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Trait specific modulatory effects of caffeine exposure on compulsive-like behaviors in a spontaneous mouse model of obsessive-compulsive disorder

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Obsessive-compulsive disorder (OCD) is a psychiatric disorder characterized by recurring intrusive thoughts and repetitive compulsive behaviors, ultimately interfering with their quality of life. The complex heterogeneity of symptom dimensions across OCD patient subgroups impedes diagnosis and treatment. The core and comorbid symptomologies of OCD are thought to be modulated by common environmental exposures such as consumption of the psychostimulant caffeine. The effect of caffeine on the expression of obsessions and compulsions are unexplored. The current study utilized mouse strains (HA) with a spontaneous, predictable, and stable compulsive-like phenotype that have face, predictive, and construct validity for OCD. We demonstrate that an acute high dose (25 mg/kg) of caffeine decreased compulsive-like nest-building behavior in the HA strains in the first hour after injection. However, nest-building scores increased in hours 3, 4, and 5 after administration finally decreasing over a 24 h period. In contrast, a high dose of chronic caffeine (25 mg/kg/d) increased nest-building behavior. Interestingly for compulsive-like digging behavior, acute exposure to a high dose of caffeine decreased the number of marbles buried, while chronic exposure had little effect. An acute high dose of caffeine decreased anxiety-like and motor activity in open field behaviors whereas chronic caffeine administration did not have any overall effect on open field activity. The results, therefore, suggest a complex role of caffeine on compulsive-like, anxiety-like, and locomotor behaviors that is dependent on the duration of exposure. ***Behavioural Pharmacology*** **31: 622–632**

Behavioural Pharmacology 2020, **31**:622–632

Keywords: anxiety-like, caffeine, compulsive-like, mice, obsessive-compulsive disorder

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Received 4 June 2019 **Accepted as revised** 16 April 2020

Introduction

Caffeine (1,3,7-trimethylxanthine) is the most common psychostimulant, consumed by 90% of the worldwide adult population (Fredholm *et al.*, 1999). Caffeine has been linked to many health-enhancing effects in humans such as increased alertness (Smith, 2002), enhanced long-term memory (Angelucci *et al.*, 2002; Borota *et al.*, 2014) and improved motor performance (Almosawi *et al.*, 2018). Animal studies have also demonstrated that caffeine improves spatial learning in a rat model of attention deficit-like behaviors (Prediger *et al.*, 2005) and decreases aggression. In mice, caffeine increases exploratory activity and aggressive-like behaviors (Valzelli and Bernasconi, 1973). At low doses, caffeine increases locomotor activity while it lowers activity at high doses (El Yacoubi *et al.*, 2000). It is believed that these modulatory effects of caffeine are due to its preferential antagonizing properties on adenosine A1 and A2A receptors in the brain (Huang *et al.*, 2005; Ballesteros-Yáñez *et al.*, 2012). Over the years, performance-enhancing effects of caffeine have been utilized as a potential market for caffeinated products targeting primarily adolescents and young adults (Claghorn *et al.*, 2017). Access to varieties of caffeinated beverages has caused a surge in the unregulated and disproportionately high consumption of caffeine among the population. It is not until recently that the harmful effects of chronic caffeine consumption have started emerging, such as cardiovascular anomalies, sleep disturbances, and substance abuse (Temple *et al.*, 2017). The modulatory effects of caffeine are further compounded in individuals with a history of mental illness. Studies on patient cohorts have indicated that caffeine consumption can produce varied effects in patients suffering from mental

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disorders, such as reduced depression, manic symptoms, and anxiety (Lara, 2010; Wang *et al.*, 2016). The specific interaction of caffeine and obsessive-compulsive disorder (OCD) is currently understudied (Koran *et al.*, 2009), and chronic caffeine treatment with a dose gradually built up to 300 mg once a day over several weeks caused a modest reduction in OCD symptoms in treatment-resistant OCD patients (Shams *et al.*, 2019).

OCD is a relatively common psychiatric disorder that affects approximately 3.5 million patients in the USA each year (Ruscio *et al.*, 2010). OCD patients have recurring obsessive thoughts, such as contamination fear, sexual or religious obsessions, or need for symmetry/order, that leads to compulsive behaviors, such as excessive hand washing, cleaning, hoarding, or counting (Murphy *et al.*, 2010). While obsessions and compulsions are cardinal features of OCD, the specific content of these obsessions and compulsions vary widely among patient cohorts (Murphy *et al.*, 2010) imparting a complex clinical heterogeneity to the disorder. Generally, four to seven dimensions are recognized that include symmetry/ordering/counting/incompleteness, hoarding, contamination/cleaning, obsessions/checking, aggression/violence, superstitions/rituals, and taboo/sexual/religious. The specific dimensions vary between studies (Mataix-Cols *et al.*, 2005; Katerberg *et al.*, 2010; Ruscio *et al.*, 2010; Torresan *et al.*, 2013; Pauls *et al.*, 2014; McCarty, 2017) which shows no consensus in the field. This heterogeneity in OCD symptoms complicates the identification of candidate genes (Pauls *et al.*, 2014) and the choice of initial pharmacotherapy, which may explain why 40–60% of OCD patients do not respond to initial selective serotonin reuptake inhibitors treatment (Pigott and Seay, 1999). This necessitates repeated intervention with different drugs until a response is observed (Jenike, 2004). OCD is often associated with other psychiatric disorders, such as major depression, social phobia, Tourette's syndrome, social and generalized anxiety, bipolar, attention deficit-hyperactivity, dysthymic, and alcohol use disorders. People with autism spectrum disorders often have OCD-like symptoms (Jacob *et al.*, 2009; Postorino *et al.*, 2017). The pattern of comorbidities is quite variable among OCD patients (Murphy *et al.*, 2013) with high drug-resistant rates (Pallanti *et al.*, 2011) that decreases the quality of life (Campos *et al.*, 2015). Considering the clinical heterogeneity among patient populations it is feasible that psychostimulants will have differential effects among various patient subgroups. In fact, OCD patients with comorbid bipolar disorders become easily addicted to caffeine indicating possible interactions between caffeine, bipolar disorder, and OCD traits (Perugi *et al.*, 2002; Masi *et al.*, 2007).

