



A ketogenic diet diminishes behavioral responses to cocaine in young adult male and female rats

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HIGHLIGHTS

- A ketogenic diet (KD) decreased cocaine-induced stereotypy in male and female rats.
- This effect was observed during repeated injections and following abstinence.
- Cocaine-induced ambulatory sensitization was also disrupted in KD-fed rats.
- KDs may have therapeutic potential for cocaine addiction.

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ABSTRACT

Ketogenic diets (KDs) are high fat, low carbohydrate formulations traditionally used to treat epilepsy; more recently, KDs have shown promise for a wide range of other neurological disorders. Drug addiction studies suggest that repeated exposure to drugs of abuse, including cocaine, results in a suite of neurobiological changes that includes neuroinflammation, decreased glucose metabolism, and disordered neurotransmission. Given that KDs positively regulate these factors, we addressed whether administration of a KD has potential as a novel therapy for drug addiction. In this study, male and female Sprague-Dawley rats were placed on a KD or a control diet (CD), beginning at five weeks of age and continuing through the end of behavioral testing. Three weeks after initiation of dietary treatments, rats received daily i.p. injections of cocaine (15 mg/kg) or saline vehicle for one week, were drug free for a subsequent week, and then all animals received a final challenge injection of 15 mg/kg cocaine. In the absence of cocaine injections, stereotyped locomotor responses were minimal and were unaffected by dietary treatment. In contrast, both males and females fed a KD exhibited decreased cocaine-induced stereotyped responses as compared to CD-fed rats. The sensitization of ambulatory responses was also disrupted in KD-fed rats. These results suggest that KDs directly impact dopamine-mediated behaviors, and hence may hold potential as a therapy for drug addiction.

1. Introduction

Addiction to psychostimulants such as cocaine is a complex, multifaceted disorder that to date has proven difficult to prevent or treat. This is in part due to the wide range of changes throughout the central nervous system that occur in addiction, such as decreased glucose metabolism, increased neuroinflammation, and altered neurotransmission. Many of these changes persist for months, if not years, into abstinence. A history of abuse of cocaine or methamphetamines is associated with persistent decreases in glucose metabolism in cortical (orbitofrontal cortex) and subcortical (striatum) brain areas implicated

in drug addiction in human addicts (Chang et al., 2007; Volkow et al., 2004), an effect that is also seen in non-human primates treated acutely with cocaine (Lyons et al., 1996). In addition, serum levels of proinflammatory cytokines/chemokines are elevated in cocaine-abusing individuals, and have been put forth as a potential biomarker of the severity of abuse/dependence (Araos et al., 2015; Moreira et al., 2016); these factors, along with reactive oxygen species, are also elevated in the cortex and striatum of rats injected with cocaine (Dietrich et al., 2005; Sorg et al., 2011). Finally, cocaine not only has lasting effects on dopamine neurotransmission within the reward and reinforcement neurocircuitry, but also impacts other neurotransmitters that directly

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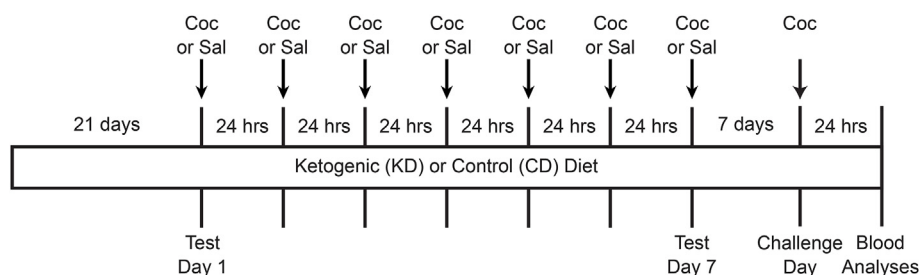


Fig. 1. Timeline of experimental manipulations. Coc = injections of cocaine (15 mg/kg; 1 ml/kg); Sal = injections of saline vehicle (1 ml/kg). Sal-Coc = animals injected with saline on Test Days 1–7; Coc-Coc = animals repeatedly injected with cocaine during this period (all animals injected with cocaine on Challenge Day). Sample sizes per group for females are as follows: CD Sal-Coc ($n = 12$); CD Coc-Coc ($n = 12$); KD Sal-Coc ($n = 12$); KD Coc-Coc ($n = 12$). Sample sizes per group for males are as follows: CD Sal-Coc ($n = 11$); CD Coc-Coc ($n = 12$); KD Sal-Coc ($n = 10$); KD Coc-Coc ($n = 12$).

and indirectly interact with dopamine, such as adenosine. For example, there is decreased extracellular availability of adenosine and decreased adenosine A_{2A} receptor (A_{2A}R) expression within the ventral striatum during cocaine withdrawal in rats (Manzoni et al., 1998; Marcellino et al., 2007), and systemic administration of an A_{2A}R agonist diminishes the sensitizing effect of repeated cocaine administration on locomotor responses in rats (Filip et al., 2006). Given this diverse set of factors impacted by psychostimulant addiction, effective therapies will likely need to be multifaceted as well.

