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Effects of Chronic Caffeine Administration on Behavioral and Molecular Adaptations to Sensory Contact Model Induced Stress in Adolescent Male Mice

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ORIGINAL RESEARCH

Effects of Chronic Caffeine Administration on Behavioral and Molecular Adaptations to Sensory Contact Model Induced Stress in Adolescent Male Mice

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Abstract

Previous studies have shown that caffeine attenuates stress-induced mood dysfunction and memory deterioration through neuronal adenosine A2A receptors antagonism. However, whether caffeine exerts this effect through modulating other molecular targets, which interfere with the resilience to social defeat stress in adolescent male mice is unknown. This study was conducted to investigate the role of caffeine in the behavioral responses to social stress induced by the sensory contact model (SCM) and the possible alteration of the gene expression level of Na/K ATPase pump. Adolescent male mice were exposed to SCM for 12 days. Caffeine was administered intraperitoneal daily for 14 days after SCM. The time spent in interaction zone, social interaction ratio, preference index to novel objects, time spent in the open arms and immobility time in forced swimming test were used to measure the locomotor activity, social avoidance, short-term memory, anxiety and depression in mice. The results showed that chronic treatment with caffeine for 14 days improved locomotor activity, reversed the avoidance of social behavior, improved preference to novel objects, and reversed depression induced by social defeat stress in adolescent male mice, suggesting the enhancement of the resilience to social defeat stress induced by caffeine. Moreover, caffeine treatment did alter gene expression levels of Na/K ATPase isoforms in both prefrontal cortex and hippocampus. Altered gene expression was significant in most cases and correlates with the observed behavioral changes. Taken together, our findings provide new insight into the effects of chronic caffeine administration on locomotor activity, social avoidance, short-term memory and depression in adolescent male mice exposed to SCM.

Keywords Caffeine · Sensory contact model · Adolescence · Na/K ATPase · Chronic stress

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Introduction

Adolescence is a transitional stage between childhood and adulthood associated with extensive structural and functional neurodevelopment and a wide range of neurobiological changes, which renders adolescents susceptible to stress-induced cognitive and emotional dysfunctions (Schneider 2013). Exposure to chronic stress during adolescence, such as exposure to social bullying and subordination, could result in long-term psychological and behavioral consequences (Spear 2000; Gunnar and Quevedo 2007). Social defeat in rodents has been used to model negative social experiences of subordination in humans where evoked confrontation between two animals results in domination of the one with aggressive behavior and subordination of the other with submissive behavior (socially defeated) (Hollis and Kabbaj 2014). Several studies have shown that repeated exposure to social defeat stress in rodents elicits a set of negative

behavioral effects like anhedonia, social avoidance, reduced exploratory behavior and locomotor activity (Venzala et al. 2012; Jin et al. 2015). Most studies of social defeat-stress have been performed in adult animals. However, exposing animals to social defeat stress during adolescence might provide significant insight into the effect of early-life exposure to stress on the future adulthood behaviors since the social defeat-induced alterations could be a reflection of neurodevelopmental and neurochemical disturbances in the neural systems implicated in emotion and cognition (Buwalda et al. 2005; Coppens et al. 2011; Zhang et al. 2016). Increased evidence suggests potential roles for Na/K ATPase in neuronal signaling pathways and gene transcription in addition to its role as an ion pump in maintaining the neuronal electrochemical gradient and proper neuronal function (Orlov et al. 2017). A functional Na/K ATPase is composed of a catalytic α-subunit and a regulatory β-subunit. The catalytic α-subunit exists in four different isoforms, α1 through α4, each with a distinct expression pattern. Alpha one (α1) isoform is ubiquitously expressed in all tissue types, as it's essential for maintaining cellular function. In addition to the housekeeping α1 isoform, α2 and α3 isoforms are expressed in the brain where α2 isoform is mainly expressed in the glial cells and α3 isoform is specifically expressed in the neuronal cells (Isaksen and Lykke-Hartmann 2016; Shrivastava et al. 2018). Na/K ATPase has been shown to be involved in neuronal synaptic transmission, memory formation and neuronal stress reactivity (de Vasconcellos et al. 2005; Pivovarov et al. 2018) as evidenced by the neurological disorders resulting from Na/K ATPase isoforms mutations (Holm et al. 2016a, b; Isaksen and Lykke-Hartmann 2016; Shrivastava et al. 2018).

