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Food restriction enhances peak corticosterone levels, cocaine-induced locomotor activity, and ΔFosB expression in the nucleus accumbens of the rat

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ABSTRACT

Chronic stress has been known to potentiate addictive behaviours in both human addicts and experimental animals. In the present study, chronic mild food restriction was used as a stressor to investigate its effect on the locomotor simulant effects of cocaine as well as FosB expression in the nucleus accumbens and caudate putamen. Chronic mild food restriction enhanced the locomotor response to the first cocaine injection, such that chronically food restricted animals showed a significant increase in activity upon an initial administration of 15 mg/kg of cocaine, an effect which only became apparent in control animals after repeated injections. Food restriction also increased expression of the 35–37 kDa isoforms of ΔFosB compared to free-fed rats. ΔFosB proteins have been previously implicated in the rewarding effects of drugs of abuse and therefore their upregulation by the prolonged stress of food restriction suggests a possible mechanism for the enhancement of addictive behaviours by stress.

1. Introduction

Chronic drug administration is a defining characteristic in the etiology of addiction. One animal model commonly used to investigate the importance of repeated drug administration in the development of addictive behaviours in experimental animals is locomotor sensitization to psychostimulant drugs. Repeated exposure to a constant dose of cocaine (Borowsky and Kuhn, 1991) or amphetamine (Piazza et al., 1989) results in a progressive enhancement of locomotor activity with subsequent administration. The behavioural effects of repeated drug administration can be mimicked by exposure to psychological stress. Exaggerated locomotor responses to psychostimulant administration are evident after a variety of stressors including restraint, forced swim, social defeat, and food restriction (Rouge-Pont et al., 1995; Bell et al., 1997; Miczek et al., 1999; Araujo et al., 2003).

The mechanism whereby stress enhances behavioural responses to psychostimulants is thought to involve stress-induced elevations in the adrenal steroid hormone, corticosterone, which in turn enhances levels of mesolimbic dopamine (Deroche et al., 1995; Rouge-Pont et al., 1995). Indeed, locomotor sensitization with repeated psychostimulant exposure has been shown to be, in part, dependent upon elevated levels of extracellular dopamine (Fibiger et al., 1973; Karler et al., 1995).

Enhanced mesolimbic dopaminergic tone is associated with the appearance of isoforms of the transcription factor, ΔFosB (Nye et al., 1995). Inducible overexpression of ΔFosB in the

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Abbreviation: FR; food restriction

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striatum of transgenic mice produces locomotor sensitization as well as augmenting other addictive behaviours, suggesting that these proteins may play an important role in the behavioural responses these drugs (Kelz et al., 1999). Chronic restraint stress has also been shown to result in persistent expression of *fos-b* proteins in both stress (Stamp and Herbert, 1999) and reward neurocircuitry (Perotti et al., 2004). This suggests that the perhaps the long- term alterations in behaviour could occur via the converging actions of both the drug and stress upon ΔFosB expression.

Chronic food restriction (FR) is a commonly used stressor and has been shown to enhance the locomotor and neurochemical effects of psychostimulants (Bell et al., 1997; Cadoni et al., 2003). Chronic FR, as a model of naturally occurring food shortages in an animal's environment, is more ethologically relevant than other commonly used experimental models of stress, such as restraint. Furthermore, corticosterone levels remain high after several weeks after FR while hypersecretion after repeated restraint rapidly adapts (Stamp and Herbert, 1999; Belda et al., 2005). This is particularly relevant in trying to model the human condition since chronic stressors have been strongly implicated in stress-related pathology (Sapolsky, 1998). One very important methodological consideration is the time of day that food is available to FR animals since this can phase shift both hormone and activity rhythms (Challet et al., 1997; Leal and Moreira, 1997). The majority of studies examining the FR on addiction have allowed access to food during the animals' inactive phase (i.e. during the light portion of the cycle) (Bell et al., 1997; Cabib et al., 2000) and therefore possibly confounding the interpretation of results.