Ketogenic diets (KDs) are high fat, low carbohydrate, adequate protein formulations that have a long-standing therapeutic history for the treatment of intractable epilepsy (Masino and Rho, 2010). Individuals on a KD experience a shift in metabolism resulting in the use of ketone bodies (e.g., acetoacetate, β -hydroxybutyrate) as a primary metabolic fuel. This switch occurs due to a decrease in available glucose, leading to a conversion of free fatty acids to ketone bodies within the liver (Hartman et al., 2007). Ketone bodies can readily substitute for glucose in cellular respiratory pathways throughout the body and brain; indeed, ketone bodies are a more efficient energy source, generating more molecules of ATP per unit oxygen vs. glucose (Hartman et al., 2007).

Research into the precise mechanisms underlying the therapeutic effects of KDs on epilepsy and other neurological disorders have revealed that these diets normalize behaviors and neural processes disrupted by disease states. For example, treatment of mice with a KD prior to kainic acid-induced seizures reduces expression of neuroinflammatory factors (e.g., COX-2 and prostaglandin E2) in the hippocampus (Jeong et al., 2011), and administration of the ketone body acetoacetate reduces hippocampal hyperactivity and extracellular glutamate levels in rats induced to have seizures by 4-aminopyridine infusions (Juge et al., 2010). A KD lowers - and perhaps equally important - stabilizes glucose levels (Noakes et al., 2006; Nuttall et al., 2015); furthermore, KD or ketone body treatment elevates ATP in healthy brain tissue (DeVivo et al., 1978; Nakazawa et al., 1983; Pan et al., 1999) and normalizes ATP availability in animal models of brain/spinal cord disorders or damage (Deng-Bryant et al., 2011; Nylen et al., 2009; Zhao et al., 2006). When considering the close inter-relationship between the purines ATP and adenosine as well as the direct link between metabolism and neuronal activity, it is not surprising that purines have been implicated in the therapeutic effects of KDs (Masino et al., 2012; Masino and Geiger, 2008). Specifically, mice lacking the adenosine A1 receptor (A₁R) exhibit spontaneous electrographic seizures, and treatment with a KD decreases seizures in mice heterozygous, but not homozygous, for the A₁R knockout (Masino et al., 2011). Similarly, recent work demonstrated a ketone ester's ability to reduce anxiety in a genetic rat model of absence seizures requires activation of A₁Rs (Kovács et al., 2018).

Existing research into mechanisms underlying the therapeutic effects of KDs suggests that metabolic therapy can impact or even normalize several factors disrupted or implicated in psychostimulant addiction. To date this hypothesis has not been tested directly. It is well established that rodents exhibit a reliable increase in ambulatory and repetitive, stereotyped locomotor responses following repeated

administration of cocaine (Robinson and Berridge, 1993). Here, we examined the impact of KD treatment on these behavioral responses to cocaine in male and female rats. We found that administration of a KD did not alter behaviors in the absence of cocaine injections; in contrast, ambulatory and stereotyped (i.e., rearing) locomotor responses induced by cocaine injections were disrupted in KD-treated male and female rats.

2. Material and methods

2.1. Animals

Male and female Sprague-Dawley rats were first-generation offspring of breeders obtained from Charles River Laboratories. Rats were pair-housed by sex at weaning (21 days) in polycarbonate cages with wire mesh tops. Animals were maintained on a 12:12 h light:dark cycle (lights on at 7 am), with all behavior testing occurring between the hours of 9 am and 2 p.m. Food and water were available *ad libitum*. Animal procedures were carried out in accordance with the Guide for the Care and Use of Laboratory Animals (8th Ed.) and approved by the Trinity College Institutional Animal Care and Use Committee.

2.2. Diet

At 5 weeks of age, individual cages of male and female rats were randomly assigned to receive either a control diet (CD; LabDiet 5001, W. F. Fisher & Son, Somerville, NJ) or a KD (F3666, Bio-Serv, Flemington, NJ) (see Fig. 1 for experimental schematic). Rats were weighed prior to placement on the diet, and then at least three times weekly during the five week period that rats remained on their assigned diets. Rodents consuming the F3666 diet self-regulate caloric intake such that it does not differ from control chow (Badman et al., 2009).

2.3. Drugs and injections

Cocaine hydrochloride (cocaine; C5776, Sigma, St. Louis, MO) was dissolved in sterile saline to the working concentration (15 mg/ml). Starting at eight weeks of age, individual cages of CD and KD male and female rats were randomly assigned to receive daily i.p. injections (1 ml/kg) of either cocaine or sterile saline vehicle. Animals received their assigned injections for seven consecutive days, and then all animals underwent a drug-free period (no injections) for seven consecutive days. All animals then received a final “challenge” injection of cocaine (15 mg/kg) following the conclusion of this drug-free period. All injections occurred in the context of the testing apparatus (see below). The dose and patterning of cocaine injections employed here have previously been shown to induce behavioral sensitization in both male and female rats (Halbout et al., 2016; Kosten et al., 1994; Martinez et al., 2014; Peterson et al., 2016; Sircar and Kim, 1999).