Utilizing mouse strains with a spontaneous, predictable and stable compulsive-like phenotype that have face, predictive, and construct validity for OCD (Greene-Schloesser *et al.*, 2011; Mitra *et al.*, 2016a; Mitra *et al.*, 2017a) can be a valuable starting point to investigate the interaction of genetic background and specific traits on psychostimulant responses (Mao *et al.*, 2015; Mitra and Bult-Ito, 2018). Hence, using the compulsive-like strains of mice (Mitra *et al.*, 2017a; Mitra and Bult-Ito, 2018) we hypothesized that exposures to acute and chronic caffeine will result in differential behavioral expressions pertaining to compulsive-like and anxiety-like domains.

Methods

This project was conducted as per the University of Alaska Fairbanks Institutional Animal Care and Use Committee approved animal care and experimental procedures (IACUC assurance numbers 911872: chronic caffeine study; 1516416: acute caffeine study). Only male mice were used.

Experimental subjects

The mouse model of OCD was developed from house mouse strains (*Mus musculus*) through bidirectional selection for nest-building behavior (Lynch, 1980; Bult and Lynch, 2000). The HS/Ibg outbred strain (McClearn *et al.*, 1980), which was developed through crossing of eight inbred strains (A, AKR, BLB/c, C3H/2, C57BL, DBA/2, Is/Bi, and RIII), served as the stock population for the selective breeding (Lynch, 1980). Bidirectional selection resulted in two compulsive-like strains (H1 and H2, Lynch, 1980; and HA1 and HA2, Bult and Lunch, 2000), which consistently exhibit a 40-fold higher level of compulsive-like nest-building behavior (Lynch, 1980; Bult and Lynch, 2000) and a three-fold higher level of compulsive-like digging in the marble-burying behavioral test (Greene-Schloesser *et al.*, 2011; Mitra *et al.*, 2017a) when compared to the two non-compulsive-like strains (C1 and C2, Lynch, 1980; and CA1 and CA2, Bult and Lunch, 2000). The two randomly-bred control strains (C1 and C2, Lynch, 1980; and CA1 and CA2, Bult and Lunch, 2000) express intermediate levels of these behaviors (Lynch, 1980; Bult and Lynch, 2000; Greene-Schloesser *et al.*, 2011; Mitra *et al.*, 2017a). This excessive, repetitive and perseverant otherwise normal nest-building and digging behaviors by the compulsive-like strains make them a good model to study compulsive-like phenotypes (Greene-Schloesser *et al.*, 2011, Mitra et al., 2016; Mitra and Bult-Ito, 2017; Mitra et al., 2017a, 2017b, 2017c; Winter *et al.*, 2018).

The originally selected lines were designated H1, H2, C1, C2, L1, and L2 (Lynch, 1980). Subsequently, the H1 and H2 strains were crossed and reselected for building big nests resulting in the HA1 and HA2 strains. The L1 and L2 strains were crossed and reselected for building small nests resulting in the LA1 and LA2 strains. The C1 and C2 strains were crossed and maintained by random breeding resulting in the CA1 and CA2 strains (Bult and Lynch, 2000). Subsequent research established that these mouse strains reflected compulsive-like (HA1 and

HA2 strains) and non-compulsive-like (LA1 and LA2 lines) phenotypes with randomly-bred CA1 and CA2 as intermediary controls (Greene-Schloesser *et al.*, 2011; Mitra *et al.*, 2017b). After 56 generations of selection, the strains were maintained by random breeding. The HA1 and HA2 strains were crossed in 2013 to yield the HA3 strain. This was done to preserve about 50% of the genetic information contained in the HA2 strain as its fertility was declining towards extinction. Subsequently, the HA1 and HA3 strains were crossed to yield the HA4 for the same reason. For the chronic caffeine experiment, the HA1 and HA3 strains were used (the BIG1 and BIG2 strains, respectively, as previously described (Mitra *et al.*, 2016a; Mitra *et al.*, 2017a; Mitra and Bult-Ito, 2018). For the acute caffeine experiment, the HA3 and HA4 strains were used due to the extinction of the HA1 strain. The HA4 strain exhibit consistent and comparable compulsive-like nest-building and marble burying phenotypes with the HA1 strain (without any inter-strain differences) and hence was a suitable model to investigate the acute effects of caffeine. The goal of the current study was to establish the role of caffeine exposure on compulsive-like and anxiety-like phenotypes in spontaneous compulsive-like mouse strains. The rationale for utilizing multiple compulsive-like strains was to examine if genotypic variations can influence the modulatory effect of caffeine on behavioral expressions pertaining to various affective domains.

Compulsive-like behavior in our mice is determined as an excessive and persistent expression of otherwise normal behaviors, that is, nest-building and digging. A rapid and repeated movement of the front paws and mouth are initiated in the compulsive-like strains when introduced to cotton (Greene-Schloesser *et al.*, 2011). This perseverant action leads to pulling of excessive amount of cotton through the cage top metal bars over prolonged periods, which shows face validity for OCD. This compulsive-like phenotype is significantly attenuated when introduced to first-line pharmacotherapies, such as fluoxetine, fluvoxamine, and clomipramine, which shows predictive validity for OCD (Greene-Schloesser *et al.*, 2011; Mitra and Bult-Ito, 2018). A similar behavioral phenotype is also seen in the compulsive-like strains when evaluated for digging behavior in the marble-burying test (Greene-Schloesser *et al.*, 2011; Mitra et al., 2016a, 2016b; Mitra et al., 2017a, 2017b; Mitra and Bult-Ito, 2018). Construct validity (Zike *et al.*, 2017) is demonstrated by desipramine having no effect on compulsive-like nest-building behavior (Greene-Schloesser *et al.*, 2011) and the involvement of the serotonergic (Greene-Schloesser *et al.*, 2011; Mitra et al., 2016a, 2016b; Winter *et al.*, 2018), cholinergic, estrogenic, oxytocinergic, and GABAergic neurotransmitter pathways (Mitra *et al.*, 2016; Mitra *et al.*, 2017; Winter *et al.*, 2018). Due to the varied levels of compulsive-like nest-building and marble burying behaviors among the strains they represent a certain level of phenotypic variation (Mitra and Bult-Ito, 2018) recapitulating some aspects of clinical heterogeneity (Katerberg *et al.*, 2010; Mataix-Cols *et al.*, 2005; McCarty, 2017; Pauls *et al.*, 2014; Ruscio *et al.*, 2010; Torresan *et al.*, 2013).

Mice were group-housed in polypropylene cages (27 × 17 × 12 cm; four mice per cage) with wood shavings and free access to food (Lab Diet Mouse Diet #5015; Purina Mills, St Louis, Missouri, USA) and water under a 12–12 light-dark cycle. Pups were weaned when they reached 19–21 days old and housed with same sex littermates. Experimental animals were aged matched (~60 days old) for the acute and chronic caffeine studies.

