilitating effects, while caffeine (Mumford, Neill, and Holtzman, 1988), low doses of apomorphine (Fouriezos and Francis, 1992; Knapp and

Kornetsky, 1996), and a mixed 5-HT1A/1B agonist (Harrison, Parsons, Koob, and Markou, 1999) have been reported to increase BSR thresholds. These are just a few examples of drug related changes in reward efficacy.

The effects of antidepressant drugs have also been evaluated on thresholds derived from rewarding brain stimulation. It has been hypothesized that interventions that relieve symptoms of depression in humans would enhance the rewarding effect of stimulation. Results of these studies have shown that at best, chronically administered antidepressants only modestly decrease BSR thresholds (Fibiger and Phillips, 1981; Hall, Stellar, and Kelley, 1990; Markou, Hauger, and Koob, 1992; McCarter and Kokkinidis, 1988), and parallel the clinical literature which suggests that antidepressants are not rewarding in “normal” individuals (Barr, Heninger, Goodman, Charney, and Price, 1997).

In order to properly verify the efficacy and time course of antidepressants using BSR as a measure of reward, exogenous manipulation of the animal in order to induce the depressive symptoms that mimic those experienced by humans has been a better approach. Investigators aimed to emulate depression by inducing behavioural changes in these animals that were reminiscent of anhedonia, a core symptom of depression. At this point, CMS had been shown to alter sucrose intake (Willner et al., 1987) and it was suggested that this deficit signaled a change in the hedonic value of the sucrose solution. Another group (Moreau et al., 1992) decided to evaluate the effects of CMS on another measure of hedonia, BSR, and found that animals showed a gradual increase in frequency thresholds during CMS exposure. Furthermore, animals pre-treated with the antidepressant desipramine did not show the expected increase in threshold (Moreau et al., 1992). Later work revealed that treatment with

mianserin, tolcapone, and electroshock treatment, administered at least two weeks after CMS induction, was able to reverse the CMS-induced increase in frequency threshold (Moreau, Borgulya et al., 1994; Moreau, Bourson et al., 1994; Moreau et al., 1995).

Notwithstanding these reports, changes in sucrose and BSR have not been consistently observed following exposure to chronic stressors, a point reported by Willner in a recent review of this literature (Willner, 1997a). With respect to BSR, two groups of investigators have been unable to replicate the CMS-induced increase in thresholds. Lin et al., (2002) reported a slight facilitation in responding for BSR while Nielsen's group (2000) found no meaningful change in thresholds during the course of a nine week stress regime in either of the two rat strains tested. However, it is interesting to note that a subgroup of two of the 11 animals tested did in fact exhibit a substantial increase in threshold starting around the third week of CMS exposure (Nielsen et al., 2000). These considerations were important in developing the experiments described in this thesis, of which there are five.

In the first, we evaluated the effects of an antidepressant on BSR thresholds. While previous studies reported, at most, small changes in thresholds associated with TCAs, we chose to assess whether paroxetine, an antidepressant of the SSRI class, would yield a greater facilitation in self-stimulation thresholds. Given that our results were only successful in replicating the modest decrease in BSR threshold reported by others, we elected to modify the venue of our research by first inducing a "depressive" state in the animals by chronic exposure to mild stressors while monitoring hedonic changes.

The last four studies of this dissertation employed the CMS procedure. Difficulties in replicating the CMS-induced behavioural changes reported in the literature led us to evaluate

the methodology involved in this procedure. The remaining studies were conducted with three main purposes. The first was to determine whether the chronic mild stressors employed were in fact stressful, as assessed by HPA axis responsivity. The second goal was to determine whether the behavioural measures typically employed to assess anhedonia were sufficiently sensitive for its detection. The third objective was to explore the interaction between genetic factors and stress and to ascertain how these may pertain to depression, as evaluated via the CMS model.

Table 1. DSM-IV-TR DIAGNOSTIC CRITERIA FOR A MAJOR DEPRESSIVE EPISODE

A. Five or more of the following symptoms have been present during the same 2-week period and represent a change from previous functioning; at least one of the symptoms is either (1) depressed mood or (2) loss of interest or pleasure. Do not include the symptoms that are clearly due to a general medical condition, or mood incongruent delusions or hallucinations.

1. Depressed mood most of the day, nearly every day, as indicated by either subjective report (e.g. feels sad or empty) or observation made by others (e.g. appears tearful).

Note, in children and infants, it can be irritable mood.

2. Markedly diminished interest or pleasure in all, or almost all activities most of the day, nearly every day (as indicated by either subjective account or observations made by others).

3. Significant weight loss when not dieting or weight gain (e.g. change of more than 5% of body weight in a month), or decrease or increase in appetite nearly every day.

Note: In children, consider failure to make expected weight gains.

4. Insomnia or hypersomnia nearly every day.

5. Psychomotor agitation or retardation nearly every day (observable by others, not merely subjective feelings of restlessness or being slowed down).

6. Fatigue or loss of energy nearly every day.

7. Feelings of worthlessness or excessive or inappropriate guilt (which may be delusional) nearly every day (not merely self reproach or guilt about being sick).

8. Diminished ability to concentrate or think, or indecisiveness, nearly every day (either by subjective account or observations made by others).

9. Recurrent thoughts of death (not just fear of dying), recurrent suicidal ideation without a specific plan, or a suicide attempt or a specific plan for committing suicide.

B. The symptoms do not meet the criteria for a mixed episode

C. The symptoms cause clinically significant distress or impairment in social, occupational, or other important areas of functioning.

D. The symptoms are not due to the direct physiological effects of a substance (e.g. drug of abuse, a medication, or other treatment) or a general medical condition (e.g. hyperthyroidism).

E. The symptoms are not better accounted for by a bereavement (i.e. after the loss of a loved one). The symptoms persist for longer than two months or are characterized by marked functional impairment, morbid preoccupation with worthlessness, suicidal ideation, psychotic symptoms, or psychomotor retardation.

Table 2. DSM-IV-TR DIAGNOSTIC CRITERIA FOR A MAJOR DEPRESSIVE DISORDER (SINGLE EPISODE OR RECURRENT)

- A. Presence of a at least one Major Depressive Episode (described above)
- B. The Major Depressive Episode is not better accounted for by Schizoaffective Disorder and is not superimposed on Schizophrenia, Schizophreniform Disorder, Delusional Disorder, or Psychotic Disorder Not Otherwise Specified.
- C. There has never been a Manic Episode, a Mixed Episode, or a Hypomanic Episode (unless these episodes were substance or treatment induced).

Specification of the clinical status or features of the Major Depressive Episode

- a) Mild, Moderate, Severe without Psychotic Features or With Psychotic Features
- b) Chronic
- c) With Catatonic Features
- d) With Melancholic Features
- e) With Atypical Features
- f) With Postpartum Features

Table 3. DSM-IV-TR CRITERIA FOR MELANCHOLIC FEATURES SPECIFIER

Specify if:

- A. Either of the following, occurring during the most severe period of the current episode:
 - 1) Loss of pleasure in all, or almost all, activities
 - 2) Lack of reactivity to usually pleasurable stimuli (does not feel much better, even temporarily, when something good happens)
- B. Three (or more) of the following:
 - 1) Distinct quality of depressed mood (i.e., the depressed mood is experienced as distinctly different from the kind of feeling experienced after the death of a loved one)
 - 2) Depression regularly worse in morning
 - 3) Early morning awakening (at least 2 hours before usual time of awakening)
 - 4) Marked psychomotor retardation or agitation
 - 5) Significant anorexia or weight loss
 - 6) Excessive or inappropriate guilt

Table 4. Drugs used or being tried as antidepressant treatments

CLASS OF ANTIDEPRESSANT	MAJOR SITE (S) OF ACTION	MAJOR ADVERSE EFFECTS	DRUG NAMES
<u>MAOI</u> <u>Classical (irreversible & nonselective)</u>	MAO-A and MAO-B enzyme	Constipation, urinary retention, dry mouth, sedation, postural hypotension, hypertensive crises related to food	Phenelzine (Nardil) Tranylcypromine (Parnate) Isocarboxazid (Marplan)
<u>RIMAs - reversible inhibitors of MAO A</u>	MAO-A enzyme	Mild nausea, dry mouth, headache	Moclobemide (Aurorix)
<u>Selective inhibitors of MAO B</u>	MAO-B enzyme		Deprenyl (selegiline; Eldepryl)
<u>Tricyclic antidepressants</u>	Presynaptic monoamine reuptake transporters	Dry mouth, confusion, memory impairments, blurred vision, drowsiness	Imipramine (Tofranil) Amitriptyline (Elavil, Endep, Tryptanol) Maprotiline (Ludiomil) Desipramine (Norpramin)
<u>SSRIs</u>	Presynaptic serotonin reuptake transporters	Anxiety, nausea, headache, sexual dysfunctions, agitation, akathisia	Fluoxetine (Prozac) Sertraline (Zoloft) Paroxetine (Paxil) Fluvoxamine (Luvox) Citalopram
<u>NDRI (norepinephrine & dopamine reuptake blockers)</u>	Presynaptic norepinephrine and dopamine transporters	Increased incidence of seizures and bulimia	Bupropion (and hydroxylated metabolite)

SNRI (serotonin-norepinephrine reuptake inhibitors)	Presynaptic serotonin and norepinephrine transporters	Menstrual irregularities, nausea, headache, dry mouth	Venlafaxine Duloxetine (clinical testing)
SARIs (Serotonin-2 antagonist/reuptake inhibitors)	5-HT _{2A} receptors, presynaptic serotonin transporters	Sedation, dry mouth, nausea, dizziness	Trazodone Nefazodone
Phosphodiesterase inhibitor	Phosphodiesterase-4 enzyme	Nausea	Rolipram
Sigma ligand	unknown	unknown	
Glucocorticoid inhibitor	unknown	unknown	Metyrapone
Natural products	Largely unknown Presynaptic serotonin, norepinephrine, dopamine, GABA, and L-glutamate transporters	Gastrointestinal symptoms, dizziness, confusion, tiredness, sedation	St. John's Wort (hypericum perforatum)
Natural Products			St. John's Wort (hypericum perforatum)

Table 5. Animal Models Used to Study Depression

	References
Pharmacological Induction Models	
1. Reserpine reversal	Bourin et al., 1983, Willner, 1984
2. Amphetamine potentiation	Willner, 1984; Little, 1988
3. 5-HT induced	Willner, 1984
Stress Induction Models	
1. Learned helplessness	Maier, 1984; Willner, 1984; Overstreet & Steiner, 1998; Bourin et al., 2001
2. Behavioural Despair	Kulkarni & Parale, 1986; Borsini & Meli, 1988; Lucki, 1997, Bourin et al., 2001
3. Chronic unpredictable stress	Katz, 1981; Willner, 1984
4. Chronic mild stress	Papp, Moryl, Willner, 1996; Willner, 1997 Overstreet & Steiner, 1998; Bourin et al., 2001
5. Primate separation model	Willner, 1984; Rosenblum & Pully, 1987
6. Separation in pair-bonded hamsters	Crawley, 1984 (2)
7. Maternal deprivation in neonates	Lehmann and Feldon, 2000
Genetic Models (bred for specific traits)	
1. Flinders sensitive line rat	Overstreet et al., 1988; Overstreet, 1993; Overstreet & Steiner, 1998; Yadid et al., 2000;
2. High DPAT sensitive line rat	Overstreet & Steiner, 1998
3. Wistar-Kyoto rat	Paré & Redei, 1993; Dugovic, Solberg, Van Reeth, & Turek, 2000; Lahmame, del Arco, Pazos, Yritia, & Armario, 1997
Genetic Models (genomic)	
1. CRH receptor subtype knockouts	Timpl, Spanagel, Sillaber, Kresse, Reul, Stalla, Blanquet, Steckler, Holsboer, Wurst, 1998
2. HPA transgenic	Montkowski, Barden, Wotjak, Stec, Ganster, Meaney, Engelmann, Reul, Landgraf, Holsboer, 1995
Miscellaneous	
1. Olfactory bulbectomy	Kelly, Wrynn, & Leonard, 1997; Bourin et al., 2001

Study 1

FEEDING AND REWARD INTERACTIONS FROM CHRONIC PAROXETINE TREATMENT

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Abstract

The self-stimulation paradigm was used to evaluate threshold changes following acute and chronic administration of the selective serotonergic reuptake inhibitor paroxetine; stimulation sites were located in medial forebrain bundle structures. Rats received daily systemic injections of one of three doses of paroxetine (2.5, 5, 7.5 mg/kg), either with or without stimulation, while the last group received the same number of vehicle injections with stimulation. Frequency thresholds were collected over a period of six hours on day 1 (acute phase); no marked difference in the values were observed over this time span. Thereafter, the animals were tested every third day (chronic phase) for a total of eleven sessions or roughly 31 days. Commencing around day 10 of the drug treatment, the higher dose of paroxetine produced a significant and persistent facilitation in self-stimulation thresholds, mimicking the delay in clinical response in humans that is well-documented. We also monitored on a daily basis the animals' weights and food intake. A large difference in the percent efficiency of food utilization, measured by calculating the ratio of weight change to food intake, was observed between the animals receiving stimulation and those that were not, exclusive to the higher dose of paroxetine. The percent efficiency of food utilization remained low in the animals only receiving the drug treatment, whereas they returned to baseline levels and above in subjects receiving both paroxetine and stimulation. Two findings emerge from these data: 1) the paradigm appears to model the human response to this class of antidepressants and 2) rewarding stimulation seems to counteract the drug-induced weight loss.

Paroxetine, Antidepressants, SSRI, Weight, Brain stimulation reward, Feeding, Medial forebrain bundle

1. Introduction

The monoamine hypothesis of depression states that this condition is caused by a functional deficit of monoaminergic neurotransmitters at certain sites in the brain (Coppen, 1967; Schildkraut, 1965). Support for this idea first came from the observation that monoamine antagonists could induce a depressive state (Harris, 1957; Sachar and Baron, 1979) and was further strengthened by pharmacological evidence that effective antidepressants facilitate norepinephrine and serotonin neurotransmission (Coppen, 1967; Schildkraut, 1965). While earlier studies concentrated mostly on abnormalities of these neurotransmitter systems, recent work suggests that dopamine may play a role in the delay of onset of the therapeutic action of antidepressants, by way of recently characterized subtypes of dopamine receptors (Collu, Poggiu, Devoto, and Serra, 1997).

The treatment of depression has changed over the years as awareness of the etiological basis of the disease has grown. The monoamine oxidase inhibitors were thought to be the most suitable treatment for depression when the more general monoamine hypothesis involving serotonin, norepinephrine, and dopamine prevailed. The hypothesis progressed to exclude dopamine, where the tricyclic antidepressants predominated, and finally to the exclusivity of serotonin, where the selective serotonin reuptake inhibitors (SSRI) were introduced as the choice pharmacological treatment for depression (Maes and Meltzer, 1995). The SSRIs increase the synaptic concentration of serotonin by desensitizing presynaptic serotonin autoreceptors on serotonin nerve terminals (Artigas, Bel, Casanovas, and Romero, 1996; Blier, De Montigny, and Chaput, 1987; Gardier, Malagié, Trillat, Jacquot, and Artigas, 1996; Hjorth and Auerbach, 1996; Lane, Baldwin, and Preskorn, 1995). Among this class of drugs, we find paroxetine (Paxil®), sertraline (Zoloft®), fluoxetine (Prozac®), and

venlafaxine (Effexor®), presented in decreasing order of selectivity for serotonin (Nemeroff, 1993). The drug's overall selectivity is also influenced by the action of its metabolites with the serotonin receptors. The central and autonomic side effects associated with tricyclic antidepressants are not observed with the selective serotonin reuptake inhibitors, due to their reduced interaction with muscarinic cholinergic and histaminergic receptors (Frazer, 1997).

There are several animal models that aim to simulate a depressed state within which to investigate aspects of depression, including among them the intracranial self-stimulation paradigm (Binks, Murchie, and Greenwood, 1979). This model of depression is based on the observation that a central symptom of depression is a decreased capacity to experience pleasure, and the assumption is that interventions that relieve symptoms of depression in humans, such as antidepressant drugs, will boost reward transmission, translating into a reduction in the threshold required to maintain self-stimulation responding in rats.

Overall, the tricyclic antidepressants when administered chronically tend to produce small or unremarkable decreases in reward threshold, as assessed using either the curve shift paradigm or other methods that do not rely on a simple rate-of-response to scale the effect (Fibiger and Phillips, 1981; Hall et al., 1990; Markou et al., 1992; McCarter and Kokkinidis, 1988; Valentino, Riccitelli, and Dufresne, 1991). However, there appears to be very little literature which deals specifically with the effects of a chronic challenge of agents with SSRI properties on thresholds for brain stimulation reward (Lee and Kornetsky, 1998). Thus, the aim of this study was to assess the threshold consequences of acute and chronic administration of the compound, paroxetine, an SSRI of importance in the clinical management of depression.

Paroxetine is primarily metabolized in the liver and none of its metabolites appear to be active (Lane et al., 1995). The most common side effects associated with paroxetine intake are somnolence and gastrointestinal dysfunctions such as nausea and a reduced appetite (Boyer & Feighner, 1991; Lane et al., 1995). A few cases of akathisia and bleeding which are attributed to the intake of paroxetine have been reported (Adler and Angrist, 1995; Ottervanger et al., 1994), although these effects are not apparent in all individuals treated with paroxetine. For these reasons, we included a group of animals that received paroxetine alone and no stimulation in order to distinguish the drug's influence on weight change from potential weight changes due to an increased activity level.

2. Methods

2.1 Subjects and Surgical Procedure

Three male Long-Evans and 32 male Sprague-Dawley rats (Charles River Laboratories) weighing between 355g-420 g at the time of surgery were individually housed in plastic cages and were allowed free access to tap water and Purina rat chow. The animals were maintained on a 12-h light-dark cycle with light onset at 0700 h.

Stereotaxic surgery was conducted as follows: each rat was anesthetized with 65 mg/kg intraperitoneally of sodium pentobarbital (Somnotol®) and when judged necessary, the animal was administered a 0.05 ml intramuscular injection of xylazine (Rompun®). To avoid discomfort due to pressure from the ear and incisor bars, a local anesthetic, lidocaine hydrochloride (Xylocaine® 2%), was topically applied inside the rats' ears and just behind the upper incisors. Their eyes were covered with ophthalmic ointment (BNP) to prevent dryness.

To avoid mucus buildup in the lungs, a subcutaneous injection was given either of 0.4 ml of glycopyrrolate (Robinul®) or 0.05 ml of atropine.

Monopolar stainless steel fixed electrodes, insulated with EpoxyLite, except at the tips, were implanted in the medial forebrain bundle bilaterally at the level of the lateral hypothalamus (LH) or unilaterally at the level of the ventral tegmental area (VTA). The flat skull coordinates, based on the rat brain atlas (Paxinos and Watson, 1986), were 2.6-3.0 mm posterior to bregma, 1.6-1.7 mm lateral to the midsagittal suture, and 8.2-8.4 mm below dura for the lateral hypothalamus and 4.8 mm posterior to bregma, 1.0 mm lateral to the midsagittal suture, and 8.2-8.3 mm below dura for the ventral tegmental area. A gold amphenol pin soldered to a fine stainless steel wire that was wrapped around four jewellers' screws anchored in the skull, served as the current return. Dental acrylic was used to firmly secure the entire assembly to the skull.

2.2 Behavioural Testing

All behavioural tests were conducted in a wood and Plexiglas chamber with dimensions of 27 X 37 X 51 cm. A rodent lever was fixed 3.5 cm above the floor on the lower right wall of the chamber. Stimulation was supplied by a constant-current amplifier (Mundl, 1980) and an integrated circuit pulse generator; the current was monitored continuously on an oscilloscope. Each lever press resulted in a 0.5 s train of square wave monophasic cathodal pulses, 0.1 ms in duration. Once the current was selected, the stimulation parameters remained fixed except for the frequency, which was varied according to the protocol described below.

Following a recovery period of seven days postsurgery, the rats were trained to lever press

using conventional shaping techniques. Starting with the minimum stimulation parameter values found to support high rates of responding (ex. a combination of 200 μA and 40 Hz), the current was held constant and the frequency decreased by 0.1 \log_{10} units per trial until little or no responding was observed. A 30-s pause separated each 60-s trial. The beginning of each trial was signalled by five trains of priming stimulation (one train per second) at the same parameter values as the subsequent 60 s trial. The frequency threshold was calculated by interpolation of the rate-frequency function and corresponded to the frequency associated with one-half of the maximum rate. This entire procedure was repeated at a second current, set at a value 0.2 \log_{10} units greater than the first (ex: 200 and 320 μA). During each session, which lasted roughly 45 minutes, four frequency thresholds were determined per current; the first one was considered a warmup and was, therefore, discarded. The presentation of the two currents was alternated from session to session. Lever-pressing was deemed stable for each rat when the frequency thresholds did not vary by more than 0.1 \log_{10} units for each current for 3 consecutive days.

Once the animals were thus stabilized, they were then randomly assigned to one of seven groups. There were two conditions overall - 1) drug and stimulation and 2) drug without stimulation. Hence, four of the groups received drug (either 0, 2.5, 5.0, or 7.5 mg/kg) and stimulation sessions, while the remaining three groups received a dose of either 2.5, 5.0, or 7.5 mg/kg but no stimulation. All animals were handled similarly, starting at their time of arrival except for the stimulation distinction. The activity of all animals was monitored before and after drug injections which included daily chronicling of weight and food intake; these values were then converted to percent efficiency of food utilization by dividing the

mean value of weight change (g) over 3 days by the mean value of food intake (g) over the same period.

2.3 Drug Treatment

Paroxetine was donated by SmithKline Beecham Pharmaceuticals (Oakville, Ontario, Canada). The powdered compound was dissolved in 21% dimethyl sulphoxide (DMSO). This concentration was needed to dissolve the highest dose of paroxetine; concentrations greater than this have been reported to be used without deleterious effects (Mobbs, Rothfeld, Saluja, and Pfaff, 1989; Zhu and McNaughton, 1994). The animals received daily IP injections of the filtered paroxetine solution at a dose of either 2.5, 5.0, or 7.5 mg/kg for a duration of 31 days. The control group (0 mg/kg) received daily IP injections of 1 ml/kg of filtered DMSO in water.

The day prior to commencing drug treatment, a baseline frequency threshold value was obtained at both currents for each rat. On the first day of drug treatment (acute phase), immediately following injection, each animal was tested at both currents (see earlier description) once an hour for a total of six consecutive hours. Thereafter, chronic tests were conducted once every third day, approximately 4-6 hours post-injection, to avoid any stress-related effects of injection and because the thresholds determined in the acute phase were observed to be most stable at this period. In summary, animals received 31 consecutive days of drug injections, during which self-stimulation sessions were executed a total of 10 times.

2.4 Histology

After testing was completed, the animals were given a lethal dose of sodium pentobarbital.

A necropsy was performed to evaluate the condition of the internal organs, because paroxetine is metabolized in the liver. The rats were then perfused intracardially with saline followed by 10% formalin. The brains were removed and stored in 10% formalin for at least 48 hours; they were then frozen at -20° and sectioned at a thickness of $40\ \mu\text{m}$ and stained with thionin. The location of the electrode tips was determined by aid of an atlas (Paxinos and Watson, 1986).

2.5 Statistical Analysis

Mean frequency thresholds for baseline and drug data of the animals receiving stimulation were determined at both currents for each of the six testing sessions in the acute phase and for each of the additional ten sessions in the chronic phase. Recall that in the acute phase, sessions were conducted once every hour on the first day of drug treatment, whereas in the chronic phase, sessions were once a day, every third day of drug treatment. The \log_{10} change in frequency threshold was then tabulated for each group. The mean threshold values were analyzed using a mixed ANOVA design with three factors- one independent (dose) and two repeated (current and session)- the latter with Huynh-Feldt corrections. To compare the total amount of stimulation across animals, a mean charge value was calculated from the frequency thresholds for each animal in the chronic phase of the experiment, using the equation where Q is the charge in μC (microcoulombs), I is the current in μA , N is the number of pulses in the stimulation train, and d is the pulse duration in seconds (Gallistel, 1978).

$$Q = I N d$$

To ascertain any performance effects induced by paroxetine, the maximal response rates recorded during the chronic phase of the experiment were analyzed using the same ANOVA design as above.

A similar analysis was carried out on the data pertaining to the efficiency of food utilization; in this case, the two factors were the groups (dose \pm stimulation) and treatment sessions.

3. Results

Figure 1 shows the tracings of the atlas plate (Paxinos and Watson, 1986) that best correspond to the sections containing the electrode tips, all of which were located within the limits of the medial forebrain bundle.

Insert Figure 1 about here

Histological results are not available for rats # 990 and 1184.

The analysis on the rate data yielded no significant difference between any of the groups or sessions.

The currents that were used to test the animals ranged from 200 μ A to 500 μ A, giving rise to overall charge values of 0.85 ± 0.41 μ C for the low currents and 0.91 ± 0.40 μ C for the high ones. Note that the variability does not reflect unstable thresholds, but rather changes in their values that accompanied the chronic drug regime. More importantly, the similarity in the charge values associated with low and high currents suggests good reciprocity between current and frequency. Finally, these values are consistent with what is usually observed with

stimulation at these sites (Gallistel, 1978).

Figure 2 shows the \log_{10} change in frequency threshold on the first day (or acute phase) of

Insert Figure 2 about here

drug administration. Each point and associated standard error represent the mean \log_{10} change from baseline across five subjects, at 1-h intervals following an initial injection of paroxetine or vehicle (DMSO). The difference in \log_{10} change in threshold from baseline and between the four groups are small, all less than 0.1 \log_{10} units, suggesting no acute effect of paroxetine administration. Note however, that of the 20 animals, approximately 20% of them, scattered throughout the four groups, did not readily self-administer rewarding stimulation during the first testing hour. The analysis of variance results indicate no main effect of either treatment or time, and no interaction of these two factors.

The \log_{10} changes in thresholds for the chronic phase of the experiment are shown in Figure 3. Because neither the three-way interaction nor the main effect of current were

Insert Figure 3 about here

significant, we chose to graphically represent the interaction between treatment and session that was significant, $F(28,84) = 1.93$, $\alpha = 0.025$; the degrees of freedom were corrected for violations of the compound symmetry assumption using Huynh-Feldt correction (Howell, 1992). Each point and associated standard error represent the mean \log_{10} change from baseline for five subjects collapsed across both currents. Note that of these 20 subjects, four of them, from three different groups (vehicle, 5.0 and 7.5), were removed from the study at different times, beginning at session 5 (or the 13th day of the study), mostly due to implant loss; one animal was withdrawn when its weight loss reached 10% of its initial body weight.

An effect of the higher dose (7.5mg/kg) of paroxetine administration is apparent from this figure, as the difference in the \log_{10} changes in threshold from baseline and between the paroxetine and vehicle groups continuously increases. The results of two linear contrasts revealed the 7.5-mg/kg group to be different from the other three groups at session 4, $F(1,9)=7.48$, $p < 0.025$, which is the last one to contain all five subjects in each group, and the final session (#11) in which only three subjects remained in the 7.5-mg/kg group, $F(1,9)=8.58$, $p < 0.025$.

Figure 4 shows the percent efficiency of food utilization for groups receiving paroxetine,

Insert Figure 4 about here

one dose with and the same dose without stimulation, as well as the group receiving the vehicle (DMSO) and stimulation. Most animals appeared healthy throughout drug treatment, with respect to weight and food intake, whether or not they received stimulation. However, some of the animals receiving the highest dose of paroxetine without stimulation were removed from the study near its completion due to a weight loss greater than 10%.

The percent efficiency of food utilization is initially poor in all groups treated with paroxetine, reflecting weight loss and a decrease in food intake; however, it appears to return to normal by the third treatment session in all animals that were part of the lower dose conditions. Following the 31 days of drug treatment, all animals that remained in the study returned to near control values of percent efficiency of food utilization. The analysis revealed significant main effects of group, $F(6,24) = 5.58$, $p < 0.001$, and session, $F(6,133) = 11.03$, $p < 0.001$), with Huynh-Feldt correction; we performed the analysis on 8 of the 10 sessions to include as much data as possible. A Dunnett's post hoc analysis was performed to evaluate

the different patterns of efficiency between the stimulated and unstimulated animals receiving the highest dose of paroxetine. As is apparent from Fig. 4, the stimulated animals began showing a significant improvement over their initially poor efficiency values around the third session, and this is maintained for the remainder of the study. The unstimulated animals show a small improvement in efficiency between sessions 3 and 6, after which it progressively deteriorates until the last session.

The necropsy report shows an increased occurrence in discoloration of the liver and kidneys as well as diffuse contouring of the liver lobes in most of the animals treated with paroxetine at a dose of 7.5 mg/kg (with and without stimulation) as compared to the animals in all other groups.

Behavioural observations were noted each day during drug treatment. Before injection, the animals receiving the vehicle and the lower dose of paroxetine were very active, primarily sniffing and rearing. They remained likewise after the injection, generally eating, drinking, grooming, or exploring their cages. Just before injection, the animals receiving the higher doses of paroxetine were very quiet, typically huddled in the back of their cages. Post-injection, these animals would remain quiet, retreat to the back of their cages, and often lie down on their sides. Rarely would they exhibit any eating or drinking behaviour immediately following the injection. Furthermore, some of the animals administered the higher dose of paroxetine developed hematomas in the lower abdominal cavity.

4. Discussion

An important finding of this study is that chronic administration of paroxetine decreases

frequency thresholds for self stimulation, which is interpreted as a facilitation in the rewarding value of the stimulation. Although some animals appeared sickly in their home cages where they were injected with paroxetine, the analysis of the rate data indicates that their self-stimulation performance was not compromised. The data show that the facilitation in thresholds begins at about the 10th day of drug treatment, similar to the well-documented delay in antidepressive effect reported in humans (Burrows, McIntyre, Judd, and Norman, 1988; Julien, 1998). In some animal studies, chronic desipramine administration has been shown to cause a gradual decrease in the required current necessary to support a fixed level of responding (Fibiger and Phillips, 1981; McCarter and Kokkinidis, 1988), although in one case, this effect was only seen when an ascending, but not descending, current schedule was presented (Fibiger and Phillips, 1981); when frequency rather than current was scaled in another report, no such effect was observed with the single dose tested (Hall et al., 1990). In a recent study (Lee and Kornetsky, 1998), chronic challenge of 5.0 mg/kg of fluoxetine (SSRI class of antidepressants) surprisingly gave rise to an increase in current threshold; it would be interesting to see if this finding persists with additional doses. The discrepancy in the results may also be due to differences in the behavioural techniques employed.

One possibility is that reward enhancement as measured by threshold will never be prodigious using this paradigm, that is, one in which subjects receive no manipulation prior to drug treatment. When subjects are chronically exposed to a mild stressor preceding antidepressant administration, larger effects are observed; presumably in this example, the drug is acting on an impaired reward system (Moreau et al., 1992). Likewise, when the antidepressant effects are evaluated in animals that are undergoing cocaine withdrawal, a

significant threshold-lowering effect is reported (Markou et al., 1992). Thus, our results may reflect an improvement in reward threshold that is near ceiling levels prior to treatment, such that substantial reductions in its value may not be realistic. There are human data to support this notion, that “normal” subjects are negligibly influenced by antidepressant treatment. For example, in one study (Barr et al., 1997) 6 weeks of drug administration failed to alter responses in controls on a number of well-known mood scales.

One mechanism for the antidepressant action on the reward substrate may be related to the proposed interaction between dopamine and serotonergic systems. There is evidence for an upregulation of mesolimbic dopamine receptors following chronic SSRI treatment (Hammer, Margulies, Lynn, and Brady, 1993), and it may be via this route that antidepressants modify reward signals.

The delay in therapeutic effect in clinical studies appears to coincide with several different physiological events. For example, it takes a few weeks for many of these compounds to reach a steady plasma concentration, although no correlation between efficacy and concentration has actually been established, particularly in the case of paroxetine (Kuhls, Schlake, Rolf, and Rudolf, 1992; Tasker, Kaye, Zussman, and Link, 1989).

A reduction in weight and food intake was observed initially in groups administered the higher doses of paroxetine (see Fig. 4). In particular, animals receiving the combination of stimulation and the 7.5 mg/kg dose showed a weight gain beginning at the fourth treatment session, and consequently, an increase in the percent efficiency of food utilization. Conversely, the paroxetine-treated animals alone exhibited a continuous weight loss, therefore giving rise to a very poor efficiency of food utilization throughout the drug

treatment. Thus, it seems that stimulation counteracts the weight loss due to paroxetine. This SSRI, which increases postsynaptic serotonergic stimulation, is known to have an anorectic effect in humans by grossly reducing caloric intake (Simansky, 1996). There is no clear explanation for the reversal in weight change and food intake by the animals receiving both paroxetine and stimulation, although alterations in the levels of peptides and immunological factors might be implicated. Cytokines, such factors, have been shown to decrease feeding by a direct action on the central nervous system (Plata-Salaman, Oomura, and Kai, 1988). Furthermore, these factors have been implicated in sickness behaviour and sickness-induced decreases in food-motivated behaviour (Kent, Bret-Dibat, Kelley, and Dantzer, 1996). Modulation of cytokine release by neurotransmitters has been shown (Tringali, Mirtella, Mancuso, Guerriero, Preziosi, and Navarra, 1997); therefore treatment with paroxetine, which increases serotonin levels in the brain, could potentially change cytokine release.

Intracranial stimulation could have altered neuropeptide Y levels in the brain, a substance shown to stimulate ingestive behaviour (Stanley, Chin, and Leibowitz, 1985). Interactions of this peptide with immunological factors could indirectly implicate neuropeptide Y in the attenuation of the initially observed anorexia and sickness behaviour (Sonti, Ilyin, and Plata-Salaman, 1996).

Another explanation for our finding of a seemingly protective effect of stimulation against the drug-induced anorexia may be related to alterations in endocrine and metabolic functions resulting from chronic stimulation. For example, it has been shown that lateral hypothalamic stimulation raises the metabolic rate by as much as 40% (Pawson, Preston, Haas, and Foster,

1987). Finally, the efficient utilization of their food suggests that the animals receiving stimulation might be more capable of converting their energy intake into muscle mass, resulting in a net weight gain.

Because metabolism of paroxetine occurs mostly in liver, a visual inspection was done to verify its condition; we found obvious discoloration of the liver, particularly in animals administered the high dose. Bleeding attributed to the intake of paroxetine and fluoxetine in humans has been documented, and it is postulated that this bleeding is caused by drug-induced blood platelet dysfunction (Aranth and Lindberg, 1992; Ottervanger et al., 1994). A similar hypothesis may explain the presence of the hematomas that we observed in the animals receiving paroxetine; shortly after drug treatment was discontinued, the hematomas disappeared.

In conclusion, this study suggests that the rewarding value of stimulation increases with chronic administration of paroxetine in a delayed fashion. Furthermore, the time course of the drug-induced increase in reward value parallels that observed in humans. Some of the adverse drug effects, which are now being reported in the clinical literature, have been demonstrated in this animal model. It would be useful to further investigate the role of rewarding electrical stimulation in the modification of body weight induced by paroxetine and whether this effect generalizes to other SSRIs.

Study 1.

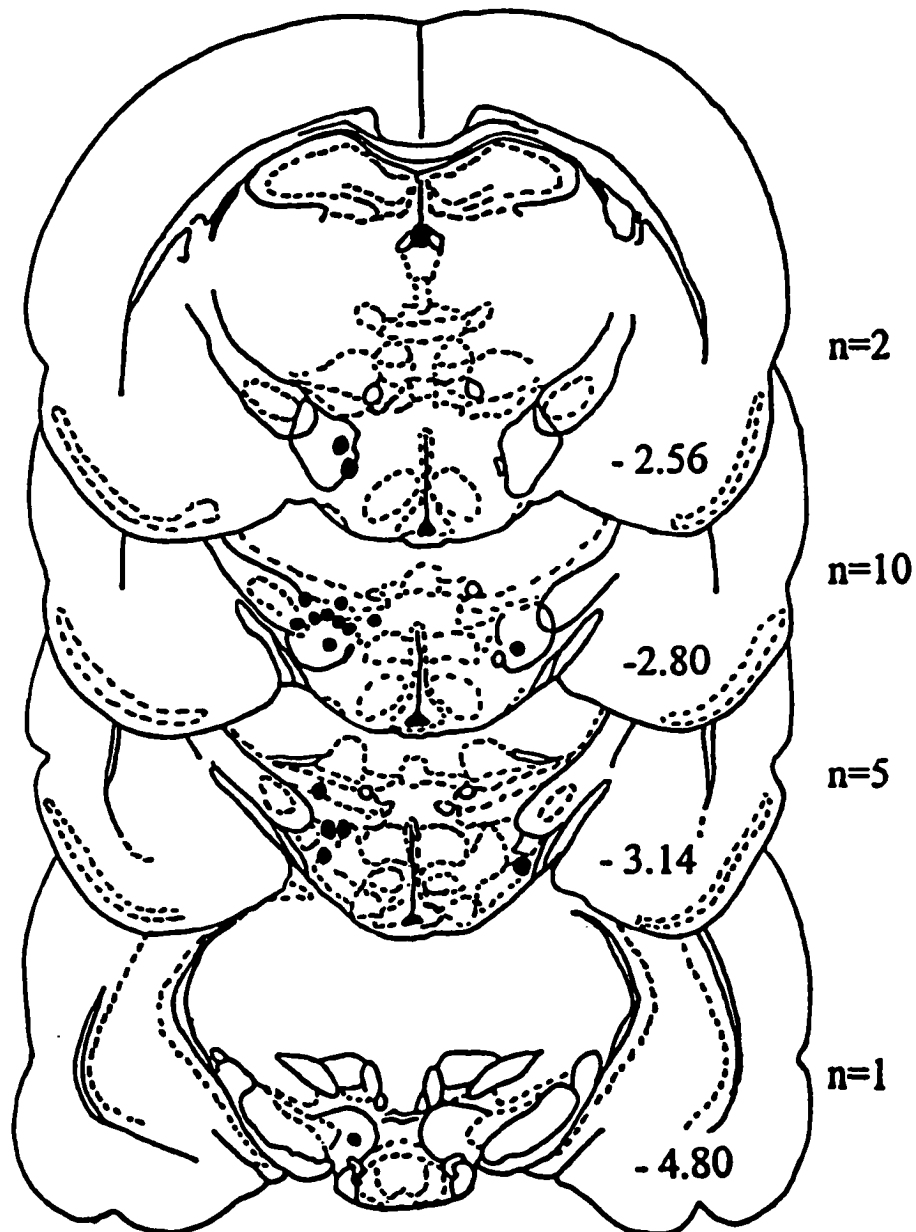


Figure 1. Tracings from the Paxinos and Watson (1986) atlas plates that best correspond to the location of the electrode tips, shown by filled circles. The antero-posterior distance behind bregma and the number of subjects with tips found at the level are indicated on the right side of the figure.

Study 1.

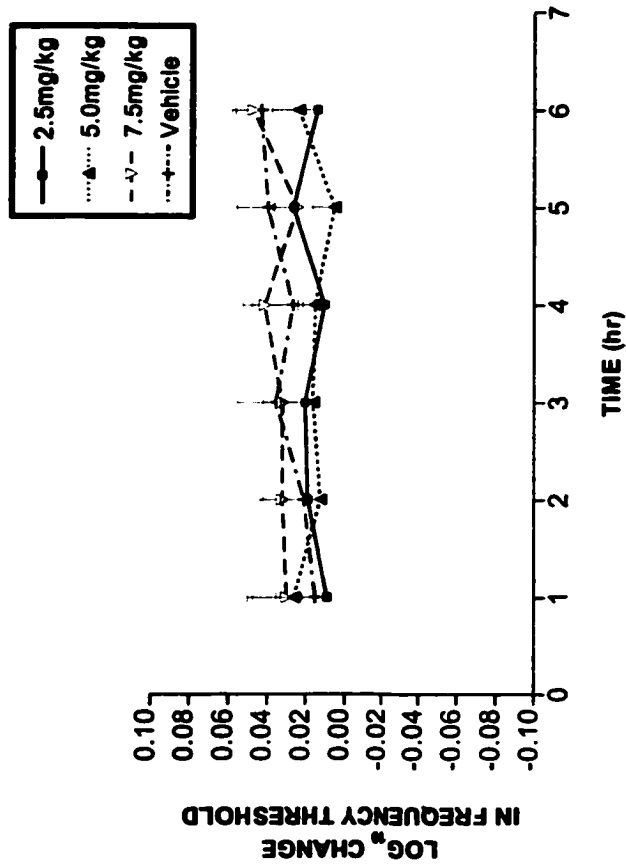


Figure 2. The acute effects of systemic administration of different doses of paroxetine or vehicle on the \log_{10} changes in frequency threshold. Each point represents the mean \pm SEM of the thresholds collected for the five animals in each group. Negative values indicate a decrease in frequency threshold, interpreted as a facilitation in the rewarding value of the stimulation.

Study 1.

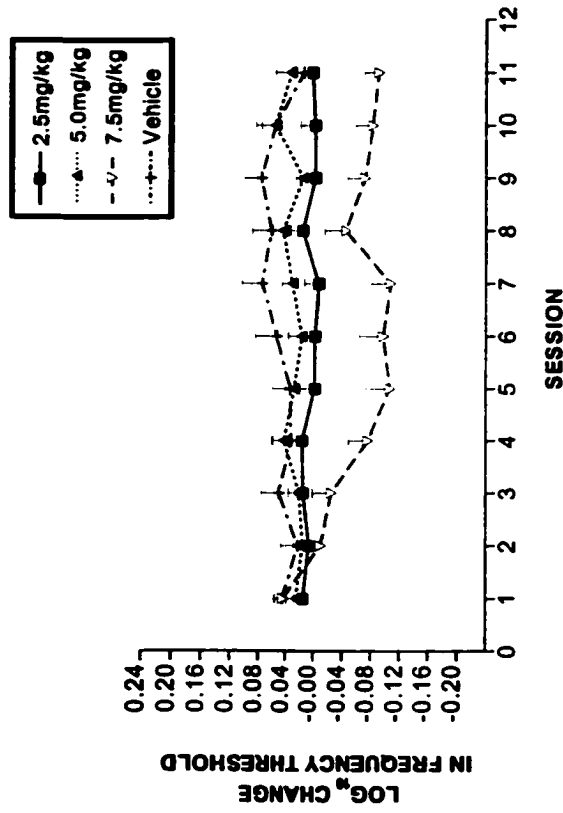


Figure 3. The chronic effects of administration of paroxetine or vehicle on the log₁₀ changes in frequency thresholds. Each point represents the mean \pm SEM of the thresholds obtained for each group of five animals over two currents. A session was conducted every third day of drug treatment. Negative values indicate a decrease in the frequency threshold, interpreted as a facilitation in the rewarding value of the stimulation while positive values mean the reverse.

Study 1.

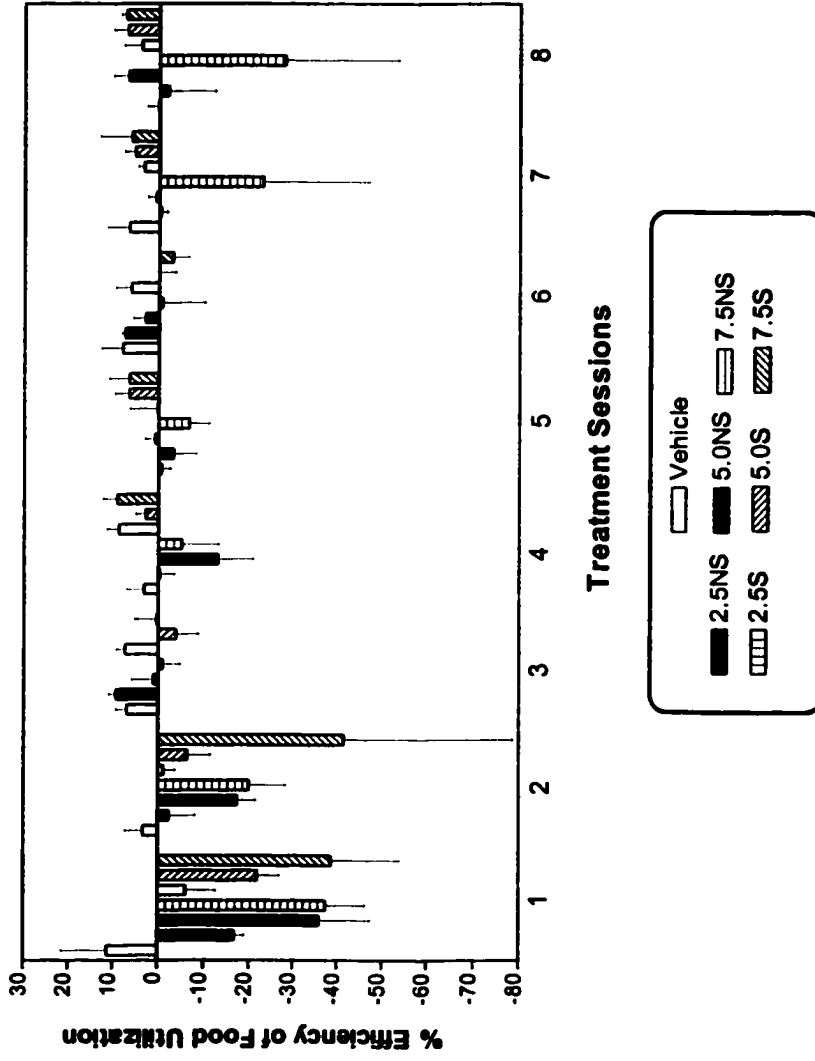


Figure 4. The graph shows each group's % efficiency of food utilization (g wt change/ g food intake). The results are expressed as cumulative group means \pm SEM over each 3 day treatment session. The legend indicates the dose of paroxetine received with stimulation (S) or without stimulation (NS).

Study 2

THE EFFECTS OF CHRONIC MILD STRESS ON MALE SPRAGUE-DAWLEY AND LONG EVANS RATS:

I. BIOCHEMICAL AND PHYSIOLOGICAL ANALYSES

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Abstract

The chronic unpredictable mild stress (CMS) is a paradigm developed in animals to model the relatively minor and unanticipated irritants that lead to a state of anhedonia in some individuals. However, the effectiveness of CMS is sometimes difficult to establish, for which unique strain sensitivities has been attributed as one contributing factor. These considerations led us to design the present study, which was an investigation of the corticosterone response to CMS in two outbred rat strains - Sprague-Dawley and Long Evans. Animals were exposed to one of two conditions - control or CMS - for three weeks during which body weight and fecal count were regularly monitored. At the end of this period, blood was sampled at a variety of time intervals following induction of a brief restraint stressor. First, a significant effect of CMS on corticosterone levels was evident at time 0 (prior to the application of the acute restraint stressor) in both strains. Second, the typical quadratic pattern of stressor-elicited fluctuations in this measure was similar in both Sprague-Dawley and Long Evans rats, with consistently elevated levels for the first hour following exposure to the acute stressor; near baseline values were observed at two hours. However, only in the Long Evans strain were CMS related values much less than that observed in the control group after restraint stress. Third, both strains showed a reduced weight gain in the CMS groups relative to control groups. Fourth, spleen and adrenal weights were similar across all groups. Fifth, fecal counts remained stable across weeks of treatment in all groups with the exception of the Long Evans rats exposed to CMS; in this group, average counts were systematically reduced over the treatment period. We conclude that a history of chronic stress significantly blunts corticosterone levels in Long Evans but not Sprague-Dawley rats

following exposure to an acute stressor. Physiological indices however are less influenced by this experience, at least when the exposure is limited to three weeks.

Key Words: corticosterone, chronic mild stress, genetic differences.

1. Introduction

Brain-stimulation reward has been proposed as a model for evaluating the effects of antidepressants (Binks et al., 1979), based on the reasoning that if a decreased capacity to experience pleasure characterizes depression in humans, treatments that relieve these symptoms should potentiate responding for rewarding stimulation. Applying this idea to threshold scaling as an index of reward efficacy (Gallistel, Shizgal, and Yeomans, 1981), drug effects would be interpreted from reductions in self-stimulation thresholds. However, this approach for evaluating compounds with antidepressant properties has had limited success. For example, only modest reductions in thresholds for rewarding brain stimulation have been reported following desipramine (Fibiger and Phillips, 1981; Hall et al., 1990; McCarter and Kokkinidis, 1988), desmethylimipramine (Markou et al., 1992; Valentino et al., 1991), and paroxetine administration (Konkle and Bielajew, 1999). In contrast, treatment with fluoxetine (Lee and Kornetsky, 1998; Lin, Koob, and Markou, 1999) produced either no change or only slight threshold increases. One interpretation for the relative lack of influence such drugs appear to have on brain-stimulation reward thresholds may be related to the fact that this model uses as its basis an “intact” or non-compromised reward system.

In order to address this problem, some investigators have modified the paradigm to include a behavioural challenge prior to drug treatment. The idea is based on the contention that

stress exposure (particularly long-term) may play a role in the etiology of depression (Anisman and Zacharko, 1982; Kendler et al., 1995; Kessler, 1997). The chronic mild stress (CMS) paradigm thus models in rodents the unexpected stressors of everyday life that some individuals handle with great difficulty. It employs ecologically relevant stressors that are chronically applied in an unpredictable manner so as to avoid habituation. The stressors used in this paradigm are ones that are known to evoke in rodents behavioural reactions related to fear and anxiety which are modified by anxiolytic agents (see Palanza, 2001; Willner, 1997 for reviews). A number of physiological and behavioural alterations accompany exposure to CMS. For instance, sleep abnormalities have been reported (Cheeta et al., 1997; Moreau et al., 1995), as have decreases in exploratory and sexual behaviour (D'Aquila et al., 1994). In addition, immunological anomalies such as an increase in the proliferative ability of splenocytes to concanavalin A (Kubera et al., 1996) as well as increases and decreases in interleukin-1 and interleukin-2 production respectively (Kubera et al., 1998). However, the main feature of CMS remains the induction of a state of anhedonia, a core symptom of depression in humans, according to the DSM IV (American Psychiatric Association, 1994). The merits and drawbacks of this paradigm have been recently reviewed (Anisman and Merali, 1997; Willner, 1997).

Several procedures have been developed to evaluate the anhedonia that arises following CMS exposure in animals. The most common is to monitor changes in consumption and/or preference for a weak sucrose solution (Willner, 1997; Willner, Muscat, and Papp, 1992; Willner et al., 1987), interpreted as an index of reward sensitivity. While some laboratories have had success in demonstrating a reduction in sucrose drinking following application of

CMS and its restoration from chronic antidepressant treatment (Monleon et al., 1995; Muñoz and Papp, 1999; Muscat et al., 1992; Papp, Moryl, & Willner, 1996; Sampson, Willner, & Muscat, 1991; Willner et al., 1987), others have failed to reliably observe any changes using this index (Nielsen et al., 2000; Willner, 1997).

Another way to interpret the anhedonic consequences that arise from a regime of CMS is to track changes in brain stimulation reward thresholds (Moreau et al., 1992). Using this paradigm, only small increases in thresholds have been reported (Moreau, Bourson et al., 1994; Moreau et al., 1996; Moreau et al., 1995); again, others see little or no effect using a similar methodology (Lin et al., 2002; Nielsen et al., 2000). Because of the large variability in such effects across animals, the importance of analyzing individual subject results has been argued (Nielsen et al., 2000). For example, this approach was used by Neilsen et al. (2000) who reported significant threshold increases in two of 11 rats subjected to a nine week regime of CMS; the grouped data of 11 rats, however, suggested no effect on thresholds.

It is well documented that stressful manipulations elicit an increase in the corticosterone response (Bhatnagar and Dallman, 1998; DeBoer, Slangen, and van der Gugten, 1988; Dhabar, McEwen, and Spencer, 1997; Hu, Gursoy, Cardounel, and Kalimi, 2000) and alterations in hypothalamic-pituitary-adrenal axis related organ weights (Burchfield, Woods, and Elich, 1980; Heiderstadt et al., 2000; Herman, Adams, and Prewitt, 1995; Pignatelli, Maia, Castro, da Conceicao Magalhaes, Vivier, and Defaye, 2000). Given the relative mild intensity of the individual manipulations that make up the CMS paradigm, the stressful aspect appears to be attributable to the unpredictability of the stressors.

The purpose of this study was to assess the biochemical and physiological alterations

following CMS exposure in order to ensure that the paradigm elicits appropriate stress responses. Behavioural data (sucrose intake and thresholds for rewarding brain stimulation) were not collected. We compared two groups of subjects - a control and a CMS group. In addition, given the evidence suggesting strain differences with respect to the effectiveness of the CMS paradigm (Nielsen et al., 2000; Willner et al., 1992), we included two different outbred strains (Sprague-Dawley and Long Evans) for which the behavioural effects of CMS have been reported by others (Marona-Lewicka and Nichols, 1996; Valverde, Smadja, Roques, and Maldonado, 1997).

2. Materials and Methods

2.1 Animals

The subjects were 31 Sprague-Dawley and 31 Long Evans rats (Charles River Laboratories, St-Constant, Québec) weighing between 370-420 g at the beginning of the study. Unless otherwise specified, the animals were individually housed in clear Plexiglas cages with food and water available freely. They were normally maintained on a 12:12h light/dark cycle with lights on at 0700 h.

2.2 Procedures

Throughout the study, all animals were weighed twice weekly, and the total number of fecal pellets in the home cage over a 24 h period was counted on a weekly basis, typically Monday morning, in order to avoid any effects of food and water deprivation (see Table 1).

Insert Table 1 about here

Handling of the animals was minimal and always carried out by the same individual.

Animals of each strain were placed in one of two groups - Control (n=11) and CMS (n=10). Those in the CMS condition were exposed to chronic unpredictable mild stressors over a three week period. The schedule and type of stressor are presented in Table 1.

2.3 Blood sampling

Immediately following the three weeks of behavioural manipulations, tail blood samples were taken by lancing the tail close to its tip and collecting a few drops of blood on an Schleicher & Schuell® filter paper (procedure adapted from Wortham and Stallings, 1997). A baseline sample was collected and then followed by an acute 10 minute restraint stress; this process was included in order to evaluate any consequences of the chronic manipulations on the typical corticosterone response pattern to an acute stressor. Note that the rats were not otherwise restrained during the blood collection procedure. Blood sampling was repeated at 15, 30, 60, and 120 min after the onset of the acute stressor; after the filter papers had dried sufficiently (approximately 4-5 hours), they were stored in a -20° C freezer for subsequent analysis. Blood samples were collected over two days between 8 and 11 AM and alternated between groups in order to minimize the effects of circadian rhythm on the results. Following the last blood sample, animals were decapitated; adrenal glands and spleen were removed and weighed.

2.4 Corticosterone assay

Blood samples were eluted from the filter paper by placing one 3.2 mm punch in a glass

tube and adding 100 μ L of Dulbecco's phosphate buffered saline (containing 0.1% gelatin) (Wortham and Stallings, 1997) to each tube. These were shaken for 1 hour at 50 rpm at room temperature, refrigerated overnight, and shaken for an additional hour the following morning.

Plasma corticosterone levels were determined using a commercially available radioimmunoassay kit (ICN Biomedicals, Costa Mesa, CA). The intra-assay variability was <10% while inter-assay variability was eliminated by running all the blood samples at one time. Total corticosterone concentration levels were determined as outlined by Wortham and Stallings (1997).

3. Results

3.1 Corticosterone

Figure 1 shows the corticosterone stress response profiles obtained in the two groups of

Insert Figure 1 about here

Sprague-Dawley and Long Evans rats, before (inset graph, time 0) and after presentation of the acute stressor (main graphs, times 15-120 min). The time 0 data have been expressed as corticosterone concentrations and the rest (times 15-120 min) as percent change from this value. Overall, the pattern that describes the corticosterone levels from 0 to 120 minutes reflects a significant quadratic trend for each group and is consistent with what others have reported (Dhabar et al., 1997; Garcia, Marti, Vallès, Dal-Zotto, and Armario, 2000). Results of the trend analyses were the following for Sprague-Dawley: control [F(1,12) = 37.94; $p < 0.00005$] and CMS [F(1,12) = 121.72; $p < 1 \times 10^{-6}$], and for Long Evans: control [F(1,18) =

73.67; $p < 1 \times 10^{-6}$] and CMS [$F(1,18) = 41.08$; $p < 5 \times 10^{-6}$]; note that these were performed on corticosterone levels before their transformation to percent change from baseline as shown in Figure 1.

A simple ANOVA was conducted on the time 0 data (graph inset) which represents corticosterone levels immediately following the three weeks of CMS exposure; a main effect of treatment [$F(1,34) = 14.43$; $p < 0.0006$] was the only significant finding.

Next, the effects of a subsequent acute stressor on corticosterone levels were investigated separately in each strain. The percent changes from baseline (time 0) corticosterone values were analyzed using a split-plot ANOVA design with one repeated (15, 30, 60, & 120 time points) and one independent factor (two groups - control and CMS); significant main effects of time were found for both strains: Sprague-Dawley [$F(1,16) = 9.29$; $p < 0.005$], Long Evans [$F(2,36) = 8.12$; $p < 0.002$]. A group effect was only evident in the Long Evans rats [$F(1,18) = 7.69$; $p < 0.02$]; in this strain, the interaction between group and time just failed to reach significance ($p < 0.063$) before Greenhouse-Geisser correction for violations of sphericity were applied. This correction is only used when repeated factors have more than two levels (Howell, 1997).

3.2 Body Weight

Figure 2 depicts the body weight profiles of the control and CMS groups during the three

Insert Figure 2 about here

weeks of treatment expressed as percent change from baseline weight (values obtained a few days before treatment). Final weights were evaluated by a two-way ANCOVA with strain and group as independent factors and baseline weight as the covariate. Results of the analysis indicate a main effect of group [$F(1,37) = 37.94$; $p < 3 \times 10^{-6}$] as well as strain [$F(1,37) = 20.22$; $p < 7 \times 10^{-5}$]; no interaction between the two factors was detected. Sprague-Dawley rats were consistently heavier than their Long Evans mates with baseline weights roughly 380 g and 350 g for each strain respectively.

3.3 Organ weight

Adrenal and spleen weights appear in Figure 3; they are expressed as mg weight/100 g of

Insert Figure 3 about here

body weight. The analysis of the spleen weight, which was based on a two factor (two strains and two groups) ANCOVA using body weight as a covariate, gave rise to no significant main effects or interaction. Adrenal gland weight was similarly analyzed with the added factor of side (left vs right). Other than a significant difference between left and right adrenals [$F(1,38) = 12.63$; $p < 0.002$], the right adrenal gland being heavier in all groups, no other notable effects were revealed. Note that the analyses were carried out on actual organ weight, and not the transformed values that appear in the figure. Analyses on transformed data such as these usually do not satisfy the assumption of isometry (Packard and Boardman, 1999).

3.4 Fecal output

The average fecal count per group, determined once weekly, is shown in Figure 4. These

Insert Figure 4 about here

data were analyzed via a mixed ANCOVA design with two independent factors (strain and group), one repeated factor (time), and body weight as a covariate; Greenhouse-Geisser correction was applied as required (Howell, 1997). The results of the analysis indicate significant two-way interactions between strain and time [$F(3,106) = 3.14$; $p < 0.04$] and between group and time [$F(3,106) = 3.94$; $p < 0.02$]. Long Evans animals showed a consistently higher fecal output than their Sprague-Dawley mates, giving rise to a significant strain difference [$F(1,36) = 12.37$; $p < 0.002$]; main effects of group [$F(1,36) = 4.42$; $p < 0.05$] and time were also detected [$F(3,106) = 3.14$; $p < 0.04$]. A post hoc analysis yielded a significant effect of time for the Long Evans CMS animals only [$F(3,111) = 8.75$; $p < 0.00003$] where a gradual decrease in fecal output is observed over the three weeks of manipulations.

4. Discussion

While CMS is valid conceptually as a model, its application has not yielded consistent results across different laboratories (Lin et al., 2002; Nielsen et al., 2000; Willner, 1997). Minor modifications in the number, type, and frequency of stressor exposure do not appear to explain the lack of reliability (Willner, 1997). In our own experience (Konkle, Kentner, Baker, Bielajew, Fouriez, and Merali, Submitted), using male Sprague-Dawley and Long Evans rats, we have been unable to show any CMS-related alterations in either self-stimulation thresholds or sucrose preference. One possibility is that these indices are not

adequately sensitive to the effects of stressors. For this reason, we chose to assess whether changes in endocrine or physiological parameters following CMS exposure would be more easily discernible.

4.1 Gross physiological measures

Our data suggest that the gross physiological measures employed here were not altered by the three weeks of stress experience. Although chronic stress exposure is often associated with adrenal gland hypertrophy (Burchfield et al., 1980; Heiderstadt et al., 2000; Herman et al., 1995; Pignatelli et al., 2000) and decreased spleen weight (Batuman, Sajewski, Ottenweller, Pitman, and Natelson, 1990; Dominguez-Gerpe and Rey-Mendez, 1997), we found no such differences. One possibility is that the elevation in corticosterone levels was not sufficient to incur significant alteration in organ weights or that the time frame of exposure to an increase in HPA-axis activity was too short. In fact, results of our preliminary data (unpublished) from a six week CMS regime suggest that gland weight is elevated. Recently, Khan et al. (1999) found adrenomedullin levels significantly elevated in a number of tissues including the adrenal glands, following application of an acute stressor. While promising as a measure of stress-related physiological activity, the role of this peptide as a long-term regulator of adrenal function needs to be evaluated.

A slower rate of body weight gain or even weight loss is often reported with exposure to chronic stressors (Burchfield et al., 1980; D'Aquila et al., 2000b; Dhabar et al., 1997; Harris et al., 1998; Harro et al., 1999; Nielsen et al., 2000; van Raaij, Dobbe, Elvers, Timmerman, Schenk, & Oortgiesen, 1997; Willner et al., 1994). In the present study, animals in the CMS

group did show a reduced weight gain relative to their control comparison. One explanation is that the food and water deprivation stressor employed contributed to this effect; however, we as well as others report similar observations - reduced weight gain associated with the stressed group only - when both control and CMS groups are deprived as part of the behavioural protocol (Baker, Kentner, Konkle, & Bielajew, 2002; D'Aquila et al., 2000b; Harris et al., 1998, Hennessy, Heybach, Vernikos, and Levine, 1979; Pacak, Palkovits, Kvetnansky, Yadid, Kopin, and Goldstein, 1995; Willner et al., 1992). Finally, an increase in defecation has been reported following acute stress (Castagliuolo et al., 1996; Ikeda, Miyata, Orita, Kubota, Yamada, and Tomioka, 1995; Morrow and Garrick, 1997). To our knowledge there have been no reports on the effects of CMS on fecal output. Although in this study defecation was typically greater in the Long Evans animals, the CMS manipulation did not increase total output. If anything, CMS exposure produced a slight decrease overall in the Long Evans strain. Perhaps any immediate change in colonic motility is restored over the duration of CMS exposure. Thus, using gross physiological measures as markers of stress reactivity does not appear to have adequate sensitivity, at least in the case of a chronic regime of mild stressors.

4.2 Biochemical measures

Given the difficulty in producing any sizable behavioural modifications with CMS (Lin et al., 2002; Nielsen, 2000; Willner, 1997), we investigated whether biochemical indices would be more sensitive to this manipulation. We do report a significant increase in corticosterone levels from CMS exposure (before the application of an acute stressor), and a strain

difference in the interaction between acute stress reactivity and previous stress experience.

Rodent strain differences in sensitivity to stress are prominent in the literature (Dhabar et al., 1997; Gómez, Lahmame, de Kloet, & Armario, 1996; Sarrieau, Chaouloff, Lemaire, & Mormede, 1998; Shanks, Griffiths, Zalcman, Zacharko, & Anisman, 1990) and the failure to consistently observe CMS related corticosterone elevations may be related to genetic differences. Chronic mild stress does not increase corticosterone secretion in either Sprague-Dawley or Lister rats (Harris et al., 1998; Willner et al., 1987), while such an effect has been detected in the Flinders lines of rats (Ayensu et al., 1995). Results are inconclusive in the Wistar strain, one group showing no difference in corticosterone levels following three weeks of stressors (Stout et al., 2000), and another reporting increases in corticosterone levels following a three but not six week administration regime (Harris et al., 1998). The mild nature of the stressors (low intensity) may also contribute to the subtleness of the HPA axis response. A few investigators have demonstrated a correlation of stressor intensity with corticosterone levels (Hennessy et al., 1979; Odio & Maickel, 1985; Pacak et al., 1995). It is possible that strain differences in susceptibility to stress interact with the low intensity of the stressors associated with this paradigm to yield CMS effects discernable only in the Long Evans strain. Caution must be taken when interpreting these data as stressor adaptation capability has been shown to differ between strains (Dhabar et al., 1997).