2.4. Behavioral testing

Rats were tested for behavioral responses induced by repeated

cocaine or saline injections using an automated system (Kinder Scientific, Poway CA). Each unit of this system was composed of a plexiglas open field chamber ($44 \times 22 \times 20.5$ cm) surrounded by a bilevel set of sensing frames. These frames generated an X–Y grid of photobeams within the chamber. Photobeam breaks were transmitted to a Windows computer running MotorMonitor software (Kinder Scientific). This software further discriminated beam breaks into ambulations (change of the animal's entire body position on the X–Y grid of the lower frame) and time spent rearing (total time spent with any photobeams broken on the upper frame). According to the original scale proposed by Creese and Iversen (1974) and modified for use with cocaine by others (e.g., Daunais and McGinty, 1994; Festa et al., 2004), general ambulatory activity is scored as a low/moderate form of psychostimulant-induced stereotypy, whereas focused/persistent rearing is considered to be a more extreme response.

Each testing session began with an initial 30 min habituation period. Rats were then removed from the testing apparatus, injected with either cocaine or saline, and then returned to the testing apparatus for a 60 min test period. Ambulation and rearing data collected by the MotorMonitor software during the test period were summed (by behavior) into individual 5-min bins. As has been reported previously (Boudreau and Wolf, 2005; Shumsky et al., 1997), we observed that ambulatory and rearing responses peaked during the first 30 min following cocaine injections in CD animals (Fig. 2); consequently, statistical comparisons were limited to responses that were summed across the first 30 min of the test period.

2.5. Blood analyses

Twenty-four hours following the Challenge Day test, rats were euthanized using isoflurane gas and blood was taken from the lateral tail vein during the procedure. Glucose and β -hydroxybutyrate levels in blood samples were determined using Precision Xtra meters (Abbott Laboratories, Bedford MA).

2.6. Statistical analyses

All data were analyzed using SPSS for Macintosh, version 24.0 (IBM Corp, Armonk, NY USA). Data were first examined for skewness and normality (Shapiro-Wilk test), in order to determine if the assumptions of parametric statistical tests were met. When identified, outliers (scores outside the interquartile range (IQR) by more than $1.5 \times \text{IQR}$) were removed from the affected data set. The effects of diet (CD vs. KD), drug history (Sal-Coc vs. Coc-Coc), day (day of testing) and sex (male vs. female) on body weight were examined via mixed-design ANOVAs. Statistically significant three-way interactions were further decomposed for the simple effect of diet on each day, within each sex, using the MANOVA script in SPSS (pooled error term used from overall ANOVA). Challenge Day weight z-scores were tested for their relationship to Challenge Day time spent rearing (separately within each sex) using Pearson's r . In order to control for between-subject effects of diet condition on weight, z-scores were calculated separately for each treatment group (i.e., each raw score was standardized using its treatment group mean and standard deviation) and then z-scores were pooled within each sex. For the week of daily cocaine or saline injections, analyses were restricted to the first (Test Day 1) and seventh (Test