Experimental design

Each male mouse remained in the same caffeine dose treatment group throughout the acute and chronic caffeine studies.

Acute caffeine study

In the acute caffeine study, male mice received a subcutaneous injection containing 0, 3, or 25 mg/kg doses of caffeine in saline. Compulsive-like and anxiety-like behaviors were measured in the compulsive-like HA mouse strains as described in the Behavioral Tests section below.

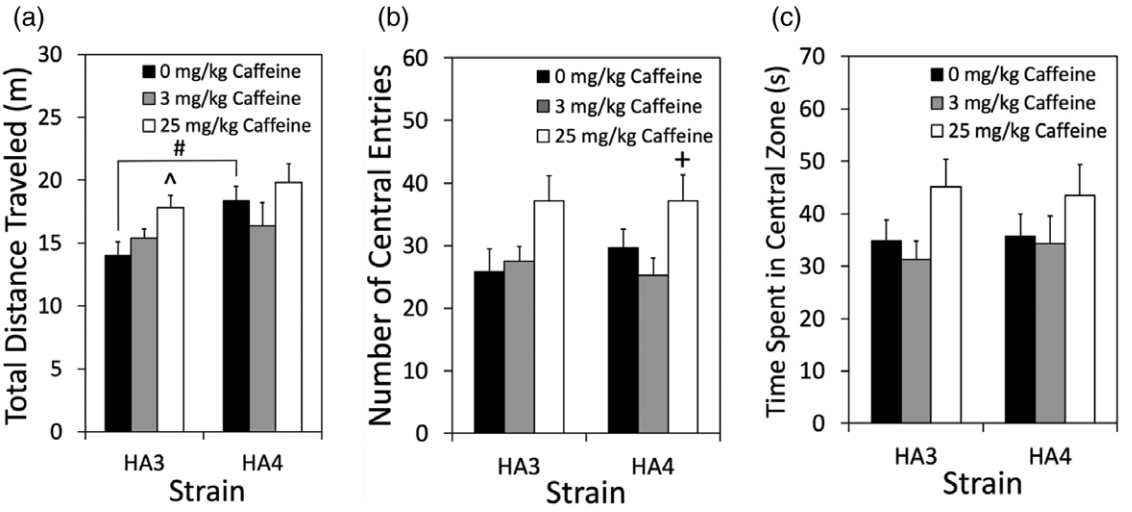
Chronic caffeine study

To investigate the chronic effects of 4 weeks of caffeine exposure in the compulsive-like mouse strains, male mice received caffeine in their drinking water at low (3 mg/kg/d) and high (25 mg/kg/d) doses of caffeine, and a 0 mg/kg tap water control. Compulsive-like nest-building and marble burying were performed once every week; that is, 1 week before treatment (week 1), four times during the caffeine treatment period (weeks 2, 3, 4, and 5), and 1 week after cessation of treatment (week 6). The anxiety-like open field test was performed before treatment (week 1), in the final week of caffeine treatment (week 5), and 1 week after cessation of treatment (week 6) to minimize exposure to the open field as successive exposures reduce open field performance (Fig. 4). Nest-building and marble burying behaviors were measured on days 2 and 3 (week 1), 12 and 13 (week 2), 19 and 20 (week 3), 27 and 28 (week 4), 34 and 35 (week 5), and 41 and 42 (week 6), respectively. The open field was conducted thrice (day 1 of week 1, day 33 of week 5, and day 40 of week 6). Day 7 was the first day of caffeine treatment and day 35 was the last day of caffeine treatment.

Drugs

Caffeine (Cat no: 24277682) was purchased from Sigma-Aldrich (St. Louis, Missouri, USA). For the acute study, caffeine was dissolved in sterile saline with a concentration to match a 3 mg/kg or 25 mg/kg dose in a subcutaneous injection volume of 0.30 ml per 40 g mouse, or 0.0075 ml per 1 g of body weight. Sterile saline was used as the control

Fig. 1



Mean (±SEM) total distance traveled for the HA3 and HA4 compulsive-like male mice (a), and number of central entries (b) and time spent in the central zone (c) in the open field test one hour after a subcutaneous injection with a 0, 3, or 25 mg/kg caffeine dose. ^$P < 0.05$ compared to the 0 mg/kg/d caffeine dose group. +$P < 0.05$ compared to the 3 mg/kg/d caffeine dose group. #$P < 0.05$ HA3 and HA4 groups at 0 mg/kg caffeine dose.

treatment. The caffeine and saline final solutions were sterilely filtered using a sterile Acrodisc 0.2 µm syringe filter into sterile 10 ml empty amber vials. The caffeine final solution was made fresh before each set of injections. For the chronic study, caffeine (3 mg/kg/d and 25 mg/kg/d) was dissolved in sucrose tap water (2.9 g/L) and given orally in graduated water bottles. The vehicle group received a sucrose solution (2.9 g/L) in tap water. Water consumption was measured for 3 days to establish baseline water intake. Based on the average water consumption, the caffeine concentration was determined to achieve the daily intake of caffeine, that is, 0, 3, or 25 mg/kg. Water volume was checked every 12 h and water bottles were changed every other day with fresh sucrose and caffeine solutions. Water consumption was measured through volume loss daily and mice were weighed once a week throughout the study, and caffeine solutions were adjusted as appropriate.

Behavioral tests

Open field behaviors

Animals were assessed for anxiety-like and locomotor activity in the open field test (Simon *et al.*, 1994; Prut and Belzung, 2003). Animals undergoing testing were transported in home cages and were housed outside the testing room prior to testing. The open field apparatus consisted of an arena (40 × 40 × 30 cm). Testing was conducted for 5-minute (acute caffeine study) or 3-minute (chronic caffeine study) durations. For the acute study, testing was started 1 h after injection. Animals were individually placed in the center of the field and allowed to explore the arena. Time spent and the total number of central entries in the inner zone were evaluated as anxiety-like measures (Mitra *et al.*, 2016a; Mitra *et al.*, 2016b; Mitra *et al.*, 2017a; Mitra *et al.*, 2017b; Mitra and Bult-Ito, 2018). Total distance traveled was considered for assessing locomotor activity (Tatem *et al.*, 2014; Mitra *et al.*, 2016a; Mitra *et al.*, 2017a; Mitra and Bult-Ito, 2018). All experimental parameters were recorded by the ANYMaze video tracking system (Stoelting Co., Wood Dale, Illinois, USA). The apparatus was cleaned with a dilute chlorhexidine solution and dried before each test. Following the test, the animals were returned to their home cages with same sex littermates.