The aims of the present study were: (1) to determine whether chronic FR alters locomotor stimulant effects of cocaine and (2) to determine whether chronic FR enhances ΔFosB in central reward circuitry. Results from previous studies have shown that psychological stress before the administration of a single challenge dose of psychostimulant, like cocaine or amphetamine, can enhance locomotor activity (Rouge-Pont et al., 1995; Miczek et al., 1999; Araujo et al., 2003; Cadoni et al., 2003). Only one study (Bell et al., 1997) has investigated whether this augmentation persists with repeated drug administration in stressed animals. The present study employed FR before and during repeated cocaine administration in order to determine whether stress can alter the development of locomotor sensitization. Moreover, FR animals were allowed access to food at the beginning of the dark cycle in order to ensure that their activity rhythms were synchronized with that of the control animals.

2. Results

2.1. FR elevates peak corticosterone levels

Plasma corticosterone levels showed the typical nycthermal rhythm with lower levels in the morning (effect of time; $F_{1,20}=16.27$, $p=0.03$). Chronic FR enhanced the plasma corticosterone levels (effect of stress; $F_{1,20}=19.13$, $p=0.02$) which was evident by an elevation in peak (evening) levels (Fig. 1A), but there was no significant effect on the daily rhythm. Thymus weights were smaller ($t_{22}=5.98$, $p<0.0001$, Fig. 1B) and adrenal weights were larger ($t_{22}=2.78$, $p=0.011$, Fig. 1C) in the FR animals, which is consistent with the hypersecretion observed in that group.

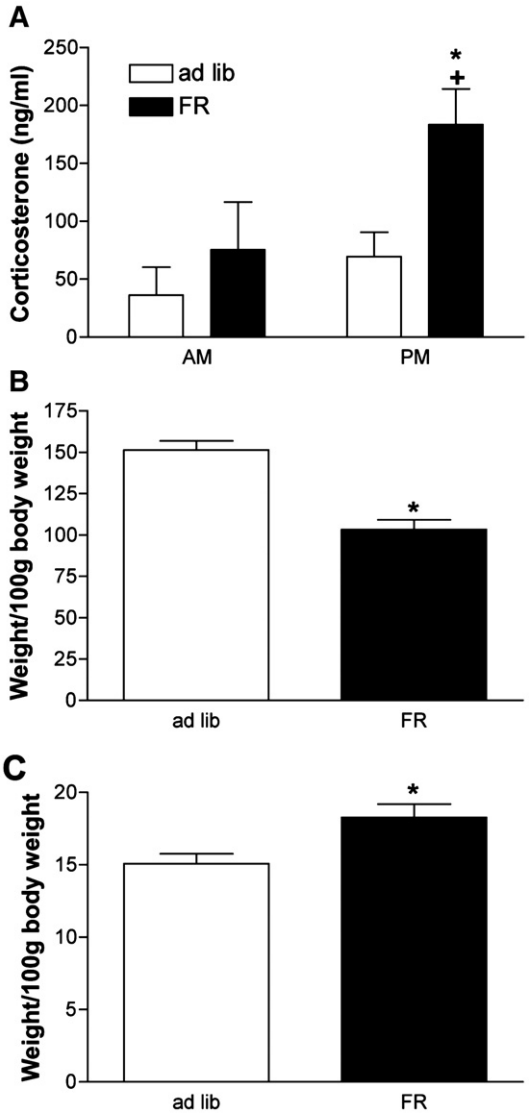

Fig. 1 – Plasma corticosterone (A), thymus and adrenal weights (per 100g of body weight) (B and C, respectively) after chronic food restriction; * = significantly different than corresponding *ad libitum* fed control group; + = significantly different than corresponding morning (AM) levels (Tukey's, $p<0.05$).