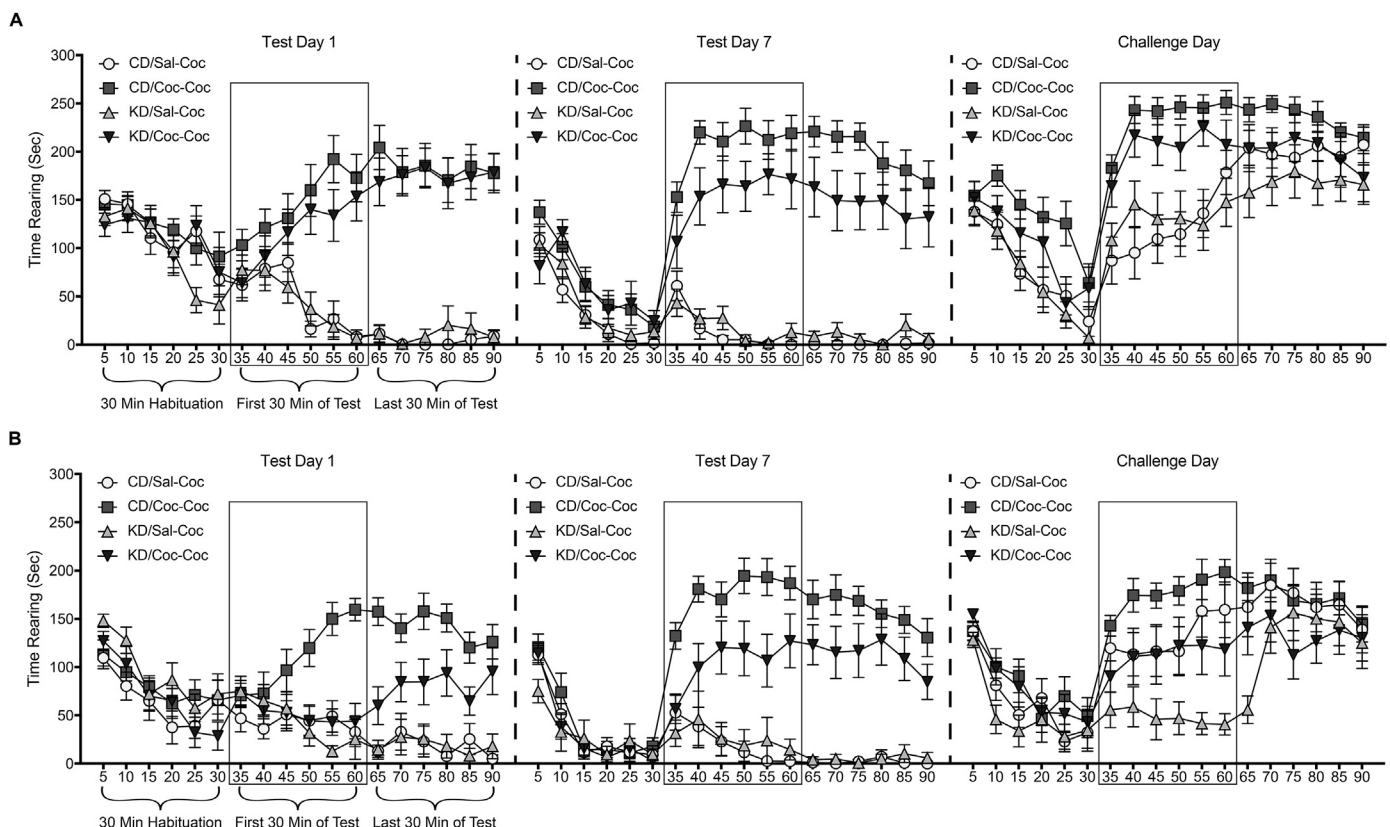


Fig. 2. Mean (\pm SEM) within-session data for time spent rearing by female (A) and male (B) rats treated with a control (CD) or ketogenic (KD) diet. Within each test (Test Day 1, Test Day 7, and Challenge Day), data were further divided into 5 min bins. The 30 min prior to injection comprised the habituation phase; the first and second 30 min periods following injection comprised the test phase. Note that the labels below each phase for Test Day 1 also apply to Test Day 7 and the Challenge Day. Rectangles enclose the first 30 min of the test phase, to further highlight the subset of the data that were summed for later statistical comparisons. Sal-Coc = animals injected with saline Test Days 1–7, and Coc-Coc = animals repeatedly injected with cocaine during this period (all animals injected with cocaine on Challenge Day).

Day 7) injection days in order to simplify the analyses. The effect of diet, sex and time (Test Day 1 vs. Test Day 7) on ambulations and time rearing were examined separately within each drug history group, using mixed-design ANOVAs. Statistically significant interactions were further decomposed for the simple main effect of day within each diet condition using mixed-design ANOVAs. Finally, data from the Challenge Day were examined for the effects of diet, drug history, and sex on ambulations and time rearing using factorial ANOVAs. Statistically significant two-way interactions were further decomposed for the simple main effect of diet within each sex using factorial ANOVAs (pooled error term used from overall ANOVA). In all cases, p -values of less than 0.05 were considered *a priori* to be significant. Effect sizes are reported as partial η^2 .

3. Results

3.1. Behavioral measures: repeated daily cocaine or saline injections

Starting at eight weeks of age, male and female rats maintained on a KD or CD were assigned to receive daily injections of cocaine or saline vehicle for one week. Analyses of ambulations and time spent rearing were restricted to Test Days 1 and 7. Descriptive, within-session data for time rearing on these test days is provided in Fig. 2. When examining summed (across the first 30 min following injection) cocaine-induced rearing responses, time spent rearing was 53% higher on Test Day 7 vs. Test Day 1 ($F(1,44) = 16.18$, $p < 0.001$, $\eta^2 = 0.27$) (Fig. 3). Although this sensitizing effect on time spent rearing was seen irrespective of diet condition, the overall levels of cocaine-induced rearing were significantly decreased by 33% in KD vs. CD animals ($F(1,44) = 17.67$, $p < 0.001$, $\eta^2 = 0.29$). Finally, females spent more time (41%) rearing compared to males ($F(1,44) = 13.072$, $p < 0.001$, $\eta^2 = 0.23$). In contrast to animals treated with cocaine, saline-treated animals showed a 51% decrease in rearing over time ($F(1,41) = 15.28$, $p < 0.001$, $\eta^2 = 0.27$). Importantly, there was no effect of diet on time spent rearing in these saline-injected animals ($F(1,41) = 0.027$, $p = 0.87$, $\eta^2 = 0.001$).