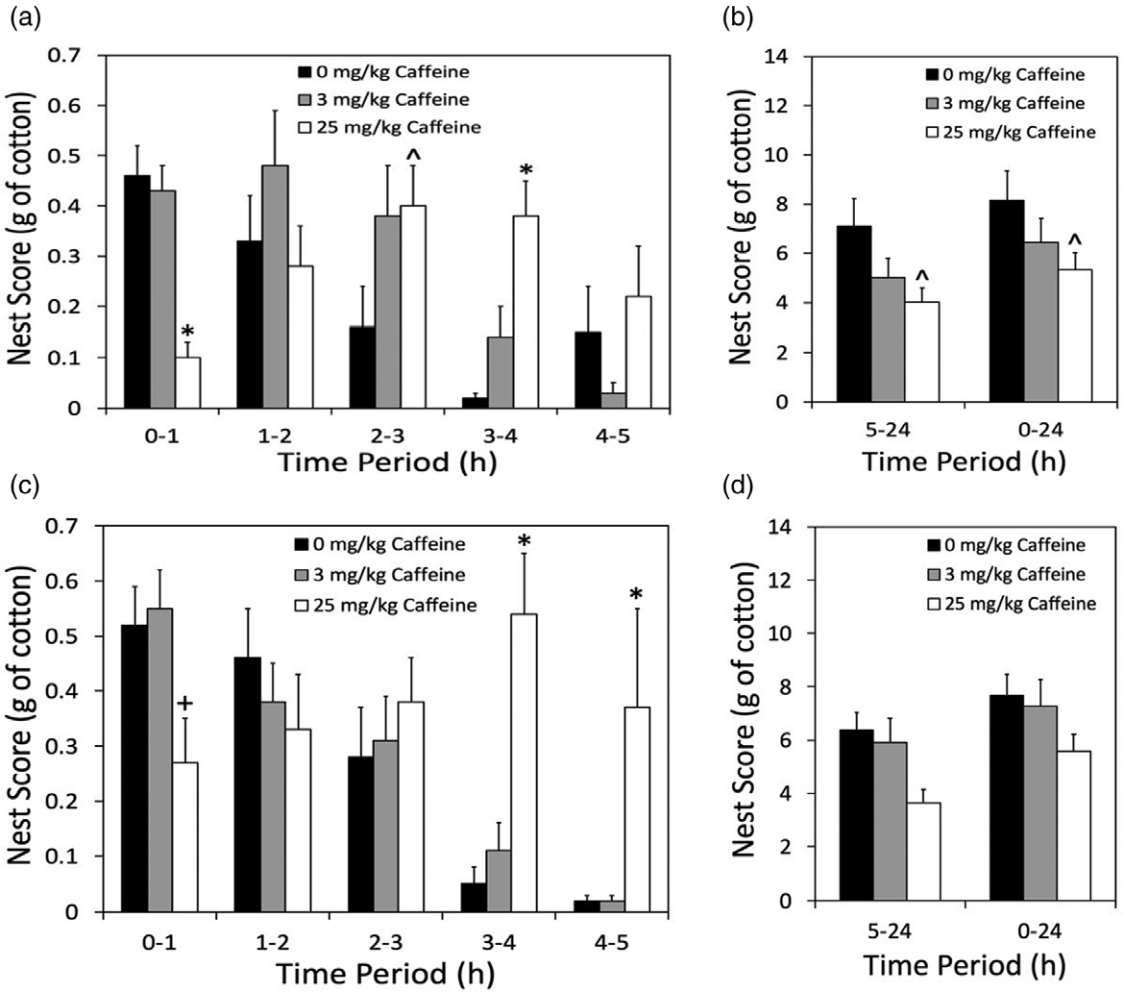
Nest-building behavior

Nest-building behavior was used to assess the compulsive-like phenotype of the mice (Greene-Schloesser *et al.*, 2011; Mitra et al., 2016a, 2016b; Mitra et al., 2017a, 2017b; Mitra and Bult-Ito, 2018). All mice were individually housed in a clean mouse cage and were allowed to access a pre-weighed cotton roll placed in the cage top food hopper. For the acute caffeine study, the amount of cotton used by the mice was determined 0–1 h, 1–2 h, 2–3 h, 3–4 h, 4–5 h, 5–24 h, and 0–24 h after the subcutaneous injection by weighing the cotton roll at 0, 1, 2, 3, 4, 5, and 24 h after injection. For the chronic caffeine study, the amount of cotton used by the mice after 24 h was determined by weighing the cotton roll at time 0 and 24 h (Bult and Lynch, 1996, 1997, 2000; Lynch, 1980). Following the test, the animals were returned to their home cages with same sex littermates.

Marble burying behavior

The marble-burying test was used to evaluate compulsive-like digging behavior (Takeuchi *et al.*, 2002; Thomas

Fig. 2



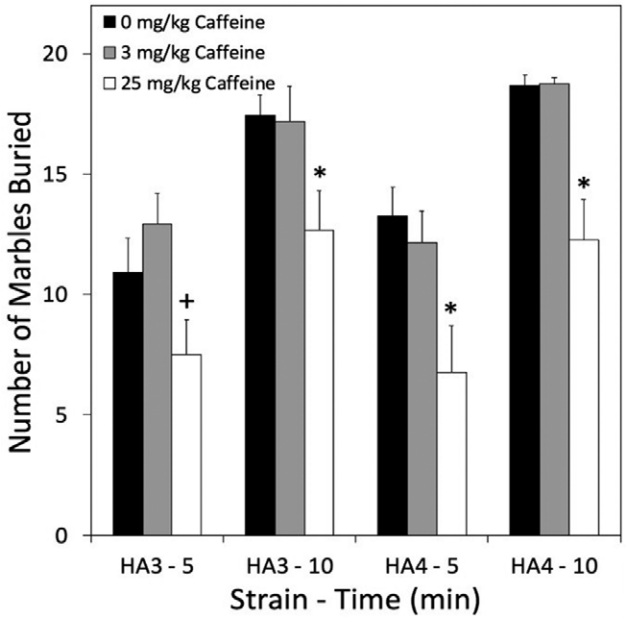
Mean (±SEM) nest building scores for HA3 (a) and HA4 (c) compulsive-like male mice during the first 5 h, and 5–24 h and 0–24 h (b – HA3, d – HA4) after a subcutaneous injection with a 0, 3, or 25 mg/kg caffeine dose. *$P < 0.05$ compared to the 0 and 3 mg/kg/d caffeine dose groups. ^$P < 0.05$ compared to the 0 mg/kg/d caffeine dose group. +$P < 0.05$ compared to the 3 mg/kg/d caffeine dose group.

et al., 2009; Greene-Schloesser *et al.*, 2011; Angoa-Pérez *et al.*, 2013). All experimental mice were individually introduced to a polypropylene cage (37 × 21 × 14 cm) containing 20 glass marbles (10 mm in diameter) evenly spaced on firmly pressed 5 cm deep wood shavings bedding with no access to food or water for 10 min. The total number of marbles buried at least 2/3 at 5 minutes and 10 minutes for the acute and 10-minute for the chronic studies were quantified as compulsive-like digging behavior (Greene-Schloesser *et al.*, 2011). For the acute study, testing was started 1 h after injection. Following the test, the animals were returned to their home cages with same sex littermates.