Interestingly, caffeine has been found to decrease the expression of renal Na/K ATPase which could be underlying its natriuretic and diuretic effect since renal Na/K ATPase plays an essential role in sodium reabsorption in the renal tubules (Lee et al. 2002). Moreover, caffeine has been reported to improve Na/K ATPase activity in the brain (Imam-Fulani et al. 2016), suggesting differential site-specific effects of caffeine on Na/K ATPase. Caffeine consumption correlates inversely with depression and memory deterioration due to its adenosine A1 and A2A receptors-mediated effects on synaptic transmission and plasticity (Lopez-Cruz et al. 2018; Lopes et al. 2019). Caffeine controlled chronic stress-induced mood dysfunction by antagonizing adenosine A2A receptors in forebrain neurons (Kaster et al. 2015). Adenosine A2A receptors have been shown to be physically associated and control the activity of Na/K ATPase in astrocytes, which underlies the regulation of astrocytic glutamate transport (Matos et al. 2013), providing a clear and evident impact on mood (Matos et al. 2015).

Notably, the effects of caffeine might differ between adults and adolescents with most studies being conducted in adults rendering caffeine impact in adolescents poorly defined (Porciúncula et al. 2013). This is of particular importance given the growing consumption of caffeine among adolescents especially college students attempting to improve their cognitive performance. Moderate caffeine consumption is generally considered safe and beneficial, while heavy caffeine consumption is associated with adverse effects, which is an emerging risk with the excessive consumption of high dose caffeine-containing energy drinks in adolescents (Reissig et al. 2009; Cho 2018). Given that caffeine effects have been largely investigated in adults, the caffeine intoxication threshold dose in adolescents is unknown. However, others have reported that caffeine consumption in adolescent rats induced behavioral and neurochemical changes including a dual effect on recognition memory and anxiety that was associated with alterations in hippocampal and cortical BDNF levels and astrocytic and nerve terminal markers (Ardais et al. 2014). In this study, we focused on determining the effect of chronic administration of caffeine on the behavioral responses to social stress induced by the sensory contact model (SCM) and the possible alteration of the gene expression level of Na/K ATPase pump in frontal cortex and hippocampus.

Materials and methods

Animals

One hundred weaned male Swiss albino mice (22 days) were brought to our facility, caged in standard plastic cages (4–6 per cage) under standard laboratory conditions and left undisturbed for 7 consecutive days for adaptation. Dedicated efforts made to minimize animal suffering and the number of animals used in accordance with the Guidelines for Animal Experiments of Faculty of Pharmacy, the British University in Egypt and approved by the Ethical Committee of Faculty of Pharmacy, the British University of Egypt with the ethical approval number is: EX-1906. Mice were isolated for 5 days to abolish any social effects (D29-D34).

Sensory contact model (SCM)

Model established according to Kudryavtseva et al. (2014). Pairs of animals of similar weight placed in stainless steel cages (28 × 14 × 10 cm) divided by a clear perforated Plexiglas partition that allows visual and olfactory but not physical contact. Pairs left undisturbed for 2 days for adaptation to the housing conditions and sensory contact. Every day (10:00–11:00 am), the partition removed for 10 min to allow physical interaction for 12 consecutive days (D36-D48). One of the partners clearly dominates the other within 2 or 3 daily encounters. During the tests, one animal seen to attack, bite,

and chase the other who only displayed defensive behavior (sideways, upright postures, withdrawal, lying on the back or freezing). Aggressive confrontations discontinued if the aggression has lasted more than 3 min. After every session, the winner remained resident in their compartments while defeated mouse paired with the winning member in a new unfamiliar cage.

Experimental design

Defeated mice randomly assigned to control group (vehicle), caffeine 50 mg/kg or caffeine 100 mg/kg, given intraperitoneal (i.p.) daily for 14 consecutive days (D48-D61) where the first dose administered 2 h after the last SCM session. Additional three group consists of mice isolated for 5 days served as normal undefeated control (vehicle), normal undefeated received caffeine 50 mg/kg and normal undefeated group received 100 mg/kg (Fig. 1).

Drugs

Caffeine (1,3,7-trimethylxanthine was purchased from Sigma Aldrich, Germany. Caffeine (50 and 100 mg kg^{-1}) was dissolved in sterile normal saline solution NaCl 0.9% (2.5 mg ml^{-1}). The solutions of drugs were prepared fresh daily and injected i.p. in a volume of 20 ml kg^{-1} every morning at 9:00 am for 14 days. Behavioral assessment tests started 24 h after the last day of chronic caffeine injection.

Behavioral assessment

Open-field test (OF)

The open field used was a black Plexiglas box (40 × 40 × 30 cm). Test was performed under dim light conditions (10 lx). Animal was placed in the center of the arena and videotaped for 5 min., the open field was wiped thoroughly after each session using 70% propanol (Weiss et al. 2004). Videos were evaluated using Any Maze® video tracking software (Stoelting co.) for the following parameters: total distance travelled, time spent in the center of the arena and total immobility time.

