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Behavioral and neurochemical consequences of a history of human -like dieting.

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BEHAVIORAL AND NEUROCHEMICAL CONSEQUENCES OF A HISTORY OF HUMAN-LIKE DIETING

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ABSTRACT

A history of weight fluctuation and cyclic restrictive dieting (CRD) in humans has been blamed for subsequent overeating, obesity, eating disorders and mood disturbances such as anxiety and depression. However, there is no experimental proof that weight fluctuation involving CRD directly impacts eating behavior and mood, and the central neurochemistry that mediates feeding, reward and mood regulation. The aim of this study was to explore behavioral and neurochemical changes using an animal model of CRD, with varying access to palatable food (PF). We found that a history of CRD in rats did not result in overeating or obesity, nor did it alter consumption of PF versus low-fat chow. However, in rats with no CRD history, serotonin (5HT) turnover and dopamine (DA) turnover in the nucleus accumbens were positively correlated, but this relationship was abolished in rats with a CRD history, regardless of whether fed chow only, chow with intermittent PF, or chow with daily PF. Another factor explored in this study, access to PF, resulted in reduced DA and DOPAC levels in an anterior section of the hypothalamus, irrespective of whether the access was intermittent or daily. Finally, rats in the intermittent PF group with a history of CRD had reduced 5HT and DA levels in the medial prefrontal cortex (mPFC), and reduced 5HT in the whole hypothalamus after feeding, compared to those with no history of CRD. These neurochemical changes may affect feeding under certain circumstances, and may alter mood regulation. Indeed, rats fed intermittent PF with a history of CRD had reduced forced swim test activity, a

validated animal model of depression, and in previous studies in our laboratory, this group would binge eat following an acute foot-shock stress. Together, these findings may help explain how a seemingly innocuous dieting-like pattern can produce chronic detrimental feeding and emotional changes that characterize some eating disorders in humans.

DEDICATION

This work is dedicated to my husband, Michael, and to my family in New Zealand for their love, encouragement, and support, which has always been given with open arms, providing me the freedom with which to live my life.

ACKNOWLEDGEMENTS

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LIST OF ABBREVIATIONS

5HIAA	5-hydroxyindoleacetic acid
5HT	5-hydroxytryptamine
CRD	cyclic restrictive dieting
CSF	cerebrospinal fluid
DHBA	dihydroxybenzylamine
DOPAC	3-4 dihydroxyphenylacetic acid
EDTA	disodium ethylenediamine tetraacetate
HVA	homovanillic acid
mPFC	medial prefrontal cortex
PF	palatable food
PVN	paraventricular nucleus
SEM	standard error of the mean
VMH	ventromedial hypothalamus

1. Introduction

The prevalence of obesity and overweight has drastically increased in Western societies in recent decades [1-3]. It should not be surprising then that people often attempt to control and limit weight gain, or reduce weight, by dieting or reducing their caloric intake. The majority of these dieting attempts are unsuccessful. Even when people are able to lose weight initially, most break their diet and recover the weight they lost. Unfortunately, this often sets up a lifestyle of weight fluctuation (i.e. "yo-yo dieting") in which people experience periods of food restriction followed by periods of eating more than what is required for energy homeostasis, and thus regaining the lost weight. It is well known that being overweight or obese inflicts health consequences, but it has also been suggested that weight fluctuation may induce physical and psychological health problems. Given that the majority of people who intentionally lose weight and eventually recover that weight are chronically overweight or obese, it is difficult to determine whether weight fluctuation itself negatively impacts physical or psychological health, independent of the effects of being overweight or obese.

The goal of this study, then, was to determine whether weight fluctuation via cyclic restrictive dieting (CRD), defined for the purpose of this study as periods of restricted caloric intake that results in weight loss followed by periods of unrestricted caloric consumption with regain of the lost weight, alters physical and psychological health. An animal model of CRD was used that induces binge-like eating in rats after an acute stressor [4-7]. This model mimics human weight fluctuation via CRD because the peri-

ods of restricted food access are interspersed with intermittent access to palatable food (PF), a type of food usually forbidden to dieters during food restriction periods [8]. Access to PF was also manipulated to determine whether PF itself, or the frequency of access to PF, contributed to behavioral and neurochemical abnormalities. Using this model, we considered whether CRD with PF access increased susceptibility to overeat and gain weight. We also examined levels of monoamines, important in both feeding and mood regulation, in brain areas controlling feeding, reward and mood. Finally, we investigated whether CRD produced other psychological consequences such as anxiety and anhedonia.

In Western societies, a large proportion of the adult population engages in weight loss and weight control practices. In interviews of U.S. adults completing the National Health Interview Survey, 24.3% of men and 37.6% of women reported trying to lose weight [9]. Among these respondents, reducing calories was the most popular method of losing weight (57.6% of men and 63% of women). In another study, 57% of women and 50% of men reported trying to control weight, and of these, over half said they were dieting or limiting their food intake [10]. Both of these studies also reported that although weight loss practices were more prevalent among the obese and overweight population, a substantial proportion of the normal weight population (particularly women) also reported a desire to lose or control weight.

Unfortunately, dieting seems to rarely be successful. In one prospective study of people who intended to lose weight, only 60% of them were able to do so and a quarter actually gained weight [11]. Furthermore, on average subjects lost less than 20% of the weight they initially planned to lose. In the long term, even if people are successful at losing weight initially, within 3 years the majority will have regained the weight they lost

or will have gained more weight than they had at their starting point [12]. Unfortunately, this sets up a pattern of yo-yo dieting or frequent weight fluctuations.

Weight fluctuation is prevalent, particularly among women. A study of young and middle-aged American women found that 21.6% were weight cyclers (intentionally losing 10 lbs or more at least 3 times in previous 4 years; [13]). In a large Finnish study, the authors found that 7% of men and 10% of women could be classified as severe weight cyclers (intentionally losing and regaining more than 5 kg at least 3 times during the previous decade) and a further 11% of men and 19% of women were mild weight cyclers (intentionally losing and regaining more than 5 kg once or twice in previous 10 years). Severe weight cycling was associated with more frequent doctor visits suggesting poorer health than in successful dieters or even obese non-dieters [14]. Unfortunately, neither of these studies described whether the intentional weight loss (that ultimately classified them as weight cyclers) was achieved by restrictive dieting, by exercise, or by a combination of these two. However, as described above, restrictive dieting is a very common tool for intentional weight loss [9, 10] and so this is the method used in the current study to induce weight loss in rats.

Some studies have found that people with a history of weight fluctuation are more susceptible to overeating or binge eating [15-18], but other studies have found no relationship between weight fluctuation and binge eating [19, 20]. Those with a history of weight fluctuation are also reported to gain more weight over the long term compared to weight stable people [15, 16, 21-23]. In animal models of weight fluctuation via CRD, no evidence of persistent overeating or increased weight gain has been found [24-27]. For example, Lu et al. [25] cycled rats through periods of food restriction while they were maintained on a high fat diet, and Lauer, Reed, and Hill [24] cycled rats identified as

obesity-prone or obesity-resistant on a high fat diet, but neither study found that weight gain increased as a result of cycling. In another study, rats experienced food restriction and refeeding cycles while maintained on a low fat diet, and then were given *ad libitum* access to a high fat diet after the CRD history, but in this case, those with a history of CRD actually gained less weight than those fed *ad libitum* throughout [27]. In rats given a choice of three diets with differing fat contents, a CRD history induced no change in diet preference or in weight gain [26].

Despite a lack of evidence to support the hypothesis that weight fluctuation or CRD specifically results in persistent perturbations in eating behavior or in increased weight gain, as aforementioned, CRD is thought to be an etiological and maintenance factor in binge eating disorder, bulimia, and some types of anorexia nervosa [28-32]. Given that up to 4.2% of American women suffer from bulimia nervosa alone [33], it is important to learn how CRD may increase one's vulnerability to, and may underlie co-morbid symptoms of, perturbed eating, such as depression and an addictive-like regard for food. CRD may change the neural substrates underlying feeding and mood regulation and in so doing, chronically alter future intake patterns, body weight, and mood. The current study addressed this possibility by attempting to identify neurochemical and behavioral changes in an animal model of weight fluctuation via CRD.

Feeding studies in animals may not yet have adequately simulated human dieting patterns. Human dieting and refeeding patterns are quite varied and are more complex than those described in the animal studies described above. For example, the availability and intake patterns of palatable, sweet, high fat food is something that varies across dieting conditions. Many people not only limit calories, but also reduce their fat consumption while dieting [9], which contrasts with the animal studies described above because in

those studies the same diet (and thus the same fat percentage) was provided during food restriction and refeeding periods.

There are reports from human studies that a history of weight fluctuation results in greater preference for food combinations of sweet taste and high fat compared to those who maintained a stable body weight [34, 35], but these did not consider whether limited access to palatable food (PF) also contributed to the increased intake of that food after restriction from it is lifted. While at least one study has found no relationship between weight fluctuation and fat intake in obese humans [36], some animal studies found that rats with a history of weight fluctuation via CRD increased their intake of fat over that of carbohydrate or protein when given a choice [37-39]. Each of these studies, however, allowed animals to consume palatable, high fat diets or to self-select from fat, carbohydrate, and protein prior to and/or during food restriction. Thus, it cannot be determined whether weight fluctuation alone, independent of restricted or intermittent access to PF, contributed to preference for PF.

There is evidence from both human and animal studies that limiting access to PF or providing an intermittent schedule of access to that food subsequently increases PF choice and intake once the restriction has been lifted [40-43]. Some researchers argue that limiting access to certain foods results in "perceived deprivation" because the actual number of calories may not be reduced [44], and thus weight fluctuation via CRD is not necessary to increase consumption after the limitations on PF have been lifted. It could be that people engaging in CRD who also limit their access to PF while dieting are both calorically deprived and hedonically deprived, and the combination of these two may induce more behavioral and psychological disturbances than either of these alone.

Therefore, while it is clear that weight fluctuation via CRD is a common practice, particularly among women of both normal and overweight, it is less clear whether CRD itself, or limitation of PF during CRD, contributes to overeating or obesity. One goal of this study was to determine whether a history of CRD without any access to PF is sufficient to increase susceptibility to overeating or weight gain. Further, PF was provided to some groups on a daily basis and to others on an intermittent basis in order to investigate whether the schedule of access to PF contributes to any subsequent abnormalities in feeding patterns or weight gain. The inclusion of a group with intermittent access to PF differs from previous models of CRD, which found no change in food intake and weight gain due to the CRD history [24-27]. We hypothesized that rats with the combination of a history of CRD and intermittent access to PF would develop greater intake of PF compared to those without a history of CRD because the combination of both caloric and hedonic (or actual and perceived) deprivation would increase drive for PF more than each type of deprivation alone would. Also, if allowed to consume PF daily, rats with a history of CRD would gain more weight than those without a history of CRD and those without daily access to PF.

There have been almost no attempts to study neurochemical changes resulting from a history of CRD. A prime candidate for disruption due to dieting is 5-HT because of its well-known role in feeding and mood regulation (for review see [45-51]). Serotonin is believed to promote satiety, and thus increases in 5-HT are associated with reduced feeding [45-47]. Individual 5-HT receptors have been identified as regulating certain meal pattern parameters. Specifically, the 1B receptor reduces meal size, and the 2C receptor reduces rate of eating [47]. With respect to mood regulation, dysregulation of 5-

HT function, or reduced 5-HT activity, has been frequently implicated as contributing to mood disturbances, particularly depression [49-51].

Dieting reduces tryptophan levels in plasma and, in women only, results in an exaggerated prolactin response to d-fenfluramine or tryptophan treatment [52-55]. This implies dieting-induced alterations to brain 5-HT function. These studies, however, were conducted in subjects made acutely hungry and it is not known whether alterations in 5-HT function persevere after a history of CRD.

In contrast to these exaggerated prolactin responses in non-disordered women when given an acute period of reduced calories, women with bulimia have reduced prolactin response to 5-HT stimulation compared to non-bulimic controls following an overnight fast [56-58]. Recovered bulimics, however, had prolactin responses similar to those of normal control women after both had experienced an overnight fast [59]. However, when tryptophan was depleted in recovered bulimics, they reported a return of negative mood and loss of control over eating symptoms [60], suggesting that diminished 5-HT may indeed trigger bulimic-like symptoms in those susceptible to this disorder.

Other studies in bulimics found those with severe binge eating frequency to have lower cerebrospinal fluid (CSF) concentrations of the 5-HT metabolite, 5-hydroxyindoleacetic acid (5HIAA), and the dopamine metabolite, homovanillic acid (HVA; [61, 62]). Upon recovery from bulimic symptoms, CSF levels of HVA normalize, but 5HIAA actually becomes elevated in comparison to controls [63]. Together, the above studies suggest that central 5-HT function is reduced during dieting, and this may result in feeding and mood disturbances, particularly in those susceptible to eating disorders. Furthermore, 5-HT function is either persistently perturbed by a history of bulimia, which typically includes CRD, or 5-HT dysfunction is a trait factor, one present before

the disorder becomes apparent and one that may contribute to its development and maintenance.

A goal of the current study was to investigate 5-HT function as a consequence of a history of CRD both indirectly in awake, behaving rats and directly by measuring their levels of 5-HT and 5HIAA in key brain areas involved in feeding, reward, and mood regulation. We firstly measured feeding responses following administration of the drug fluoxetine, which blocks reuptake of 5-HT from the synapse, in rats with a history of CRD and those without. We then dissected and assayed levels of 5-HT and 5HIAA in the hypothalamus, a key regulator of feeding (for review see [64-66]), in the nucleus accumbens, which is involved in reward mediation (for review see [67-69]), and in the medial prefrontal cortex (mPFC), a central regulator of mood (for review see [70-72]). Because dopamine is also involved in reward regulation, and is known to interact with 5-HT, we measured dopamine and the dopamine metabolite, 3-4 dihydroxyphenylacetic acid (DOPAC), in these areas to identify whether this neurochemical is perturbed by a history of CRD. We hypothesized that a history of CRD would reduce 5-HT levels in the hypothalamus even if rats had recovered normal energy balance. Further, we hypothesized that CRD would disrupt dopaminergic signaling and the 5-HT-dopamine interaction in the nucleus accumbens, contributing to symptoms of reduced behavioral activity and anhedonia [73-75]. In the PFC, where human depression is characterized by reduced blood-flow and glucose metabolism [73-75], and where depressed patients have a suppressed response to the 5-HT-releasing drug, fenfluramine [76], we hypothesized that rats with a history of CRD would have reduced 5-HT levels.

Just as the effect of CRD on neurochemistry is unknown, it is also unclear whether weight fluctuation or CRD contributes to significant mood disturbances such as

anxiety and depression. There are numerous reports that eating disorders, which, as discussed above, involve CRD, are associated with anxiety [77-80] and depression [80-82]. But no direct causal link between CRD and mood disorders has been established. A small number of studies have associated CRD with psychological distress and depression [21, 83, 84]. One study discovered slightly, but not significantly, elevated ratings of depression and anxiety in people with a history of weight fluctuation, but binge eating severity was more clearly associated with depression and anxiety than weight fluctuation [85]. However, others claim that a history of CRD or weight fluctuation does not adversely impact psychological health [20, 86]. It is possible that only a subset of people who experience weight fluctuation or CRD, those who also engage in binge eating, report more anxiety and depression. Indeed, Kensinger et al., [87] discovered that people with a history of weight fluctuation who also had severe binge eating episodes reported more depression and psychological distress than weight cyclers who did not engage in binge eating. A number of other studies corroborate the possibility that binge eating is associated with depression [88-91] and anxiety [31, 91].

Together, these studies suggest that it is not the history of CRD itself that renders people susceptible to mood disturbances, such as anxiety and depression, but these problems are only found in those who engage in binge eating behavior that is consequent to CRD. It is also possible that underlying anxiety and depressive traits precede weight fluctuation and render some people susceptible to binge eating. To date, no one has investigated the effects of CRD on mood in an animal model. While we cannot directly measure anxiety and depression in animals, there are tests of behavior under certain challenging conditions that have been validated as animal models of anxiety and depression because they predict the efficacy of anxiolytics and antidepressants in humans. Specifi-

cally, in the elevated maze and open field tests, rats prefer to remain in enclosed areas or close to walls rather than be in open spaces. This is used as an animal model of anxiety, and anxiolytics are effective at increasing the time rats spend in open areas [92-95]. The forced swim test has also been used extensively as an animal model of helplessness, a characteristic of human depressive behavior [96, 97]. In this test, helplessness or depression is defined as inactivity or lack of struggling, and this is minimized by antidepressants [98-100]. In a 2-bottle choice test between sucrose and water reduced intake of sucrose is interpreted to mean that there is a decrease in the reward value of this normally preferred solution, and thus also serves as a model of anhedonia, a central feature of depression [99, 101-103].

Rats that have experienced a history of CRD were subjected to these tests in order to investigate whether CRD contributes to behaviors validated to represent mood disturbances. We hypothesized that rats with a history of CRD would have reduced open area activity in the elevated maze and open field tests, reduced struggling in the forced swim test, and reduced intake of sucrose solution.

2. General methods

2.1. Animals

A total of 105 female Sprague-Dawley rats (Harlan: Indianapolis, IN) were used for these experiments. The Institutional Animal Care and Use Committee at the University of Alabama at Birmingham approved all procedures. Rats were 90 days old on arrival and were housed individually in clear polypropylene woodchip-bedded cages under a 12/12 hour light-dark schedule (lights out at 12 noon). Rats were allowed to acclimate to colony conditions with *ad libitum* standard rat chow and water for a minimum of one week after arrival. After acclimation, rats were assigned to CRD and no-CRD groups. Group assignment was performed on the basis of body weight immediately prior to the start of the CRD protocol; ensuring groups within an experiment had approximately equal means and ranges of body weights. During the CRD protocol, rats were given *ad libitum* water at all times (except where noted) and food intake, corrected for spillage, was measured daily with fresh food given immediately prior to lights out. Body weights were recorded on at least 3 occasions during each cycle.

2.2. Cyclic Restrictive Dieting (CRD) Protocol

Each cycle lasted 12 days. The first 5 days were food restriction days, during which rats in the CRD groups were given 66% of the total chow calories that the entire group was consuming prior to the start of cycling, while rats in no-CRD groups were provided *ad libitum* food. Within these CRD and no-CRD groups were 3 subgroups that

varied in the amount of PF food they were given: the Chow Only group received 66% of chow on restriction days; the Intermittent PF group also received 66% of chow on restriction days; and the Daily PF group received the same amount of calories as the 66% amount given to the other groups, but half of the calories came from chow and half from PF (see Table 1). Rats in the no-CRD groups were fed *ad libitum* chow throughout. Days 6 through 12 were refeeding days with only chow or chow and PF given as detailed in Table 1. The Intermittent PF groups were given PF to simulate breaking their diet and then given PF again on the last day of each cycle. On occasion, if body weight and food intake of CRD rats had not recovered to controls' level (i.e. mean body weight within 2% of no-CRD group and mean food intake within 5% of no-CRD group) by day 12, an extra day was added to the *ad libitum* refeeding portion of the cycle (i.e. days 8-11), to allow complete recovery of food intake and body weight. After Day 12, the rats began the next cycle starting again with Day 1.

<table>
<tr><th rowspan="2">Diet Groups</th><th rowspan="2">Restriction Subgroups</th><th rowspan="2">Days 1-5 "Restriction"</th><th>Days 6-7</th><th>Days 8-11</th><th>Day 12</th></tr>
<tr><th colspan="3">"Refeeding"</th></tr>
<tr><td rowspan="2">Chow Only</td><td>No-CRD</td><td>Ad lib chow</td><td colspan="3" rowspan="2">Ad lib chow</td></tr>
<tr><td>CRD</td><td>66% chow</td></tr>
<tr><td rowspan="2">Intermittent PF</td><td>No-CRD</td><td>Ad lib chow</td><td rowspan="2">Ad lib Chow + Ad lib PF</td><td rowspan="2">Ad lib chow</td><td rowspan="2">Ad lib Chow + Ad lib PF</td></tr>
<tr><td>CRD</td><td>66% chow</td></tr>
<tr><td rowspan="2">Daily PF</td><td>No-CRD</td><td>Ad lib Chow + Ad lib PF</td><td colspan="3" rowspan="2">Ad lib chow + Ad lib PF</td></tr>
<tr><td>CRD</td><td>66% Chow + Ad lib PF</td></tr>
</table>

Table 1: Cyclic restrictive dieting (CRD) protocol. This protocol was used in all experiments except for experiments 2A, 3A and experiment set 4, wherein only the Intermittent PF condition (with and without CRD) was tested.