To our knowledge, there are no reports in the literature of the potential effects of previous exposure to CMS on an animal's endocrine response to an acute insult. In this study, all animals exhibited the usual quadratic pattern (Dhabar et al., 1997; Garcia et al., 2000)- an initial rise in corticosterone level with maximum values observed between 15 and 30 min

following the stressor, after which the levels gradually decreased to near basal values. Of interest is the fact that in the Sprague-Dawley rats, the differences between the groups were negligible, while in the Long Evans strain, they were substantial. Even at time 0, Long Evans rats in the CMS group displayed corticosterone levels that were almost twice that observed in the equivalent Sprague-Dawley group and about four times its own control group (see insets of Figure 1). In fact, viewed in this way, the differences between the control and CMS groups over time are roughly proportional to their baseline difference. For example, at time 15, the CMS associated level increased by about 550% while the data from the control group were elevated by 2200%, a difference of four times. (Note that the graphs display differences from baseline following exposure to the acute stressor rather than the absolute corticosterone values). One explanation that would fit this pattern is that the corticosterone levels in Long Evans animals are reaching ceiling values, preventing the expression of acute stressor induced effects between the two groups. While we find no literature to support this idea, given strain differences in biochemical profiles, the possibility that our data reflect a scaling artifact should be acknowledged. The other argument, that in the Long Evans strain, these differences reflect the reduced sensitivity to an acute stressor following a chronic history of stressor exposure, is dealt with in the next section.

One concern with the time course data expressed as percent change from baseline is the fact that initial values, in absolute terms, between the CMS and control groups (time 0) were different; values for control groups were roughly one quarter that of treatment groups. Consequently we also evaluated group differences on the basis of absolute corticosterone levels; the data are represented in this manner in Figure 5. Viewed this way, we observed no

Insert Figure 5 about here

difference between control and CMS Long Evans rats in their reactivity to an acute stressor. However, a strain difference persists in that Sprague-Dawley rats exposed to CMS appear to be more reactive to the acute challenge than their control counterparts, indicating that the CMS experience facilitates the HPA response to a novel stressor. Of interest is the fact that six weeks of stress experience does not elicit the same response, that is both Spague-Dawley and Long Evans male and female rats fail to display any interaction between CMS and exposure to a novel stressor (Konkle, Baker, Kentner, Bielajew, 2002). The behavioural data comparing the effects of CMS on both strains of male rats provide further support for this gender difference, with increased stress vulnerability in Long Evans rats; CMS only produced a deficit in sucrose intake in Long Evans male rats (Konkle, Kentner et al., Submitted) while the effects of six weeks of stress had a greater impact on activity levels in this strain (Bielajew, Konkle, Stewart, Hutchins, Santa-Maria Barbagallo, and Kentner, Submitted).

4.3 Genetic factors

The view that strain differences contribute to stress susceptibility is perhaps a more parsimonious explanation for the patterns observed in our data. If the data in Figure 1 reflect genuine changes in corticosterone levels, then one interpretation of the pattern is that Long Evans rats adapt more readily to acute stress following prolonged exposure to a variety of mild stressors, relative to Sprague-Dawley rats that have been similarly treated. Others have also reported strain differences in comparing Sprague-Dawley, Fischer 344, and Lewis rats

(Dhabar et al., 1997). Using a somewhat different protocol, this group found that restraint stress administered three times over 10 days gave rise to significantly different corticosterone levels across strains. In addition, adaptation to the stressor was highest in the Lewis rats and the lowest in the Fischer 344 rats (Dhabar et al., 1997). These data and that of others (Dhabar et al., 1997; Gómez et al., 1996; Shanks et al., 1990) indicate that different strains are characterized by unique stress responses, at least as interpreted by corticosterone levels, and that these are differentially affected by previous stress experience.

Strain differences have been reported employing the CMS paradigm using behavioural measures - sucrose intake and/or preference (reviewed in Willner, 1997) and brain-stimulation reward (Nielsen et al., 2000) to characterize the effects of CMS. For example, one group (Valverde et al., 1997), found a reduction of about 30% in sucrose consumption in Long Evans male rats following CMS while another (Dunčko, Brtko, Kvetnanský, & Jezová, 2001; Dunčko, Kiss et al., 2001) observed a 50% decrease in sucrose consumption in Sprague-Dawley rats after the CMS experience. Note that the first study (Valverde et al., 1997) did not express consumption as a function of body weight. If our interpretation that Long Evans rats are adapting more readily to the acute stressor as a result of their CMS experience is valid, then we would predict a similar pattern, sucrose consumption closer to control levels in the case of Long Evans but not Sprague-Dawley rats. Using brain stimulation thresholds as a measure of CMS induced anhedonia, Nielsen et al. (2000) found, unexpectedly, minor improvements in the thresholds associated with PVG hooded but not Wistar rats. However, when the values of individual animals were examined, most of the Wistar rats showed no threshold change over time while in two animals, there were sizable

increases, suggesting that individual variability as well as strain difference needs to be examined in such studies.

To date, this paradigm has mostly been exploited for its macro-environmental manipulations; a richer picture may emerge when genetic factors are taken into account, such as animal strain, as well as the consideration of individual differences in susceptibility. Indeed, same-strain animals are now being bred for differences in their behavioural repertoire, for example, rats that are either high or low in motor activity in the forced swim test (Weiss, Cierpial, and West, 1998), or rats that are bred for their susceptibility to amygdala kindling (Slow vs Fast) which is correlated with differences in their reactivity to stressors (McIntyre, Kent, Hayley, Merali, & Anisman, 1999). In order for a paradigm to properly model a human disorder, it would seem reasonable to take into account the spectrum of human differences. The level of stress reactivity between and within different strains as well as gender (Palanza, 2001) should be considered when assessing stress susceptibility.

Table 1.
Schedule of chronic mild stressors administered over a seven day period and repeated for three weeks.

	Monday	Tuesday	Wednesday	Thursday	Friday
0800 am	Confinement 1 h	Novel cage 1 h	Restricted food 2 h	Exposure to empty bottle 1 h	Novel cage 1 h
Noon		Confinement 1 h		Confinement 1 h	Confinement 1 h
0200 pm	Confinement 1 h	Confinement 1 h	Confinement 1 h	Confinement 1 h	
0400 pm	Overnight illumination	Overnight food & water deprivation	Overnight water deprivation	Overnight pairing	Reversed Light/Dark cycle over weekend

Study 2.

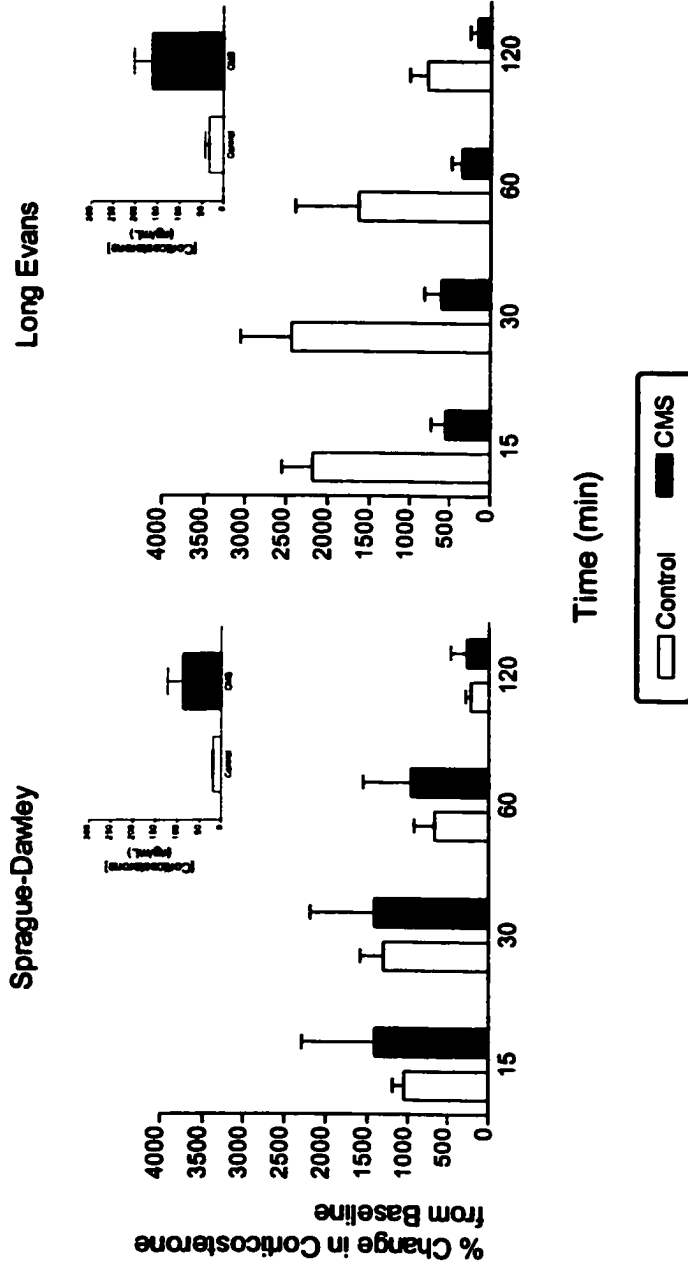


Figure 1. The large graphs depict the average change in percentage \pm standard error of the mean from baseline corticosterone level (time 0) across time for each strain. The small graphs show the actual corticosterone value \pm standard error of the mean (ng/dL) that was obtained for each group at time 0. The unfilled bars represent the control (n=11) groups and the hatched bars, the CMS (n=10) groups. Time 0 represents the level obtained immediately following three weeks of CMS treatment after which an acute stressor was administered and the corticosterone levels evaluated at times 15-120 min.

Study 2.

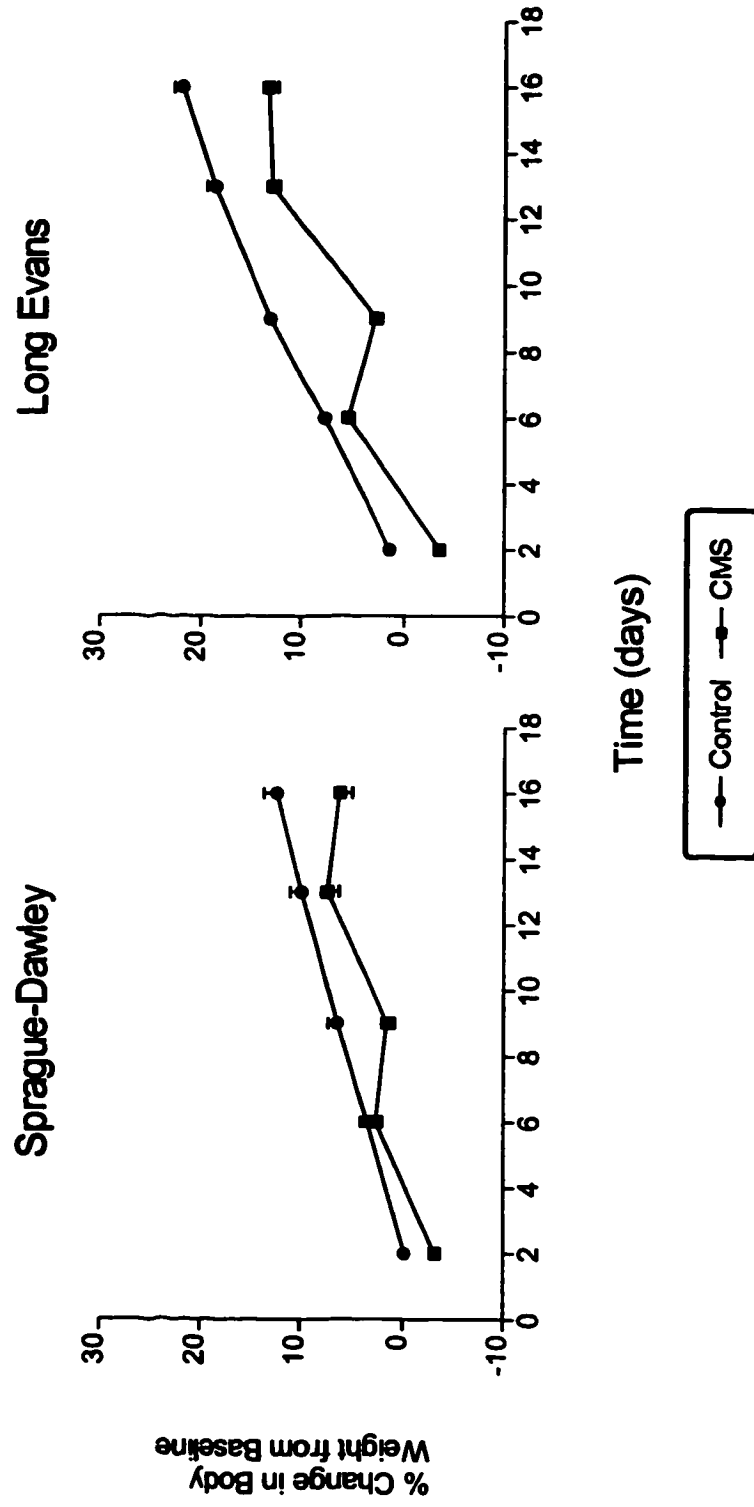


Figure 2. The percent change in body weight over time for each group (control, n=11 and CMS, n=10) with the data obtained from the Sprague-Dawley rats on the left and the Long Evans on the right. Error bars represent standard errors of the mean. Before the start of the CMS treatment, weights were monitored for several days.

Study 2.

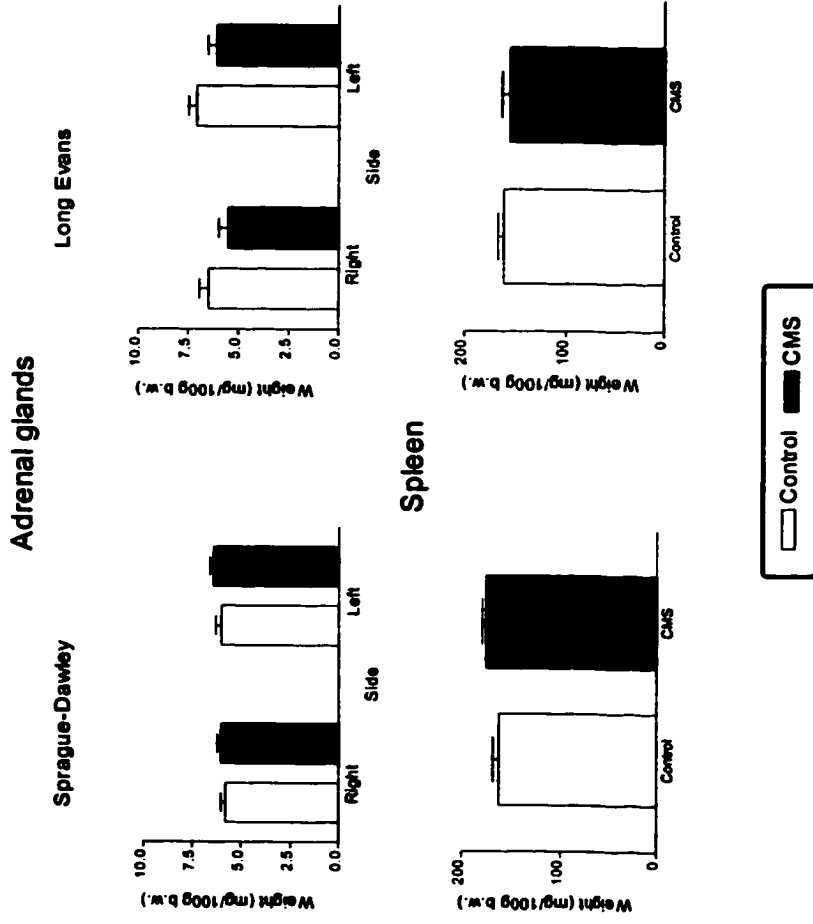


Figure 3. The left (Sprague-Dawley) and right (Long Evans) panels represent the data from each strain. In the top panel is shown the left and right adrenal gland weights expressed as the adrenal wet weight in mg per 100 g of body weight. The bottom plots represent the spleen weights (wet weight in mg per 100 g of body weight) of each group. The groups are identified at the bottom of the graph. 94

Study 2.

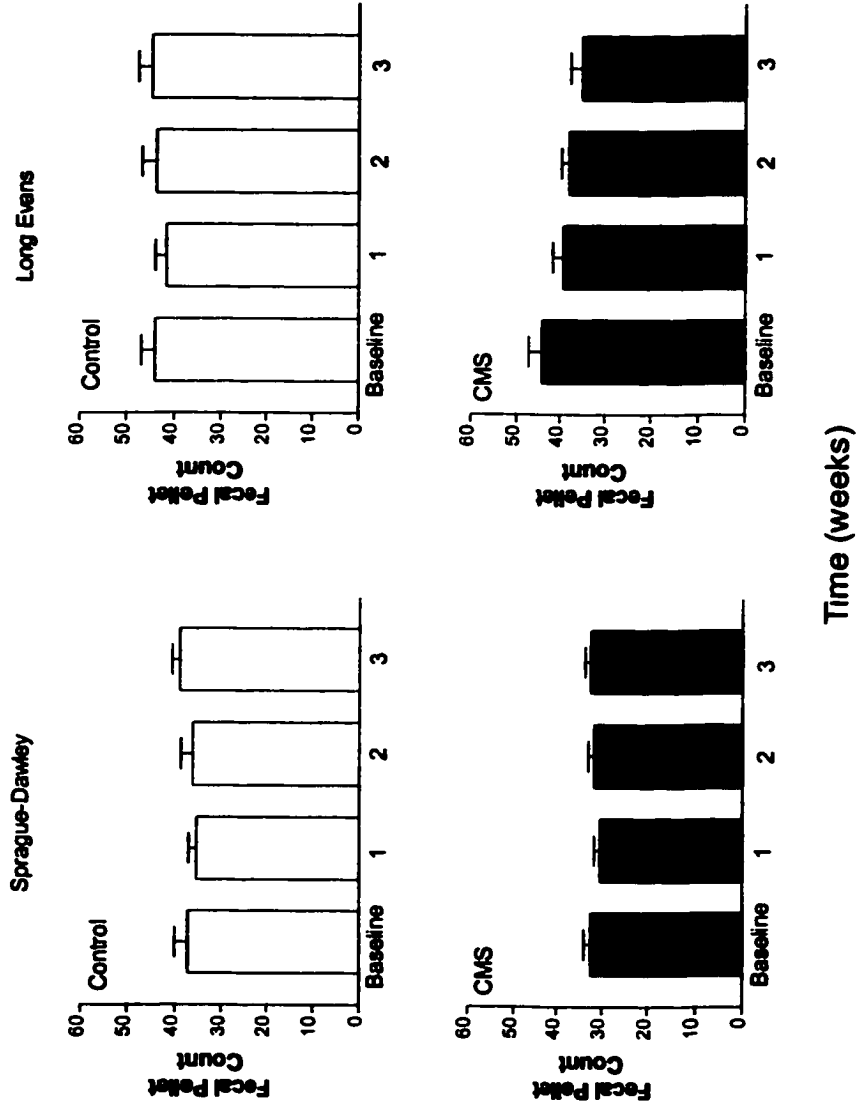


Figure 4. The left and right panels represent the data from each strain, Sprague-Dawley and Long Evans, respectively. From top to bottom are shown the average fecal pellet count \pm standard error of the mean across animals for a 24 hour period during the week preceding and each week of the treatment phase in the control and CMS groups.

Study 2.

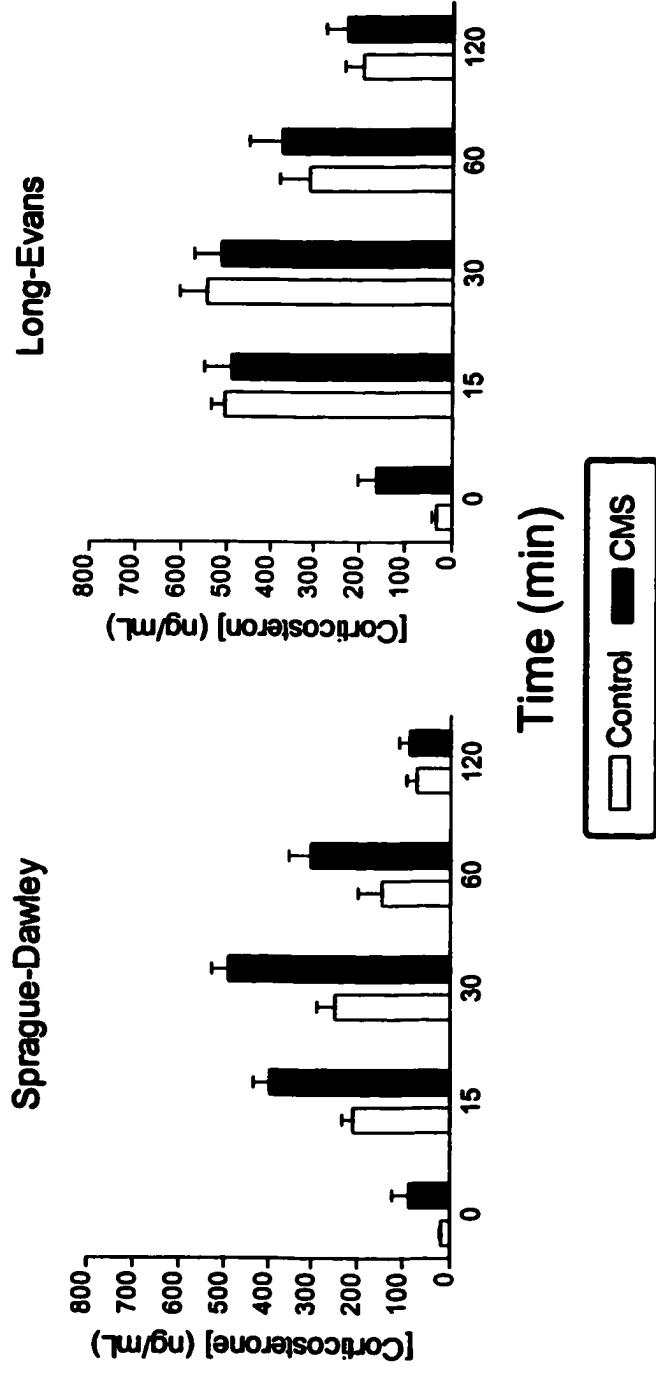


Figure 5. Average corticosterone levels \pm standard error of the mean (ng/mL) per group, in two female outbred rat strains. The Sprague-Dawley related data appear on the left half and that of the Long Evans rats on the right half of the figure. Time 0 represents the corticosterone levels obtained immediately following the six weeks of treatment after which an acute stressor was administered and the corticosterone levels evaluated at times 0-120 min.

Study 3

THE EFFECTS OF CHRONIC MILD STRESS ON MALE SPRAGUE-DAWLEY AND LONG EVANS RATS:

II. COMPARISON OF BEHAVIORAL MEASURES OF ANHEDONIA

**Submitted to Behavioral Neuroscience as: Konkle, A.T.M., Kentner, A.C., Baker, S.L., Bielajew, C.,
Fouriezos, G., and Merali, Z. The effects of chronic mild stress on male Sprague-Dawley and Long Evans
rats: II. Self-stimulation thresholds and sucrose preference measures of anhedonia.**

Abstract

The chronic mild stress (CMS) paradigm has been validated on numerous occasions, but efforts to replicate its findings have been problematic. To address this issue, we evaluated the anhedonic changes in both sucrose consumption and brain-stimulation reward, the two behaviours typically employed in such studies, during a six week regime of CMS in Sprague-Dawley (SD) and Long Evans (LE) male rats. Physiological (body weight, adrenal and spleen weight) and biochemical (plasma corticosterone levels) indices were also assessed. While no behavioural effects of the stressors were evident in SD animals, we found a significant short-lived reduction in 1h sucrose consumption in LE rats, that was less evident when the intake was monitored over 24h. Sucrose preference was not altered in either strain. Physiological and biochemical measures yielded some notable strain differences in stress reactivity. Weight gain was markedly reduced in stressed SD rats and there was an attenuated corticosterone response to an acute stressor in LE rats. These results underscore the importance of strain differences in studies employing the CMS paradigm.

1. Introduction

In humans, exposure to unpredictable stressors over an extended duration has long been thought to be associated with the development of depressive symptomatology (Anisman and Zacharko, 1982; Kendler et al., 1995; Kessler, 1997). Based on this premise, paradigms employing a variety of chronic and unpredictable mild stressors have been developed for use in animal studies in order to produce a pattern of behaviours that resembles human depression. Although the focus of early studies was to demonstrate the effectiveness of anti-depressant agents in restoring impaired activation induced by stress exposure (Katz and Sibel, 1982; Roth and Katz, 1981), appetitive related deficits were also observed. For example, Katz (1982) demonstrated substantial reductions in sucrose consumption, as an index of anhedonia, in animals chronically exposed to severe stressors. Building on this theme, Willner et al. (1987) designed a procedure that consisted of the chronic delivery of a variety of mild stressors, presented in random sequence, intended to model more specifically the development of human melancholic behaviour, or a decreased responsiveness to pleasurable stimuli. Retaining the chronicity but weakening the intensity of the stressors was shown to result in not only a generalized decrease in reward reactivity, but gave rise to many of the same symptoms - weight loss, abnormal sleep patterns, reduced locomotor activity, and endocrine disturbances - associated with a major depressive disorder. There are a variety of measures used to assess changes in hedonic state as a consequence of chronic mild stressors (CMS), including intake and preference of a mild sucrose solution (Willner et al., 1987) and thresholds for brain-stimulation reward (BSR) (Moreau et al., 1992). Although there is little issue with the validating criteria underlying the CMS paradigm, replicability of the

behavioural alterations induced by the protocol has not been consistent.

Consumption of a mild sucrose solution has been reported to be decreased with CMS exposure and restored with the concurrent administration of antidepressant compounds (Benelli et al., 1999; Cheeta et al., 1994; Dziedzicka-Wasylewska et al., 1997; Genedani et al., 2001; Grippo et al., 2002; Luo and Tan, 2001; Willner, et al., 1987). Others have found a reduction in sucrose preference, electing to use this transformation of the intake measure as it takes into account total liquid consumption (Benelli, et al., 1999; Dunčko, Brtko, et al. 2001; Dunčko, Kiss et al., 2001). However, reports of failure to show a decrease in either consumption or preference for a weakly sweet solution have also surfaced (D'Aquila, Newton, & Willner, 1997; Nielsen et al., 2000). In our hands, we have observed strain differences in female rats as a consequence of CMS exposure on measures of sucrose intake and preference (Baker et al., 2002). Stress appears to inhibit the increase in the 1h sucrose intake measure that is observed in singly housed control Sprague-Dawley females, while it decreases sucrose intake and preference in the 24h measure in the same strain. However, the Long Evans CMS-treated females only show a slight reduction in 1h intake versus their control counterparts.

The results of studies using BSR as an index of anhedonia have proven to be no less discordant. While one group has consistently reported an increase in frequency thresholds following repeated stressor exposure (Moreau, et al., 1992; Moreau et al., 1994; Moreau et al., 1996; Moreau, Bourson et al., 1994; Stout et al., 2000), others have shown a slight facilitation in thresholds (Lin et al., 2002). Nielsen et al. (2000) report no effect in group thresholds and only a slight increase in a subgroup of two of these animals. We have been

unable to observe any change in frequency threshold after one to six weeks of CMS exposure in two strains of female rats, Sprague-Dawley and Long Evans (Kentner, Baker, Konkle, Fouriez, & Bielajew, 2002).

Our failure to observe any notable behavioural changes as a consequence of CMS led us to investigate whether the chronic manipulations would evoke an increased endocrine response typically associated with stressor application. We (Bielajew, Konkle, & Merali, 2002) have recently reported elevated corticosterone levels in CMS animals versus their control counterparts following a three week stressor regime. Furthermore, in that study, application of a subsequent severe acute stressor yielded a strain difference in the corticosterone response with Sprague-Dawley associated values greater than that observed in Long Evans rats. Rate of weight gain in CMS groups was significantly reduced in both strains.

In the present paper, we continue our exploration of genetic factors in stress susceptibility, expanding the scope to include a lengthier stress regime of six weeks and assessments of behavioural, physiological, and biochemical responses in two male outbred rat strains - Sprague-Dawley and Long Evans. Frequency thresholds for BSR are reported in the first study and sucrose intake and preference for a 1 hour and a 24 hour test in the second study.

EXPERIMENT 1. CHANGES IN BRAIN STIMULATION-REWARD AS A MEASURE OF ANHEDONIA

2. Method

2.1 Subjects and Surgery

Twenty eight male rats (14 Long Evans and 14 Sprague-Dawley) weighing between 300-

505 g at the time of surgery were employed in this study. Rats were randomly assigned to either control or CMS groups. They were singly housed and maintained on a 12h light/12h dark cycle with lights on at 0700. Food and water were available freely. These conditions prevailed unless otherwise specified in the stressor schedule. Animals were weighed twice a week.

Stereotaxic surgery was carried out under continuous administration of the inhalant anesthetic halothane. Just prior to surgery, animals were given a subcutaneous injection of atropine sulfate in order to minimize respiratory distress. Bilateral electrodes were aimed at the level of the ventral tegmental area (VTA), using the coordinates: 4.8 mm posterior to bregma, 0.7 mm lateral to the midsagittal suture, and 8.4 mm below the skull surface reading at bregma (Paxinos and Watson, 1998). The electrodes were fashioned from stainless steel wire, 250 μm in diameter, and insulated with epoxylite or Formvar to the rounded tips; electrodes were made in house or purchased from Plastic Products. A fine stainless steel wire was wrapped around four stainless steel skull screws and served as the current return. The entire assembly was secured to the skull with dental acrylic.

2.2 Behavioural Tests

Following a minimum of 7 days recovery from surgery, the animals were trained to press a lever to obtain brain stimulation using traditional operant shaping techniques. Tests were conducted in a Plexiglas/wood cage, 27 cm deep X 37 cm wide X 51 cm high. A constant-current amplifier (Mundl, 1980) and an integrated pulse generator (built in-house) were used to supply the stimulation. Each depression of the lever resulted in the delivery of a 500 ms

train of rectangular cathodal pulses of 100 μ s duration; a reduced train duration of 300 ms was employed in one rat due to unstable responding at the higher value. The lowest current and pulse number to elicit minimum responding of approximately 30 bar presses/minute and avoid severe motor artifacts were determined in individual animals. The stimulation parameters were continuously monitored on an oscilloscope by reading the voltage drop across a 1 k Ω precision resistor in series with the animal.

Once reliable bar responding was established, the testing protocol was begun. A descending method of limits was employed to produce rate-number functions. These were obtained using a constant current and decreasing the pulse number by 0.1 \log_{10} units at the beginning of each 60 sec trial from a value that gave rise to near maximal responses to one that yielded little or no response. The beginning of each trial was signaled by three priming stimulations, set at the same stimulation parameters as the subsequent 60 s trial. The number threshold was obtained by interpolation of the rate-number curve and was defined as the number of pulses corresponding to half of the maximum response rate. Four rate-number curves were generated per session, the first of which was considered a warm-up and not retained. Thus, an average threshold was calculated at each session, based on three rate-number functions. The animal was deemed stable when the average rate-number threshold did not differ by more than 0.1 \log_{10} units on three consecutive test days. A baseline session was conducted a few days prior to commencing delivery of the stressors; tests were conducted twice a week during the CMS procedure. Each number threshold was converted to a frequency threshold or the number of required pulses per second (Hz) and presented as such in this paper; all analyses were conducted on this measure.

The CMS schedule is presented in Table 1. Following the six weeks of its administration,

Insert Table 1 about here

the animals were given a lethal dose of sodium pentobarbital and perfused intracardially with 0.9% saline followed by sucrose-buffered 10% formalin. The brains were removed and stored in sucrose-buffered 10% formalin until sectioned. The tissue was cut in 40 μ m sections, floated onto gel-covered slides, and stained with cresyl violet to locate the electrode tips (Paxinos and Watson, 1998).

2.3 Statistical Analyses

The maximum rate and frequency threshold data were analyzed separately by strain using a mixed ANCOVA design. There was one repeated factor, time (12 levels), and one independent factor, group (control vs CMS) with the baseline measure employed as a covariate. The weight data were similarly analyzed; although weight was chronicled bi-weekly, we excluded from the analysis those time points that were taken on the day following food deprivation. Thus only six time points for weight are reported. Finally, a two-way ANOVA was applied to the baseline weight data to determine if there were differences between groups and strain. The Greenhouse-Geisser or Hundt-Felt correction for violations to the assumption of sphericity was applied to all effects associated with the repeated measure (Howell, 2002).

Given that the minimum current necessary to elicit BSR differed between animals and the fact that one animal was tested at a lower train duration than the others (300 ms instead of 500 ms), we elected to calculate the total charge value associated with each threshold

determination, allowing comparison of the total amount of stimulation delivered across animals. A mean charge value was calculated for each animal using the equation:

$$Q = INd$$

where Q is the charge in μC , I is the current in μA , N is the required number of pulses in the stimulation train, and d is the pulse duration in s (Gallistel, 1978). Statistical analysis of these data was carried out in the same manner as that for the rate and threshold values.

3. Results

Histological analyses verified all electrode tips to the medial forebrain bundle, from the posterior hypothalamus to the ventral tegmental area.

A summary of the statistical results are shown in Table 2, with those pertinent to the

Insert Table 2 about here

BSR groups reported on the top half of the Table; only significant values are reported. Note that in order to facilitate data presentation, the values in the associated figures are expressed as percent change from baseline; however, all analyses were conducted on the raw data.

Figure 1 shows the average weight change across animals for each group. As expected,

Insert Figure 1 about here

weights in both strains were significantly elevated over the course of the six week treatment phase. However, group and interaction differences were only obtained in the Sprague-Dawley animals. The analysis of baseline weights alone indicated no dissimilarities between groups, strain, or their interaction.

The top portion of Figure 3 depicts the average maximum response rate expressed as a

Insert Figure 2 about here

change from the maximum baseline rate over the 12 testing sessions. These were not affected by the CMS manipulation. The bottom portion of this figure shows the percent change from baseline in BSR frequency thresholds for each group. In the Long Evans animals there was a tendency for the thresholds associated with the CMS group to drift towards lower values thereby giving rise to a significant difference relative to the control group. Indeed in both strains, CMS group thresholds were systematically less than that of the control groups.

The minimum currents necessary to elicit stable bar pressing ranged from 200-630 μA across animals. These produced overall charge values in Sprague-Dawley rats of 0.11-0.72 μC and 0.24-1.22 μC for the control and CMS groups respectively, while for the other strain, we calculated charge values of 0.21-1.27 μC for the control and 0.19-1.31 μC for the CMS animals. The same pattern of statistical results was obtained as that for the threshold data.

EXPERIMENT 2. CHANGES IN SUCROSE CONSUMPTION AND PREFERENCE AS A MEASURE OF ANHEDONIA

4. Method

4.1 Subjects

The subjects were 16 Sprague-Dawley and 16 Long Evans male rats (Charles River Laboratories) weighing between 297-582 g at the start of baseline data collection. Housing conditions were the same as those described in Experiment 1.

4.2 Procedure

Body weight was chronicled weekly. Animals of each strain were assigned to one of two groups - Control (n=16) or CMS (n=16). See Table 3 for the schedule of stressors employed.

Insert Table 3 about here

4.2.1 Behavioural tests

Animals were first acclimatized to a two bottle test paradigm in which a 1% sucrose solution was made available in one bottle and water in the other. Following that, all groups were food and water deprived the day before the test was conducted; deprivation was used to ensure that the animals would drink during the one hour test (Willner, 1997). The two bottles were then reintroduced; sucrose and water intake were measured 1-h and 24-h later.

Preference for sucrose was obtained by calculating the ratio of sucrose/ total fluid intake.

This test was conducted five times over several weeks in order to obtain baseline intake and preference measures. During CMS exposure, these tests were administered weekly for the duration of the experiment (six weeks).

4.2.2 Blood Sampling

At the end of the six weeks of CMS, tail blood droplets were collected on a Schleicher and Schuell® filter paper. A first sample was acquired after which the animals were subjected to a 10 min restraint stress. Blood samples were then taken 15, 30, 60, and 120 min following the onset of the stressor. Animals were selected from alternate groups in order to minimize the effects of the circadian rhythm on the results. Adrenal glands and spleen were dissected

the following day at the time of sacrifice.

4.2.3 Corticosterone Assay

Blood samples were eluted from the filter paper by placing one 3.2 mm punch in a glass tube and adding 100 μ L of Dulbecco's phosphate buffered saline (containing 0.1% gelatin) (Wortham and Stallings, 1997) to each tube. These were shaken for 1 hour at 50 rpm at room temperature, refrigerated overnight, and shaken for an additional hour the following morning.

Plasma corticosterone was determined by radioimmunoassay using a commercially available reagent kit (ICN Biomedicals, Costa Mesa, CA). The samples were all run at one time so as to eliminate any inter-assay variability, while the intra-assay variability was <10%. Total corticosterone concentration was determined using the methods described by Wortham and Stallings (1997).

4.3 Statistical Analyses

All analyses were conducted separately for each strain (Statistica, 1998). A mixed ANCOVA design was employed for the analysis of body weight and the sucrose intake and preference data. Time was the repeated factor, group the independent one, and body weight served as the running covariate for the sucrose data while baseline weight served as the fixed covariate for the analysis of body weight. Baseline differences in body weight between group and strain were assessed separately using a two-way ANOVA design. Adrenal gland weights were analyzed using a mixed ANCOVA design with factors group (independent), side

(repeated) and body weight (covariate) at the time of sacrifice. Likewise, body weight was employed as a covariate in the analysis of spleen weight. A comparison of the corticosterone levels between the control and CMS treatments at time 0 was achieved using an independent t-test. The endocrine data pertaining to times 0-120 min were analyzed via a mixed ANOVA design with group (control vs CMS) the independent factor and time (0, 15, 30, 60, and 120) repeated one. The entire profile (0 to 120 min) for each group was subjected to trend analysis. Greenhouse-Geisser or Hundt-Felt correction was applied where appropriate - repeated factors with more than two levels (Howell, 2002)

5. Results

A summary of the significant statistical findings associated with the data obtained in Experiment 2 (sucrose tests) is shown on the bottom half of Table 2.

The analyses of body weight, data shown at the top of Figure 3, yielded treatment and time

Insert Figure 3 about here

effects in the case of both strains; only the Sprague-Dawley data included a significant interaction. The middle of Figure 3 shows the adrenal gland weights for each group and strain. A side difference was revealed in the Sprague-Dawley animals while a treatment effect was found in the Long Evans strain; in all cases, CMS related weights were greater than that of control animals. No differences in spleen weights were obtained; these data appear on the bottom of Figure 3.

The 1h sucrose intake (top) and preference (bottom) data are shown in Figure 4. Analyses

Insert Figure 4 about here

of the data revealed no statistical effects in the Sprague-Dawley animals, whereas in the Long Evans rats, we found a significant effect of time and an interaction between group and time for the 1h intake measure. With respect to the 1 hr preference data, the tendency for preference to gradually increase over the course of the study in the Long Evans groups resulted in a significant time effect.

Analyses of the 24 h sucrose intake data, shown at the top of Figure 5, yielded an effect of

Insert Figure 5 about here

time and a group/time interaction in Long Evans animals and no significant findings in Sprague-Dawley rats. The preference data associated with the 24h sucrose intake only gave rise to a significant effect of time in Long Evans animals.

Figure 6 depicts the corticosterone profiles for both rat strains. Statistical analyses

Insert Figure 6 about here

revealed a significant group difference at time 0 exclusively in the Long Evans strain. Further analyses of the changes in corticosterone levels following the acute restraint stress revealed a time effect in both strains of rats. The quadratic trend in the corticosterone response from time 0 to 120 min, typical of such data (Dhabar et al., 1997; Garcia et al., 2000), was significant in all groups.

6. Discussion

The aim of these two studies was to evaluate the physiological, biochemical, and behavioural consequences of CMS in two male outbred rat strains. The findings indicate that

the two strains are differentially affected by the six week regime of stressors as indexed by the rate of weight gain and plasma corticosterone levels. The results of the behavioural measures employed - sucrose and BSR tests - were less impressive in distinguishing the effects of CMS; reasons for this are discussed below, including the possibility that different rat strains are a contributing factor.

6.1 Methodological Issues

6.1.1 Sucrose consumption

Chronic mild stress studies that evaluate changes in sucrose intake as a measure of anhedonia typically administer a low concentration solution (0.7-2.0 %) for 1h following a period of food and water deprivation. Even though deprivation prior to the test ensures some degree of intake, a concern that has been expressed (Matthew et al., 1995) is that the 1h response measures both metabolic and hedonic capacity, thereby making it difficult to isolate treatment differences. Some investigators have instead monitored 24 h intake in order to bypass the immediate effects of deprivation as a potential confound (Ayensu et al., 1995; Hatcher et al., 1997).

Another concern is the observation that sucrose consumption increases in unstressed or control animals (Katz, 1982; Kioukia et al., 2000; Willner et al., 1987). Thus, differences in sucrose intake between treated and untreated groups may result from a failure to increase sucrose intake in stressed animals. Bearing these issues, it has been recommended that preference for a sucrose solution rather than its intake be evaluated (Grote and Brown, 1971). This takes into account the total amount of fluid consumed (water and sucrose) and may

allow for a better distinction between alterations in drinking related to physiological thirst versus those associated with the hedonic properties of the solution. Some investigators employ the two-bottle procedure, measuring amount of both water and sucrose consumed (D'Aquila, Newton et al., 1997; Dunčko, Kiss et al., 2001; Grippo et al., 2002; Hatcher et al., 1997), while others more simply monitor water intake periodically during the experiment (Muscat et al., 1992; Willner et al., 1994). Finally, there are differences across studies in terms of the frequency of behavioural tests during the CMS administration phase. For example, some studies report the results of weekly measures (Benelli et al., 1999; Nielsen et al., 2000; Papp, Nalepa, Antkiewicz-Michaluk, Sánchez, 2002); others report the results associated with one or two time points (Ayensu et al., 1995; Dunčko, Kiss et al., 2001). In order to avoid these problems but still have comparable data, we compulsively included all of these measures in our experiment - weekly assessments of 1 and 24h sucrose intake and preference. Furthermore, all analyses of the data pertaining to sucrose intake were conducted with body weight as a covariate; because food deprivation can alter the rate of weight gain, it has been suggested that this factor is related to reductions in sucrose intake (Matthews et al., 1995).

In the present study, whereas no difference in 1 and 24h sucrose intake was observed between Sprague-Dawley groups (CMS & control), we found a group-related difference in both of these measures in the Long Evans animals. An initial decrease in 1h intake was observed after one week of CMS in this strain; however, this returned to near basal levels by the fourth week of CMS administration. No clear pattern was apparent for the 24h sucrose intake measure. The lack of a significant difference between control and CMS Sprague-

Dawley animals appears to be related to the fact that both groups decreased their sucrose consumption, at least initially, which may have been associated with the food deprivation condition. It has been reported that CMS induces a greater reduction in 1h sucrose intake when measured during the dark versus the light phase of the rat's diurnal cycle (D'Aquila, Newton et al., 1997). The 24h measure employed here reflects intake during both of these and as a result is not specifically influenced by the animal's activity level.

Expressing the same data as preference for sucrose solution negated the group/time interaction that was obtained for both intake measures; this transformation shows that both sucrose and water intake are similarly affected in both strains whether monitored for 1 or 24h. Others have reported either no effect (D'Aquila, Newton et al., 1997; Matthews et al., 1995) or slight reductions in 1h preference (Benelli et al., 1999; D'Aquila, Newton et al., 1997; Dunčko, Brtko et al., 2001; Dunčko, Kiss et al., 2001; Grippo et al., 2002; Kopp, Vogel, Rettori, Delagrange, and Misslin, 1999; Willner et al., 1987), findings that are sometimes limited to specific time points in the experiment. A recent study explored individual differences in spontaneous sucrose consumption in rats (Brennan, Roberts, Anisman, and Merali, 2001) and found that animals that consume low levels of solid sucrose at baseline - Low group - typically earned less reinforcements than the High group when performance for sucrose was evaluated using the progressive ratio method. However, the break-point in progressive ratio performance, a measure akin to threshold, was similar in both groups (Brennan, et al., 2001). When 1h sucrose preference was evaluated following a regime of CMS, a comparison of the baseline consumption levels across studies revealed no difference (Baker et al., 2002; D'Aquila, Newton et al., 1997; Dunčko, Kiss, et al., 2001; Kopp et al.,

1999; Grippo et al., 2002; Willner et al., 1987). This suggests that the effects of CMS on preference that are reported in some studies are not related to the animals' baseline preference levels. In order to properly address this concern in our own work, the profiles of individual animals were evaluated and the differences in sucrose preference over time were found to be unrelated to baseline preference levels which ranged from roughly 40 to 90% across groups and strains. Thus, pre-treatment preference levels, regardless of their magnitude, do not appear to interact with the effects of CMS.

6.1.2 Brain-stimulation reward

Brain-stimulation reward has also been explored as a behavioural endpoint of CMS. Even though this method is more laborious than the sucrose consumption test, its appeal lies in the assessment of CMS effects via a measure related to the direct activation of the substrate underlying reward pathways, thus avoiding the potential confound of weight loss related to food deprivation (Forbes, Stewart, Matthews, and Reid, 1996). The results of a recent study have shown that chronic mild food deprivation fails to alter thresholds for BSR (Lin et al., 2002), providing support for the contention that deprivation is not a confound when changes in BSR thresholds are used as an index of hedonic changes.

Stressors, in the form of acute and severe footshock, have been shown to increase current thresholds for BSR in mice (Bowers, Zacharko, and Anisman, 1987; Zacharko et al., 1990). However, the consequences of mild stressors on thresholds for BSR have been less consistent. One group has reliably shown an increase in frequency thresholds during CMS exposure, interpreted as a decrease in the rewarding value of the stimulation, followed by a

gradual return to near basal levels once the stressors are discontinued (Moreau et al., 1992; Moreau, Borgulya et al., 1994; Moreau, Bourson et al., 1994; Moreau, et al., 1996). We and others have been unable to replicate this effect (Kentner et al., 2002; Lin et al., 2002; Nielsen, et al., 2000). In the present study we show no effect of CMS on thresholds and charge values for BSR in Sprague-Dawley rats and a slight if significant facilitation in the Long Evans strain. Furthermore, we obtain no effect when the identical procedure is applied to the same strains of female rats (Kentner et al., 2002). Others have also reported either no effects (Nielsen et al., 2000- male Wistar rats) or a slight decrease in frequency (Lin et al., 2002) or current (Nielsen et al., 2000) thresholds at certain time points during the chronic stress procedure (male Wistar and PVG rats). However, Nielsen et al. (2000) did find increases in thresholds for BSR in a subgroup of animals, two Wistar and one PVG rat. Thus inspired, we examined the thresholds in individual animals but observed no such distinction. The profile of frequency thresholds for the grouped data presented in the bottom portion of Figure 2 does not typify that observed for all animals. In Figure 7, we show the thresholds

Insert Figure 7 about here

over time of two CMS animals of each strain, one in which thresholds were stable during the stress administration period (9/14 rats) and a second in which thresholds gradually decreased (5/14 rats). A notable increase in thresholds was observed in one control animal of each strain.

In order to address a specific concern related to the scaling of thresholds for BSR (Bielajew, 1983), we re-calculated the thresholds for a few animals using a different procedure. The thresholds that are presented in this experiment represent the average of

three, one for every rate-frequency curve generated on each testing day. A second method of threshold determination involves first averaging the rate at each frequency for the three functions followed by interpolation of the threshold from this mean rate-frequency function; thresholds calculated with this method appear to be more sensitive to change when the shape of the individual rate-frequency curves differ (Bielajew, 1983). We show no deviation from our original findings when frequency thresholds are instead scaled in this manner, further supporting our claim that thresholds for BSR are relatively insensitive to CMS-elicited anhedonia. Furthermore, the small group difference that is reported in the Long Evans animals appears to be related to a pattern in which thresholds increase in the control and decrease in the CMS rats when the data are grouped. Although statistically significant, these small variations in thresholds over time, from 5-10%, are probably not meaningful. When thresholds are monitored over time in unchallenged animals, small variations of this nature are likewise observed (Konkle, Bielajew, Fouriez, & Thrasher, 2001). One group did report a difference in responsiveness to an acute stress challenge that was site dependent, with dorsal VTA stimulation more effective in producing threshold alterations than ventral sites (Zacharko et al, 1990). Our placements ranged were scattered along the medial forebrain bundle from the posterior lateral hypothalamus to the VTA. However, there was not enough variability in the threshold data to correlate these values with electrode location.

Taken together, the results of our behavioural tests suggest that neither measure is a powerful tool for assessing the anhedonic consequences of CMS. Slight variations in the CMS schedule do not appear to explain the failure to obtain reliable and threshold and/or sucrose differences between groups (Benelli et al., 1999; Moreau et al., 1992; Willner et al.,

1987). The importance of an interaction between genetic differences and stressor exposure in discerning the susceptibility of an animal to this environmental challenge is presented in the following section.

6.2 Genetic issues

Strain differences in behavioural reactivity to stress are well documented. Mouse strains differ in their escape performance in a shuttle apparatus following exposure to uncontrollable footshock (Shanks and Anisman, 1993) as well as in the magnitude and duration of a stress-induced reduction in the consumption of a highly palatable diet (Griffiths, Shanks, and Anisman, 1992). Wistar, Wistar-Kyoto, and Fischer rats have been shown to differ in their responses in two passive-avoidance tasks (Paré, 1993), tests used to assess behavioural inhibition. However, stress reactivity is also different between “normal” outbred strains. Faraday (2002) has recently shown a reduction in activity in the open field apparatus in female Sprague-Dawley but not Long Evans rats chronically exposed to immobilization stress, as well as an incongruous startle response between these two strains following stressor exposure, in both males and females.

In this paper, we report a strain difference in behavioural response to CMS on two measures of reward, with Long Evans rats slightly more reactive, at least in sucrose tests, than their Sprague-Dawley counterparts. A review of the CMS literature yielded very few studies that compare the effects of CMS between rodent strains. While one study shows a reduction in the consumption of a mildly sweet solution in both Flinders Sensitive and Resistant lines of rats exposed to CMS (Ayensu et al., 1995), another reports a decrease

exclusively in the Sensitive line (Pucilowski et al., 1993). A comparison of sucrose consumption in common rat strains revealed a decrease in preference in Sprague-Dawley rats, as well as a slight reduction in sucrose intake without a change in preference in the Wistar strain (Kioukia et al., 2000). Although the chronic stressors did not influence sucrose consumption in Wistar rats in a second study, PVG rats showed a slight deficit on this measure when compared to unstressed animals (Nielsen et al., 2000). In the same study, assessment of the effects of CMS on BSR thresholds yielded a slight facilitation in CMS animals in both strains in one instance and no effect in the Wistar animals in another (Nielsen et al., 2000). In our comparison of Sprague-Dawley and Long Evans female rats, we have observed a strain difference in sucrose measures (Baker et al., 2002), but no effect whatsoever on BSR thresholds (Kentner et al., 2002).

Harris et al. (1998) assessed plasma corticosterone concentrations as an index of the stress response and report elevated levels in Sprague-Dawley but not Wistar rats exposed to six weeks of stress; note that a three week stress regime did increase corticosterone levels in Wistar rats. In the present study, we also observed a strain difference in reactivity to six weeks of CMS, with Long Evans male rats appearing more vulnerable to the stressors than the Sprague-Dawley animals; a reverse trend was found in female rats (Konkle et al., 2002). However, we observed in another study elevated corticosterone levels in male rats of both of these strains following three weeks of stress exposure (Bielajew et al., 2002). Taken together, these findings suggest that Sprague-Dawley male rats habituated more quickly to the stress procedure than their Long Evans counterparts. However, note that both Sprague-Dawley and Long Evans male and female rats exposed to six weeks of CMS show a

corticosterone response to an acute stressor that is similar to that obtained in control animals. Following a three week CMS experience in male rats, a strain difference in reactivity to a subsequent acute stressor was observed; whereas the same response pattern in the absolute corticosterone levels was observed between CMS and control Long Evans rats, an enhanced response was reported in Sprague-Dawley rats (Bielajew et al., 2002).

Stress has also been shown to influence body weight and these effects appear to be strain and gender dependent (Burchfield et al., 1980; Dhabar et al., 1997; van Raaij et al., 1997). For example, Faraday (2002) has recently reported a greater effect of chronic restraint stress on weight gain in Long Evans versus Sprague-Dawley male rats but very little difference between female strains. Chronic application of the mild stressors has also typically altered the rate of weight gain (Barr and Phillips, 1998; Bielajew et al., 2002; Brotto et al., 2001; D'Aquila, Monleon et al., 1997; D'Aquila et al., 2000b; Dunčko, Kiss et al., 2001; Dziejzicka-Wasylewska et al., 1997; Harris et al., 1998; Harro et al., 1999; Hatcher et al., 1997; Matthews et al., 1995; Muscat et al., 1988; Sánchez and Papp, 2000; Willner et al., 1994), which our data in the present study support. In both experiments reported here, male CMS-Sprague-Dawley rats showed a slower rate of weight gain than their associated control groups, about a 10 % difference between groups. Long Evans animals on the other hand appeared less sensitive in this respect to the CMS exposure, showing a significant decrease in weight gain in only one of the two experiments (2% difference from the Control group in Experiment 1 and 8% difference from the Control group in experiment 2).

Finally, Long Evans but not Sprague-Dawley treated rats displayed adrenal gland hypertrophy, suggesting an increase in HPA axis activity in these animals. This finding is

consistent with the strain difference in the corticosterone profile that was found following the application of the acute restraint stress. One explanation is that Long Evans animals display an adaptive response to the acute stressor, presumably due to previous CMS exposure; chronic activation of the HPA axis would lead to an increase in adrenal gland weight. Their rate of body weight gain is consistent with this interpretation; an initial decrease in weight in CMS animals followed by a rate of weight gain that is similar to that of the control animals (see Figure 3, graph b). For example, the slopes associated with each function are 0.70 (Control) and 0.66 (CMS).

7. Conclusion

In summary, we have shown strain differences in stress reactivity on physiological and biochemical grounds but fail to consistently demonstrate any effects of these stressors on behavioural measures of reward. We do report a reduction in sucrose consumption in the 1h test for the Long Evans animals; however, sucrose levels appear to quickly return to basal levels. This short-lived effect provides support for our claim that Long Evans rats rapidly adapt to the stressor experience.

In evaluating the influence of CMS on multiple behavioural endpoints, our aim was to provide some insight as to which measure is the most appropriate. The results of the present study support the view that CMS induces a reduction, albeit short-lived, in sucrose intake monitored for 1h, at least in Long Evans animals. While the 1h preference data failed to meet statistical significance, the pattern is consistent with this interpretation - an initial decrease in preference. No advantage was served by the longer monitoring period in either strain.

Finally, thresholds for BSR were significantly decreased in Long Evans rats, contrary to the expectation that CMS elevates reward thresholds (Moreau et al., 1992; Moreau, Borgulya et al., 1994; Moreau, Bourson et al., 1994; Moreau, et al., 1996). Given the different sensitivities to stressors in individual animals, even within the same strain (Nielsen et al., 2000), and the relatively modest group effects generally reported, it would be informative to evaluate different behavioural endpoints in the same animal, in order to better understand the mechanisms underlying anhedonia and chronic stress.

Table 1. Schedule of chronic mild stressors for Experiment 1 administered over a seven day period and repeated for six weeks.

	Monday	Tuesday	Wednesday	Thursday	Friday
0800 am	Confinement 1 h	ICSS	Exposure to empty bottle 1 h	Restricted food 2 h	ICSS
Noon		Confinement 1 h	Confinement 1 h	Confinement 1 h	Confinement 1 h
0200 pm	Confinement 1 h	Confinement 1 h		Confinement 1 h	
0400 pm	Overnight illumination with 30° cage tilt	Overnight water deprivation	Overnight food & water deprivation	Overnight pairing in wet bedding	Reversed Light/dark cycle over weekend

Table 2 - Summary of Results of Statistical Analyses

<u>Experiment 1 (Brain-stimulation reward groups)</u>		
<u>Measure</u>	<u>Sprague-Dawley</u>	<u>Long Evans</u>
Body weight:	Group (F _{1,11} = 13.24; p<0.004)	
	Time (F _{2,29} = 32.84; p<1X10 ⁻⁵)	(F _{2,18} = 133.14; p<1X10 ⁻⁵)
	Interaction (F _{2,29} = 6.38; p<0.004)	
BSR maximum rates:		
BSR thresholds:	Group	(F _{2,18} = 133.14; p<1X10 ⁻⁵)
Charge values:	Group	(F _{1,5} = 8.72; p<0.04)
<u>Experiment 2 (Sucrose groups)</u>		
Body weight:	Group (F _{1,14} = 24.40; p<3X10 ⁻⁴)	(F _{1,14} = 5.85; p<0.03)
	Time (F _{2,24} = 50.79; p<1X10 ⁻⁵)	(F _{1,20} = 92.20; p<1X10 ⁻⁵)
	Interaction (F _{2,24} = 5.01; p<0.03)	
Adrenal weight:	Group	(F _{1,14} = 5.70; p<0.04)
	Side (F _{1,15} = 5.49; p<0.04)	
Spleen weight:		
1 hr sucrose intake:	Time	(F _{3,45} = 5.27; p<0.003)
	Interaction	(F _{4,48} = 6.66; p<4X10 ⁻⁴)
1 hr preference:	Time	(F _{6,79} = 2.55; p<0.03)
24 hr sucrose intake:	Time	(F _{6,78} = 2.47; p<0.031)
	Interaction	(F _{6,78} = 2.44; p<0.033)
24 hr preference:	Time	(F _{4,56} = 5.82; p<5.7X10 ⁻⁴)
<u>CRT assay:</u>		
Time 0	Group	(t ₁₅ = 3.10; p<0.007)
Time 0-120 min	Time (F _{2,24} = 51.85; p<1X10 ⁻⁶)	(F _{2,18} = 21.44; p<1X10 ⁻⁵)
Quadratic trend	Control (F _{1,10} = 67.52; p<9X10 ⁻⁶)	(F _{1,8} = 29.81; p<7X10 ⁻⁴)
	CMS (F _{1,10} = 60.41; p<1.5X10 ⁻⁵)	(F _{1,8} = 25.00; p<0.002)

Table 3. Schedule of chronic mild stressors for Experiment 2 administered over a seven day period and repeated for six weeks.

	Monday	Tuesday	Wednesday	Thursday	Friday
0800 am	Confinement 1 h	Exposure to empty bottle 1 h	Novel cage 1h	Restricted food 2 h	Novel cage 1h
Noon	Confinement 1 h	Confinement 1 h		Confinement 1 h	Confinement 1 h
0100pm				Introduce Sucrose	Measure 24h Sucrose Consumption
0200 pm	Confinement 1 h	Confinement 1 h		Measure 1h Sucrose Consumption, provide restricted food for ½ h	
0400 pm	Overnight water deprivation	Overnight pairing in wet bedding	Overnight food & water deprivation	Overnight illumination with 30° cage tilt	Reversed light/dark cycle over weekend

Study 3.

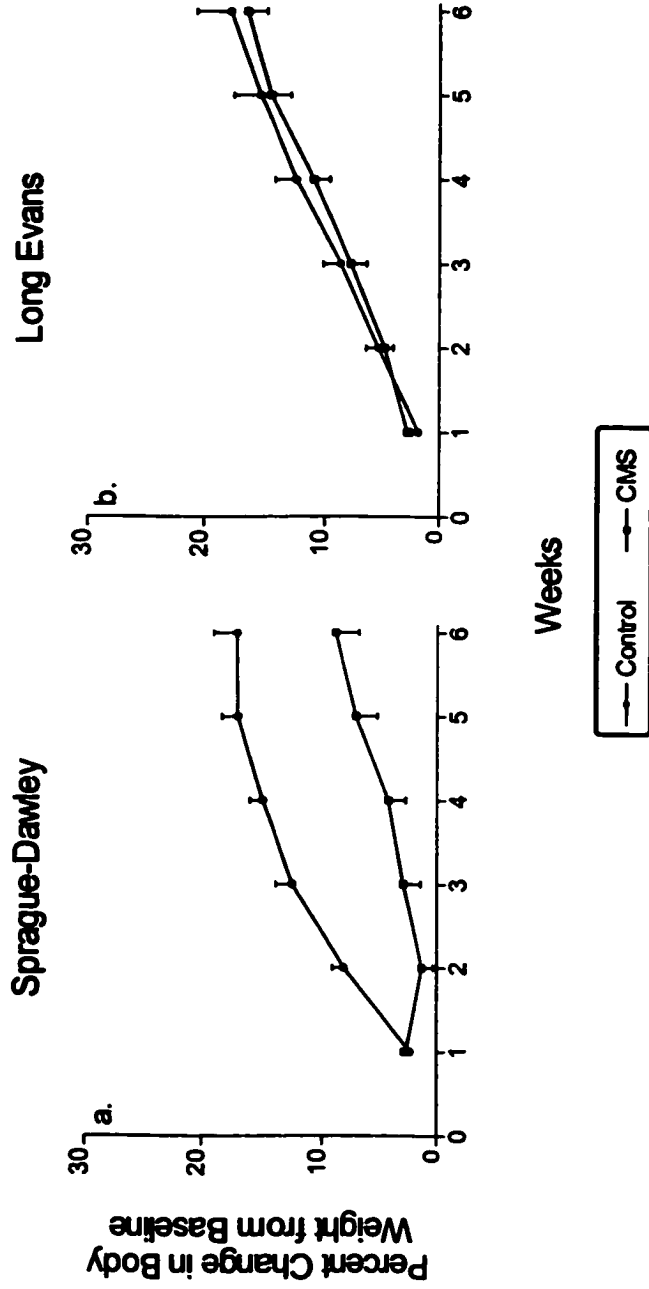


Figure 1. The graphs show the average percent change from baseline weight (\pm SEM) per group over the six week testing period for animals in Experiment 1. Sprague-Dawley animals are shown in graph a and Long Evans in graph b. Circles represent the control (n=7) and squares the CMS groups (n=7).

Study 3.

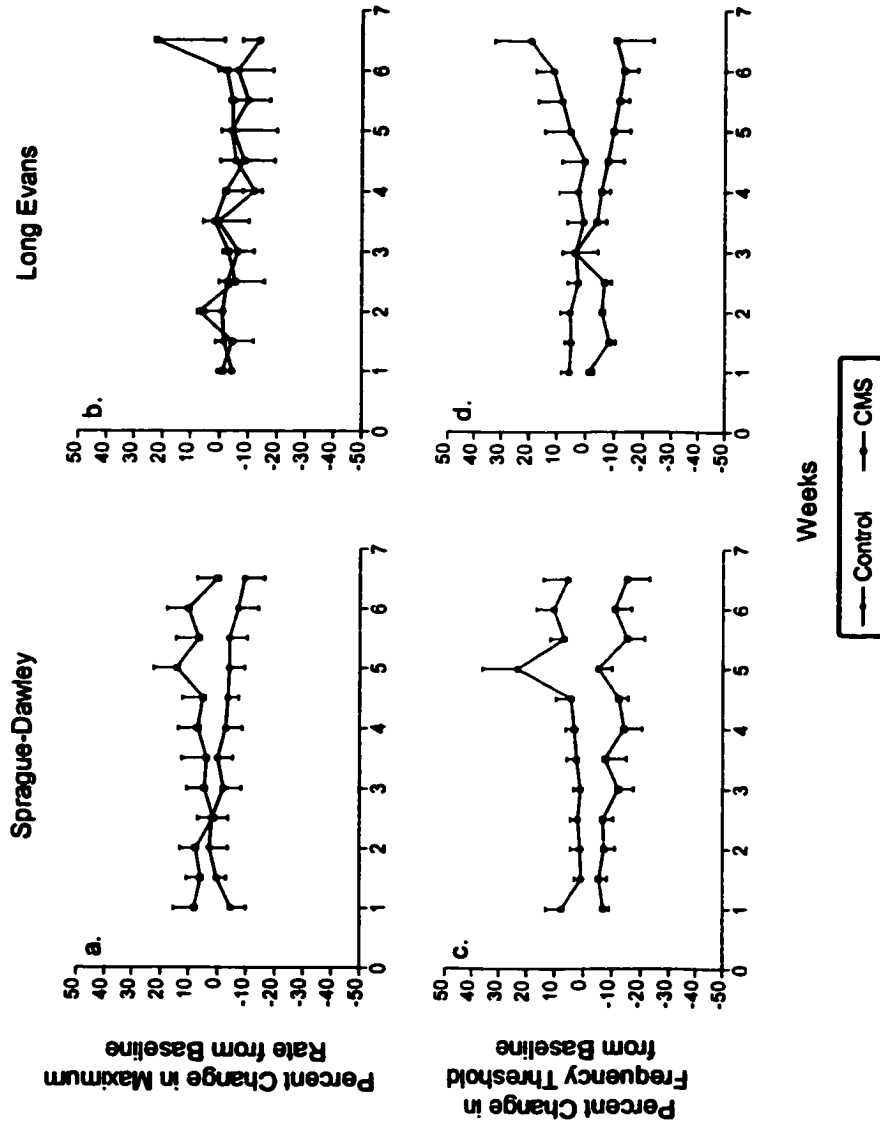


Figure 2. The data represent the percent change from baseline in maximum response rates (graphs a & b) and frequency thresholds (graphs c & d) over the six weeks of CMS. Each point represents the average value \pm SEM for seven animals. Note that no baseline differences were detected between the groups in either measure. The Sprague-Dawley associated data are shown in the left graphs, a & c, and Long Evans on the right b & d. Circles represent the control and squares the CMS groups.