Social avoidance test (SA)

Social avoidance was performed 3 h after the open field-test as described elsewhere (Krishnan et al. 2007). In brief, the test was performed test in the same open field boxes subjects were previously habituated during the open field test under low light conditions (10 lx). The test consisted in two consecutive sessions of 150 s each. In session one, mice were placed in the open field containing an empty circular wire mesh cage (18 × 9 cm) located at the center of one side of the arena. In the second session, the circular cage contained a naïve mouse (target). A virtual interaction zone (area projecting 8 cm around wire cage) was delimited, and the time spent in this zone was scored during both sessions using a video tracking system (Any maze®). Social interaction behavior was estimated as interaction ratio: (time spent in the interaction zone in the presence of target/time spent in the interaction zone in the absence of target).

Novel object recognition (NOR)

Each mouse was habituated to the arena for a 10 min session followed by a 5 min familiarization session in which mouse was allowed to explore 2 identical objects placed in a fixed position (Hale and Good 2005). Two minutes later, mouse was subjected to a 3 min test phase in which one of the familiar objects was replaced by a novel one (Ennaceur 2010). The position of the novel object was counterbalanced between subjects. Both familiarization and test sessions were videotaped and assessed for the total exploratory time of familiar and novel objects, defined as directing the nose toward the object at a distance less than or equal to 2 cm. The preference index was calculated as follow: (time spent exploring the novel object - time spent exploring the familiar object)/time exploring both novel and identical objects (Ennaceur and Delacour 1988).

Elevated plus maze test (EPM)

The apparatus was established according to Bourin et al. 2007. In brief, the apparatus consisted of an elevated (40 cm) central platform (5 × 5 cm) with two opposite open arms

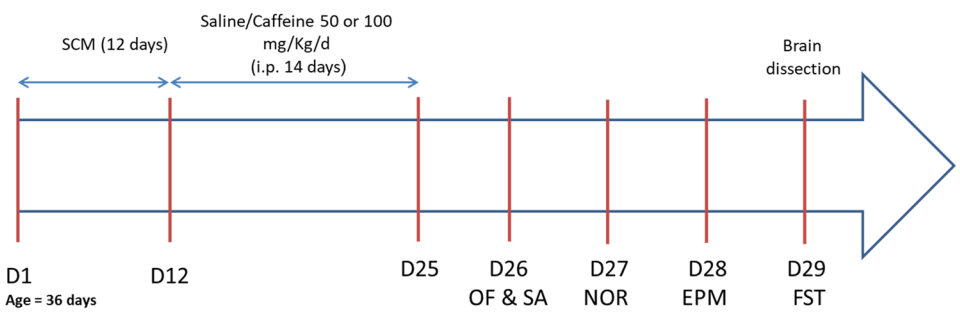

Fig. 1 Experimental timeline

(5 × 30 cm) and 2 opposite closed ones (5 × 30 × 15 cm). Animal was placed on the central platform and videotaped for 5 min. Videos were evaluated for the time spent in either open or closed arms.

Forced swimming test (FST)

The test was performed as described previously (Yankelevitch-Yahav et al. 2015). The apparatus used is a glass cylinder (20 cm diameter, 50 cm height) filled with tap water at 25 ± 1 ºC to a level of 30 cm so that it cannot touch the bottom of the container with its hind legs. Each mouse was videotaped for 6 min. where the first 2 min were considered as pretest and the last 4 min (test period) were analyzed for total immobility time. At the end of each session, each mouse removed from the container, dried thoroughly using paper towels, and placed under heat lamb to recover its body temperature before being returned to home cage. Water was changed after every session to avoid any influence on the next mouse.

Quantitative real time PCR (qRT-PCR)

Tissue extraction

One day after behavioral testing, mice were decapitated and brains removed and snap frozen for hippocampal and prefrontal cortex mRNA extraction. RNA was isolated from the frontal cortex and hippocampus of the mice after microdissection of the brain as described previously (Chiu et al. 2007) 1 day after the last behavioral test (FST). Once the frontal cortex or the hipocampus were isolated from the animal, they were snap-frozen in liquid nitrogen and stored in − 80 °C for further processing. RNA was extracted from either a lobe of the frontal cortex or the hippocampus using Trizol Reagent (Invitrogen, USA) as per instruction of the manufacturer. Isolated RNA was quantified spectrophotometrically using UV–Vis Spectrophotometer Quawell q5000 (Quawell, USA) and RNA purity was detected using 260/280 ratio. RNA was treated with RNAse-Free DNAse (Thermo Scientific, USA) to remove any contaminating genomic DNA. Then, cDNA was prepared from DNAse treated RNA using RevertAid First Strand cDNA Synthesis Kit (Thermo Scientific, USA). Gene expression was done by qRT-PCR using gene specific primers (sequence of the primers used are given in Table 1), and Maxima SYBR Green qPCR Master Mix, 2X (Thermo Scientific, USA). All qRT-PCR experiments were done on StepOnePlus Real time PCR system (Applied Biosystems, USA). The relative RNA expression was calculated from threshold cycle (Ct) detected by the instrument using the formula $2^{-\Delta Ct}$., relative to β-actin as the house keeping gene. The cycling conditions were as follows: initial denaturation at 95 °C for 10 min, followed by 40 amplification cycles of 95 °C for 15 s and combined annealing/extension at 60 °C for 1 min.