2.3. Diets

Two diets were used in this study: regular rat chow (Harlan-Teklad, Indianapolis, IN) and Double-Stuf Oreo cookies (Nabisco, Hanover, NJ) as the highly palatable food (PF). The rat chow is composed of 3.5% kcals from fat, 70% kcals from carbohydrate, 17% kcals from protein, 9.5% moisture, and contains 3.74 kcals / gram. The Oreo cookies are composed of 43% kcals from fat, 57% kcals from carbohydrate, trace protein (0.02% kcals), and contain 4.83 kcals / gram.

2.4. Data analysis

Dependent variables, including food intake, body weight, and monoamine levels, were analyzed by within and between group analyses of variance (ANOVAs) and Bonferroni post-hoc tests or independent group t-tests if only two groups were included in the analysis. Independent group t-tests were used to analyze differences in behavioral test scores for Experiment 4. Pearson's r correlation coefficient was calculated to assess the associations between monoamine levels and ratios (for turnover measures), and to measure the inter-rater reliability scores for the behavioral test ratings. For all experiments, the alpha level was set at $p < 0.05$ and 2-tailed tests were conducted unless a directional hypothesis was made previously (1-tailed tests are noted in the Results section). Food intake is expressed as mean kcals ± SEM. Neurochemical measures are represented as mean ng / mg of wet brain tissue.

2.5. Euthanasia

At the time of euthanasia, the rats were transported individually in a familiar container, which had been used throughout the experiments to weigh them in, to another

room where each was euthanized rapidly via unanaesthetized guillotine decapitation (IACUC approved). All rats were euthanized on the last day of the cycle, when the CRD rats had returned to normal food intake and body weight. Rats were euthanized under non-food deprived conditions before lights went out except where otherwise noted.

3. Experiment 1: Does a history of CRD render rats susceptible to overeating and weight gain?

3.1. Methods

This experiment explored the impact of a history of CRD in combination with varying access to PF on susceptibility to overeat and gain weight. Following acclimation, 48 rats were weight-matched into the 6 groups (N = 8 per group) described in Table 1, and the CRD cycling began. Twenty-four hour food intake was recorded each day immediately prior to lights out, and body weight was recorded immediately prior to and following restriction (cycle days 1 & 6) and on the last day of the cycle (day 12). Food intake measures were taken at 1, 4, and 24 hours after lights out during the first day of refeeding and on the last day of each cycle. The rats were cycled through 12 CRD cycles.

3.2. Results

To confirm that the number of calories provided to rats during the restriction period throughout cycling was indeed less than they would normally consume under *ad libitum* circumstances, comparisons were made of the intake of CRD versus no-CRD groups during cycle 1 and then again during cycle 11. A repeated measures ANOVA including each of the 5 restriction days during cycle 1 indicated that there was no difference across the restriction days. There was, however, a diet by restriction interaction ($F(2,42) = 71.42, p < 0.001$), a main effect of restriction ($F(1,42) = 669.40, p < 0.001$), and a main effect of diet ($F(2,42) = 74.75, p < 0.001$). Follow up comparisons across the 6 groups confirmed that, as we expected, each of the no-CRD groups consumed more than the

CRD groups during restriction (p values < 0.05), and the Daily PF/no-CRD group consumed more than any other group (p values < 0.001; Fig. 1A). On cycle 11, a repeated measures ANOVA again confirmed no difference across the restriction days and this time there was no diet by restriction interaction, and no main effect of diet. As expected, a main effect of restriction was evident ($F(1,42) = 44.56, p < 0.001$; Fig. 1A), confirming that the CRD groups were still consuming less food during restriction than the no-CRD groups.

To investigate whether access to PF increased intake further in addition to the expected hyperphagia that took place when the diet was broken, comparisons were made across the diet and CRD groups for the first refeeding day. During cycle 1 there was no interaction between diet and restriction on the first refeeding day, but there were main effects of diet ($F(2,42) = 15.547, p < 0.001$) and of restriction ($F(1,42) = 25.347, p < 0.001$). Post hoc tests revealed a difference in the mean intakes of the Chow Only group (collapsed across CRD and no-CRD conditions) versus the intakes of both the Intermittent PF, and the Daily PF groups (p values < 0.01). As Fig. 1A shows, the Chow Only group consumed less than the other diet groups, which had access to PF. Intake of the CRD groups was greater than that of their no-CRD control groups, confirming that CRD did induce rebound hyperphagia following restriction, as expected. On the first refeeding day of cycle 11, there was a marginal interaction between diet and restriction ($F(2,42) = 3.24, p = 0.05$), and main effects of both diet ($F(2,42) = 12.624, p < 0.001$) and restriction ($F(1,42) = 19.360, p < 0.001$). As indicated by Fig. 1A, and confirmed by comparisons across the 6 groups, intake of the Intermittent PF/CRD group was the same as that of the Intermittent PF/no-CRD group, and was also the same as the Chow Only/CRD and Daily

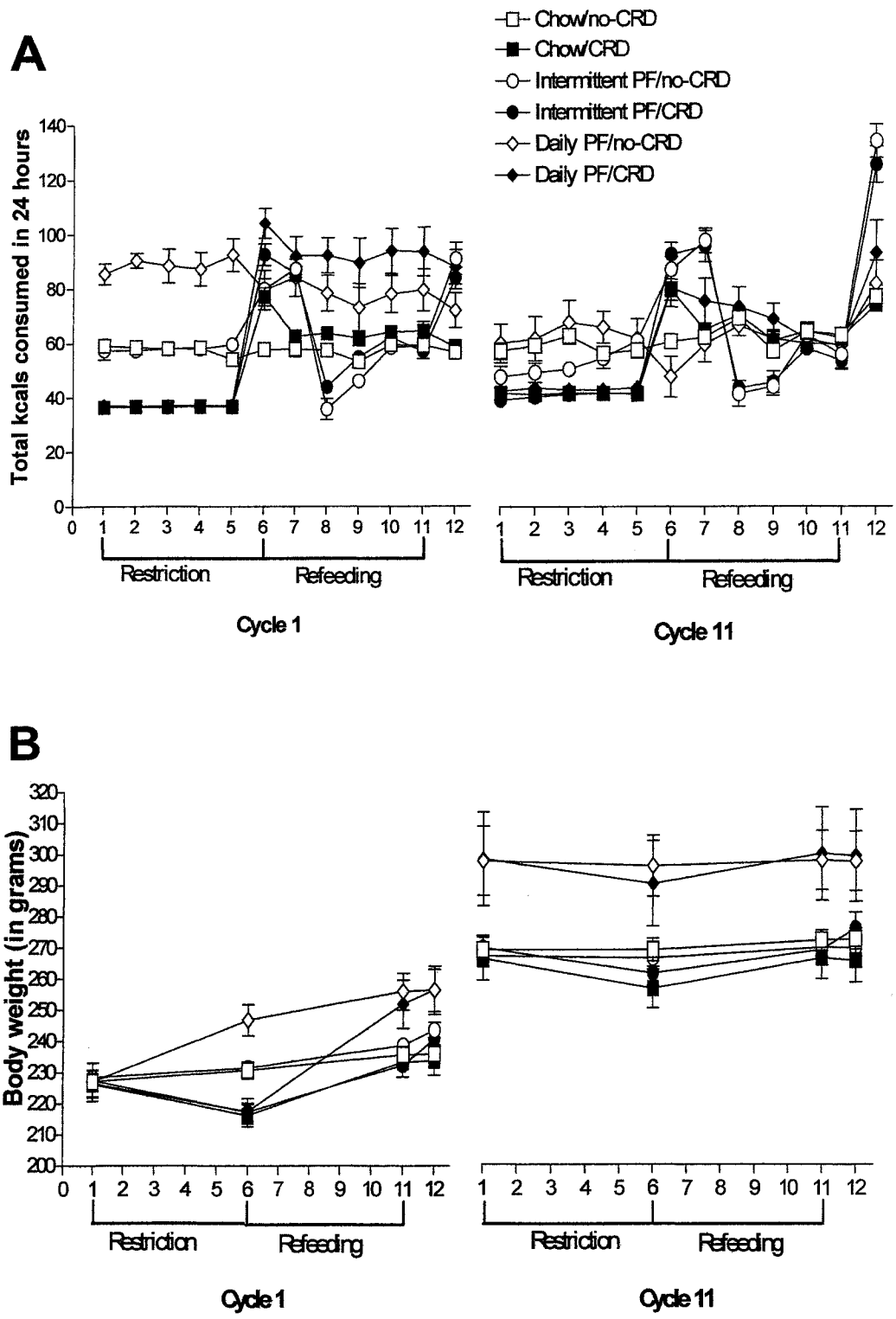

Fig. 1. Experiment 1. Total 24-hour caloric intake (A) and body weight in grams (B) for each day of cycle 1 and of cycle 11.

PF/CRD groups. This suggests that access to PF has increased intake of the Intermittent PF/no-CRD group to the same level as hungry rats following a food restriction period.

To investigate whether, after refeeding to recover normal body weight and food intake, Intermittent/CRD rats would consume more than any other group when PF was once again provided to them, analyses were made of intake during the last test day of cycle 1 and of cycle 11. On both cycles, no interactions between diet and restriction were evident. Importantly, there was not a main effect of restriction, confirming that rats with a history of CRD had recovered their intake to the same level as that of their control groups. There was however a main effect of diet for each of these test days (Cycle 1: $F(2,42) = 20.23, p < 0.001$; Cycle 11: $F(2,42) = 29.751, p < 0.001$). On cycle 1, test day intake of the Chow Only group was lower than that of the other two groups which had access to PF, while on Cycle 11, intake of the Chow Only group equaled that of the Daily PF group, but the Intermittent PF group consumed more than each of these groups (p values < 0.05). The fact that the Daily PF group was consuming the same amount as the Intermittent PF group during the test day of Cycle 1, but less than the Intermittent PF group during the test day of Cycle 11, suggests that either intake of the Daily PF group was reduced after multiple cycles, or that the intake of the Intermittent PF group was increased after multiple cycles.

To investigate this last point, a repeated measures ANOVA comparing intake on the test day of cycle 1 with intake on the test day of cycle 11 revealed a cycle by diet group interaction ($F(2,41) = 9.463, p < 0.001$). As can be seen in Fig. 1A, and as was verified by an ANOVA of the difference in intake between the two time points, the Intermittent PF group had a greater increase in intake from cycle 1 to cycle 11 compared to both the Chow Only and the Daily PF groups (p values < 0.01). Therefore, to conclude

the above analyses, intermittent access to PF combined with a history of CRD did not increase intake to any greater degree than did intermittent access to PF alone. However, as the number of cycles increased, intermittent access to PF did increase total caloric intake on days when PF was provided.

Despite this increase in caloric intake of the Intermittent PF group during days when PF was provided, the Intermittent PF group weighed no more than the Chow Only group at the end of the experiment. Those with daily access to PF, however, gained more weight than each of the Chow Only and Intermittent PF groups (end of experiment body weights in grams: Chow Only/no-CRD = 273.1 ± 3.6, Chow Only/CRD = 267.4 ± 6.5, Intermittent PF/no-CRD = 271.6 ± 3.3, and Intermittent PF/CRD = 270.6 ± 5.2 versus Daily PF/no-CRD = 306.5 ± 10.3, and Daily PF/CRD = 308.8 ± 20.3 grams; $F(2,44) = 9.284$, $p < 0.001$; Fig. 1B). The fact that the Intermittent PF group gained no more weight than the Chow Only group during the course of the experiment, despite overeating on certain days, can be explained by the fact they ate the same total calories across each cycle as the Chow Only group (Fig. 2). In contrast, the Daily PF group consumed more calories across cycle 1 than did the other diet groups ($F(2,44) = 29.661$, $p < 0.001$; Daily PF vs. Chow Only and Intermittent PF, p values < 0.001; Fig. 2). Despite this initial overeating, the total intake of the Daily PF group during cycle 11 was equal to that of the Chow Only and Intermittent PF groups (Fig. 2).

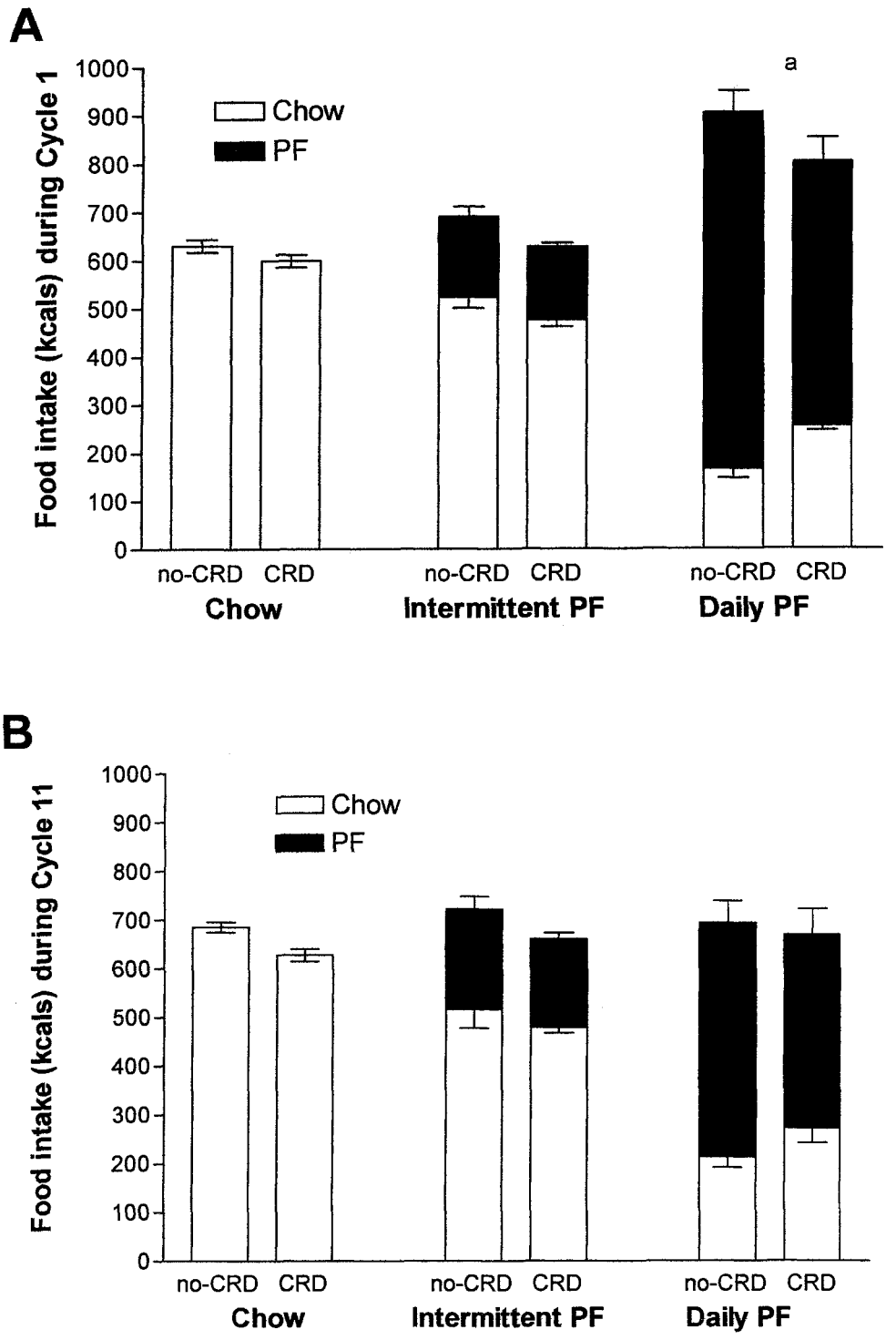

Fig. 2. Experiment 1: Total calories consumed for the Chow Only, Intermittent PF, and Daily PF groups across Cycle 1 (A), and Cycle 11 (B). Total intake of the Daily PF group was greater than that of the Chow Only and of the Intermittent PF groups (p values < 0.001).

4. Experiment 2: Does a history of CRD induce altered feeding response to the serotonin reuptake inhibitor fluoxetine?

4.1. Drug

Fluoxetine was used for this experiment because we were primarily interested in 5-HT's activity in the synapse, and its signaling of receptors, in response to acute food intake. Fluoxetine blocks 5-HT reuptake, and thus increases synaptic levels of 5-HT [104], promoting a satiety response. Fluoxetine has also been given clinically and found to alleviate binge eating symptoms in bulimics [105, 106].

Fluoxetine hydrochloride was purchased from Sigma (St. Louis, MO) and dissolved in physiological saline (the control solution). Fluoxetine was administered i.p. in a 3 mg/kg dose. A multiple dose study in rats of the same sex and similar age with access to the same PF and chow revealed that this was the minimum dose required to decrease food intake to a significant degree ($p < 0.05$; refer to [107]).

4.2. Methods

4.2.1. Experiment 2A: Effect of fluoxetine on food intake of rats with intermittent PF and CRD or no-CRD history in a binge-eating context

This experiment was undertaken to explore the possibility that a history of CRD affects serotonergic regulation of food intake in animals with intermittent access to PF. A separate group of 25 rats were used for this experiment. These rats were cycled through the CRD (or no-CRD protocol) outlined in Table 1 within the Intermittent PF group only (no-CRD: N = 12; CRD: N = 13). Six of the rats from the no-CRD group and

seven from the CRD group were additionally exposed to an acute foot-shock stress on the last day of each cycle to produce binge-eating in the CRD group as has been described elsewhere [6].

On the 20th and 21st cycle, after binge eating was well established in CRD+S rats (Figure 3, saline bars), each group (i.e. no-CRD only, no-CRD + S, CRD only, CRD + S) received either saline or fluoxetine in counterbalanced order over two consecutive cycles, 2 hours after the stress (or no-stress). By this time, lights had been out for 2 hours and rats had been feeding *ad libitum* on chow and PF for 2 hours. Given that fluoxetine has central effects within 30 minutes but not beyond 90 minutes since peripheral administration [108], we administered fluoxetine immediately prior to the time at which binge eating of CRD + S rats becomes apparent, which was 2 hours after foot-shock stress. To ensure rats did not experience food deprivation during this time, they were provided with *ad libitum* access to chow and PF as they were accustomed to following foot-shock stress, and intake was recorded for the 2 hours prior to injections and at 1, 2, 4, and 24 hours after injections.

4.2.2. Experiment 2B: Effect of fluoxetine on food intake of rats with and without CRD history with varied diets (chow only, intermittent PF, and daily PF)

Because the previous experiment indicated that CRD altered the rats' feeding response to fluoxetine, this experiment sought to determine the influence of intermittent PF (as opposed to consistent chow only or daily PF diets) on the CRD-induced exaggerated response to fluoxetine. Thus, fluoxetine was tested in a group of rats with and without CRD history; some with access to chow only, access to intermittent PF, or access to daily PF. In addition, to rule out the possibility that intake prior to fluoxetine treatment might

affect the efficacy of fluoxetine we administered fluoxetine prior to food consumption in this experiment. The same group of rats (N = 48) used in Experiment 1 was used for this experiment. Rats received either saline or fluoxetine on Day 12 of cycles 4 & 5 in a complete within subjects design. Prior to the injection, food was removed for 1 hour and then immediately after the injection pre-weighed chow or chow and PF (according to their diet condition) was presented, which coincided with lights out. Food remaining was recorded at 1, 2, 4, and 24 hours post injection.

4.2.3. Experiment 2C: Effect of fluoxetine following a food trigger on food intake of rats with and without a history of CRD with varied diets (chow only, intermittent PF, and daily PF)

The results of Experiments 2A and 2B differed, possibly due to the fact that in Experiment 2A rats were allowed to eat for 2 hours prior to fluoxetine administration while in Experiment 2B rats were not given food prior to fluoxetine. To resolve this discrepancy, the same rats as those used in Experiment 2B again received either saline or fluoxetine, but they were given a small morsel of food prior to drug treatment in an attempt to trigger 5-HT release in central feeding regulation sites, which might magnify the fluoxetine-induced differences in feeding response between groups. On cycles 6 & 7, food was removed from rats' cages 1 hour prior to lights out, and a trigger of 8.2 kcals of food was given immediately at lights out. The size of the food trigger was selected based on data from previous CRD cycles showing that at least this amount was consumed by rats in each of the diet conditions during the first hour after lights out. Rats in the chow only condition received chow (2.2 grams) as the trigger, while those in the intermittent or daily PF conditions received a morsel of chow and of PF (1 gram of each) as the trigger. The rats were then made to wait one hour without any food after which time they were

injected with fluoxetine or saline and given a premeasured amount of food: only chow for the Chow groups, and chow and PF for the Intermittent and Daily PF groups. Intake was recorded at 1, 2, 4, and 24 hours post injection. The rats were given either saline or fluoxetine on cycle 6 and then received the opposite treatment on cycle 7 to complete a within subjects design.