Cocaine-induced ambulations were also summed across the first 30 min following injections and compared across treatment groups. There were no significant effects of test day ($F(1,42) = 1.19$, $p = 0.28$, $\eta^2 = 0.028$) or diet ($F(1,42) = 0.059$, $p = 0.81$, $\eta^2 = 0.001$) on ambulations; however, there was a significant diet \times test day interaction ($F(1,42) = 7.77$, $p = 0.008$, $\eta^2 = 0.16$) (Fig. 4). For CD animals, the expected sensitizing effect (increasing ambulations from Day 1 to Day 7) was observed ($F(1,23) = 8.57$, $p = 0.008$, $\eta^2 = 0.27$). In contrast,

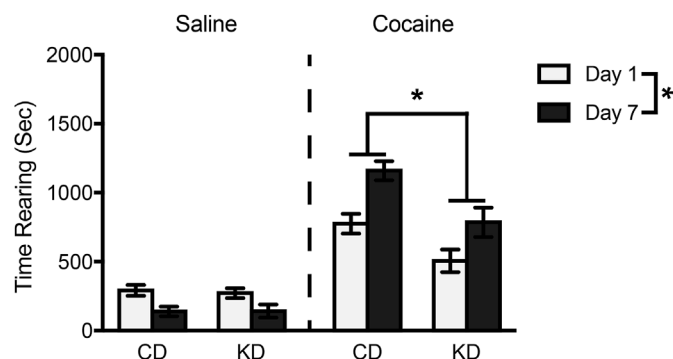


Fig. 3. Mean (\pm SEM) time spent rearing summed across the first 30 min of the test phase on Test Day 1 and Test Day 7 by rats on a control (CD) or ketogenic (KD) diet, following injections of either saline or cocaine. Data were collapsed across sex (diet \times sex interaction was non-significant). Cocaine-induced rearing increased significantly from Test Day 1 to Test Day 7. Furthermore, rearing time was significantly lower when comparing KD to CD animals. $*p < 0.05$, Test Day 1 vs. Test Day 7 for cocaine- and saline-injected animals, as well as CD vs. KD comparisons in cocaine-injected animals.

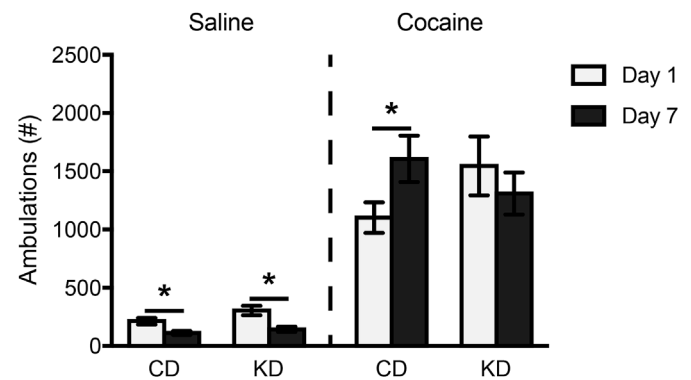


Fig. 4. Mean (\pm SEM) ambulations summed across the first 30 min of the test phase on Test Day 1 and Test Day 7, by rats on a control (CD) or ketogenic (KD) diet, following injections of either saline or cocaine. Data were collapsed across sex (diet \times sex interaction was non-significant). There was a significant diet \times test day interaction, such that the expected increase in cocaine-induced ambulations over time was observed in CD, but not KD, animals. $*p < 0.05$, Test Day 1 vs. Test Day 7 for cocaine- (CD only) and saline- (both CD and KD) injected animals.

KD-treated animals failed to show this sensitizing response across test days ($F(1,21) = 1.512$, $p = 0.23$, $\eta^2 = 0.067$). Similar to rearing results, females engaged in more ambulations (44%) compared to males irrespective of diet condition or test day ($F(1,42) = 4.27$, $p = 0.045$, $\eta^2 = 0.092$). In animals repeatedly injected with saline, ambulations decreased by 52% across test days ($F(1,40) = 28.85$, $p < 0.001$, $\eta^2 = 0.42$). There was no significant effect of diet ($F(1,40) = 3.28$, $p = 0.078$, $\eta^2 = 0.076$), diet \times test day interaction ($F(1,40) = 1.37$, $p = 0.25$, $\eta^2 = 0.033$), or effect of sex ($F(1,40) = 1.94$, $p = 0.17$, $\eta^2 = 0.046$) in these animals.

3.2. Behavioral measures: challenge day

Following a drug-free (no injections) period of one week, male and female rats were observed for cocaine-induced behaviors on a subsequent challenge day. Descriptive, within-session data for the challenge day rearing can be seen in Fig. 2. When comparing animals receiving their first injection of cocaine (Sal-Coc) vs. animals with a history of cocaine injections (Coc-Coc), summed (first 30 min) time spent rearing was 68% higher in Coc-Coc animals ($F(1,88) = 33.18$, $p < 0.001$, $\eta^2 = 0.27$) (Fig. 5). Similar to results obtained during daily