Statistical analyses

Data obtained from behavioral tests were expressed as mean ± confidence interval 95% (CI 95%), while the data of the qRT-PCR was expressed as means ± standard error of mean (SEM). Multiple comparisons among groups were analyzed using one-way analysis of variance (ANOVA) followed by Tukey test as a post-hoc test. The probability level less than 0.05 was considered to indicate statistical significance. All Statistical procedures were performed using IBM SPSS version 17 computer package, USA. Graphs were sketched using GraphPad Prism version 5 software (GraphPad Software Inc., USA).

3. Results

Behavioral analysis

Five tests were performed in order to investigate exploration, anxiety, memory and stress-coping behavior (OF, SA, NOR, EPM and FST) (Fig. 2).

Open-field test (OF)

The OF was used to quantify behavioral responses such as locomotor activity, explorative behavior and anxiety. SCM significantly decreased locomotor activity manifested by a significant decrease in the total distance traveled compared to the control group (Fig. 3a, $F(5, 48) = 21.1$, $P < 0.0001$), with a corresponding increase in immobility time (Fig. 3c, $F(5, 48) = 10.93$, $P < 0.0001$), which can be interpreted as defeated mice were more likely to freeze in a novel environment. However, caffeine treatment of defeated groups did not improve any of that. Interestingly, caffeine treatment of normal subjects, not exposed to SCM, did have a significant

Table 1 Primers sequences used in qRT-PCR experiment:

Gene	Forward primer	Reverse primer
ATP1α2	5′-GTCCCTGAGGATCTCATCCA-3′	5′-TGTGGGCATCATATCAGAGG-3′
ATP1α3	5′-GATGATACCCACACCCTTGG-3′	5′-TCACCACAGACAACCTTTGC-3′
β-actin	5′-CTTGCTCTGGGCCTCGTC-3′	5′-GGCTGTATTCCCCTCCATC-3′

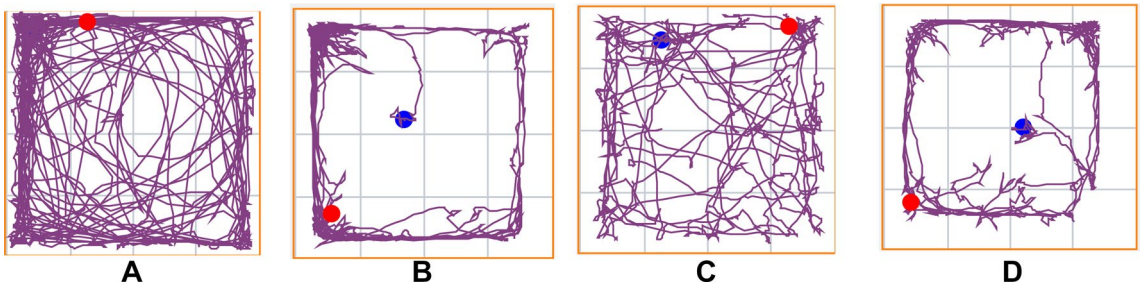

Fig. 2 Graphical representation of locomotive pathway of mice in OF. **a** Control, **b** Defeated, **c** Defeated + C50, **d** Defeated + C100. Control group showed extensive locomotion and prolonged existence in the center of the arena while defeated ones showed sigmotaxic behavior with minimal existence in the center of the arena. 50 mg/kg dose of caffeine but not 100 mg/kg dose, exerted partial reversal of such effect

reduction in the total distance traveled when compared to control group (Fig. 3a). On the other hand, caffeine administration of 50 mg/kg did have an anxiolytic effect as it significantly increased the time spent in the center of the OF arena (Fig. 3b, $F(5, 48) = 25.27$, $P < 0.0001$).

Social avoidance test (SA)

Exposure to SCM significantly decreased the interaction ratio in the SA test when compared to control group (70% reduction, $F(5, 48) = 9.895$, $P < 0.0001$). Only defeated mice that received 100 mg/kg treatment of caffeine did have a significant increase in the social interaction ratio when compared to the defeated group (Fig. 4). In other words, caffeine treatment improved sociability in male mice exposed to chronic stress.