4.3. Results

4.3.1. Experiment 2A: Effect of fluoxetine on food intake of rats with intermittent PF and CRD or no-CRD history in a binge-eating context

Prior to injections, the body weights and 24-hour chow intakes of rats in the CRD group were confirmed to have returned to no-CRD control levels. Foot-shocked CRD rats treated with saline ate more than twice the kcals of PF than the other groups by 2 hours post injection (or 4 hours post-stress, $F(3,21) = 19.091, p < 0.001$; Fig. 3). There was a significant drug by group by stress interaction for PF ($F(1,21) = 17.66, p < 0.001$) and for total intake ($F(1,21) = 6.56, p < 0.05$) at 2 hours post injection. By 4 hours post injection, however, this effect had diminished. As shown in Fig. 3, fluoxetine reduced intake for both CRD groups compared to the no-CRD groups, but the effect was much stronger for rats in the CRD + S group than for those in the CRD alone group. An ANOVA with post hoc tests of the difference scores (between saline- and fluoxetine-induced intake) confirmed that the anorectic effect of fluoxetine was greater in the CRD + S group than any other group (p values < 0.01). The anorectic effect of fluoxetine in the CRD + S group was still evident at 4 hours post-injection (not shown).

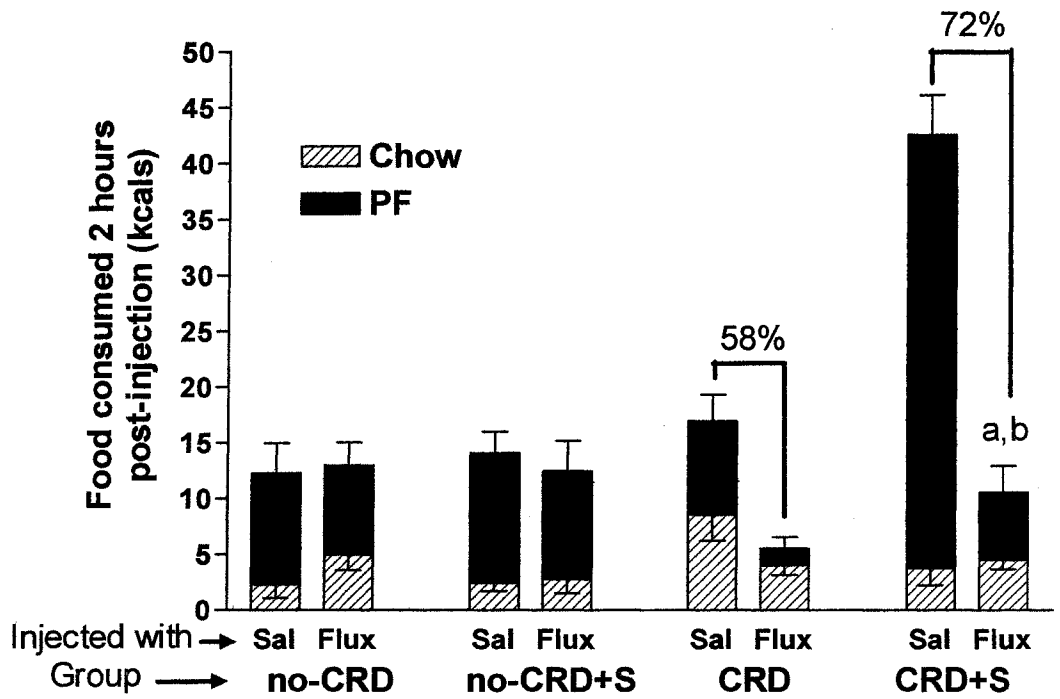

Fig. 3. Experiment 2A. The effect of fluoxetine (i.p. 3 mg/kg) on intake of no-CRD and CRD groups with intermittent access to PF, with and without exposure to an acute stressor. A drug by CRD history by stress interaction was observed (a: $p < 0.05$), and analysis of the difference scores between saline- and fluoxetine-induced intake showed that fluoxetine had the greatest effect in the group with the history of CRD with stress (b: $p < 0.01$) that overate following the stress.

4.3.2. Experiment 2B: Effect of fluoxetine on food intake of rats with and without CRD history with varied diets (chow only, intermittent PF, and daily PF)

Under saline conditions, the Intermittent PF group consumed more calories than both the Chow Only and the Daily PF groups (1H: Chow Only = 7.667 ± 0.697, Intermittent PF = 13.454 ± 1.824, Daily PF = 6.425 ± 0.826 kcals, $F(2,45) = 9.393$, $p < 0.001$; 2H: Chow Only = 12.786 ± 1.128, Intermittent PF = 21.571 ± 1.982, Daily PF = 12.660 ± 1.135 kcals, $F(2,45) = 12.066$, $p < 0.001$; data not shown). There was no interaction between drug, diet group, and CRD history, and there was also no interaction between drug and CRD history. There was, however, an interaction between diet group and drug at

both 1 and 2 hours post injection (1 hour: $F(2,42) = 3.53$, $p < 0.05$; 2 hours: $F(2,42) = 4.613$, $p < 0.05$; data not shown). Follow-up analyses of the difference scores between saline- and fluoxetine-induced total intake at 1 hour revealed a trend for the anorectic effect of fluoxetine to be greater in the Intermittent PF group (collapsed across CRD and no-CRD groups) compared to the other Chow Only and Daily PF groups (p values < 0.08). At 2 hours, the effect of fluoxetine was greater in the Intermittent PF group compared to the Chow Only group ($p < 0.05$). There were no differences in saline-treated intake and in the anorectic effect of fluoxetine between the no-CRD and CRD subgroups within the Intermittent PF group.

4.3.3. Experiment 2C: Effect of fluoxetine following a food trigger on food intake of rats with and without a history of CRD with varied diets (chow only, intermittent PF, and daily PF)

When fluoxetine was administered 1 hour after a food trigger, a marginal drug by diet group by CRD history interaction was discovered at 1 hour post injection for intake of PF ($F(1, 27) = 4.023$, $p = 0.055$; Fig. 4). As shown in Fig. 4, fluoxetine appeared to be more potent in the Intermittent PF/CRD group, compared to all other groups, although an ANOVA of the difference scores between saline- and fluoxetine-induced intakes failed to indicate a significant difference between the groups. The interaction between diet group and CRD history might be due to the fact that the food trigger induced overeating in only the Intermittent PF/CRD group (Intermittent PF/no-CRD total kcals = 12.73 ± 1.93; Intermittent PF/CRD total kcals = 19.91 ± 3.00; $t(13) = 1.944$, $p_{(1\text{-tailed})} < 0.05$). Fluoxetine essentially normalized this trigger-induced overeating in the Intermittent PF/CRD group. By 2 and 4 hours after the injection there was still an overall drug effect (2 hours: $F(1,41) = 35.06$, $p < 0.001$; 4 hours: $F(1,41) = 23.91$, $p < 0.001$) but no interactions and no differ-

ences were evident between the groups (data not shown). By 24 hours post-injection, there was no longer an effect of fluoxetine.

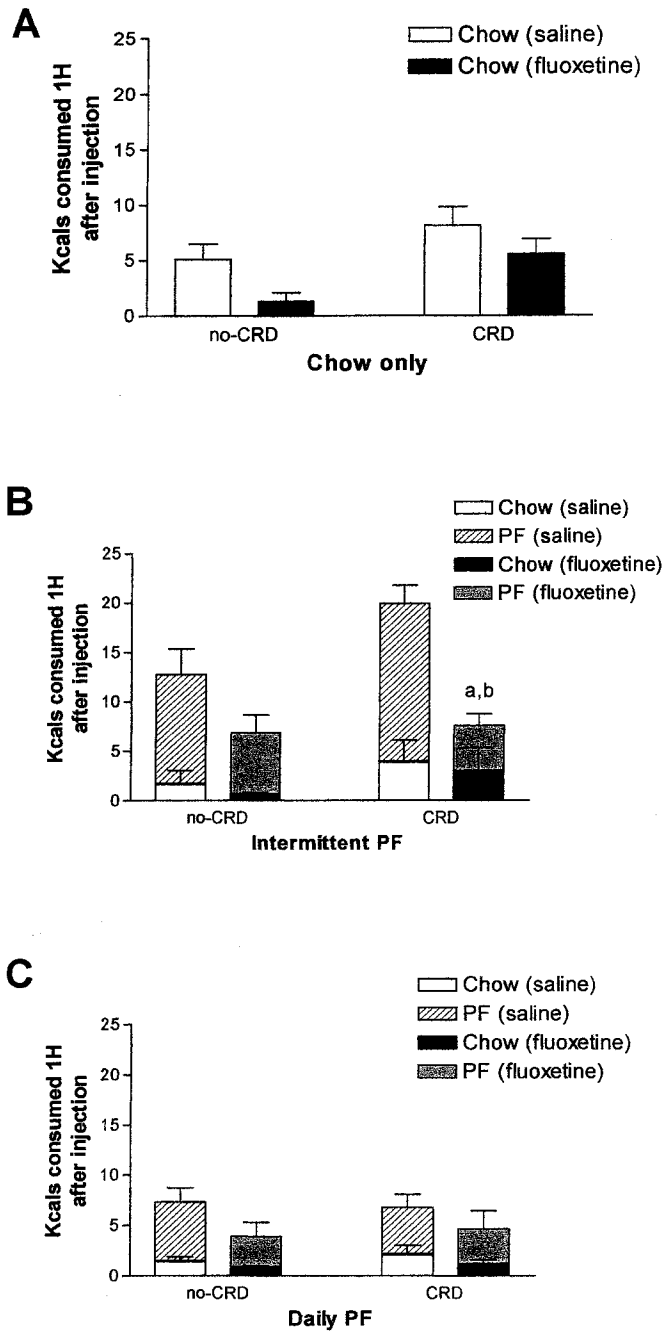

Fig. 4. Experiment 2C. The effect of fluoxetine (i.p. 3mg/kg) on trigger-induced 1 hour intake of Chow Only (A), Intermittent PF (B), and Daily PF (C) groups with and without a history of CRD. There was a drug by diet group by CRD history interaction (a: $p = 0.055$), and the trigger itself induced overeating in the Intermittent PF/CRD group compared to the Intermittent PF/no-CRD group (b: $p < 0.05$).

5. Experiment 3: Does a CRD history alter levels of monoamines in brain regions known to regulate feeding, reward, and mood?

5.1. Methods

5.1.1. Experiment 3A: Effect of CRD (or no-CRD) history on hypothalamic and nucleus accumbens serotonin and dopamine in rats previously maintained on intermittent PF

Given the results of Experiment 2 above, we wanted to investigate whether levels of 5-HT in the hypothalamus (involved in feeding) were reduced by a history of CRD, and whether levels of 5-HT and dopamine in the nucleus accumbens (involved in reward) were altered by a history of CRD. The same N = 25 rats used in Experiment 2A were used for this post-mortem experiment. On the day of euthanasia, the average body weight across groups (Intermittent PF/no-CRD and Intermittent PF/CRD) did not differ. On Day 12 of cycle 25, rats underwent stress (or no stress) during the light and then were euthanized by guillotine between 2 and 4 hours after lights out, while sated, therefore matching the conditions under which they had been treated with fluoxetine on cycles 20 and 21. Brains were rapidly extracted and dissected over ice. Brains were placed ventral side up in a cutting block (Plastics One: Roanoke, VA) and the hypothalamus and nucleus accumbens were dissected using the methods adapted from Heffner et al. [109] and co-ordinates of Paxinos & Watson [110]. The hypothalamus was dissected from a section taken from just anterior of the optic chiasm to just posterior of the mamillary bodies. A square region was cut from the ventral-medial segment of this section (approximately 2 mm off each side of the 3rd ventricle). Bilateral nucleus accumbens was taken from a

section made from anterior of the optic chiasm to anterior of the olfactory tubercles and 2 mm punches taken around the anterior commissure on each side.

5.1.2. Experiment 3B: Effect of CRD (or no-CRD) history on anterior hypothalamic, nucleus accumbens, and medial prefrontal cortex serotonin and dopamine in previously maintained on chow only, intermittent PF, or daily PF

This experiment was conducted in order to expand on findings of the previous experiment, that CRD had reduced hypothalamic 5-HT and 5HIAA and had abolished the association between 5-HT turnover and dopamine turnover in the nucleus accumbens of rats fed intermittent PF. This time, we used rats with and without CRD history, maintained on chow only, intermittent PF, and daily PF, to investigate the effect of these diets on monoamine levels in the hypothalamus and their association in the accumbens. The same rats used for Experiments 1, 2B, and 2C (N = 48) were used for this post-mortem experiment. At the end of the experiment, body weights of the Chow Only and Intermittent PF groups (including both CRD and no-CRD subgroups) did not differ. However, rats in the Daily PF group (both no-CRD and CRD) were heavier (Daily PF = 307.63 ± 10.99, Chow Only = 270.25 ± 3.66, Intermittent PF = 271.07 ± 3.067, $F(2,44) = 9.284$, $p < 0.001$). One rat from the Intermittent PF/CRD group was not included in the post-mortem measures due to sickness prior to euthanasia. On Day 12 of cycle 12, rats were euthanized by guillotine decapitation during the last 4 hours of daylight. Food was removed from the rats at least one hour prior to euthanasia and so, unlike Experiment 3A, rats were not allowed to feed immediately prior to euthanasia. Because they were euthanized during the light when rats do not normally consume large meals, removal of food for a brief period of time immediately prior to euthanasia did not constitute food deprivation. By not allowing animals to eat prior to euthanasia, we ruled out the possibility that

differences in monoamine levels between the groups were due to differences in food intake.

Brains were extracted rapidly and then tissue was dissected on ice. Dissection procedures matched those used in Experiment 3A except this time only an anterior section of the hypothalamus was dissected, and the mPFC was also dissected. The brains were placed ventral side up in a cutting block (Plastics One: Roanoke, VA) and the anterior hypothalamus, nucleus accumbens, and mPFC were dissected using the methods adapted from Heffner et al. [109] and with guidance from the Rat Atlas of Paxinos & Watson [110]. The hypothalamus was dissected from a section taken from just anterior to the optic chiasm to 2 mm posterior of that first slice. A square region was cut from the ventral-medial segment of this section (approximately 2 cubic mm off each side of the 3rd ventricle). Bilateral nucleus accumbens was taken from a section made from anterior to the optic chiasm to anterior of the olfactory tubercles and 2 mm punches taken around the anterior commissure on each side. The mPFC was taken from the anterior-most slice made to dissect out the nucleus accumbens extending to 2 mm anterior to that section. From that 2 mm thick section, a 2 mm-wide punch was taken medially, beginning approximately 1 mm below the brain surface. Tissue was immediately weighed and placed into a 0.05 N perchloric acid solution containing 10 ng/200 μl of dihydroxybenzylamine (DHBA: served as the internal standard), then was homogenized and centrifuged. The supernatant from each sample was then filtered through a 0.2 μm syringe tip filter into a 1.5 ml conical microcentrifuge tube and stored in a -20°C freezer until analysis (which occurred no more than 14 days after harvest of the tissue).

5.2. Measurement of monoamines

Serotonin, dopamine, and the metabolites 5HIAA and DOPAC, were measured using high performance liquid chromatography (HPLC) with electrochemical detection (4-channel CoulArray EC detector; ESA, Inc., MA). The samples were injected using an ESA Model 542 autosampler. The mobile phase contained 20% methanol, 32.5 mg/L of sodium dodecyl sulfate, 0.1 mM disodium ethylenediamine tetraacetate (EDTA), 30 mM citric acid, and 60 mM monobasic sodium phosphate, and adjusted with phosphoric acid to pH 2.8. Analytes were separated by a C-18 reverse-phase silica-bead column (Varian Inc., Lake Forest, CA) and detected by electrode potentials set to reduce at -100 mV, and to oxidize at 150, 250, and 450 mV. Data were integrated using CoulArray® for Windows®[32] software (ESA, Inc., MA). Raw data were then corrected for tissue weight and quantified using standard calibration curves developed from standards of known quantity that were interspersed with the tissue samples.

5.3. Verification of brain dissection

To verify that the dissection methods used captured the regions intended, an additional dissection was carried out on another female rat of the same age and weight. Using the dissection procedure described in section 5.1.2., the anterior, posterior and lateral borders of each region dissected were compared to the rat atlas [110]. The anterior section of the hypothalamus matched sections –0.26 to –2.12 mm relative to bregma, with the lateral cut made 2 mm either side of the 3rd ventricle, and the dorsal cut made across the top border of the 3rd ventricle (Fig. 5).

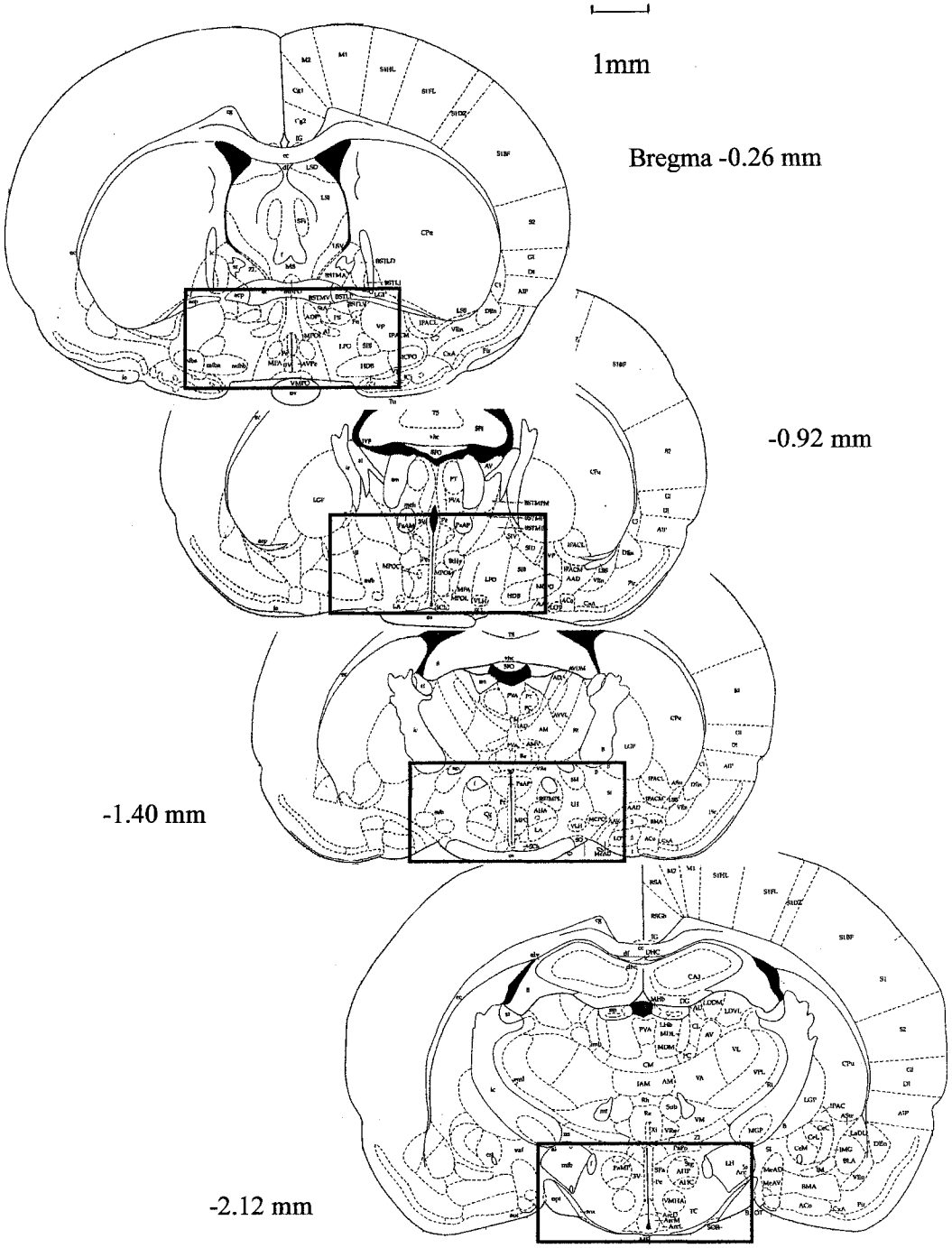

Fig. 5. Experiment 3. Outlined area dissected as the anterior hypothalamus. The slice encompassed an anterior-posterior region –0.26 to –2.12 mm relative to bregma, a lateral section 2 mm of either side of the 3rd ventricle, and a ventral-dorsal section from just beneath the top border of the 3rd ventricle to the base.