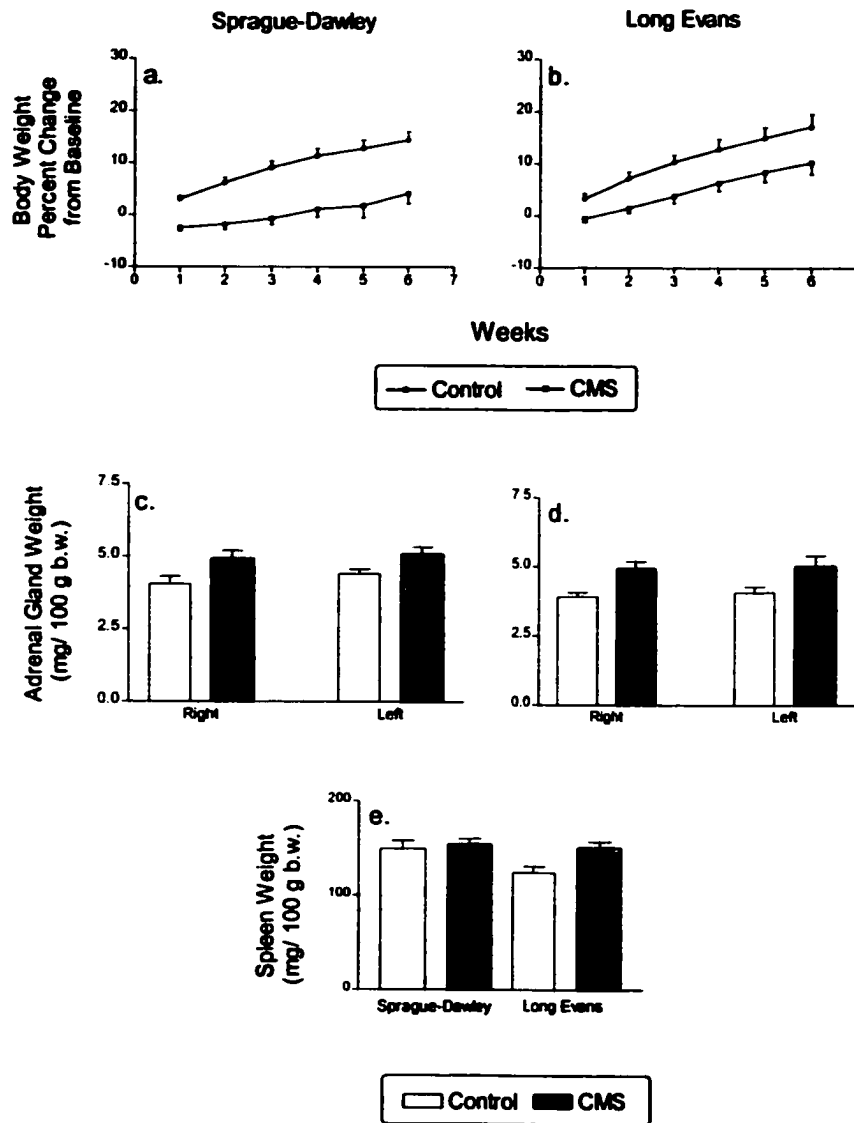


Figure 3. Graphs a and b represent the mean percent change in body weight from baseline values (\pm SEM) during the six weeks of CMS administration for animals in Experiment 2. Adrenal and spleen weight as a function of treatment are shown in the remaining graphs. Adrenal glands appear in the middle, Sprague-Dawley (graph c) and Long Evans (graph d); note that the right and left adrenal glands are shown separately for each group. Spleen weights for both strains are shown in graph e. Each bar represents the mean \pm SEM weight for 8 animals.

Study 3.

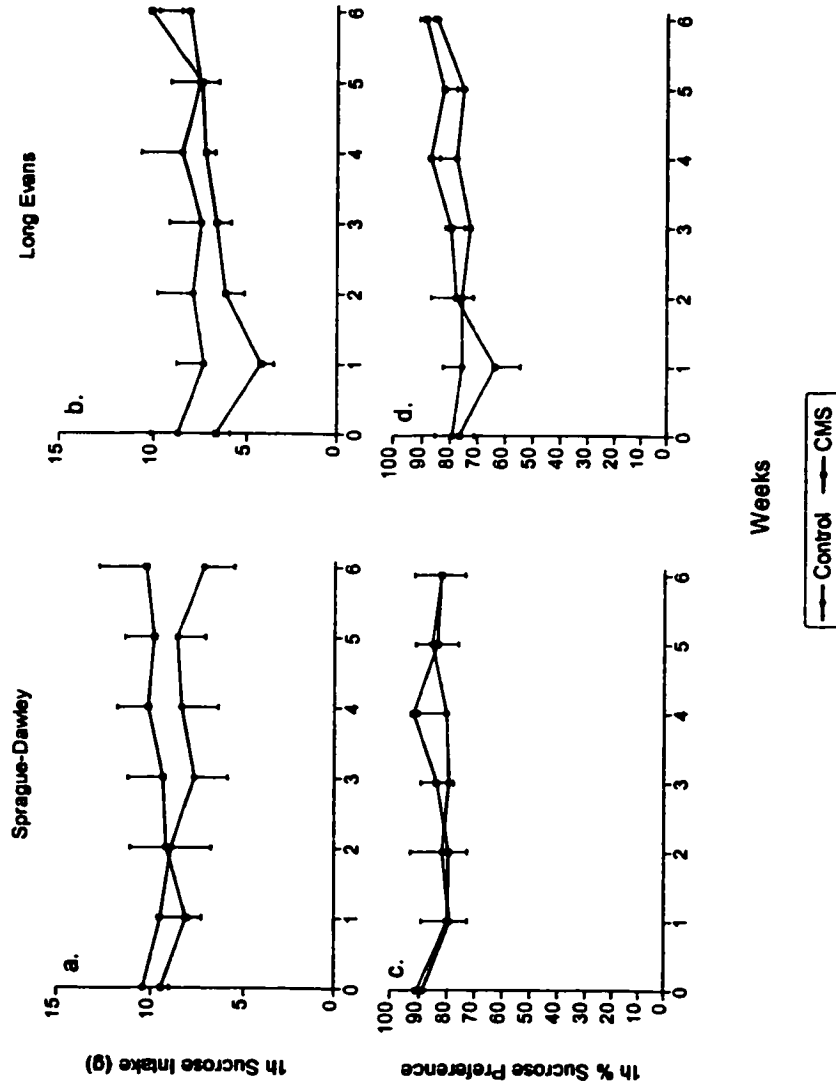


Figure 4. Data pertaining to the 1-h measure of 1% sucrose consumption and preference, over six weeks of CMS administration. Values are means \pm SEM for each group. Intake measures are shown on graphs a (Sprague-Dawley) and b (Long Evans) while preference is displayed on graphs c (Sprague-Dawley) and d (Long Evans). Circles represent the control (n = 8) and squares the CMS groups (n = 8).

Study 3.

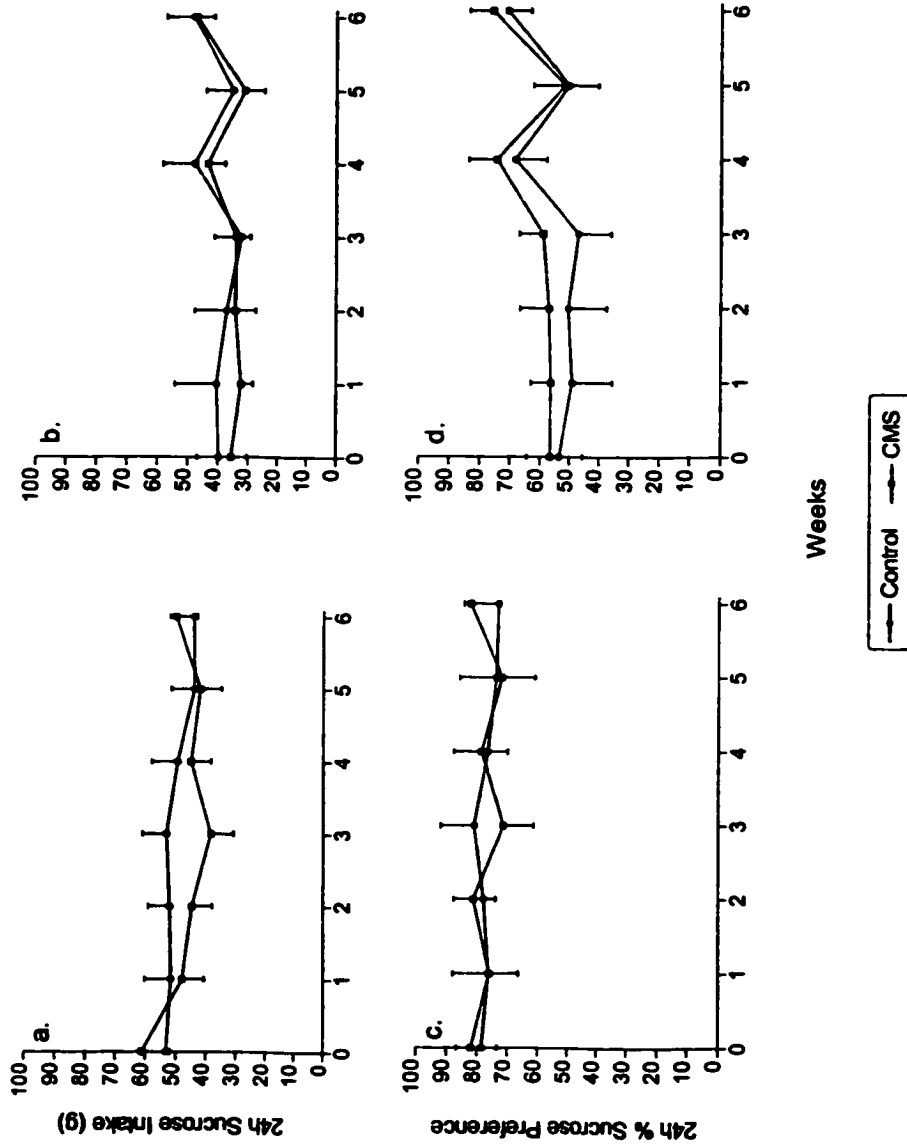


Figure 5. The graphs show the effect of CMS on the consumption and preference for a 1% sucrose solution monitored over 24 hours. Mean intake and preference \pm SEM for Sprague-Dawley animals ($n = 8/\text{group}$) are shown on graphs a & c respectively. Those for the Long Evans strain ($n = 8/\text{group}$) appear on graphs b & d (intake & preference, respectively).

Study 3.

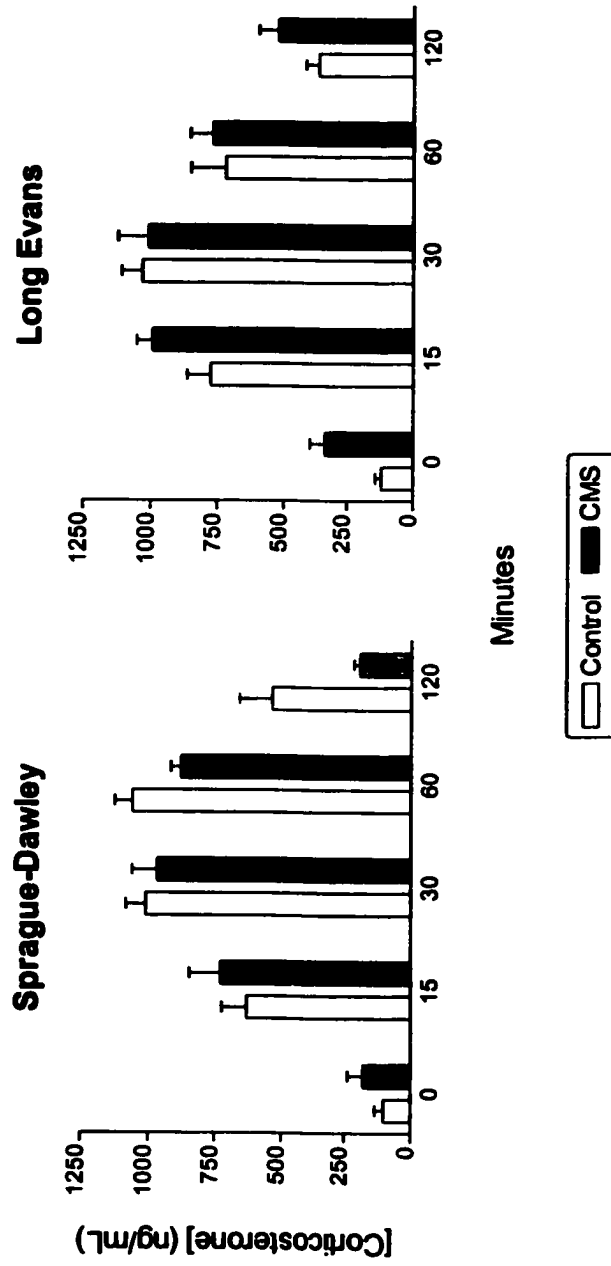


Figure 6. Average corticosterone levels \pm standard error of the mean (ng/mL) per group, in two male outbred rat strains. The Sprague-Dawley related data appear on the left and that of the Long Evans on the right half of the figure. Time 0 represents the corticosterone levels obtained immediately following the six weeks of treatment after which an acute stressor was administered and the corticosterone levels evaluated at times 0-120 min.

Study 3.

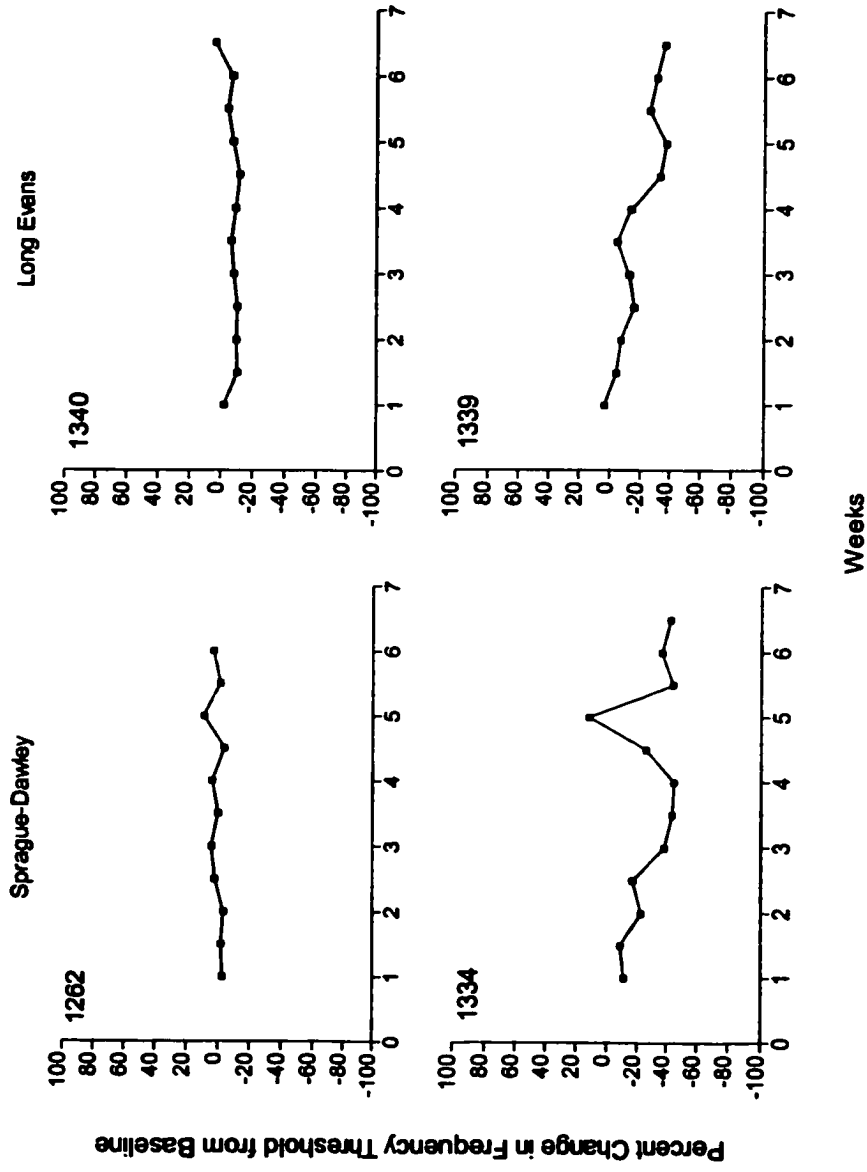


Figure 7. The graphs show the data from two individual rats (CMS group) of each strain, Sprague-Dawley on the left and Long Evans on the right, in Experiment 1. Numbers 1340 & 1262 represent rats associated with frequency thresholds that were stable over the six week CMS exposure period while 1334 & 1339 are characteristic of rats that showed a gradual decline in the frequency threshold over time relative to baseline values.

Study 4

EVALUATION OF THE EFFECTS OF CHRONIC MILD STRESSORS IN TWO FEMALE RAT STRAINS

Submitted to Brain Research as: Konkle, A.T.M., Baker, S.L., Kentner, A.C., Bielajew, C., Santa-Maria Barbagallo, L., Fouriezios, G., and Merali, Z. Evaluation of the effects of chronic mild stressors in two female rat strains. (Manuscript submitted to Brain Research).

Abstract

The chronic mild stress (CMS) paradigm was developed in order to simulate in animals, the symptom of anhedonia, a major feature of depression. Typically, changes in hedonic status are interpreted from a decrease in either intake or preference for a mild sucrose solution or by an increase in the threshold for brain-stimulation reward (BSR). Although the incidence of depression is significantly higher in women versus men, the response of male rodents have typically been examined in this and other animal models of depression. The purpose of this study was to examine the effects of six weeks of CMS administration in two strains of female rats - Sprague-Dawley and Long Evans, using physiological (body, adrenal, and spleen weight) and biochemical (plasma corticosterone levels) indices of stress as well as evaluations of BSR thresholds and 1 and 24 hour tests of sucrose intake and preference. Estrous cycle was tracked throughout the study. Our results indicate a slower rate of weight gain in animals exposed to the chronic stress regime. Furthermore, CMS was shown to disrupt regular estrous cycling, predominantly in the Long Evans strain of rats. Evaluation of the behavioural measures revealed no effect of CMS on BSR thresholds in either strain. The main behavioural finding was a significant reduction in 24h sucrose intake in Sprague-Dawley animals; this decrease was modest in the Long Evans strain. Corticosterone levels were elevated in CMS treated animals relative to the singly-housed control groups, but a strain difference in sensitivity to a subsequent stressor was revealed. Gender specific effects of chronic stressors were obtained, suggesting an interaction between genetic and environmental factors in the etiology of depression, as modeled by this paradigm.

Keywords: Chronic mild stress, females, sucrose intake and preference, brain-stimulation reward, anhedonia, estrous, corticosterone

1. Introduction

It is well recognized that there are sex differences in the prevalence of many psychopathologies with women being relatively disadvantaged in the case of anxiety (Seeman, 1997; Wittchen and Essau, 1993) and mood disorders (Seeman, 1997; Sprock and Yoder, 1997). This may be partly due to the fact that a variety of mood disorders include problems with an inherent gender component, for example, depressive disorder with postpartum onset, menstrual dysphoric disorder, and post-menopausal syndrome (American Psychiatric Association, 1994). Although these illustrate only a subset of the mood disturbances, their association with fluctuations in gonadal hormone levels has prompted theorizing as to the implication of gonadal endocrine factors in depression. Despite the fact that the incidence of depression in women is estimated at twice that in men (American Psychiatric Association, 2000; Sprock and Yoder, 1997; Weissman, Bland, Joyce, Newman, Wells, and Wittchen, 1993) and the relatively recent inclusion of females in clinical studies pertaining to this disorder, a gender bias towards male responses dominates the research in animal models of depression.

A reason for this neglect of female data in animal studies is the potential confound related to cyclic hormone levels (Blanchard, Griebel, and Blanchard, 1995). In the past, unless the work dealt with questions directly associated with reproduction, most studies were carried out on male subjects. Since then, important gender distinctions have come to light. A critical finding has been the gender dimorphism in the pharmacokinetic and pharmacodynamic profiles of many drugs. For example, males and females differ in their rate of absorption of certain compounds, due in part to distinct gastrointestinal tract physiologies. Furthermore,

drug distribution is now known to be affected in part by the availability of plasma binding proteins, of which females have lower levels of one such protein - α 1-acid glycoprotein - its concentrations affected by endogenous estrogen levels (Beierle, Meibohm, and Derendorf, 1999). The profile of adverse drug effects is also related to gender as some compounds have been shown to interact with estrogen resulting in noxious consequences (briefly reviewed in Godfroid, 1999).

There exists a variety of animal paradigms that model depression. Some are generally used for the purpose of evaluating novel antidepressants, while others emulate the human condition in order to study the neurobiological underpinnings of the disorder. The versatility of the chronic mild stress (CMS) model addresses both. This paradigm involves the administration of unpredictable mild stressors (Willner et al., 1987) designed to mimic the daily hassles that reportedly aggravate the onset of depression in humans (Anisman and Zacharko, 1982; Kendler et al., 1995; Kessler, 1997). While this paradigm has been extensively validated with male rodents (Willner, 1997a, 1997), very few laboratories have examined the anhedonic consequences of CMS in female animals (Benelli et al., 1999; Dunčko, Brtko et al., 2001; Dunčko, Kiss et al., 2001; Lanfumey et al., 1999).

The importance of evaluating the effects of stressors in both sexes is demonstrated by the results of a study suggesting that female rats are more vulnerable than males to the effects of chronic stress (Kennett et al., 1986). In that study, female rats were less affected by a single exposure to 2h of restraint stress versus their male counterparts; however, whereas male rats adapted to chronic restraint, females failed to show any adaptive response following 5 days of exposure.

A review of the literature indicates that there is limited information concerning the effects of CMS in female animals. Assessment of biochemical indices has revealed an increase in tyrosine hydroxylase gene expression in the adrenal glands (Dunčko, Brtko et al., 2001) and a decrease in the locus coeruleus (Dunčko, Kiss et al., 2001) of female rats exposed to CMS. Corticosterone levels have been found to be elevated following CMS in female mice (Lanfumeijer et al., 1999) but unaffected in rats (Dunčko, Kiss et al., 2001). While there appears to be little consequence of exposing female rats to CMS when evaluated for place preference conditioning (Benelli et al., 1999) or in the open field paradigm (Benelli et al., 1999; Dunčko, Kiss et al., 2001), a large reduction in sucrose intake has been shown in animals exposed to three weeks of stress versus their control mates (Benelli et al., 1999; Dunčko, Kiss et al., 2001; Genedani et al., 2001); only one study has shown a difference in sucrose preference as a result of chronic exposure to stressors (Benelli et al., 1999). Note that most of the reviewed data pertains to ovariectomized female rats. In the case in which the effects of CMS on sucrose intake were compared in intact versus gonadectomized females, a decrease in sucrose intake in unstressed-ovariectomized animals similar in magnitude to that of the intact CMS treated female rats was found (Dunčko, Brtko et al., 2001).

The aim of this study was to evaluate whether the effects of CMS on behavioural, biochemical, and physiological indices are unique in intact female rats compared to what has been reported in male rats. Therefore, we compared the consequences of CMS on two commonly employed behavioural measures of anhedonia - sucrose intake and preference and brain-stimulation reward (BSR); to our knowledge, no one has yet done the same analysis on female rats. Daily vaginal swabbing allowed us to determine whether estrous cycling

remained regular throughout the study. At the end of the study, plasma corticosterone levels were determined and adrenal glands and spleens weighed. In the first experiment in which sucrose intake and preference were measured, we included a group receiving CMS for six weeks, and two control groups - singly-housed and group-housed animals. The latter group was added in order to discern whether housing conditions would be a confounding factor; female rats reportedly thrive much better in a constant social environment versus an isolated one (Brown and Grunberg, 1995; Palanza, 2001) compared to male rats. Finally, because of our earlier findings of strain differences in reactivity to stress (Bielajew et al., 2002), we decided to employ the two common outbred rat strains, Sprague-Dawley and Long Evans, in which this distinction was found.

EXPERIMENT 1. CMS EFFECTS ON SUCROSE INTAKE AND PREFERENCE

2. Materials and Methods

2.1. Subjects

A total of 24 Sprague-Dawley and 24 Long Evans female rats (Charles River Laboratories, St-Constant, Qc), weighing between 228-286g at the start of the experiment, were used. They were assigned to one of three groups: singly-housed control (n=16), group-housed control (n=16), or singly-housed CMS (n=16). Singly-housed animals were kept in standard sized plastic cages while group housed were assigned four to a cage that was twice the surface area of the standard cages; Purina rat chow and water were available freely unless stated otherwise. Rats were maintained on a 12h light/12h dark cycle with lights on at 0700 h. Throughout the study, all animals were weighed weekly and vaginal swabs were collected

5 days/week at approximately the same time of day in order to assess changes in the estrous cycle.

2.2. Procedure

Animals were first acclimatized to a 1% sucrose solution for 48h and then to the two bottle paradigm - sucrose and water. Five baseline values were collected in the following manner. Animals from all groups were food deprived overnight. Those group-housed were placed in individual cages 1h prior to testing; each animal was assigned a cage that was re-used on subsequent test days. One and 24 h after the sucrose and water bottles were introduced, fluid intake of each animal was recorded; note that only 1h consumption was evaluated in group-housed control animals. Food was returned to the home cage following the 1h measurement. Preference for sucrose was determined by the ratio of sucrose (g) to sucrose and water intake (g) converted to a percent score. Tests were then conducted weekly for the next six weeks of CMS according to the above procedure; a detailed description of the CMS procedure is provided in Table 1.

Insert Table 1 about here

2.3. Blood Sampling

Following the six weeks of CMS, tail blood samples were collected in each rat by lancing the tail close to its tip and collecting a few drops of blood on an Schleicher & Schuell® filter paper (procedure adapted from Wortham and Stallings, 1997) After the first sample was taken, the rat was placed in a rodent restraint cone for 10 min; this condition was included in

order to evaluate any consequences of CMS on the typical corticosterone response pattern to an acute stressor. Note that the rats were not otherwise restrained during the blood collection procedure. Blood sampling was repeated at 15, 30, 60, and 120 min after the onset of the acute stressor. After the filter papers had dried sufficiently (approximately 4-5 hours), they were stored in a -20°C freezer for subsequent analysis. Blood samples were collected over two days between 8 and 11 AM and alternated between groups in order to minimize the effects of circadian rhythm on the results. Following the last blood sample, animals were decapitated and adrenal glands and spleen dissected and weighed.

2.4. Corticosterone assay

Blood samples were eluted from the filter paper by placing one 3.2 mm punch in a glass tube and adding 100 µL of Dulbecco's phosphate buffered saline (containing 0.1% gelatin) (Wortham & Stallings, 1997) to each tube. These were shaken for 1 hour at 50 rpm at room temperature, refrigerated overnight, and shaken for an additional hour the following morning.

Plasma corticosterone levels were determined using a commercially available radioimmunoassay kit (ICN Biomedicals, Costa Mesa, CA). The intra-assay variability was <10% while inter-assay variability was eliminated by running all the blood samples at one time. Total corticosterone concentration levels were determined as outlined by Wortham and Stallings (1997).

2.5. Statistical Analyses

All statistical analyses were carried out using the Statistica software package (Statistica, 1998). The results associated with each strain were assessed separately. Estrous cycle regularity was analyzed via a Chi-square goodness-of-fit test on each strain. Changes in body weight were evaluated using a mixed ANCOVA design with time as the repeated factor, group as the independent one, and baseline weight as the fixed covariate. Sucrose intake was similarly analyzed with body weight at each time designated a running covariate. A one-way ANCOVA was employed to analyze spleen weights, with group as the independent factor and body weight at the time of sacrifice as the covariate. The same analysis was carried out on the data pertaining to adrenal gland weights with the addition of side as a repeated factor. Finally, corticosterone levels at time 0 were analyzed via a simple ANOVA design, with group as the independent factor. Trend analyses were conducted on each group in order to evaluate the pattern of corticosterone levels over time. The data at time 0-120 min were analyzed by a mixed ANOVA design, with group the independent factor and time the repeated one.

3. Results

Examination of the estrous cycles associated with the Sprague-Dawley rats revealed no significant difference from the expected pattern among groups. However, the results of the analyses pertaining to the Long Evans animals showed that cycling activity was significantly reduced in the CMS group, compared to control values; these data appear in Figure 1.

Insert Figure 1 about here

A summary of the results of the remaining analyses pertaining to Experiment 1 is presented in the top portion of Table 2.

Insert Table 2 about here

Analyses of the body weight data, presented in Figure 2a & b, revealed significant effects

Insert Figure 2 about here

of group and time in both rat strains, with CMS groups showing the slowest rate of weight gain compared to their control counterparts. A significant side difference in adrenal gland weight, shown in Figure 2c & d, was obtained in both strains. Recall that the analysis was conducted using body weight as a covariate so that the side difference is not readily apparent in the figure. An overall difference in spleen weight between groups was found for the Long Evans rats only (Figure 2e), and according to the results of pair-wise post-hoc analyses, due to heavier spleens in the CMS group relative to both singly-housed ($F_{1,20} = 4.94$; $p < 0.04$) and group-housed ($F_{1,20} = 15.40$; $p < 8.4 \times 10^{-4}$) control animals; note that spleen weights for singly- and group-housed animals did not differ from each other.

No difference in 1h sucrose intake, presented in Figure 3a & c was found in either rat

Insert Figure 3 about here

strain; however, when the weight covariate was eliminated, a significant time effect was revealed (Figure 3 b & d). Analysis of the 1h sucrose preference data gave rise to a significant group difference in both strains (Figure 3e & f), although no meaningful pattern is evident.

The 24h sucrose intake data were found to be significantly different in the Sprague-Dawley

animals (Figure 4 a & b), due to reduced intake associated with CMS animals, but only when

Insert Figure 4 about here

the covariate was excluded from the analysis. In Long Evans rats (Figure 4c & d), however, a significant interaction between group and time was found when the covariate was included, but not otherwise. Analysis of the sucrose preference data revealed no findings related to the 24h measures (Figure 4e & f).

While group differences in corticosterone levels were found for both strains at time 0 (Figure 5), further probing of these data via pair-wise analyses revealed that the

Insert Figure 5 about here

difference was related to higher corticosterone levels in Sprague-Dawley CMS and group-housed control animals relative to the singly-housed control group. In Long Evans rats, this difference was related to elevated corticosterone levels in the group-housed control animals only.

A trend analysis of the corticosterone data from times 0 to 120 revealed, as expected, significant quadratic patterns in corticosterone levels following the application of an acute stressor (Bielajew et al., 2002; Dhabar et al., 1997; Garcia et al., 2000). Analysis of the data obtained at time intervals 0-120 min yielded a significant effect of group and time in both strains. Further analyses showed no significant difference between singly housed control and CMS animals of either strain.

EXPERIMENT 2. CMS EFFECTS ON BRAIN STIMULATION REWARD THRESHOLDS

4. Materials and Methods

4.1. Animals

The animals consisted of 14 Sprague-Dawley and 14 Long Evans female rats (Charles River Laboratories) weighing between 220-300 g at the time of surgery. In this study, rats were divided into singly-housed control (n = 14) and CMS (n = 14) groups; note that a group-housed control condition was not included in this experiment. All animals had free access to Purina rat chow and tap water unless stated otherwise. Rats were maintained on a 12h light/12h dark cycle. During the baseline phase of the study and thereafter, body weights were chronicled biweekly and vaginal swabs were collected on week days.

4.2. Surgery

Immediately prior to surgery, rats were administered atropine sulfate (0.05 ml) to prevent excessive bronchopulmonary secretion and then anesthetized by continuous administration of the inhalant anesthetic halothane. The electrodes were aimed bilaterally at the ventral tegmental area using the coordinates 4.8 mm posterior to bregma, 0.7 mm lateral to the midsagittal suture, and 8.0-8.4 mm below the skull surface (Paxinos and Watson, 1998).

The electrodes (Plastics 1 Inc.) were fashioned from stainless steel wire, 250 μm in diameter and insulated with polyimide to the polished tip. A flexible stainless steel wire wrapped around four stainless steel skull screws served as the current return. The entire assembly was secured to the skull with dental acrylic.

4.3. Behavioural Tests

Following a minimum seven day recovery period, the rats were trained to lever-press for brain stimulation on a continuous reinforcement schedule. All tests were conducted in a wood and Plexiglas box with dimensions 27 cm deep X 37 cm wide X 51 cm high.

Stimulation was supplied by a constant-current amplifier (Mundl, 1980) and an integrated circuit pulse generator built in-house. Each depression of the lever resulted in the delivery of monophasic, cathodal pulses of 100 μ s duration with a train duration of either 300 or 500 ms; a lower train duration was used in animals for which the higher value led to unstable behaviour. The lowest current and pulse number to elicit approximately 30 lever presses/min were determined for each animal. The parameters were continuously monitored on an oscilloscope by reading the voltage drop across a 1 k Ω precision resistor in series with the rat.

Animals were introduced to the threshold procedure once lever pressing was found to be reliable. Rate-number functions were determined using a descending method of limits. Each 60s trial consisted of the presentation of a descending sequence of pulse numbers, 0.1 \log_{10} units apart, starting at a value that supported a high rate of responding and ending at a pulse number for which little or no responding was obtained. The current was held constant and ranged from 200 to 630 μ A across animals. The beginning of each trial was signaled by three priming stimulations, set at the same stimulation parameters as the subsequent 60 s trial. A number threshold, interpolated from the rate-number function and defined as the value that supported half the maximum response rate, was determined for each curve. Four rate-number functions were generated during each session; the first was considered a warm

up curve and discarded. The average threshold was based on the remaining three functions. Thresholds were deemed stable for each rat when they did not vary by more than $0.1 \log_{10}$ units for three consecutive test days. Following the collection of one baseline session, the animals were administered six weeks of CMS and threshold tests conducted bi-weekly; the CMS schedule is presented in Table 1. Each number threshold was converted to a frequency threshold or the number of required pulses per second (Hz) and presented as such in this paper; all analyses were conducted on this measure.

4.4. Histology

Following completion of the experiment, rats were administered a lethal dose of sodium pentobarbital (Somnotol). They were perfused intracardially with saline followed by buffered formalin containing 10% sucrose. The brains were removed and stored in the formalin solution at 6°C for a minimum of 24 h. Coronal sections were placed on gel coated slides and stained with cresyl violet in order to better locate the electrode tips using the Paxinos and Watson atlas (1998).

4.5. Statistical analyses

Data pertaining to the estrous cycle were analyzed via a Chi-square goodness-of-fit test on each strain. The response rates, frequency thresholds, and body weight data were analyzed separately by strain using a mixed ANCOVA design. Group was the independent factor with two levels, time the repeated one with 12 levels, and the associated baseline value the fixed covariate. Note that although body weight was chronicled bi-weekly, we excluded from the

analyses those values that were collected on a day following food deprivation. The Greenhouse-Geisser or Huynh-Feldt correction for violations to the assumption of sphericity were applied to all results pertaining to the repeated measure (Howell, 2002).

Finally, given individual differences in the train duration and current parameters, frequency thresholds were transformed to charge values in order to better compare the total amount of stimulation delivered across animals. A mean charge value was calculated for each animal with the formula:

$$Q = INd$$

where Q is the charge in μC , I is the current in μA , N is the required number of pulses in the stimulation train, and d is the pulse duration in s (Gallistel, 1978). These data were analyzed in the same manner as those pertaining to rate and frequency threshold.

5. Results

Electrode placements ranged from the posterior lateral hypothalamus to the anterior portion of the nigrostriatal bundle, with most tips in or near the ventral tegmental area.

Analyses of the estrous cycle data revealed no difference between the groups for either strain; however, note that the frequency of regular cycles were low in both the control and CMS animals, irrespective of strain (Figure 1b).

Body weight analyses for both strains yielded a significant effect of group and time, as well as an interaction between these two factors. Inspection of Figure 6 reveals a slower rate of

Insert Figure 6 about here

weight gain in all CMS animals.

Graphs a and b in Figure 7 depict the average maximum response rates while graphs c & d

Insert Figure 7 about here

show the mean frequency thresholds over time; no effect of CMS in either measure was detected; the same result was obtained in the case of charge values (data not shown).

6. General Discussion

These experiments were designed to evaluate, in female rats, the physiological, biochemical, and behavioural consequences of a six week regime of mild stressors. Results of our analyses revealed that chronic stress reduced the rate of weight gain in Sprague-Dawley and Long Evans female rats. While 1h sucrose intake and preference measures were little affected by the chronic stress regime, a gradual CMS-induced reduction in 24h sucrose intake was apparent in both strains. No effect of CMS was detected when BSR thresholds were instead evaluated as a measure of anhedonia. Assessment of corticosterone levels as an index of the stress response revealed higher levels in animals exposed to CMS; note that grouped housing also significantly increased corticosterone values relative to animals in the singly-housed control condition.

6.1 Behavioural Indices

Chronic mild stress has previously been shown to decrease the intake and preference for a mild sucrose solution in female rats (Benelli et al., 1999; Genedani et al., 2001; Dunčko, Brtko, et al., 2001). However most of these tests have been conducted in ovariectomized females, and the surgical procedure itself has been associated with a fall in consumption

(Dunčko, Brtko, et al., 2001). In intact females, Duncko, Kiss et al., (2001) have reported a decrease in intake but not preference for sucrose during the course of a three week stress period. In our hands, CMS was ineffective in reducing sucrose consumption in the 1h measures. While 1h sucrose intake increased in the Sprague-Dawley control rats that were singly housed, the amount of sucrose consumed over the test period remained relatively constant in the CMS and group-housed control rats. Results for the Long Evans strain suggest a slight increase in sucrose intake in all three groups. Even though a group difference was detected in the 1h preference test, no meaningful pattern of CMS-induced alterations can be discerned. Results for the 24h measures indicated a gradual reduction in sucrose intake induced by CMS; no changes were found when preference was instead evaluated. In studies employing male subjects, a reduction in the intake (for example see Cheeta et al., 1994; Dunčko, Kiss et al., 2001; Grippo et al., 2002; Willner et al., 1987) and preference (Grippo et al., 2002; Kioukia et al., 2000; Kopp et al., 1999; Willner et al., 1987) for a mildly sweet solution has been reported. However, some groups have noted no change in preference following a chronic stressor regime (Hatcher et al., 1997; Matthews et al., 1995).

To our knowledge, evaluation of BSR thresholds as an index of anhedonia following CMS has never been investigated in female animals. Results from male rats have not been consistent. While one group of investigators regularly reports a gradual increase in frequency thresholds as a consequence of CMS (Moreau et al., 1992; Moreau, Borgulya et al., 1994; Moreau, Bourson et al., 1994; Moreau et al., 1996;), others have shown either a slight facilitation in self-stimulation thresholds (Lin et al., 2002) or no change in this value (Nielsen

et al., 2000). However, individual differences in stress susceptibility have been observed. Nielsen et al. (2000) reported an increase in BSR thresholds in a subgroup of two of their 11 animals. When BSR thresholds were evaluated in female rats in the present study, no CMS-induced changes were detected. When more severe stressors are employed, the results are similarly ambiguous, with footshock yielding either an increase (Bowers et al., 1987; Zacharko et al., 1990) or no effect (Zacharko et al., 1990) on BSR current thresholds. It has been suggested that these differences are related to the site of electrical stimulation (Zacharko et al., 1990). In our data, no such correlation was observed. Indeed, the BSR thresholds, which were obtained from stimulation of a diversity of placements, displayed unwavering stability over time, a pattern that was observed in all animals. Performance variables - maximum response rates - were likewise unaffected by the stress regime, remaining at constant levels in all rats across the six weeks of administration. Whether evaluated in males (Konkle, Kentner et al., Submitted) or females, Sprague-Dawley or Long Evans, we are unable to observe the development of anhedonia, as measured by thresholds for rewarding brain stimulation.

6.2 Biochemical Indices

A positive correlation between plasma corticosterone and estrogen has been shown in that female rats show peak corticosterone levels in the proestrous phase of the estrous cycle (Atkinson and Waddell, 1997), the period associated with the greatest estrogen levels. In the present study, we assessed the relationship between estrous phase (using an ordinal scale to code estrous level) and corticosterone concentrations using the Spearman Rank test and

found no significant correlation, suggesting that corticosterone levels were not biased to a particular estrous phase.

As expected, at time 0, corticosterone levels were elevated in CMS rats versus singly-housed control animals, but failed to reach significance in the Long Evans animals. We have previously reported a strain difference to a six week CMS experience in male rats, but showed the reverse trend, that is Long Evans rats displayed an increased sensitivity to CMS (Konkle et al., 2002); however, we found no such distinction following a three week stress regime (Bielajew et al., 2002). While strain differences in reactivity to stress have been studied in male rats (Dhabar et al., 1997; Gomez et al., 1996), little information exists regarding the interaction of strain with gender. In a recent study, Faraday (2002) reported that rats exposed to chronic restraint stress exhibit levels of prepulse inhibition in the acoustic startle paradigm that is gender and strain dependent. For example, Sprague-Dawley female rats exhibited greater prepulse inhibition than did their male counterparts; the reverse was true for the Long Evans animals, suggesting that the combination of gender and strain are important considerations in studies assessing the effects of stressors. Our finding of a gender difference in susceptibility to chronic stress, at least as assessed by corticosterone levels, is also supported by Kennett et al.'s (1986) work that showed a gender difference in the vulnerability to acute versus chronic stress exposure. Although the differences in corticosterone levels between control and CMS Long Evans groups did not reach statistical significance, this strain does appear to be generally reactive to stressors, a claim supported by our finding of a greater disruption of estrous cycle regularity in this strain.

In the present study female group-housed control rats also showed elevated levels relative

to singly-housed animals. While male rats reportedly show stress responses as a result of crowding and grouped housing, females fare better in such social groups (Brown and Grunberg, 1995; Palanza, 2001). Thus differences in corticosterone levels at time 0 between the two control groups may reflect the contribution of activity levels. For example, repeated voluntary running on a vertical wheel or horizontal disc has been shown to increase corticosterone levels in female hamsters (Borer, Bestervelt, Mannheim, Brosamer, Thompson, Swamy, and Piper, 1992). High baseline corticosterone levels in the group-housed control animals may also have prevented the detection of any acute stressor responses, due to ceiling effects. Consequently, the comparison of biochemical data was restricted to the singly-housed control and CMS animals.

6.3 Physiological Indices

While we report here a reduced gain in body weight following six weeks of stress administration, other studies have shown no effect of a three week exposure in ovariectomized Lister rats (Benelli et al., 1999) and (intact) Sprague-Dawley female rats (Dunčko, Kiss et al., 2001). This discrepancy may be due to a difference in the duration of the stress regime, a suggestion that is supported by our data. For example, in Figures 1a and 1b and 6, it is apparent that the rate of weight gain is similar between the control and stressed animals for at least the first three weeks of manipulations. With one exception, all CMS groups show a significant decline in their rate of weight gain compared to control groups starting at approximately the fourth week. In male rats, as little as three weeks of CMS exposure has been sufficient to change the rate of body weight gain in some studies (Bielajew

et al., 2002; Dunčko, Kiss et al., 2001; Matthews et al., 1995; Nielsen et al., 2000).

The consequences of CMS on other physiological indices are less clear. In the present study, we report no effect of CMS on adrenal gland weight, a finding that supports the results of previous experiments conducted with female (Dunčko, Kiss, et al., 2001) and male rats (Bielajew et al., 2002; Harris et al., 1998; Harro et al., 1999). However, the application of severe stressors has been associated with an increase in adrenal gland weight (Burchfield et al., 1980; Herman et al., 1995; Pignatelli et al., 2000) suggesting that the intensity of the stressors employed here was not sufficient to produce long term changes in adrenal gland morphology. We have shown in a previous study that spleen weight was unaffected by a three week regime of stress in male Sprague-Dawley and Long Evans rats (Bielajew et al., 2002). We now report slightly heavier spleens in chronically stressed Long Evans female rats compared to the singly- or group-housed groups. While most investigators have failed to show any effect of stressors on spleen weight (Dhabar et al., 1997; Pittman, Ottenweller, Pritzel, Natelson, McCarty, and Tapp, 1995; van Raaij et al., 1997), others have reported a decrease (Batuman et al., 1990; Dominguez-Gerpe and Rey-Mendez, 1997). Whether this difference between males and females is a gender specific phenomenon needs to be further investigated.

In previous studies evaluating the effects of CMS in female animals, regularity of the estrous cycle was not an issue, because subjects were ovariectomized prior to stressor challenge. However, disruption of cyclicity has been observed with administration of a chronic variable regime of moderately severe physical stressors (Rodriguez Echandia et al., 1988) and chronic mild food deprivation (Tropp and Markus, 2001); note that strain

differences in susceptibility of the estrous cycle to food deprivation were found. In our first experiment, we report that CMS significantly disrupted cycling in Long Evans rats. Sprague-Dawley rats were similarly affected albeit to a lesser extent; the difference in the total frequency of regular cycles over the six week study was not significantly different from their control cohorts. Chronic exposure to physical stressors has been shown to induce a constant diestrous phase in female Holtzman rats (Rodriguez Echandia et al., 1988). Animals exposed to CMS in the present experiment were not preferentially stalled in a particular phase of the estrous cycle; however, individual animals tended to remain in the same phase throughout. While there would be an adaptive advantage to remaining in diestrous when faced with a threat (stress), it is difficult to comprehend the reason for which the estrus (sexually receptive) phase would predominate in other animals exposed to the same threat.

In the second experiment, irregular cycling predominated in all animals. Given that control and CMS rats showed the same degree of cycle disruption, which was observed even during baseline tests, it appears that stimulation, and not necessarily activation of specific reward-relevant neurons, interfered with regular cycling. The literature concerning this phenomenon is quite sparse. Stratmann and Craft (1997) have reported no effect of BSR on estrous cycling, and in two other groups, BSR-induced disruptions in cycling for some but not all animals (Bless, McGinnis, Mitchell, Hartwell, and Mitchell, 1997; Drewett and Herberg, 1975). Drewett and Herberg (1975) have even described a state of pseudopregnancy in a subset of their animals which were stalled in diestrous for 12-13 days followed by a day in which thick mucosal secretions were detected. While the effects of estrogen on BSR have been documented (Bless et al., 1997; Meyerson, Wilkins, and Sawyer, 1969), the

the consequences of rewarding stimulation on estrous cycling have not. Given the location of these placements, often in estrogen rich structures, an interaction between stimulation and estrous activity might be expected. Further exploration of this phenomenon would be warranted.

6.4 Conclusion

Given gender differences in the prevalence of depression, the aim of this work was to examine the responses of females using an established animal model of depression. The results of the behavioural measures employed were very modest. Only a weak behavioural response was obtained, that of a reduction in 24h sucrose intake in the Sprague-Dawley rats. Any interpretation of anhedonia is diminished by the fact that 24h preference was unaffected indicating that both water and sucrose intake were similarly reduced in these animals.

The slower weight gain reported for female rats in this study mirrors that typically reported for male animals. However, evaluation of the stress response by measuring plasma corticosterone levels following the six weeks of stress exposure, revealed a strain-specific pattern of CMS-induced reduction in stress sensitivity that was different than that reported in males.

An intriguing finding was the significant effect of CMS on estrous activity. A stressor-induced chronic disruption in cycling is related to alterations in gonadal hormone levels or ratios. These changes could interfere with the functioning of systems receiving gonadal hormone input.

Taken together, the work presented here and that reported earlier based on male rats (Bielajew et al., 2002; Konkle, Kentner et al., Submitted) suggests that CMS significantly

modifies physiological and biochemical reactions, as observed by appropriate alterations in weight gain, estrous cycle, and corticosterone levels. However, hedonic changes do not consistently accompany these effects and are only modest at best. The usefulness of this model will require the development of more reliable behavioural measures of assessing changes in anhedonia and identifying vulnerable individuals.

Table 1. Summary of the stressors applied during each of the six weeks of CMS exposure and the frequency of weekly exposure in each experiment.

Stress regimen	Experiment 1 (Sucrose Tests)	Experiment 2 (BSR Tests)
<u>Daytime stressors</u>		
Confinement (1h)	7	8
Novel cage (1h)	2	-
Exposure to empty bottle (1h)	1	1
Restricted food	1	1
<u>Overnight stressors</u>		
Food deprivation	1	1
Water deprivation	1	1
Pairing in wet bedding	1	1
Illumination with 30° cage tilt	1	1
Weekend reversed		
light/dark cycle	1	1

Table 2 - Summary of Results of Statistical Analyses

<u>Measure</u>	<u>Experiment 1 (Sucrose groups)</u>		<u>Long Evans</u>
	<u>Sprague-Dawley</u>		
Estrous cycle:			($X^2_2 = 8.14$; $p < 0.006$)
Body weight:	Group	($F_{2,29} = 6.63$; $p < 0.007$)	($F_{2,20} = 6.59$; $p < 0.007$)
	Time	($F_{3,54} = 130.75$; $p < 1 \times 10^{-5}$)	($F_{2,37} = 49.05$; $p < 1 \times 10^{-5}$)
Adrenal weight:	Side	($F_{1,21} = 4.45$; $p < 0.05$)	($F_{1,21} = 5.63$; $p < 0.03$)
Spleen weight:	Group		($F_{2,20} = 7.70$; $p < 0.004$)
1 hr sucrose intake:	Time	($F_{3,73} = 2.76$; $p < 0.05$)*	($F_{3,57} = 4.46$; $p < 0.008$)*
1 hr preference:	Group	($F_{2,20} = 3.94$; $p < 0.04$)	($F_{2,18} = 6.08$; $p < 0.01$)
24 hr sucrose intake:	Group	($F_{1,14} = 4.62$; $p < 0.05$)*	
	Interaction		($F_{3,41} = 3.63$; $p < 0.02$)
24 hr preference:			
<u>CORT assay:</u>			
Time 0	Group	($F_{2,20} = 5.61$; $p < 0.01$)	($F_{2,20} = 4.94$; $p < 0.02$)
Time 0-120 min	Group	($F_{2,17} = 8.50$; $p < 0.003$)	
	Time	($F_{3,48} = 46.78$; $p < 1 \times 10^{-6}$)	($F_{3,37} = 45.33$; $p < 1 \times 10^{-6}$)
	Interaction		($F_{5,37} = 3.17$; $p < 0.02$)
Quadratic trend	Single	($F_{1,17} = 91.92$; $p < 1 \times 10^{-6}$)	($F_{1,14} = 34.38$; $p < 4 \times 10^{-5}$)
	Grouped	($F_{1,17} = 59.32$; $p < 1 \times 10^{-6}$)	($F_{1,14} = 5.76$; $p < 0.04$)
	CMS	($F_{1,17} = 91.41$; $p < 1 \times 10^{-6}$)	($F_{1,14} = 25.16$; $p < 2 \times 10^{-4}$)

* indicates analyses carried out without the covariate

Experiment 2 (Brain-stimulation reward groups)

Estrous cycle:			
Body weight:	Group	($F_{1,11} = 21.35$; $p < 7.4 \times 10^{-4}$)	($F_{1,7} = 12.84$; $p < 0.0089$)
	Time	($F_{3,40} = 50.92$; $p < 1 \times 10^{-6}$)	($F_{4,31} = 28.86$; $p < 1 \times 10^{-6}$)
	Interaction	($F_{3,40} = 2.95$; $p < 0.04$)	($F_{4,31} = 6.45$; $p < 7.3 \times 10^{-4}$)

No significant results were found for the BSR maximum rates, BSR thresholds, or charge values.

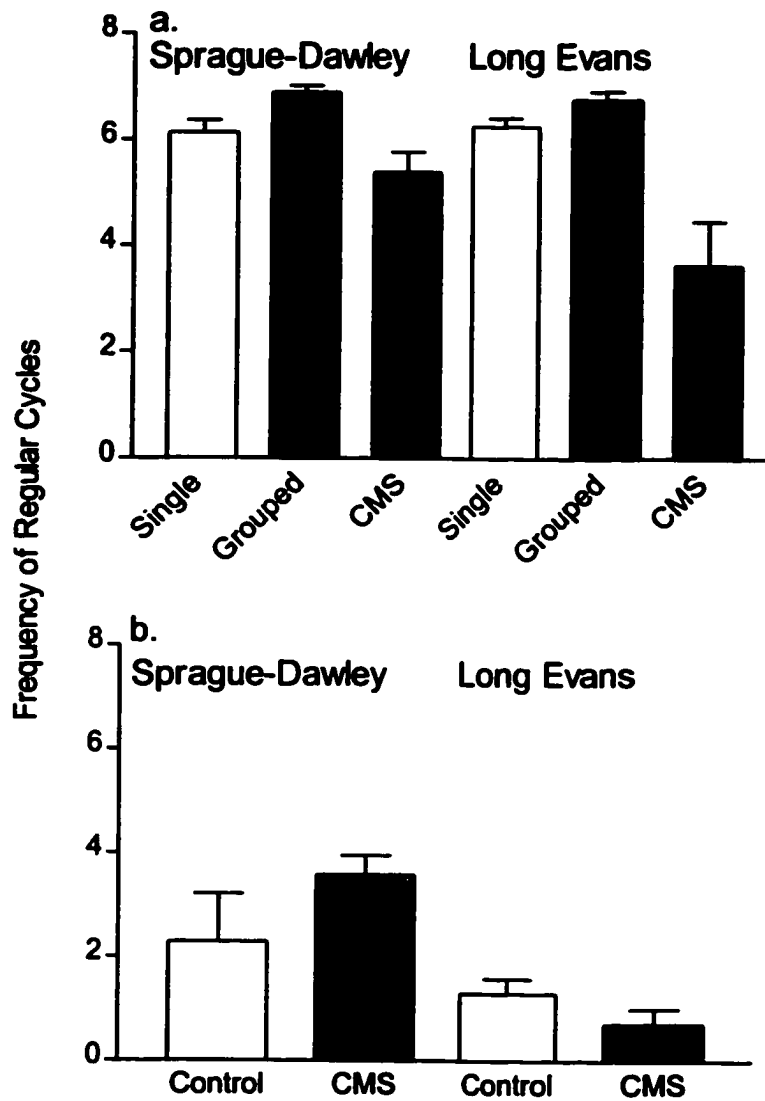


Figure 1. Average number of weeks of regular cycles exhibited by each group over the course of the study (out of a total of seven). Graph a represents the values for animals in Experiment 1 while graph b, animals in Experiment 2.

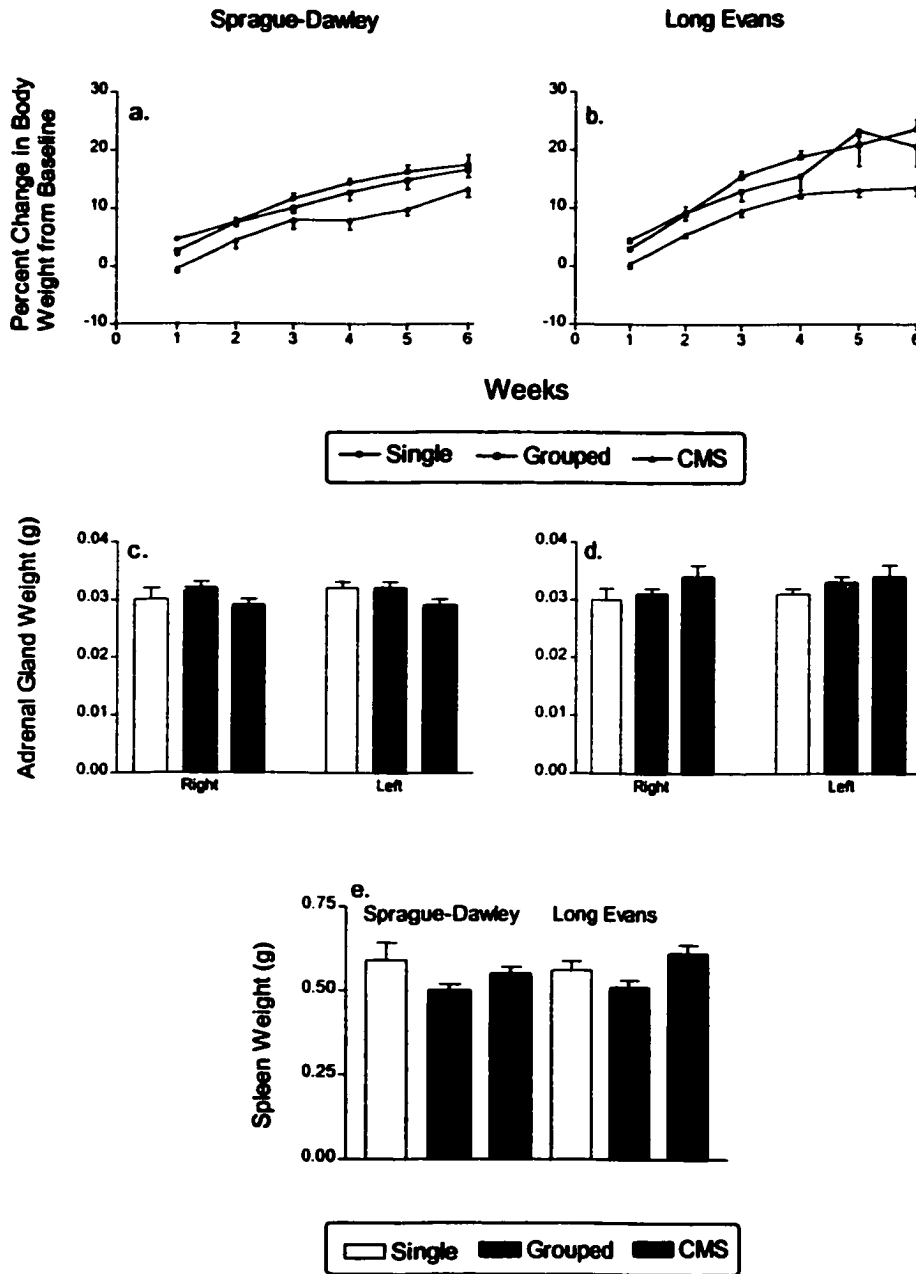


Figure 2. The graphs pertain to the weight data associated with animals in Experiment 1. Graphs a & b display the average percent change from baseline in body weight for each group over time. Average adrenal gland weights, expressed as wet weight in mg per 100g of body weight are shown in graphs c & d. Spleen weights (wet weight in mg/ 100 g body weight) appear in graph e. The respective groups are identified at the bottom of the graphs.

Study 4.

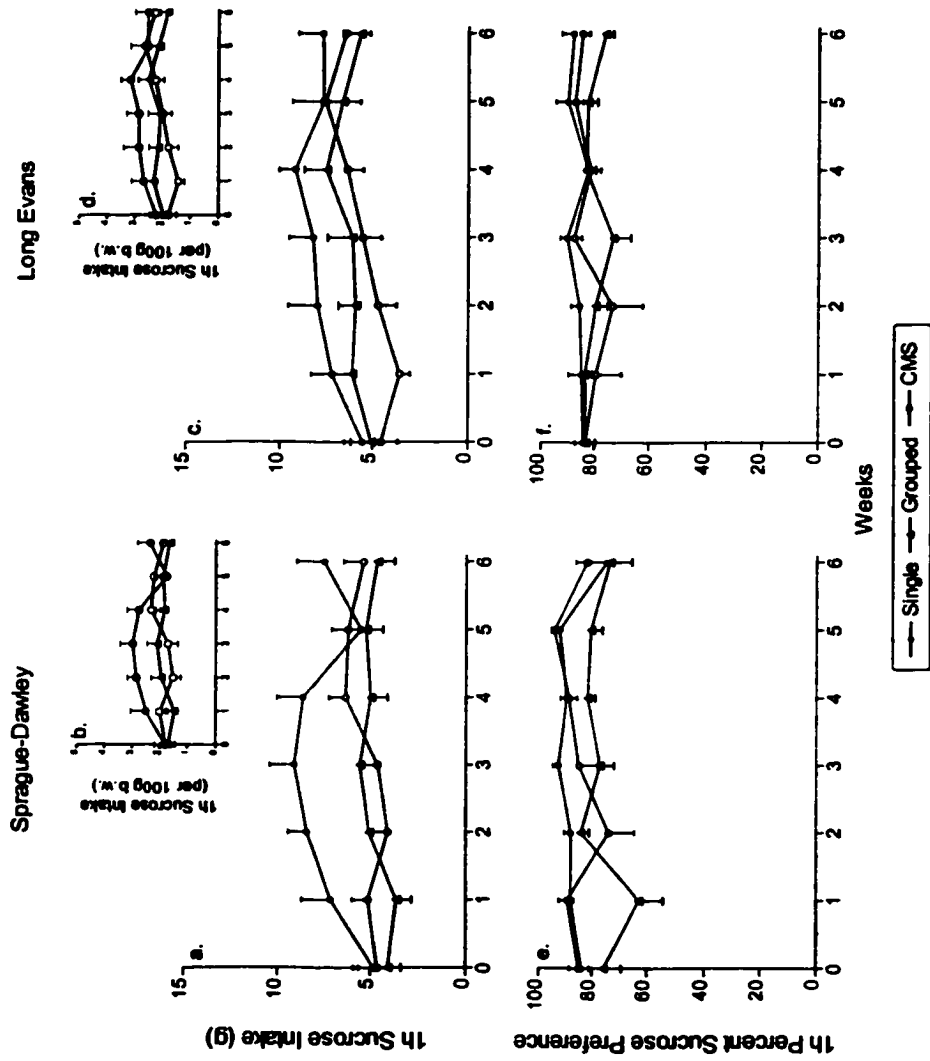


Figure 3. The average 1h sucrose intake (g) \pm standard error of the mean for each group is shown in graphs 3a, & c; note that inset graphs (b & d) display the same data corrected for every 100g of body weight. Graphs e & f show the mean 1h preference values by group. Data from the Sprague-Dawley and Long Evans animals appear in the left and right columns respectively. The legend for the groups is shown at the bottom of the figure.

Study 4.

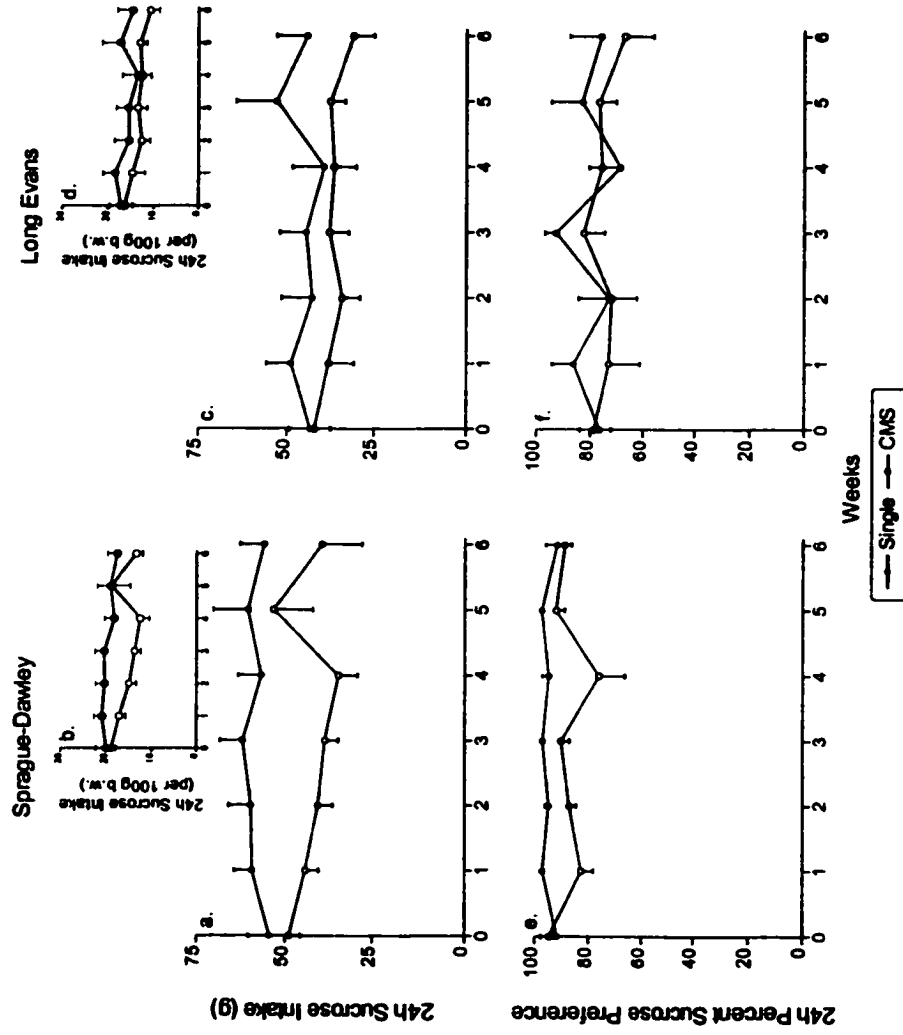


Figure 4. Graphs a & c show the average sucrose intake over a 24h period for each group while the inset graphs (b & d) display the same data as a function of body weight. Twenty-four hour preference values are shown in graphs e & f. The data pertaining to Sprague-Dawley animals appear on the left and those for the Long Evans animals on the right side. The groups are identified at the bottom of the figure.