Statistical analysis

All statistical analyses were performed with Statistical Analysis Software (SAS version 9.4, Cary, NC). For the acute caffeine study, open field behaviors and nest building behavior during the 5-25 h and 0-24 h periods were tested in a general linear model (GLM) analysis of variance (ANOVA) for effects of strain (HA3 and HA4), caffeine dose (0, 3, and 25 mg/kg), and interaction effect (strain by caffeine dose). Nest building during the first 5 h after injection were tested in a repeated measure (time: nesting 0-1 h, 1-2 h, 2-3 h, 3-4 h, and 4-5 h) GLM ANOVA for effects of strains (HA3 and HA4), caffeine dose (0, 3, and 25 mg/kg), and interaction effects (strain by caffeine dose, strain by time, time by caffeine dose, and strain by caffeine dose by time). Marble burying behavior was tested in a repeated measure (time: 5 and 10 min) GLM ANOVA for effects of strains (HA3 and HA4), caffeine dose (0, 3, and 25 mg/kg), and interaction effects (strain by caffeine dose, strain by time, time by caffeine dose, and strain by caffeine dose by time). For the chronic caffeine study, open field, marble burying, and nest building behaviors were tested in a repeated measures (time: open field: weeks 1, 5, and 6; marble burying and nest building: weeks 1, 2, 3, 4, 5, and 6) GLM ANOVA for effects of

Fig. 3



Mean (± SEM) number of marbles buried after 5 and 10 minutes for the HA3 and HA4 compulsive-like male mice one hour after a subcutaneous injection with a 0, 3, or 25 mg/kg caffeine dose. *$P < 0.05$ compared to the 0 and 3 mg/kg caffeine dose groups. +$P < 0.05$ compared to the 3 mg/kg/d caffeine dose groups. Each mean at the 10-minute time point was significantly higher compared to its equivalent mean at the 5-minute time point ($P < 0.05$).

strains (HA1 and HA3), caffeine dose (0, 3, and 25 mg/kg/day), and interaction effects (strain by caffeine dose, strain by time, time by caffeine dose, and strain by caffeine dose by time). Wherever significance was found an appropriate post hoc pair-wise comparison was conducted using the Tukey's Studentized Range Test. For nest-building behavior, the amount of cotton used in grams was square root transformed to obtain a more normal distribution (Bult and Lynch, 1996, 1997, 2000; Lynch, 1980). All values are expressed as mean ± SEM and statistical significance was set at a probability level of $P < 0.05$.

Results

Anxiety-like behaviors

An acute high dose of caffeine increased locomotor activity and decreased anxiety-like behaviors in the compulsive-like HA3 and HA4 mouse strains

The HA4 mice had significantly higher locomotor active levels in the open field than the HA3 mice ($F_{1,65} = 6.84$, $P < 0.012$) as measured by the distance traveled (Fig. 1a), while the acute 25 mg/kg caffeine group of the HA3 strain was more active than the 0 mg/kg caffeine dose group (Fig. 1a), which explains the significant dose-effect ($F_{2,65} = 3.57$, $P < 0.034$). The non-significant strain by dose interaction effect ($F_{2,65} = 1.13$, $P > 0.32$) demonstrated that the strains responded similarly to caffeine (Fig. 1a). The acute 25 mg/kg dose increased the number of central entries (Fig. 1b) and the time spent in the central zone (Fig. 1c) compared to the 0 and 3 mg/kg doses ($F_{2,65} = 6.48$, $P < 0.003$). The strain, strain by dose interaction effects for the number of central entries ($F_{1,65} = 0.07$, $P > 0.79$; $F_{2,65} = 0.44$, $P > 0.64$, respectively) and the time spent in the central zone ($F_{1,65} = 0.05$, $P > 0.83$; $F_{2,65} = 0.13$, $P > 0.87$, respectively) were NS as shown in Fig. 1b and c, respectively.

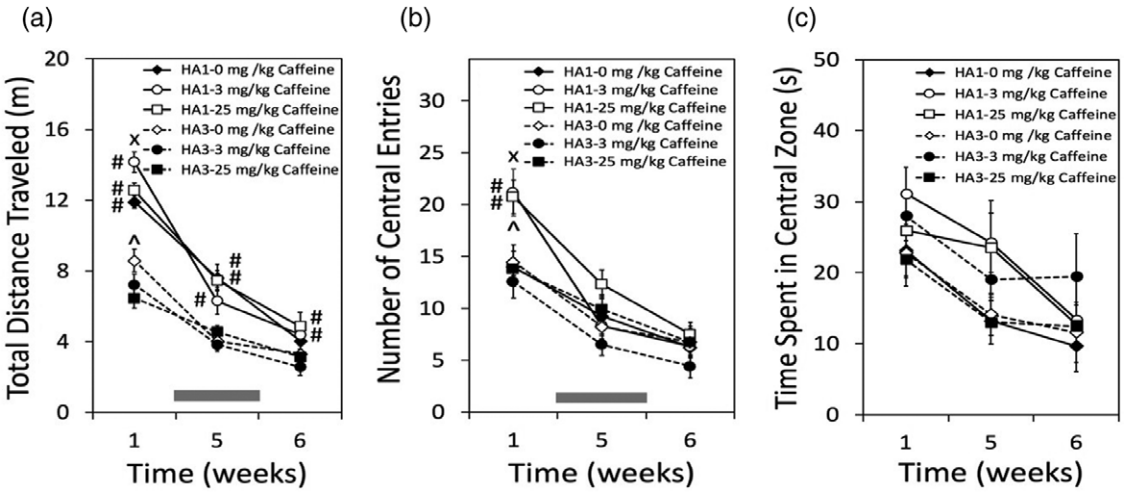
Chronic caffeine administration did not influence activity and anxiety-like open-field behavior in the HA1 and HA3 compulsive-like strains markedly