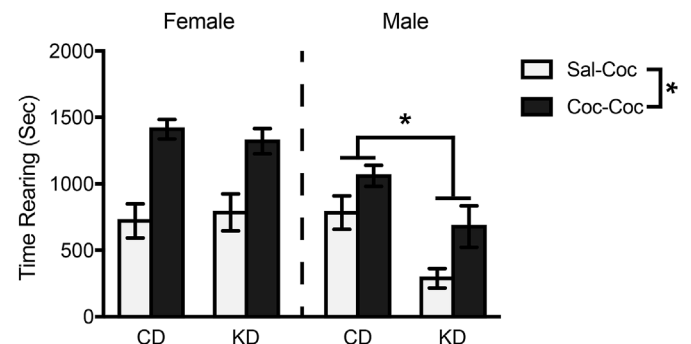


Fig. 5. Mean (\pm SEM) time spent rearing during the first 30 min of the test phase on Challenge Day by female and male rats on a control (CD) or ketogenic (KD) diet, following injections of cocaine. Irrespective of sex, animals with a prior history of cocaine injections (Coc-Coc) spent more time rearing vs. those with a prior history of saline injections (Sal-Coc). A significant diet \times sex interaction revealed that in males, KD treatment resulted in decreased time rearing compared to CD treatment. This effect was not seen in females. $*p < 0.05$, Sal-Coc vs. Coc-Coc comparison, as well as CD vs. KD comparison within males.

Table 1
Ambulations data (challenge day injections).

	Sal-Coc	Coc-Coc
Female		
CD	1909 ± 216	2288 ± 230
KD	2562 ± 306	2626 ± 210
Male		
CD	1776 ± 288	1726 ± 187
KD	1213 ± 149	1661 ± 286

Mean (± SEM) ambulations during the first 30 min following cocaine injections on the Challenge Day. Female and male rats were maintained on either a control (CD) or ketogenic (KD) diet, and had a prior history of either saline (Sal-Coc) or cocaine (Coc-Coc) injections.

injections of cocaine, animals treated with the KD exhibited decreased time spent rearing (22%) compared to CD animals ($F(1,88) = 7.54$, $p = 0.007$, $\eta^2 = 0.079$), and females spent 46% more time rearing compared to males ($F(1,88) = 18.87$, $p < 0.001$, $\eta^2 = 0.18$). A significant diet × sex interaction ($F(1,88) = 6.70$, $p = 0.011$, $\eta^2 = 0.071$) indicated that the effect of diet was conditional on sex. Indeed, a significant decrease in time spent rearing (46%) was observed in KD-vs. CD-treated males ($F(1,88) = 13.67$, $p < 0.001$, $\eta^2 = 0.13$), but not females ($F(1,88) = 0.013$, $p = 0.91$, $\eta^2 < 0.001$).

Cocaine-induced ambulations on the Challenge Day differed across the sexes ($F(1,86) = 9.53$, $p = 0.003$, $\eta^2 = 0.10$), with females showing 42% more ambulations compared to males irrespective of diet or drug history (Table 1). No significant effects of diet ($F(1,86) = 0.005$, $p = 0.94$, $\eta^2 < 0.001$), drug history ($F(1,86) = 0.44$, $p = 0.51$, $\eta^2 = 0.005$) or interaction effects (all $p > 0.05$) were observed.

3.3. Physiological measures

Weight of rats assigned to a KD or a CD were assessed at several time points: prior to the diet condition assignment (Initial Weight); prior to their first (Test Day 1) and seventh (Test Day 7) daily injections of cocaine or saline; and prior to cocaine injections on the Challenge Day. Weight increased by 77% over this time period ($F(3,258) = 2285.63$, $p < 0.001$, $\eta^2 = 0.96$) (Table 2). This effect of time varied across diet condition and sex, as evidenced by significant diet × day × sex interaction ($F(3,258) = 195.54$, $p < 0.001$, $\eta^2 = 0.70$). Within each sex, there were no differences in weight between diet groups initially (females: $F(1,65.45) = 0.68$, $p = 0.41$, $\eta^2 = 0.01$; males: $F(1,52.07) = 0.14$, $p = 0.71$, $\eta^2 = 0.003$); however, weight was significantly decreased in KD vs. CD animals at every other timepoint in both females (Test Day 1: $F(1,65.45) = 183.62$, $p < 0.001$, $\eta^2 = 0.74$; Test Day 7: $F(1,65.45) = 196.49$, $p < 0.001$, $\eta^2 = 0.75$; Challenge Day: $F(1,65.45) = 248.34$, $p < 0.001$, $\eta^2 = 0.79$) and males (Test Day 1: $F(1,52.07) = 494.43$, $p < 0.001$, $\eta^2 = 0.91$; Test Day 7: $F(1,52.07) = 602.92$, $p < 0.001$, $\eta^2 = 0.93$; Challenge Day: $F(1,52.07) = 856.48$, $p < 0.001$, $\eta^2 = 0.94$).

Table 2
Weight data.

	Baseline	Day 1	Day 7	Challenge Day
Females				
CD	147.8 ± 3.4	241.6 ± 3.4	252.5 ± 3.6	275.9 ± 4.0
KD	142.9 ± 3.5	161.2 ± 4.6*	169.3 ± 4.6*	182.3 ± 5.1*
Males				
CD	168.8 ± 5.0	358.6 ± 5.2	385.1 ± 5.2	436.3 ± 5.7
KD	165.9 ± 4.9	184.0 ± 6.2*	192.3 ± 6.2*	206.5 ± 7.1*

Mean (± SEM) weight taken at Baseline (just prior to diet assignment), and just prior to Day 1, 7, and Challenge Day injections. Female and male rats were maintained on either a control (CD) or ketogenic (KD) diet. * $p < 0.05$, within column comparison of diet within each sex.