Study 4.

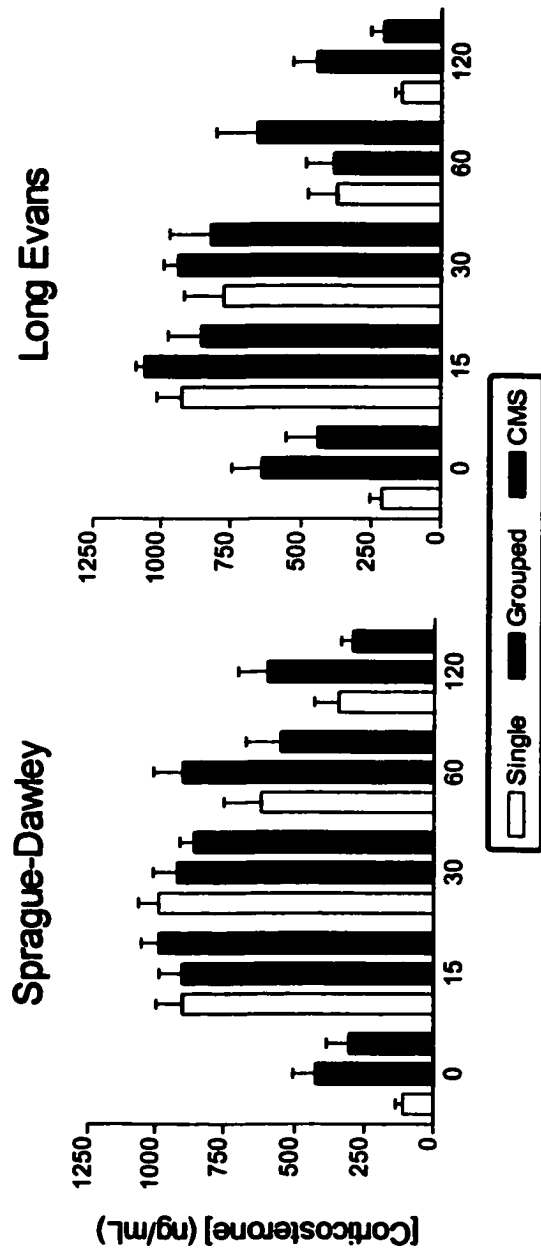


Figure 5. Average corticosterone levels \pm standard error of the mean (ng/mL) per group, in two female outbred rat strains. The Sprague-Dawley related data appear on the left and that of the Long Evans on the right half of the figure. Time 0 represents the corticosterone levels obtained immediately following the six weeks of treatment after which an acute stressor was administered and the corticosterone levels evaluated at times 0-120 min.

Study 4.

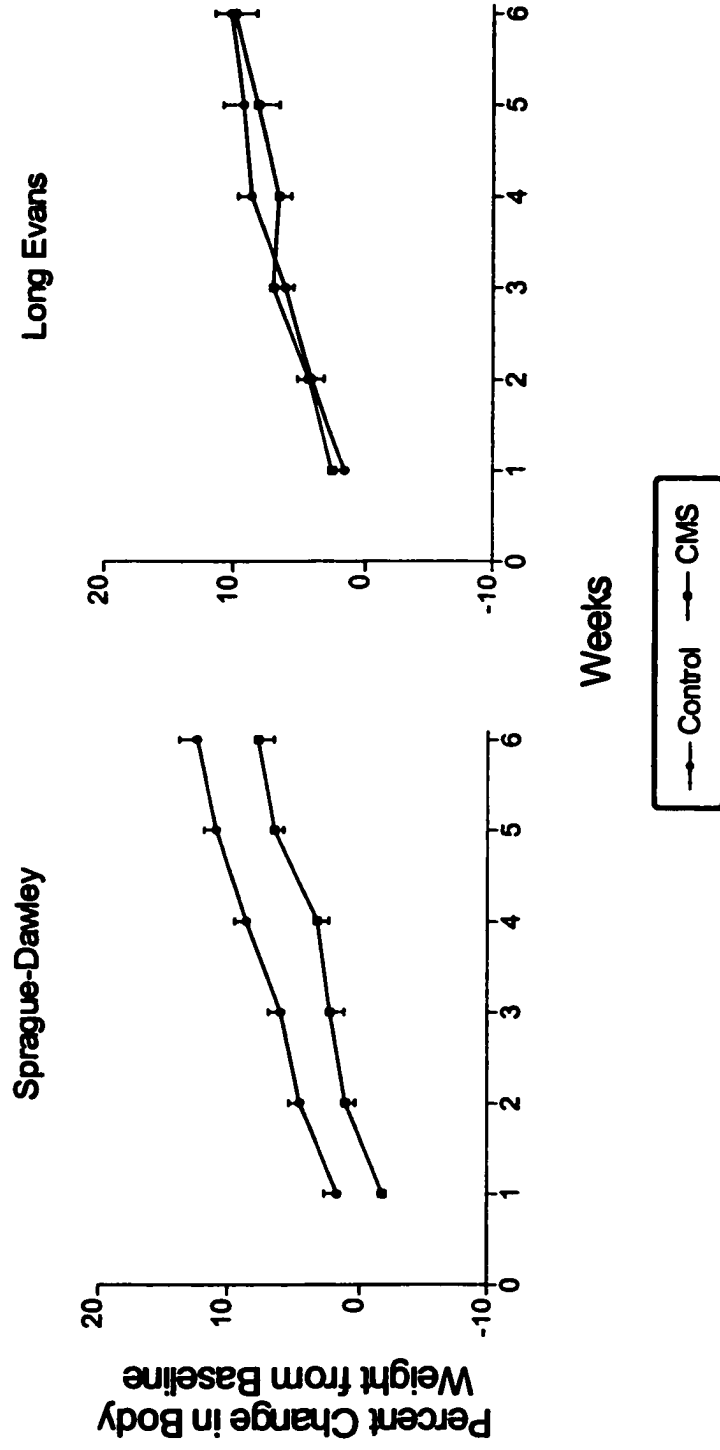


Figure 6. The average change in body weight from baseline for animals in Experiment 2 are shown. Graph a depicts the Sprague-Dawley while graph b the Long Evans animals. The error bars represent the standard error of the mean.

Study 4.

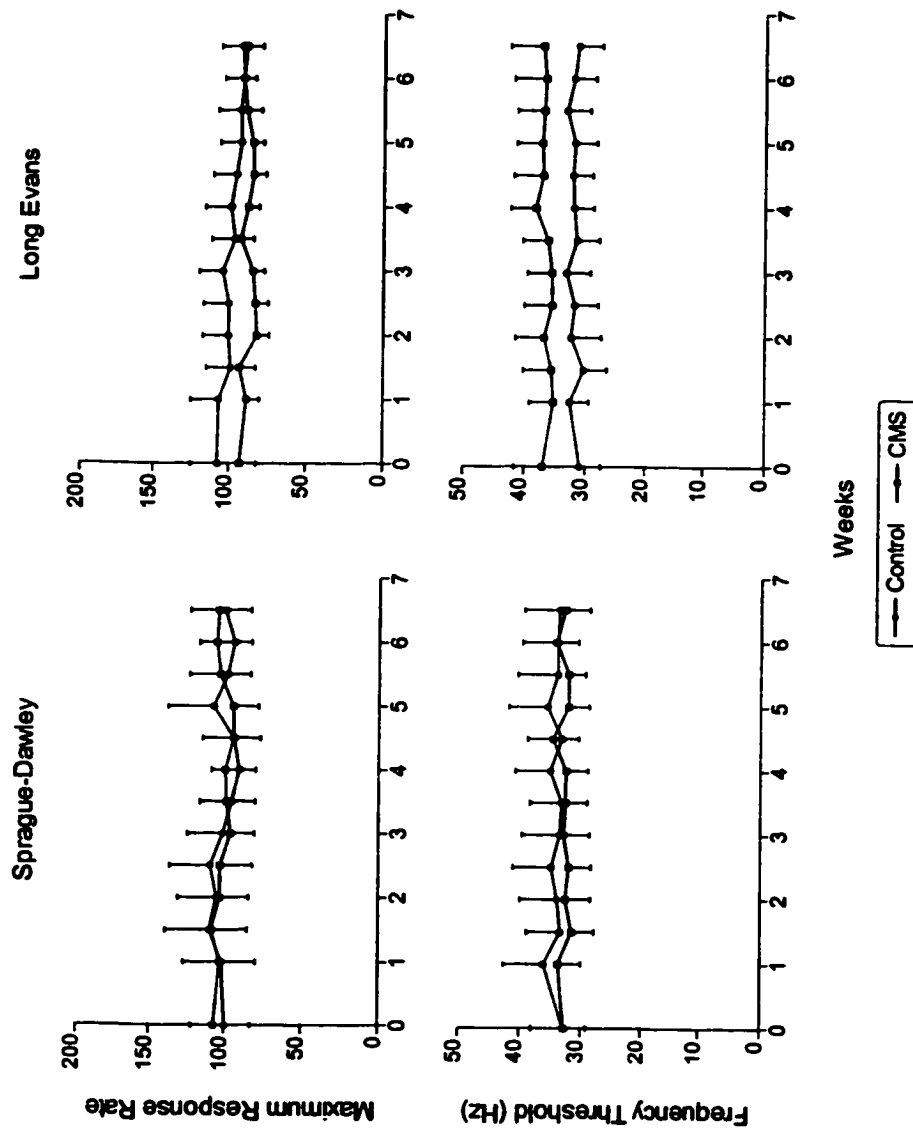


Figure 7. Average change from baseline values in maximum response rates \pm standard error of the mean are shown in graphs a & b. Average frequency thresholds \pm error term, expressed as a change from baseline appear in graphs c & d. The right column represent the Sprague-Dawley while the left the Long Evans animals. The groups are identified at the bottom of the figure.

Study 5

COMPARING STRAIN AND GENDER DIFFERENCES IN REACTIVITY IN THE FORCED SWIMMING TEST: EFFECTS OF PREVIOUS STRESS EXPOSURE

Submitted for publication to Stress as: Bielajew, C, Konkle, A.T.M., Stewart, A., Hutchins, A.A., Santa-Maria Barbagallo, L, and Kentner, A.C. Gender and strain specific effects of chronic mild stress on forced swimming behavior.

Abstract

The chronic mild stress (CMS) procedure was developed in rodents to target anhedonia, the core symptom of depressive melancholia. Stress exposure has been shown to induce a variety of physiological, biochemical, and behavioural alterations relevant to depression, although its anhedonic consequences as indexed by either sucrose intake and preference or thresholds for brain stimulation reward are less reliably observed. In the present study, we assessed the effects of six weeks of CMS on two strains of male and female rats subsequently challenged with an acute psychophysical stressor, forced swimming; their behaviour in the swimming cylinder was evaluated on two consecutive days. Overall, significant differences in forced swim behaviours were observed between male control and CMS groups. In particular, male Long Evans rats with a history of CMS, showed the greatest decrease in the duration of forced swim activity on the second test day, a pattern not observed in the Sprague-Dawley strain of rats, or in any of the female groups. The results suggest that the effects of depressogenic manipulations are strain and gender dependent, with male Long Evans rats most susceptible, as demonstrated by the selective reduction of struggling behaviours. Inclusion of multiple measures, including the forced swim test, would provide a better understanding of the psychopathological profile engendered by chronic exposure to mild stressors and its genetic specificity.

Key words: chronic mild stress, forced swim test, gender differences, animal models, depression

1. Introduction

Animal models of psychopathologies are most often employed for the purpose of screening and developing new therapeutic drugs. The forced swim test (FST) is an example of one such model used for evaluating potential antidepressant compounds (Porsolt, Anton, Blavet and Jalfre, 1978; Porsolt, Bertin, and Jalfre, 1977; Porsolt, Le Pichon et al., 1977). The procedure involves monitoring a rodent's behaviour in a forced swim situation (immersion in a water filled cylinder) before and after drug challenge; activity is typically scored as struggle or immobility. The model is based on the reasoning that initial exposure will produce escape-like responses or behaviours related to struggle, after which defeat or despair-like responses (immobility) will predominate. Re-exposure to this environment quickly induces the characteristic immobile posture in most animals. However, drug tests have shown that following antidepressant administration, immobility is either prevented or delayed (Porsolt, Bertin, and Jalfre, 1978).

This procedure has proved successful in screening such antidepressants as tricyclics (Porsolt et al., 1978; Rénéric and Lucki, 1998), monoamine oxidase inhibitors (Ferigolo, Barros, Marquardt, and Tannhauser, 1998; Porsolt, Anton et al., 1978), selective serotonin reuptake inhibitors (Bourin, Colombel, Malinge, and Bradwejn, 1991; Detke and Lucki, 1996; Rénéric and Lucki, 1998), and some atypical antidepressants such as venlafaxine (Rénéric and Lucki, 1998) bupropion (Hemby, Lucki, Gatto, Singh, Thornley, Matasi, Kong, Smith, Davies, and Dworkin, 1997) and mianserin (Cryan and Lucki, 2000). Furthermore, the test has been shown to adequately discriminate between antidepressants and anxiolytics or

neuroleptics (Kawashima, Araki, and Aihara, 1986; Nishimura, Ida, Tsuda, and Tanaka, 1989; Porsolt, Bertin et al., 1977). One criticism is the lack of a chronic behavioural induction phase such as that typically associated with clinical depression. Instead, the paradigm assesses the acute effects of therapeutic agents, which are not usually assessed in humans.

In addition to being a reliable predictor of antidepressant activity, evaluation of endocrine measures suggest that the FST procedure is a powerful stressor; plasma corticosterone levels have been reported to increase by as much as five times that of basal levels following exposure to FST (Connor, Kelly, and Leonard, 1997) with rapid return to pre-stress levels.

Another animal model developed to simulate a depressive state is one based on the consequences of exposure to chronic mild stressors (CMS). A history of experience with low intensity stressors is thought to parallel the development of depressive symptomatology in humans, in whom for example, a positive relationship between daily life strains and depression has been reported (Anisman and Zacharko, 1982; Kendler et al., 1995; Kessler, 1997).

The effects of CMS on a variety of physiological, biochemical, and behavioural endpoints have been assessed in rodents. The chronic regime of stressors is typically associated with alterations in sleep (Cheeta et al., 1997; Moreau et al., 1995), and frequently weight loss or decrease in weight gain in stressed animals (Bielajew et al., 2002; D'Aquila, Monleon et al., 1997; Dunčko, Kiss et al., 2001; Harris et al., 1998; Harro et al., 1999; Hatcher et al., 1997; Matthews et al., 1995; Muscat et al., 1988; Nielsen et al., 2000; Willner et al., 1994) as well as adrenal gland hypertrophy (Baker et al., 2002) and a decrease in thymus weight (Kubera et

al., 1998).

Assessment of biochemical changes associated with CMS have been reported as elevations in plasma corticosterone levels (Bielajew et al., 2002; Harris, et al., 1998; Konkle, et al., 2002), although some investigators have failed to differentiate between CMS and control animals on the basis of this measure (Harris, et al., 1998; Stout et al., 2000; Willner et al., 1987). However, we have recently shown in one rat strain (Long Evans) exposed to an acute stressor following a history of CMS, a corticosterone response indicative of adaptation to stress exposure (Bielajew et al., 2002), suggesting a strain-specific phenomenon.

The effects of CMS measured using behavioural indices has been reported to both reduce (Harro et al., 1999) and increase (Benelli et al., 1999; Harris et al., 1998) rearing frequency in the open field apparatus as well as decrease motility in some reports (D'Aquila et al., 2000b).

The anhedonic consequences of CMS have been demonstrated by a decrease in intake or preference for a mild sucrose solution in animals exposed to the chronic regime of stressors (Willner et al., 1987, Baker et al., 2002; D'Aquila, Newton et al., 1997; Nielsen et al., 2000). When hedonic status following a history of mild stressors is evaluated instead by determining the thresholds for brain-stimulation reward, one group consistently demonstrates an increase in frequency thresholds following CMS (Moreau et al., 1994; Moreau, et al., 1995; Moreau et al., 1996; Stout et al., 2000), while we and others show either no difference (Kentner et al., 2002; Nielsen et al., 2000) or a slight facilitation in thresholds (Lin et al., 2002) in CMS animals.

The difficulty to replicate CMS induced effects experienced by some investigators has created doubt as to its usefulness as an animal model (Willner & Mitchell, 2002). An

interaction between the low intensity of the stressors and the robustness of the behavioural measures as well as strain differences in stress susceptibility may explain the lack of consistency, especially in the behavioural data, across laboratories.

Thus, we decided to employ a different strategy, and evaluate the effects of CMS on responsiveness to a potent psychogenic stressor - forced swimming. To our knowledge, only one other group (Harro et al., 1999) has pursued this question. In their study, while the behaviour of intact CMS animals was not different from their control counterparts, animals that were first treated with the noradrenergic denervation agent DSP-4 decreased their period of immobility in FST compared to appropriate control animals, suggesting an antidepressant role for CMS under these conditions.

In view of the fact that some strains appear to adapt to the chronic stress regime, at least when subjected to an acute novel stressor, (Bielajew et al., 2002), we evaluated the FST in two outbred rat strains, Sprague-Dawley and Long Evans. Male and female rats were included in order to contrast their responses.

2. Methods

2.1 Animals

Forty six rats were used in this study, 26 Sprague-Dawley and 20 Long Evans. These were further divided into control and CMS groups.

2.2 Behavioural Tests

Following six weeks of CMS administration (see schedule of stressors in Table 1), animals

Insert Table 1 about here

were introduced to a modified version of the FST. They were placed in an opaque acrylic cylinder (62.5 cm in height, 32.5 cm in diameter) filled with water (24°C) up to 14 cm from the rim (or to a depth of 48.5 cm). The wall of the cylinder was shrouded with black plastic. Each rat was immersed in the cylinder for a 5 min trial, then removed, dried, and returned to a heated cage. The procedure was repeated the next day. Both tests were conducted between 0800 and 1000 h.

Test sessions were videotaped (Sony color videocamera) and later viewed by an investigator unaware of the treatment conditions. Scoring via the aid of customized software (Stewart Software, Inc.) consisted of identifying which behaviours each animal exhibited during consecutive one minute bins; note that in addition to frequency scores, the duration associated with each behaviour was also recorded. The documented behaviours are described in Table 2.

Insert Table 2 about here

3. Results

The intra-rater reliability was estimated at 0.96. A second scorer evaluated roughly 15% of the sessions in order to assess inter-rater reliability; consistency of scoring based on a Pearson correlation coefficient was 0.97 (Howell, 2002).

In order to evaluate the length of the swim test as adequate for detecting changes in swim behaviours, we compared the percent of total frequency and duration engaged in active behaviours (swimming with struggle, climbing, and diving) in the first one minute (bin 1) to

the remaining four minutes (bins 2-5) of the swim session. The percent difference in active behaviours between bin one and bins 2-5 in each session (referred to as Day 1 and Day 2) are shown in Table 3.

Insert Table 3 about here

With one exception, activity levels were always greater in the first bin as shown by positive difference values.

In all groups, behaviour 3 - swimming with struggle - was the most prominent. The behavioural pattern in male control and CMS groups was similar on the first test day whereas on the second test day, animals in the CMS group tended to replace behaviour 3 with an increase in floating and swimming without struggle. In female rats, control and CMS animals exhibited similar behavioural patterns that remained relatively unchanged between the first and second test days. In order to facilitate the analyses and the comparison of our data with that of others, we grouped the seven behaviours into clusters of active, passive, or other behaviours. The active cluster comprised swimming with struggle, climbing, and diving, and the passive cluster, floating and sinking; because swimming without struggle and grooming were not defined as escape or despair behaviours, they were classified as others. These data are shown in Figure 1 (male Sprague-Dawley and Long Evans) and Figure 2

Insert Figures 1 & 2 about here

(female Sprague-Dawley and long Evans) expressed as frequency (top half) and duration (bottom half). Each cluster was analyzed separately by strain and gender via a mixed ANOVA design with group as the independent factor group (control vs CMS) and day (1 vs 2) as the repeated factor (Howell, 2002). Analysis of the frequency values associated with

the active cluster of behaviours recorded in male rats indicated significant main and interaction effects in the Sprague-Dawley groups (group: $F(1,10)=11.98$, $p<.007$; day: $F(1,10)=12.02$, $p<.007$; group x day: $F(1,10)=6.20$, $p<.04$) and a significant interaction between group and day in Long-Evans animals ($F(1,6)=6.68$; $p<.05$); the main effect of day just failed to reach significance. The analysis of the frequency scores of the passive and other behaviours revealed no differences in the male groups of rats. The results based on the duration of active behaviours yielded significant effects of day in both strains (Sprague-Dawley: $F(1,10)=7.38$, $p<.03$ and Long Evans: $F(1,6)=17.69$, $p<.006$) but no group effect. However, the interaction in the case of the Long Evans groups was significant ($F(1,6)=18.79$, $p<.005$). Behaviours classified as “others” also gave rise to a significant interaction in Long Evans rats ($F(1,6)=9.99$, $p<.02$) and main effects of day in both strains (Sprague-Dawley: $F(1,10)=7.77$, $p<.02$; Long Evans: $F(1,6)=8.13$; $p<.03$).

The analyses of the data obtained from female groups only revealed a difference in day based on the frequency and duration of active behaviours in Sprague-Dawley rats (frequency: $F(1,12)=13.05$, $p<.004$; duration: $F(1,12)=6.38$, $p<.03$). In the same strain, the duration of passive behaviours yielded a significant interaction between group and day ($F(1,12)=7.89$, $p<.02$) and finally, a main effect of day in the case of other behaviours ($F(1,12)=5.89$, $p<.04$). Otherwise, no other results were statistically significant.

4. Discussion

There were two main findings in the present study. First, the effects of previous exposure to CMS on forced swimming behaviours were gender dependent, that is, more evident in

male than in female rats. Second, these differences were strain dependent, more prominently observed in Long Evans male rats. Activity levels, as expressed by frequencies, were generally higher on the first test day in males of all groups, especially in Sprague-Dawley rats with a history of CMS experience. However, re-exposure to the test produced a dramatic decrease in “escape-like” behaviours in Long Evans rats previously exposed to chronic stressors.

In the past, a single variable - change in immobility scores - was often used to evaluate the effectiveness of antidepressants (Porsolt, Bertin, et al., 1978). In recent years, it has become more common to assess drug effects on the basis of at least two categories of FST behaviour, immobility and activity (Bourin, Redrobe, and Baker, 1998; Einat, Karbovski, Korik, Tsalah, and Belmaker, 1999; Maj, Rogóż, Skuza, and Koiodziejczyk, 1997; Redrobe and Bourin, 1998). In this study we were able to easily discriminate seven different behaviours, for which rater reliability was very high - 97%.

A second modification was the length of the first swim exposure. Many investigators subject animals to 15 min of forced swim on the day prior to drug tests, while in the present study the first session was shortened to 5 min. In order to verify if the session length was sufficient to detect changes in active behaviours (swimming with struggle, climbing, and diving), we compared separately the percent frequency and duration of the total time spent in active behaviours during the first minute (bin 1) with the last four minutes (bins 2 - 5) of the trial on each test day; the difference between bins is shown in Table 3. Except for one case, animals were always more active in the first bin than the last four, the difference ranging from 15.5 to 50.8%, suggesting that activity declined rather quickly in the first session and

that 5 min of swimming served as a sufficient psychophysiological stressor. Furthermore, Armario et al., (1988) have reported no overall differences in struggling, swimming, or immobility between animals subjected to 5 or 15 min of forced swimming, and this on either of three consecutive test days.

In this study, the ratio of active to passive behaviours was notably different from that typically reported in the literature. Animals spent less than 10% of their time in passive behaviours on each test day, whereas others have observed as much as a 70% duration of immobility. This discrepancy may be due to the difference in the session length. Other factors such as recording of immobility time (Thornton, Evans, and Harris, 1986), water depth (Detke and Lucki, 1996), age (Yates, Panksepp, Ikemoto, Nelson, and Conner, 1991), and circannual rhythms (Abel, 1995) have also been shown to influence FST behaviour.

The results of the analyses on the active cluster of FST behaviours indicate that the effects of previous exposure to CMS were much less evident in female rats; very little difference in either frequency or duration of active behaviours was discernable between the two treatment groups on both swim tests. Furthermore, activity levels in female rats exposed to CMS did not change on the second day of observation compared to the first in either strain employed. There may be several reasons why differences in female groups were little apparent. First, females may be less vulnerable to the effects of CMS. Due to the limited literature exploring the behavioural effects of CMS in females, it is difficult to determine whether this is in fact the case. On measures typically employed to evaluate hedonic changes related to CMS, we and another group have shown a modest reduction in sucrose intake (Dunčko, Kiss et al., 2001; Konkle, Baker et al., submitted) but fail to find any behavioural consequence of CMS

on thresholds for brain-stimulation reward (Konkle, Baker, et al., submitted). However, biochemical and physiological responses are more evident. For example, we have shown elevated corticosterone levels, a reduction in weight gain, and abnormal estrous cycling related to CMS exposure (Konkle, Baker et al., submitted), suggesting that the stressors are effective, at least as indexed by these measures.

Second, female rats are generally more active in such paradigms (reviewed in Barros and Ferigolo, 1998), showing increased signs of struggle compared to male animals. This might produce difficulty in discerning any behavioural deficits related to previous stress exposure; however, female offspring of dames exposed to prenatal restraint show greater immobility than their control counterparts (Alonso, Arevalo, Afonso, Rodríguez, 1991). Gender differences in reactivity to forced swimming are also observed in drug tests; administration of the antidepressant imipramine in male rats decreases the duration of immobility whereas in female rats, the frequency, but not duration, of immobility and climbing behaviours is altered by the treatment (Barros and Ferigolo, 1998).

In male rats, although the group differences in the frequency of active behaviours were much greater in the Sprague-Dawley strain, the reduction in active behaviours during the second swim test was roughly proportional between strains. The Sprague-Dawley rats in the CMS group tended to alternate between swimming with struggle and climbing much more than the comparable Long Evans group, which accounts for their high frequency scores on the first test day. However, the analysis of the duration of each cluster suggests that Sprague-Dawley animals exposed to CMS showed little alteration in their pattern of responding from first to second session, similar to the pattern observed in the corresponding control group.

The time spent in active behaviours was likewise similar within each session. One interpretation is that this strain is less vulnerable to chronic stressors; their response is similar to what is observed in animals re-exposed to forced swimming following administration of an anti-depressant agent. Alternatively, the six weeks of CMS in these animals may have conditioned them against further stressors. For example, in support of this argument, one group has observed a reduction in immobility time on the second test day in male Wistar rats exposed to 15 days of CMS (Harro et al., 1999); however, it is unknown whether these animals instead showed a compensatory increase in struggle or other behaviours.

The Long Evans CMS group on the other hand was distinctly different on the second forced swim test, reducing duration of active behaviours by more than 50% and spending more time engaged in both passive and other behaviours. Given this profile, the significant reduction in the duration of active behaviours from the first to second session does not suggest that the animals were demonstrating only behavioural despair as active behaviours were replaced equally by grooming and swimming without struggle (other behaviours) and sinking and floating or passive behaviours indicative of despair. The fact that animals engaged in both passive and other behaviours may indicate some degree of adaptation to novel stressors as a result of previous stressor experience. Passive and immobile responses are usually characteristic of learned helplessness. This would occur because animals would learn from their first exposure to forced swim that attempting to escape is futile. If we evaluated the behavioural profiles on the basis of struggle versus no struggle categories, then the differences in responding between the two test days in Long Evans rats with a history of chronic stress is particularly evident. Previous work from our laboratory also supports a

strain difference between Sprague-Dawley and Long Evans rats with respect to biochemical consequences of CMS. Male Long Evans rats exposed to six weeks of CMS show elevated plasma corticosterone levels compared to control animals (Konkle, Baker et al., submitted), while little difference between the comparable Sprague-Dawley rats is observed.

Strain differences in reactivity in the FST have been well documented. For example, C57 and DBA mice naive to the procedure displayed drastically different basal levels of activity and immobility upon their first exposure whereas a subsequent experience similarly altered the time spent immobile and simply swimming (Alcaro, Cabib, Ventura, Puglisi-Allegra, 2002). Another group showed a greater frequency of immobile behaviours and lower frequencies of swimming and climbing behaviours in Wistar-Kyoto rats compared to Sprague-Dawley (López-Rubalcava and Lucki, 2000).

In summary, the data presented here lend further support to the growing literature on genetic variation with respect to stress susceptibility. The FST appears to be sensitive to detecting the behavioural consequences of CMS in males distinguishing strain differences whereas in female rats, stress history does not alter swim behaviour. These genotype-related distinctions lend themselves well to modeling the susceptibility of some humans to develop depressive symptomatology as a consequences of daily life hassles. In these individuals, subsequent exposure to a more severe stressor may elicit a learned-helplessness type of response, a characteristic symptom of depression. These findings speak to the importance of utilizing multiple and varied measures in the evaluation of the effects of stressors.

Table 1. Schedule of chronic mild stressors administered over a seven day period and repeated for six weeks prior to FST exposure.

	Monday	Tuesday	Wednesday	Thursday	Friday
0800 am	Confinement 1 h	ICSS	Exposure to empty bottle 1 h	Restricted food 2 h	ICSS
Noon		Confinement 1 h	Confinement 1 h	Confinement 1 h	Confinement 1 h
0200 pm	Confinement 1 h	Confinement 1 h		Confinement 1 h	
0400 pm	Overnight illumination with 30° cage tilt	Overnight water deprivation	Overnight food & water deprivation	Overnight pairing in wet bedding	Reversed light/dark cycle over weekend

Table 2. A description of each of the seven behaviours scored during FST.

<u>Behaviour</u>	<u>Description</u>
1. Floating	The animal remaining motionless with no movement of the limbs
2. Swimming without struggle	Rat making swimming motions necessary to keep its head above water
3. Swimming with struggle	Rat making swimming motions with vigorous movements of all paws
4. Climbing	Rat making vigorous movements of its forepaws in and out of the water directed at the walls
5. Diving	Rat's body is submerged under water, typically head towards the bottom
6. Grooming	Face washing with paws, head shaking
7. Sinking	A typical falling motion such that the rat is submerged under water with its head facing upward

Table 3. A comparison of bin 1 and bins 2-5 of the 5 in trial in the frequency and duration of active behaviours.

<u>Males</u>				
	<u>Sprague-Dawley</u>		<u>Long Evans</u>	
	<u>Control</u>	<u>CMS</u>	<u>Control</u>	<u>CMS</u>
<u>Difference (bin 1 vs bins 2-5) in the percent frequency of active behaviours</u>				
<u>Day 1</u>				
Difference	21.5	20.1	15.5	19.7
<u>Day 2</u>				
Difference	19.4	29.2	20.9	26.6
<u>Difference (bin 1 vs bins 2-5) in the percentage of time spent in active behaviours</u>				
<u>Day 1</u>				
Difference	38.0	20.4	29.5	22.5
<u>Day 2</u>				
Difference	<u>33.8</u>	<u>35.4</u>	<u>17.6</u>	<u>39.6</u>
<u>Females</u>				
<u>Difference (bin 1 - bin 2-5) in the percent frequency of active behaviours</u>				
<u>Day 1</u>				
Difference	33.6	28.1	26.2	39.1
<u>Day 2</u>				
Difference	30.7	33.9	17.6	33.2
<u>Difference (bin 1 - bin 2-5) in the percentage of time spent in active behaviours</u>				
<u>Day 1</u>				
Difference	41.0	50.8	20.6	25.9
<u>Day 2</u>				
Difference	44.0	41.7	- 1.4	20.5

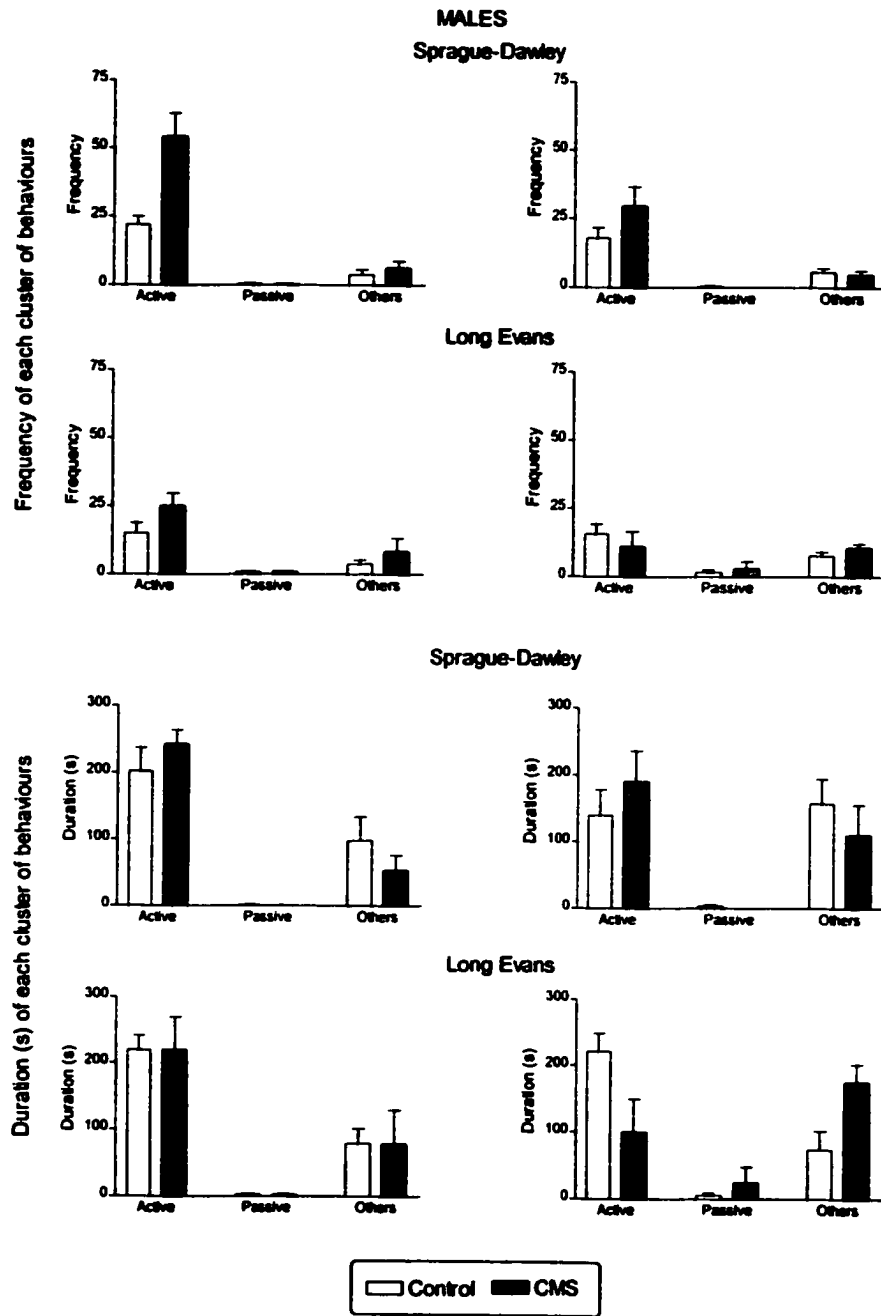


Figure 1. Data pertaining to male Sprague-Dawley and Long Evans rats. The figure depicts the frequency of occurrence (top two rows) and duration (bottom two rows) of the seven behaviours (divided into three clusters of active, passive, and other behaviours) that were monitored on each day (Day 1 on the left and Day 2 on the right) of forced swim. A description of each behaviour is presented in Table 2.

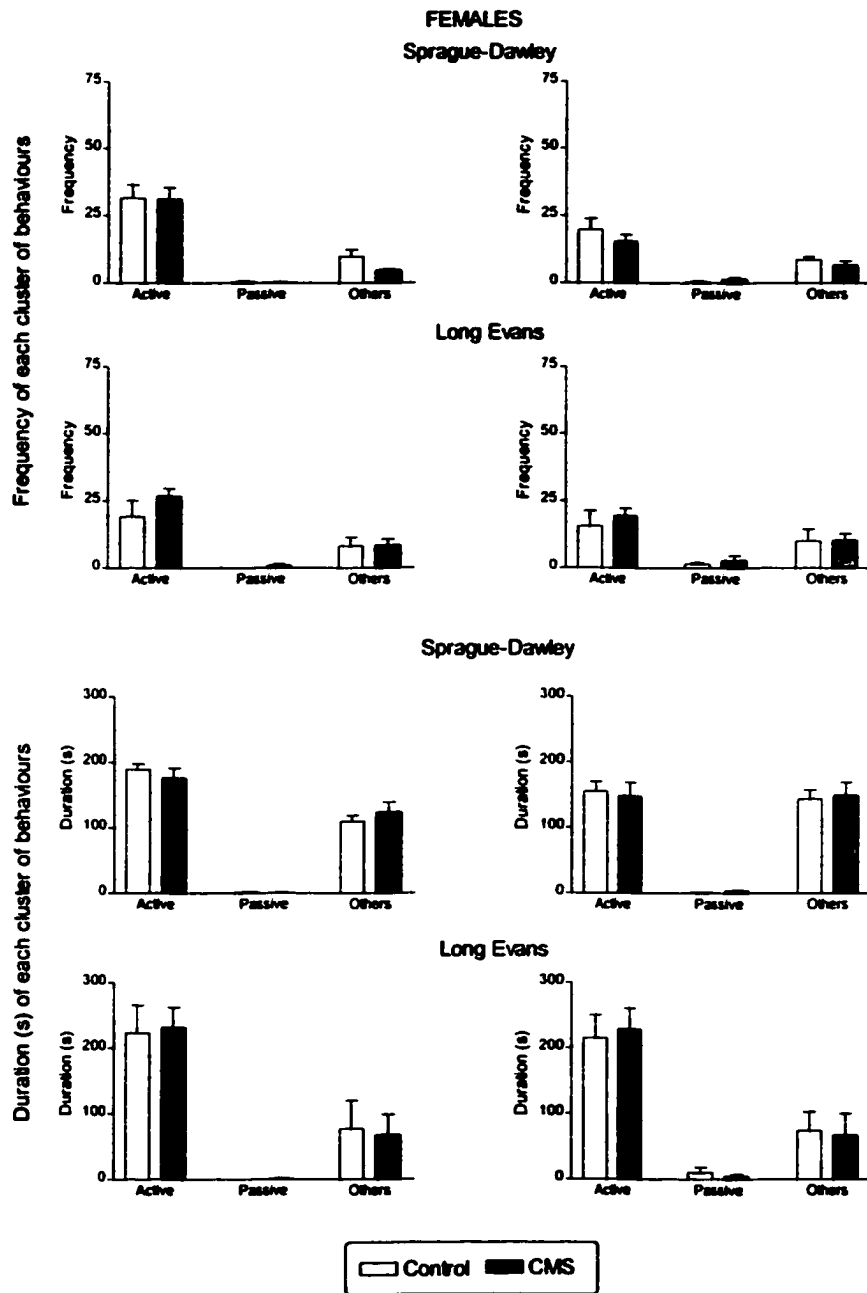


Figure 2. Data pertaining to female Sprague-Dawley and Long Evans rats. The figure depicts the frequency of occurrence (top two rows) and duration (bottom two rows) of the seven behaviours (divided into three clusters of active, passive, and other behaviours) that were monitored on each day (Day 1 on the left and Day 2 on the right) of forced swim. A description of each behaviour is presented in Table 2.

GENERAL DISCUSSION

The goal of the studies reported in this thesis was to evaluate the behavioural, physiological, and biochemical consequences of CMS, considering potential genetic differences in vulnerability to stressors. This investigation was prompted by the first finding that the antidepressant, paroxetine, only produced a modest decrease in BSR thresholds, similar to what has been reported with TCAs (Fibiger and Phillips, 1981; Hall et al., 1990; Markou et al., 1992; McCarter and Kokkinidis, 1988). The small drug effect is likely related to the fact that the animals were “normal”, and the results similar to what is observed in the clinical population when control (or non-depressed) individuals are administered an antidepressive agent (Barr et al., 1997). Given this finding, we explored manipulations that would produce effects modeling clinical depression.

The CMS paradigm was developed by Willner to model clinical depression in animals by inducing anhedonia, a core symptom of depression (Willner et al., 1987). While some investigators have consistently shown CMS-induced deficits in behavioural indices of anhedonia (Moreau, Borgulya et al., 1994; Moreau, Bourson et al., 1994; Moreau et al., 1996; Muscat et al., 1992; Papp et al., 1996), others have reported difficulties in replicating these findings (Lin et al., 2002; Nielsen, et al., 2000; Harris et al., 1998; Matthews et al., 1995).

As a first step we chose to evaluate the behavioural indices typically used to assess the effects of CMS in order to better understand the reliability issues associated with the model and how these affect its replication. The behavioural measures included BSR thresholds, sucrose consumption, and activity in the FST. A six week exposure to mild stressors produced relatively no change in BSR thresholds, in either male or female, Sprague-Dawley

or Long Evans rats. The effects of CMS on another behavioural measure, the intake and preference for a mild sucrose solution yielded modest results. In male rats we detected a reduction over time in 1h sucrose intake and in female rats, a similar pattern in the 24h intake measure; note that preference was relatively unaffected by CMS. Behavioural differences in stress reactivity to an acute psychophysiological stressor, a 5 min exposure to forced swimming, were also evaluated in rats with and without prior stress experience. While most CMS groups displayed a slightly higher frequency of active behaviours on the first test day compared to control animals, only male Long Evans rats showed a substantial reduction in these types of behaviours compared to their control mates on the second test day. Whereas time spent in any cluster of behaviours did not differ between the groups on the first day, again, male Long Evans rats previously exposed to CMS displayed a marked shift from the time spent in active, or struggling types of behaviours to those reflecting a learned helplessness profile. The next step was to determine if the stressors were effective as such, by assessing CMS-induced changes on physiological and biochemical endpoints. The physiological measures included rate of body weight gain, adrenal gland and spleen weights, and estrous cycle regularity in female rats. Overall, weight gain was slower in both female and male rats exposed to the stressors and the estrous cycle was significantly disrupted with chronic exposure to these stressors, more prominently in the Long Evans rats, suggesting an increased vulnerability to stress in females of this strain.

The biochemical analyses were based on plasma corticosterone levels following first, chronic exposure to stressors and second, subsequent administration of an acute stressor. Results of these analyses revealed that exposure to three or six weeks of CMS significantly

elevated corticosterone levels in stressed rats relative to control groups. However, the difference in reactivity between control and CMS animals to a subsequent 10 min restraint stress revealed no significant group differences.

The inconsistent behavioural consequences of CMS exposure, particularly with respect to the induction of anhedonia, the core symptom of depression that it is purported to model, suggests that it is of limited utility as an animal model in which to study depression.

However, even though the effects of stress are not reliable as indexed by typical behavioural measures of anhedonia, its effects on physiological and biochemical ones appear to be more dependable. This may suggest that the chronic application of mild stressors does in fact induce anhedonia, but that the current methodology is not appropriate or sensitive to the hedonic changes. This hypothesis is further explored in the sections that follow.

EFFECTIVENESS OF THE STRESSORS

These findings are generally in accordance with what has been reported by others. Evaluation of adrenal glands and spleen weight revealed no overall effect of CMS; adrenal gland hypertrophy was only found in male Long Evans rats exposed to six weeks of stressors. While severe stressor exposure is typically associated with an increase in adrenal gland weight (Burchfield et al., 1980; Hennessy et al., 1979; Pignatelli et al., 2000), the effects of mild stressors such as those employed in the CMS procedure have not been generally evaluated. One group reported no effect of three weeks of CMS on adrenal gland weight in male or female rats (Dunčko, Kiss et al., 2001); to our knowledge, no one has previously evaluated the effects of CMS on spleen weights.

The CMS literature is inconsistent with respect to the effects of mild stressors on plasma corticosterone levels. Some investigators have reported no change in corticosterone values with CMS exposure (Azpiroz et al., 1999; Dunčko, Kiss, et al., 2001; Stout et al., 2000; Willner et al., 1987), while others have shown an increase related to stressor application (Ayensu et al., 1995; Lanfumey et al., 1999). However, one group has demonstrated a strain difference between Wistar and Sprague-Dawley rats in susceptibility to a CMS-induced increase in corticosterone levels following a three week exposure to the mild stressors (Harris et al., 1998). In the present studies, male rats exposed to three weeks of CMS showed elevated corticosterone levels. However, analyses of corticosterone levels following a six week exposure to mild stressors revealed a CMS-induced increase dependent on strain and gender; in female rats, CMS was associated with elevated corticosterone levels in the Sprague-Dawley but not the Long Evans strain, while this pattern was reversed in males.

We were also interested in evaluating whether CMS exposure would alter the corticosterone response to a novel acute stressor. Monitoring the time course of the effect of restraint stress on corticosterone levels revealed no discernable CMS effect; a peak in corticosterone levels was evident 30 min following application of the acute stressor in both control and CMS animals and levels typically returned to near basal ones by 120 min.

The main finding from these studies is a strain difference in stress vulnerability. In synthesizing the results of the four studies based on the CMS paradigm we conclude that Long Evans rats are more susceptible to the effects of stressors. A review of the data indicates first, a reduction in the hedonic value of a mild sucrose solution in both male and female rats, second, a tendency to reduce struggle behaviours in the forced swim test, third,

an increase in corticosterone levels associated with three and six weeks of stressor exposure, indicating enhanced HPA axis activity, four, adrenal gland hypertrophy in male rats following six weeks of CMS expected with HPA axis hyper-responsivity, and five, a greater disruption in estrous cycle regularity in female Long Evans rats, a phenomenon that is well-known in the clinical literature of depressed women.

Taken together, the results of the physiological and biochemical assessments suggest that the chronic manipulations are producing a disturbance, at least in the strains examined in these studies. However, whether anhedonia is a significant consequence of stressor exposure or the behavioural tools employed capable of detecting it is unclear.

BEHAVIOURAL INDICES REVISITED

Most investigators evaluate the anhedonic effects of CMS on intake and/ or preference for a mildly sweet solution, although there have been criticisms regarding their use in this context (Matthews et al., 1995; Forbes et al., 1996). These have been discussed in detail earlier (see study 3) so they will only be briefly referred to in this section. The first issue is the use of sucrose in solution, given that its caloric value, albeit minute, may play an incentive role in consumption in food deprived animals. To counter this argument, the use of calorie-free saccharin was substituted and did not alter the results reported using sucrose (Harris et al., 1998; Hatcher et al., 1997; Pucilowski et al., 1993; Willner et al., 1987). A second issue is the necessity for the inclusion of a period of food deprivation prior to intake tests. Hatcher et al. (1997) have in fact demonstrated that the CMS-induced reduction in saccharin intake is dependent on the animal's feeding status. In one experiment, they showed no CMS-induced

decrease in saccharin intake when food but not water deprivation was omitted from the CMS procedure. Furthermore, they demonstrated that increasing the interval between food deprivation and intake tests abolished the CMS-induced reduction in saccharin intake (Hatcher, Bell, Reed, and Hagan, 1997).

Our concern was related in that we felt that the 1-h sucrose intake test immediately following a period of overnight food deprivation would be confounded by the deprivation procedure. Thus, we included a 24h intake measure with food replacement in order to better discern any CMS effects due to anhedonia rather than the animal's physiological state. Whereas no deficit in sucrose intake was apparent for male Sprague-Dawley rats, an initial decrease was found in the Long Evans strain in both the 1 and 24h measures. In females, a gradual decrease was apparent in the 24h measure in Sprague-Dawley and to a lesser extent in Long Evans animals. However, when sucrose preference was instead evaluated, these CMS-induced effects, in male and female rats, were no longer detectable. Given that both sucrose and water intake were decreased, it is unlikely the intake deficits reflect uniquely a state of anhedonia. Willner (1997b) has argued that evaluating water intake separately from the sucrose or saccharin test may be a better practice, rather than presenting both sucrose and water solutions together as is typically done to assess preference. The reasoning is that since basal water intake is typically very low compared to sucrose, conducting two-bottle tests may lead to false positive results. That is, sucrose preference will be interpreted due to a decrease in sucrose intake and no change in water intake due to a floor effect. Given this concern, it may be best to modify this behavioural test in such a way as to enable investigators to scale sucrose preference. One way to achieve this could be to conduct a multi-bottle test, each

containing a different concentration of sucrose. Scaling concentration preference in this way may provide a better indication of the appetitive nature of the solutions.

In evaluating changes in BSR thresholds associated with CMS exposure, only one group has consistently observed the expected pattern (Moreau et al., 1992; Moreau, Borgulya et al., 1994; Moreau, Bourson et al., 1994; Moreau et al., 1995; Moreau et al., 1996). They report a gradual threshold increase, reaching a maximum of 30% change from baseline by the end of the second week of stress exposure. Others, however, including ourselves, fail to detect similar threshold changes; Nielsen et al. (2000) show no overall effect of CMS in Wistar or PVG rats, while a slight facilitation in the rewarding effect of the stimulation has been reported by another group (Lin et al., 2002). We found no change in threshold in females of either strain and male Sprague-Dawley rats; however, Long Evans male rats showed an overall reduction in threshold, predominantly due to small threshold changes that were observed in a couple of animals. These effects tended to be less than the criterion for stability so are not felt to be meaningful. Parenthetically, we have recently conducted pilot work in female rats in which we evaluated the consequences of three weeks of CMS on BSR thresholds and sucrose consumption in the same animal. While CMS had no effect on BSR thresholds, a substantial reduction in sucrose intake on the 24h measure was found, suggesting that the behavioural indices are differentially sensitive to the effects of chronic stressors.

The difficulty of altering BSR thresholds with exogenous manipulations is well known. While some drugs produce large changes (see Stellar and Stellar, 1985 for review) others such as antidepressants only do so modestly (Fibiger and Phillips, 1981; Hall et al., 1990;

McCarter and Kokkinidis, 1988). Even the application of intracerebral lesions, used to evaluate the functional connections of the reward pathway, generally only produce small alterations in BSR thresholds, at best (Arvanitogianis, Waraczynski, and Shizgal, 1996; Gallistel, Leon, Lim, Sim, and Waraczynski, 1996; Johnson and Stellar, 1994; Simmons, Ackermann, and Gallistel, 1998). In fact, other than drug challenges, few manipulations are successful in altering BSR thresholds. For example, we have examined the effects of chronic enrichment and found no change over time in thresholds (see appendix 1). One treatment that did result in attenuated reward thresholds was an increase in the difficulty of bar pressing by the addition of excessive weight to the self-stimulation lever (Fouriezos, Bielajew, and Pagotto, 1990). Finally, exposure to stressors, in the form of footshock has also produced increases in BSR thresholds. However, these changes have been shown to be dependent on the stimulation site, that is, animals with dorsal ventral tegmental electrode placements exhibited a small threshold shift while those with more ventral placements were unaffected by the stressors (Zacharko et al., 1990). We have previously reported a lack of such a relationship for the animals that contributed to the studies reported in this dissertation, and refer you to appendices 3 and 4 for the thresholds obtained in individual animals and the histological diagrams, respectively.

The lack of consistency and difficulty in replicating CMS-induced behavioural deficits may suggest that we lack the proper tools to evaluate hedonic changes, assuming that such changes occur as a consequence of chronic stress exposure. In fact anhedonia is one of two core symptoms of depression, and not necessary for its diagnosis in humans nor is it specific to major depressive disorder as it has been associated with schizophrenia and other

neurological pathologies (Bermanzohn and Siris, 1992; Koob, 1992). It may be that we see the same phenomenon in our animal models of depression and that like the clinical literature, depression is not always accompanied by anhedonia. Because of these issues, some groups are turning toward a more ethological perspective for developing appropriate, that is, species specific stressors and assessment techniques. In humans, depression develops from exposure to psychological stressors and not generally physical ones. Likewise, modeling depression in animals should include stressors that are psychologically relevant to the species under study and for which a stress response has an evolutionary basis. In rats, for example, some of the best studied psychogenic stressors include isolation, crowding, and threat of a predator (Brown and Grunber, 1995; Haller et al., 1999; Martinez, Calvo-Torrent, and Pico-Alfonso, 1998; Perrot-Sinal, Ossenkopp, and Kavaliers, 1999). However, gender differences in reactivity to such stressors have been reported (Brown and Grunber, 1995; Haller et al., 1999), leading to further ethological considerations when modeling a human disorder in animals (see rev. Palanza, 2001).

NEW PERSPECTIVES ON BEHAVIOURAL INDICES

In an attempt to explore novel behavioural measures in which we could more easily discern CMS-induced changes as they pertain to depression, we evaluated the effects of CMS on forced swim behaviour. Female rats of both strains failed to display any behavioural alterations as a consequence of CMS exposure, while the effects in male rats were strain specific. Sprague-Dawley rats exposed to the chronic stress regime consistently showed a higher frequency of active behaviours than their control counterparts. However, the

comparable Long Evans group shifted from a profile of mostly active behaviours on the first test day to a nearly equal frequency of “other” behaviours on the second test day.

Furthermore, these rats displayed a clear reduction in the time spent in active behaviours upon re-exposure to the test and exhibited an increase in passive and other behaviours. One interpretation is that previous stress exposure produces adaptation to novel challenges resulting in less active behaviours. Alternatively, it may be that this group of rats display learned helplessness; having been exposed to forced swim on the first test day, Long Evans male rats previously exposed to CMS learn more quickly that escape is futile. While this test is useful in distinguishing the effects of stress on subsequent challenges, it is not directly addressing the issue of anhedonia.

Feeding and reproduction are behaviours that are survival necessities in rats as in other species and the stimuli related to these activities function as primary rewards. When animals are faced with a threat, bodily mechanisms involved in feeding and reproduction will be shut down in order for energy to be directed towards restoring homeostasis. This mechanism may explain the initial decrease in sucrose consumption typically found in animals exposed to CMS. However, with continued exposure to stressors, energy reserves are depleted and thus require further nourishment in order to maintain basal functional levels. In this respect, availability of a mildly sweet solution will not hold the same incentive for non-stressed animals as those exposed to a homeostatic threat; for control animals, the sucrose solution has an appetitive value but for CMS exposed animals, it is simply a source of nutrients necessary for survival, thus a consummatory value. Reports of reduced sexual behaviour in male rats exposed to CMS (Brotto et al., 2001; D’Aquila et al., 1994) may also be related to a

shift in energy focus rather than anhedonia. The chronic CMS-induced disruption in estrous cycle regularity reported in the fourth study of this thesis also supports the idea that nonessential bodily functions are curtailed in the face of a stressor.

Although several investigators have proposed modifications to the sucrose measure, for example, the use of saccharin versus sucrose, evaluating preference versus intake, or assessing changes in the break point in the progressive ratio paradigm (Barr and Phillips, 1998), all of these measures will be inappropriate if the inherent hedonic property of a palatable solution is lost and now simply interpreted as a source of nutrients.

Given this hypothesis, it may be necessary to employ secondary rewards as behavioural measures of anhedonia. Drugs would constitute one such source and the effects of CMS on their rewarding property have been successfully investigated with place preference conditioning. However its use is limited due to the fact that only few tests can be conducted; habituation has been shown to occur with repeated exposure to the test apparatus. The evaluation of BSR thresholds would be ideal in this respect except that it appears to be too robust to be influenced by CMS (Nielsen, et al., 2000; Lin et al., 2002).

Other paradigms related to enrichment, social contact, or play behaviour would be potential candidates given that these behaviours are thought to be rewarding (Calcagnetti and Schechter, 1992; Pellis and McKenna, 1995; von Frijtag, Van den Bos, and Spruijt, 2002). For example, some investigators have evaluated the effects of chronic emotional or physical stressors on rat behaviour in an enriched environment; a difference in active interactions, in the form of sniffing, rearing on, and crawling on the enrichment objects, for example, has been suggestive of motivational deficits (Rodriguez Echandia et al., 1988; Cabrera,

Rodriguez Echandia, Jatuff, and Foscolo, 1999). While evaluating the effect of CMS on an environmental enrichment test may prove to be enlightening, it may also be useful to assess any CMS-induced changes in a socially enriched environment. This could include the evaluation of alterations in the social affinity of the experimental animal toward its group mates by using a partition to separate animals and monitor rearing and sniffing behaviour at the partition. It could also encompass the assessment of changes in play behaviour, such that the experimental animal would be allowed contact with a familiar mate and any alterations in play behaviour would be quantified. Changes in these types of behaviours during CMS exposure might be a better indication of anhedonia; deficits would suggest a lack of interest in the formerly pleasurable activity rather than simply a change induced from homeostatic imbalance.

In addition, genetic variables must also be carefully considered in work dealing with the influence of stress; we show here, strain and gender differences in susceptibility to CMS. Furthermore, it has recently been suggested that the breeding facility from where the research animals are obtained may play a role in stress susceptibility. Such differences could be related to breeding practices or peculiarities in the environmental conditions specific to these breeders. Indeed Willner (1997a) has acknowledged difficulties in replicating his own CMS-induced behavioural deficits following a change in the breeding facility from which animals were obtained in earlier studies. Other investigators have evaluated stress-related behaviours of the same strain of rats obtained from different sources. These studies revealed differences in immobility in the open field paradigm, as well as on measures of free exploration and social interaction (Paré and Kluczynski, 1997; Rex, Sondern, Voigt, Franck, and Fink, 1996).

Even though different rearing practices may be responsible for these discrepancies, these findings have led some investigators to consider the importance of inter-individual differences. Similar to humans, it may be that particular animals of a given strain are more susceptible to the stressors employed. Although we examined our data for individual differences, as previously reported no clear CMS-induced individual differences were found in sucrose intake, preference, or BSR thresholds (please see appendices 2, 3, and 4). Nielsen et al. (2000) also reported no overall change in BSR thresholds from CMS exposure, but they did find a substantial increase in thresholds in two of their rats, showing a particular sensitivity of these animals to the stressors. This suggests that it may be useful to develop screens for stressor vulnerability before any manipulations are conducted, as the more susceptible animals may model characteristics of the clinical population of interest. Even though most humans experience daily stressors, the majority do not develop major depressive disorder. One screening method could be to evaluate resting state fecal hormone levels (Harper and Austad, 2000; Miller, Hobbs, and Sousa, 1991; Wasser, Bevis, and Hanson, 1997) and on this basis categorize the animals as high, intermediate, or low stress susceptibility and correlate these data with subsequent behavioural tests.

A final thought, given our current understanding of these disorders, is that the modeling of human psychopathologies in animal work may require the use of multiple measures, including physiological and biochemical stress indices, as well as a variety of diverse behavioural endpoints. Although comprehensive test batteries are typically used to evaluate human psychopathologies, they have also proven useful in some animal models; for example,

the mouse defensive test battery is often employed in the study of anxiety and drugs for its treatment (Griebel, Rodgers, Perrault, and Sanger, 1999; Blanchard, Griebel, and Blanchard, 2001). This practice in evaluation of the CMS model, in addition to monitoring inter-individual differences in stress vulnerability, may result in a more consistent picture of the effects of stressors and ultimately a better understanding of the mechanisms underlying depression.

*Ce cafard qui envahit mon être
N'a d'égal que ma volonté de puiser
Au fond de moi-même le courage
De retrouver le ciel bleu*

L. Konkle, 2002



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Appendix 1

**EVALUATING THE INFLUENCE OF CHRONIC ENRICHMENT
ON BEHAVIOURAL INDICES OF REWARD AND STRESS IN
MALE SPRAGUE-DAWLEY AND LONG EVANS RATS**

1. METHODOLOGY

1.1 Subjects

The subjects were Sprague-Dawley (SD) and Long-Evans (LE) male rats (Charles River Laboratories, St-Constant, Québec). Upon arrival, animals were separated into control or enriched conditions. Control animals were individually housed in clear Plexiglas cages with food and water available freely; animals in the enriched groups were housed four or five per large cage (about twice the surface area of a standard cage). They were normally maintained on a 12-h light - 12h dark cycle with lights on at 0700 h.

The enrichment environment was a large three story cage, with dimensions 1.175 x 0.050 x 0.095 m, in which the animals were kept from 8 AM until 4 PM from Monday to Friday. The cage was equipped with large playing tubes, a running wheel, a climbing robe, a hammock, and grid-like sides, also for climbing. Food and water were available at all times in the cage.

1.2 Procedure

1.2.1 Experiment 1 (Effects of three weeks of environmental enrichment on plasma corticosterone levels)

Twenty one SD and 21 LE rats with a starting weight between 326 and 407 g were used in this study. Throughout the experimental phase, body weight was chronicled bi-weekly. Following the three weeks of enrichment, blood sampling, corticosterone determination and all other procedures were conducted as described in the methods section of the second study of the thesis.

1.2.2 Experiment 2 (Effects of six weeks of environmental enrichment on sucrose intake and preference and endocrine measures)

In this study, we employed 16 SD and 16 LE male rats. Their weights at the start of the experiment ranged from 360 to 597 g. Body weight was chronicled weekly. Animals from all groups were food deprived overnight before the test. Those group-housed were placed in individual cages 1h prior to the test; each animal was assigned a cage that was re-used on subsequent test days. A description of the 1h sucrose testing procedure can be found under the methods section of the third study of the thesis.

1.2.3 Experiment 3a (Effects of six weeks of environmental enrichment on thresholds for brain-stimulation reward)

A total of 28 male rats (14 SD & 14 LE) were used in this experiment. Their body weight at the start of the experiment ranged from 333 to 645 g. Surgery, behavioural tests, and sacrifice were conducted as described in Study 3 of the thesis.

1.2.4 Experiment 3b (Activity in the forced swimming test of animals exposed to six weeks of environmental enrichment)

Animals that had received six weeks of environmental enrichment were tested in the FST, according to the procedure described in the last study of the thesis.

2. Results

2.1 Statistical analyses

All statistical analyses were carried out as described in the three parallel experiments that were conducted as part of the thesis that compared CMS to control animals.

Table 1. SUMMARY OF STATISTICAL RESULTS

* unless otherwise noted, all analyses involving a repeated factor with more than 2 levels were corrected for potential violations to the assumption of sphericity using the Greenhouse-Geiser correction to the degrees of freedom.