No significant chronic caffeine dose ($F_{2,66} = 0.13$, $P > 0.87$) and strain by dose interaction ($F_{2,66} = 1.95$, $P > 0.15$) effects were found on the distance traveled, while the HA1 strain was more active than the HA3 strain ($F_{1,66} = 149.67$, $P < 0.0001$) at most time points (Fig. 4a). Both strains reduced locomotor activity with successive open field tests ($F_{2,132} = 280.14$, $P < 0.0001$), but the activity of the HA1 strain decreased more rapidly than the activity of the HA3 strain ($F_{2,132} = 26.29$, $P < 0.0001$) (Fig. 4a). The dose by time ($F_{4,132} = 2.83$, $P < 0.03$) and strain by dose by time interaction ($F_{4,132} = 3.21$, $P < 0.015$) effects were significant, due to different dose groups responding differently over time and by strain (Fig. 4a).

For the number of central entries, the chronic 3 and 25 mg/kg/d dose groups of the HA1 strain by chance started with higher values compared to the 0 mg/kg/d dose group and all dose groups if the HA3 strain ($F_{1,66} = 12.21$, $P < 0.001$) (Fig. 4b). The chronic 3 and 25 mg/kg/d dose groups of the HA1 strain decreased more over successive open field tests than the 0 mg/kg dose ($F_{2,66} = 3.45$, $P < 0.038$), while the HA3 strain did not show a clear dose-effect, which explains the significant strain by dose interaction effect ($F_{2,66} = 3,14$, $P < 0.05$). Both strains reduced the number of central entries over time ($F_{2,132} = 93.81$, $P < 0.0001$), although the chronic 3 and 25 mg/kg/d dose groups of the HA1 strain did this more quickly over time than the dose groups of the HA3 strain (Fig. 4b) resulting in a significant strain by time interaction effect ($F_{2,132} = 4.57$, $P < 0.014$). The dose by time ($F_{4,132} = 1.89$, $P > 0.12$) and strain by dose by time ($F_{4,132} = 1.68$, $P > 0.16$) interaction effects were NS.

For the time spent in the central zone, the strain effect ($F_{1,66} = 0.66$, $P > 0.41$), strain by dose ($F_{2,66} = 0.75$, $P > 0.47$), strain by time ($F_{2,132} = 1.51$, $P > 0.22$), and strain by dose by time ($F_{4,132} = 0.34$, $P > 0.85$) interaction effects were NS as shown in Fig. 4c. A significant dose effect was observed ($F_{2,66} = 3.97$, $P < 0.024$), due to the chronic 25 mg/kg/d dose group tending to have higher values at all timepoints compared to the 0 mg/kg/d dose group, with the 3 mg/kg/d dose group having intermediate values (Fig. 4c). As the rate of decrease in the time spent in the central zone was similar for the three-dose groups ($F_{2,132} = 0.14$, $P > 0.96$), no true caffeine effect

Fig. 4



Mean (± SEM) total distance traveled (a), number of central entries (b), and time spent in the central zone (c) for HA1 and HA3 compulsive-like male mice after oral treatment with chronic 0, 3, and 25 mg/kg/d caffeine. ^$P < 0.05$ and $^{x}P < 0.05$ compared to the 0 mg/kg/d caffeine dose group. #$P < 0.05$ compared to the HA3 group with the same caffeine dose at the same time point. The gray bar indicates when caffeine was administered. Week 5 represents the fourth week of caffeine treatment.

was observed. Both strains and all dose groups decreased their time spent in the central zone with successive open field tests ($F_{4,132} = 15.60$, $P < 0.0001$).

Compulsive-like behaviors

Acute caffeine administration initially decreased nest-building behavior which was followed by a rebound effect in the HA3 and HA4 compulsive-like strains

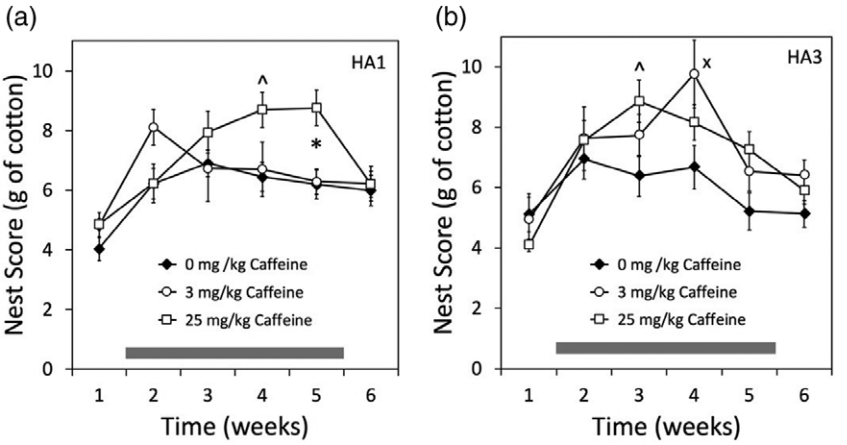
Nest building behavior was significantly suppressed by an acute 25 mg/kg caffeine dose in the first hour after injection while it was significantly increased during the third, fourth, and fifth hour after injection compared to the saline control (0 mg/kg caffeine) and 3 mg/kg caffeine groups (Fig. 2a), with a significant dose-effect ($F_{2,66} = 14.15$, $P < 0.0001$) and no significant strain ($F_{1,66} = 0.32$, $P > 0.51$) or strain by dose interaction ($F_{2,66} = 0.52$, $P > 0.59$) effects (Fig. 2a). The significant time by dose interaction effect ($F_{8,264} = 16.29$, $P < 0.0001$) was due to the saline control and 3 mg/kg caffeine dose groups starting out with relatively high nesting scores at the beginning of the 0–5 h time period and decreasing nesting scores over time, while the 25 mg/kg caffeine dose group started out with low nesting scores which increased over time. The strain effect, the strain by dose, time by strain ($F_{1,66} = 0.39$, $P > 0.53$), and time by strain by dose ($F_{2,66} = 1.38$, $P > 0.25$) interaction effects were NS as shown in Fig. 2a.