The 2 mm nucleus accumbens section included sections –0.26 through to 2.2 mm relative to bregma and a 2 mm punch cut from around the anterior commissure encompassed both shell and core of the nucleus accumbens (Fig. 6). The 2 mm slice for the mPFC included sections 2.2 through to 4.7 mm relative to bregma, and the medial 2 mm punch encompassed all of the mPFC (i.e. that region referred to as prelimbic cortex by Paxinos & Watson [110]; Fig. 7). Of note, the dissections for both the nucleus accumbens and the mPFC resulted in slightly more area than the 2 mm intended being included along the anterior-posterior plane, but this was most likely due to the smaller size of these female rats used in this study, in comparison to the larger (270-310 grams) male rats used to create the Paxinos & Watson rat atlas. However, even with the slightly larger area included in the dissection, the desired areas were obtained with minimal tissue from other regions (see Fig. 6 and 7).

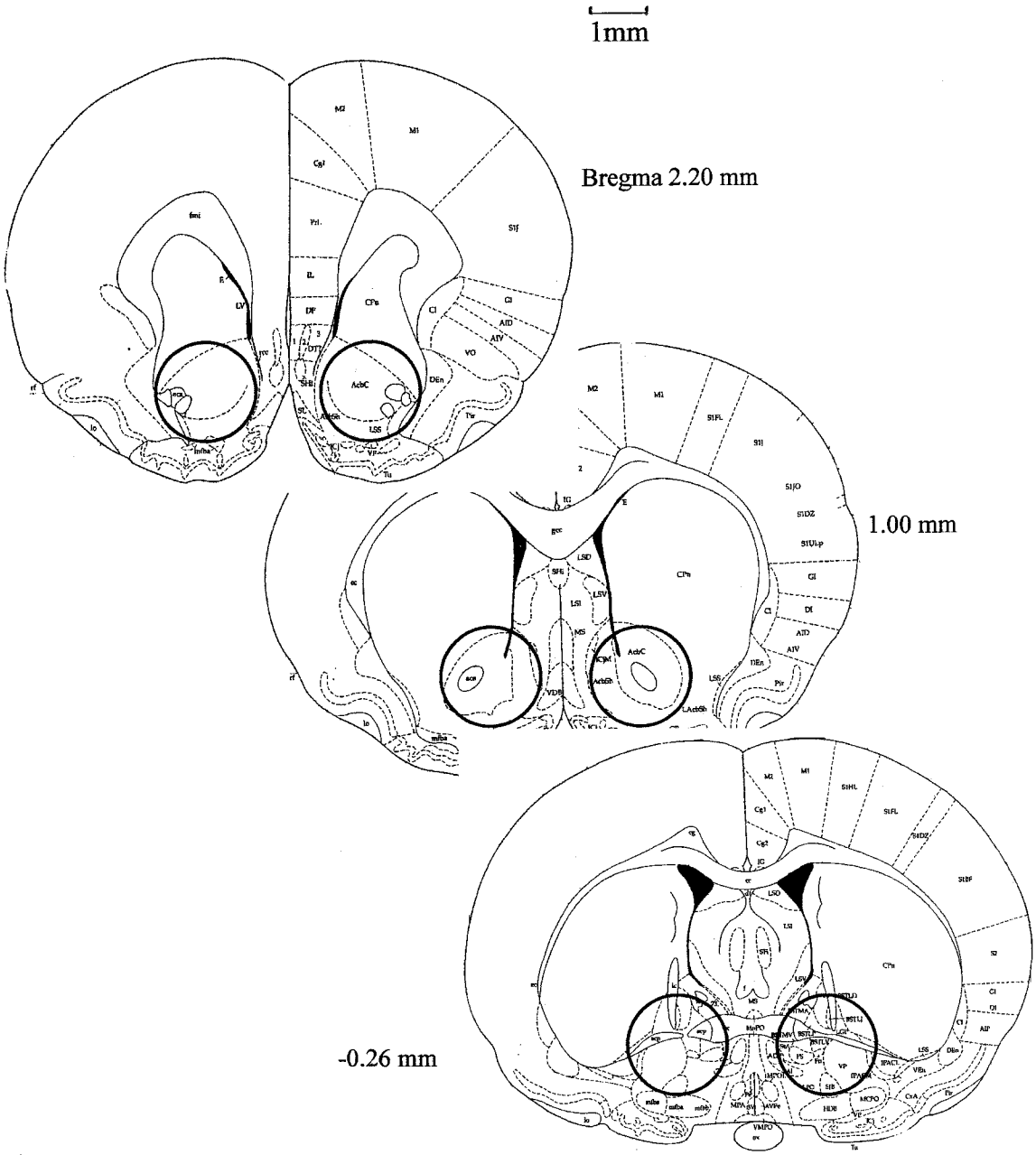

Fig. 6. Experiment 3. Outlined area dissected as the nuclei accumbens. The slice encompassed the anterior-posterior section –0.26 to 2.2 mm relative to bregma, and then bilateral 2 mm-wide tissue punches were made around the anterior commissure to encompass both the shell and core of the nuclei accumbens.

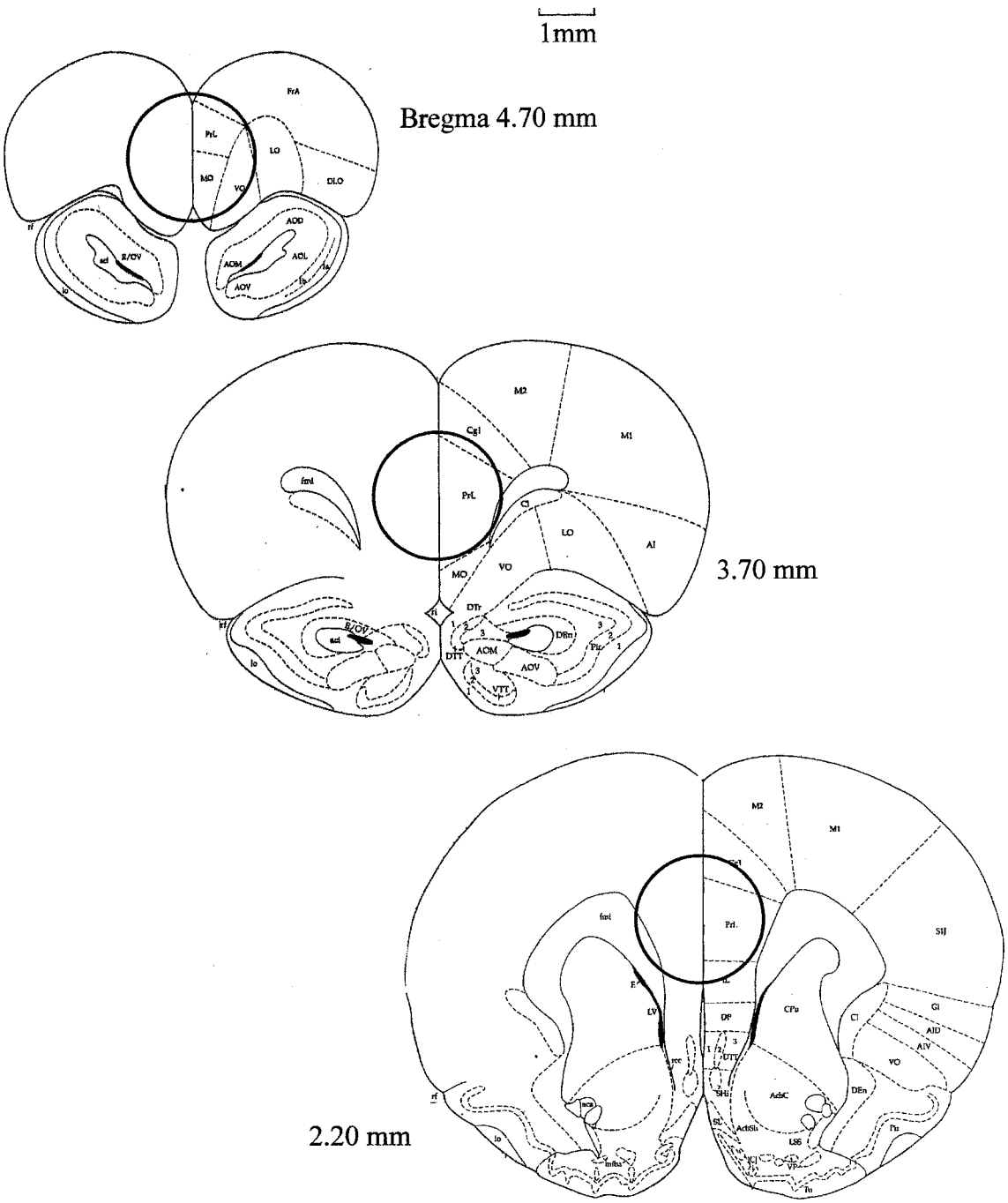

Fig. 7. Experiment 3. Outlined area dissected as the mPFC. The slice encompassed the anterior-posterior section 2.2 to 4.7 relative to bregma and a medial 2 mm tissue punch was made to encompass all of the mPFC.

5.4. Results

5.4.1. Experiment 3A: Effect of CRD (or no-CRD) history on hypothalamic and nucleus accumbens serotonin and dopamine in rats previously maintained on intermittent PF

Analyses of variance comparing 5-HT and 5HIAA levels across the four groups yielded no significant differences between the groups. However, given our hypothesis that a history of CRD would reduce hypothalamic 5-HT levels compared to rats with no history of CRD, our primary comparison of interest was between these two groups. Furthermore, it has previously been shown that stress transiently increases hypothalamic 5-HT [111] and so we did not collapse the stress and no stress groups together for this comparison. Rats with a history of CRD alone had lower whole hypothalamic levels of 5-HT and 5HIAA compared to those in the no-CRD group (5-HT: no-CRD = 0.76 ± 0.04; CRD = 0.63 ± 0.05 ng/mg; $t(9) = 2.062, p < 0.05$ $_{(1\text{-tailed})}$, 5HIAA: no-CRD = 0.64 ± 0.02; CRD = 0.56 ± 0.04 ng/mg; $t(9) = 2.045, p < 0.05$ $_{(1\text{-tailed})}$, Fig. 8A & B). As shown in Table 2, 5-HT levels in the CRD+S group greater than those of the CRD alone group and were the same as those of no-CRD groups. Dopamine, DOPAC and turnover ratios (measured as metabolite/monoamine) of dopamine and 5-HT did not differ between groups (Table 2).

In the nucleus accumbens, there were no differences in tissue levels of 5-HT, dopamine, their metabolites, or their turnover ratios (Table 2). However, a strong positive correlation was evident between 5-HT turnover and dopamine turnover in the no-CRD group, an association that was completely absent in the CRD group (no-CRD: $r = 0.86, p < 0.01$; CRD: r = -0.026, ns; Fig. 9A & B). This relationship between 5-HT turnover and dopamine turnover was true of both the stressed and non-stressed no-CRD rats but absent

in both the stressed and non-stressed CRD rats and so appears to be a salient effect specific to CRD history.

	5-HT	5HIAA	DA	DOPAC	5HIAA/ 5-HT	DOPAC/ DA
Hypothalamus						
no-CRD (*N*)	0.76±0.04 (6)	0.64±0.02 (6)	0.27±0.03 (6)	0.08±0.01 (6)	0.85±0.02 (6)	0.28±0.02 (6)
no-CRD+S (*N*)	0.77±0.04 (5)	0.60±0.08 (5)	0.31±0.02 (5)	0.08±0.01 (5)	0.78±0.09 (5)	0.27±0.05 (5)
CRD (*N*)	0.63±0.05 (5)	0.56±0.04 (5)	0.25±0.02 (5)	0.08±0.01 (5)	0.90±0.07 (5)	0.31±0.02 (5)
CRD+S (*N*)	0.82±0.08 (6)	0.60±0.08 (6)	0.35±0.04 (6)	0.09±0.02 (6)	0.73±0.04 (6)	0.25±0.02 (6)
Nucleus Accumbens						
no-CRD (*N*)	0.59±0.07 (6)	0.44±0.05 (6)	6.29±0.67 (6)	1.73±0.17 (6)	0.28±0.02 (6)	0.75±0.03 (6)
no-CRD+S (*N*)	0.60±0.05 (5)	0.46±0.05 (5)	6.31±0.30 (5)	1.98±0.33 (5)	0.32±0.06 (5)	0.79±0.12 (5)
CRD (*N*)	0.61±0.04 (5)	0.41±0.03 (5)	6.28±0.50 (5)	1.79±0.18 (5)	0.29±0.03 (5)	0.69±0.07 (5)
CRD+S (*N*)	0.64±0.04 (7)	0.45±0.04 (7)	5.74±0.69 (7)	1.97±0.28 (7)	0.34±0.03 (7)	0.70±0.06 (7)

Table 2. Levels of 5-HT, 5HIAA, dopamine (DA), DOPAC, and their turnover ratios in whole hypothalamus and in the nucleus accumbens for rats maintained on intermittent PF with and without a history of CRD. Data are mean ± SEM ng / mg and *(N)* = sample size. CRD + S = CRD with stress group.

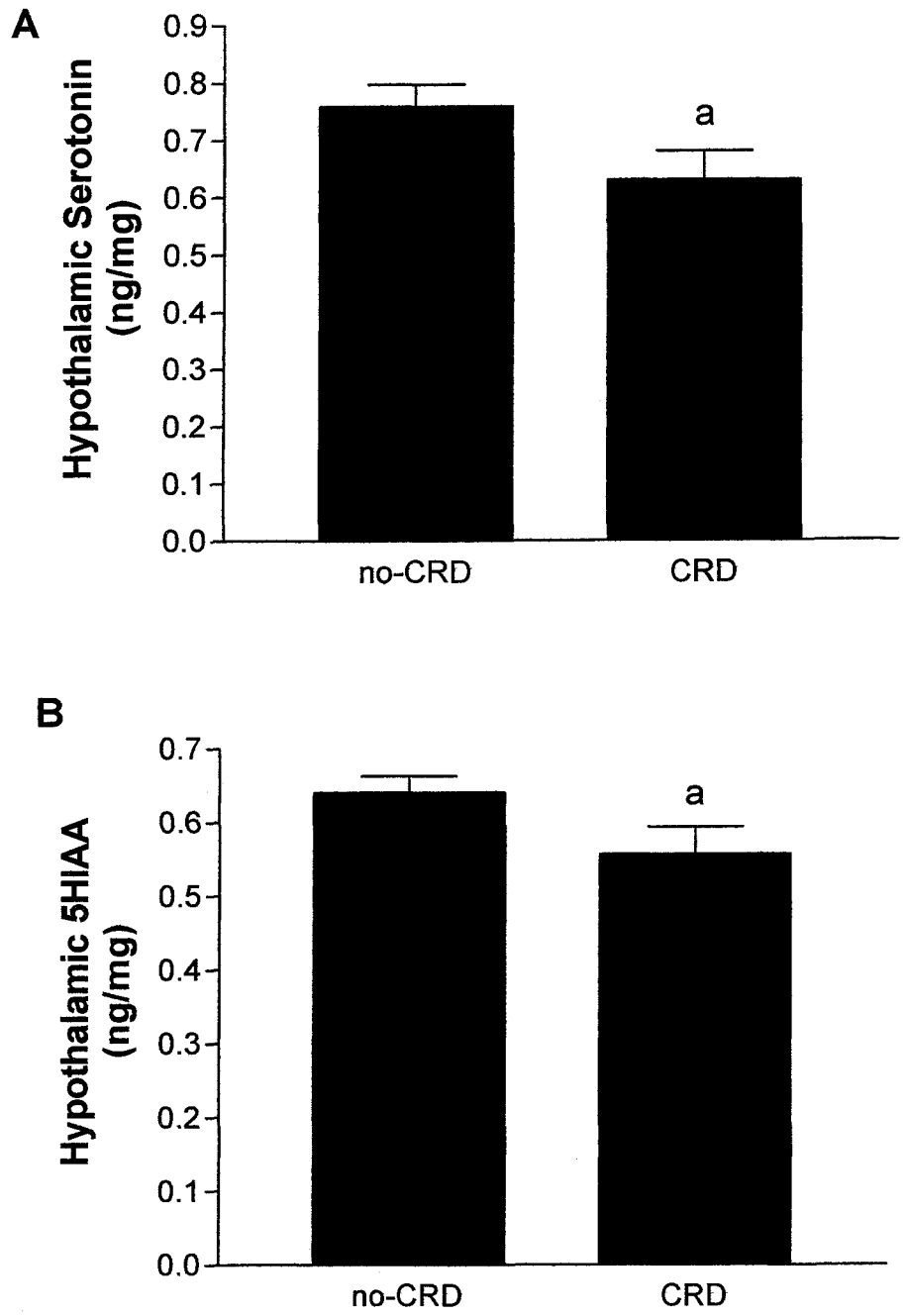

Fig. 8. Experiment 3A. The effect of a history of CRD vs. no-CRD on whole hypothalamic tissue levels of 5-HT (A) and 5HIAA (B) in groups maintained on intermittent PF (a: Intermittent PF/no-CRD vs. Intermittent PF/CRD, $p < 0.05$).

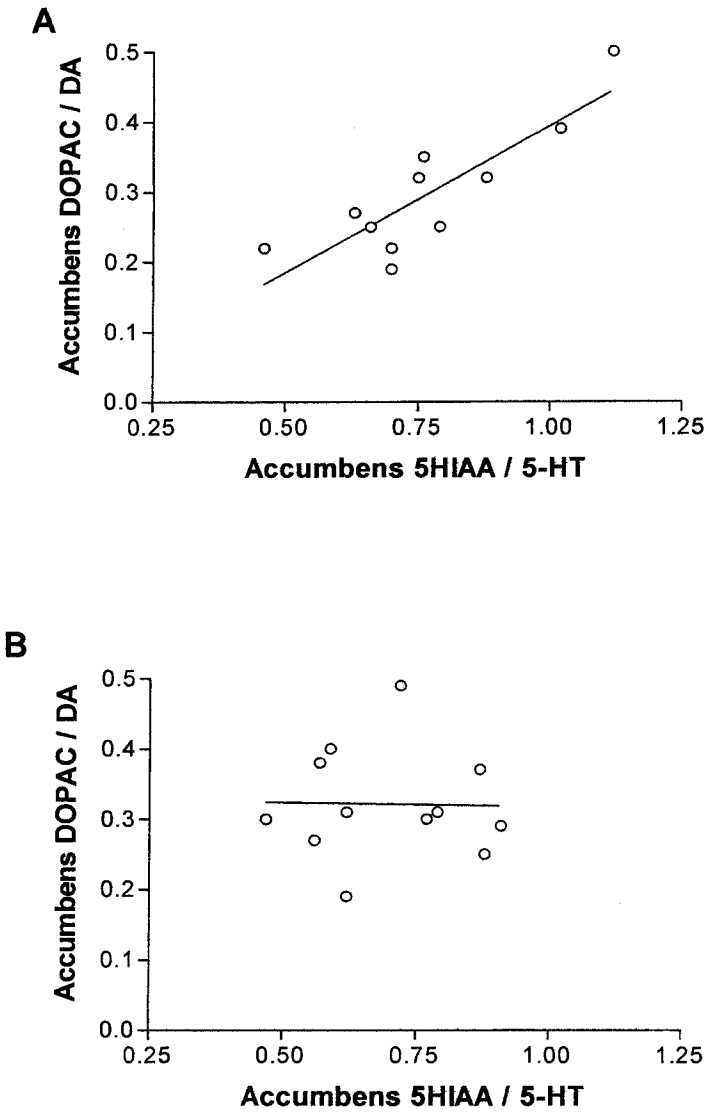

Fig. 9. Experiment 3A. The effect of a history of CRD vs. no-CRD on the relationship between 5-HT turnover and dopamine (DA) turnover in the nucleus accumbens of rats fed intermittent PF. For no-CRD controls (A), 5-HT turnover and DA turnover were positively correlated ($r = 0.86$, $p < 0.01$) but this relationship was completely absent for those with a history of CRD (B: $r = -0.026$, ns).