Experiment 1. Three weeks of enrichment

	Treatment: Time:	SD F(2,29) = 157.38; p=0.000000	LE F(1,18) = 20.42; p=0.00027 F(1,25) = 389.7; p=0.000000
Weight Change (baseline served as the covariate)			
Feces (body weight served as running covariate)	Treatment: Int:	F(1,18) = 4.66; p=0.045 F(3,46) = 8.38; p=0.00032	F(2,44) = 3.70; p=0.025
Cort time 0	Treatment:	F(1,13) = 18.03; p=0.00095	
Cort Time 0-120 min	Time:	F(2,16) = 30.16; p=0.00001	F(3,52) = 61.27; p=0.000000
Adrenal glands (body weight served as the covariate)	Side:	F(1,19) = 6.77; p=0.018	F(1,19) = 7.77; p=0.012
Spleen (body weight served as the covariate)			

Experiment 2. The effects of six weeks of enrichment on sucrose consumption, corticosterone, and physiological measures

<u>Sucrose animals</u>	<u>SD</u>	<u>LE</u>
Weight changes (Baseline served as covariate)	Time: Interaction: F(2,23) = 103.87; p=0.00000	F(1,18) = 141.40; p=0.00000 F(1,18) = 5.08; p=0.028
Cort time 0	Treatment: F(1,14) = 9.11; p=0.0092	F(1,14) = 13.35; p=0.0026
Cort Time 0-120 min	Time: Int: F(3,29) = 20.53; p=0.00000 F(3,29) = 5.31; p=0.0050	F(2,24) = 32.26; p=0.00000 F(2,24) = 3.50; p=0.039
Adrenal glands (body weight served as covariate)	Treatment: Side: F(1,14) = 8.55; p=0.011	F(1,13) = 6.62; p=0.023
Spleen (body weight served as covariate)		
1h sucrose intake (body weight served as running covariate)	Time: F(3,44) = 3.21; p=0.027	
1h sucrose pref		

Experiment 3a. The effects of six weeks of enrichment on brain-stimulation reward

Bsr animals

LE

SD

**Weight changes
(Baseline served
as covariate)**

Time: F(2,21) = 61.28; p=0.0000

F(3,28) = 144.89; p=0.000000

**Rate
(Baseline served
as covariate)**

**Freq Thresholds
(Baseline served
as covariate)**

Treatment:

F(1,5) = 6.03; p=0.058

**Charge
(Baseline served
as covariate)**

Interaction:

F(11,66) = 1.88; p=0.058 (not corrected)

Experiment 3b. The effects of six weeks of enrichment on forced swimming behaviour

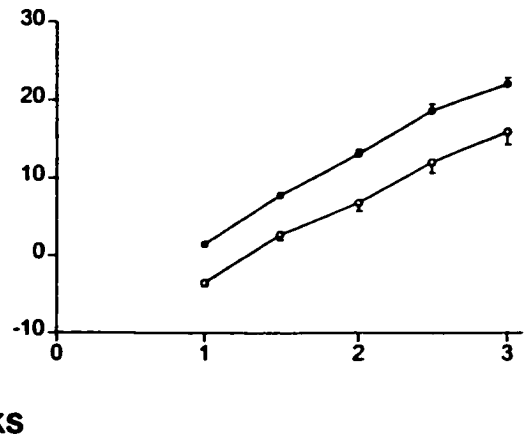
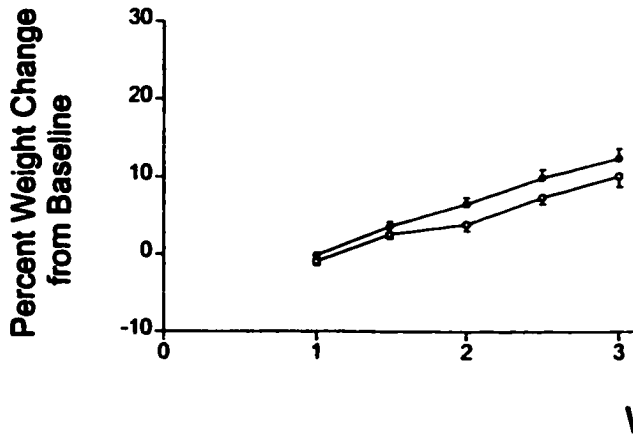
<u>FST animals</u>	<u>SD</u>	<u>LE</u>
<u>Frequency</u>		
Active Cluster	Group: F(1,12) = 8.22; p=0.014	
Passive Cluster	Day: F(1,12) = 8.70; p=0.012	F(1,10) = 21.90; p=0.00087
Others Cluster		
<u>Duration</u>		
Active Cluster	Day: F(1,12) = 17.97; p=0.0011	F(1,10) = 9.05; p=0.013
Passive Cluster	Interaction:	F(1,10) = 9.73; p=0.011
Others Cluster	Day: F(1,12) = 16.85; p=0.0015	F(1,10) = 10.28; p=0.0094
	Interaction:	F(1,10) = 12.41; p=0.0055

Experiment 1. Change in Body weight, adrenal and gland weight

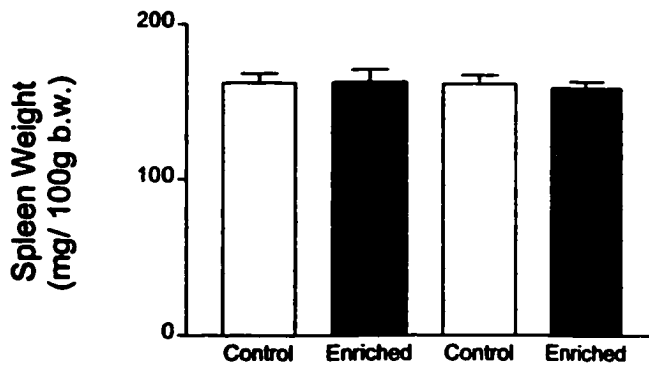
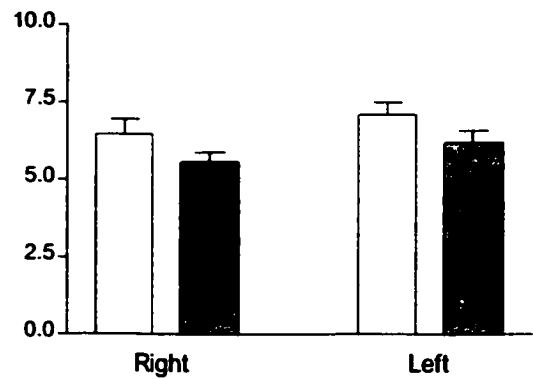
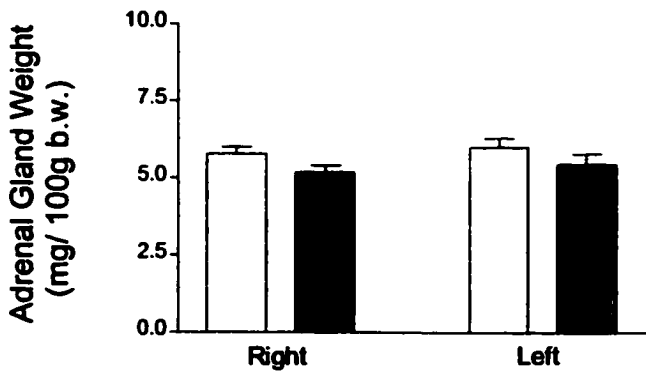
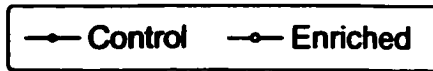
Sprague-Dawley

Long Evans

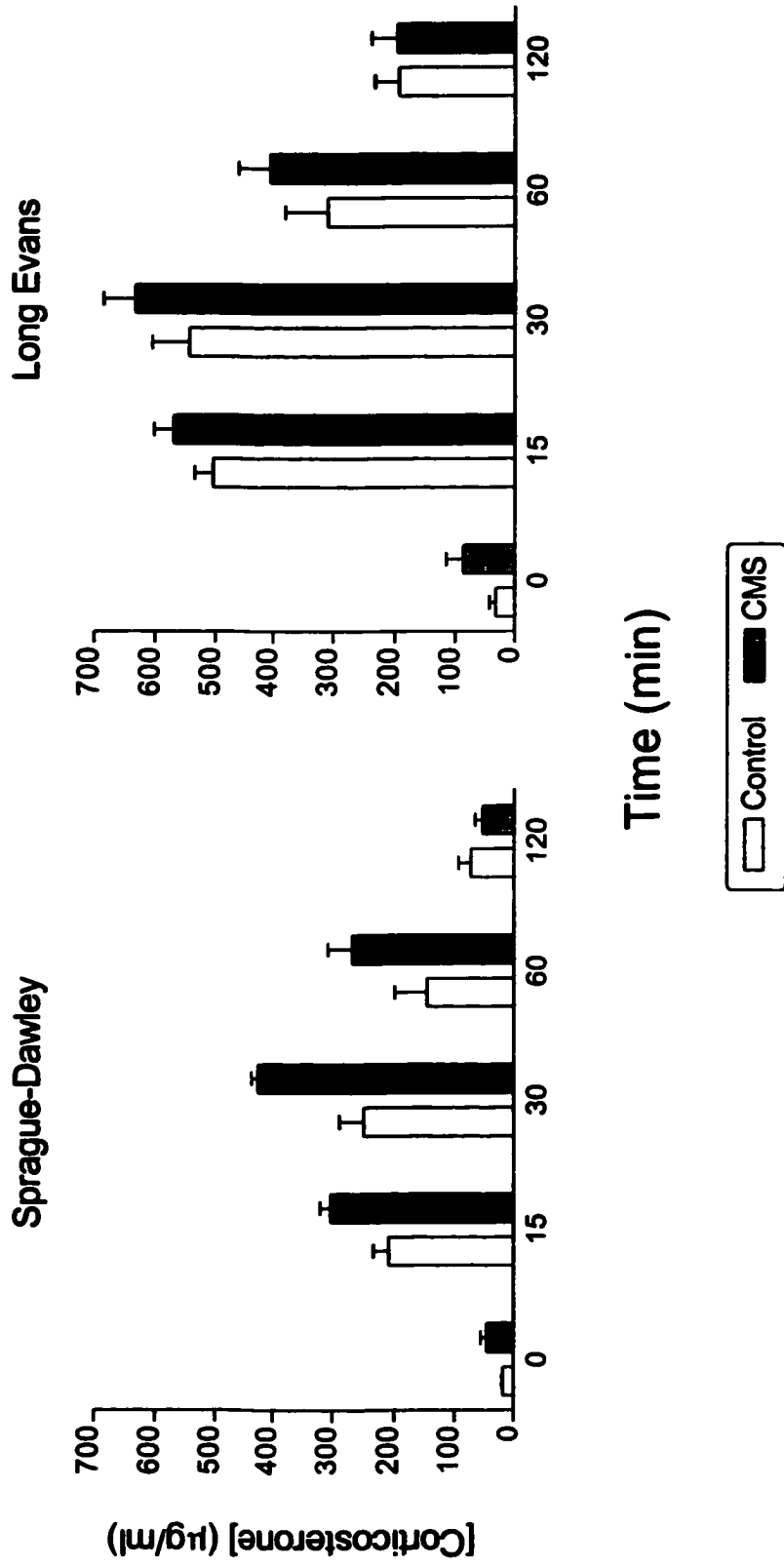
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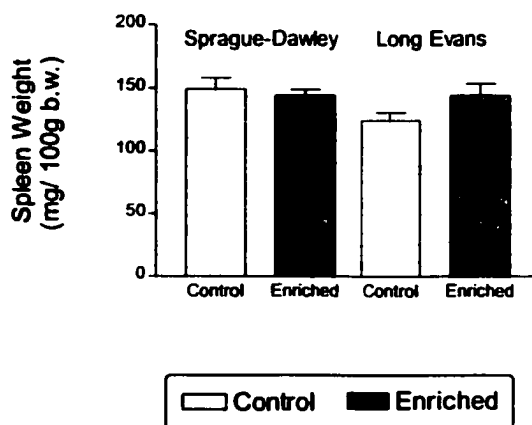
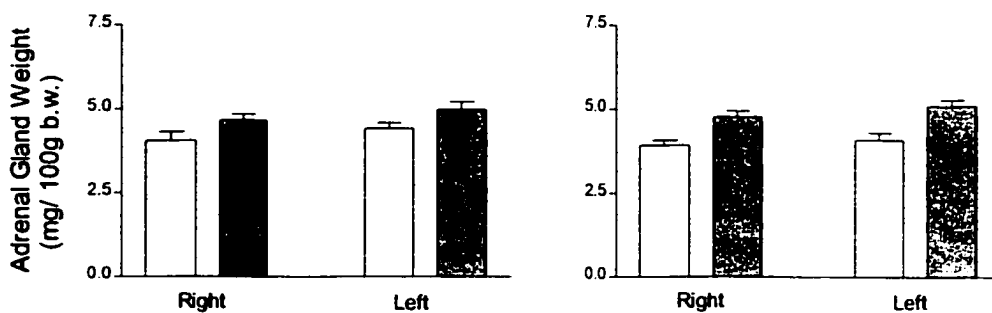
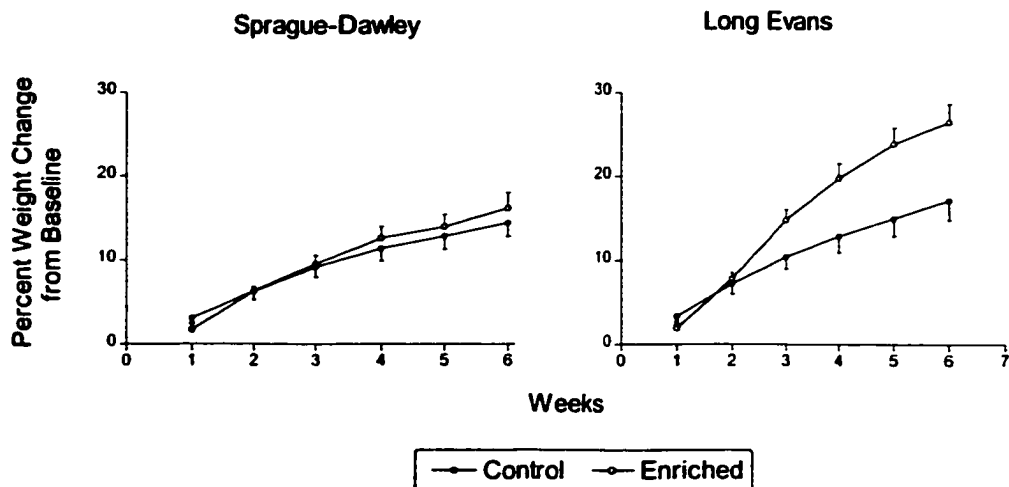
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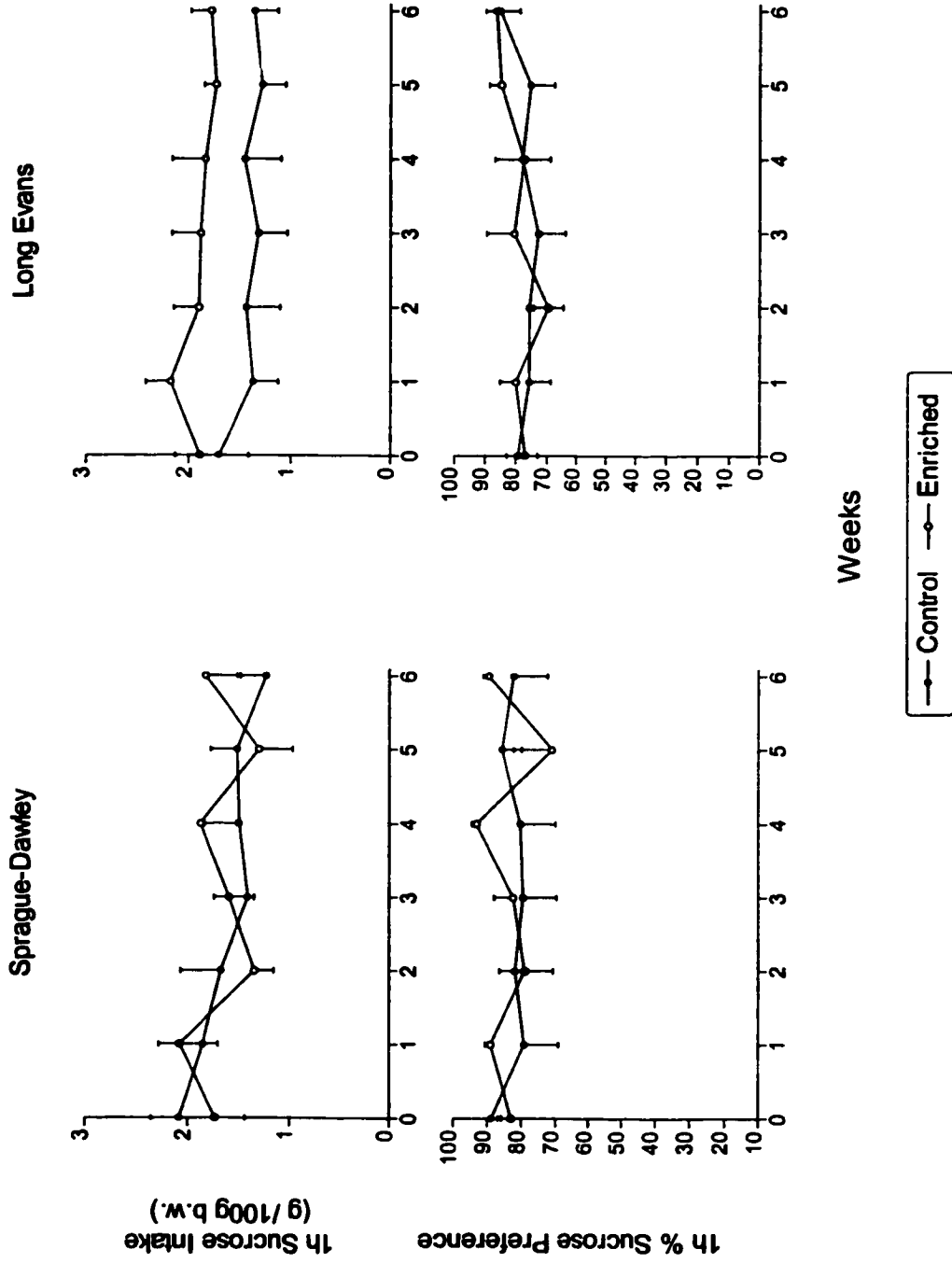
Experiment 1. Corticosterone levels



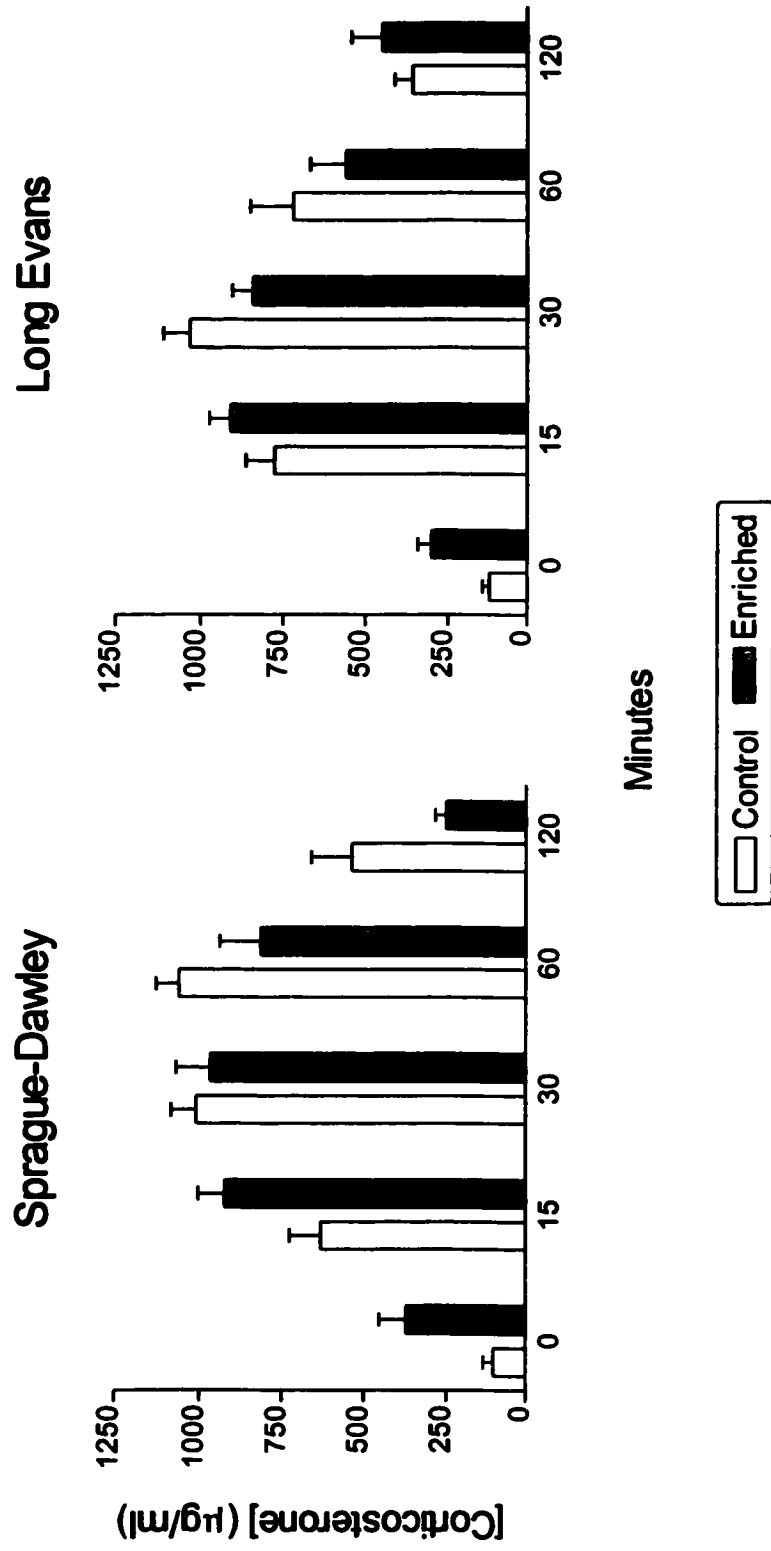
Experiment 2 - Change in body weight, and adrenal gland & spleen weight



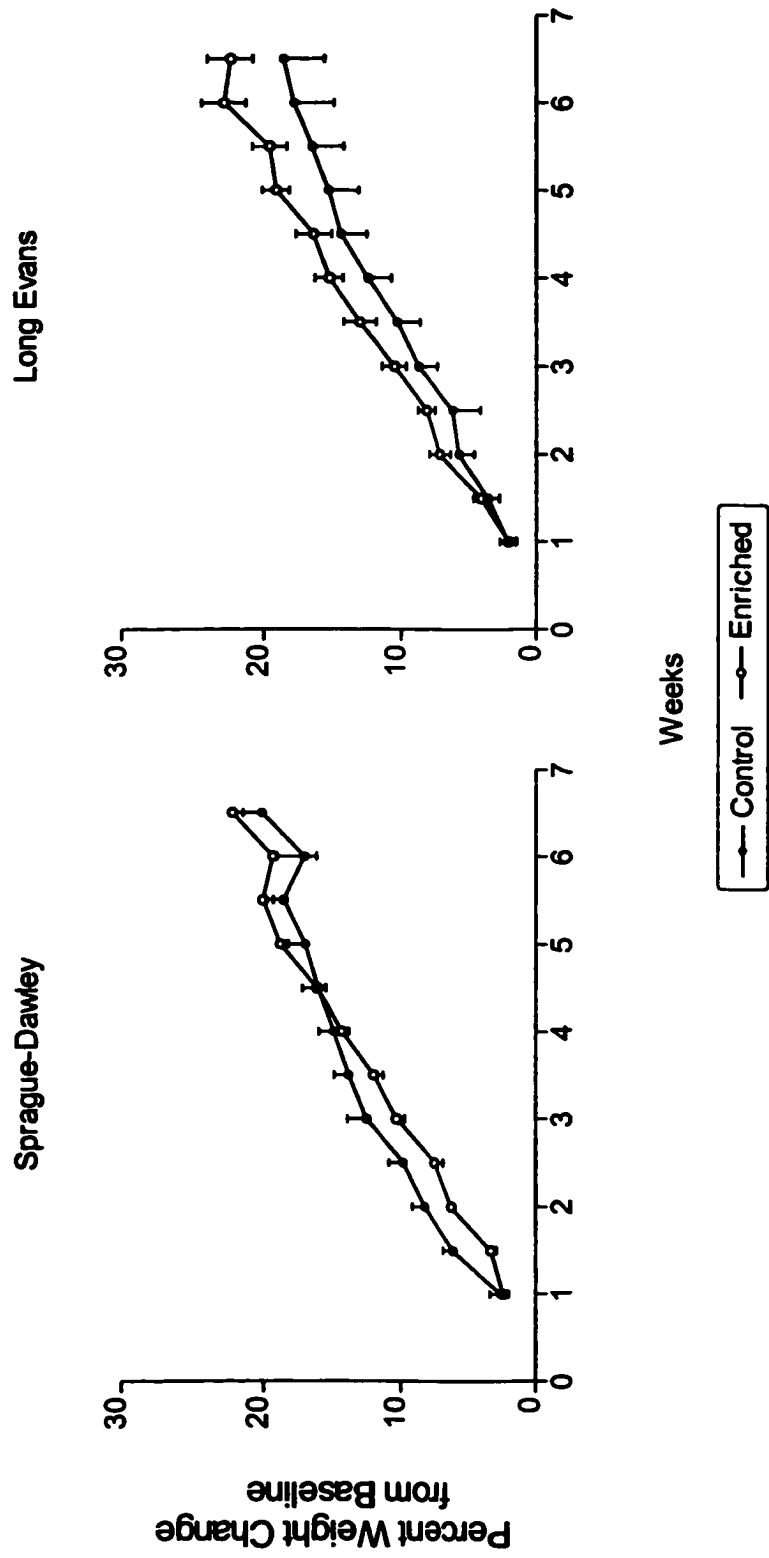
Experiment 2 - Sucrose intake & preference on same page



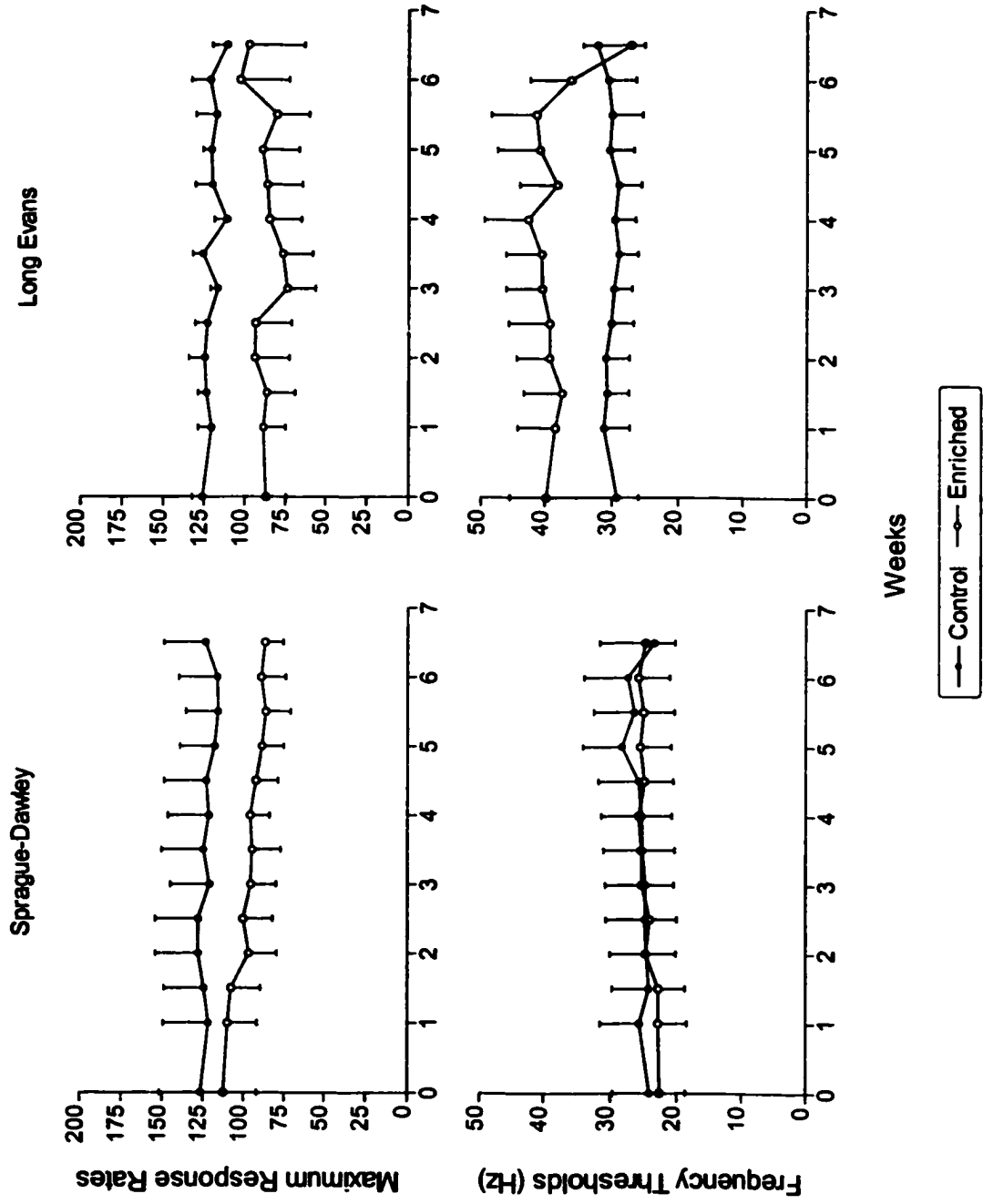
Experiment 2 - Corticosterone levels



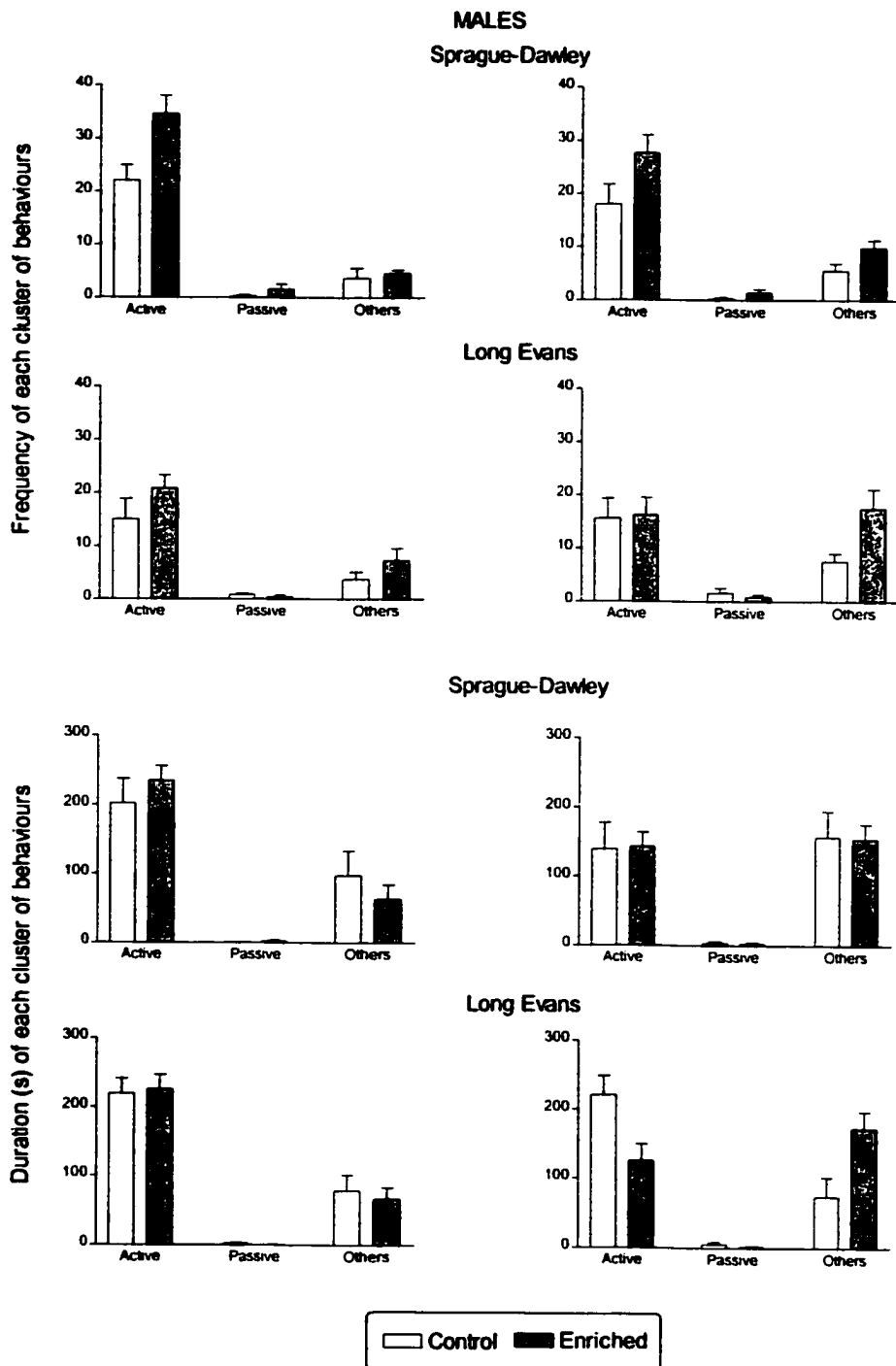
Experiment 3 - Percent Change in Body Weight from Baseline



Experiment 3 - Maximum response rates & thresholds



Experiment 4 - FST data



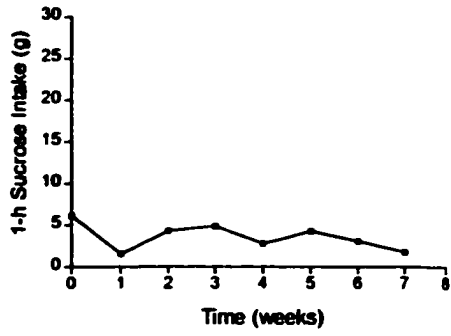
Appendix 2

**One and 24h sucrose intake and preference data
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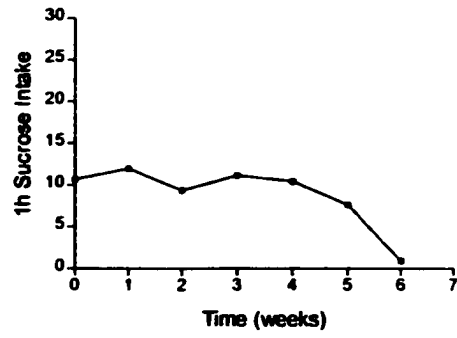
SD Control Males 1h Sucrose Intake

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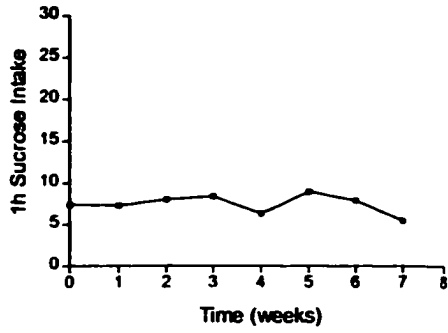
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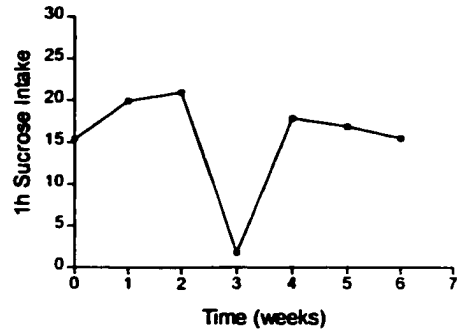
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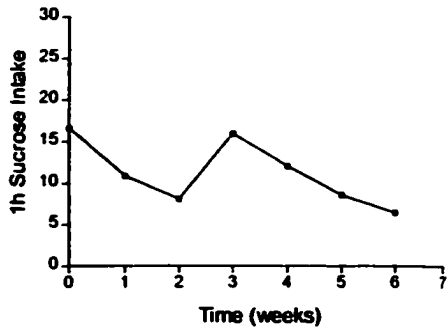
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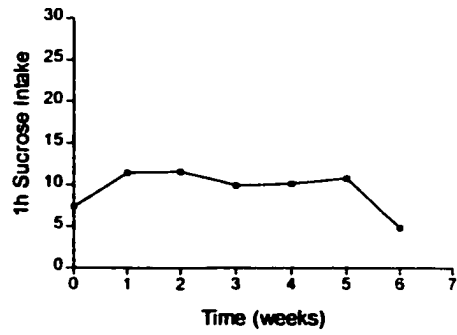
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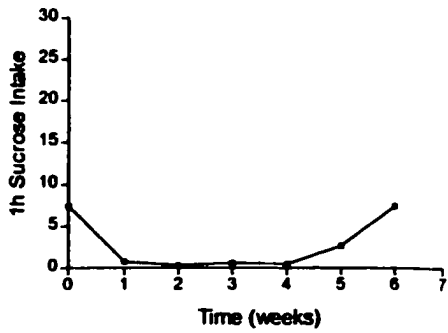
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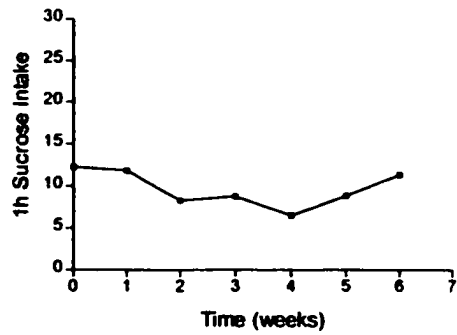
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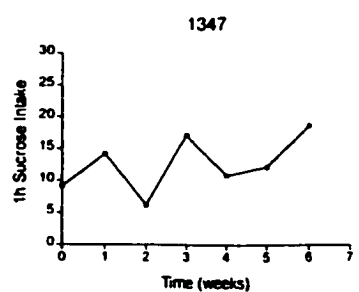
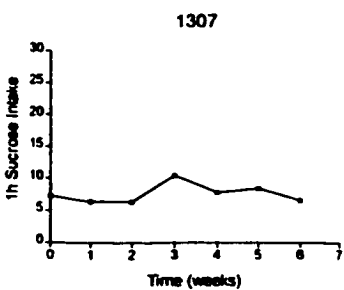
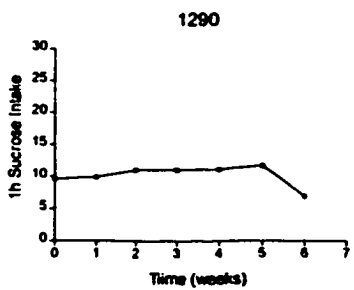
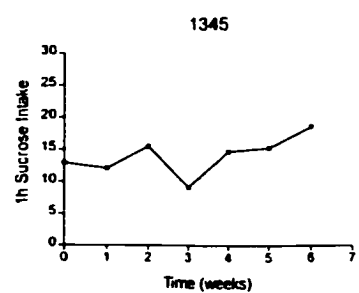
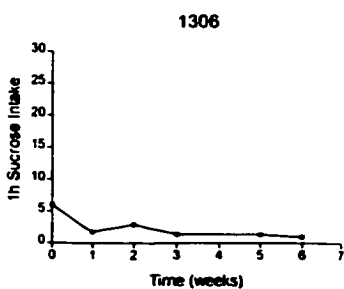
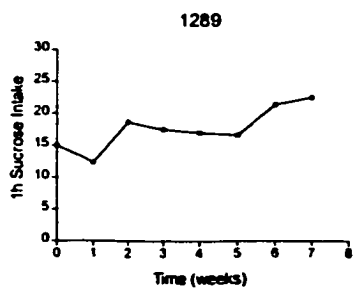
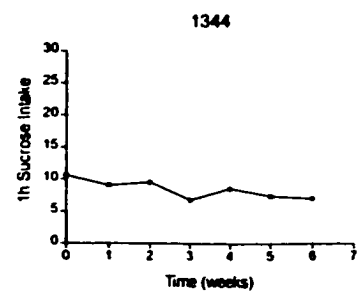
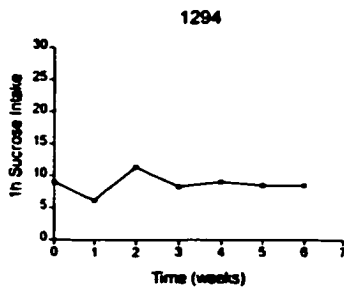
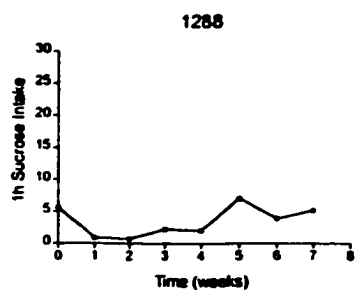
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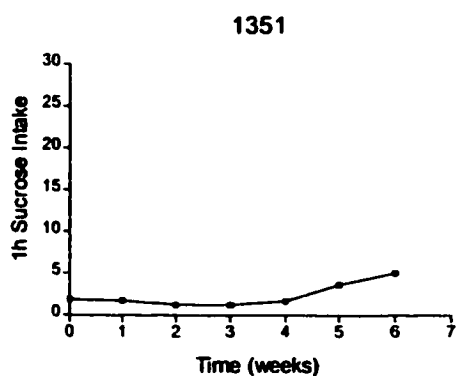
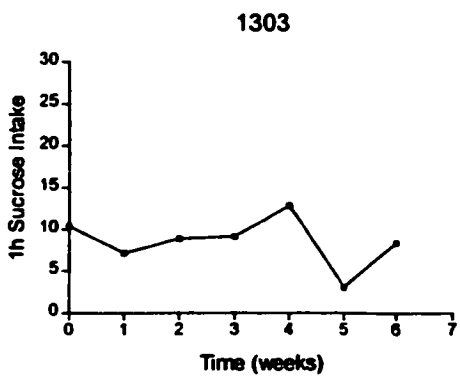
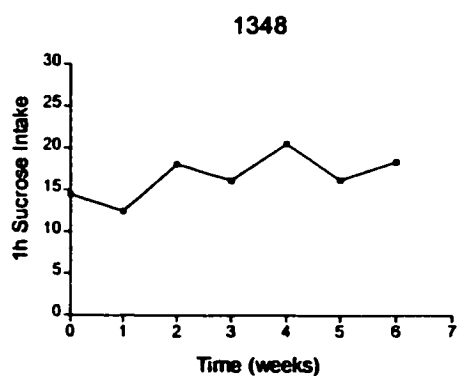
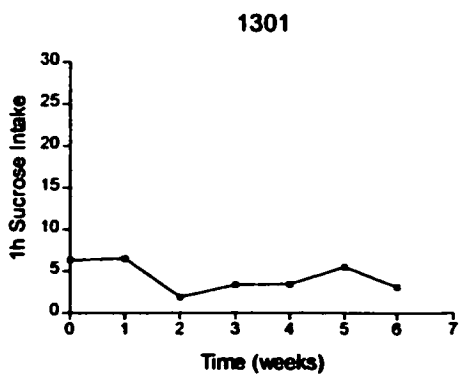
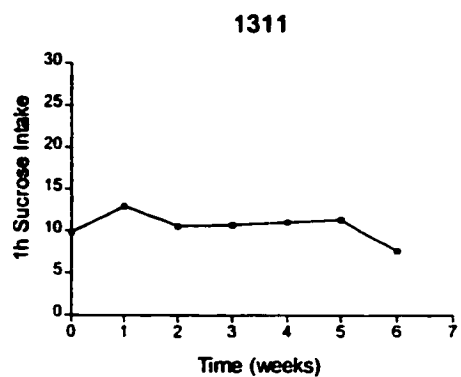
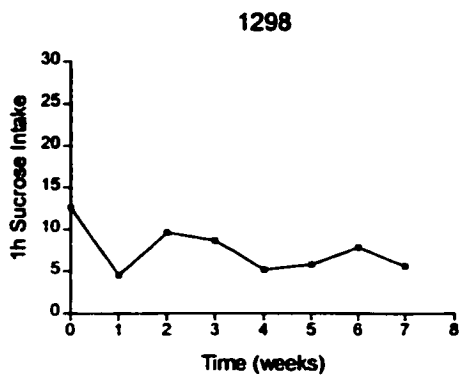
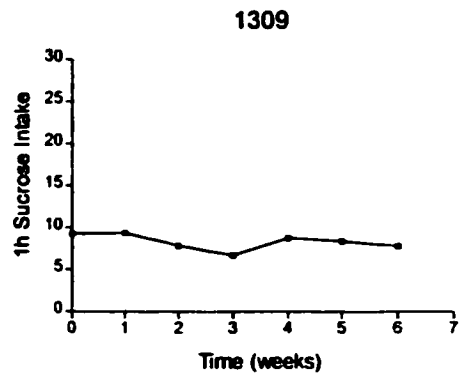
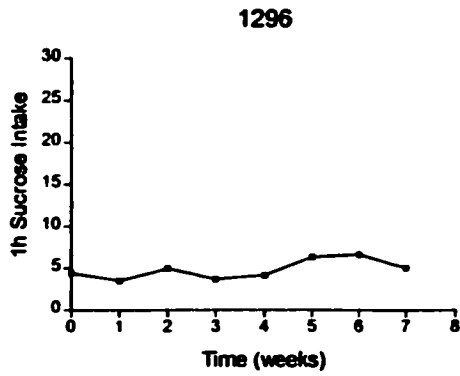


SD CMS Males 1h Sucrose Intake



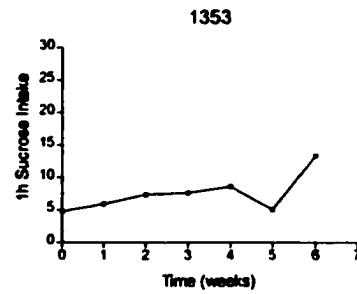
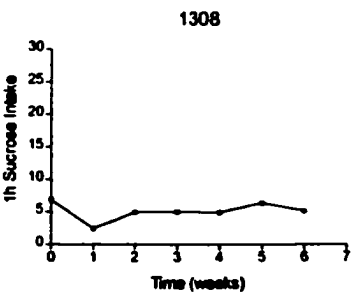
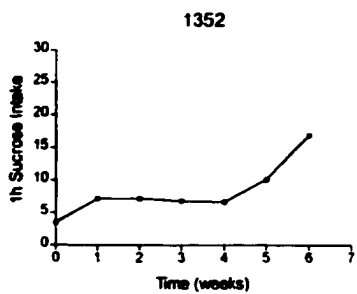
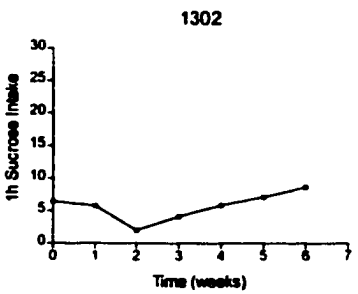
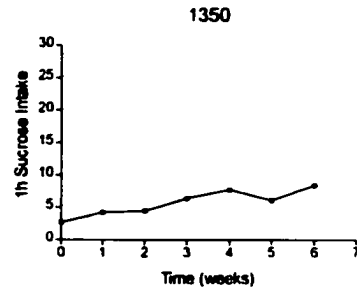
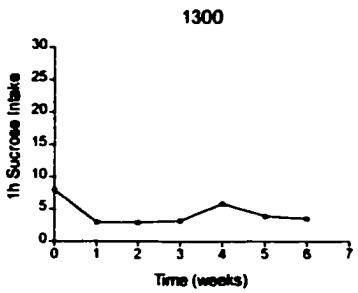
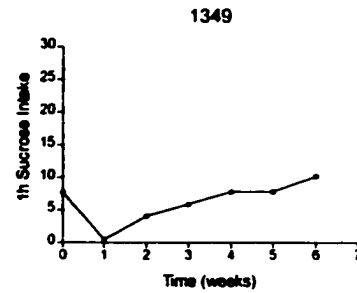
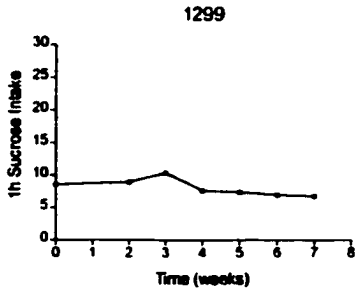
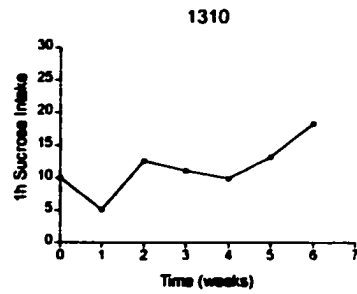
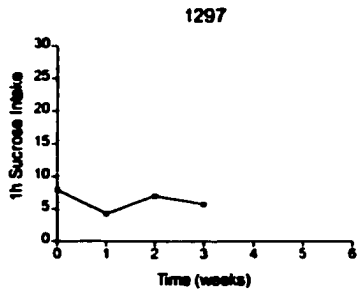
LE Control Males 1h Sucrose Intake

251



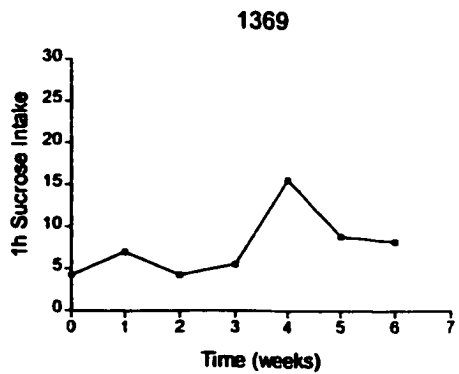
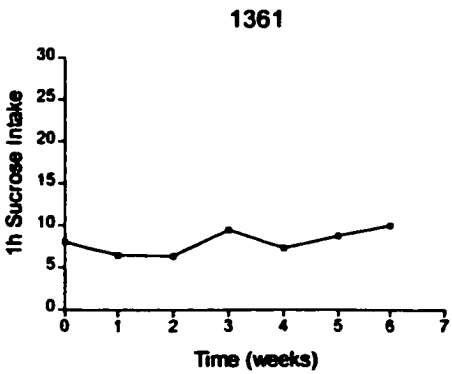
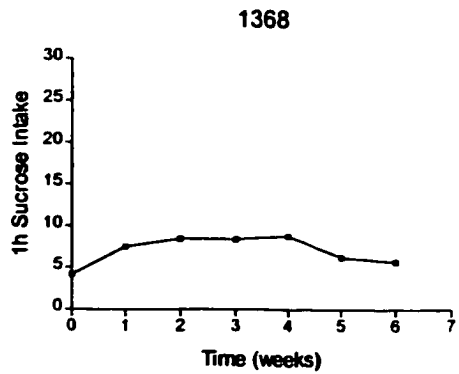
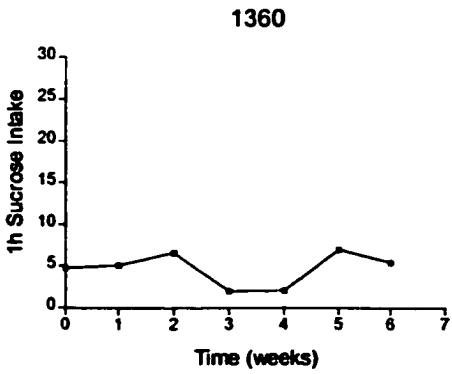
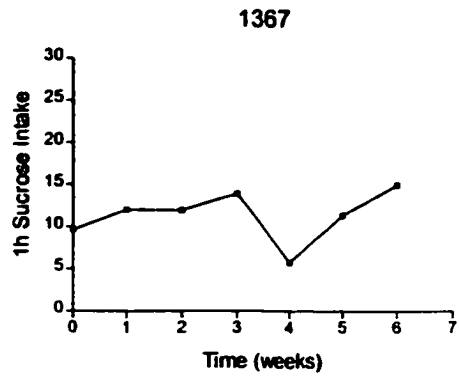
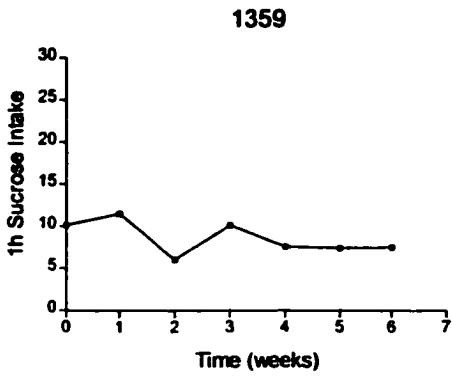
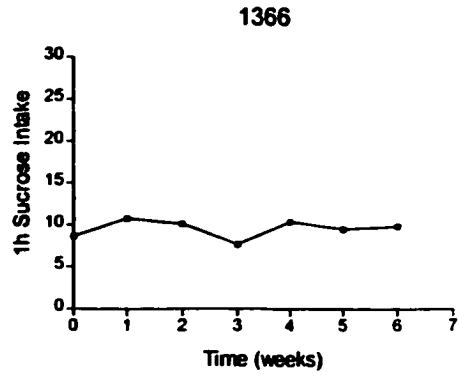
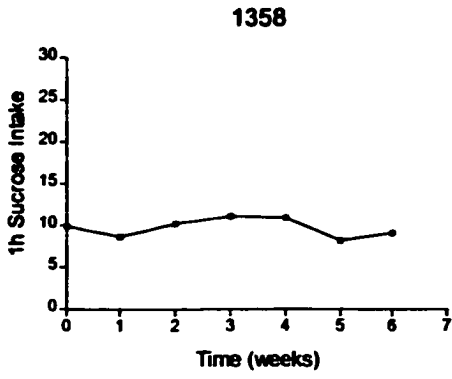
LE CMS Males 1h Sucrose Intake

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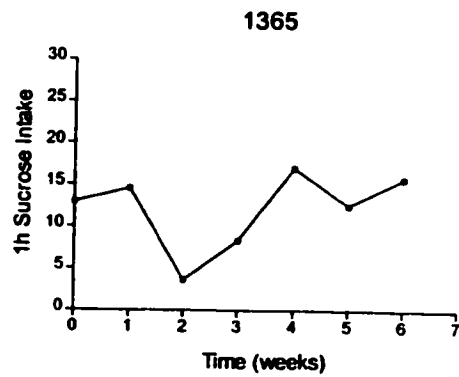
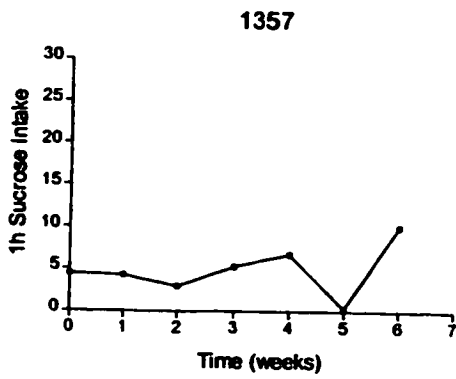
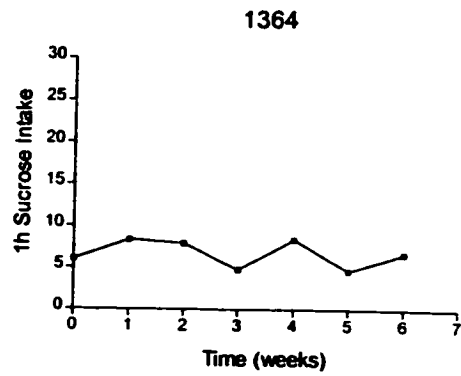
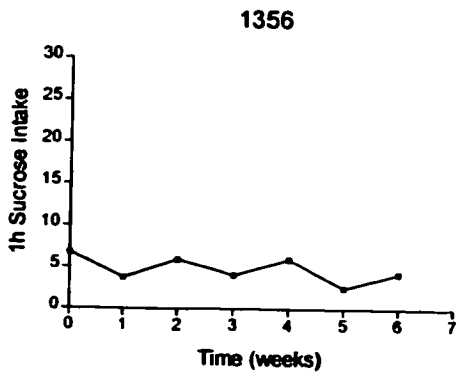
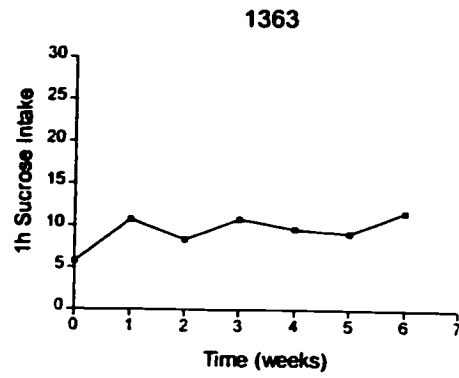
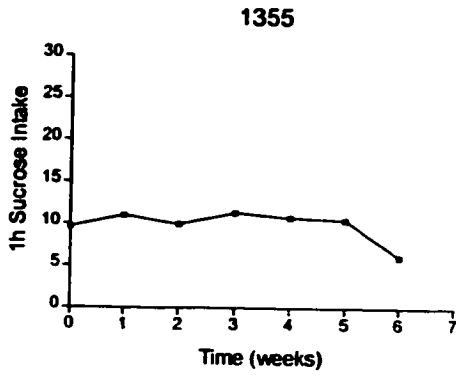
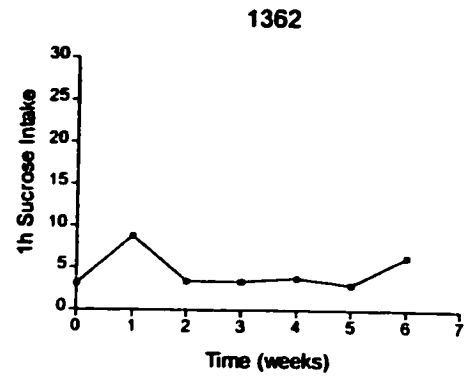
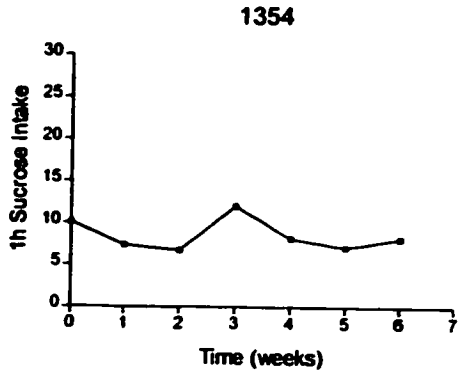
LE Enriched Males 1h Sucrose Intake

253



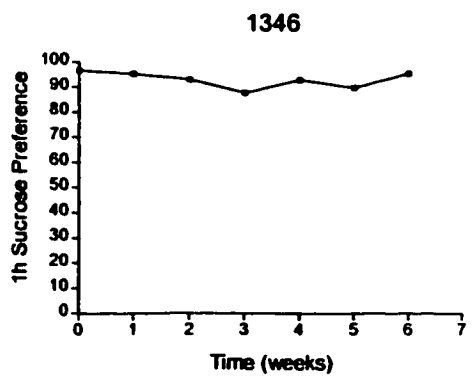
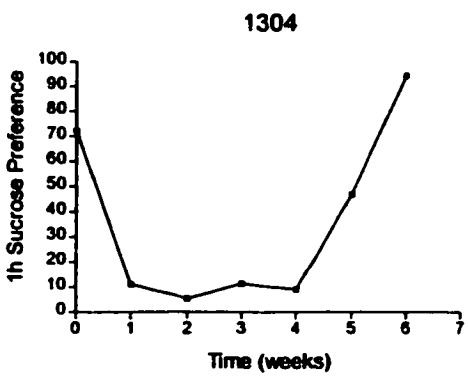
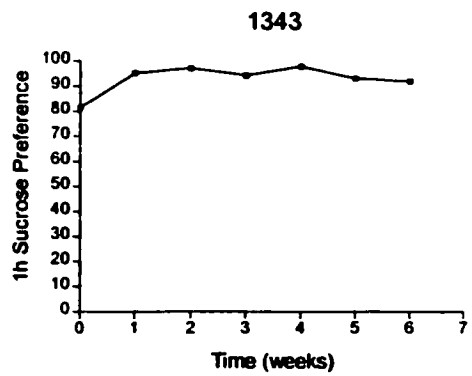
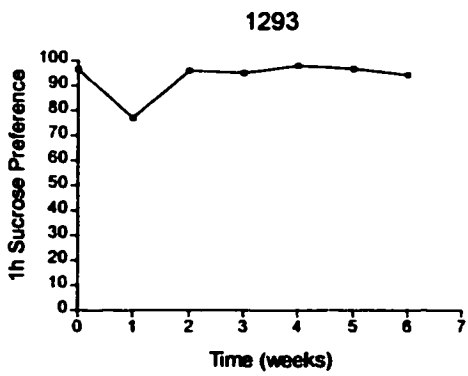
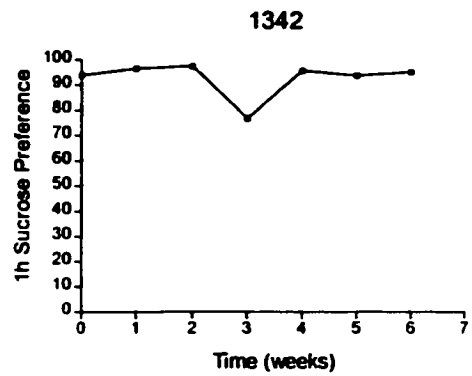
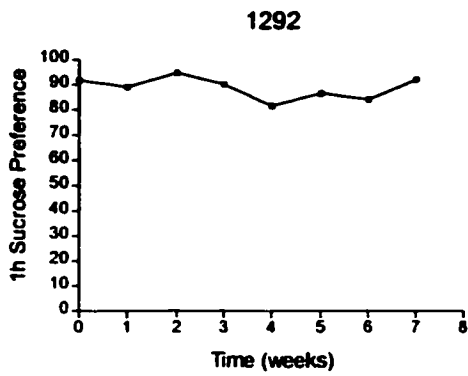
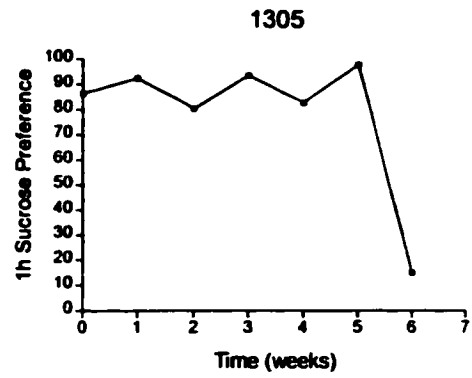
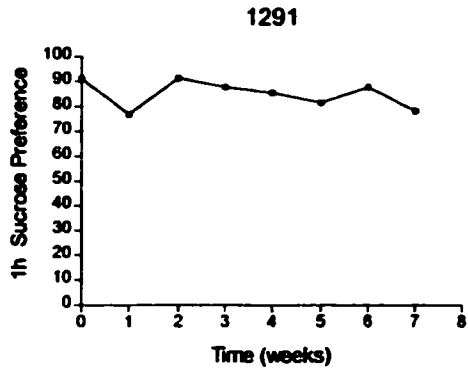
SD Enriched Males 1h Sucrose Intake

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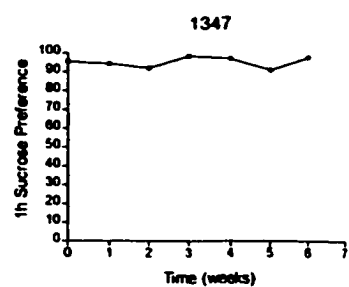
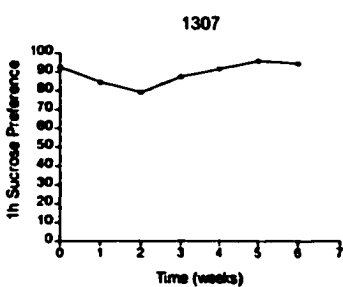
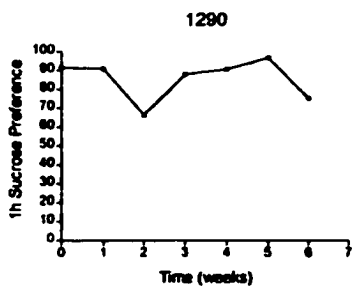
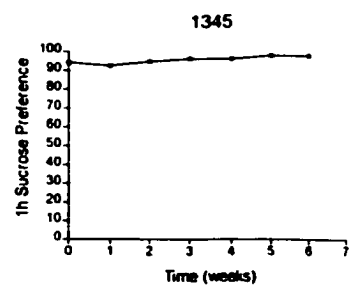
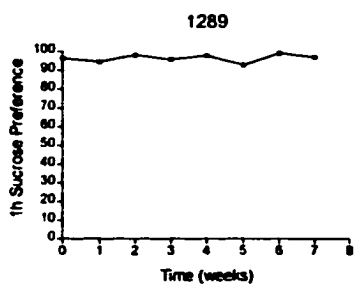
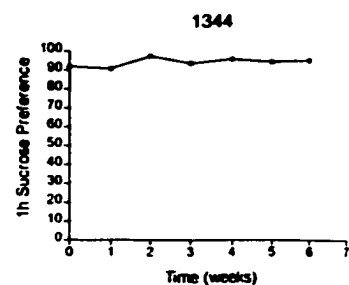
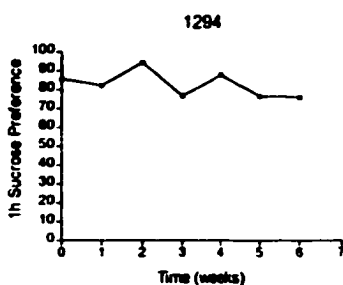
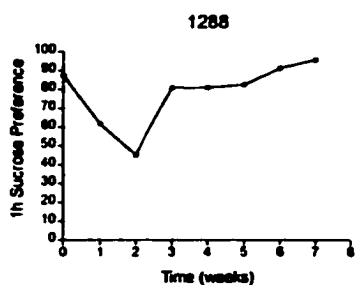