During the 5–24 h and 0–24 h time periods, the acute injection of 25 mg/kg caffeine reduced nest-building behavior significantly compared to the saline control group ($F_{2,66} = 7.76$, $P < 0.0009$; $F_{2,66} = 4.04$, $P < 0.0023$, respectively), while the acute 3 mg/kg caffeine group showed intermediate values, but not significantly different from the other groups (Fig. 2b). The strain ($F_{1,66} = 0.01$, $P > 0.91$; $F_{1,66} = 0.13$, $P > 0.71$, respectively) and strain by dose interaction ($F_{2,66} = 0.46$, $P > 0.63$; $F_{2,66} = 0.25$, $P > 0.78$) effects were not significantly different for the 5–24 h and 0–24 h periods as shown in Fig. 2b. The 5–24 h and 0–24 h dose groups showed similar patterns (Fig. 2b), due to the fact that the total amount of cotton used during the 0–5 h period were similar among the groups (Fig. 2a).

Chronic caffeine administration increased compulsive-like nest-building in the HA1 and HA3 compulsive-like strains

Chronic caffeine administration significantly increased nest-building behavior in the HA1 and HA3 strains ($F_{2,66} = 3.15$, $P < 0.05$), without strain ($F_{1,66} = 0.06$, $P = 0.81$) and strain by dose interaction ($F_{2,66} = 0.50$, $P > 0.60$) effects. In general, the dose groups of the two strains had an initial increase in their nest scores followed by a decrease, which explain the significant time effect ($F_{5,330} = 39.85$, $P < 0.0001$) (Fig. 5). The strains showed some detailed responses that were different, which explain the significant strain by time ($F_{5,330} = 2.50$, $P < 0.031$) and strain by dose by time ($F_{10,330} = 3.09$, $P < 0.001$) interaction effects. For the HA1 strain, the chronic 0 and 3 mg/kg/d dose groups showed very similar nest scores, which were below the chronic 25 mg/kg/d dose group during the caffeine treatment period (Fig. 5a). In contrast, for the HA3 strain, the chronic 3 and 25 mg/kg/d dose groups showed very similar nest scores, which were above the 0 mg/kg/d dose group during the caffeine treatment period (Fig. 5b).

Fig. 5



Mean (± SEM) nest building score for HA1 (a) and HA3 (b) compulsive-like male mice after oral treatment with chronic 0, 3, and 25 mg/kg/d caffeine. *$P < 0.05$ compared to the 0 and 3 mg/kg/d caffeine dose groups. #$P < 0.05$ compared to the HA3 group with the same caffeine dose at the same time point. The gray bar indicates when caffeine was administered.

An acute high dose of caffeine reduced digging behavior in the HA3 and HA4 compulsive-like strains

Marble burying behavior was significantly suppressed by an acute 25 mg/kg caffeine dose 1 h after injection ($F_{2,66}$ = 14.15, $P < 0.0001$) compared to the saline control and 3 mg/kg caffeine groups (Fig. 3). The strain effect ($F_{1,66}$ = 0.32, $P > 0.51$) and strain by dose ($F_{2,66}$ = 0.52, $P > 0.59$), time by strain ($F_{1,66}$ = 0.39, $P > 0.53$), and time by strain by dose ($F_{2,66}$ = 1.38, $P > 0.25$) interaction effects were NS as shown in Fig. 3. A significant time effect ($F_{1,66}$ = 173.87, $P < 0.0001$) reflected a higher marble-burying score at 10 minutes compared to 5 minutes at all doses (Fig. 3).

Chronic caffeine administration revealed minimal effects on digging behavior of the HA1 and HA3 compulsive-like strains

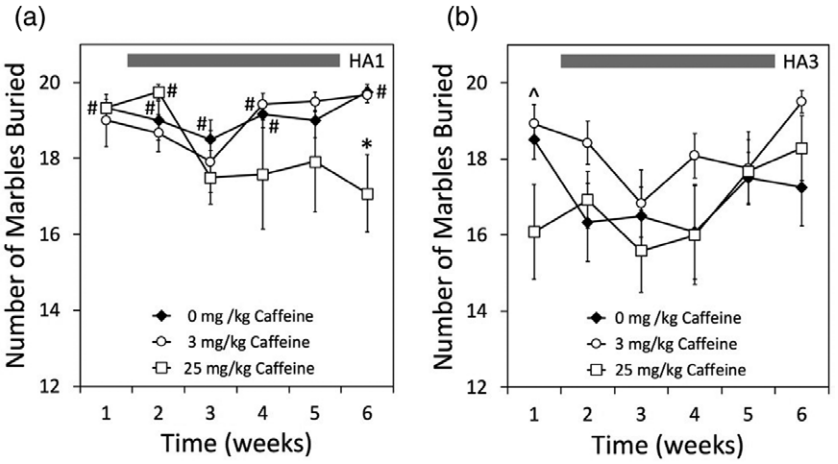
For compulsive-like marble-burying, the HA1 strain buried more marbles than the HA3 strain, at several time points of the chronic caffeine dose groups ($F_{1,66}$ = 17.92, $P < 0.0001$) (Fig. 6a and b). A significant dose-effect ($F_{2,66}$ = 3.92, $P < 0.3$) was observed, which was due to the number of marbles buried by the mice in the chronic 25 mg/kg/d generally being lower than the other dose groups for both strains (Fig. 6), which also explains the non-significant strain by dose interaction effect ($F_{2,66}$ = 1.25, $P > 0.29$), however, because significance was only reached in the HA1 strain at week 6 when no caffeine was administered (Fig. 6a), the overall effect of caffeine on digging behavior was minimal. The strain by time ($F_{5,330}$ = 1.00, $P > 0.41$), dose by time ($F_{10,330}$ = 0.86, $P > 0.55$), and strain by dose by time ($F_{10,330}$ = 1.60, $P > 0.10$) interaction effects were NS, indicating that the strains and dose groups generally responded in a similarly throughout the testing period.

Discussion

Several studies have indicated the association between caffeine and aggravation of mood and psychiatric disorders (Hedges *et al.*, 2009; Cerimele *et al.*, 2010; Wang *et al.*, 2016), but the role of caffeine in influencing obsessions and compulsions in OCD is currently not well understood (Koran *et al.*, 2009; Shams *et al.*, 2019). Since heterogeneity in OCD symptoms complicates the identification of candidate genes and the choice of initial pharmacotherapy (Mataix-Cols *et al.*, 2005; Katerberg *et al.*, 2010; Ruscio *et al.*, 2010; Torresan *et al.*, 2013; Pauls *et al.*, 2014; McCarty, 2017), it is plausible that the complex heterogeneity among patient subgroups and their responses to pharmacotherapy (Jenike, 1998; Pigott and Seay, 1999; Jenike, 2004) can produce differential responses when exposed to common psychostimulants such as caffeine.