2.2. Dose response for cocaine-induced locomotor sensitization

Locomotor sensitization to cocaine differed depending on dose administered (dose × day, repeated measures ANOVA, $F_{3,15}=7.30$, $p=0.003$, Fig. 2). On Day 1 of cocaine administration, only the 25 mg/kg dose significantly elevated locomotor activity, and did not increase further upon repeated administration. The 15 mg/kg dose, however, did not produce an increase in locomotor activity on Day 1, but significantly increased locomotor activity on Day 5. Administration of either saline or 5 mg/kg cocaine did not produce hyperlocomotion on Day 1,

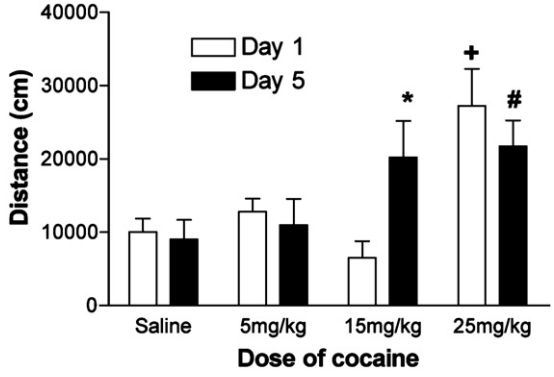

Fig. 2 – Total locomotor activity on after a single (Day 1) or repeated (Day 5) injections of cocaine; * = significantly different than same dose on Day 1; + = significantly different than other doses on Day 1; # = significantly different than Day 5 saline and 5 mg/kg (Tukey's, $p<0.05$).

nor did they produce a sensitized response on Day 5. Therefore a dose of 15 mg/kg was used as a threshold sensitizing dose in subsequent experiments.

2.3. *FR enhancement of cocaine-induced locomotor activity*

As expected, cocaine treatment (15 mg/kg) increased overall locomotor activity (main effect of drug, $F_{1,27}=43.4$, $p<0.0001$). This cocaine-induced elevation in locomotor activity in FR animals was evident after the first injection (on Day 1) while this was only apparent on Day 5 in the *ad lib* control animals (repeated measures ANOVA, day × stress × drug; $F_{2,54}=3.43$, $p=0.04$, see Fig. 3 for post hoc comparisons).

2.4. *FR enhances ΔFosB in the nucleus accumbens*

Given that the effect of FR on the locomotor activating effects of cocaine was most pronounced on Day 1, levels of ΔFosB in the caudate putamen and nucleus accumbens were assessed by Western blotting in drug naive animals. Chronic FR resulted in a small but significant increase in band intensity at 35–37 kDa in the nucleus accumbens ($t_6=3.17$, $p<0.02$, Fig. 4A) but not the caudate putamen (Fig. 4B). The band intensity in the nucleus accumbens of FR animals was most pronounced for the smaller 35 kDa band, whereas repeated cocaine-induced ΔFosB expression (see positive control in Fig. 4) showed up mostly as the upper 37 kDa band.

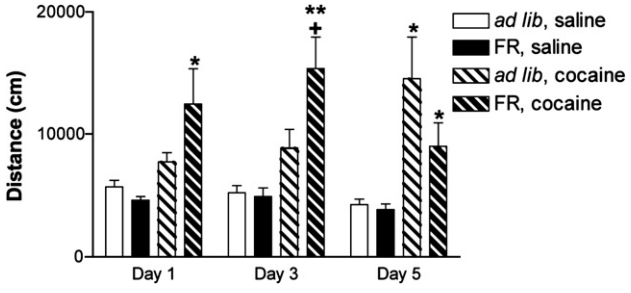

Fig. 3 – Effect of food restriction on cocaine-induced locomotor sensitization; significantly different than corresponding saline control * = $p<0.01$; ** = $p<0.0001$; + = different than corresponding unstressed cocaine group $p<0.05$ (Tukey's).