SD Control Males 1h % Sucrose Preference

255

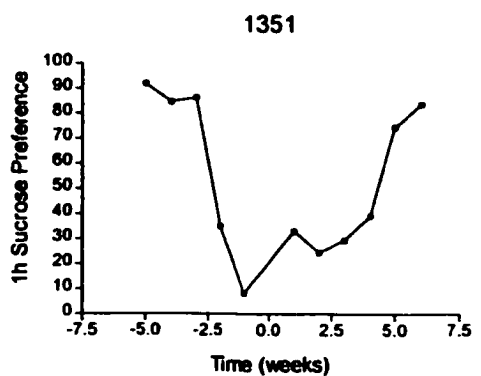
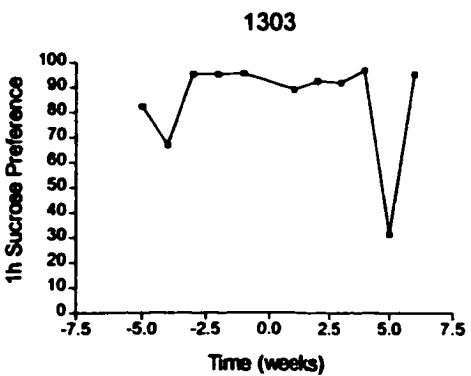
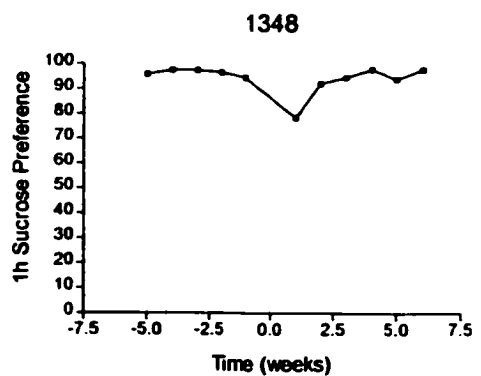
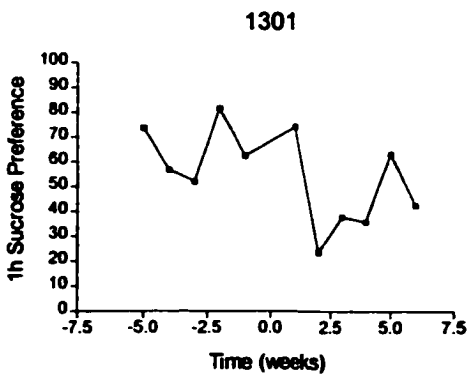
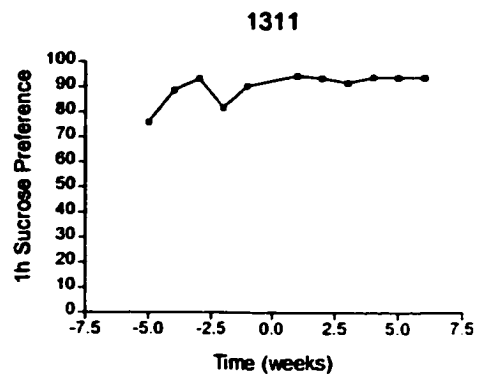
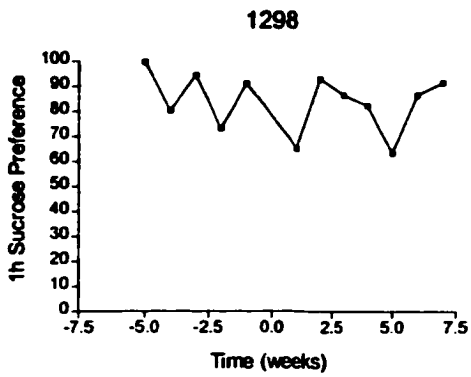
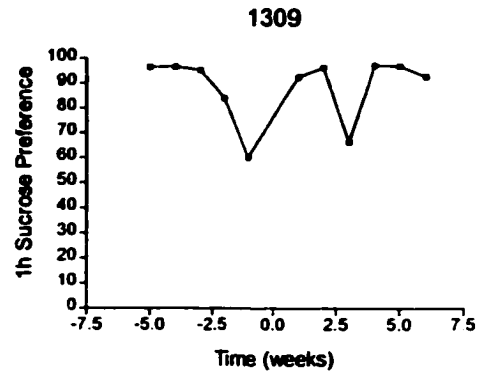
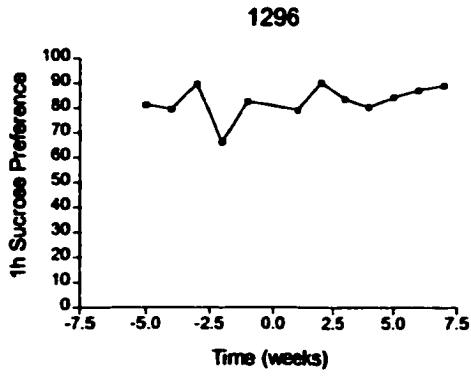


SD CMS Males 1h % Sucrose Preference

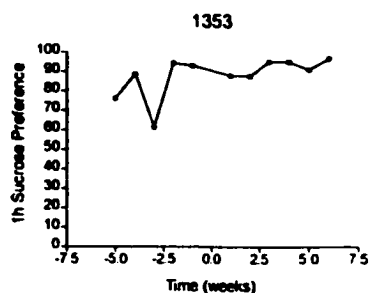
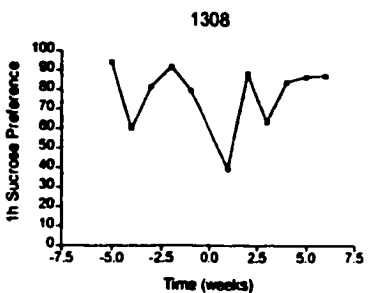
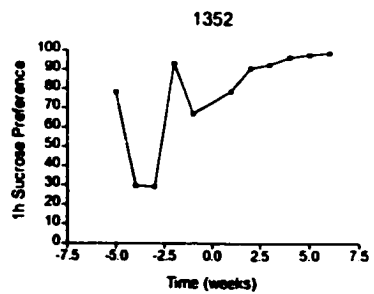
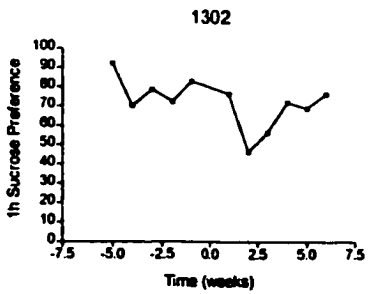
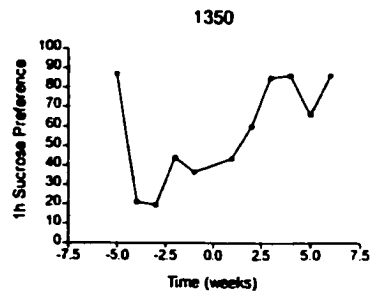
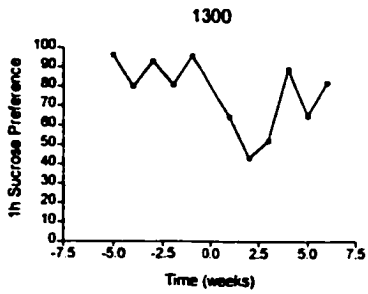
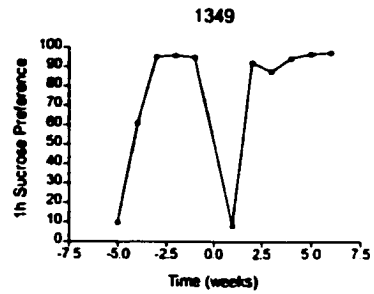
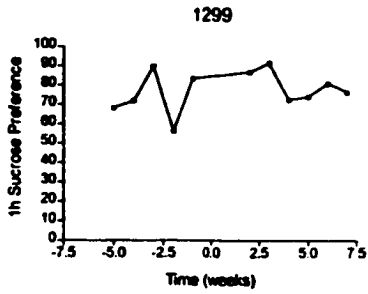
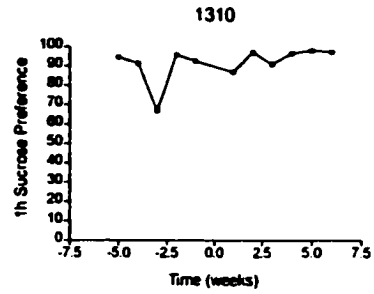
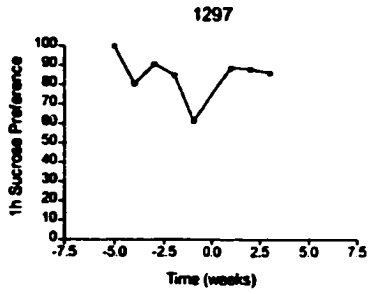


LE Control Males 1h % Sucrose Preference

257

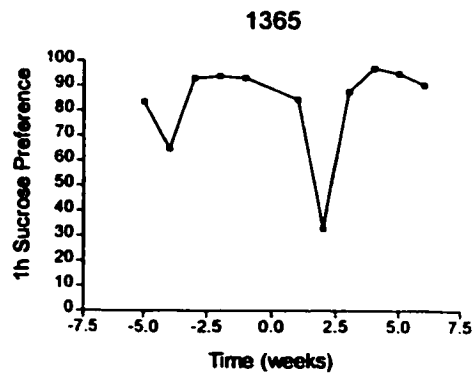
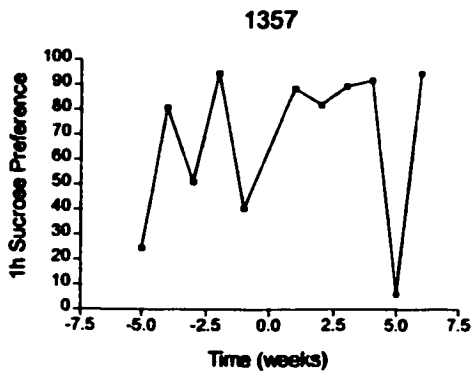
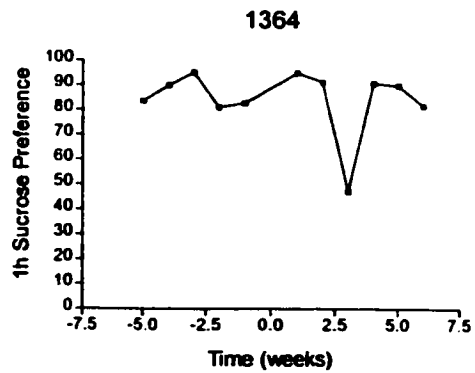
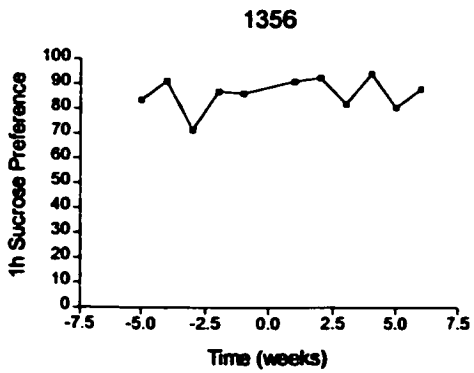
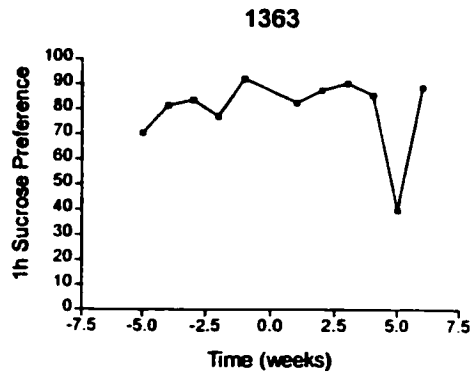
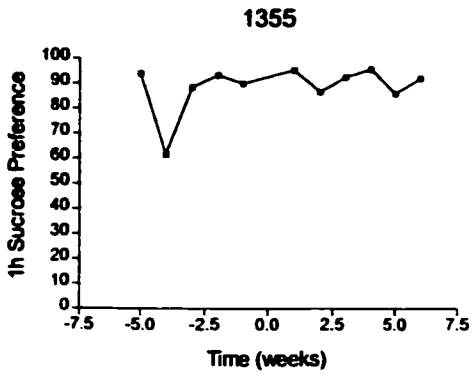
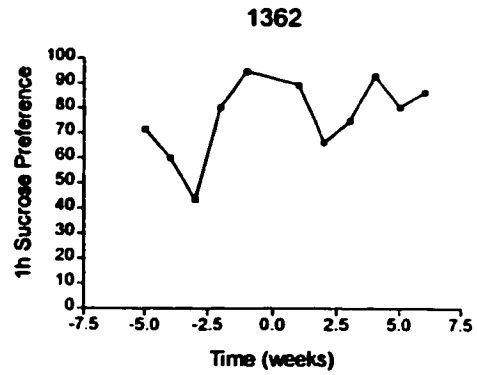
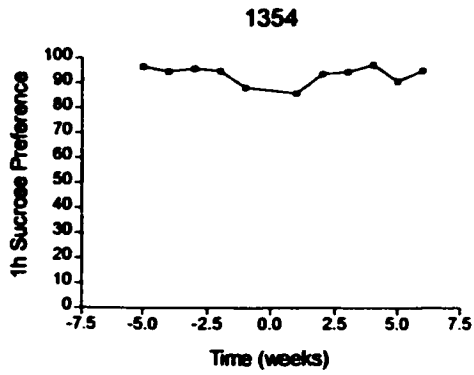


LE CMS Males 1h % Sucrose Preference



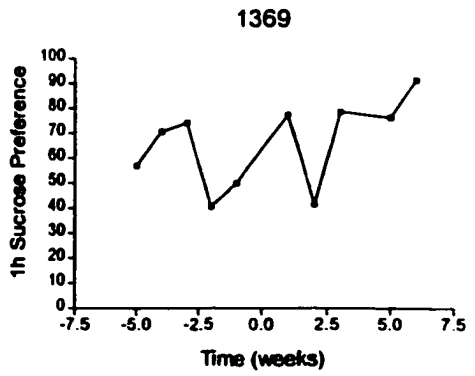
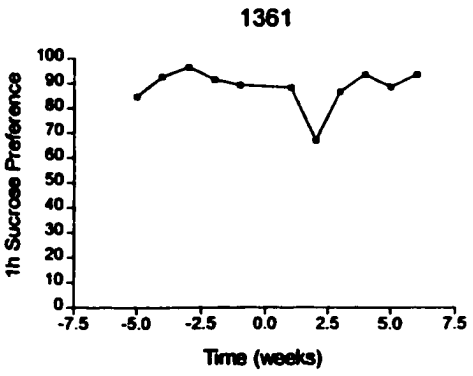
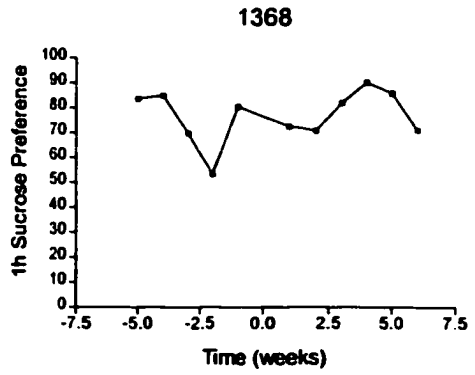
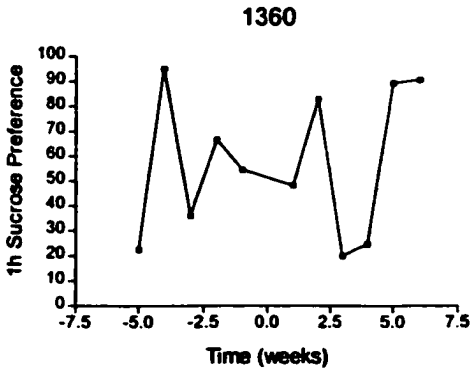
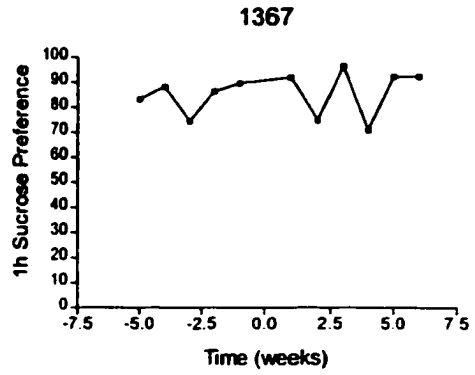
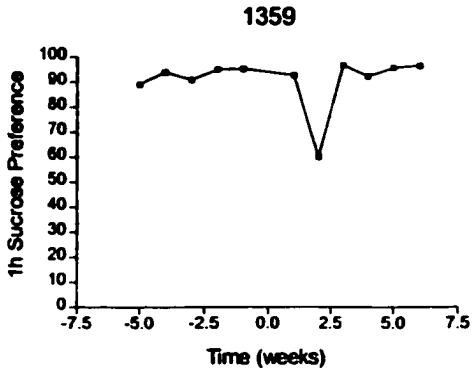
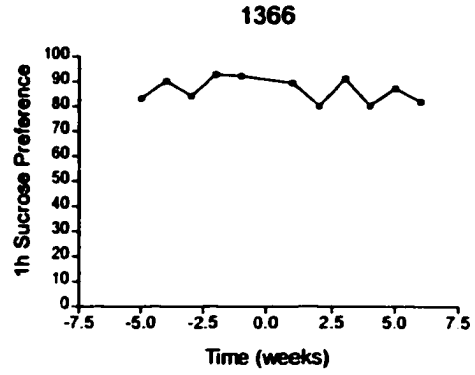
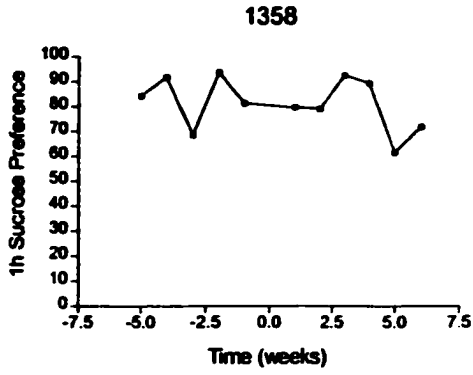
SD Enriched Males 1h % Sucrose Preference

259



LE Enriched Males 1h % Sucrose Preference

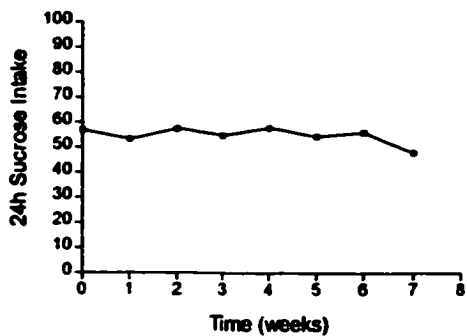
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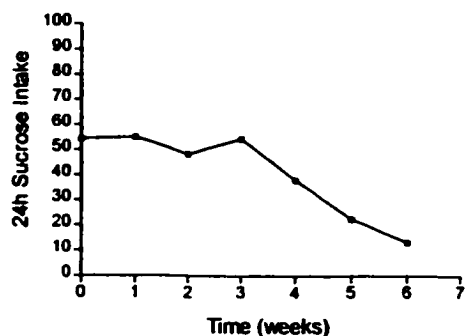
SD Control Males 24h Sucrose Intake

261

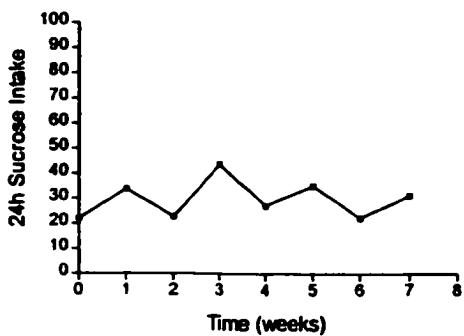
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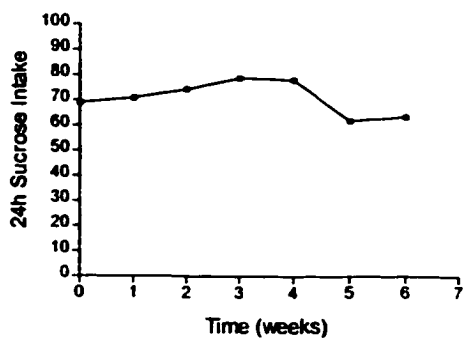
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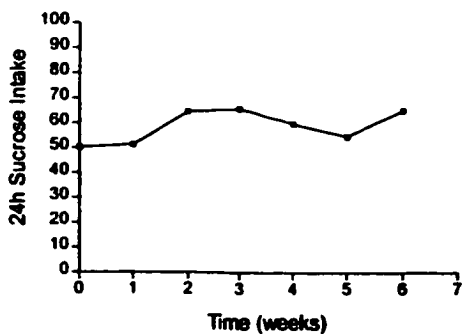
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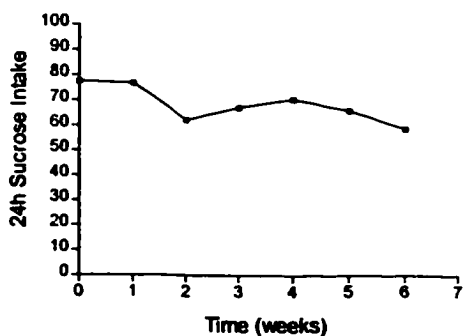
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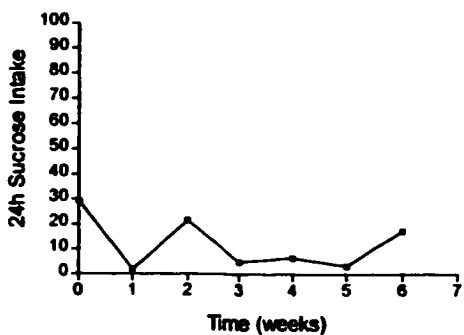
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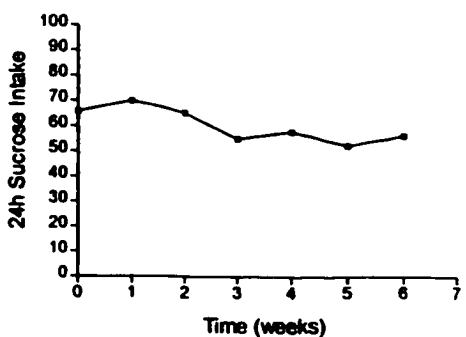
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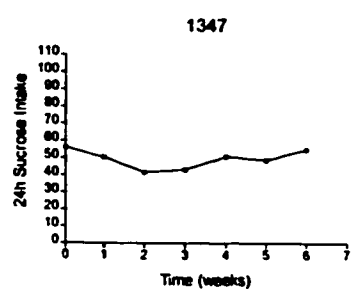
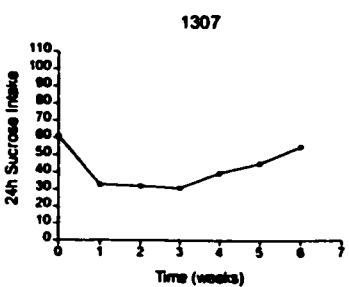
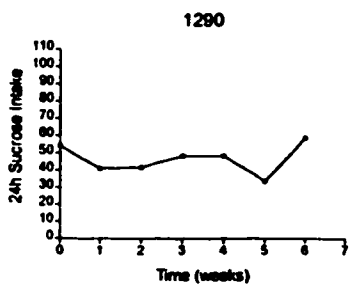
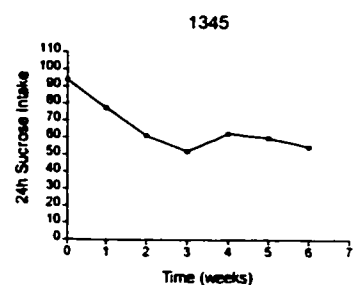
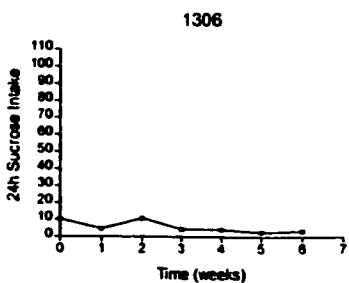
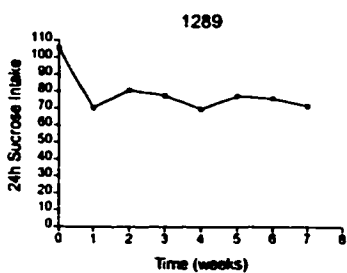
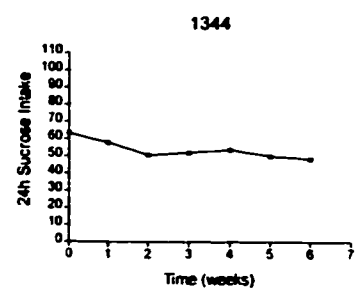
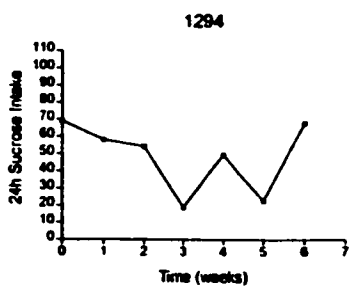
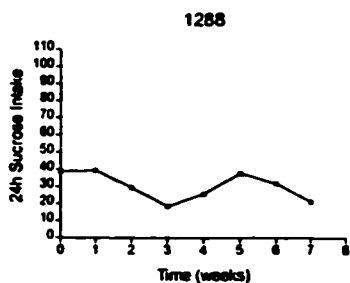
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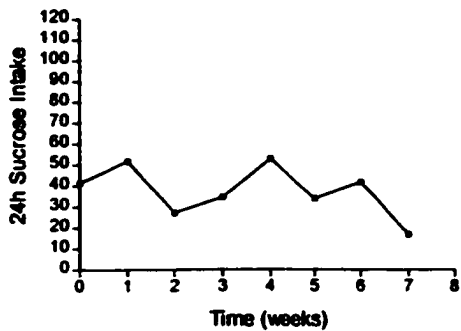


SD CMS Males 24h Sucrose Intake

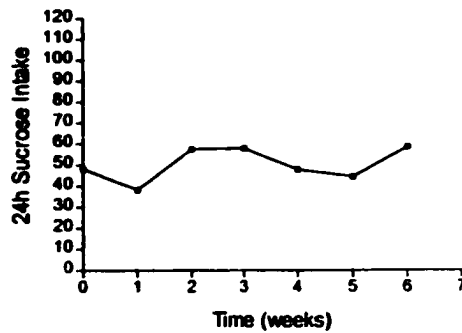


LE Control Males 24h Sucrose Intake

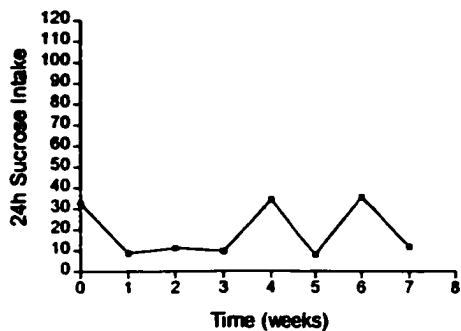
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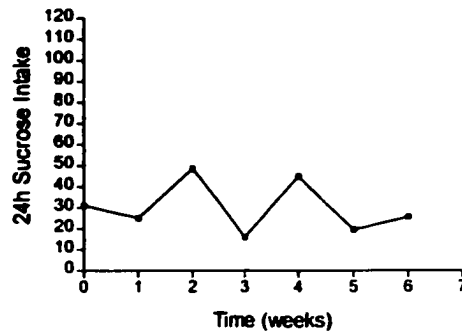
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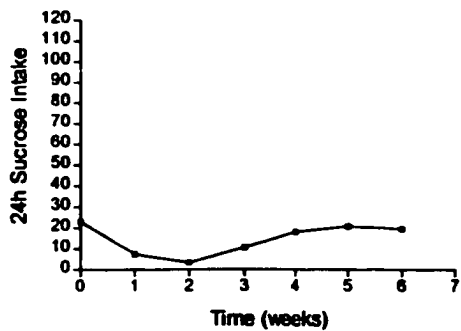
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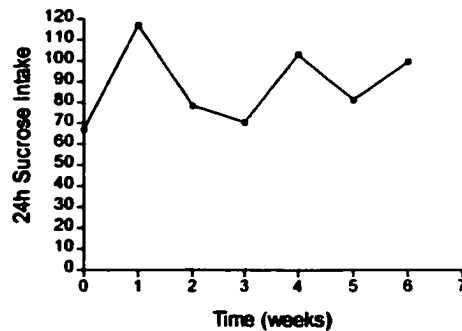
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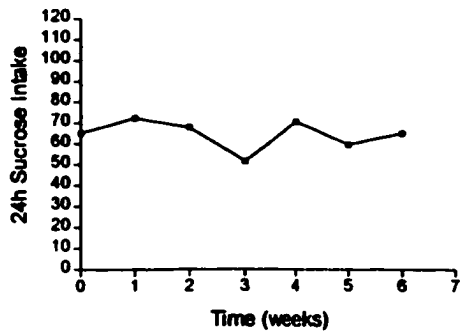
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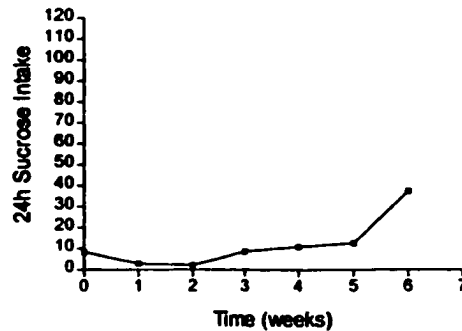
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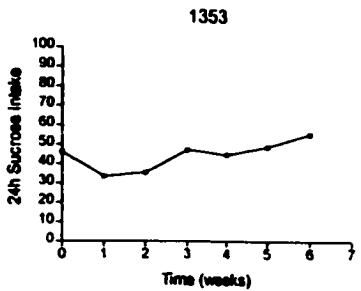
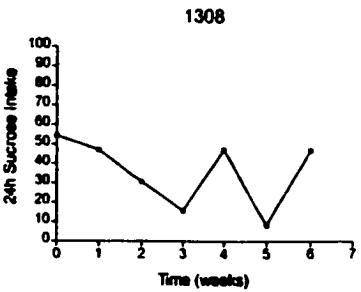
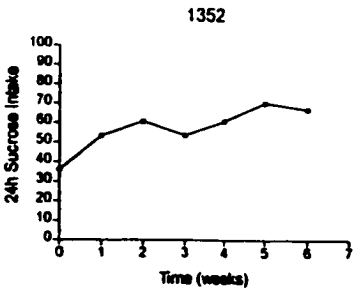
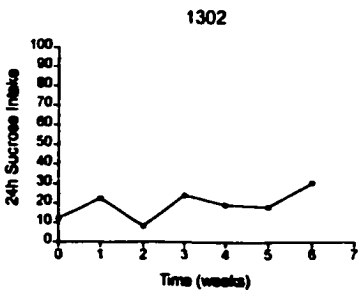
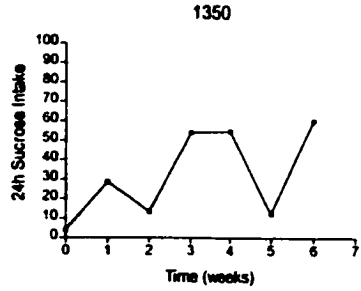
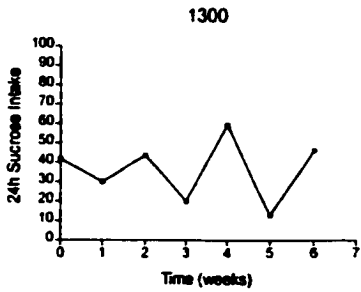
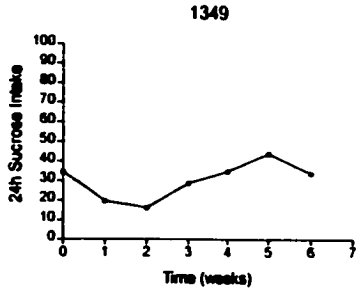
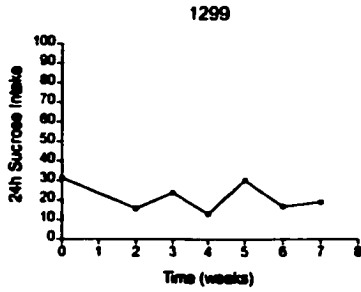
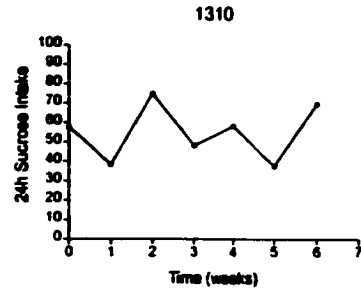
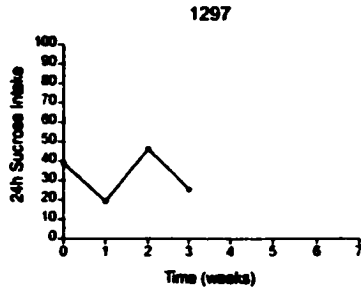
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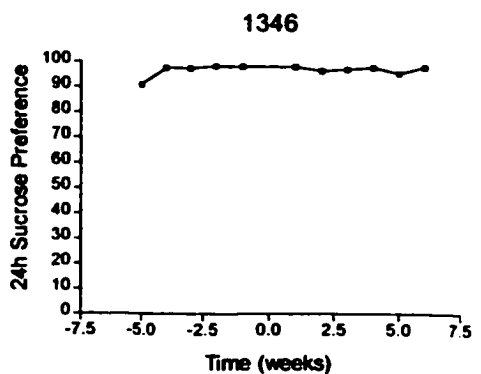
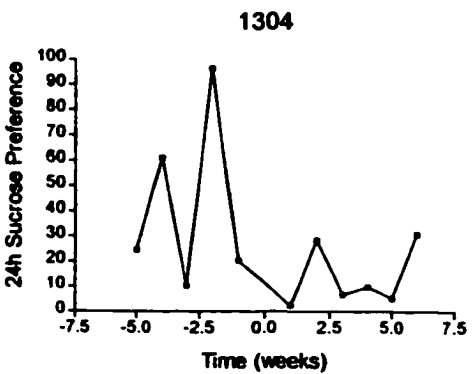
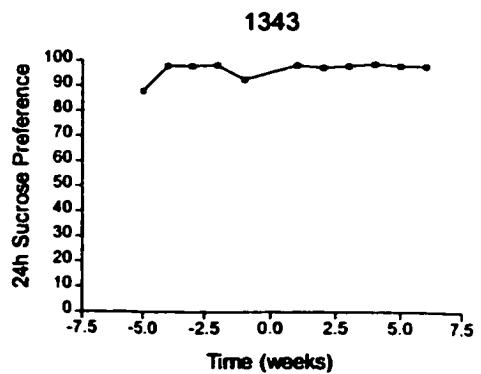
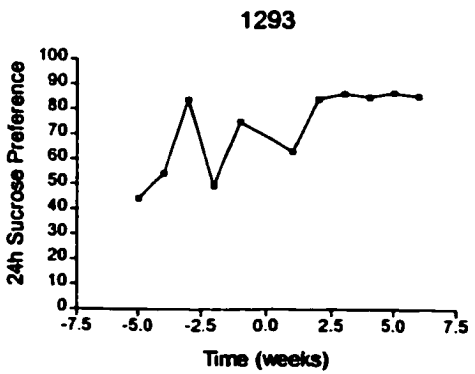
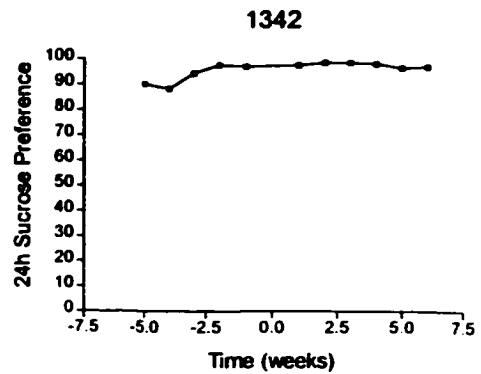
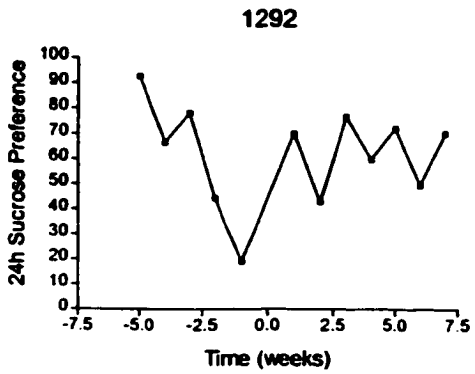
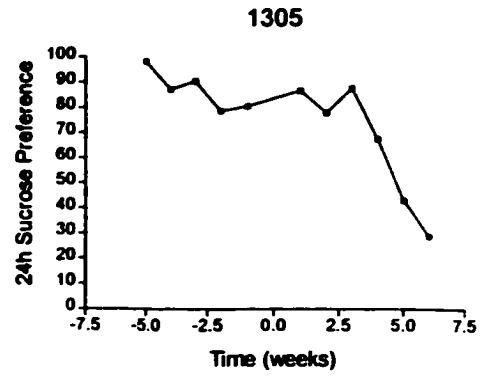
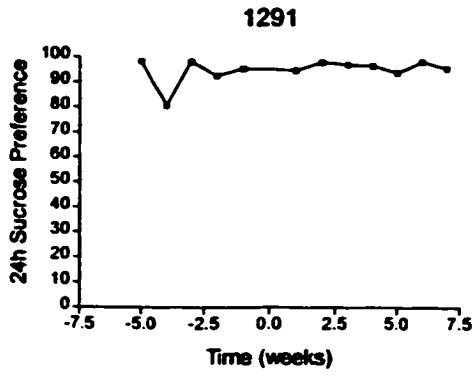


LE CMS Males 24h Sucrose Intake

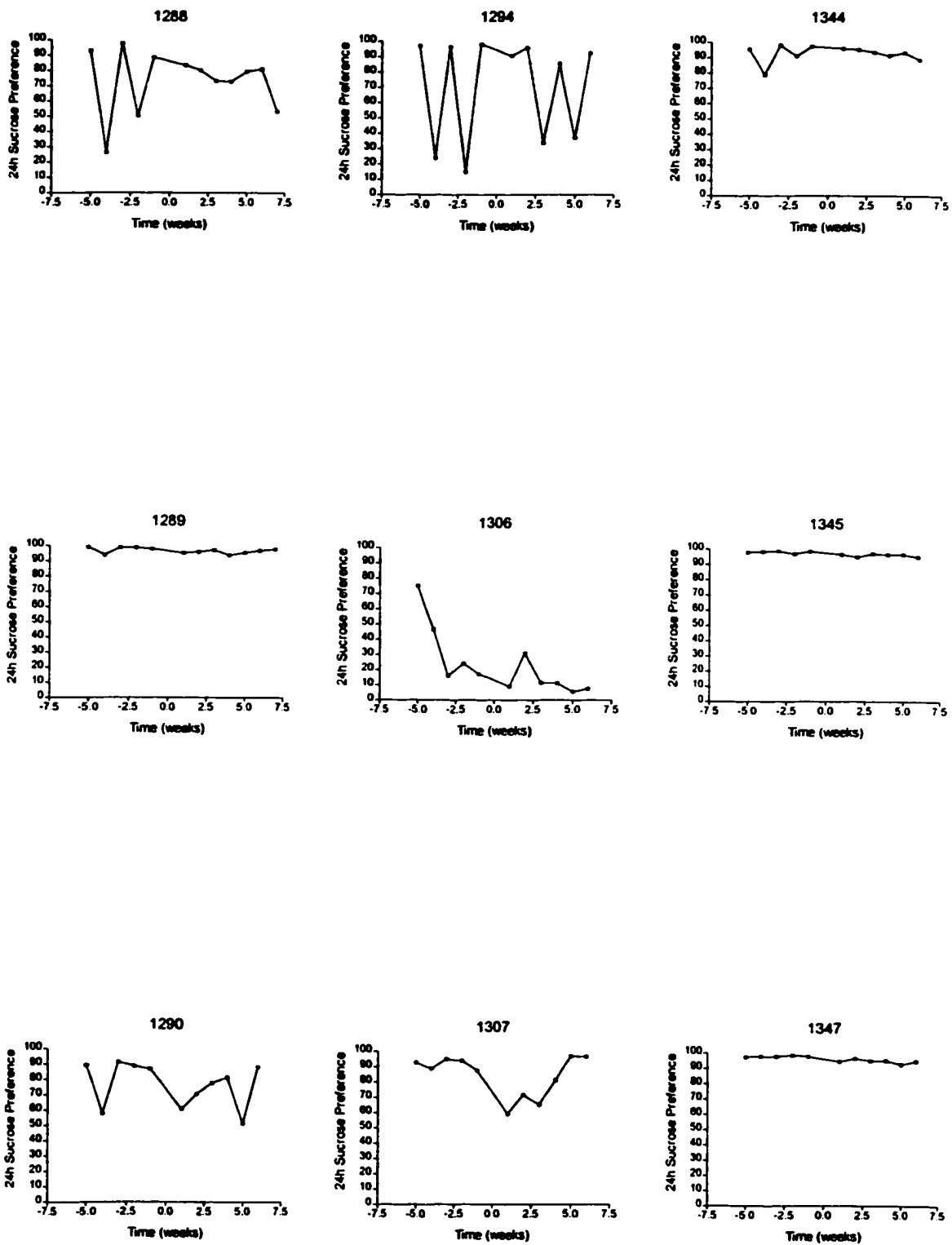


SD Control 24h % Sucrose Preference

265

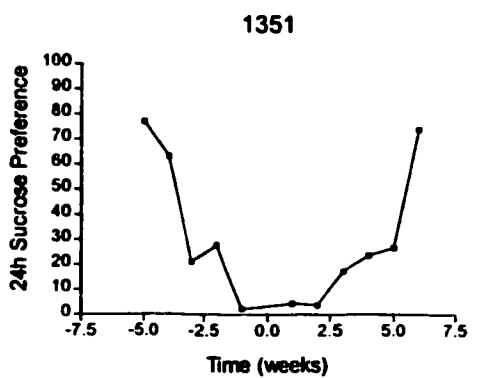
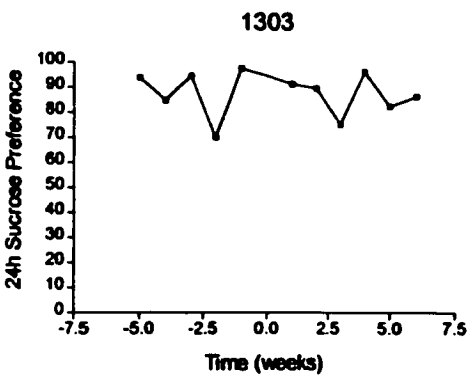
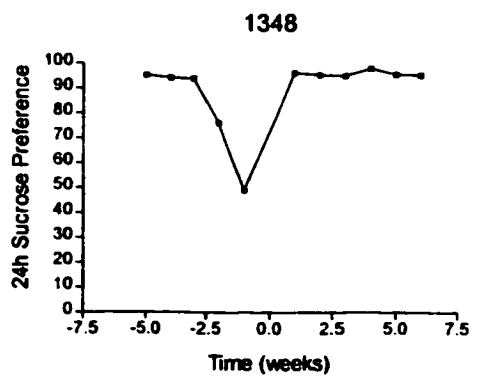
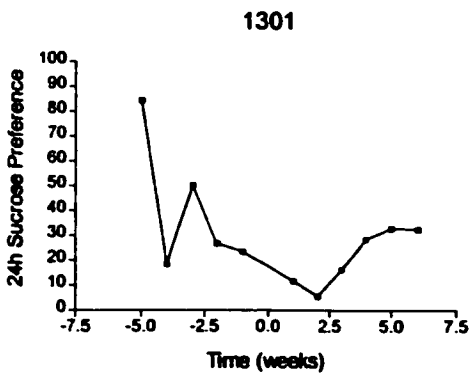
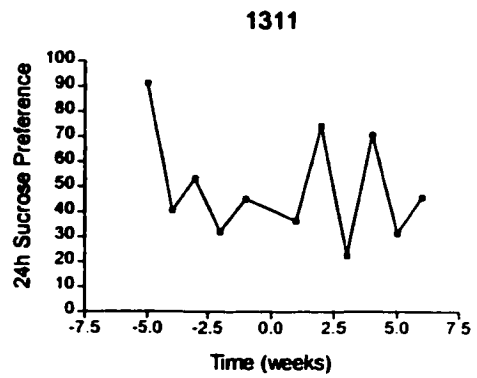
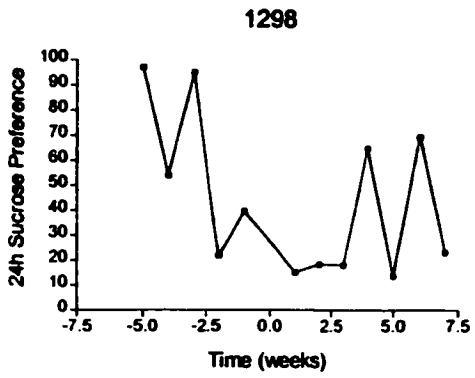
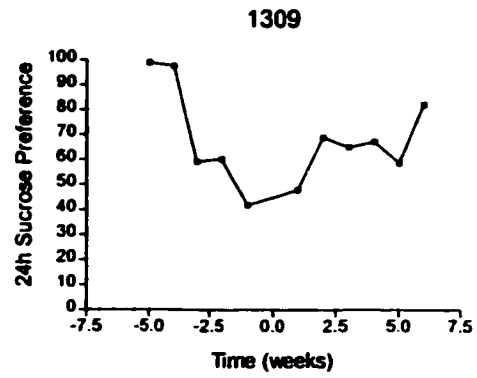
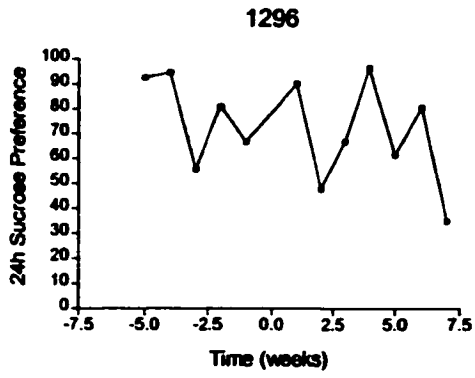


SD CMS 24h % Sucrose Preference

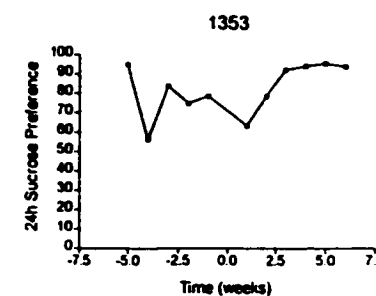
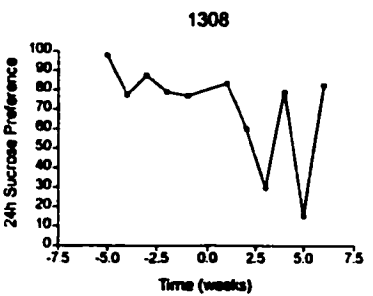
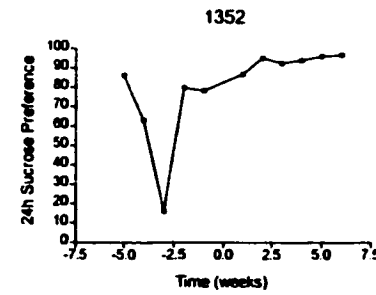
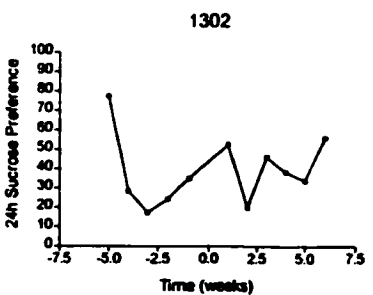
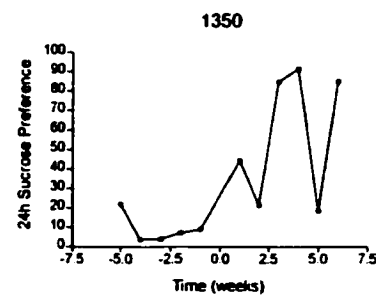
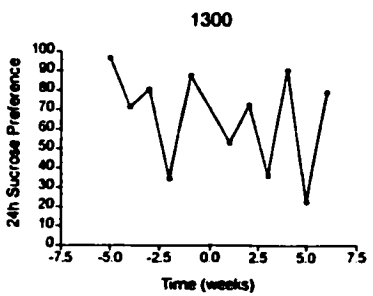
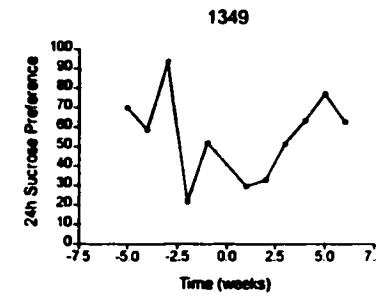
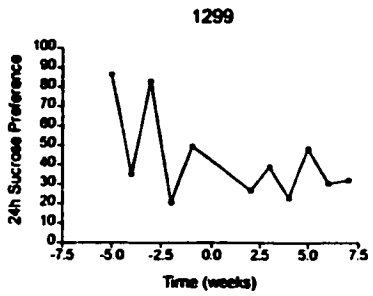
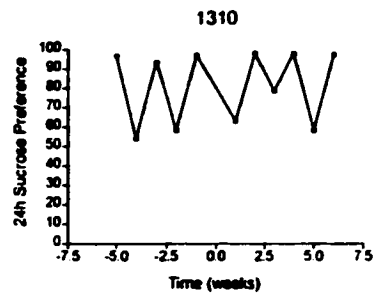
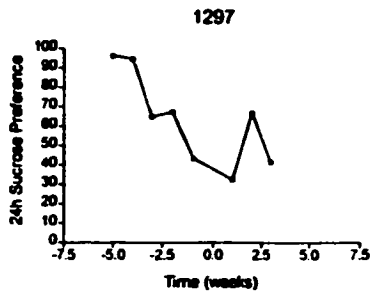


LE Control 24h % Sucrose Preference

267

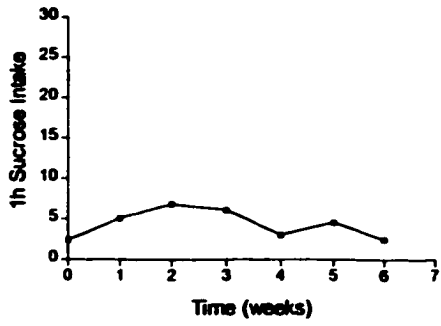


LE CMS 24h % Sucrose Preference

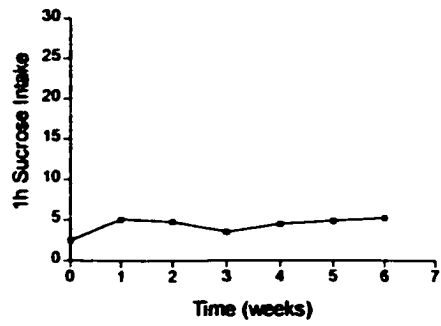


SD Control Females 1h Sucrose Intake

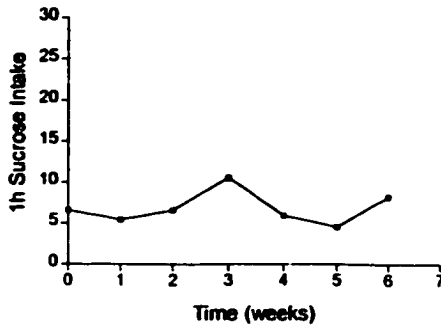
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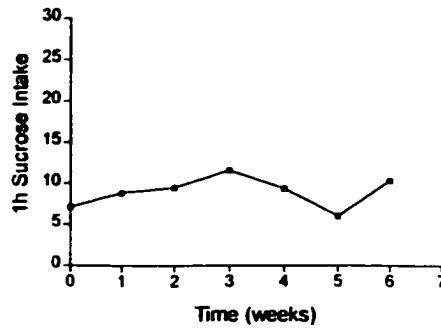
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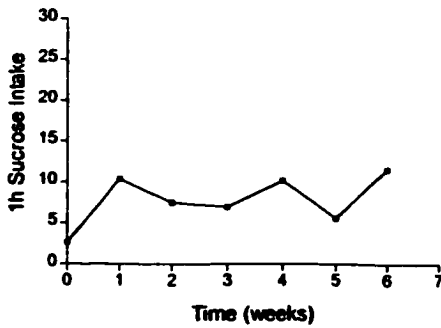
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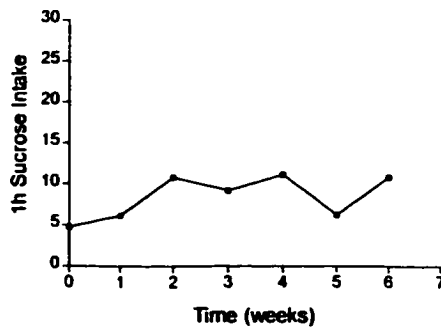
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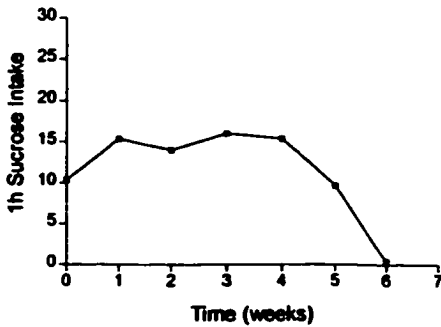
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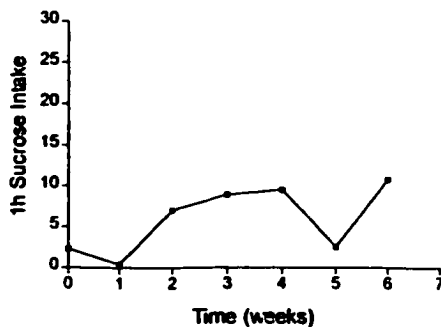
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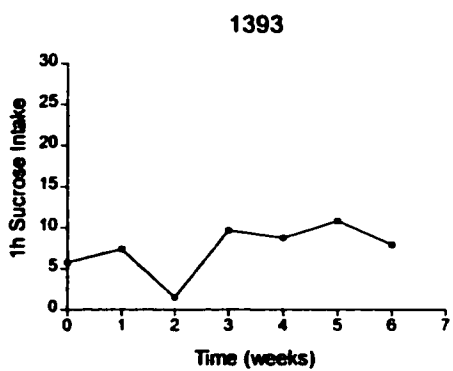
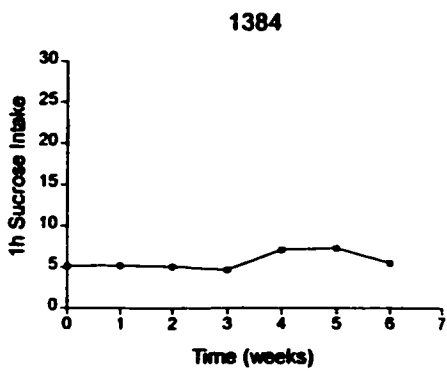
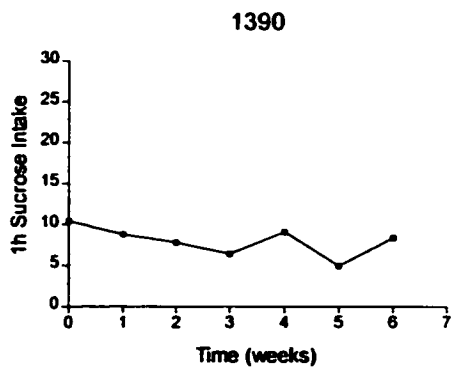
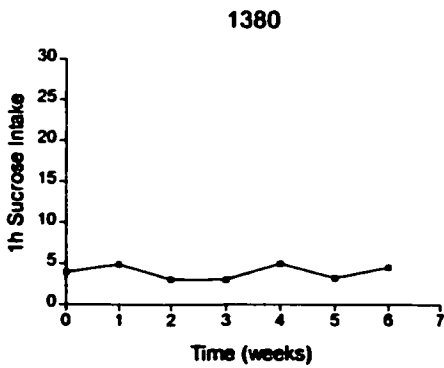
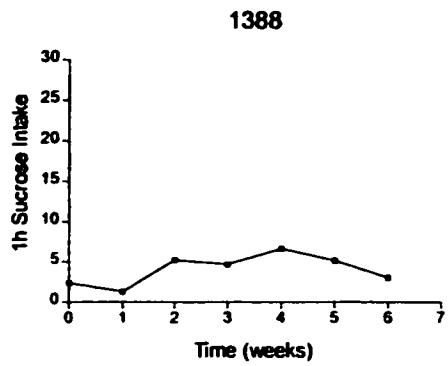
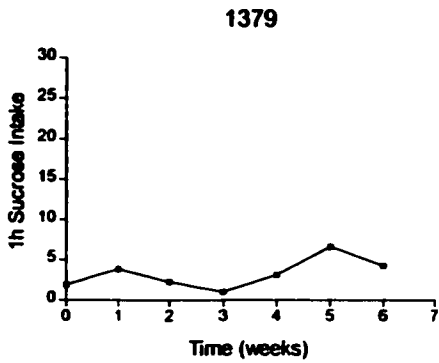
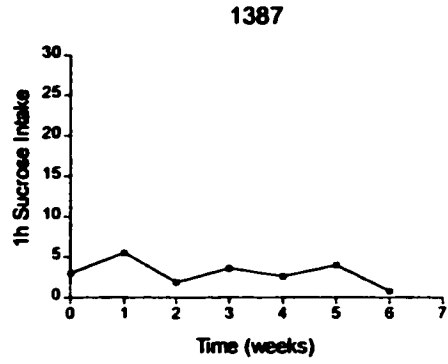
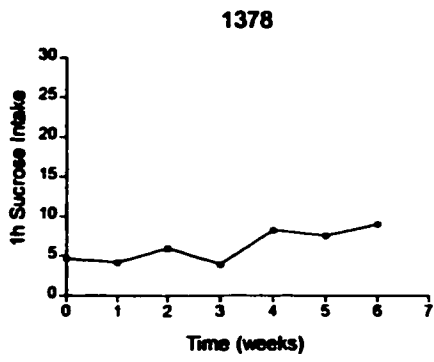


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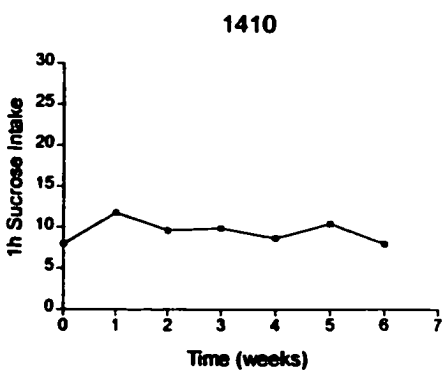
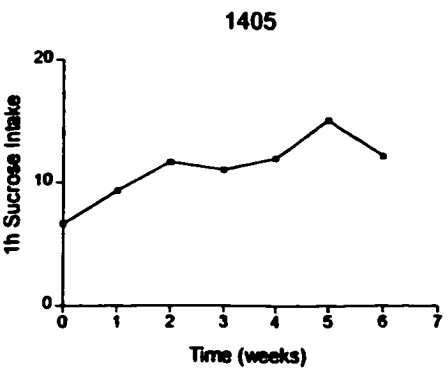
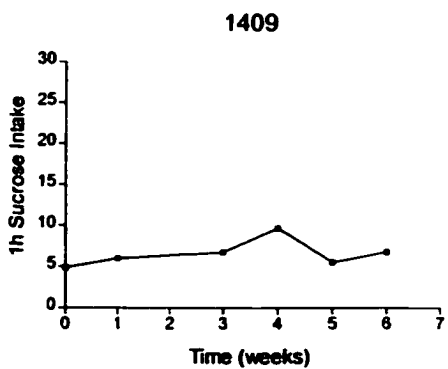
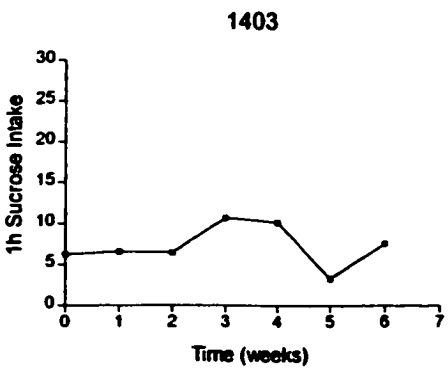
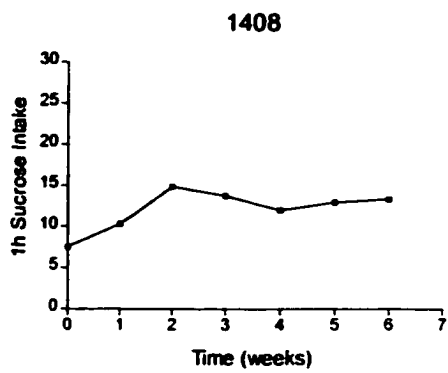
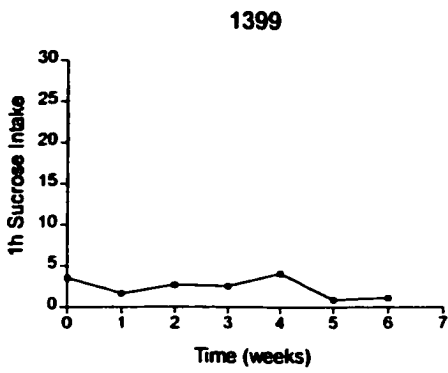
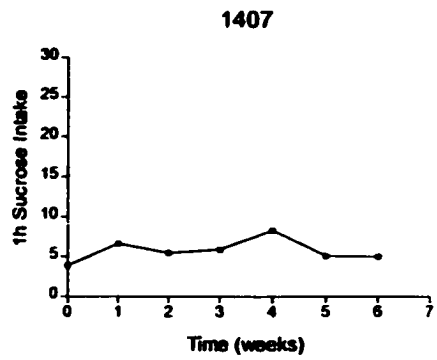
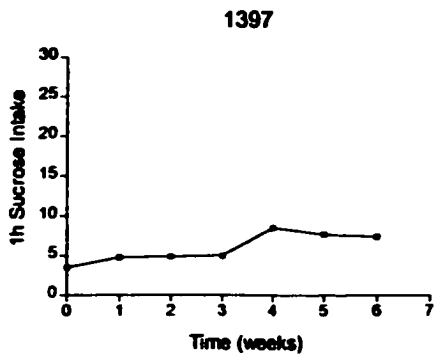


SD CMS Females 1h Sucrose Intake

270



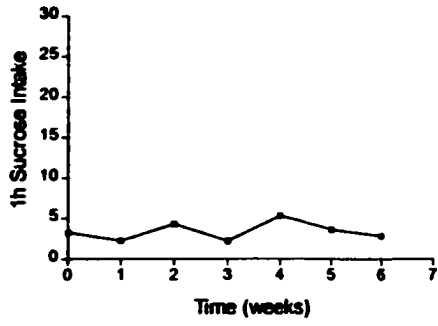
LE Control Females 1h Sucrose Intake



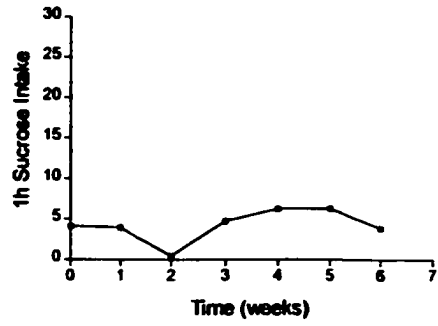
LE CMS Females 1h Sucrose Intake

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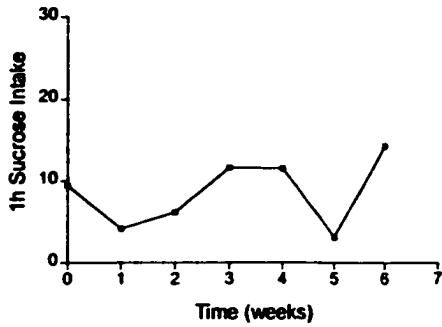
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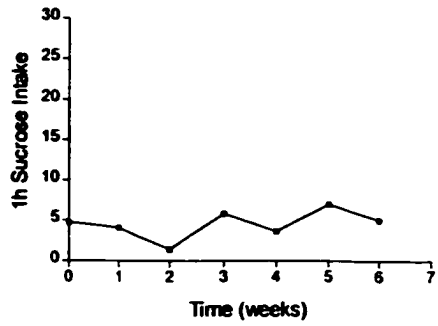
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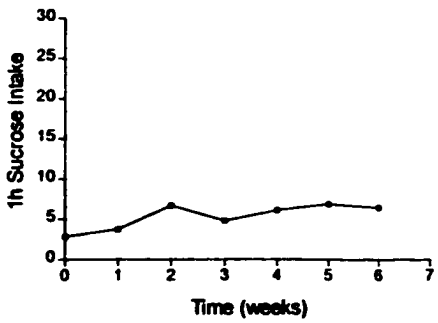
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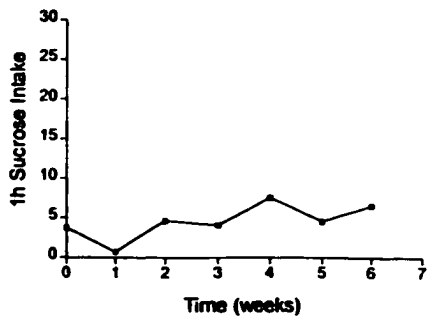
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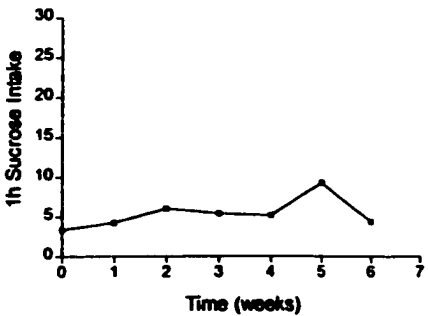
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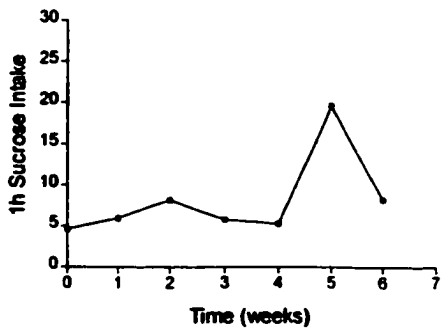
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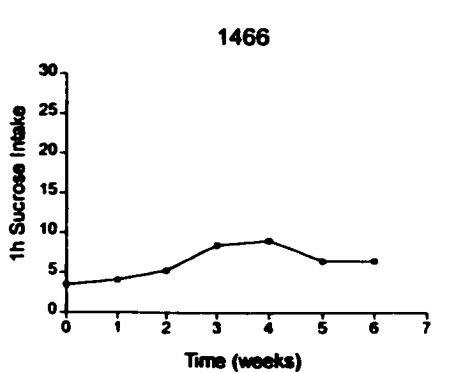
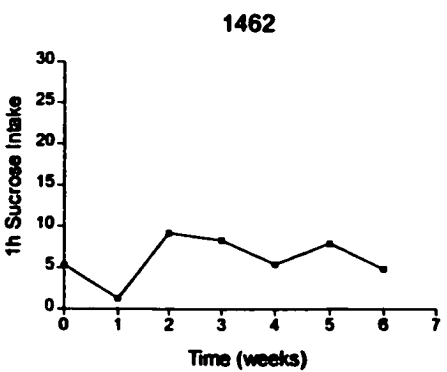
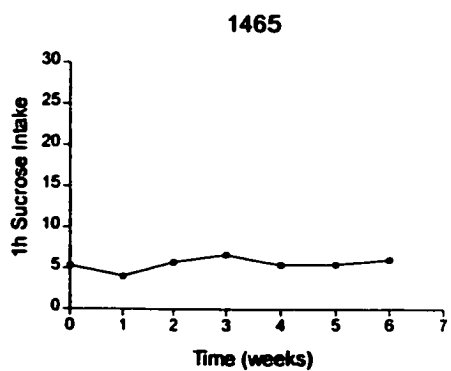
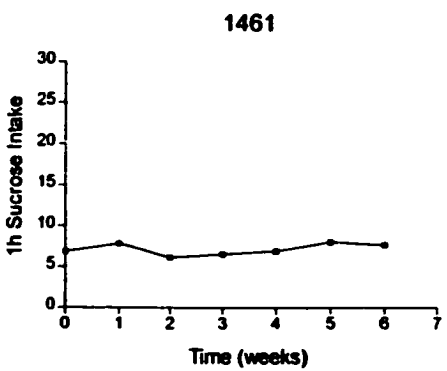
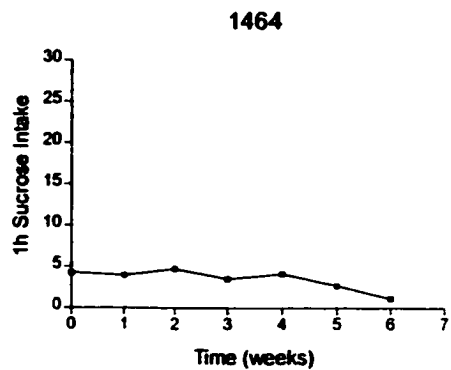
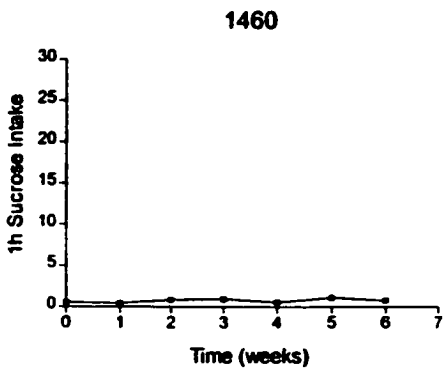
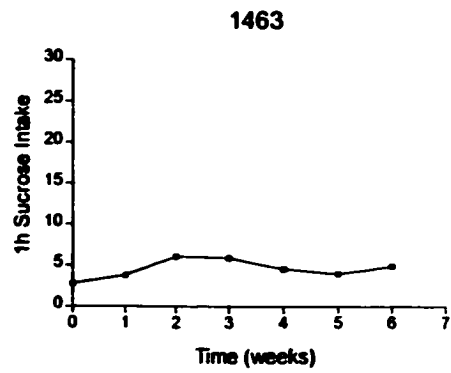
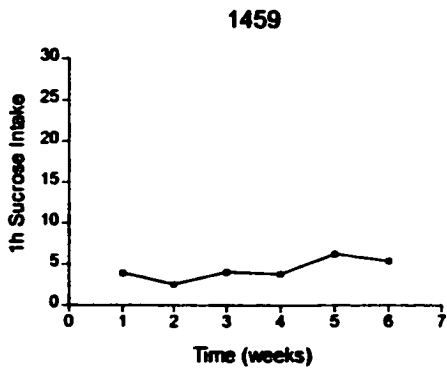