5.4.2. Experiment 3B: Effect of CRD (or no-CRD) history on anterior hypothalamic, nucleus accumbens, and medial prefrontal cortex serotonin and dopamine in previously maintained on chow only, intermittent PF, or daily PF

Following from the results of Experiment 3A, a smaller portion of the hypothalamus was dissected in an effort to further define the region in which decreased 5-HT lev-

els existed in CRD rats compared to no-CRD rats. The region dissected was an anterior portion of the hypothalamus, and included the paraventricular nucleus (PVN), the anterior hypothalamus, the anterior portion of the lateral hypothalamus, and the medial preoptic region. Assay of this section however, yielded no differences between 5-HT or 5HIAA in CRD and no-CRD rats (Table 3). While there were no differences as a function of CRD history, there was a difference in dopamine and DOPAC levels in this region as a function of diet group (dopamine: $F(2,35) = 4.322$, $p < 0.05$; DOPAC: $F(2,37) = 3.516$, $p < 0.05$). Bonferroni post hoc tests revealed that the groups with intermittent access to PF (pooled across the no-CRD and CRD condition) had lower dopamine and DOPAC levels than the Chow Only group, and there was a strong trend for rats with daily access to PF to also have lower dopamine and DOPAC levels in this region of the hypothalamus (dopamine: Chow Only = 3.48 ± 0.46, Intermittent PF = 1.94 ± 0.30, Daily PF = 2.24 ± 0.38 ng / mg, DOPAC: Chow only = 0.34 ± 0.04, Intermittent PF = 0.20 ± 0.03, Daily PF = 0.23 ± 0.04 ng / mg; Fig. 10A & B).

	5-HT	5HIAA	DA	DOPAC	5HIAA/ 5-HT	DOPAC/ DA
Hypo-thalamus						
Chow/ no-CRD (*N*)	1.12±0.26 (8)	0.28±0.09 (7)	3.38±0.67 (7)	0.33±0.05 (8)	0.21±0.03 (7)	0.11±0.01 (7)
Chow/CRD (*N*)	1.13±0.11 (6)	0.21±0.05 (5)	3.59±0.70 (6)	0.37±0.07 (6)	0.20±0.04 (5)	0.11±0.01 (6)
Int./ no-CRD (*N*)	0.98±0.12 (5)	0.21±0.06 (5)	1.78±0.44 (5)	0.19±0.06 (5)	0.20±0.05 (5)	0.10±0.01 (5)
Int./CRD (*N*)	0.83±0.12 (7)	0.14±0.05 (7)	2.06±0.43 (7)	0.21±0.04 (7)	0.15±0.03 (7)	0.11±0.01 (7)
Daily/ no-CRD (*N*)	0.75±0.16 (6)	0.14±0.05 (6)	2.26±0.47 (6)	0.21±0.06 (6)	0.19±0.06 (6)	0.09±0.01 (6)
Daily/CRD (*N*)	0.80±0.12 (8)	0.13±0.03 (7)	2.23±0.61 (7)	0.25±0.07 (8)	0.17±0.04 (7)	0.09±0.01 (7)
Nucleus accumbens						
Chow/ no-CRD (*N*)	0.59±0.12 (5)	0.18±0.03 (4)	10.54± 1.47 (5)	1.10±0.18 (5)	0.25±0.04 (4)	0.10± 0.003 (5)
Chow/CRD (*N*)	0.55±0.13 (6)	0.14±0.03 (6)	10.97± 1.03 (6)	1.22±0.09 (6)	0.35±0.11 (6)	0.11± 0.005 (6)
Int./ no-CRD (*N*)	0.70±0.09 (6)	0.14±0.02 (6)	11.46± 1.18 (6)	1.17±0.16 (6)	0.24±0.05 (6)	0.10± 0.006 (6)
Int./CRD (*N*)	0.54±0.11 (7)	0.13±0.03 (7)	9.93±0.73 (7)	1.08±0.07 (7)	0.23±0.04 (7)	0.11± 0.003 (7)
Daily/ no-CRD (*N*)	0.59±0.07 (7)	0.13±0.02 (7)	10.76± 0.93 (7)	1.12±0.12 (7)	0.24±0.05 (7)	0.10± 0.004 (7)
Daily/CRD (*N*)	0.42±0.10 (8)	0.11±0.02 (8)	9.92±1.11 (8)	0.99±0.12 (8)	0.42±0.14 (8)	0.10± 0.005 (8)
mPFC						
Chow/ no-CRD (*N*)	0.25±0.07 (5)	0.04±0.01 (5)	0.26±0.01 (5)	0.08±0.02 (5)	0.21±0.04 (5)	0.31±0.06 (5)
Chow/CRD (*N*)	0.36±0.10 (7)	0.08±0.01 (6)	0.51±0.14 (7)	0.15±0.03 (7)	0.27±0.08 (6)	0.37±0.07 (7)
Int./ no-CRD (*N*)	0.42±0.09 (7)	0.09±0.03 (5)	0.48±0.10 (7)	0.11±0.02 (7)	0.22±0.04 (5)	0.25±0.05 (7)
Int./CRD (*N*)	0.12±0.03 (5)	0.03±0.01 (4)	0.20±0.03 (5)	0.07±0.02 (5)	0.28±0.07 (4)	0.33±0.08 (5)
Daily/ no-CRD (*N*)	0.24±0.04 (8)	0.07±0.01 (8)	0.29±0.02 (8)	0.06±0.01 (8)	0.41±0.16 (8)	0.22±0.04 (8)
Daily/CRD (*N*)	0.31±0.07 (6)	0.08±0.02 (6)	0.29±0.03 (5)	0.12±0.04 (6)	0.30±0.08 (6)	0.31±0.05 (5)

Table 3. Levels of 5-HT, 5HIAA, dopamine (DA), DOPAC, and their turnover ratios in anterior hypothalamus, nucleus accumbens, and medial PFC for rats maintained on chow only, intermittent PF, or daily PF, with and without a history of CRD. Data are mean ± SEM ng / mg.

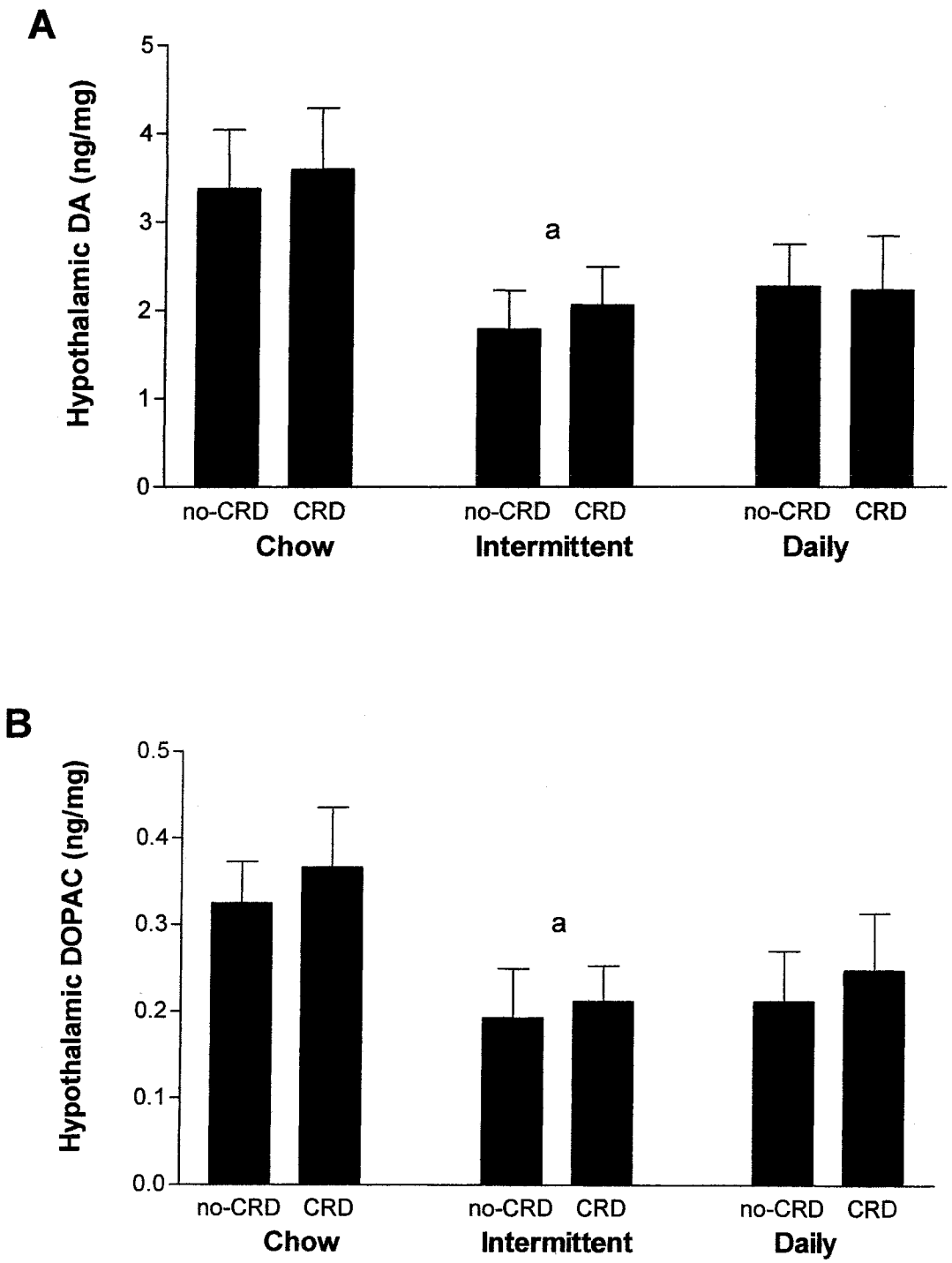

Fig. 10. Experiment 3B. Differences in tissue levels of dopamine (A) and DOPAC (B) from an anterior section of the hypothalamus in rats fed chow only, intermittent PF, and daily PF for rats with and without a history of CRD (a: Intermittent PF/no-CRD and CRD combined vs. Chow Only/no-CRD and CRD combined, $p < 0.05$). There was no effect of a history of CRD.

In the nucleus accumbens, we confirmed again that no differences in tissue levels of 5-HT, dopamine, or their metabolites existed between rats with and without a history of CRD. There were also no differences as a function of diet condition (i.e. Chow Only, Intermittent PF or Daily PF). However, the result from Experiment 3A in which the no-CRD group exhibited a strong positive association between 5-HT turnover and dopamine turnover, while the CRD group had no such correlation, was replicated here. Furthermore, this pattern occurred irrespective of diet conditions. Given that diet condition made no difference to the result, we collapsed the diet conditions together to yield overall no-CRD and CRD correlations (no-CRD: $r = 0.71$, $p < 0.01$; CRD: $r = -0.061$, ns; Fig. 11A & B). There were a couple of marginal outliers with high 5HIAA/5-HT ratios in the CRD group, but even if these were removed from the data set, there was still no correlation between dopamine turnover and 5-HT turnover for the CRD group. Also of note, in the earlier experiment comparing the no-CRD and CRD groups from just the Intermittent PF condition, the turnover ratio for DOPAC/dopamine was much greater than that from the second experiment (even if those in the Intermittent PF group are considered alone). The ratio in the first experiment ranged between 0.4 and 1.1 ng / mg, whereas in the second experiment the ratio was between 0.08 and 0.13 ng / mg. In contrast, the majority of the ratios for 5-HT turnover fell between 0.1 and 0.5 ng / mg in both experiments. Possible reasons for this discrepancy are discussed below.

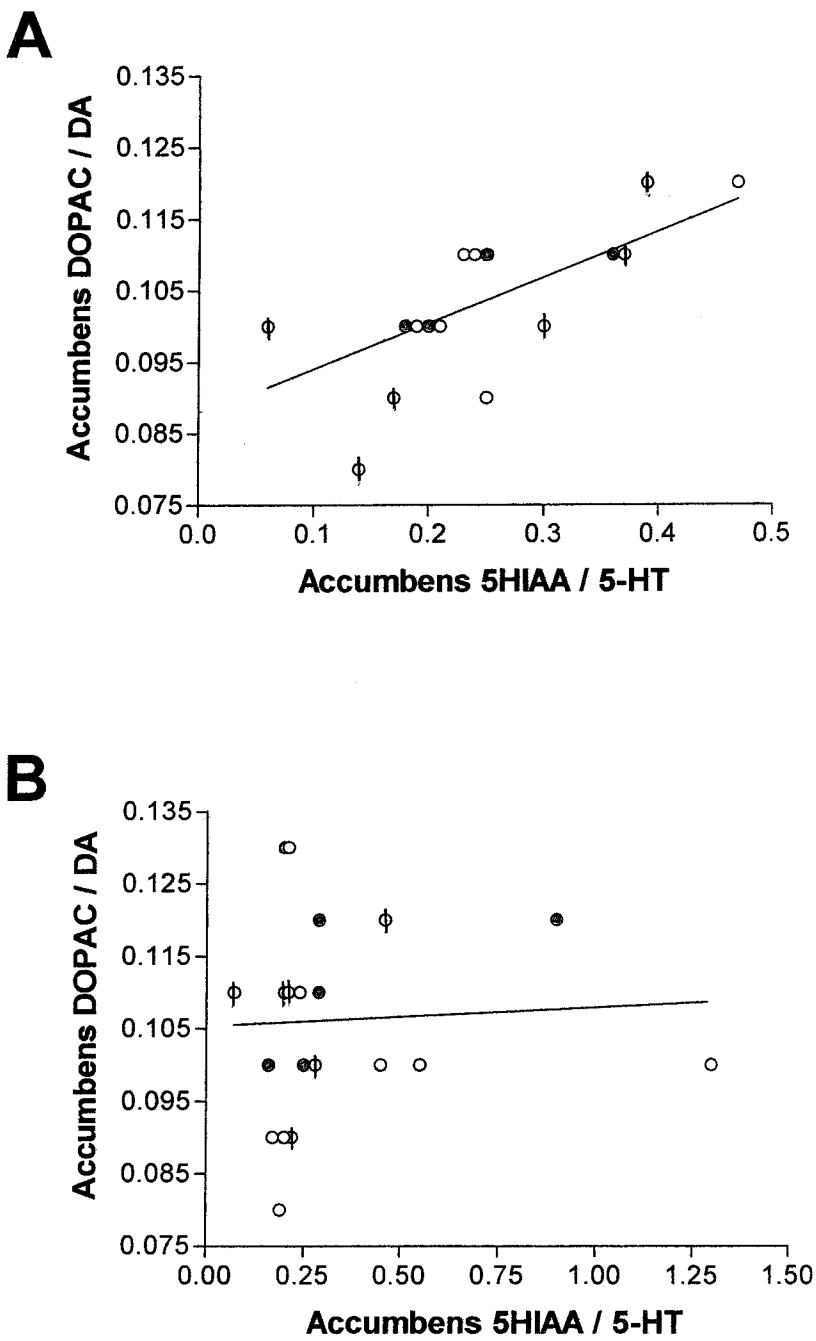

Fig. 11. Experiment 3B. The effect of a history of CRD on the relationship between 5-HT turnover and dopamine (DA) turnover in the nucleus accumbens. For no-CRD controls (A), 5-HT turnover and DA turnover were positively correlated ($r = 0.71$, $p < 0.01$) but this relationship was completely absent for those with a history of CRD (B: $r = -0.061$, ns). ● = Chow Only, ϕ = Intermittent PF, ○ = Daily PF.

In the mPFC, there was no interaction between diet and CRD history, nor were there any main effects of each of these on monoamine or metabolite levels. However, when the Intermittent PF group was considered alone, those with a history of CRD had lower tissue levels of 5-HT and dopamine levels than the no-CRD group (5-HT: no-CRD = 0.418 ± 0.087, CRD = 0.122 ± 0.013 ng / mg, $t(10) = 3.376$, $p < 0.05$; dopamine: no-CRD = 0.477 ± 0.105, CRD = 0.199 ± 0.026 ng / mg, $t(10) = 2.575$, $p < 0.05$; Fig. 12A & B). As shown, this effect is carried both by a modest reduction of monoamine levels in the CRD group as compared to all other groups, but also by a non-significant increase in monoamine levels of the no-CRD Intermittent PF group compared to the other diet condition groups. Levels of DOPAC and 5HIAA were also lower in the intermittent PF/CRD group compared to the no-CRD group of the same diet condition, but these differences did not obtain statistical significance.

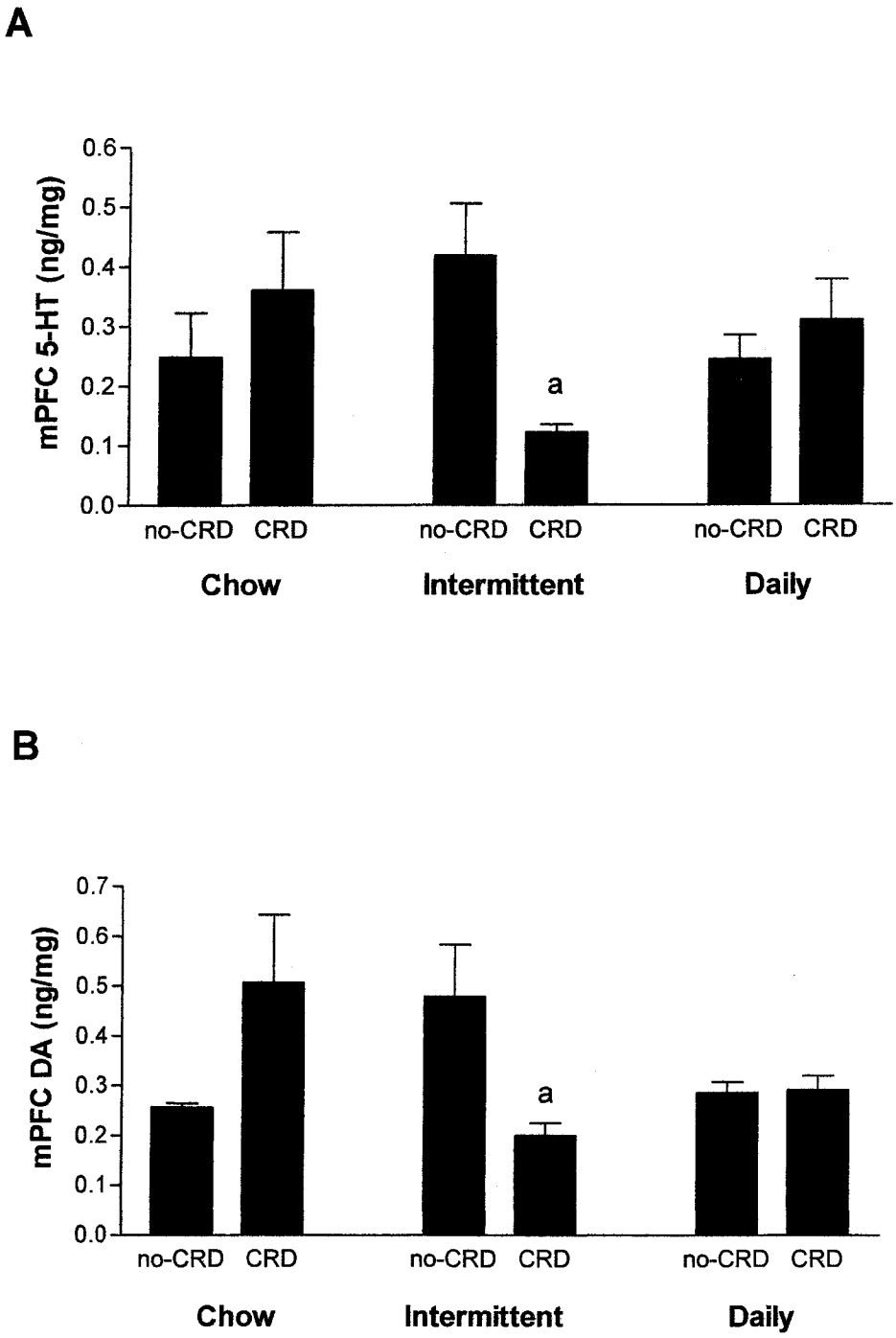

Fig. 12. Experiment 3B. Tissue levels of 5-HT (A) and dopamine (B) in the mPFC of rats fed chow only, intermittent PF, and daily PF with and without a history of CRD (a: Intermittent PF/CRD vs. Intermittent PF/no-CRD, $p < 0.05$).

6. Experiment 4: Does a history of CRD increase behaviors consistent with animal models of anxiety or depression?

6.1. Methods

Given the previous findings that a history of CRD with intermittent access to PF induced a reduction of 5-HT and dopamine in the mPFC and was associated with a lack of association between 5-HT turnover and dopamine turnover in the nucleus accumbens, this set of experiments was conducted to determine whether rats with such a history exhibited behaviors consistent with animal models of anxiety or depression. A new group of N = 32 rats were used for this set of experiments. Rats were weight-matched into Intermittent PF/no-CRD and Intermittent PF/CRD groups (N = 16 per group), and proceeded through the cycling protocol for the Intermittent PF group outlined in Table 1.

6.1.1. Experiment 4A: Elevated maze test

Eight of the rats from each of the no-CRD and CRD groups were randomly assigned to undergo the elevated maze test (the other half were used in the open field test). Testing was conducted on the last 2 days of cycle 7, during the 3 hours prior to lights out. Rats were not deprived of food prior to testing. The rats were tested in counterbalanced order, with half from each group being tested on the first test day and half on the second test day. They were maintained on chow during these test days. One rat at a time was carried in its home cage covered with a sheet, from the colony room to an adjacent room (same light and temperature conditions) for testing.