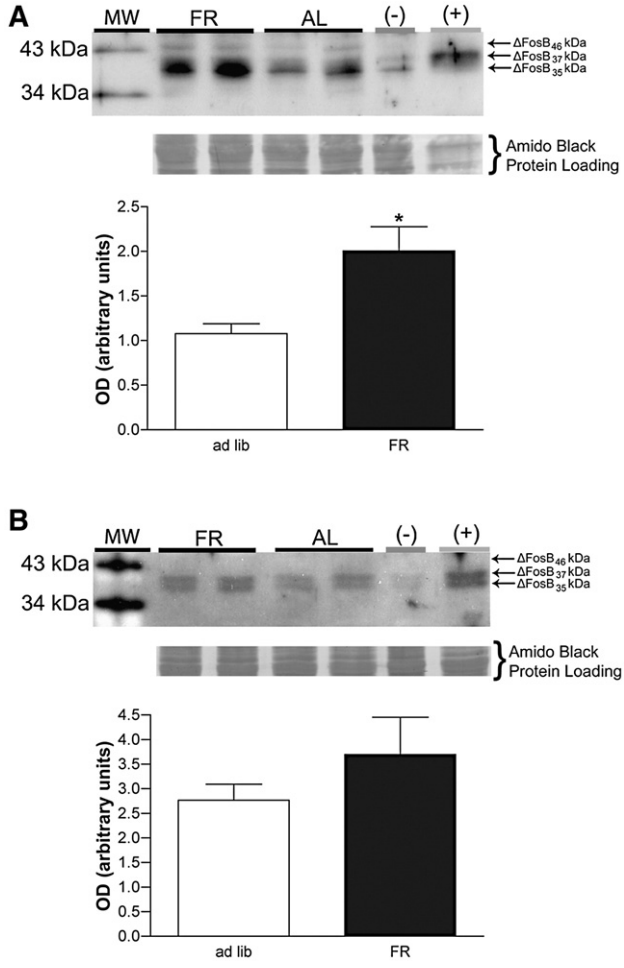

Fig. 4 – Chronic FR upregulates FosB-immunoreactive bands at approximately 35 and 37 kDa in the nucleus accumbens (A) but not the caudate putamen (B) in comparison to *ad libitum* (AL) rats. These bands were the same as those present after five days of repeated cocaine (15 mg/kg, (+) = positive control). Negative control tissue was excised from the cerebellum ((–) = negative control) which has been previously reported to express very low levels of this protein (Herdegen et al., 1995). MW indicates molecular weight marker. * = significantly different from *ad libitum* fed control group (student's t-test, $p<0.02$).

3. Discussion

The results of the present study confirm and extend findings by previous investigators. Previous reports using various models of stress, such as restraint, forced swim, and social aggression, have reported enhanced locomotion to an acute cocaine challenge (Bell et al., 1997; Miczek et al., 1999; Araujo et al., 2003). However, few studies have addressed whether this enhancement persists with repeated cocaine exposure. This is particularly important since the pattern of human addiction is characterized by repeated administration and previous stress has been implicated as a risk factor (Sinha, 2001).

One study by Bell et al. (1997) examined the effect stress on sensitization to repeated cocaine. They found a progressive increase in locomotor activity in rats that were administered cocaine every second day, an effect that was more pronounced in chronically food deprived rats. However, the FR animals in this study exhibited a 28% increase in general activity which could be due to shifted activity rhythms since the animals were given access to food in the late portion of the light cycle. We are confident that the animals' activity rhythms in the present study were phase synchronized since there was no difference in unstimulated (cocaine-free) locomotion between control and FR rats. Despite the discrepancy in locomotor activity of control animals, there are similarities in the findings from the two studies. In accordance with Bell et al. (1997) as well as other reports (Deroche et al., 1995; Rouge-Pont et al., 1995; Cadoni et al., 2003), we found that FR enhanced the initial locomotor activating response to cocaine. However, Bell et al. (1997) found a pronounced, progressive increase in the locomotor activating effects of cocaine in FR rats, whereas the hyperlocomotion observed in our cocaine-treated FR rats remained fairly stable over the five days of testing. The reason for this discrepancy is not clear, however methodological differences between the two studies could account for this. In the report by Bell et al. (1997), rats were food restricted to 80% of their free-feeding weight, whereas the present study used a milder food restriction to maintain rats at 90% of their free-feeding weight. It is possible that more severe food restriction is necessary in order to observe a potentiation of locomotor activity over a prolonged period of time. Another methodological difference was the dose of cocaine used. The highest dose used by Bell et al. (1997) was 10 mg/kg, whereas the present study used a slightly higher dose of 15 mg/kg, therefore resulting in a ceiling effect on locomotor activity. This dose was chosen because it produced significant increases in locomotor activity *only* after repeated injections in free-fed rats and was therefore considered a "threshold sensitizing dose" (dose response assessment in Experiment 2). A dose of 5 mg/kg did not produce hyperlocomotion, either upon initial exposure or after repeated injections, while a dose of 25 mg/kg produced hyperlocomotion after the first cocaine injection. Since we were interested in determining whether FR would influence the *development* of behavioural sensitization, the 15 mg/kg dose was most appropriate.