Novel object recognition test

As shown in Fig. 5, control group and normal animals in all caffeine treatment groups spent similar exploratory time for two identical objects during each training session. Exposure to SCM significantly reduced the recognition index during retention test sessions when compared to control group ($F5, 48 = 6.847$, $P < 0.0001$). The post hoc comparisons of groups also showed that the defeated males in the 100 mg/kg caffeine-treated group exhibited a higher recognition index than in the defeated- and defeated + 50 mg/kg caffeine-treated groups. These results indicate that exposure to SCM resulted in impairment of the recognition memory functions and only 100 mg/kg treatment of caffeine brought it back to normal.

Elevated plus-maze test

In the EPM, defeated group spent a significantly less time ($p < 0.05$) in the open arms than control group (Fig. 6), indicative of a higher state of anxiety. Such effect remained unchanged even after 50 mg/kg of caffeine treatment. Although statistically insignificant, 100 mg/kg of caffeine treatment did have an additive anxiogenic effect through decreasing the time spent in the open arms (45% decrease) compared to the defeated group as shown in Fig. 6. Interestingly, 100 mg/kg of caffeine treatment in normal mice did show an anxiogenic effect expressed by the significant reduction of time spent in open arms when compared to the control group ($F(5, 48) = 7.087$, $P < 0.0001$).

Forced swimming test

Figure 7 presents mean total immobility time for control and treatment groups. The defeated group showed depressive response in the FST manifested by a significant increase in the total immobility time when compared to control group. Such effect was abolished in the defeated group that received 50 mg/kg treatment of caffeine. On the other hand, both doses of caffeine did have an anti-depressive effect on normal mice as they significantly reduced the total immobility time when compared to control group ($F(5, 48) = 47.55$, $P < 0.0001$)

Gene expression analysis

In order to examine the role of the genes involved in the behavioral assessment, we studied the expression pattern of Na/K ATPases in brain frontal cortex and hippocampus regions at RNA level using qRT-PCR.

mRNA expression level of Na/K-ATPase alpha isoforms in hippocampus and prefrontal cortex

As shown in Fig. 8a, SCM showed significant elevation in expression of ATPase-α2 in hippocampus. This elevated expression was reversed by treatment with either 50 mg/kg or 100 mg/kg dose of caffeine. Treatment of normal mice with either dose showed no effect on ATPase- α2 expression. On the other hand, SCM showed no change in the expression

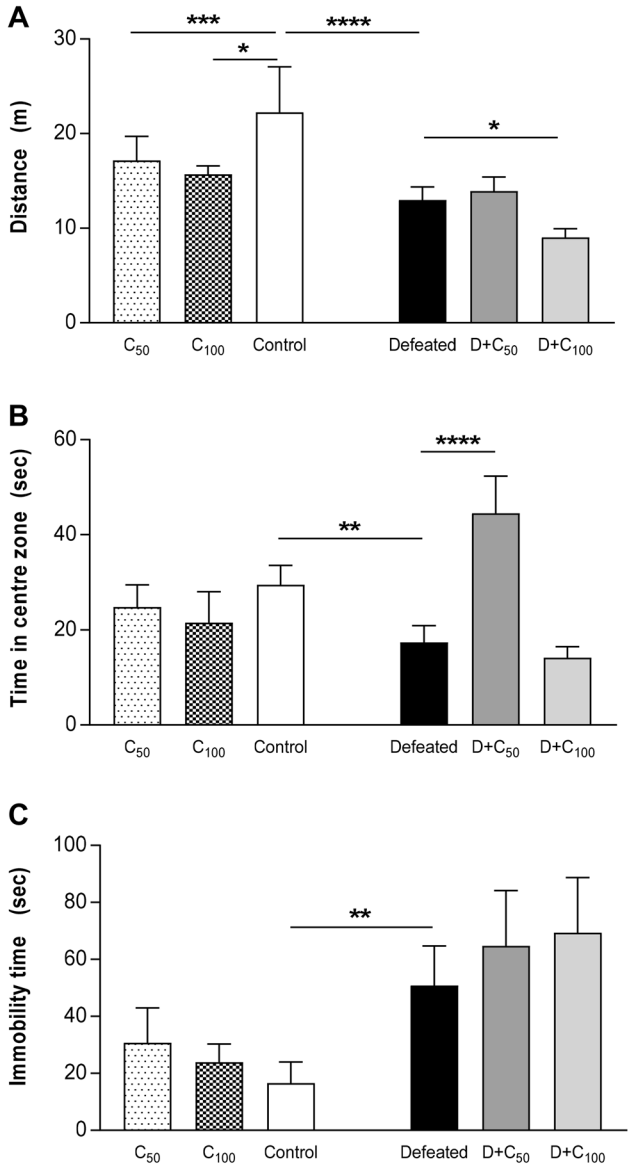

Fig. 3 Effect of 14 days' caffeine administration (50, 100 mg/kg/day i.p) on the behavior of normal and socially defeated mice in the open field. **a** Total distance travelled by the mouse during 5 min. observation session (F (5, 48) = 21.1 P < 0.0001), **b** time spent in the center of the arena (F (5, 48) = 25.27 P < 0.0001), **c** total time was the mouse immobile (F (5, 48) = 10.93 P < 0.0001). Data expressed as mean ± 95% CI (n = 08–10). *p ≤ 0.05, *p ≤ 0.01, ***p ≤ 0.001 ****p ≤ 0.0001. C_{50}: caffeine 50 mg/kg, C_{100}: caffeine 100 mg/kg