We used a simplified "V" version of the elevated plus maze that is commonly used to measure rodent activity in open versus closed spaces. The elevated maze consisted of a wooden platform 50 cm tall, painted black, with 2 arms at a 90-degree angle to each other. One arm was open (length: 50 cm by width: 10 cm), with a low (1 cm) ledge on the sides; the other arm was of similar dimensions but enclosed (length: 50 cm by width: 10 cm by height: 28 cm), and arms were connected by a 10 cm^2 extension of the open arm (Fig. 13). The maze was placed on the floor. Rats were initially placed on the corner of the elevated maze and positioned so as not to be preferentially oriented toward either the enclosed or open arm. Activity of each rat was recorded for 5 minutes by a DVD recorder positioned to view both arms of the elevated maze. The rat was then immediately returned to its home cage and then to its home colony room. The elevated maze was cleaned thoroughly with chlorhexiderm in between rat trials.

The DVD recordings were subsequently given to blind raters to record number of closed and open arm entries (defined as all 4 paws entering the arm), time spent in closed and open arms, frequency of "peeping out" of the closed arm (defined as when the head and/or shoulders emerge from the closed arm without a complete open arm entry), frequency of head dips over the side of the open arm (defined as when the head and shoulders are dipped over the side of the open arm), frequency of rearing (both forepaws raised off the ground), and frequency of grooming.

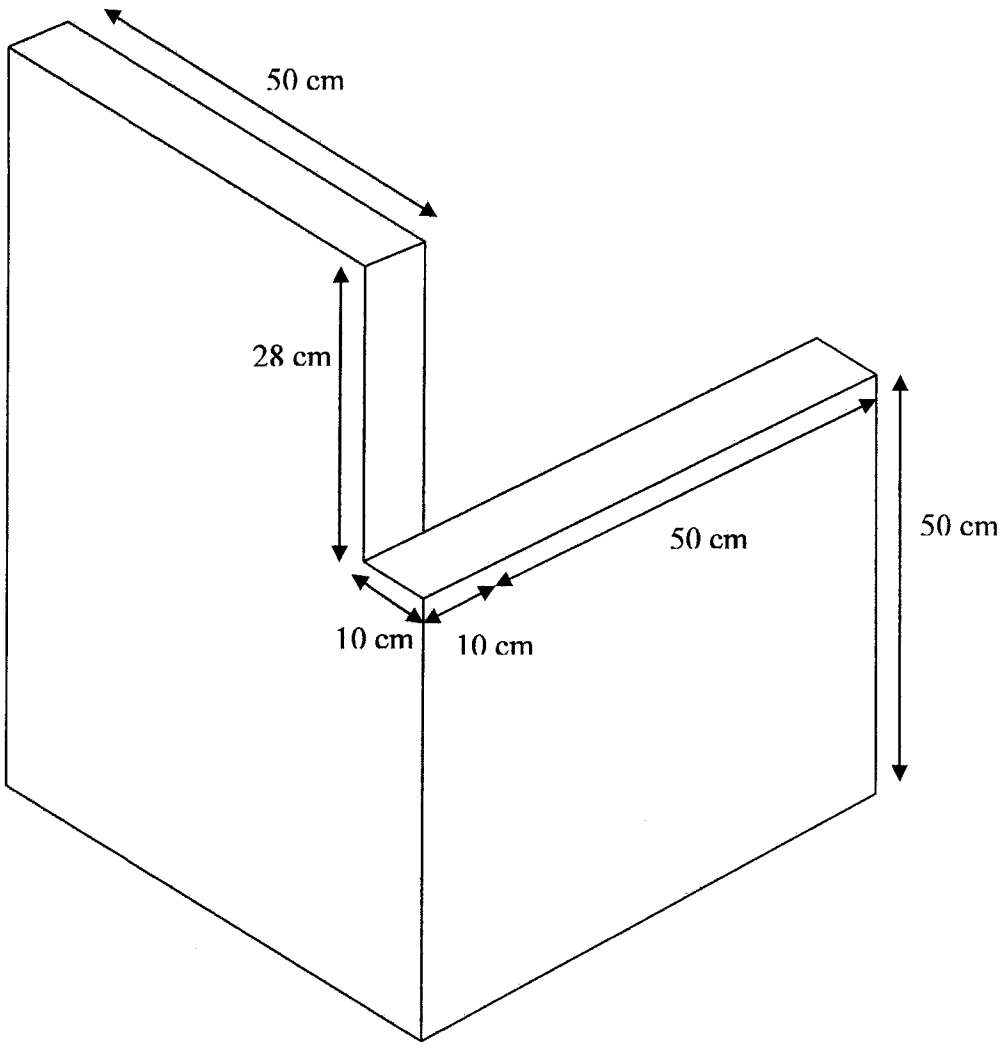

Fig. 13: Experiment 4A: Diagram of the elevated maze.

6.1.2. Experiment 4B: Open field test

The eight rats from each of the no-CRD and CRD groups that were not used for the elevated maze test underwent the open field test. Testing was conducted on the last 2 days of cycle 7, during the 3 hours prior to lights out under non-food deprived conditions. The rats were tested in counterbalanced order, with half from each group tested on the first test day, and half on the second test day. As with the elevated maze test, one rat at a

time was carried in its home cage covered with a sheet from the colony room to an adjacent room (same light and temperature conditions) for testing.

The open field was a 61 cubic cm clear polycarbonate box with no lid, and was placed on the floor on top of white paper. On the underside of the box, black electrical tape delineated a grid with 36 equal-sized squares (i.e. 6 rows of 6 squares each). Each rat was initially placed in the same one corner of the open field, facing out toward the center of the field. Its activity was recorded for 5 minutes using a DVD recorder positioned above the arena. Following the test, the rat was immediately returned to its home cage and colony room. The arena was cleaned thoroughly with chlorhexiderm after each rat was tested.

The DVD recordings were subsequently given to blind raters to record the number of line crosses (defined as all 4 paws crossing a line), number of entries into the center (entry into any square not flanked by a wall), duration spent in the center and in the periphery (any square flanked by a wall), frequency of rearing (2 forepaws off ground), and frequency of grooming.

6.1.3. Experiment 4C: Forced swim test

Eight of the rats from each of the CRD and no-CRD groups were selected for the forced swim test. They were counterbalanced for the type of anxiety test in which they had previously served, and for whether or not they had received sucrose solution (see Experiment 4D) in the past. This test was conducted in daylight, during the 4 hours prior to lights out. Rats were taken one by one in their covered individual home cages from the colony room to an adjacent room for testing (light and temperature conditions were the same as in their home colony room). The forced swim test was conducted in a clear glass

cylinder (height: 43 cm, diameter: 20 cm), filled with water to a depth of 30 cm and at a temperature of 25 ± 1 Celsius. Rats were placed in the water for 15 minutes during a pre-test phase and 24 hours later were again placed in the water for the 5-minute test. This procedure is standard for the forced swim test [98, 99]. After being taken out of the water, rats were towel-dried and then placed in a dry non-bedded cage in another adjacent room, under a heat lamp for 30 minutes until they were completely dry, then returned to their home cage and their colony room. The water in the test cylinder was replaced after each rat's test.

Activity during the pretest and test phases was recorded on a DVD recorder positioned directly above the cylinder for subsequent rating by blind raters. Raters recorded latency to become completely immobile (defined as no body or forepaw movement and no contact with the sides of the cylinder), duration spent completely immobile, duration of active struggling/climbing (defined as vigorous movements of the forepaws, breaking the surface of the water), duration of swimming (defined as diving or vigorous movement through the water to move to another quadrant). A value for time spent inactive was calculated by subtracting duration of active struggling and swimming from 5 minutes.

6.1.4. Experiment 4D: Sucrose solution test

Prior to the beginning of CRD cycling, half of the rats from each of the no-CRD and CRD groups were randomly selected to participate in a 2-bottle sucrose solution versus water choice test periodically during cycling. Two baseline tests were conducted before cycling started, and as the results of these tests were the same, the tests were averaged to give an overall baseline score for sucrose and water intake. These same rats were tested again after the refeeding period during cycles 1, 2, 5, and 10. Experience with this

sucrose test was taken into account when assigning groups to the subsequent elevated maze, open field, and forced swim tests.

Sucrose was diluted in tap water to make a 1% solution and was freshly prepared on each day of testing. On the test days, food and water was removed 60-90 minutes prior to lights out and at lights out, pre-weighed chow, water bottles, and sucrose bottles were given to each rat. The sucrose was given in a bottle identical to their regular water bottles and care was taken to ensure there was no leakage from the bottles. Sucrose was firstly placed in the location usually held by the rats' water bottles, while their more familiar water bottle was moved to the other side of the cage. Intake of sucrose solution, water, and chow was recorded at 1, 4, and 24 hour intervals after presentation of the sucrose. At each intake interval, the water and sucrose bottles were swapped to ensure the rats did not drink more from one bottle due to location familiarity.

6.1.5. Experiment 4E: Novelty interaction test

Rats that did not serve in the sucrose test described above were given a second novel bottle containing only water during the sucrose test of cycle 5. This test was conducted only once on cycle 5 after a history of CRD or no-CRD cycles had been experienced. Procedures for placement of the bottles and swapping them at each intake reading matched those of the sucrose solution tests. Water intake from each bottle (the familiar bottle and the novel bottle) was recorded at 1, 2, 4, and 24 hours.

6.2. Results

6.2.1. Experiment 4A: Elevated maze test

The inter-rater reliability was 0.97 and 0.95 on ratings of number of entries to the open and closed arms, respectively; was 0.92 for time spent in the open and time spent in the closed arms; was 0.87 on ratings of rearing and 0.75 on ratings of peeping out and grooming. There were no significant differences between the Intermittent PF/no-CRD and Intermittent PF/CRD groups in any of these parameters. Rats spent an average of 222 ± 12.20 seconds in the closed arm versus just 77.97 ± 12.20 seconds in the open arm.

6.2.2. Experiment 2B: Open field test

The inter-rater reliability was 0.93 for ratings of number of line crosses; was 0.89 for number of center entries; and was 0.89 for time spent in the center versus periphery. No differences between the CRD groups were found on any of these parameters. Rats spent more time in the periphery versus the center, with an average of 286.37 seconds in the periphery and 13.63 ± 2.02 seconds in the center. The number of line crosses during the first 30 seconds and over the entire 5 minutes was the same across groups (first 30 seconds: no-CRD = 19.13 ± 3.13, CRD = 15.56 ± 1.85; 5 minutes: no-CRD = 146.31 ± 8.46; CRD = 152.75 ± 9.67).

6.2.3. Experiment 4C: Forced swim test

Rats from no-CRD and CRD groups were at the same body weight at the time of testing (non-CRD: 281.25 grams ± 1.86, CRD: 278.88 ± 1.34 grams). Two raters blind to group membership, rated the DVD recordings. The inter-rater reliability for "latency to become immobile" was low at only 0.25 and so this measure was not considered. The

rats spent very little time swimming (e.g. < 5 seconds) and so this was added to the climbing/struggling time to give an overall "active/struggling" score. The inter-rater reliability for active/struggling score was 0.85. The scores from each rater were averaged and showed that the no-CRD rats spent a greater amount of time in active swimming and struggling behaviors than did the CRD rats (no-CRD: 103.75 ± 13.124, CRD: 72.38 ± 9.792 seconds; $t(14) = 1.916$, $p_{(1\text{-tailed})} < 0.05$; Fig. 14). There were no differences in the number of fecal boli excreted between the two groups of rats.

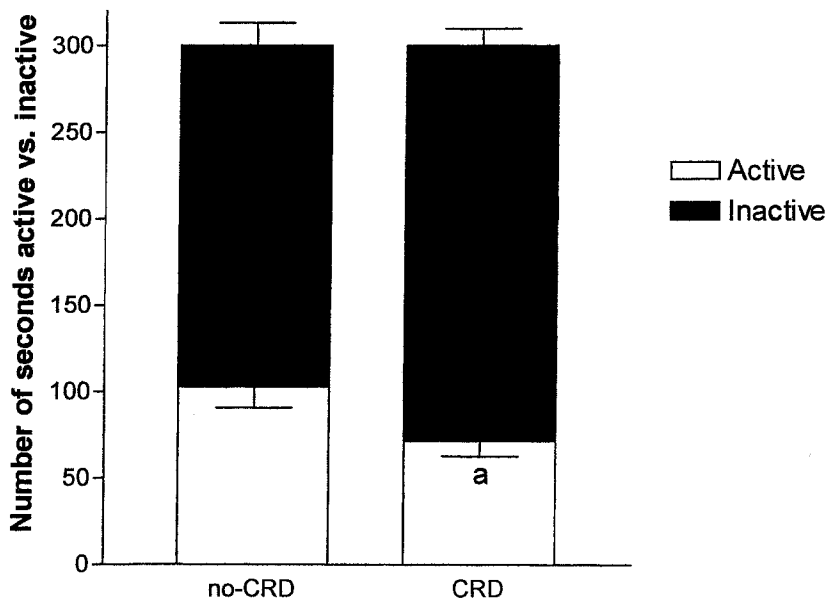

Fig. 14. Experiment 4C. The effect of a history of CRD on active vs. inactive behavior during the forced swim test (a: Intermittent PF/CRD vs. Intermittent PF/no-CRD, $p < 0.05$).

6.2.4. Experiment 4D: Sucrose test

During baseline tests, there was no difference in the amount of sucrose consumed between rats that subsequently were assigned to no-CRD and CRD groups. A clear preference for sucrose was observed, with rats drinking 50.9 ± 4.11 mls of sucrose solution

versus just 3.7 ± 0.23 mls of water in 24 hours. Rats were tested again at the end of cycles 1, 2, 5, and 10. During all cycles except for cycle 10, rats progressively increased the volume of sucrose they drank, while water intake was unaffected. Although there was a persistent trend for rats with a history of CRD to consume less sucrose than rats in the no-CRD group, this difference obtained statistical significance only during cycle 10. At 4 hours during cycle 10, the CRD rats drank significantly less sucrose than the no-CRD rats (no-CRD = 42.24 ± 3.88 mls; CRD = 31.86 ± 3.73 mls; $t(14) = 1.93, p < 0.05$ hours; Fig. 15), but water intake was the same.

6.2.5. Experiment 4E: Novelty interaction test

There was a significant group by bottle interaction at the 1, 2, and 4 hour measurements showing that rats without a CRD history drank more from the novel bottle and less from the familiar water bottle, while rats with a CRD history drank more from the familiar bottle and less from the novel bottle (1H: $F(1,14) = 13.036, p < 0.01$; 2H: $F(1,14) = 11.356, p < 0.01$; 4H: $F(1,14) = 7.514, p < 0.05$; Fig. 16A, B, & C). By 24 hours since presentation of the novel water bottle, there were no differences between the no-CRD and CRD groups in terms of intake from the water bottle or the novel bottle, but both groups were drinking more from the novel bottle than from the familiar water bottle.

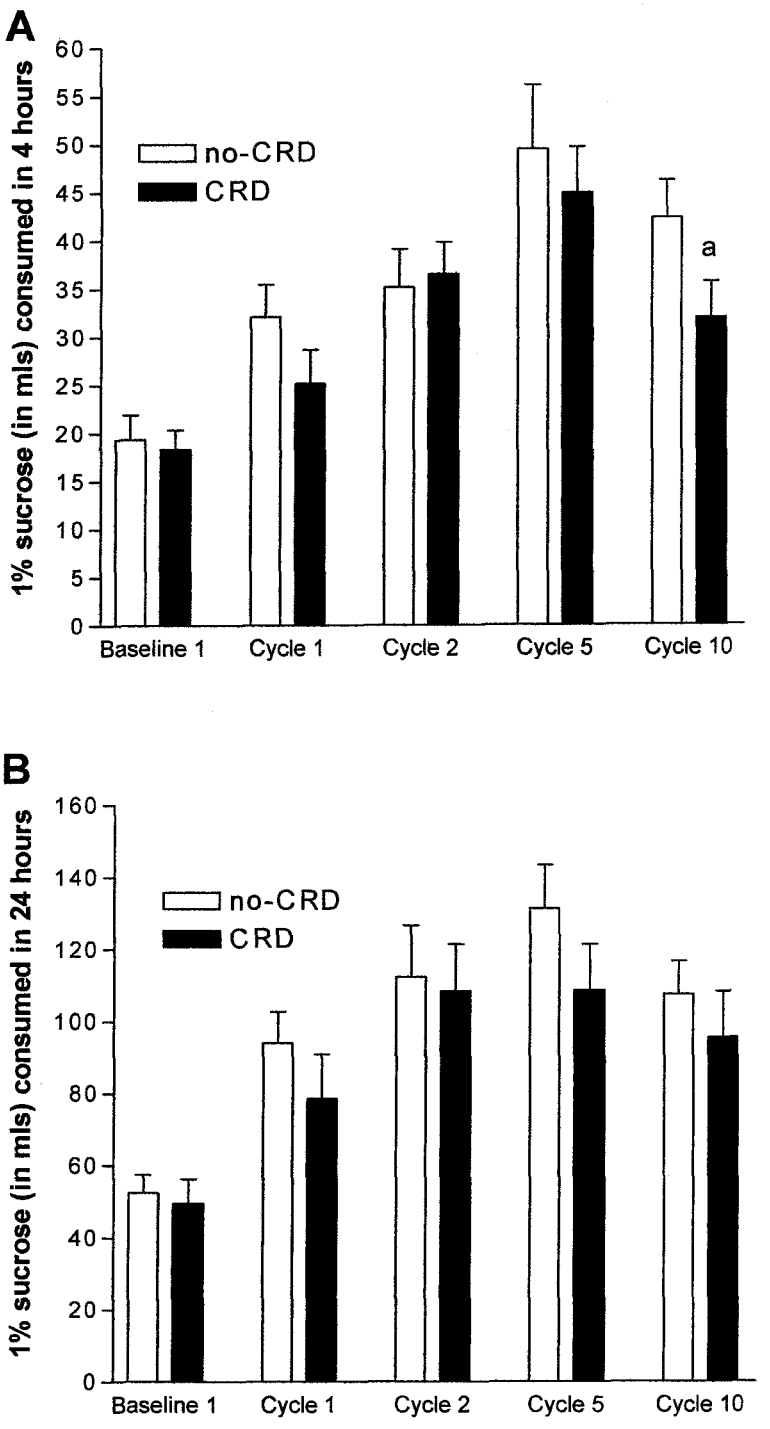

Fig. 15. Experiment 4D. The effect of a history of CRD on water vs. sucrose intake over 4 hours (A) and 24 hours (B) during a 2-bottle choice test (a: Intermittent PF/CRD vs. Intermittent PF/no-CRD, $p < 0.05$).

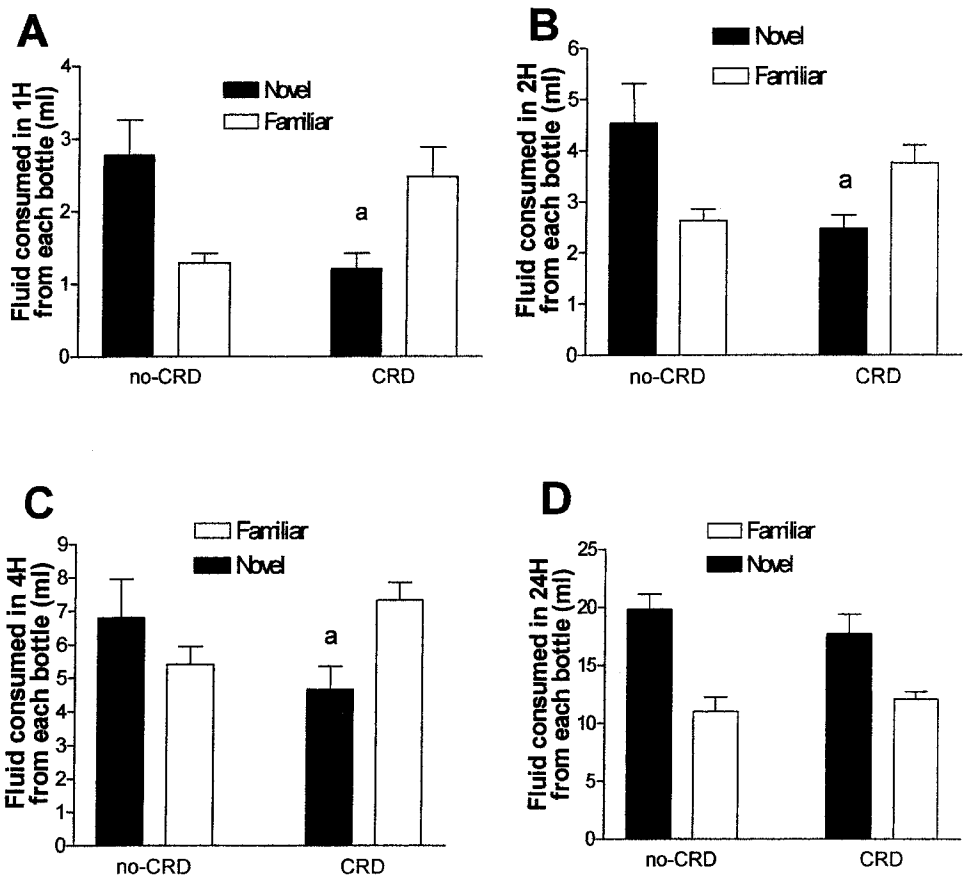

Fig. 16. Experiment 4E. The effect of a history of CRD on water intake from a familiar versus a novel bottle, at 1 hour (A), 2 hours (B), 4 hours (C), and 24 hours (D). An interaction between bottle and CRD history occurred at 1, 2, and 4 hours (a: $p < 0.05$) such that those with a history of CRD drank less from the novel bottle and more from the familiar bottle, but controls drank more from the novel bottle and less from the familiar bottle.