The hyperlocomotion observed in FR rats on day 1 of cocaine administration was similar to that observed in *ad libitum* fed cocaine-treated rats on day 5. Thus the behaviour of the FR rats resembles those that have had prior drug exposure. This finding is also complemented by the appearance of ΔFosB proteins in the nucleus accumbens in drug naive chronic FR rats. This is in accord with a recent study demonstrating elevated levels of ΔFosB proteins in the nucleus accumbens of rats exposed to repeated restraint stress (Perotti et al., 2004). Although previous research has shown that FR enhances amphetamine-induced Fos-like immunoreactivity in many reward-associated areas (Carr and Kutchukhidze, 2000), to our knowledge this is the first study to demonstrate that FR specifically augments FosB immunoreactivity.

FosB proteins, specifically the 35 and 37 kDa isoforms observed in this study, are evident after chronic administration of many drugs of abuse including cocaine, amphetamine, nicotine, and opiates (see Nestler et al., 1999 for review) as well as following naturally rewarding stimuli such as chronic wheel running (Werme et al., 2002). In the present study, we found that the effects of FR on FosB immunoreactivity were mainly manifested as an elevation in the 35 kDa band. Different treatments induce different combinations of ΔFosB isoforms, for instance chronic electroconvulsive shock predominantly upregulates the 37 kDa band (Hope et al., 1994). The functional consequences of differential upregulations of the different FosB isoforms is unknown, however it has been shown that the 33, 35 and 37 kDa ΔFosB isoforms have much longer half lives than the parent FosB protein (Chen et al., 1997). Therefore the appearance of these proteins could influence the time course of AP-1 mediated transcriptional activity in the cell. Furthermore, the increase in ΔFosB isoforms is thought to be sufficient to enhance reward-related behaviour since inducible overexpression in the dorsal striatum (caudate putamen) of transgenic mice enhances voluntary wheel running as well as self-administration of cocaine (Werme et al., 2002; Colby et al., 2003).

One notable characteristic of the ΔFosB expression in the present study is the area specificity. The enhancement was evident in the nucleus accumbens of FR rats but not the caudate putamen. This finding differs from the more widespread pattern of expression after repeated restraint and chronic unpredictable stress employed by Perotti et al. (2004), which produces elevations in 35–37 kDa isoforms in both the nucleus accumbens and the caudate putamen. The reason for this discrepancy is unclear, however, it might reflect a difference in stressor type. Different types of stressors induce similar, but not identical patterns of immediate early gene expression in stress neurocircuitry. This is evident with the stressor specific pattern of expression observed for *c-fos* and its corresponding protein, Fos. Chronic food restriction alone (in drug naive animals) increases Fos-like immunoreactivity in the bed nucleus of the stria terminalis while Fos immunoreactivity in other forebrain structures, such as the nucleus accumbens and caudate putamen, is augmented by FR only after amphetamine administration (Carr and Kutchukhidze, 2000). The pattern of Fos expression for other chronic stressors also differs. For instance, repeated social defeat produces persistent Fos expression in the medial nucleus of the amygdala (Chung et al., 1999) while this is not present after repeated restraint (Stamp and Herbert, 1999).