of hippocampal ATPase-α3 expression as compared to control group. However, treatment of social defeated mice with 50 mg/kg caffeine dose showed elevated expression of ATPase-α3, an effect that was not shown with 100 mg/kg caffeine dose. Treatment of normal mice with either 50 or 100 mg/kg caffeine doses showed no change in ATPase-α3 expression as compared to normal untreated mice (Fig. 8b).

As for the frontal cortex, as shown in Fig. 8c, ATPase-α2 showed significant elevation upon administration of either

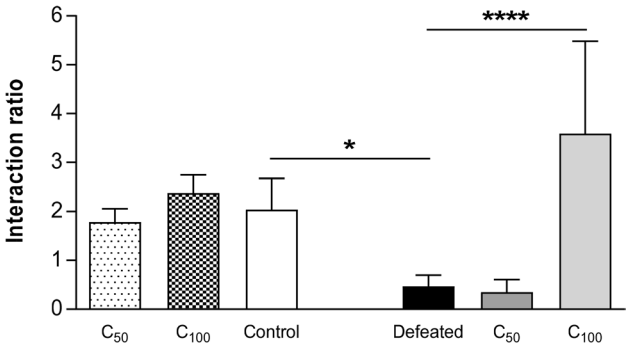

Fig. 4 Effect of 14 days' caffeine administration (50 and 100 mg/kg/day i.p) on the interaction ratio of normal and socially defeated mice in social avoidance test (F (5, 48) = 9.895, P < 0.0001). Data expressed as mean ± 95% CI (n = 08–10). *p ≤ 0.05, ****p ≤ 0.0001 C_{50}: caffeine 50 mg/kg, C_{100}: caffeine 100 mg/kg

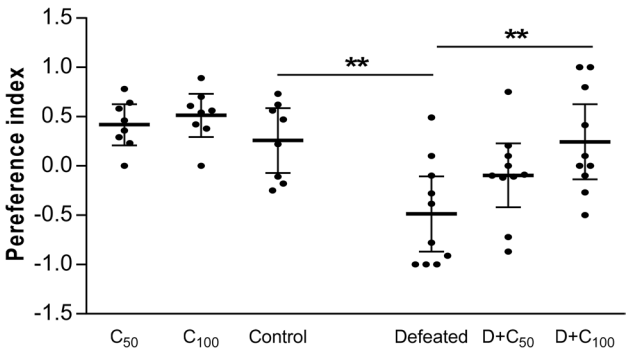

Fig. 5 Effect of 14 days' caffeine administration (50 and 100 mg/kg/day i.p) on the preference index of normal and socially defeated mice in novel object recognition test (F (5, 48) = 6.847, P < 0.0001). Data expressed as mean ± 95% CI (n = 08–10). **p ≤ 0.001, C_{50}: caffeine 50 mg/kg, C_{100}: caffeine 100 mg/kg

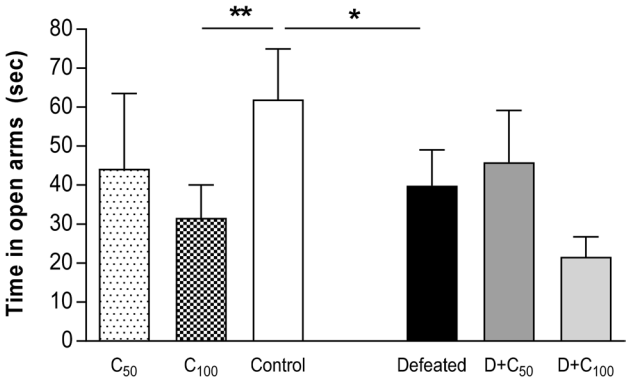

Fig. 6 Effect of 14 days' caffeine administration (50 and 100 mg/kg/day i.p) on the time spent in open arms by normal and socially defeated mice in elevated plus-maze test (F (5, 48) = 7.087, P < 0.0001). Data expressed as mean ± 95% CI (n = 08–10). *p ≤ 0.05, **p ≤ 0.01 C_{50}: caffeine 50 mg/kg, C_{100}: caffeine 100 mg/kg