We demonstrate here that a high acute dose of caffeine (25 mg/kg) significantly reduced nest-building behavior during the first hour after injection. Nesting scores rebounded during the third, fourth, and fifth hours of injection but were depressed for the 5–24 h and 0–24 h time periods. The 3 mg/kg caffeine dose had no significant effects when compared to the saline control. Although a direct comparison to the clinical condition is speculative, it can be postulated that a high dose of caffeine, such as found in several cups of coffee over a short period of time, might provide initial attenuation of compulsivity followed by several hours of exacerbated responses and, further, longer periods of moderate suppression. The effects of a 25 mg/kg dose 1 h after injection on marble-burying behavior were similar to that seen for nest building during the first hour after injection. It

Fig. 6



Mean (±SEM) number of marbles buried for the HA1 (a) and HA3 (b) compulsive-like male mice after oral treatment with chronic 0, 3, and 25 mg/kg/d caffeine. *$P < 0.05$ compared to the 0 and 3 mg/kg/d caffeine dose groups. #$P < 0.05$ compared to the HA3 group with the same caffeine dose at the same time point. The gray bar indicates when caffeine was administered.

is possible that this initial reduction might have been followed by an increase in digging behavior, as seen for nest-building behavior, but this was not measured.

Chronic exposure to a high caffeine dose resulted in trait-specific responses for compulsive-like behaviors. At the dose of 25 mg/kg, caffeine increased compulsive-like nest-building behavior in both compulsive-like strains. For marble-burying behavior, caffeine produced no overall marked effect on compulsive-like behaviors. This finding adds to previous findings in the compulsive-like mice strains showing behavioral and drug response heterogeneity, which is consistent with the evidence of clinical heterogeneity in compulsive domains (Mataix-Cols *et al.*, 2005; Katerberg *et al.*, 2010; Ruscio *et al.*, 2010; Torresan *et al.*, 2013; Pauls *et al.*, 2014; McCarty, 2017). To what extent OCD patients show similar heterogeneity in the effects of caffeine remains to be determined. For example, it is possible that the modest reduction in OCD symptoms after chronic high dose caffeine treatment in treatment-resistant OCD patients (Shams *et al.*, 2019) might not occur in other OCD patient subgroups. Considering that the HA strains consistently exhibit compulsive-like phenotypes (Greene-Schloesser *et al.*, 2011; Mitra *et al.*, 2016a; Mitra *et al.*, 2017a; Mitra and Bult-Ito, 2018), the behavioral effects of caffeine exposure on these strains indicate a possible interaction between genetic background and caffeine. Since caffeine's half-life (typically between 3 and 7 h) has been shown to vary widely among individuals (Nehlig, 2018), it is also possible that metabolism could play a role in retaining caffeine in the system thereby prolonging its effects. Thus, the difference in the metabolic profiles of the HA strains could lead to differential levels of caffeine and our observed effects. Caffeine's influence on the marble-burying behavior has been studied with relationship to an anxiety-like phenotype. In this regard, low levels of marble-burying (average of 5–10 marbles) can be a good indicator for anxiety-like responses, and caffeine administration often increases the number of marbles buried (Okuro *et al.*, 2010). However, marble burying (average of 15–20 marbles) in the HA strains represents a compulsive-like phenotype due to its excessive and perseverant nature which is accompanied by marked changes in motoric responses, including the rapid movement of the front and the back paws (Greene-Schloesser *et al.*, 2011; Mitra *et al.*, 2016b; Mitra et al., 2017a, 2017b; Mitra and Bult-Ito, 2018). The fact that acute but not chronic caffeine exposure reduced compulsive-like marble burying in both mouse strains suggests a trait specific effect that is influenced by exposure time and genotype.

An acute high dose of caffeine potentiated locomotor responses in the HA strains, which shows that the effects of caffeine on compulsive-like nest-building behavior were behavior-specific. Chronic caffeine doses did not have marked effects on open field anxiety-like and locomotor behaviors. These behavioral differences between acute and chronic caffeine regimens have been previously reported and may be due to the differential development of locomotor sensitization and tolerance under the two regimens (Holtzman, 1983; Holtzman and Finn, 1988; Holtzman *et al.*, 1991; Lau and Falk, 1994; Hsu *et al.*, 2009). Tolerance to chronic caffeine in the HA strains could be due to alterations in the adenosine receptor levels, as reported previously (Holtzman *et al.*, 1991). On the other hand, tolerance may reflect the contribution of genetic background. Thus, prior studies have demonstrated that there is a significant influence of strain on the anxiogenic effects of caffeine (Hughes and Hancock,

2016; Hughes and Hancock, 2017). In general, caffeine failed to produce anxiety-like behavior in the PVG/c rat strain, which exhibit reduced baseline levels of anxiety-like behavior but was effective in Wistar and Long Evans strains, which exhibit higher levels of baseline anxiety (Hughes and Hancock, 2016; Hughes and Hancock, 2017). Considering that the HA strains generally exhibit reduced anxiety-like behaviors (Greene-Schloesser *et al*., 2011; Mitra *et al*., 2016a; Mitra *et al*., 2017a), it is plausible, therefore, that genetic background contributed prominently to the finding that chronic caffeine failed to produce motoric and anxiety-like behavior in the present studies.

In conclusion, mice with a spontaneous compulsive-like phenotype provide an exciting opportunity to further our understanding of environmental exposures in modulating disease pathophysiology. Considering that the HA strains exhibit consistent and predictable compulsive-like nest-building and digging behaviors, it can be inferred that caffeine's effects were trait and exposure specific. Hence, the current findings provide a preclinical basis for investigating how psychostimulants act differentially among patient subgroups. In turn, this may lead to more effective therapeutic measures or avoidance of drugs that they have the potential to worsen symptoms.

Acknowledgements

We thank the Biological Research and Diagnostics (BiRD) Facility animal quarters staff for animal care.

The research reported in this publication was funded by grants from the National Institute of General Medical Sciences of the National Institutes of Health (1) under an Institutional Development Award (IDeA; grant number P20GM103395) and (2) under a Building Infrastructure Leading to Diversity Award (BUILD; three linked grants numbered RL5GM118990, TL4GM118992, and 1UL1GM118991). The work is solely the responsibility of the authors and does not necessarily represent the official view of the National Institutes of Health.

All authors performed behavioral experiments. Statistical analysis was performed and the manuscript was written by S.M. and A.B.-I.

Conflicts of interest

There are no conflicts of interest.

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