The mechanism for the upregulation of ΔFosB in the nucleus accumbens by chronic FR is yet unknown but one possibility is the modulation of mesolimbic dopamine by corticosterone. Elevations in ΔFosB proteins in the nucleus accumbens after repeated cocaine are mimicked by dopamine agonists and attenuated by administration of the D1 antagonist, SCH 23390, implicating D1 dopamine receptors in the appearance of ΔFosB (Nye et al., 1995). The effect of FR on basal levels of dopamine in the nucleus accumbens remains equivocal with some reports showing no change (Cadoni et al., 2003) and others actually reporting a decrease (Pothos et al., 1995). However, FR increases dopamine content in the nucleus accumbens (Pothos et al., 1995) and increases dopamine receptor signaling (Carr et al., 2003). Restraining cortiosterone levels with metyrapone (a corticosterone synthesis inhibitor) attentuates the elevations in accumbens dopamine by cocaine in FR animals (Rouge-Pont et al., 1995). It is therefore possible that the enhanced ΔFosB observed after FR is due to enhanced dopamine function resulting from hypersecretion of corticosterone. FosB-like immunoreactivity

after repeated restraint in the lateral septum and paraventricular nucleus are sensitive to manipulations in levels of this hormone. Stress-induced levels of corticosterone appear to restrain the FosB induction in these areas since: (1) upregulation is enhanced by adrenalectomy and (2) replacement with high levels of corticosterone is required to normalise this response (Stamp and Herbert, 2001). Although there is strong evidence for a role for corticosterone in FR-induced changes in dopamine function, other humoral factors, such as insulin and leptin may also be involved (Shalev et al., 2001; Carr, 2002).

While it is well known that stress levels of corticosterone contribute to addiction, the biological function of this phenomenon is not known. It has been proposed that one of the key roles of glucocorticoids during stress is to suppress responses elicited by the stressor (Sapolsky et al., 2000). This has been shown for immune, cognitive, and anorexic responses to stress but it is not known whether this is also true for emotional changes associated with stress. However, one hypothesis is that by stimulating central reward pathways, high levels of corticosterone can reduce aversiveness of the stressor (Piazza and Le Moal, 1997). Indeed, rats will actually self- administer high doses of corticosterone (Deroche et al., 1993; Piazza et al., 1993). Therefore it is possible that the stress facilitates reward signaling in order to reduce the negative emotional state associated with stress exposure. This process could conceivably aid in coping with a potentially threatening situation, however it becomes detrimental when an individual has the opportunity to administer drugs of abuse since stress actually potentiates their reinforcing effects.

The precise role of FosB isoforms in the development of addictive behaviours is unclear though the persistent expression after chronic administration of various drugs of abuse is intriguing. To date, two downstream targets of FosB have been identified though they have opposing effects on drug induced locomotor behaviour. Inducible overexpression of FosB upregulates the GluR2 glutamate receptor subunit, which itself enhances conditioned place preference for cocaine (Kelz et al., 1999). Cyclin-dependent kinase 5 (Cdk5) is also upregulated after either chronic cocaine or inducible overexpression of FosB, however its role in addictive behaviours is uncertain. Inhibition of this protein kinase have produced mixed results; one study found an enhanced locomotor response to cocaine (Bibb et al., 2001), while another reported an attentuated locomotor response to methamphetamine (Chen and Chen, 2005). Chronic stress upregulates GluR2 levels in the hippocampus (Rosa et al., 2002), but there have not been any reports of a similar effect in reward-related neurocircuitry. Similarly, acute stress increases Cdk5 in cholinergic septohippocampal neurons and is thought to be involved in fear conditioning (Fischer et al., 2002). It would be interesting to determine whether similar stress-induced changes in these proteins are evident in reward-related neurocircuitry.

3.1. *Conclusions*

The results of the present study confirm the finding that FR enhances locomotor stimulant effects of cocaine. Furthermore, the upregulation of ΔFosB isoforms by FR implicate these proteins as a possible molecular mechanism for stress-enhancement of reward or relapse. Future studies should also address the role of corticosterone and ΔFosB isoforms in other behavioural measures of reward, mainly conditioned place preference and self-administration. In addition the influence of stress on the expression of proteins downstream of FosB is worthy of further study.