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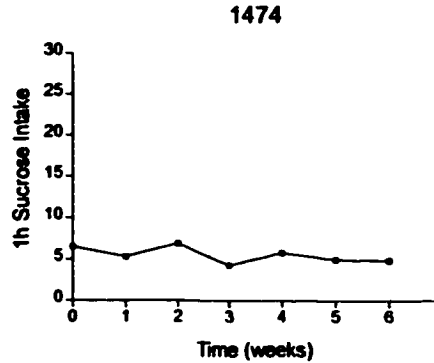
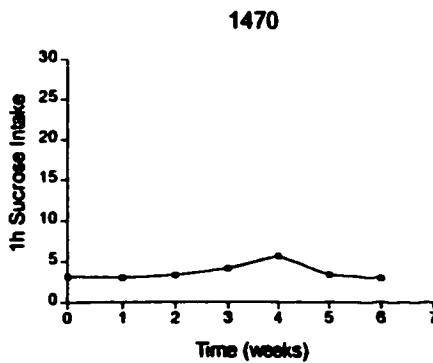
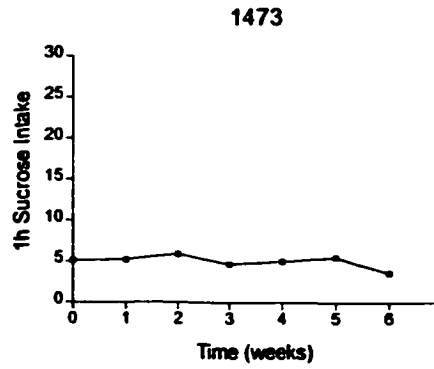
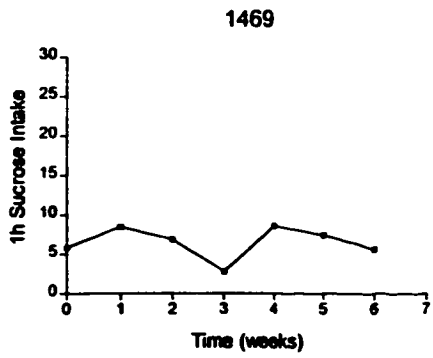
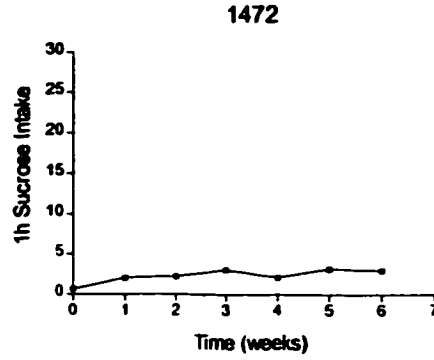
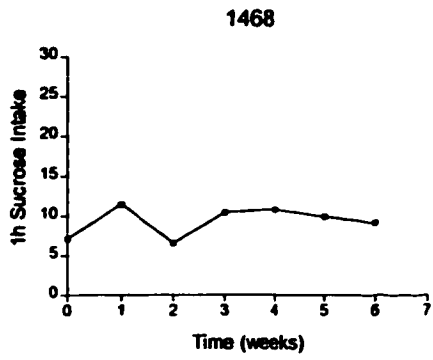
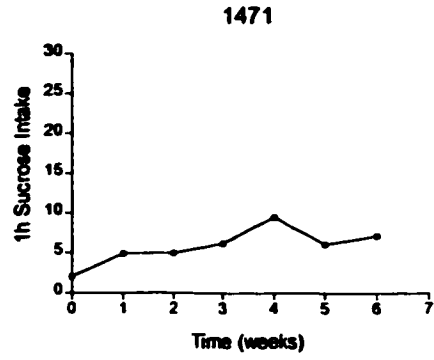
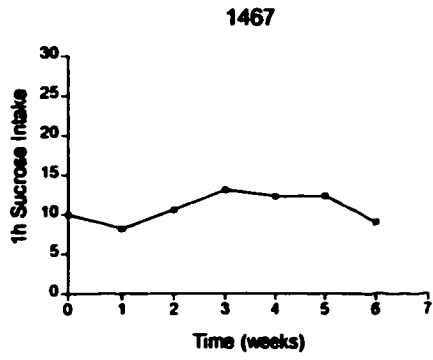
SD Grouped Females 1h Sucrose intake

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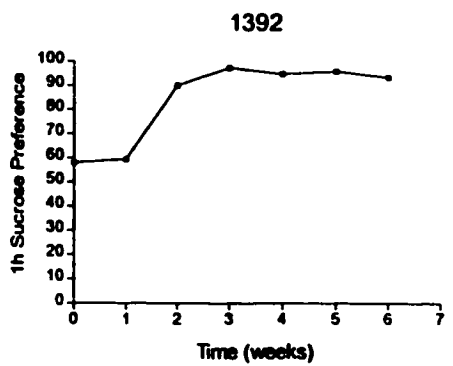
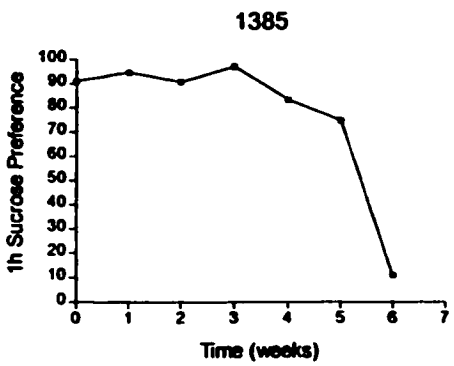
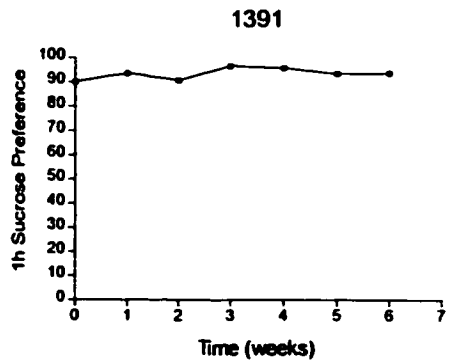
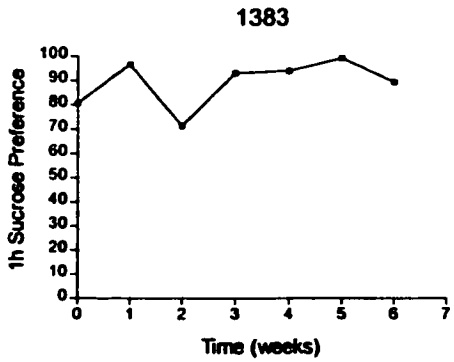
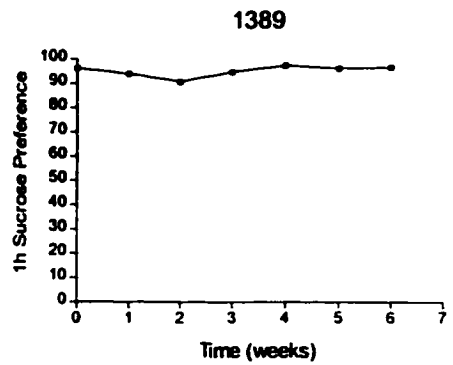
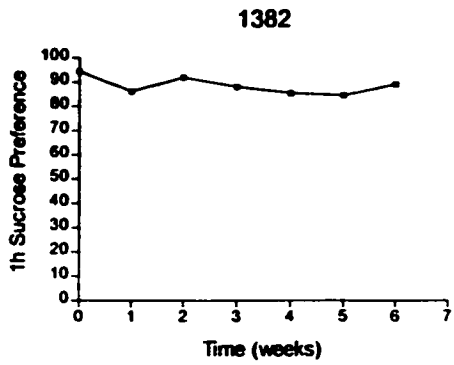
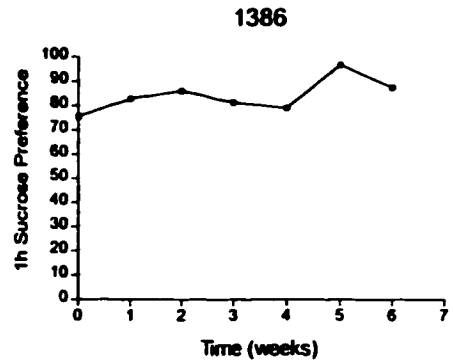
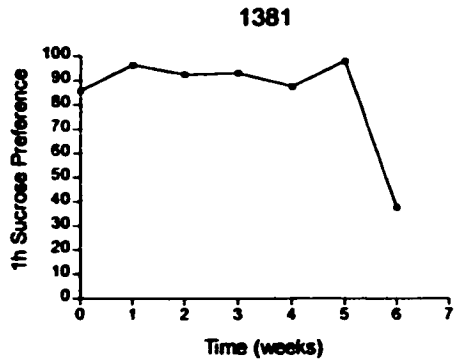
LE Grouped Females 1h Sucrose Intake

274



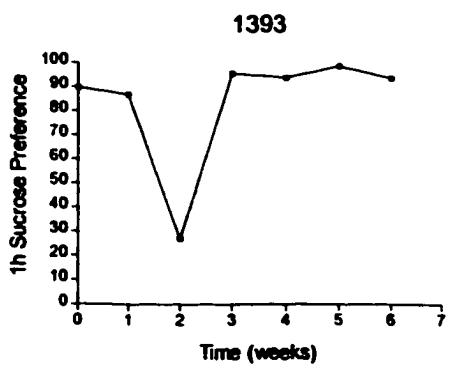
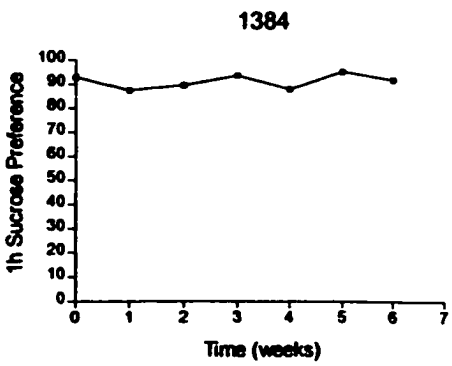
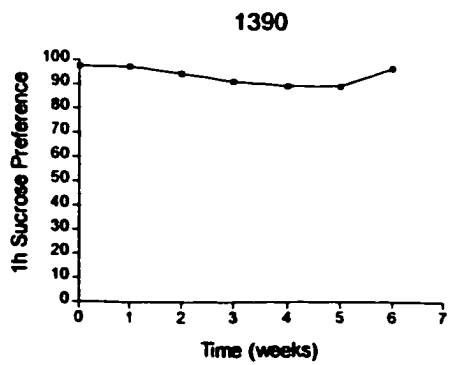
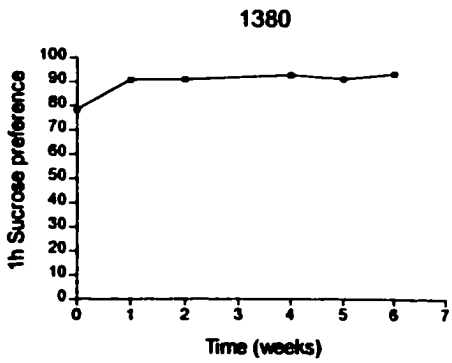
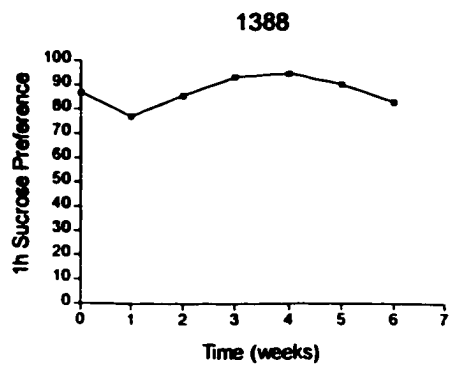
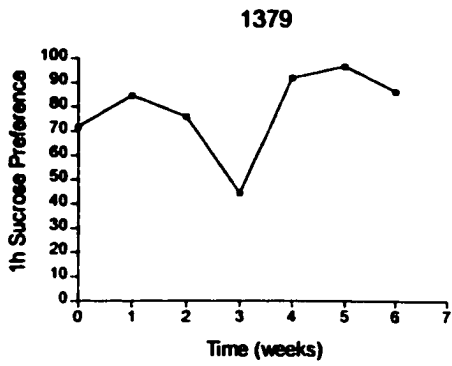
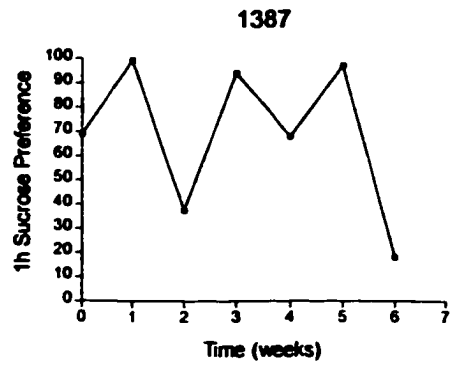
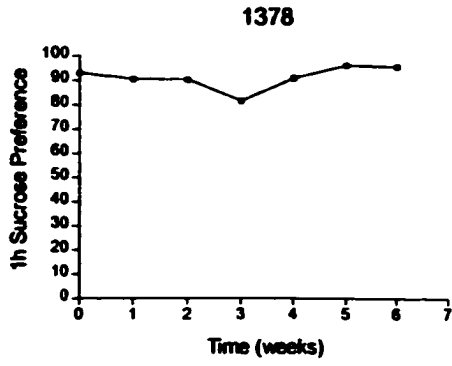
SD Control Females 1h % Sucrose Preference

275



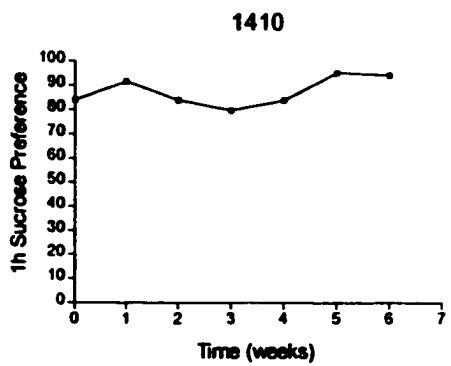
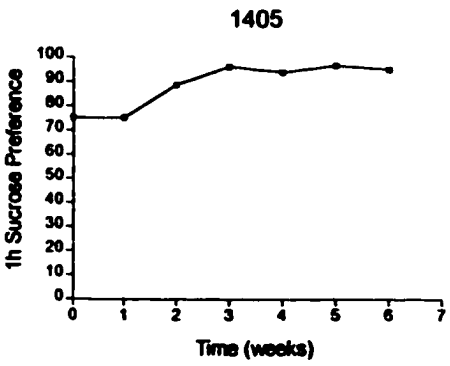
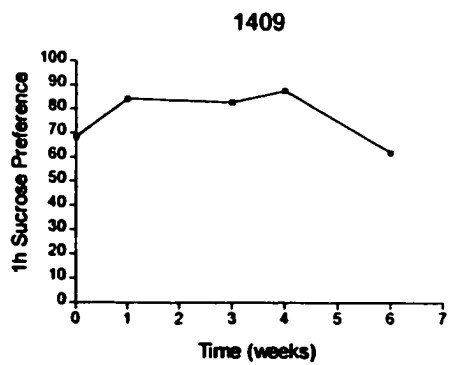
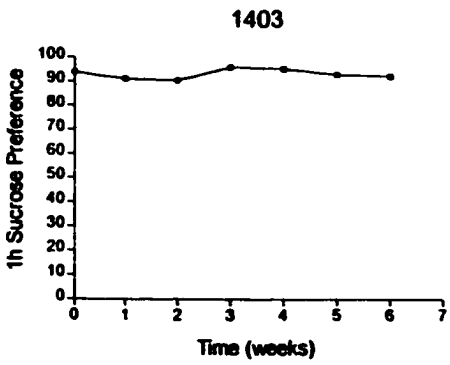
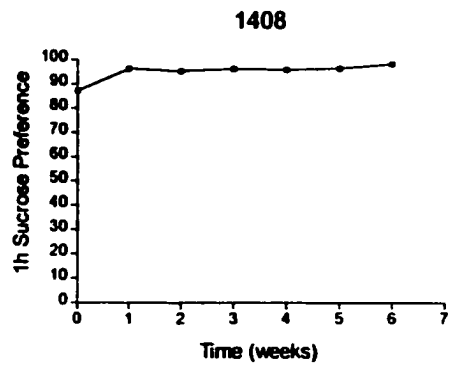
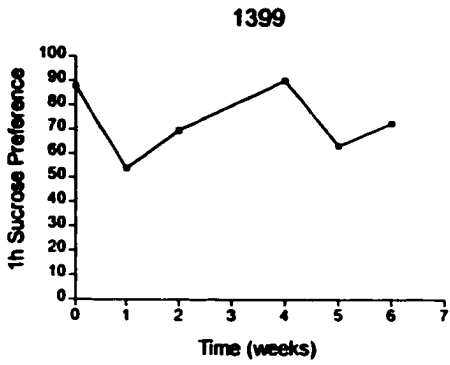
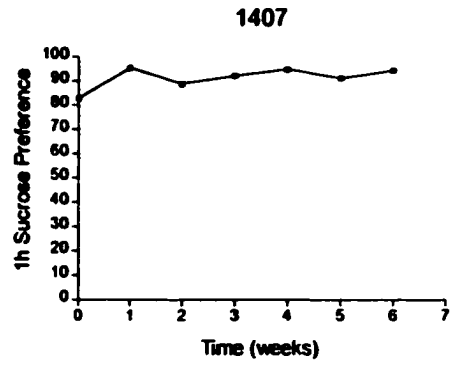
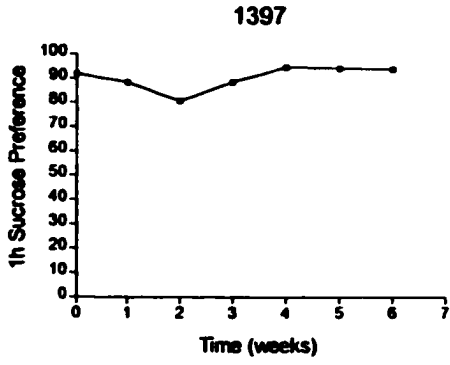
SD CMS Females 1h % Sucrose Preference

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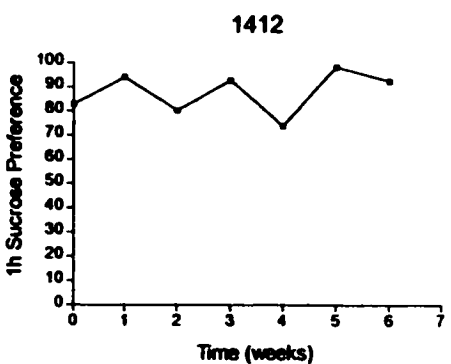
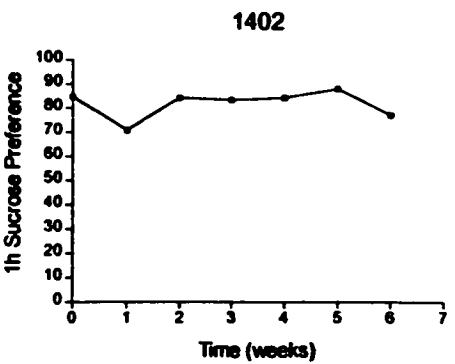
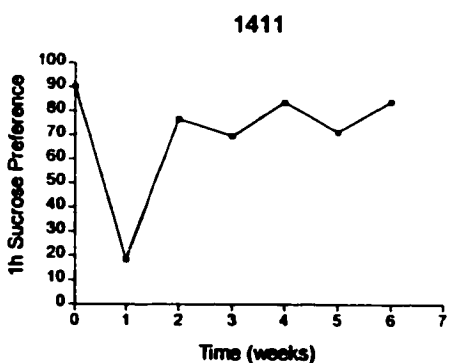
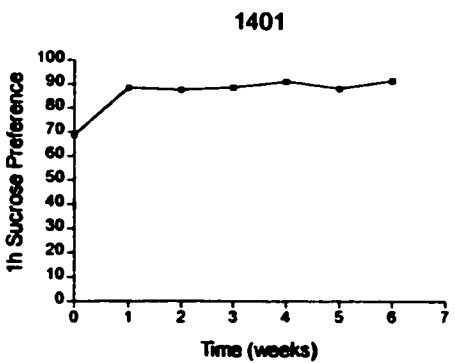
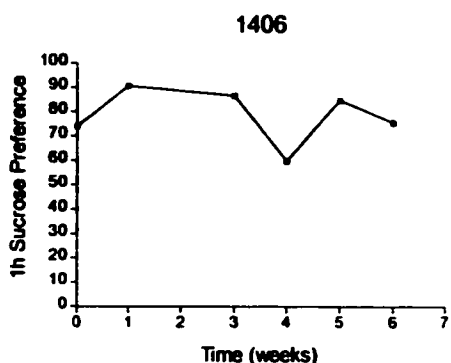
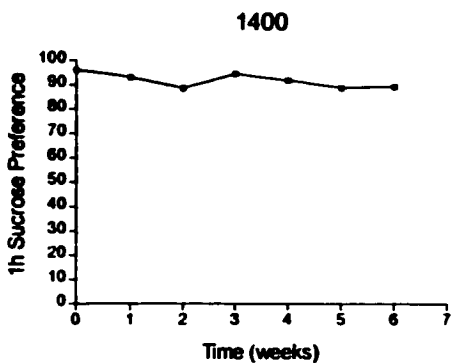
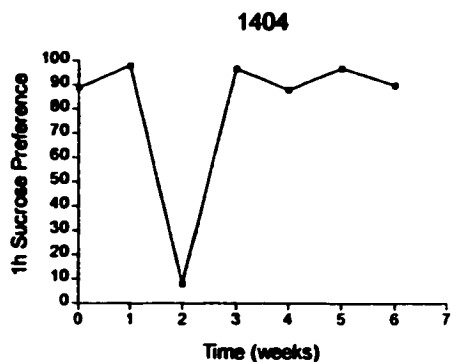
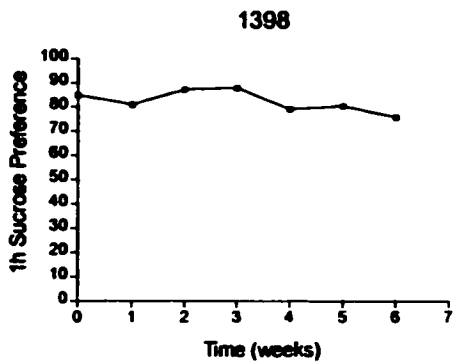
LE Control Females 1h % Sucrose Preference

277



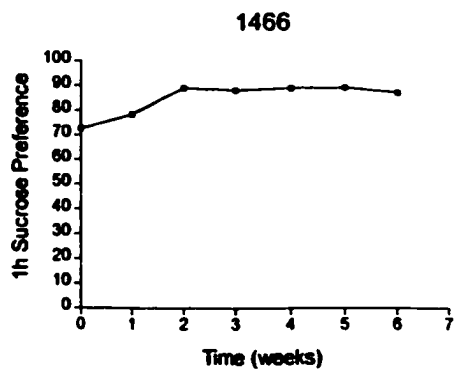
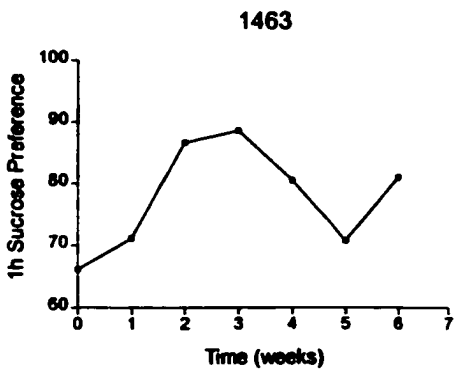
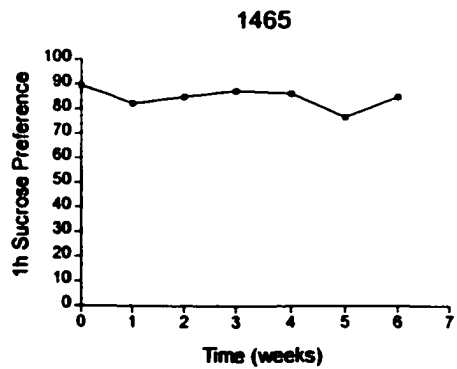
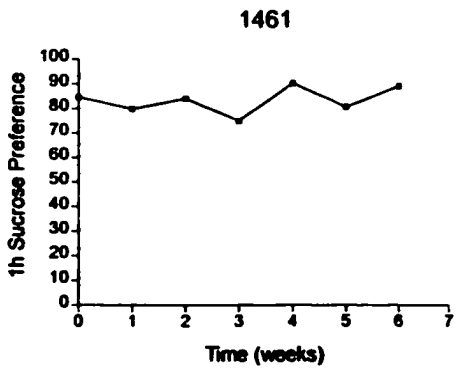
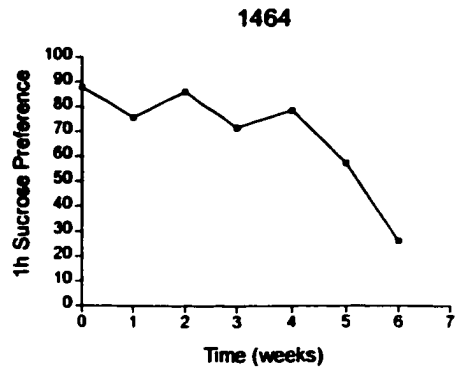
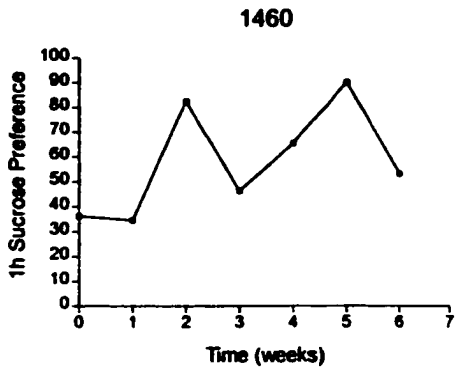
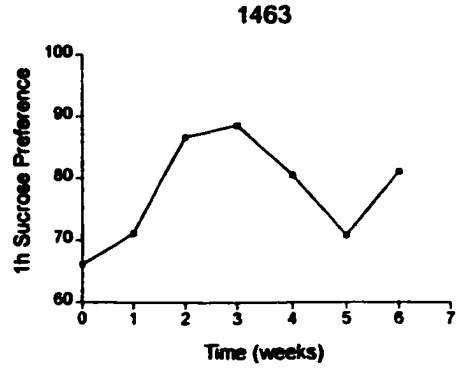
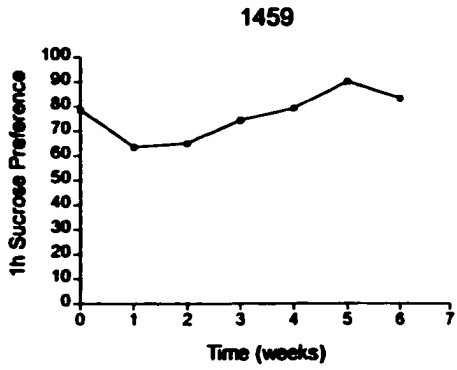
LE CMS Females 1h % Sucrose Preference

278



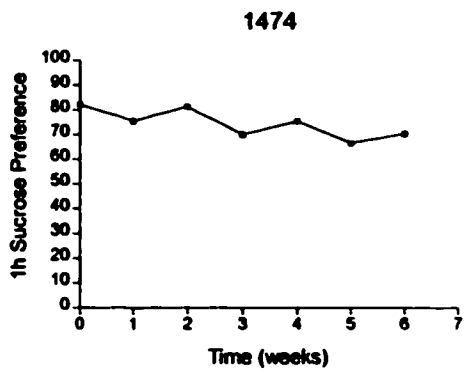
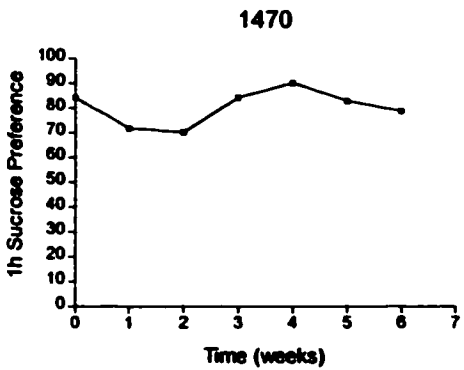
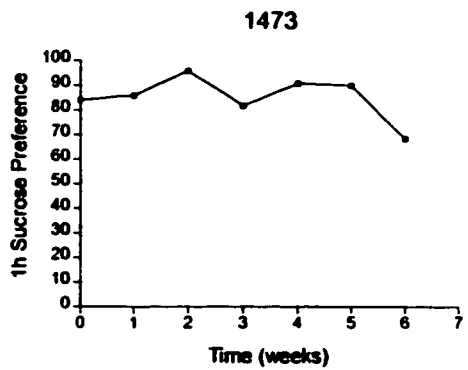
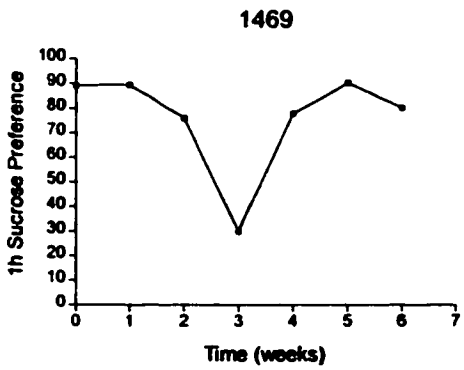
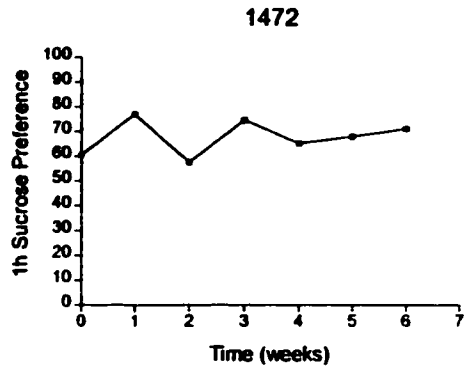
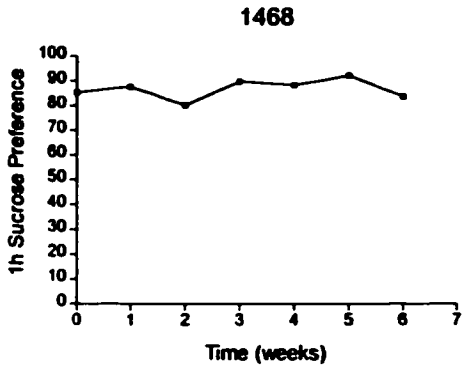
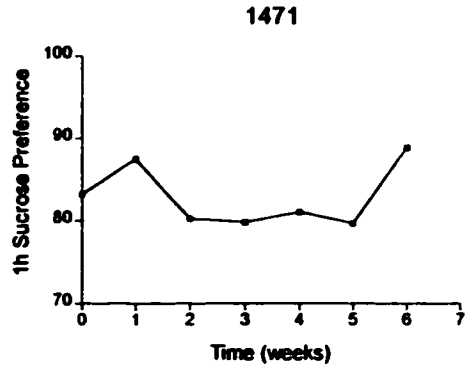
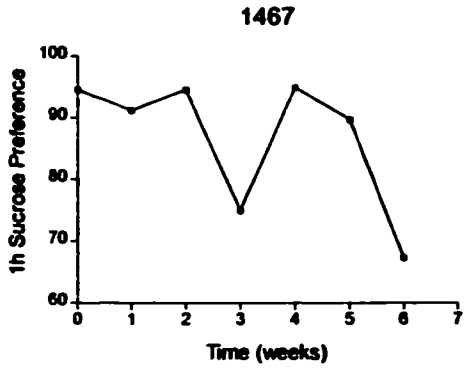
SD Grouped Females 1h % Sucrose Preference

279



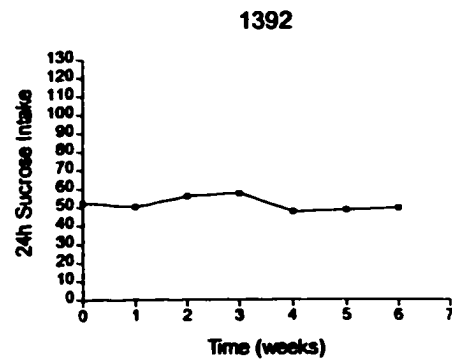
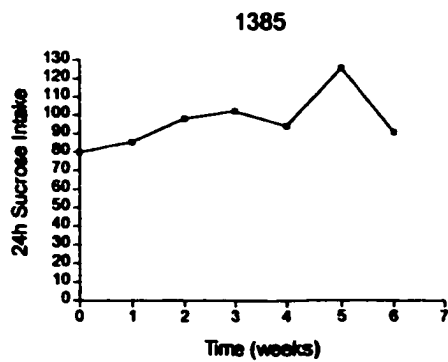
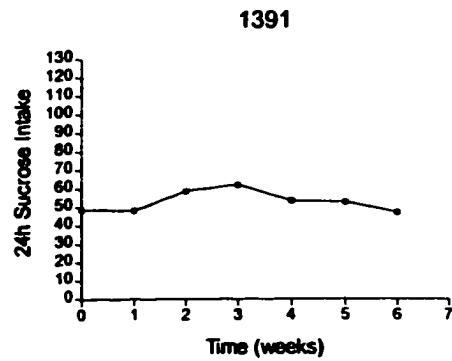
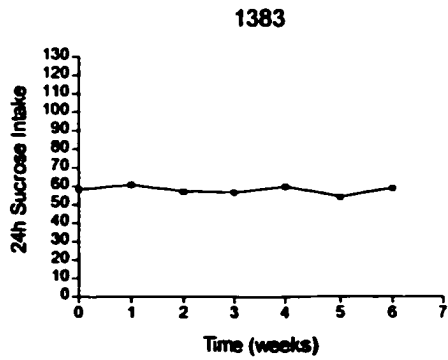
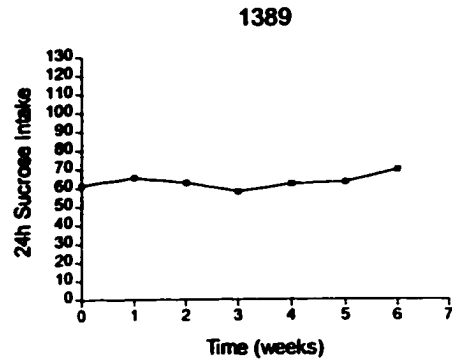
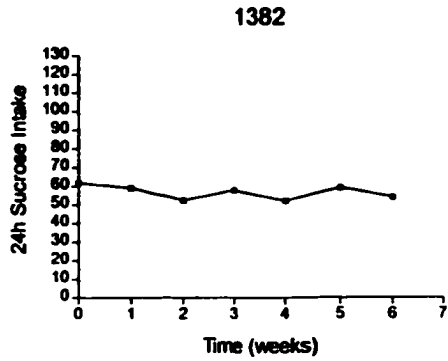
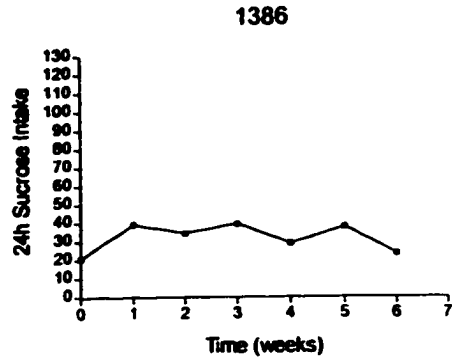
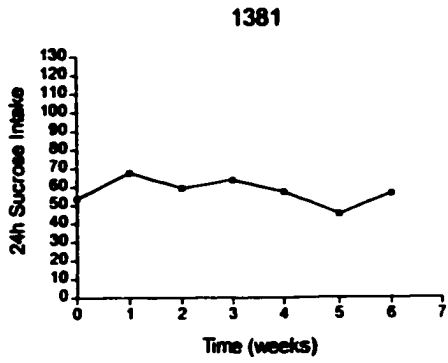
LE Grouped Females 1h % Sucrose Preference

280



SD Control Females 24h Sucrose Intake

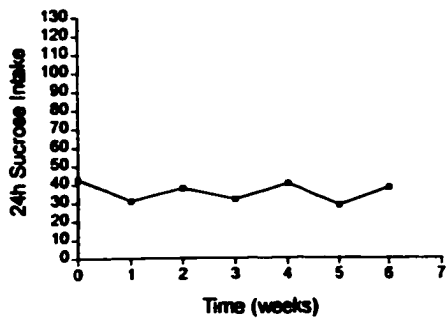
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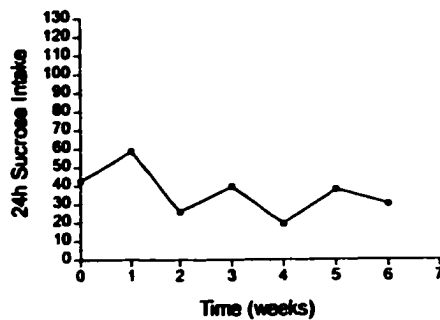
SD CMS Females 24h Sucrose Intake

282

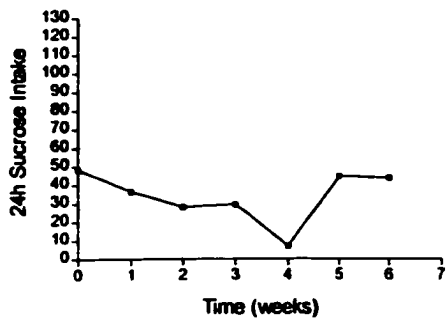
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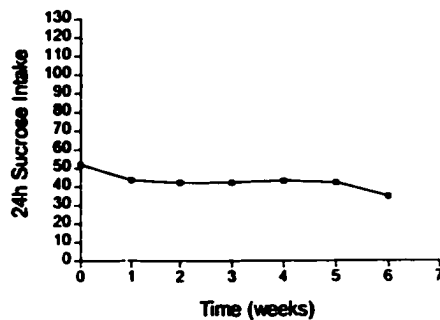
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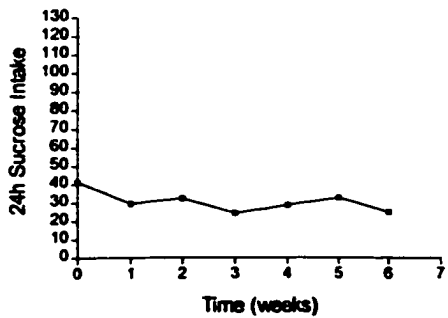
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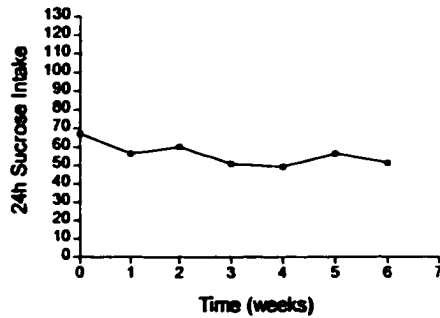
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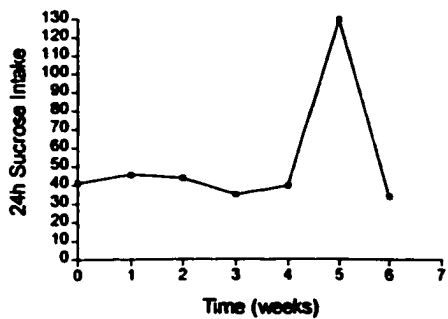
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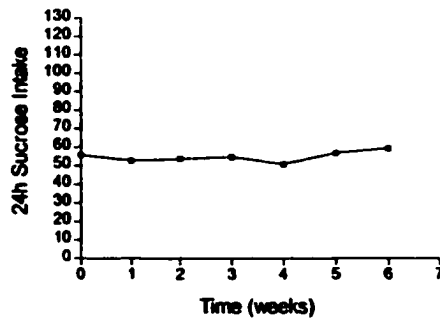
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1384

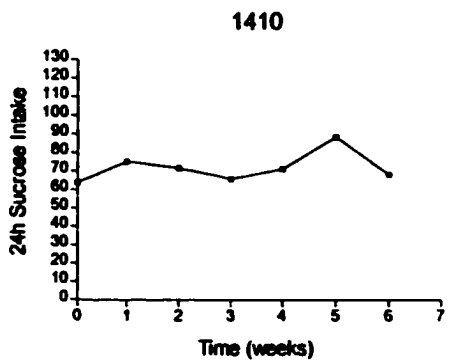
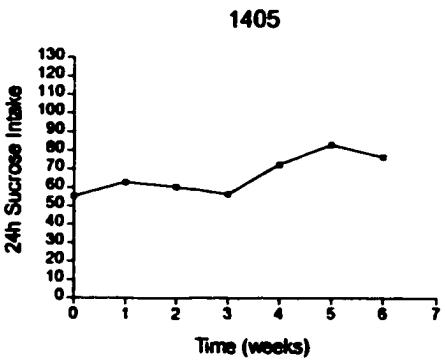
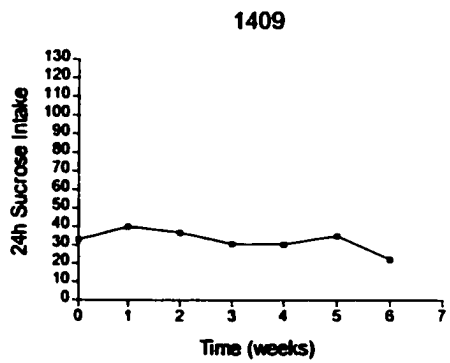
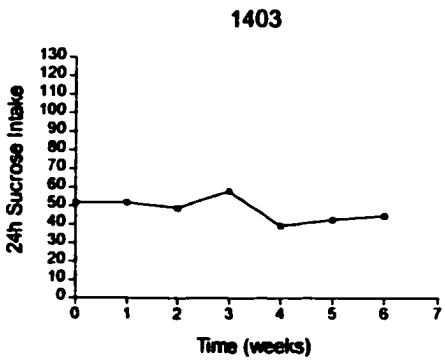
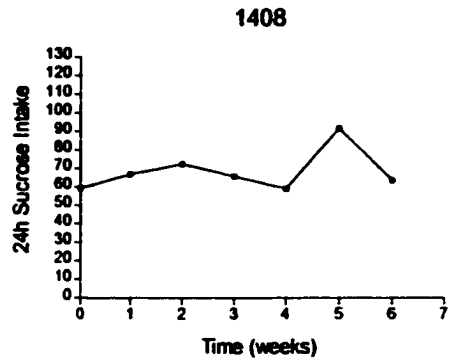
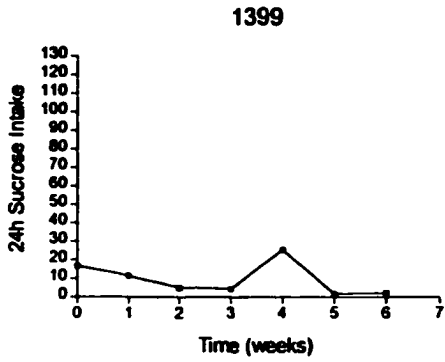
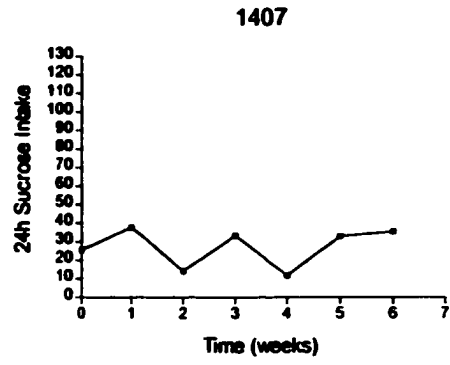
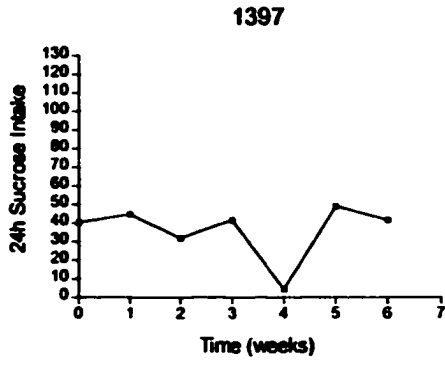


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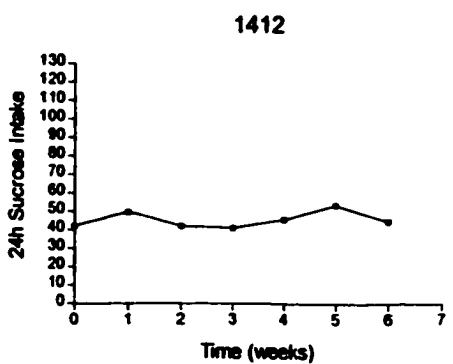
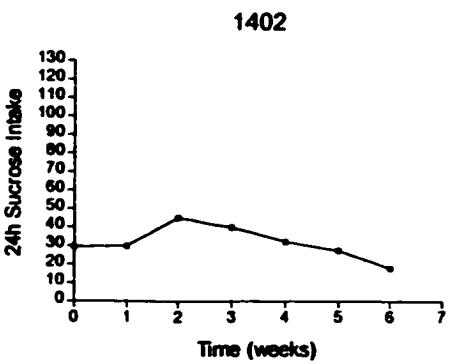
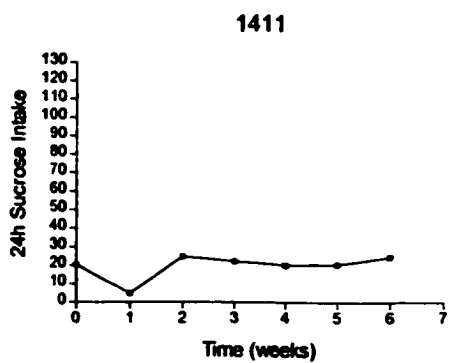
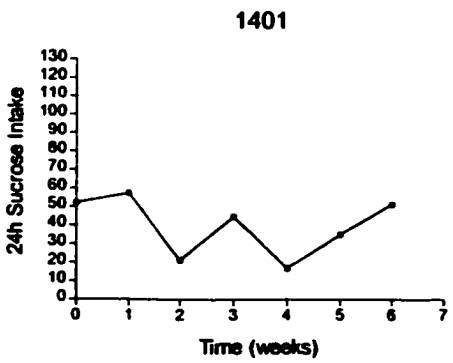
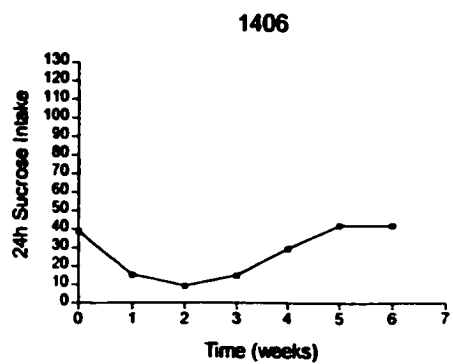
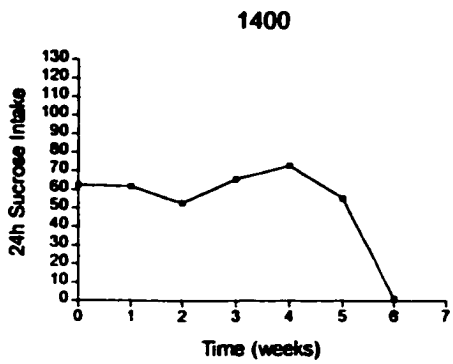
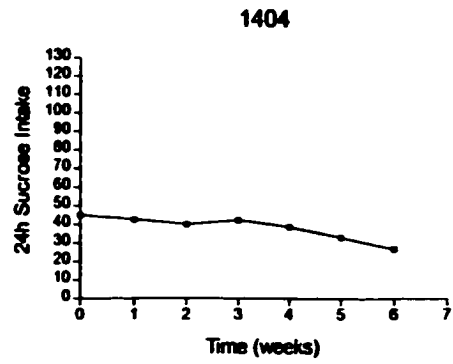
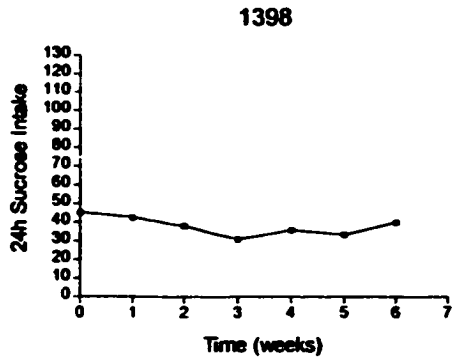
LE Control Females 24h Sucrose Intake

283



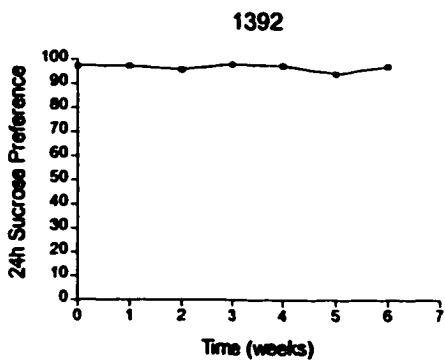
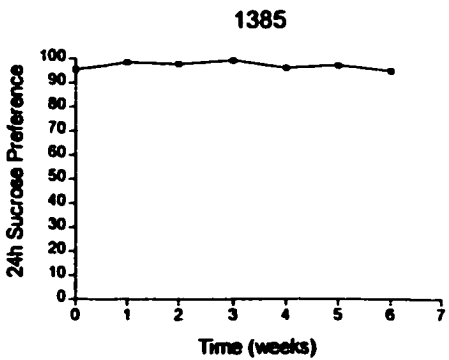
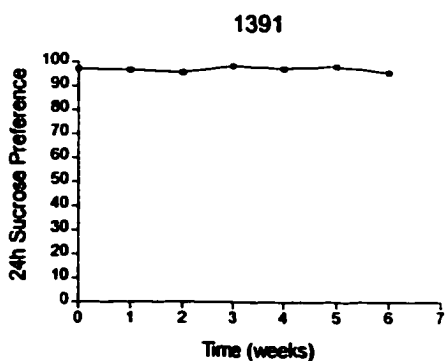
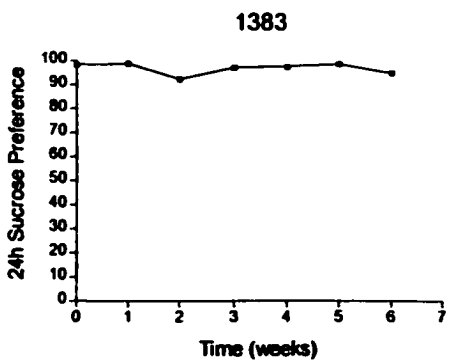
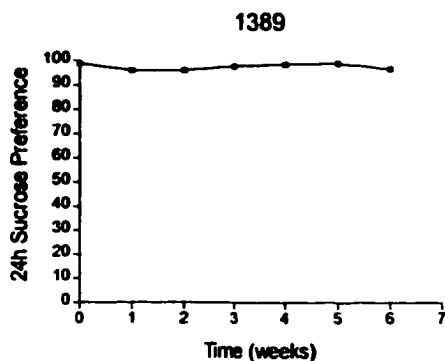
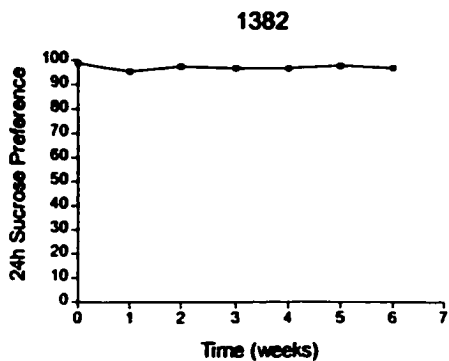
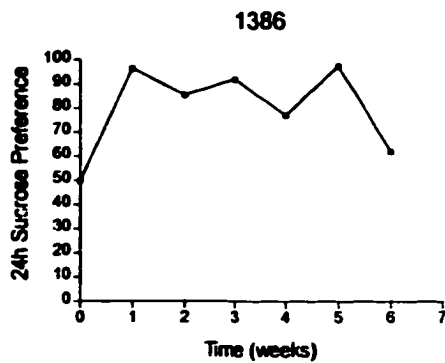
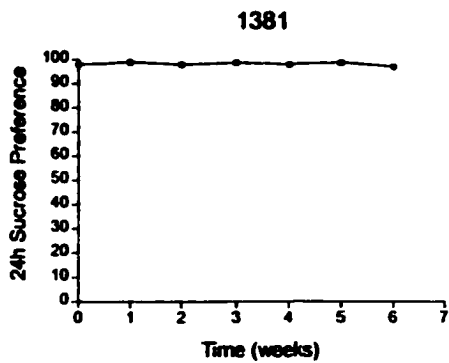
LE CMS Females 24h Sucrose Intake

284



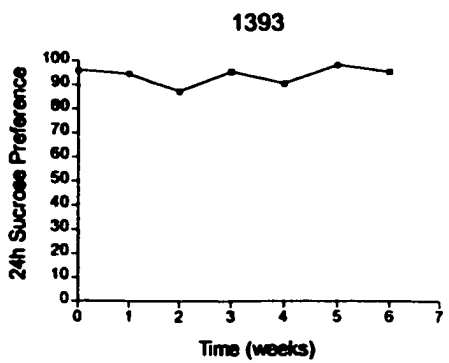
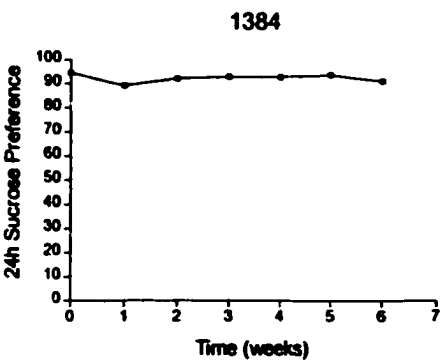
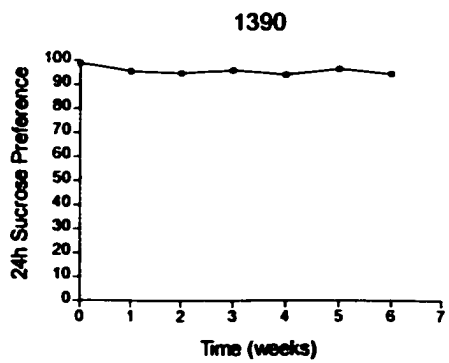
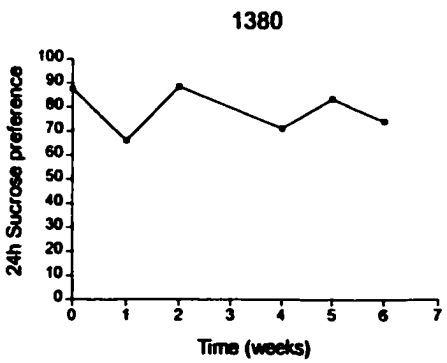
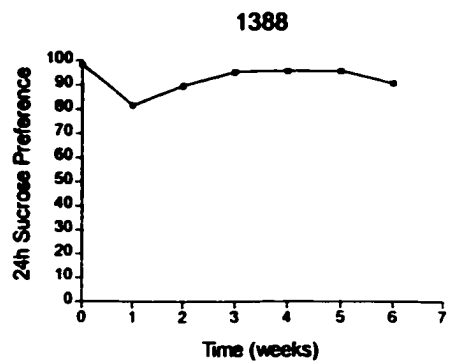
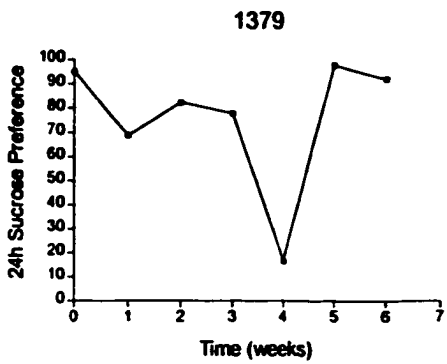
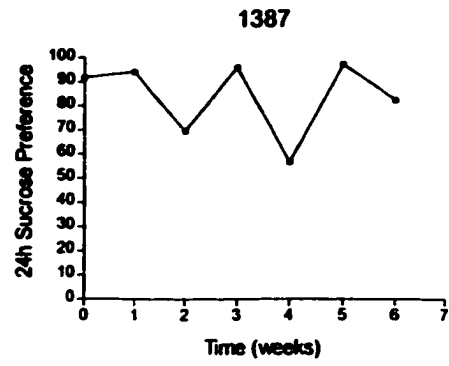
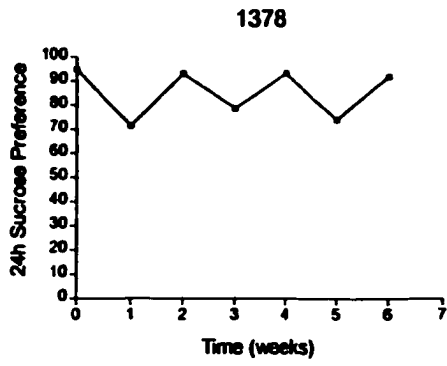
SD Control Females 24h % Sucrose Preference

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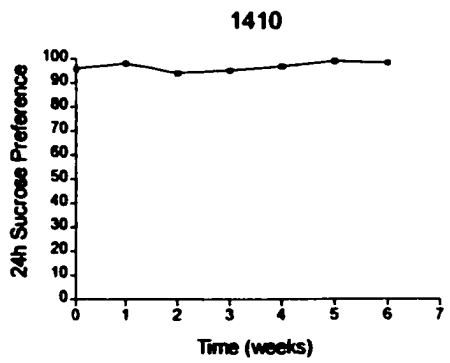
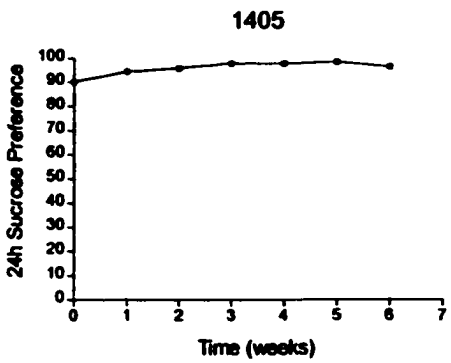
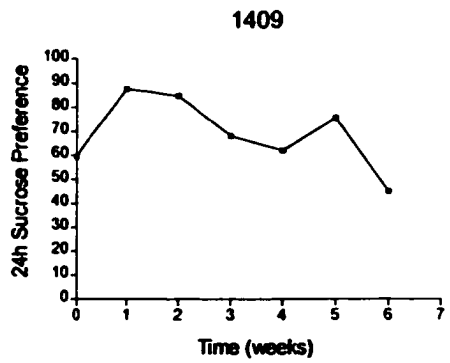
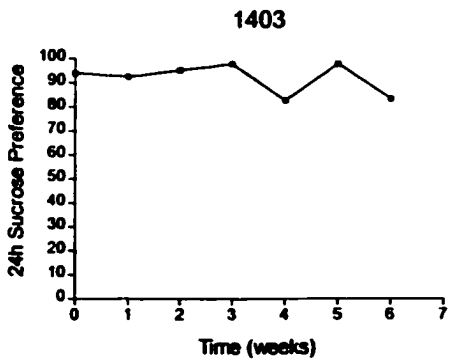
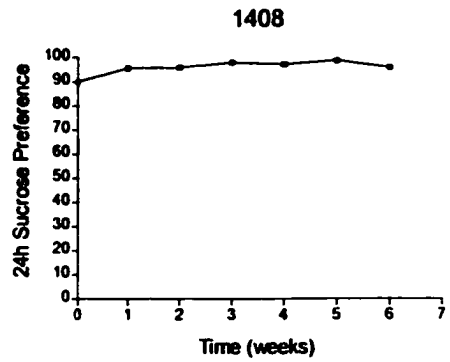
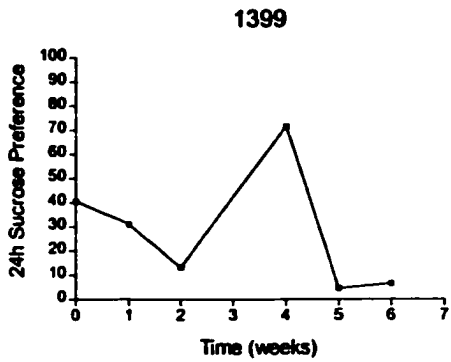
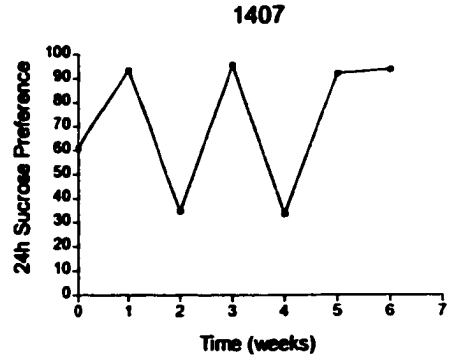
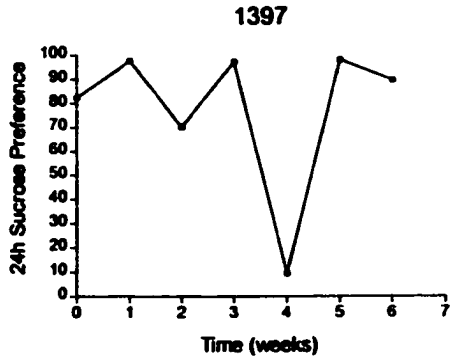
SD CMS Females 24h % Sucrose Preference