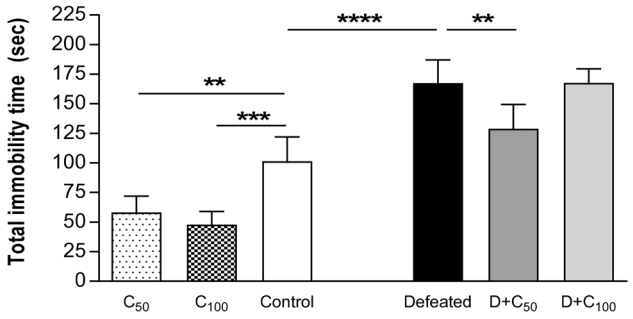

Fig. 7 Effect of 14 days' caffeine administration (50 and 100 mg/kg/day i.p) on the total immobility time of normal and socially defeated mice in the forced swimming test ($F(5, 48) = 47.55$ $P < 0.0001$). Data expressed as mean ± 95% CI (n = 08–10). **p ≤ 0.01; ***p ≤ 0.001, ****p ≤ 0.0001, C_{50}: caffeine 50 mg/kg, C_{100}: caffeine 100 mg/kg

50 or 100 mg/kg doses of caffeine as compared to control group. Social defeated mice, either alone or upon administration of 50 or 100 mg/kg doses of caffeine showed no change in ATPase-α2 as compared to control group. On the other hand, ATPase-α3 showed significant elevation in social defeated mice as compared to control group either alone or with 50 or 100 mg/kg doses of caffeine. In addition, further elevation was shown in defeated mice with 50 mg/kg caffeine administration, while 100 mg/kg dose of caffeine, although significantly elevated from control groups, however showed no significant difference from defeated mice group (Fig. 8d).

Discussion

The effect of chronic caffeine administration on stress-induced behavioral and molecular modifications was assessed in male Swiss albino mice after 12 days of SCM. To assess explorative behavior and locomotor activity, open field test was done on D26, after 2-week treatment with either caffeine or its vehicle in socially defeated mice,