4. Experimental procedures

4.1. *Animals*

Male Long–Evans rats (initial weights 300–350 g, Charles Rivers, Que.) were housed in pairs under a 12:12 h reverse light/dark cycle with lights off at 9 am. Animals were handled and weighed daily for a week before the onset of experimental procedures in order to reduce any non-specific stress responses. For behavioural testing, animals were pre-exposed to the locomotor activity boxes daily for an hour for at least three days before the onset of experimental procedures. All animals were handled in accordance with the guidelines of the Canadian Council on Animal Care and the experimental protocol was approved by the Dalhousie University Committee on Laboratory Animals.

4.2. *Food restriction*

On the first day of food restriction animals were singly housed and given 12 g of food pellets (Prolab 3000 RMH, Corridor Coop) until they reached 90% of their weight on the last free-feeding day (approximately 6 days). Food was given the same time each day at lights off, which is the normal peak feeding time in rodents. Animals were weighed daily and amount of food given was adjusted to maintain weight at 90% of free-feeding levels. Following 10 days of chronic food restriction, animals were either killed (experiments 1 and 2) or tested in the open field apparatus for locomotor responses to cocaine or saline for five days (experiment 3). Food restriction continued throughout the period of behavioural testing. *Ad libitum* fed control animals were handled and weighed daily, but were given free access to food.

4.3. *Blood collection and radioimmunoassay*

Animals were rapidly anaesthetized with CO_2 and blood was collected by cardiac puncture. Animals were then sacrificed by cervical dislocation. Blood was collected into heparinized tubes and centrifuged at 800 ×*g* and the plasma collected. Samples were stored at −20 °C until assay. Corticosterone was measured using a commercially available kit (ICN Biomedicals Inc., CA). Adrenal and thymus glands were also dissected and weighed after sacrifice since these indices are sensitive to levels of circulating corticosteroids.

4.4. *Sample preparation and Western blotting*

The brains were removed and the caudate putamen and nucleus accumbens were dissected from 2 mm and 1 mm slices, respectively (as described by Heffner et al. (1980)). Tissue was immediately frozen in liquid nitrogen, and stored at −70 °C until processed for FosB immunoreactivity using Western blotting. Nucleus accumbens samples from two brains in the same condition were pooled to generate enough tissue for western blotting. Tissue samples were homogenized

with a motorized mortar and pestle in buffer containing 0.25 M sucrose, 10 mM Tris, 1 mM EDTA, 17 μg/ml PMSF, and Complete Mini™ protease inhibitor (prepared as per manufacturer's instructions, Roche). Samples were then centrifuged at 2000 ×*g* at 4 °C for 10 min and the supernatant (cytoplasmic fraction) removed. The remaining pellet was then incubated at 4 °C in a solution containing 400 mM KCL, 0.5% Triton X-100, 1 mM PMSF and Complete Mini ™ protease inhibitor for 1 h. Following this incubation, samples were centrifuged at 8000 ×*g* at 4 °C for 15 min and the supernatant (containing nuclear proteins) removed and stored at −20 °C until Western blotting.

Protein concentration was determined using a Bio-Rad Protein Assay kit. Aliquots (containing 20 μg of protein) were denatured by heating to 95 °C for 5 min and then subjected to one-dimensional SDS-polyacrylamide gel electrophoresis with 10% acrylamide/0.27% bis- acrylamide in resolving gels. Proteins were transferred electrophoretically to PVC membrane (Immobilon), blocked in 1% non-fat milk in PBS-Tween 80 for 1 h at 37 °C, and incubated in primary antibody (1:200, rabbit polyclonal anti-FosB, Santa Cruz SC-48) at 4 °C overnight. The following day, blots were incubated in horseradish peroxidase conjugated goat anti-rabbit secondary antibody (1:3000, Santa Cruz) for 1 h at 37 °C. Immunoreactivity was visualized using enhanced chemiluminescence techniques. After bands were visualized, the membranes were checked for equal protein loading by staining for total protein using Amido Black Staining Solution (Sigma-Aldrich). The optical density of bands was expressed relative to the intensity of Amido Black stain. All bands were quantified using densitometry and expressed as arbitrary optical density (OD) units.