286



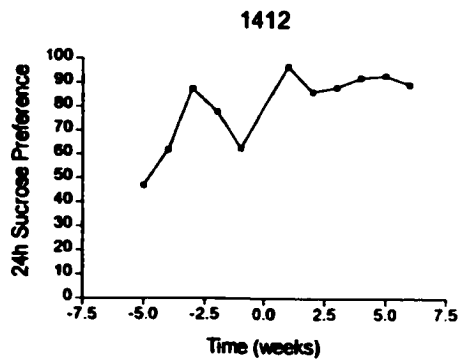
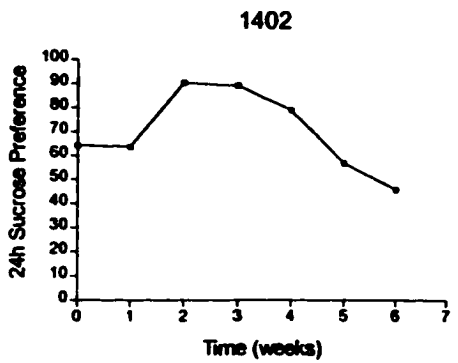
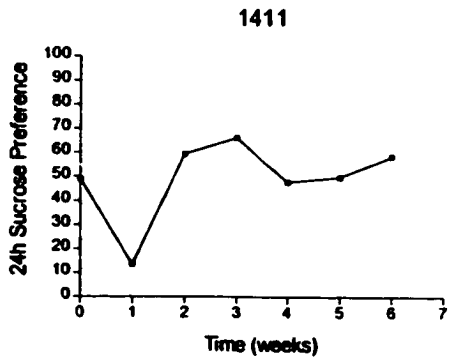
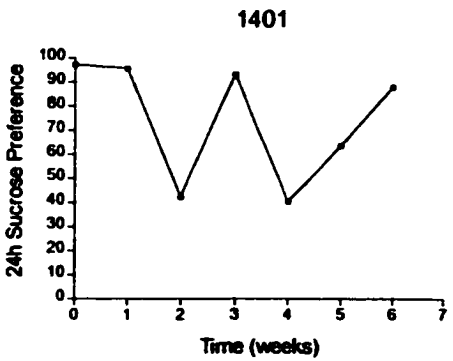
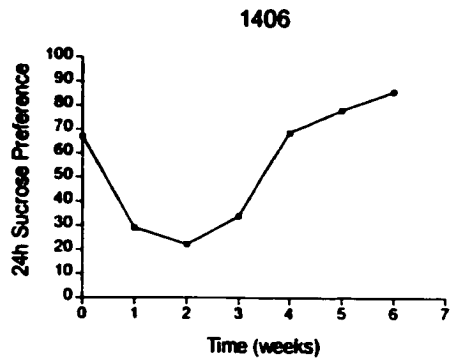
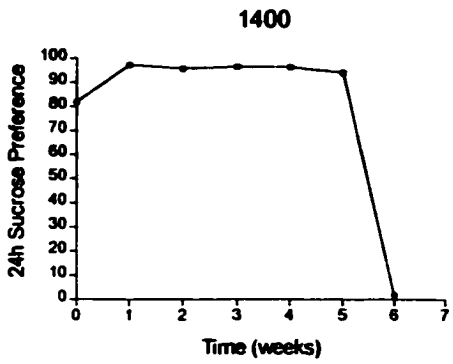
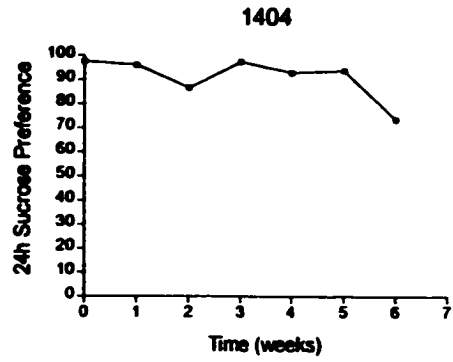
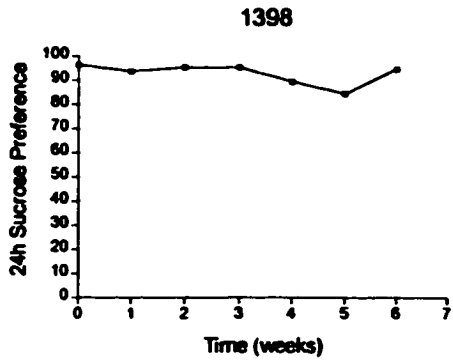
LE Control Females 24h % Sucrose Preference

287



LE CMS Females 24h % Sucrose Preference

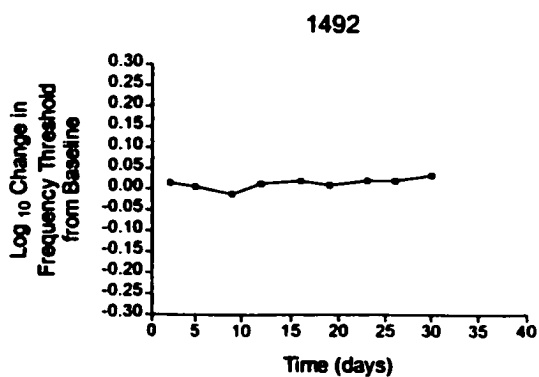
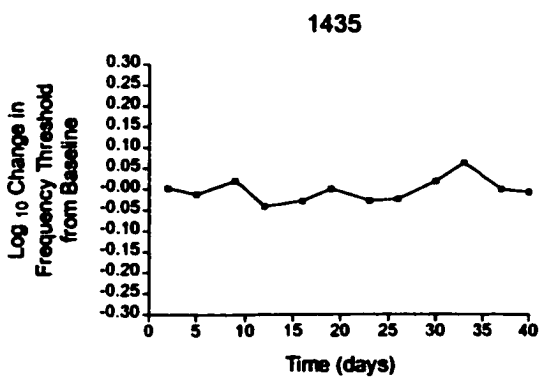
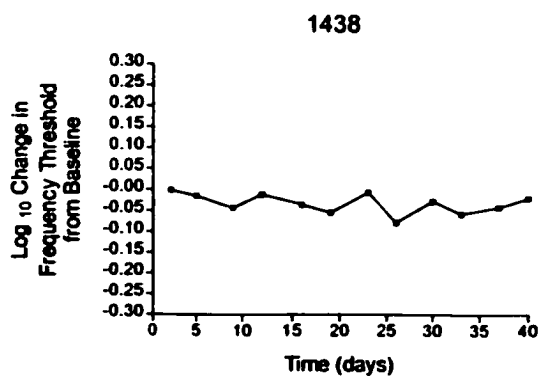
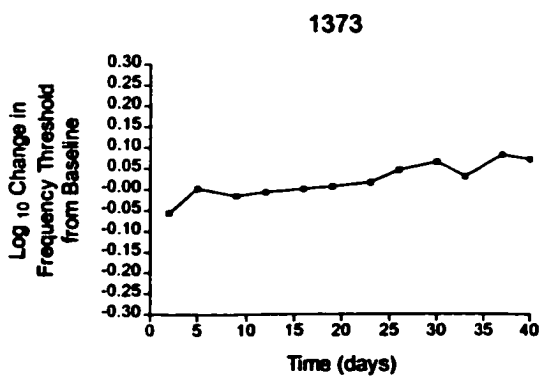
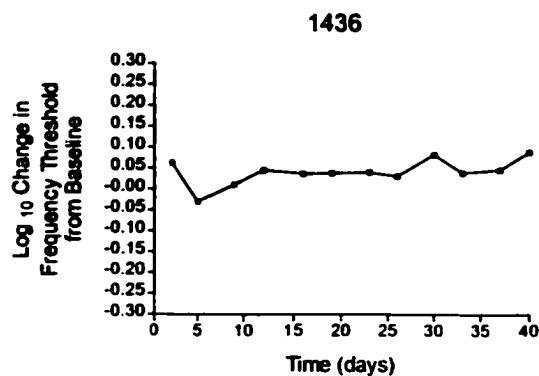
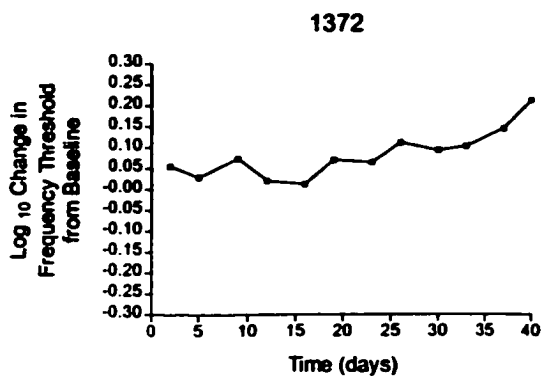
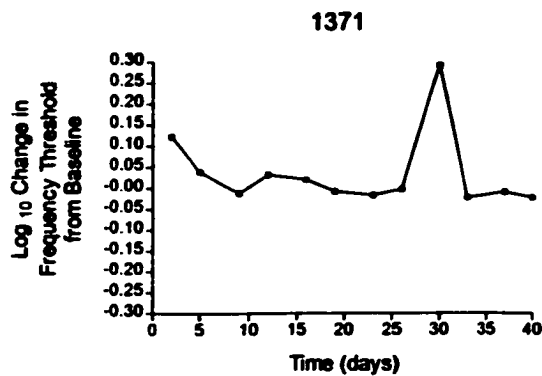
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Appendix 3

Brain-stimulation reward thresholds for individual male and female rats

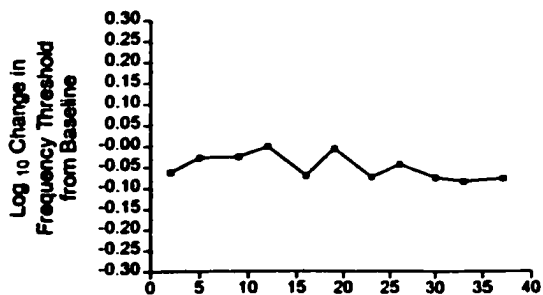
Sprague-Dawley
Control
Males



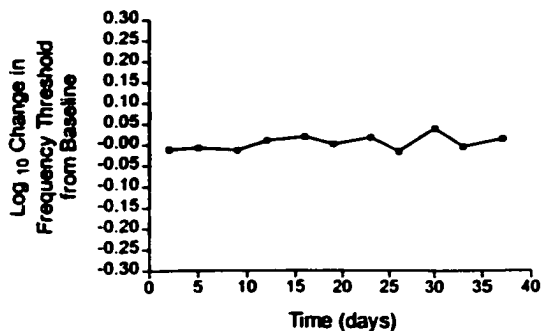
Sprague-Dawley CMS

Males

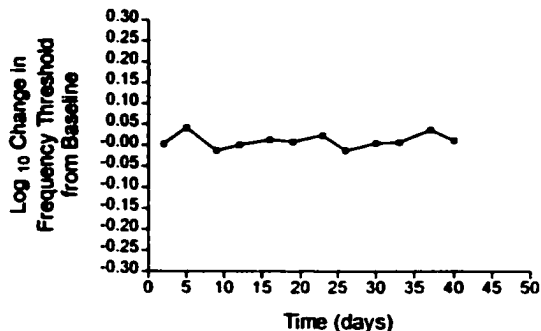
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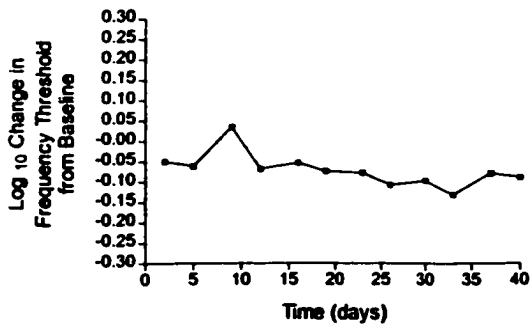
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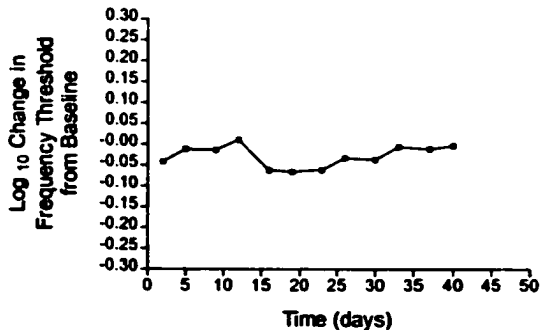
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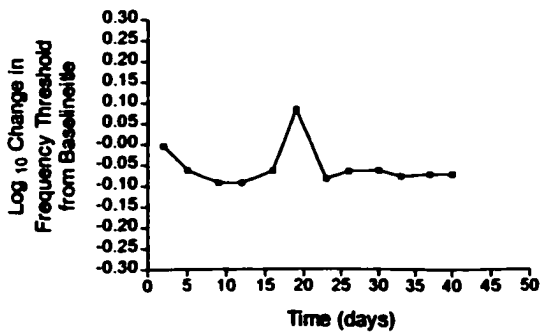
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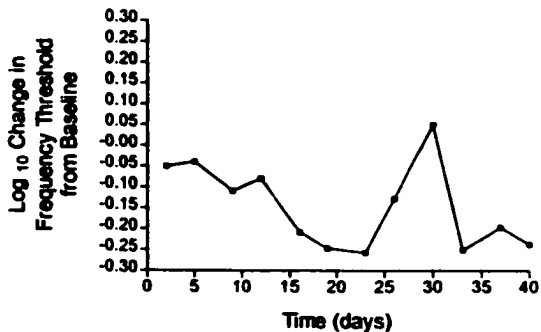
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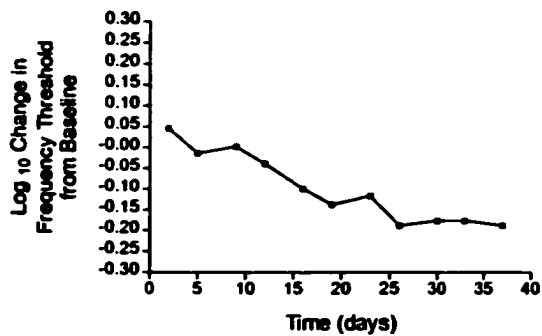
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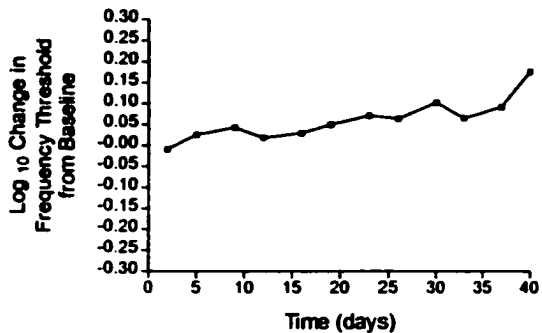
Long Evans Control

Males

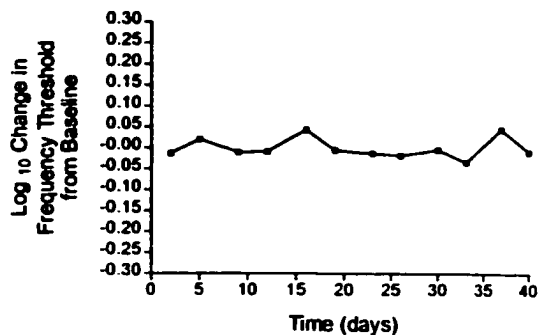
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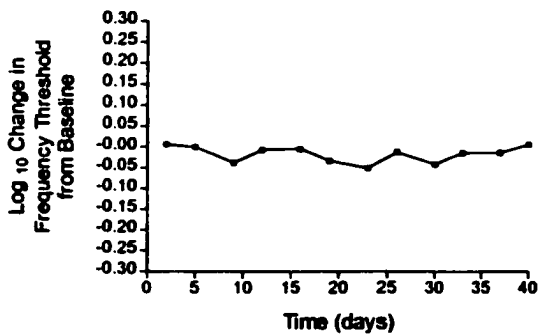
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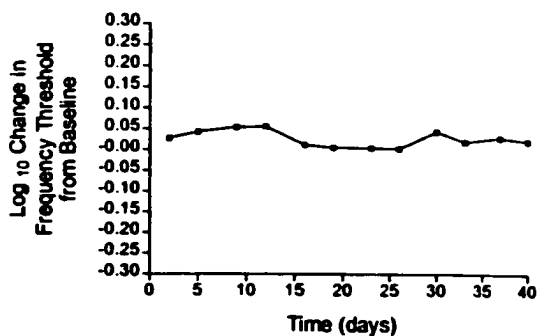
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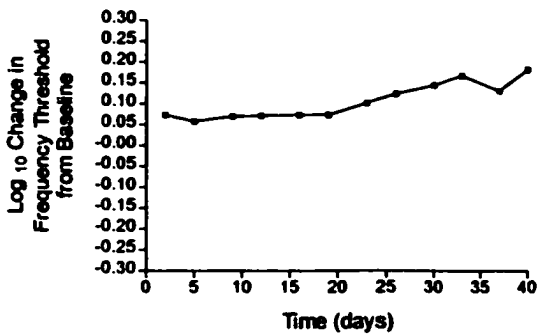
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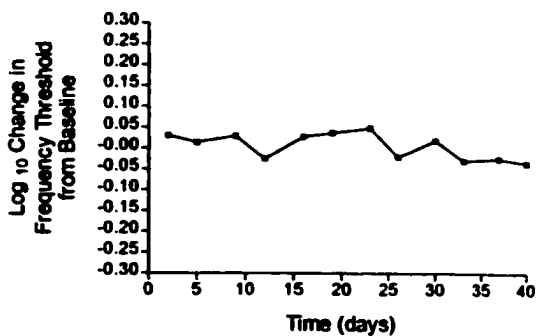
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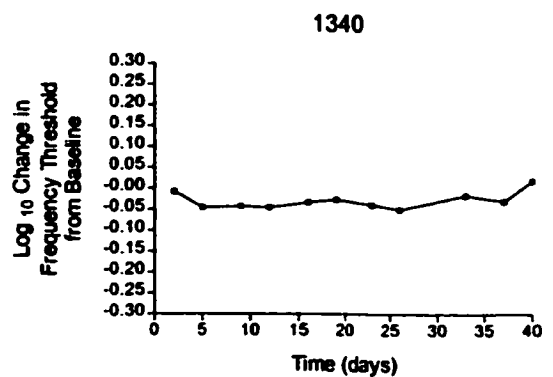
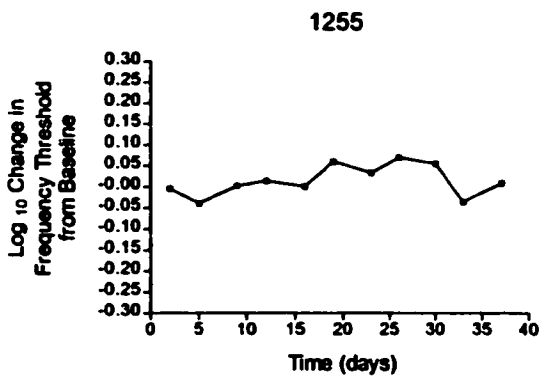
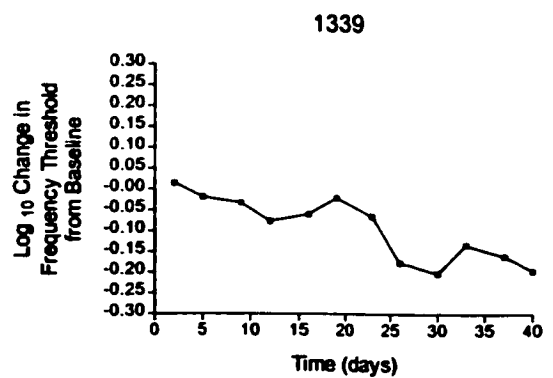
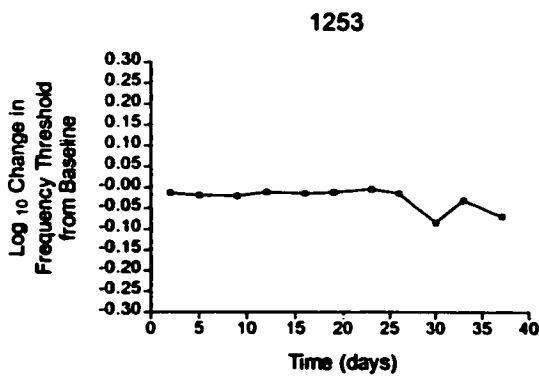
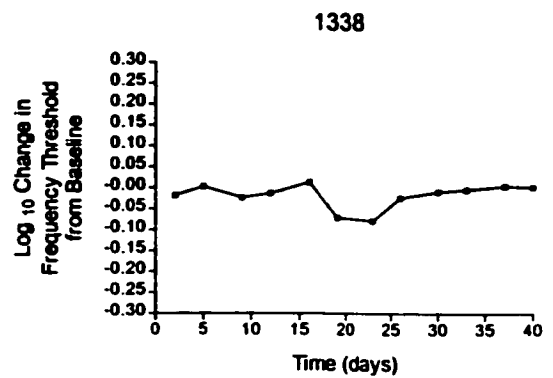
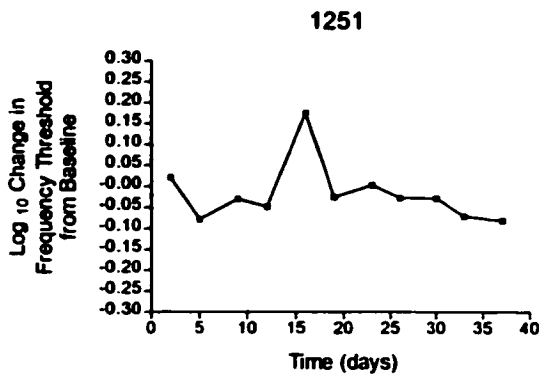
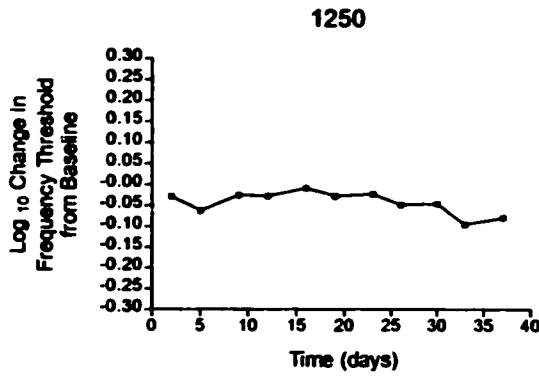
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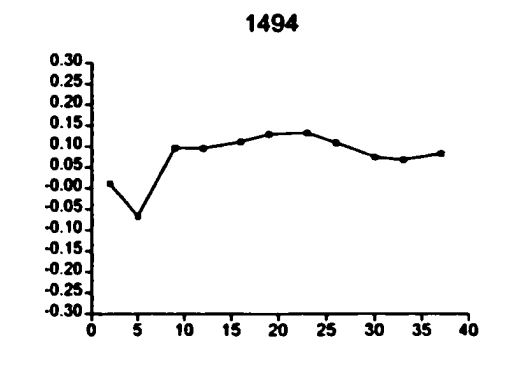
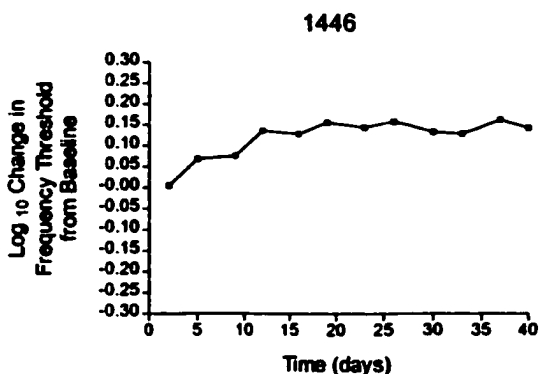
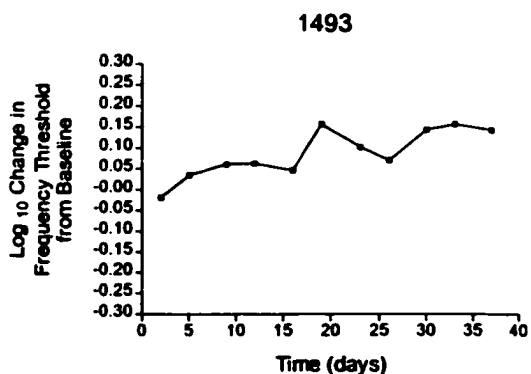
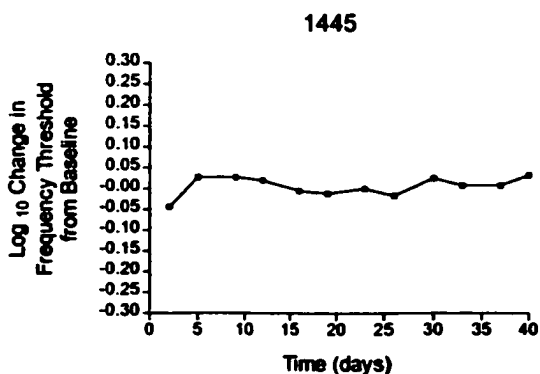
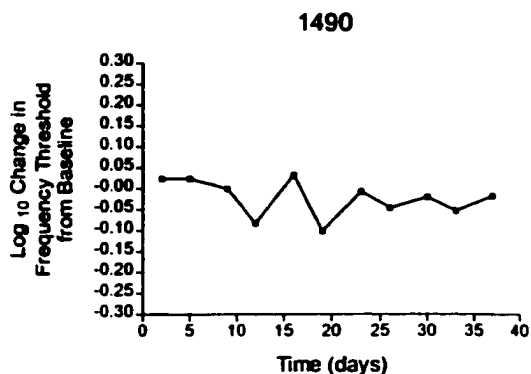
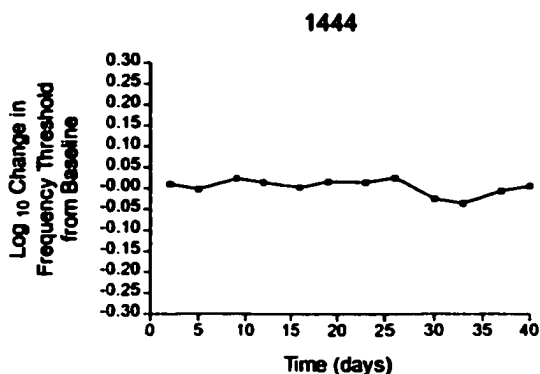
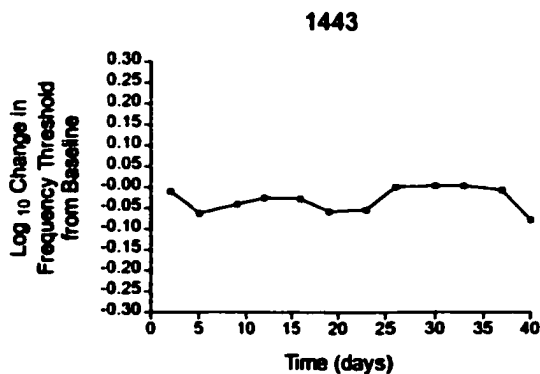
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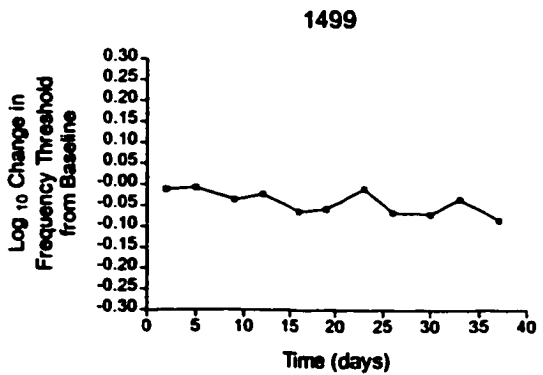
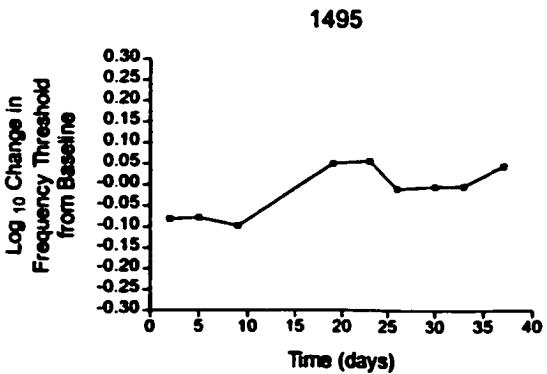
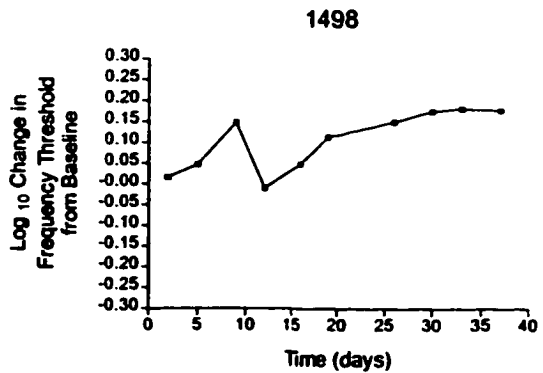
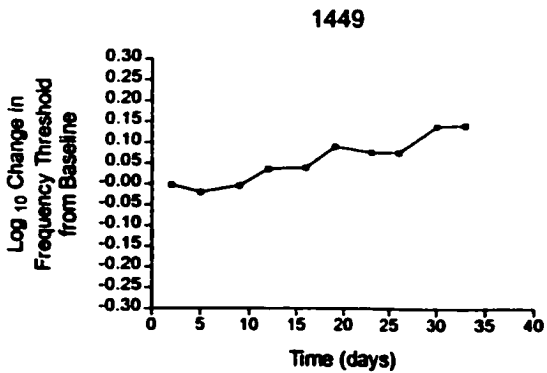
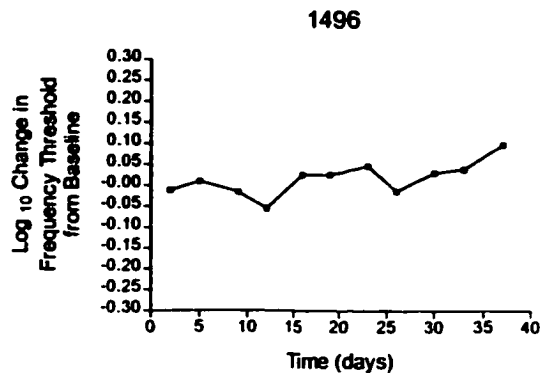
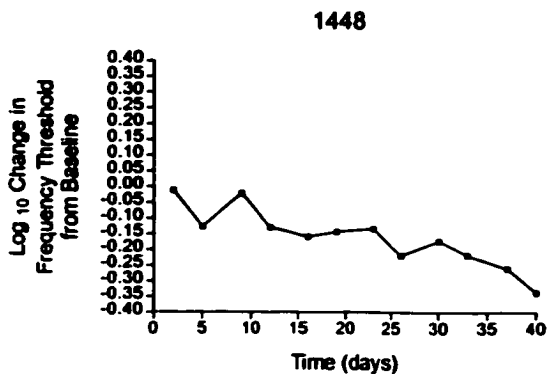
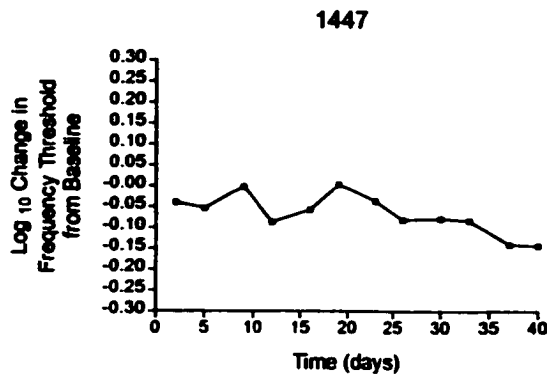
Long Evans CMS Males



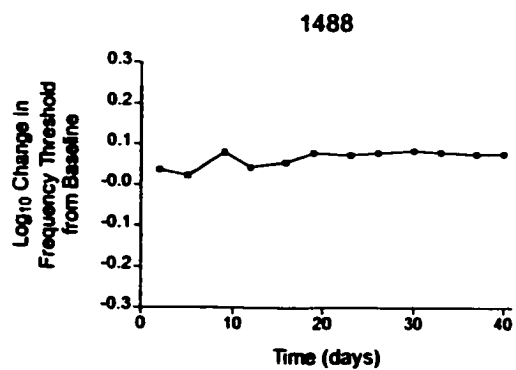
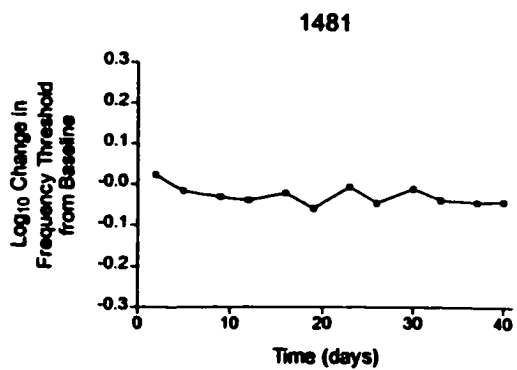
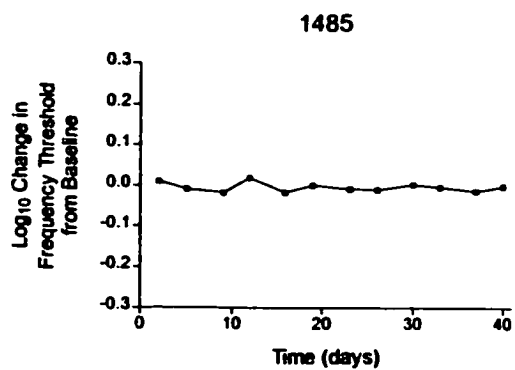
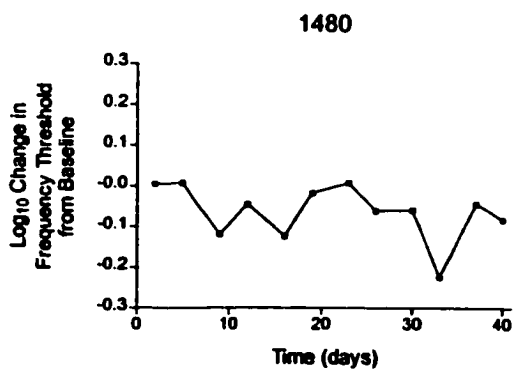
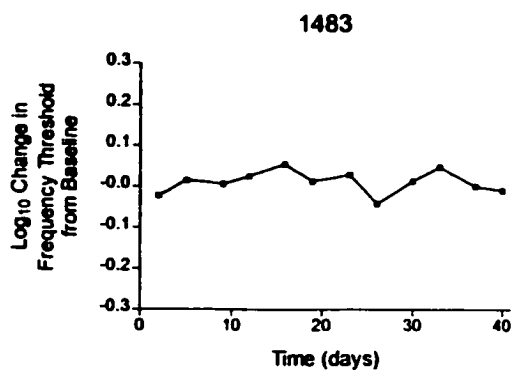
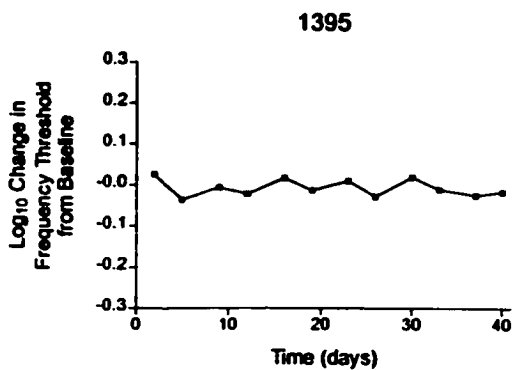
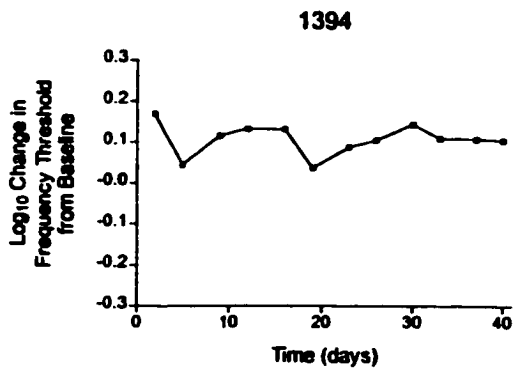
Sprague-Dawley Enriched Males



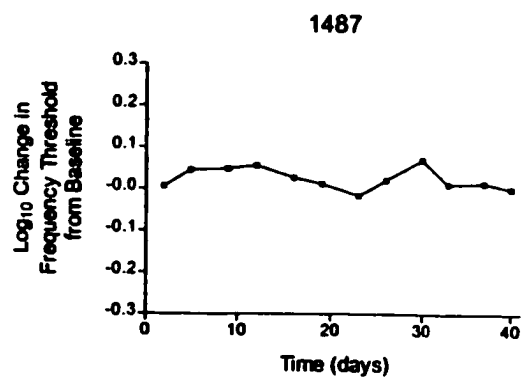
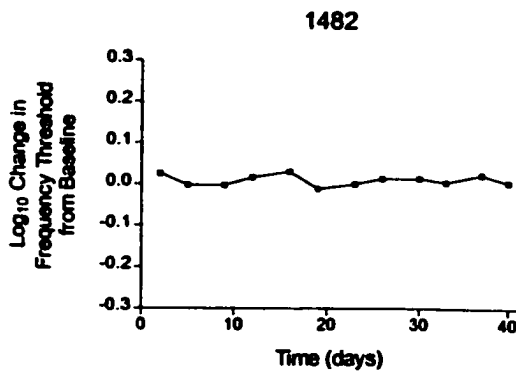
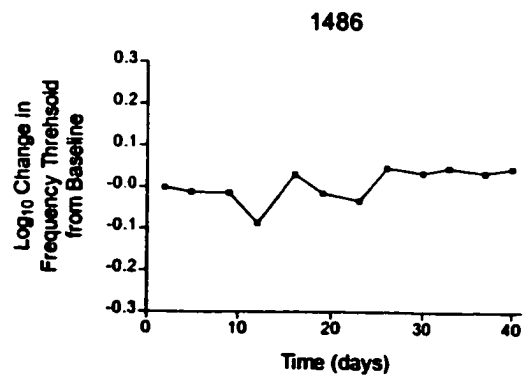
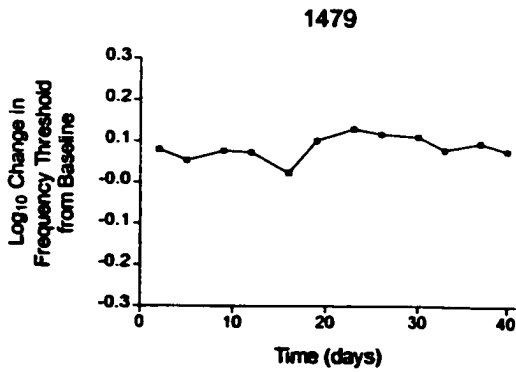
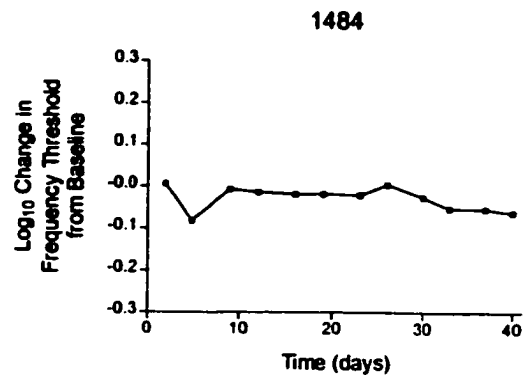
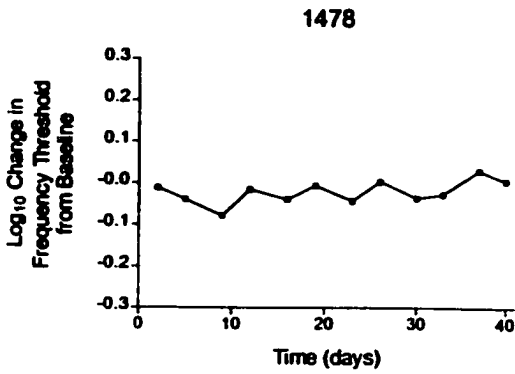
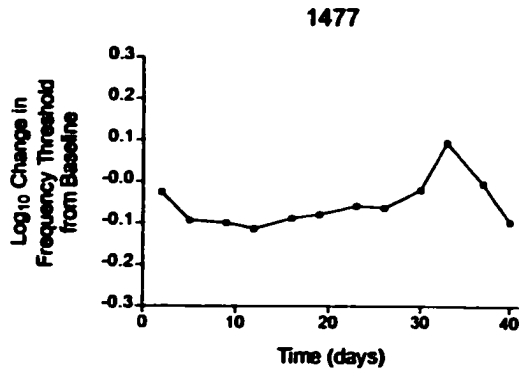
Long Evans Enriched Males



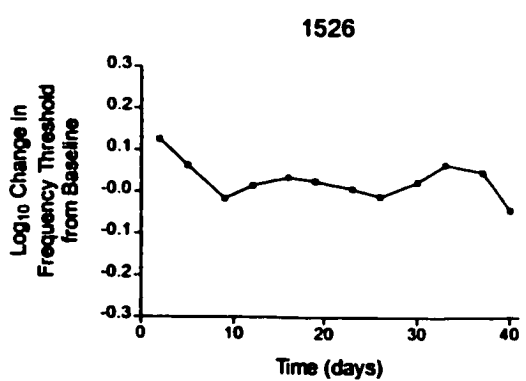
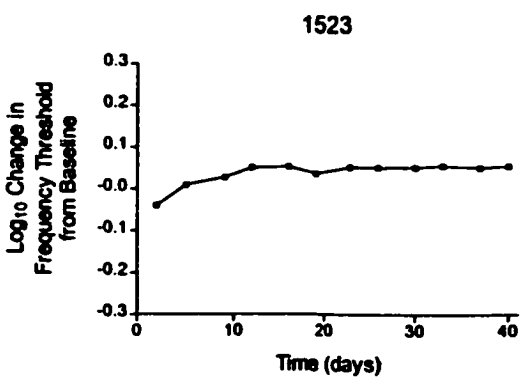
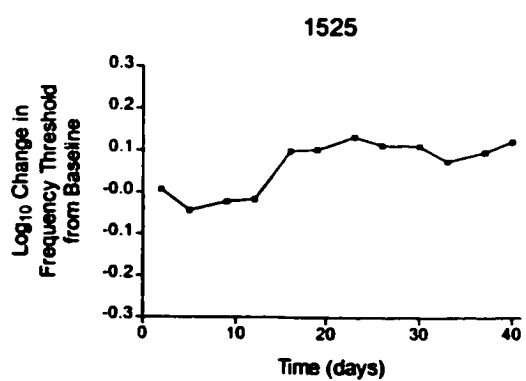
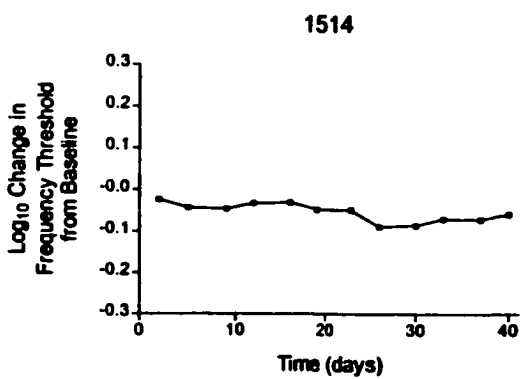
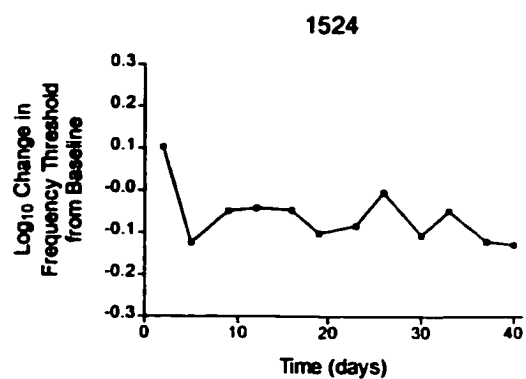
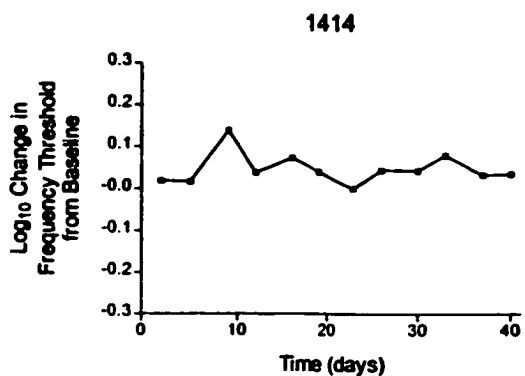
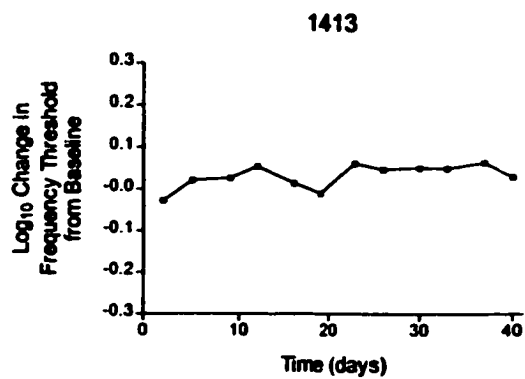
**Sprague-Dawley
Controls
Females**



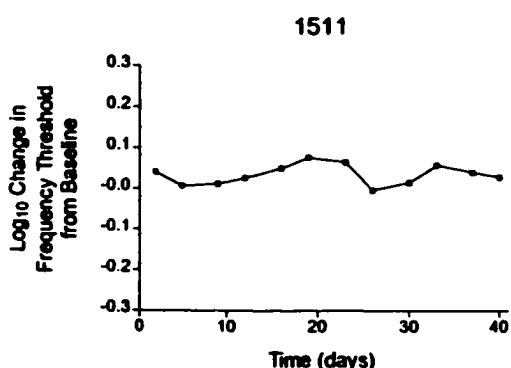
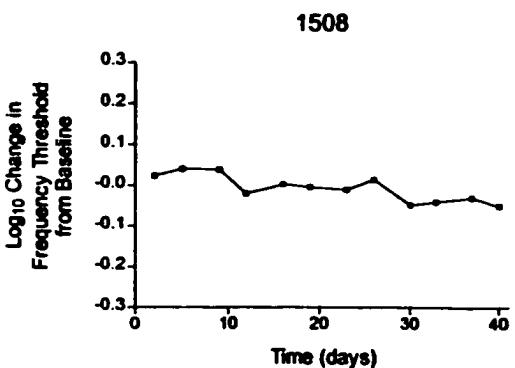
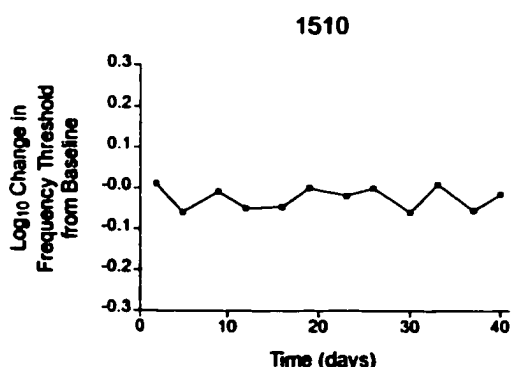
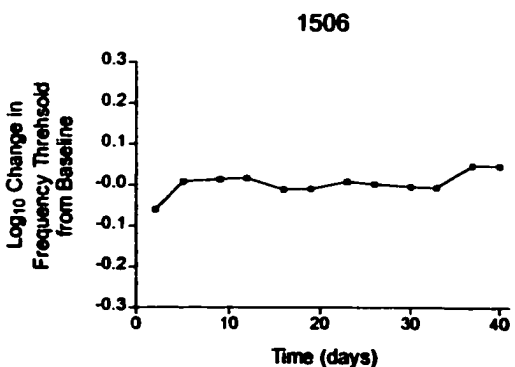
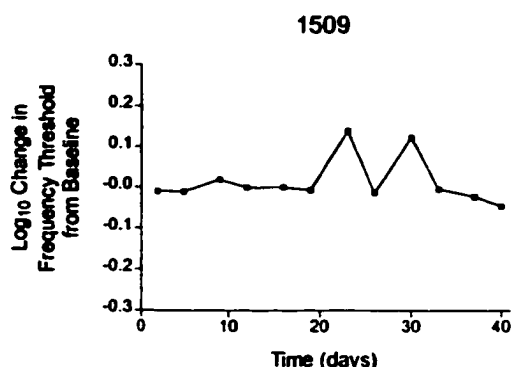
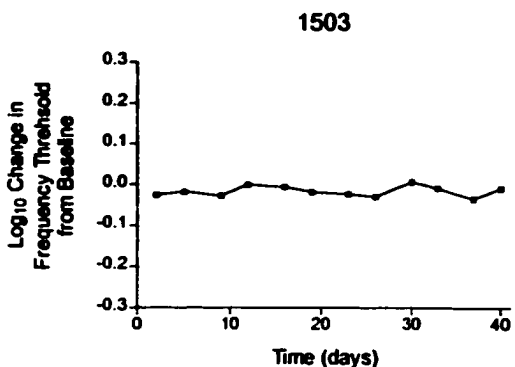
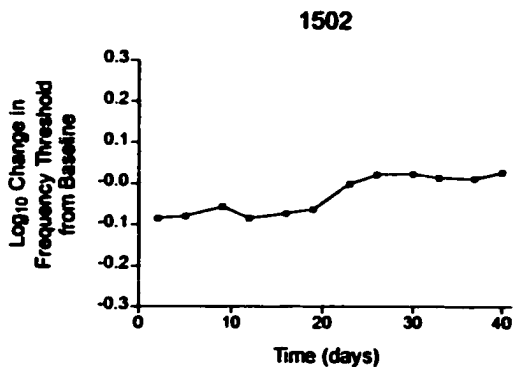
Sprague-Dawley CMS Females



Long Evans
Controls
Females

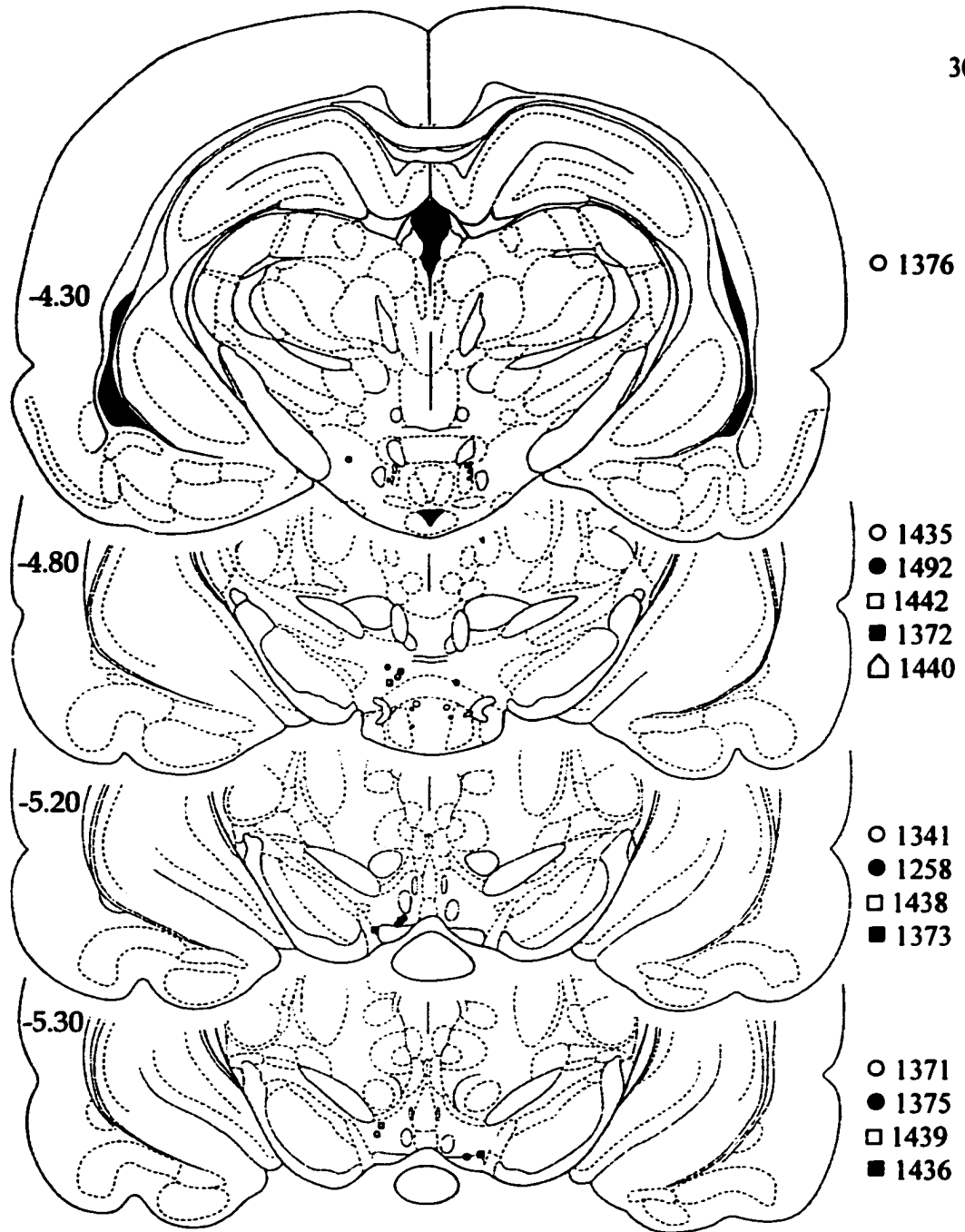


Long Evans CMS Females

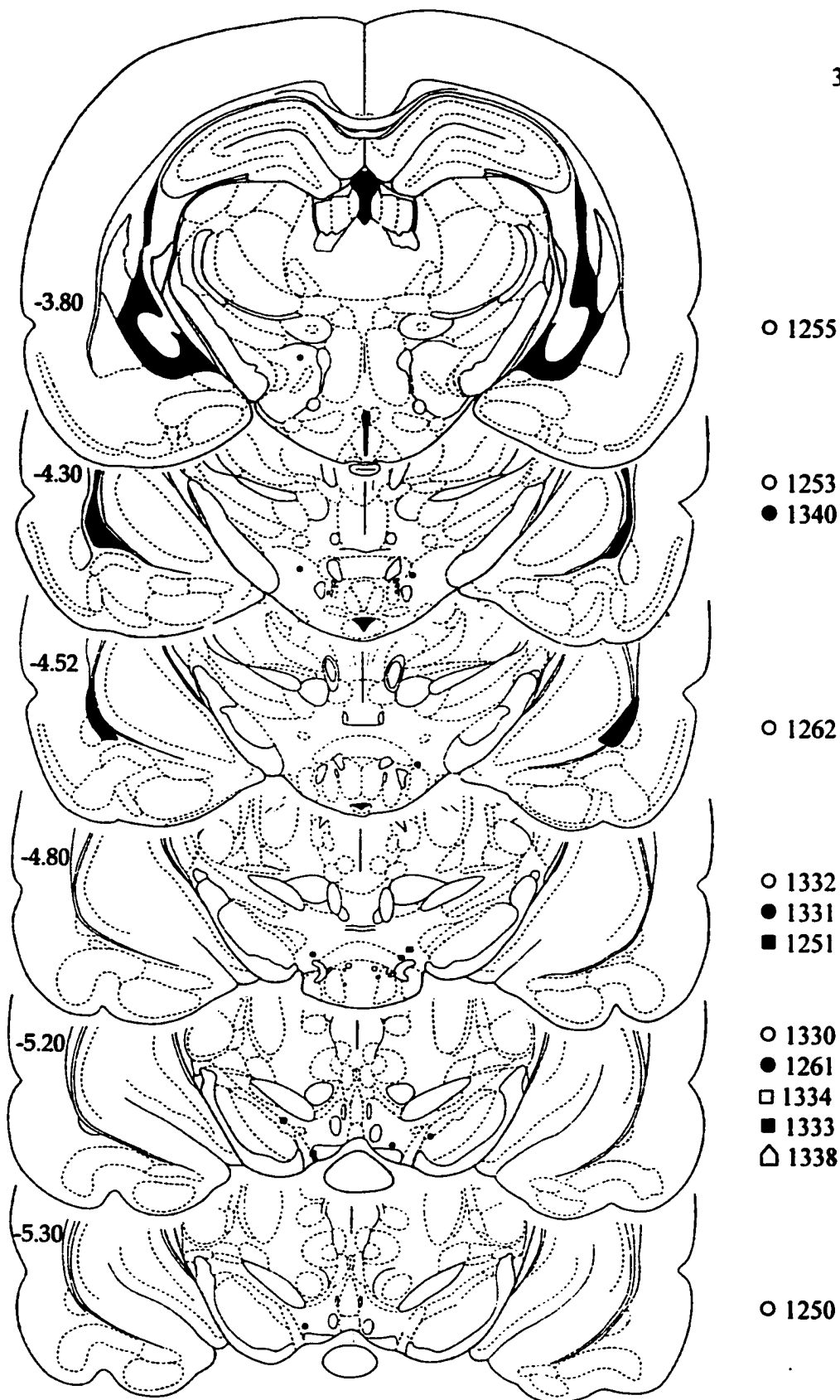


Appendix 4

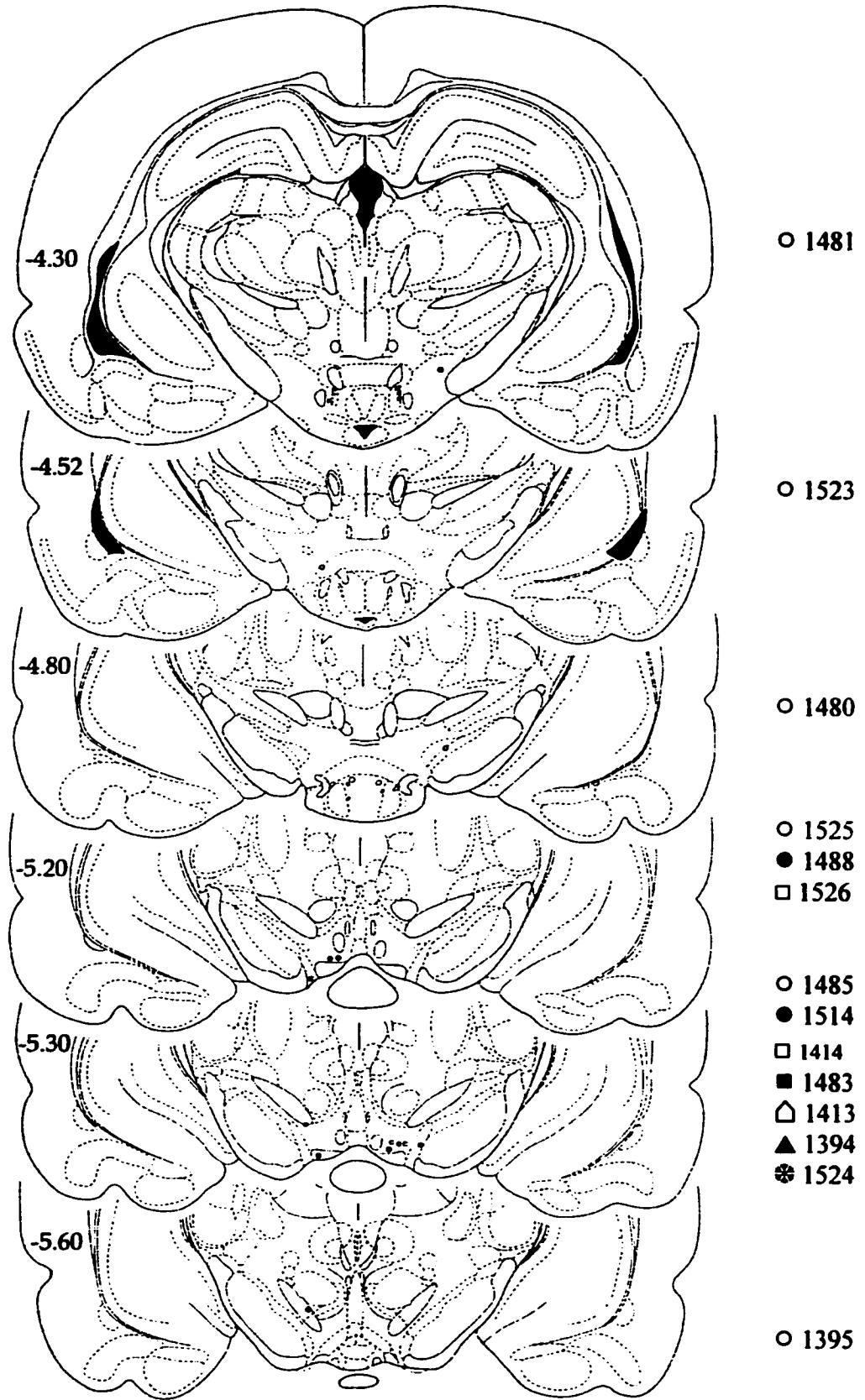
These figures show the location of the electrode tips for the subjects in the third and fourth studies. The sections are reproductions from the Paxinos and Watson (1998) atlas and depict the plates that best represent the electrode tip placement. On the left of each plate is the antero-posterior coordinate. The symbols representing the electrode tip for individual animals are to the right of the respective drawing. Note that the electrode placement for the male rat # 1339 is not available.



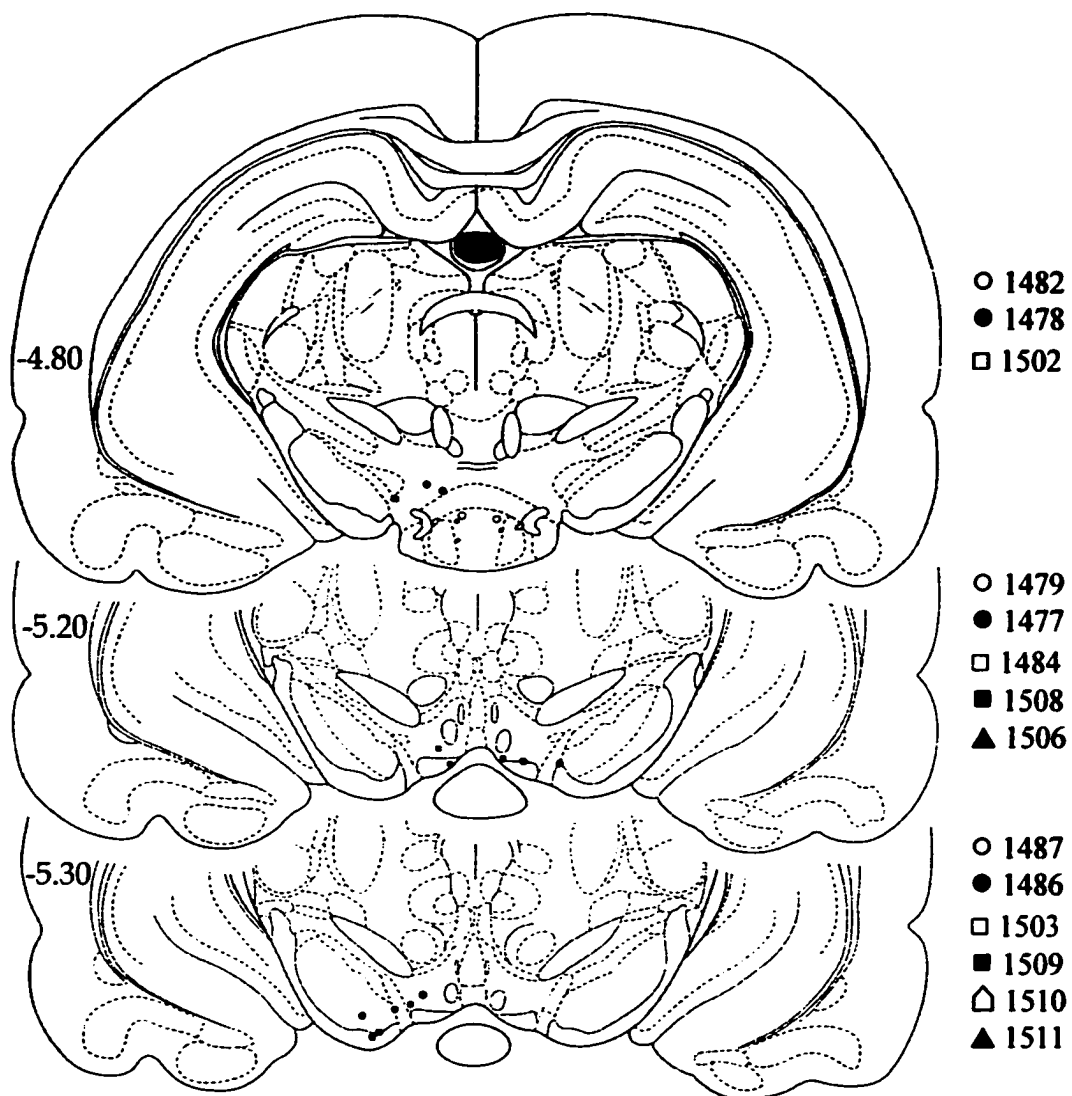
Electrode placements for control male rats



Electrode placements for CMS male rats



Electrode placements for control female rats



Electrode placements for CMS female rats