7. Discussion

The goal of this study was to determine whether a history of human-like CRD with refeeding (i.e. "yo-yo" dieting) increased susceptibility to abnormal eating patterns, weight gain, mood disturbances, and induced neurochemical changes contributing to feeding and mood changes. We investigated these possibilities using an animal model of CRD that has previously rendered rats susceptible to binge eating following an acute stressor [4-7]. The experiments provided a number of interesting results. Firstly, rats with a history of CRD were no more susceptible to overeating or weight gain than those without a history of CRD, but if the daily diet they consumed included PF, rats overate and gained weight regardless of their CRD history. Secondly, we found evidence of neurochemical changes in the hypothalamus, nucleus accumbens, and medial PFC produced by intake of PF, a history of CRD, and the combination of CRD with intermittent PF, respectively. Finally, while we did not find any evidence of behaviors consistent with animal models of anxiety due to a CRD history, these rats did exhibit less activity in a swim test challenge, and less intake of a palatable sucrose solution, which are consistent with animal models of depression. These rats also consumed less water from a novel bottle compared to the amount consumed by rats without a CRD history, and reduced novelty interaction is also indicative of depression-like symptoms. An overall and significant aspect of these findings is that they were observed in sated animals (i.e. not in energy deficit) that had experienced a history of caloric restriction and refeeding. While the body of knowledge on feeding behavior and brain control of feeding behavior is rife with studies

that involve under-feeding and over-feeding or anorexia and obesity, subjecting animals to a history of CRD that includes PF and recovery of normal body weight is unique to this study. This is surprising given the common practice among humans to control weight by food restriction, and their subsequent tendency to indulge in non-nutritious PF along with more nutritious foods during refeeding and recovery of lost weight.

The finding that a history of CRD was not associated with subsequent overeating and weight gain supports earlier research using animal models [24-27]. However, as mentioned previously, these earlier studies did not provide intermittent access to PF, which may contribute to subsequent overeating [40-43], and so we added to a group to investigate the possibility that intermittent PF access in combination with a CRD history would induce overeating. Occasional eating of non-nutritive PF is veridical of human dieting patterns because humans typically limit their intake of very palatable, high fat food while dieting and then consume these foods once the diet is terminated or "broken" [8, 112]. When PF was presented to the Intermittent PF group after those in the CRD subgroup had been refed to normal body weight and food intake, intake of PF and chow in the CRD subgroup equaled that of the no-CRD subgroup. Interestingly, after multiple cycles, intermittent access to PF resulted in greater caloric consumption of the PF by both CRD and no-CRD subgroups, than it did earlier in cycling (i.e. test day consumption of cycle 1 versus cycle 11). This supports findings of Corwin et al. [41] that over time, intermittency of access to PF increases intake. We did not, however, find support for our hypothesis that the history of CRD in combination with intermittent access to PF would render rats more susceptible to overeating PF than those without a history of CRD.

The groups that gained the most weight throughout the duration of the study were those fed PF on a daily basis. By the end of the experiment, this group was significantly

heavier and had greater body fat, but the same lean mass as rats in the other diet conditions (unpublished findings). Interestingly, after 12 CRD cycles, the end-point body weight of those in the Daily PF/CRD group was the same as the Daily PF/no-CRD group. During every refeeding period, those with a history of CRD recovered the body weight lost during CRD to match that obtained by the no-CRD group. This is interesting because it suggests that palatability or macronutrient content of the food available (i.e. increased fat per kcal) may alter an intrinsic settling point for body weight such that, if PF is consistently available, animals are driven to obtain and maintain a higher body weight than they would if maintained on chow only, or if provided with intermittent access to PF. Others have shown that rats fed a very palatable liquid diet will gain weight but will not defend that weight if they are then returned to a less palatable chow diet, but upon return of the more palatable diet, they will recover weight to previous levels maintained on that diet [113]. Similarly, rats in the Intermittent PF group did not obtain a greater body weight than those on the chow diet because once PF was removed and they were left with only chow, they reduced their intake. It was surprising that only a small amount of PF provided to the Daily PF/CRD group during food restriction days was enough to shift their settling point for body weight upwards. This is an important point to consider for human dieting conditions because it suggests that even if quantity of PF is restricted, consistent or daily access to PF may inhibit normal homeostatic control of body weight. The increased weight gain during non-restricted access to PF may be due to hedonic drive to overeat the PF, which superseded normal metabolic need. On the basis of these data then, the background diet, rather than any history of CRD, was the important factor influencing overeating and weight gain in the rats. A history of CRD either alone or in com-

bination with intermittent access to PF, did not appear to contribute to susceptibility towards obesity.

Serotonin is a key regulator of both feeding and mood (for review see [45-51]) and so was worthy of investigation using this animal model of CRD. Given that 5-HT function can be perturbed by acute periods of food restriction in humans [52-55], and 5-HT responses to drug challenges are abnormal even in recovered bulimics who have a history of CRD [59, 60, 114], we wanted to investigate whether rats with a history of CRD displayed a differential response to treatment with fluoxetine, as an indirect indicator that experience with CRD can change 5-HT function. In rats maintained on an intermittent PF regimen with and without exposure to stress (which induced binge eating in those with a history of CRD [6]), fluoxetine was administered after 2 hours of *ad libitum* feeding on chow and PF. Fluoxetine suppressed intake of groups with a CRD history (i.e. both stressed and non-stressed groups), thereby effectively eliminating the binge eating of the CRD rats that received the stressor, but was without effect in the no-CRD rats. Due to the fact that fluoxetine suppressed intake not only of the CRD with stress group that normally binge eats following the stressor, but also suppressed intake of the CRD only group, the history of CRD alone may be sufficient to alter serotonergic regulation of feeding. Peripheral administration of fluoxetine induces c-fos in multiple brain regions including the PVN, the ventromedial hypothalamus, and the posterior hypothalamic nucleus [108], and increases extracellular levels of 5-HT in the PVN [104]. Fluoxetine administered directly into medial hypothalamic nuclei such as the paraventricular, ventromedial, dorsomedial and suprachiasmatic nuclei, suppresses food intake [115]. Thus, hypothalamic nuclei, and in particular the PVN, are likely targets for the anorectic effect

of peripherally administered fluoxetine. Hypothalamic 5-HT and 5HIAA levels were subsequently measured in this group (discussed below).

The above finding of an exaggerated anorectic effect of fluoxetine in rats with a history of CRD suggests that CRD may reduce synaptic levels of 5-HT, resulting in post-synaptic receptor upregulation. Thus, when these rats are treated with fluoxetine, which, as a reuptake inhibitor, increases synaptic levels of 5-HT, they may be more sensitive to its anorectic effect. In support of this possibility, food deprivation reduces 5-HT metabolism [66] and we found reduced hypothalamic 5-HT levels in CRD rats (Figure 8). This explanation will need to be investigated in future studies to identify extracellular levels of 5-HT before and after feeding, and density of 5-HT receptors following a history of CRD.

To follow up this finding in Intermittent PF rats with a history of CRD and expand it to determine the extent to which diet affects the increased anorectic response to fluoxetine in CRD rats, we tested the same dose of fluoxetine in groups of rats maintained on chow only, on chow with intermittent access to PF, and on chow with daily access to PF, all with and without CRD history. This time, fluoxetine was given immediately prior to food presentation to rule out the possibility that previous food intake affected the anorectic effect of fluoxetine. Without previous feeding, fluoxetine reduced only PF intake, and only in the rats maintained on intermittent PF (regardless of whether they had a history of CRD or not). This group had received PF for the first time in four days immediately after fluoxetine treatment and subsequently ate more than the Chow Only or Daily PF groups. Given that both the no-CRD and CRD groups overate on PF, we can be sure that the overeating was not driven by hunger, and so it may be that fluoxetine suppressed hedonic drive for PF rather than metabolic drive for food. Also, as there was no other diet condition besides Intermittent PF in the previous experiment, we

cannot rule out the possibility that even for rats without CRD, intermittency of access to PF may augment the anorectic effect of fluoxetine.

Nevertheless, this result contradicted the effect shown in the earlier experiment because we did expect a stronger anorectic response in rats with a history of CRD compared to those without a history of CRD. There was an important difference in the timing of the injections given in each of the two experiments. In the first experiment, fluoxetine was administered after two hours of feeding, while in the second fluoxetine was administered immediately prior to food presentation. Given that food intake increases 5-HT release and metabolism in the hypothalamus [45, 66], it may be that the discrepant results between the two experiments resulted from the increased synaptic availability of 5-HT produced by two hours of feeding immediately prior to treatment in the first experiment. In an attempt to reconcile this difference, the Chow Only, Intermittent PF, and Daily PF groups were tested again, but this time they were given a small morsel, or trigger of food, one hour prior to the injection and *ad libitum* food. Since we were now comparing rats fed only chow to those fed chow and PF, we chose a small portion of food to ensure rats in all diet conditions would consume all of it prior to fluoxetine administration. This morsel of food was less than the amount of chow and PF consumed during the 2 hours of *ad libitum* feeding before fluoxetine was given in the first fluoxetine experiment. Under control conditions (saline treatment), the rats maintained on intermittent PF with a history of CRD overate in the first hour of *ad libitum* feeding following this trigger compared to those without a history of CRD. When fluoxetine was given, it suppressed this trigger-induced overeating, thus providing a greater degree of food suppression in this group compared to any other. This result is interesting for a number of reasons. Firstly, just a morsel of food was sufficient to trigger overeating, but only for the Intermittent PF/CRD

group. This is significant because this group is the most veridical of bulimics, who typically overeat following a preload or trigger [8, 116], and of non-clinical restrictive dieters, who eat more PF than non-dieters after breaking their diet or even after just smelling PF [117, 118]. The terms "counter-regulation" and "disinhibition" have often been used to describe the trigger-induced overeating that dieters engage in, meaning that they think they've broken their diet by eating a "forbidden" or fattening food and so go on to consume more food [116]. It is highly unlikely that animals would engage in this type of higher cognitive processing, so there may be a more basic drive or mechanism contributing to overeating triggered by PF. This mechanism may reflect changes in neurochemical signaling that are discussed below. The fact that the rats in the current model of CRD overate following a food trigger despite their chow intake and body weight being the same as that of controls, suggests that the history of CRD has induced a chronic change in the animals' hedonic response to PF that may increase intake after intermittent deprivation from that PF. The second result of interest in this experiment was that while fluoxetine did not differentially affect non-triggered food intake in the intermittent PF with CRD group, it did reduce their trigger-induced overeating, suggesting that such overeating is mediated by an altered 5-HT mechanism. Thirdly, dysregulation of 5-HT activity may only exist in rats with a combined history of CRD and intermittent access to PF, but this dysregulation is not evident under normal feeding conditions, only in trigger-induced or stress-induced feeding conditions.

The combination of CRD with intermittent access to PF may increase susceptibility to PF craving, such that, if the animals are not able to become sated on this food because only a small amount is provided as with a trigger, this increases their drive to consume this food later. Some studies have shown that just the smell and/or sight of PF was

sufficient to cause dieters to increase or lose control over their subsequent intake, but if no food smell was given prior to the meal or if the food was not a preferred food, dieters maintained control over their intake [117, 119]. Even though the rats in the current study were no longer dieting and were back to normal energy balance, the history of CRD may have induced a chronic susceptibility to overeat following a trigger. The craving for PF and loss of control over subsequent intake may be mediated by negative affect. Withdrawal from addictive drugs induces negative affect [120], and negative affect is associated with craving for addictive drugs or PF [121-123] and is also commonly cited as a trigger for drug and food binges in humans [18, 124, 125]. Furthermore, a history of intermittent access to PF has previously been found to induce opiate withdrawal-like symptoms in animals deprived of PF [126]. There is some evidence that reduced 5-HT availability and/or release may be at least partially responsible for food and drug craving [50], possibly via its effect on mood regulation. Together, this information suggests that the trigger of PF induced a drug withdrawal-like state in rats accustomed to intermittent PF with a history of CRD. This withdrawal state may be characterized by negative affect, which may also be mediated by a suppression of 5-HT function, thus allowing overconsumption to occur once the PF is freely available. It would be interesting in the future to conduct the forced swim test or other similar tests of depression-validated symptoms following a PF trigger in these rats. If our conclusions drawn from these results and the existing knowledge base are correct, we would expect rats with a history of CRD, if tested following a trigger, to spend more time inactive in the forced swim test, compared to those in the no-CRD group and compared to non-triggered CRD history rats.

The results of this drug experiment, then, implicate a change in 5-HT function. An important site of serotonergic feeding control in the brain is believed to be in the hy-

pothalamic nuclei, particularly the PVN which has been studied extensively with respect to meal patterns and inhibition of food intake [64-66]. Therefore, PVN 5-HT function may be highly influenced by not only acute food restriction but by a history of food restriction despite recovery of normal body weight. As previously mentioned, anorexic and bulimic patients persist in exhibiting abnormal responses to 5-HT challenges even years after recovery from their symptoms. Future tests with the CRD protocol should test to see how long after CRD rats continue to show abnormal responses to 5-HT challenges. This would aid investigations as to whether 5-HT abnormalities are truly trait or state mediated in eating disordered persons.

It is well established that acute total food deprivation and/or acute caloric restriction reduces hypothalamic serotonergic signaling in various nuclei [66, 127]. However, given that our CRD rats had been allowed to free-feed and return to normal body weight and food intake, we were not certain if a difference in hypothalamic 5-HT levels or turnover would be found. When rats fed intermittent PF were allowed to feed for 2 hours prior to euthanasia, we found reduced 5-HT and 5HIAA levels in the whole hypothalamus of those with a history of CRD. Given that 5HIAA levels in the PVN are reduced in animals deprived of food [66], we hypothesized that the specific location of this reduction in 5-HT and 5HIAA was likely to be the PVN.

Thus, for the subsequent experiment using rats from three different diet conditions: Chow Only, Intermittent PF, and Daily PF, just the anterior portion of the hypothalamus was dissected; a portion that included the PVN, the anterior hypothalamus, the anterior portion of the lateral hypothalamus, and the medial preoptic nucleus. This time however, no differences in 5-HT and 5HIAA levels were found between those with and without CRD history. It is possible that the decrease in hypothalamic levels of 5-HT oc-

curred in one of the nuclei excluded by this particular dissection. The more posterior nuclei not included in this second dissection included the ventromedial hypothalamus (VMH), dorsomedial hypothalamus, arcuate nucleus, median eminence, and much of the posterior portion of the lateral hypothalamus. Infusions of 5-HT into both the PVN and VMH reduce intake [128], and fluoxetine directly in the PVN, VMH, dorsomedial and suprachiasmatic nuclei also reduces food intake [115]. In the arcuate nucleus, 5-HT activates POMC neurons, a melanocortin mechanism for reducing food intake [129]. In the median eminence, if access to food is limited to just 2 hours per day, 5-HT is reduced [127]. Future studies will need to dissect more discrete regions, particularly the VMH, arcuate nucleus, and median eminence, to determine whether 5-HT is chronically decreased in any of these regions by a history of CRD. Another possibility for the discrepancy between the first and second dissections is that, immediately prior to the first dissection, rats were allowed to eat both chow and PF for at least 2 hours, whereas at the second dissection, rats were not food deprived although food was removed for 1 hour during the light prior to euthanasia, and rats in the intermittent PF group had not received PF for the previous 4 days. As with the results of the fluoxetine experiment, any chronic changes in levels of 5-HT and 5HIAA in rats with a history of CRD that are no longer food deprived may become evident only after stimulation by recent food intake. Given that feeding increases 5-HT release [45, 130, 131] and breakdown [66], we might expect greater 5HIAA levels in the hypothalamus of rats euthanized in a "fed" state, compared to those euthanized before a meal was taken. Unfortunately, a direct comparison of these 2 different groups cannot be made because of the different regions dissected. We would also expect that stores of 5-HT at the terminal would need to be replenished. It may be that the synthesis and transport of 5-HT is altered by a history of CRD leading to the reduced hypo-

thalamic levels of 5-HT observed in CRD rats after feeding. These multiple hypotheses to explain the discrepant 5-HT findings will need to be investigated further. It will be important to consider multiple time points and to examine both extracellular and pre-synaptic intracellular levels of 5-HT to determine the exact nature of any feeding-related perturbations in 5-HT activity.

Despite finding no difference in levels of 5-HT and 5HIAA in this dissection of the anterior portion of the hypothalamus, a difference was found in dopamine and DOPAC levels as a function of baseline diet condition. Specifically, rats with access to PF had reduced levels of dopamine and DOPAC compared to the rats fed chow only. Relatively little is known about dopamine in this region, which included the PVN, anterior hypothalamus, medial preoptic nucleus, and the anterior portion of the lateral hypothalamus, and so this finding is difficult to interpret. However, the lateral hypothalamus may be the most likely location for the observed decrease in dopamine and DOPAC levels to occur. The lateral hypothalamus has been implicated in body weight set point [132], meal size regulation [133], and palatable food-induced reward [134]. Lesions of the lateral hypothalamus result in a reduction of body weight that rats will defend even if a more PF is provided, suggesting a permanent change to homeostatic regulation of body weight [132]. This explanation may apply to those fed daily PF because they gained more weight than those in other groups, and the CRD group defended this higher body weight as shown by their rapid recovery of it following each restriction period. However, given that intermittent access to PF did not alter body weight, it seems unlikely that the reduction of dopamine in all rats with any access to PF reflects a permanent change in body weight set point.

It may be that the reduction of dopamine reflects a difference in meal size regulation. If dopamine is directly infused into the lateral hypothalamus, meal size is reduced [133] and endogenous dopamine release in the lateral hypothalamus increases in proportion to food consumed during a meal [135]. Given that animals provided with PF will eat larger meals than those without PF [136], it is possible that in the current experiment, those given any access to PF became accustomed to consuming larger meals and having less available dopamine in the lateral hypothalamus may be a mechanism by which animals are capable of eating larger portions. Unfortunately, we do not have any meal size data from the current experiment, but this is something that could be investigated in the future.

The nucleus accumbens was dissected from the same group of rats with intermittent access to PF, with and without a history of CRD, which was used to dissect the whole hypothalamus. These rats had been allowed to feed on chow and PF for at least 2 hours prior to euthanasia. No differences were found in 5-HT, dopamine, 5HIAA, DOPAC levels, or in turnover ratios. We did learn, however, that in those rats without a history of CRD, 5-HT turnover and dopamine turnover were strongly and positively correlated. Conversely, in rats with a history of CRD, this association was completely absent.

In order to determine whether this effect was due to a history of CRD alone, or due to CRD in combination with intermittent access to PF, this dissection procedure was repeated in rats maintained on different diets: Chow Only, Intermittent PF, and Daily PF. Again, no differences were found in absolute levels of accumbens 5-HT, dopamine, 5HIAA, DOPAC, or in turnover ratios for each monoamine as a function of CRD history or as a function of baseline diet. However, once again, the strong positive correlation be-

tween 5-HT turnover and dopamine turnover was present in the nucleus accumbens of rats without a history of CRD, but this relationship was entirely absent in rats with a history of CRD, irrespective of their baseline diet. Therefore, the lack of association between 5-HT turnover and dopamine turnover occurs as a result of a history of CRD and is not dependent on the availability of PF, or on the schedule of access to PF.