4.5. *Drug administration and behavioural testing*

Cocaine hydrochloride (Sigma) was dissolved in sterile 0.9% saline. Doses of 5, 15, and 25 mg/kg were used in this study, with 0.9% saline as a control injection. Animals were given at least 10 min to adapt to the testing box before any injections were given. Following this baseline period, animals were injected with either saline or one of the above doses of cocaine and locomotor activity was recorded for 1 h. This was repeated daily for five days.

The apparatus consisted of one of four 35×45×46 Plexiglas™ boxes placed below a video camera. Behavioural testing was performed as described previously (Bird et al., 2001). Briefly, animals were videotaped during the dark (active) phase and locomotor activity was processed online using Ethovision software (Noldus Information Technologies, Wageningen, The Netherlands). Each day's activity record was analyzed by the program to determine the total distance moved.

4.6. *Statistical analysis*

Differences between thymus and adrenal weights and band intensities on western blots were determined using Student's *t*-test. Plasma corticosterone was analyzed by a two way ANOVA with time of sacrifice and feeding (*ad lib* or FR) as factors. Locomotor behaviour was analyzed by repeated measures ANOVA with day as the within subjects factor and dose (Experiment 3), and FR (Experiment 4) as between subjects factors. Post hoc comparisons were made using Tukey's test. Significance was accepted at the $\alpha = 0.05$ level.

4.7. *Experiments*

4.7.1. *Experiment 1: Effect of FR on plasma corticosterone levels*

One group of animals ($n = 24$) was used to determine the effects of chronic FR on basal corticosterone concentrations. Twelve animals were food restricted for 12 days, the majority of which reached 90% of their free-feeding weight after 6 days of FR. The remaining 12 control animals were handled and weighed daily but had free access to food. Six FR and control animals were rapidly anaesthetized with CO2 during the first 30 min of the light cycle (daily trough of corticosterone) and the remaining six in each condition were killed during the first 30 min of the dark cycle (daily peak in corticosterone). Blood was collected as described above and corticosterone levels were assessed by RIA. Thymus and adrenal glands were dissected from each animal, weighed and values were expressed relative to total body weight.

4.7.2. *Experiment 2: Dose response for cocaine locomotor sensitization*

Twenty animals were used for this experiment. On the fourth day (following three days of habituation to the open field apparatus) animals were randomly assigned to one of four groups and injected with one of the following: physiological saline, 5 mg/kg, 15 mg/kg, or 25 mg/kg of cocaine hydrochloride ($n = 5$/group). Animals were placed in the testing apparatus for 30 min before injection, and then locomotor behaviour was recorded for 60 min following injection. This was repeated daily for five days with behaviour recorded on Days 1 and 5. Sensitization was defined as an increase in locomotor activity on Day 5 without an initial hyperlocomotive effect on Day (i.e. as compared to Day 1 saline controls).

4.7.3. *Experiment 3: Effect of FR on locomotor sensitization after repeated cocaine*

Thirty-two rats were used for this experiment. Following this acclimatization period, animals were divided into two groups: FR and *ad libitium* (control) feeding ($n = 16$/group). After one week of food restriction, animals were introduced to the testing apparatus for 1 hr daily for three days. On the fourth day animals in each group were further subdivided to yield four groups and injected daily with either physiological saline or 15 mg/kg cocaine (*ad lib*, saline; FR, saline; *ad lib*, cocaine; FR, cocaine; $n = 8$/group). Animals were placed in the testing apparatus for 10 min before injection, and then locomotor behaviour was recorded for 60 min following injection. This was repeated daily for five days with behaviour recorded on Days 1, 3, and 5.

4.7.4. *Experiment 4: Effect of FR on ΔFosB expression in the caudate putamen and nucleus accumbens*

Sixteen animals were used for this experiment. Eight animals were food restricted for 12 days while the remaining eight served as controls. On the last day of FR animals from both groups were anaesthetized with sodium pentobarbital and decapitated. The caudate putamen ($n = 8$/group) and nucleus accumbens ($n = 4$/group, pooled tissue) were excised from tissue slices and processed for Western blotting.

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