The effect of diet on weight gain raises the possibility that diet-induced weight changes may be a causal factor driving the reported behavioral effects. To address this issue, we first generated Challenge Day weight z-scores (each score standardized to its treatment group mean and standard deviation). These scores were then pooled within each sex and compared to time spent rearing following cocaine injections on the Challenge Day. We found that there was no correlation between weight z-scores and time spent rearing in either female ($r = -0.043$, $p = 0.77$) or male ($r = 0.0022$, $p = 0.99$) animals (Fig. 6).

Twenty-four hours following the Challenge Day test, rats were sacrificed and blood glucose and ketone levels were assessed. Blood ketone levels were 107% higher in animals on a KD vs. those on a CD ($F(1,68) = 59.94$, $p < 0.001$, $\eta^2 = 0.47$) (Fig. 7). In contrast, blood glucose levels were 11% lower in KD-vs. CD-treated animals ($F(1,68) = 9.39$, $p = 0.003$, $\eta^2 = 0.12$).

4. Discussion

Here we show that ambulatory and stereotyped locomotor responses are disrupted in cocaine-injected male and female rats administered a KD. Sensitization of cocaine-induced ambulatory activity was not observed in KD-fed animals; in addition, animals on a KD showed reduced rearing during the initial week of repeated cocaine injections (both sexes) as well as when challenged with cocaine one week following their last injection (males only). In contrast, there was no effect of KD administration on behavioral responses in animals injected with saline. Considered together, these data are the first to show that KD treatment can modify behavioral responses to psychostimulants, and suggest that KDs hold potential as a novel therapy for the treatment of psychostimulant addiction.

Therapy with a KD may reduce behavioral responses to psychostimulants via multiple mechanisms. These diets are associated with decreased neuroinflammation, stabilized glucose levels, and improved mitochondrial function (Masino and Rho, 2010) – all of which represent positive effects on factors that are negatively impacted by psychostimulant addiction (Chang et al., 2007; de Oliveira and Jardim, 2016; Moreira et al., 2016). Hallmarks of KD administration are increased blood ketones and reduced blood glucose, although these metabolic effects are not necessarily coupled tightly to other beneficial effects or behavioral changes. For example, the KD-induced decrease in stereotyped responses we observed during the week of daily cocaine injections was not maintained on the challenge day in both sexes (i.e., it persisted in males but not females) – despite no differences in the diet-induced increase in ketones or decrease in glucose across the sexes. We and others have found dissociations between metabolic and behavioral results in other models (Dallérac et al., 2017; Ruskin et al., 2013; Schoeler et al., 2017; Simeone et al., 2017; Viggiano et al., 2016), and we have previously identified behavioral effects of this diet that are sex specific (Ruskin et al., 2011, 2017a, 2017b; Ruskin, 2016). There are limited other studies on sex differences in KD effects (Chun et al., 2018; Zengin et al., 2016), but to our knowledge, no additional laboratories have published sex differences in behavioral effects of KD. Notably, sex differences in the ability of both diet and non-diet therapies to modulate behavioral responses to cocaine have been reported previously (Collins et al., 2015; Poland et al., 2016; Sershen et al., 1998).

As a link between metabolism, neural activity and behavior, KD-induced increases in the neuromodulator adenosine (Kawamura et al., 2014; Lusardi et al., 2015), potentially consequent to increasing ATP availability in the brain (Masino et al., 2009), may be particularly relevant to psychostimulants and dopaminergic systems. While some adenosine-mediated effects may be receptor-independent (Williams-Karnesky et al., 2013), it is well established that high-affinity adenosine A₁R and A_{2A}R receptor subtypes are found within the striatum and form heterodimers with dopamine receptor subtypes that would be activated by cocaine administration (e.g., A_{2A}R with dopamine D₂ receptors