Nucleus accumbens dopamine is associated with reward and motivation, and thus we might expect this to be altered in our animals with a history of CRD, which reduced intake of palatable sucrose and consumed more food than controls did following a PF trigger. It is perhaps surprising that accumbens dopamine levels were not affected by the history of CRD but it may be that while overall tissue levels of dopamine were unchanged by the CRD history, extracellular release of dopamine may well have been reduced in these rats. This possibility will need to be investigated further in the future.

It is also possible that the lack of association between dopamine and 5-HT turnover reflects a perturbation in the interaction between 5-HT and dopamine to control affect. There is an accruing body of evidence to suggest a functional interaction between 5-HT and dopamine in the nucleus accumbens. A number of studies have shown that local or systemic application of a non-specific 5-HT-2 receptor agonist increases nucleus accumbens dopamine release [137-139] and blockade of 5-HT-2A receptors diminishes accumbens dopamine release [139, 140]. Blockade of 5-HT-2C receptors however, enhances accumbens dopamine release [141]. Infusion of a 5-HT-1B agonist into either the nucleus accumbens or the ventral tegmental area will also increase accumbens dopamine release [142]. In short, there appears to be a complex interaction between the 5-HT and dopamine pathways that is mediated via multiple receptors. Both 5-HT-2A and 5-HT-2C

receptors are found in the mesolimbic dopamine pathway [143-146], where both can influence dopamine function [147].

The interaction between dopamine and 5-HT in the accumbens is functionally important. The Flinders Sensitive Line (FSL) is a genetically bred animal model of depression, wherein rats exhibit behavioral symptoms such as reduced activity in the forced swim test. When 5-HT is infused into the nucleus accumbens of wild-type rats, extracellular accumbens levels of dopamine increase, but infusion into the nucleus accumbens of FSL rats has no effect on dopamine levels [148]. Interestingly, after treatment with antidepressants, FSL rats increase their activity in the forced swim test, and intra-accumbens 5-HT treatment induces an increase in extracellular dopamine release [148]. As mentioned earlier, the 5-HT-2C receptors have an inhibitory effect on dopamine release [141], and this effect is greater in the FSL compared to wild-type rats [149]. Given that increases in dopamine release in the accumbens are associated with reward value of a stimulus (for review, see [69]), this enhanced inhibition of dopamine release may explain the anhedonia evident in depressed patients. Indeed, treatment of FSL rats with antidepressants improves behavioral symptoms of depression in the FSL rats, and reduces dopaminergic inhibition mediated via the 5-HT-2C receptor [149, 150].

Both a history of CRD and depression are factors commonly associated with eating disorders such as bulimia [80-84]. For the first time, we have uncovered behavioral evidence in an animal model of CRD that is consistent with animal models of depression. It will be interesting to investigate in the future whether antidepressants are able to reduce these behavioral disturbances in rats with a history of CRD, and whether this effect is indeed mediated via a perturbation in 5-HT receptor activity, in particular, that of the 5-HT-2C receptor, which has an inhibitory effect on dopaminergic signaling in the mesolimbic

reward pathway. Although we found no decrease in accumbens dopamine as a function of CRD, we found that turnover rate of 5-HT in controls correlated with turnover rate of dopamine in this region, but not for rats with a history of CRD. It is possible that 5-HT turnover rates affect dopamine turnover rates, given the known interactions between these monoamines at the amine release and receptor level. However, the mechanism for the turnover interactions is unknown but certainly worth investigating further.

The mPFC was dissected from groups of no-CRD and CRD rats that had been maintained on chow only, intermittent PF, and daily PF. This region was included because of its well-known role in mood regulation (for review, see [70-72, 151]). In addition, the mPFC controls activity of midbrain 5-HT neurons [152-154] and, thus, any disruption to the mPFC may alter serotonergic signaling throughout the brain. Furthermore, dopaminergic activity in the mPFC is believed to have an inhibitory influence on nucleus accumbens dopaminergic transmission [155]. Disturbances in the PFC have been found in people suffering from depression [156, 157], schizophrenia [158, 159], anxiety disorders such as obsessive-compulsive disorder [160], and attention deficit disorder [161, 162]. Furthermore, both dopamine and 5-HT in the PFC are believed to be involved in cognitive performance [163] and impulsive choice [164, 165].

When this region was assayed in the current study, those with a history of CRD and intermittent access to PF had reduced 5-HT and dopamine levels compared to those on the same diet but without CRD history. This pattern was evident only for those maintained on intermittent PF, but not for groups in the other diet conditions. There are no studies investigating the effects of the combination of reduced 5-HT and dopamine in the mPFC and so the significance of finding is difficult to interpret. Separately, depletion or reduction of dopamine in the mPFC impairs cognitive performance of rats [166], and 5-

HT depletion in the PFC results in perseverative responding to a stimulus that has previously been rewarded and extinguished, suggesting cognitive inflexibility similar to behavior seen in humans with obsessive-compulsive disorder [167]. People suffering from eating disorders have been found to be more likely than the general population to suffer from obsessive-compulsive disorder and a rigid style of thinking [79, 168] and mPFC activity is associated with increases in these symptoms in people with eating disorders [169]. Given that a history of CRD and intermittent access to PF is similar to eating patterns of humans suffering from bulimia [4, 5, 170, 171], and that depletion of 5-HT in the medial PFC was found in only this group of rats, we might expect to find behavioral evidence of obsession such as perseverative responding in these rats, if tested.

Dopamine depletion in the PFC induces hyperactivity [172], and increases swimming and active behaviors in the forced swim test [173], indicating less depression-like behavior. Conversely, PFC 5-HT depletion reduces exploratory behavior [174], and lowers consumption of sucrose-ethanol solutions [175], both of which are symptoms usually found in models of depression. In the FSL rats that serve as an animal model of depressive symptoms, PFC dopamine levels are the same as in wild-type rats [176], while 5-HT levels are much higher than in wild-type rats [177], which is opposite of what would be expected given their behavioral depression-like symptoms. However, chronic antidepressant treatment is successful at normalizing both 5-HT and dopamine levels. Given that neurochemical disturbances of FSL rats are not limited to the PFC or to the nucleus accumbens, and that treatment with antidepressants seems to correct multiple neurochemical problems, it is difficult to determine which of these directly contributes to the depression-like symptoms. Equally, each of the neurochemical disturbances may be consequent to a common underlying dysfunction that is corrected by antidepressant treatment. With

respect to the current study and knowledge available, we have no conclusive evidence that the decrease in 5-HT and dopamine in the mPFC contributes to the behaviors uncovered in rats with a history of CRD that are consistent with depression models.

There is evidence to suggest that reductions in monoamines in the mPFC observed in CRD rats may change the reward value they attribute to a stimulus. For example, rats with mPFC dopamine depletions self-administer lower doses of cocaine, suggesting enhanced sensitivity to the drug [178]. However, despite the availability of rewarding PF, rats in the current study with a history of CRD consumed the same amount of PF as rats without CRD history. If the reduction in mPFC dopamine induced by a history of CRD with intermittent access to PF played a role in sensitizing the reward system, we would have expected to see a decrease in PF consumption in this group, but that was not the case. There is some evidence that despite little or no change to food consumption, underlying differences in neurochemical transmission may still exist. Di Chiari and Tanda [179] found that even though intake of PF was unaffected, rats with a history of chronic stress had altered dopamine response to PF consumption in both the nucleus accumbens and mPFC. Granted, it will be important in the future to establish whether the specific neurochemical differences shown in this study have any functional significance, and it may be that any differences are only important in the event of unusual feeding challenges.

While very little may be known about the importance of reduced 5-HT and dopamine levels in the mPFC, it is important to acknowledge that any perturbation in transmission here could have far-reaching consequences throughout the brain due to the extensive connections between the mPFC and other areas. Excitatory efferents from the mPFC synapse onto dopamine neurons in the VTA that project back to the PFC, but not those

that project to the nucleus accumbens [180], although other neurons in the VTA do project to the nucleus accumbens [181], possibly providing an indirect route for mPFC regulation of accumbens dopamine transmission. As mentioned previously, dopamine transmission in the PFC may have an inhibitory effect on dopamine transmission in the nucleus accumbens [155], and the mPFC controls activity of midbrain 5-HT neurons [152-154]. Thus, via these connections, activity within the mPFC may help to regulate activity in areas throughout the brain, but this function of the mPFC may not be realized unless certain environmental conditions or challenges exist to engage it. For this reason, rats with a history of CRD and intermittent access to PF may appear to behave normally unless challenged by a stressor or other variation in their normal environment.

Mood regulation is controlled by activity in the PFC and limbic areas (for review see [70-72]). Therefore, we investigated the possibility that a history of CRD induces behaviors consistent with animal models of anxiety and depression. Rats maintained on intermittent PF with and without a history of CRD were subjected to validated animal models of anxiety and depression. The elevated maze provides rats with a novel environment in which they exhibit phobic tendencies such as hypervigilance, a trait common to anxiety in humans [182]. In the elevated maze test, rats tend to prefer the enclosed arms to the open arms and being confined to the open arms reduces locomotion, increases freezing behavior, increases fecal boli excretion, and increases plasma corticosterone, compared to being in the closed arm [93]. In addition, if rats are treated with anxiolytics, they enter the open arms more frequently and spend more time there compared to untreated rats [183-187]. Rats that are fearful of the open and elevated space therefore are characterized as being more anxious, and would be expected to stay within the enclosed arm and have fewer entries into and spend less time in the open arm. Rats with a history

of CRD entered the open arm and spent the same amount of time there as rats without CRD history, suggesting that a history of CRD does not necessarily increase fearfulness or anxiety during an environmental challenge.

The open field test is another measure of fearfulness of open spaces and typically, rats prefer to stay close to the walls of the open arena, rather than venture into the center of the arena [95, 100, 187, 188]. As with the elevated plus maze, treatment with anxiolytics prior to the open field test increases activity in the open area [189, 190]. We found no differences in overall locomotion, or time spent in the center versus periphery of the open field between rats with and without a history of CRD. This confirms the finding of the elevated maze test in that the history of CRD does not suppress exploration of novel, open areas, and thus does not appear to increase fearfulness or anxiety.

The forced swim test requires placing the rats in a cylinder of water for a 15-minute pretest and then 24 hours later, replacing them in the water under the same conditions for a 5-minute test. As rats cannot touch the bottom of the cylinder, they are required to struggle, swim, or otherwise float for the duration of their time in the cylinder. Latency to become inactive or immobile, and duration of time spent immobile during the 5-minute test have been used to imply a state of helplessness in the rats, which is used as a model of depression [98-100, 191]. However, not all researchers agree on the interpretation that reduced activity implies helplessness or depression. West [192] argues that reduced activity in the forced swim test is indicative of better coping and energy conservation during this physical challenge. Given that CRD rats have a history of cyclic energy deprivation, conservation of energy may be something they have adopted and now are able to apply to this situation.

In the current experiment, rats with a history of CRD spent more time floating and inactive in the water than rats without a history of CRD. Unfortunately, because of low inter-rater reliability for the "latency to become inactive" score, we cannot definitively say that rats with a CRD history became inactive in a shorter period of time, but both raters did have a trend in their data to suggest this. On the basis of this result alone, we cannot exclude the possibility that CRD rats spent more time inactive because they were better able to conserve energy as purported by West [192]. But, immobility, or total time immobile remains the most commonly used method to assess depression in animal models [98, 193, 194] and so the fact that rats with a history of CRD show behaviors consistent with this supports the hypothesis that a history of CRD may contribute to depression.

In addition, another response consistent with depression in humans and measured in animal models of depression is anhedonia, or the loss of pleasure and liking for something otherwise regarded as rewarding [28, 195, 196]. This has previously been measured in rodents by giving them access to a bottle of a mild sucrose solution in addition to their usual water to determine what proportion of sucrose they drink [99, 101-103]. In models of chronic stress, animals drink less sucrose over time compared to those not stressed, and this is interpreted as anhedonia [99, 102, 197]. However, this finding has not been consistent across all models of chronic stress [198-200]. We tested the CRD and no-CRD groups at baseline and then after refeeding during multiple CRD cycles. There was considerable variability in the amount and proportion of sucrose solution to water consumed by individual rats, but the overall trend was for the CRD group to consume less sucrose than the no-CRD group, and during the 10^{th} cycle this difference was statistically significant. We might have expected this result to be much stronger than it was, particularly given that these rats exhibited less activity in the forced swim test in an

earlier cycle. However, this is the first time the 2-bottle sucrose versus water test has been used in rats with a chronic history of CRD, or in rats with a history of intermittent PF access. This may not be the most sensitive test with which to measure changes in hedonic responses in rats for which feeding has been manipulated. However, decreased sucrose drinking in CRD rats is consistent with reduced activity in the forced swim test, and together, support the assertion that a history of CRD increases behaviors consistent with animal models of depression. Our CRD protocol is not unlike the chronic stress models cited above in which rats are exposed daily to differing mild stressors. In addition to reduced sucrose consumption, rats undergoing chronic stress also exhibit more inactivity in the forced swim test [99, 201]. It is possible therefore that the CRD regimen is stressful and this stress induces behaviors consistent with depression models. Stress may be produced by the fact that the rats have no control over the amount of food available to them during food restriction periods, creating a type of learned helplessness that generalizes to affect their behavior during other challenges including the forced swim test.

A final behavioral finding was that rats with a history of CRD were less likely to drink from a novel water bottle than rats without a history of CRD. Rats often interact more with novel objects if presented in a familiar environment [195, 202]. On the other hand, novel food items may be avoided [203]. Some researchers have speculated that interaction with novel objects is a rewarding behavior, particularly given findings that rats exhibit a conditioned place preference for locations in which they have repeatedly been exposed to novel objects [204, 205]. This raises the possibility that the CRD rats' reduced water intake from the novel bottle may be due to reduced interest and decreased reward from such an activity (i.e. anhedonia). Another possibility is that reduced intake from the novel water bottle represents greater anxiety or neophobia directed at that new

object. In the absence of any other evidence to suggest heightened anxiety in the CRD rats however (i.e. same level of activity in the elevated maze and open field, both also novel environments), this result may be more indicative of reduced reward value from novel objects in those with a history of CRD.

These behavioral tests were only conducted in rats maintained on intermittent PF. As such, we do not know the extent to which the combination of intermittent access to PF and CRD (versus CRD alone) contributed to these results. This diet condition was used because it is most representative of human dieters and of persons with bulimia nervosa with binge eating [4, 5, 170, 171]. The latter are often diagnosed with comorbid anxiety and/or depression disorders [77-82]. In addition, this intermittent PF diet condition with a history of CRD induced changes to both the relationship between 5-HT and dopamine turnovers in the nucleus accumbens, and to levels of 5-HT and dopamine in the mPFC, both areas implicated in mood dysfunction. It will be interesting in the future to investigate whether a history of CRD irrespective of PF access contributes to symptoms of depression or whether access to PF is a necessary contributor to these symptoms.

In conclusion, this study has been the first to explore both behavioral and neurochemical changes induced by a history of CRD. These findings are particularly important given that all tests were conducted at a time when food intake and body weight were back at control levels, suggesting that a history of CRD, particularly if combined with intermittent access to PF, induces chronic changes in behavior and neurochemical activity. We have learned that food intake regulation is not necessarily perturbed by a history of CRD, but if the CRD is combined with intermittent access to PF, certain challenging situations such as the availability of just a morsel of PF, can increase subsequent feeding. Given that the 5-HT reuptake inhibitor, fluoxetine, corrected this trigger-induced overeating, we

expected to find central serotonergic discrepancies in the CRD group compared to those without CRD. This was confirmed with lower 5-HT levels in the whole hypothalamus of rats with a CRD history and intermittent access to PF, but only after 2 hours of feeding. In a subsequent experiment, this same group had 5-HT levels that matched those of controls but it is not clear whether this was because of differences in feeding state prior to the dissection or because of differences in the region dissected. It will be important in future studies to dissect discrete hypothalamic nuclei to identify the precise site of any perturbations caused by a history of CRD and also to investigate extracellular differences in monoamine levels to describe dynamic changes during and after feeding.

The comorbidity between eating disorders and mood disturbances such as depression and anxiety, along with the prevalence of weight fluctuation that occurs in many eating disorders, suggested that a history of CRD might contribute to mood changes. For the first time, we have evidence from an animal model that a history of CRD combined with intermittent access to PF induces behavior that reflects depression. In addition, we have evidence that a lack of interaction between 5-HT and dopamine in the nucleus accumbens may contribute to these symptoms. The functional importance of this 5-HT-dopamine interaction is a topic of much investigation currently, and the fact that a history of CRD, which involves nothing more than manipulating food availability, is able to induce a change in this interaction is an important contribution to existing knowledge. It will be interesting in the future to determine whether antidepressants can normalize both the behavioral symptoms evidenced here and the association between 5-HT and dopamine in the nucleus accumbens. Finally, we obtained evidence of reduced hypothalamic tissue levels of dopamine due to access to PF, and we found reductions of both 5-HT and dopamine in the mPFC tissue of rats fed intermittent PF with a history of CRD. The

meaning of these perturbations is not entirely clear but well worth future investigations because of the critical role these centers and neurotransmitters play in feeding and mood regulation.

This CRD model with a comparison of different schedules of access to PF was very useful for parsing out the contributions of a history of CRD and of intermittent access to PF, two factors that are commonly found in human dieters. While each of these factors is capable of chronically altering neurochemistry in central feeding, reward and mood regulation areas, the combination of these two factors together extends the neurochemical perturbations into the mPFC, which has a variety of functions. Given the changes in both behavior and neurochemistry evidenced here, future studies employing this model of CRD are warranted and will provide a valuable contribution to the literature.

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APPENDIX

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE APPROVALS

THE UNIVERSITY OF ALABAMA AT BIRMINGHAM

Office of the Provost

NOTICE OF APPROVAL

DATE: November 23, 2004

TO: Mary M. Boggiano, Ph.D.
CH 325 1170
FAX: 975-6110

FROM: Suzanne M. Michalek, Ph.D., Vice Chair
Institutional Animal Care and Use Committee

SUBJECT: Title: Neurochemistry of Stress-Induced Binge Eating
Sponsor: NIH
Animal Project Number: 041107030

On November 19, 2004, the University of Alabama at Birmingham Institutional Animal Care and Use Committee (IACUC) reviewed the animal use proposed in the above referenced application. It approved the use of the following species and numbers of animals:

Species	Use Category	Number in Category
Rats	B	40
Rats	C	120

Animal use is scheduled for review one year from November 2004. Approval from the IACUC must be obtained before implementing any changes or modifications in the approved animal use.

Please keep this record for your files, and forward the attached letter to the appropriate granting agency.

Refer to Animal Protocol Number (APN) 041107030 when ordering animals or in any correspondence with the IACUC or Animal Resources Program (ARP) offices regarding this study. If you have concerns or questions regarding this notice, please call the IACUC office at 934-7692.

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THE UNIVERSITY OF ALABAMA AT BIRMINGHAM

Office of the Provost

NOTICE OF APPROVAL

DATE: November 9, 2005

TO: Mary M. Boggiano, Ph.D.
CH 325 1170
FAX: 975-6110

FROM: Judith A. Kapp, Ph.D., Chair
Institutional Animal Care and Use Committee

SUBJECT: Title: Neurochemistry of Stress-Induced Binge Eating
Sponsor: NIH
Animal Project Number: 051107030

On November 8, 2005, the University of Alabama at Birmingham Institutional Animal Care and Use Committee (IACUC) reviewed the animal use proposed in the above referenced application. It approved the use of the following species and numbers of animals:

Species	Use Category	Number in Category
Rats	C	108

Animal use is scheduled for review one year from November 2005. Approval from the IACUC must be obtained before implementing any changes or modifications in the approved animal use.

Please keep this record for your files, and forward the attached letter to the appropriate granting agency.

Refer to Animal Protocol Number (APN) 051107030 when ordering animals or in any correspondence with the IACUC or Animal Resources Program (ARP) offices regarding this study. If you have concerns or questions regarding this notice, please call the IACUC office at 934-7692.

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GRADUATE SCHOOL
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DISSERTATION APPROVAL FORM
DOCTOR OF PHILOSOPHY

Name of Candidate Paula Chandler-Laney

Graduate Program Psychology

Title of Dissertation Behavioral and Neurochemical Consequences of a History of Human-Like Dieting

I certify that I have read this document and examined the student regarding its content. In my opinion, this dissertation conforms to acceptable standards of scholarly presentation and is adequate in scope and quality, and the attainments of this student are such that she may be recommended for the degree of Doctor of Philosophy.

Dissertation Committee:

Name		Signature
Mary M. Boggiano	**, Chair**	
Edward Castañeda		
James E. Cox		
Timothy R. Nagy		
Alan Randich		

Director of Graduate Program

Dean, UAB Graduate School

Date JUN 16 2006