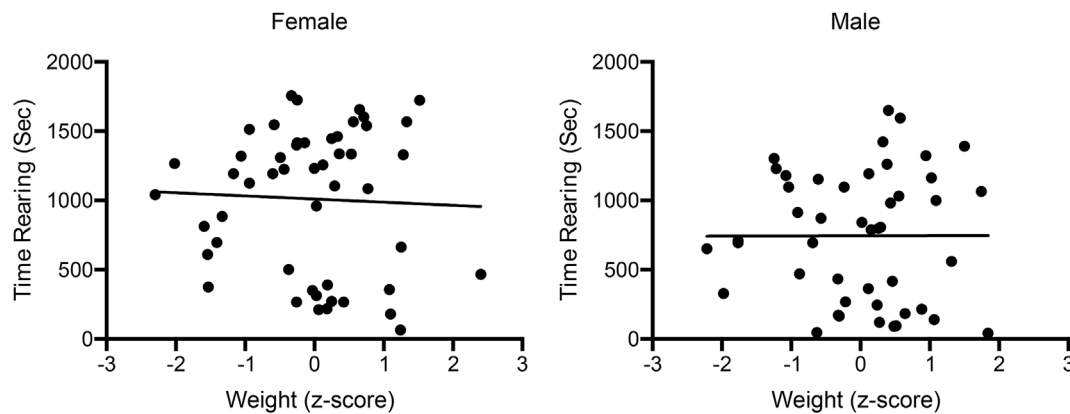


Fig. 6. Time spent rearing and standardized (within treatment group) weight of individual female and male rats on the Challenge Day. No significant relationship was observed between these two variables, in either sex. For each graph, the line of best fit (linear) is superimposed.

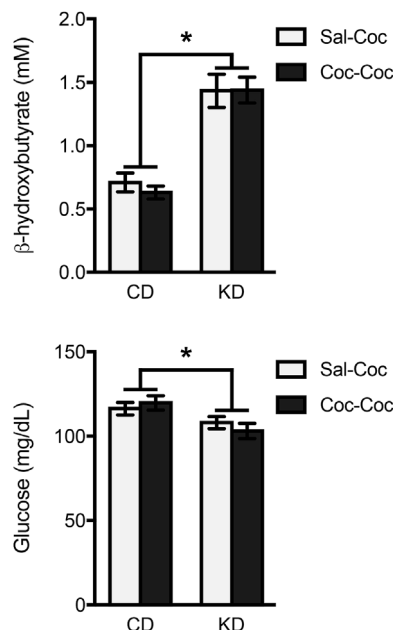


Fig. 7. Mean (\pm SEM) blood ketone (β -hydroxybutyrate) and glucose levels taken at the conclusion of the experiment. Data were collapsed across sex (diet \times sex interaction was non-significant). Rats treated with a ketogenic diet (KD) had significantly higher blood ketone levels and significantly lower blood glucose levels vs. control diet (CD)-treated rats. * $p < 0.05$, CD vs. KD comparison.

(D₂R); A₁R with dopamine D₁ receptors (D₁R)) (Ferré et al., 2008, 1997). Accordingly, KD-mediated changes in adenosine may have therapeutic potential via actions at adenosine-dopamine receptor heterodimers (Filip et al., 2012). In support of this idea, selective disruption of the A_{2A}R-D₂R heterodimer was found to prevent the reduction in cocaine self-administration normally seen following injections of the A_{2A}R agonist CGS 21680 (Borrito-Escuela et al., 2018).

Some details of the present findings merit discussion. First, the link between treatment with a KD and decreased weight in both sexes potentially complicates the dissociation of KD effects on behavior (e.g., rearing) from its effects on weight. However, our analyses suggest that these effects can indeed be dissociated. KD treatment did not affect cocaine-induced rearing (females) or ambulatory activity (both sexes) on the Challenge Day, despite the significant lower weight observed in KD vs. CD animals at this time point. Importantly, we also found no correlation between Challenge Day rearing and weight (standardized within each treatment group) in either sex. Taken together, these results suggest that weight is unlikely to be a causal factor driving the

behavioral differences observed across diet conditions. Second, the present study utilizes behavioral sensitization to cocaine as an initial means of assessing interactions between a KD and psychostimulant-induced behavior. While the cocaine administered in sensitization protocols is non-contingent (i.e., drug delivery is under experimenter, rather than animal, control), this model and the positive effects of the KD may be relevant to addiction for several reasons. Repeated injections of cocaine induce lasting neurobiological responses commonly associated with psychostimulant addiction, including enhanced cocaine-induced dopamine release (Hooks et al., 1994; Kalivas and Duffy, 1990) and structural plasticity (Ferrario et al., 2005; Robinson and Kolb, 1999) within the ventral striatum. Furthermore, a previous history of cocaine injections augments the acquisition of cocaine self-administration (Childs et al., 2006). Additional studies will be required to test directly how the KD specifically impacts the development and progression of psychostimulant addiction, particularly in relation to the rewarding/reinforcing effects of these drugs. Third, all behavioral testing was performed while KD treatment was ongoing. There are indications that the anticonvulsant effects of the KD outlast the diet itself (Bough et al., 2006; Caraballo et al., 2011; Lusardi et al., 2015; Martinez et al., 2007; Patel et al., 2010); whether its impact on cocaine-induced responses are similarly persistent remains to be explored.

In summary, we found that ambulatory and stereotyped locomotor responses induced by cocaine were disrupted in KD-treated male and female rats. This work aligns with a broadening therapeutic scope for metabolic therapies, as well as a significant and growing interest in the relationship among purines, energy homeostasis, and drug addiction (Lindberg et al., 2015). The impact of the KD on acute and chronic dopamine-mediated behaviors observed here suggests that this diet may hold therapeutic potential for neuropsychiatric and neurodegenerative diseases beyond drug addiction. Future work will be required to determine if this is indeed the case.

Declarations of interest

None.

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