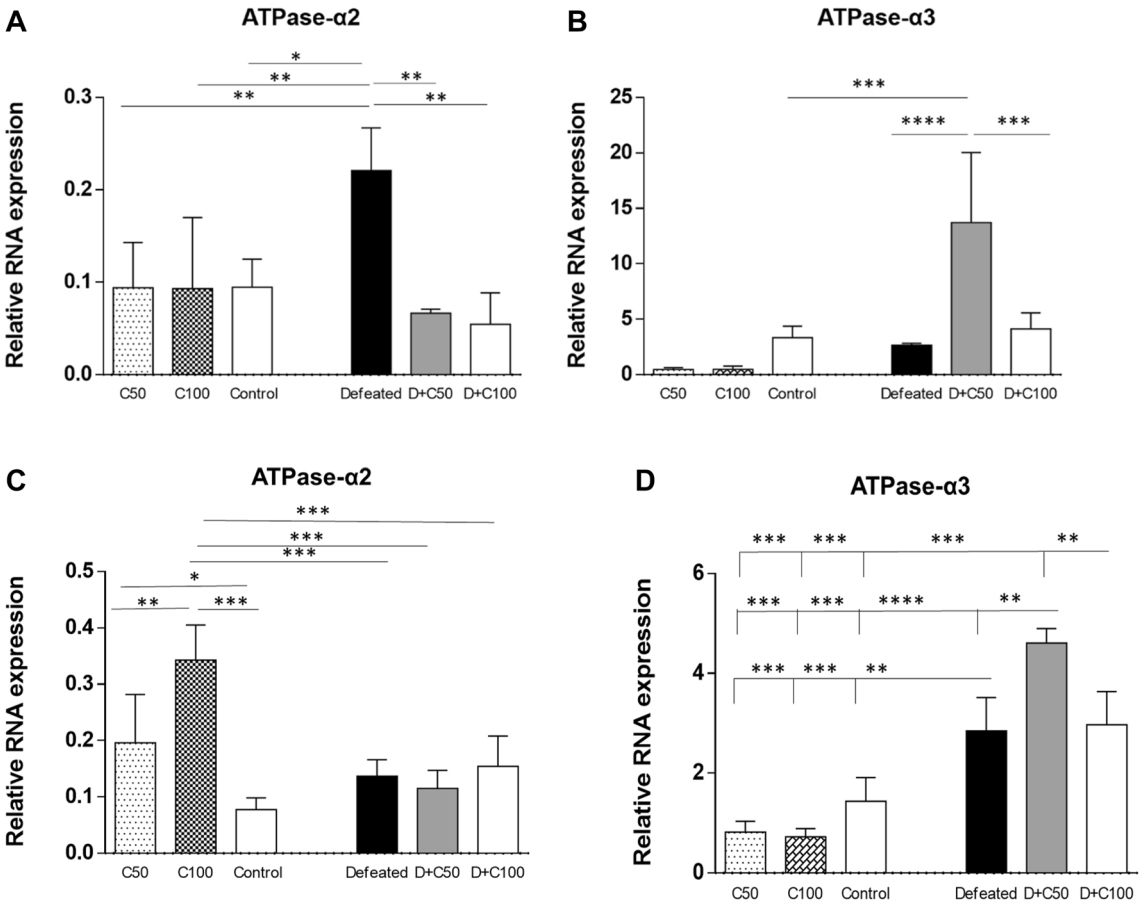

Fig. 8 Effect of 14 days' caffeine administration (50 and 100 mg/kg/day i.p) on the mRNA expression levels of ATPase-α2 and ATPase-α3 in hippocampus **a** and **b** and frontal cortex **c** and **d** of normal and socially defeated mice treated with caffeine. Data are expressed as mean ± SEM (n = 05). **p ≤ 0.01; ***p ≤ 0.001, ****p ≤ 0.0001, C_{50}: normal mice treated with caffeine 50 mg/kg, C_{100}: normal mice treated with caffeine 100 mg/kg, D + C50: socially defeated mice treated with 50 mg/kg caffeine; D + C100: socially defeated mice treated with 100 mg/kg caffeine

or vehicle in control mice. After this 2-week treatment, defeated mice cut significantly shorter distance and spent less time in the center of the open field as compared to their undefeated peers while demonstrated much longer immobility time. On the other hand, 50 mg/kg dose of caffeine was able to ameliorate the anxiogenic state elicited by SCM as it significantly increased the time spent in the center of the open field arena in contrast to 100 mg/kg dose of caffeine which did not alter any of the examined aspects in OF. However, 50 mg/kg dose of caffeine failed to restore both distance traveled and immobility time in OF to normal values (Fig. 3). It has been reported that chronic social defeat stress results in depressive behavior and downregulation of the hippocampal MAPK cascade (Iio et al. 2011). Xie et al. (2003), has reported that MAPK activation is downstream signaling of Na/K ATPase pump. The α_3 isoform of the Na/K ATPase catalytic subunit, encoded by the ATP1A3 gene, is specifically expressed in the neurons of the basal ganglia, cerebellum, and hippocampus (Isaksen and Lykke-Hartmann 2016) where Na/K ATPase alpha 3 alteration resulted in despair and stress induced depression (Holm et al. 2016b). Interestingly, our data shows that frontal cortex ATPase-α3 isoform mRNA level was significantly increased in both chronically stressed mice and after chronic caffeine treatment compared to normal subjects (both treated and untreated) (Fig. 8).

Social avoidance test, done on D26 as well, showed that defeated subjects exhibited significantly less interaction ratio in SA test when compared to the negative control group, which was unchangeable after 50 mg/kg dose of caffeine treatment. Interestingly, 2-week 100 mg/kg dose of caffeine treatment resulted in an enormous increase in the interaction ratio as it raised its value to double fold that of untreated control group (Fig. 4). A properly functioning and regulated Na/K ATPase is crucial for normal brain function as evidenced by the neurological disorders like enhanced fear and anxiety and reduced sociability resulting from mutations in the ATP1A2 and ATP1A3 genes encoding α2 and α3 isoforms (Holm et al. 2016a, b; Isaksen and Lykke-Hartmann 2016; Shrivastava et al. 2018). We have found that SCM brought a significant increase in hippocampal ATPase-α2 mRNA expression while caffeine treatment reduced it to normal levels (Fig. 8). Moreover, 50 mg/kg dose of chronic caffeine treatment did bring a significant increase in hippocampal ATPase-α3 isoform expression level (Fig. 8). To assess the effect of SCM on short-term memory, NOR test was carried out on D27. We have found that SCM altered short-term memory in affected animals evidenced by a steep decrease of preference index compared to normal subjects. 100 mg/kg dose of caffeine treatment was able to restore preference ratio of treated animals into normal values (Fig. 5).

Socially defeated mice suffered from an anxiogenic state manifested by significantly less time spent in open arms in EPM test carried out on D28, when compared to control group, while caffeine treatment failed to bring an improvement (Fig. 6). Moreover, socially defeated mice showed depressive response in the FST manifested by a significant increase in the total immobility time. Such effect was abolished after 50 mg/kg dose of caffeine treatment but not higher doses (Fig. 7).

Stress vulnerability stems from failure of individuals to adopt stress-coping responses where exposure to chronic stress especially during adolescence could result in long-term psychological and behavioral consequences (Spear 2000; Gunnar and Quevedo 2007). Hence, enhanced resilience to social defeat stress upon chronic caffeine treatment could provide clues about potential underlying neurobiological processes associated with resilience to stress which would be of great importance for the development of successful stress-coping interventions (Feder et al. 2009). Caffeine is the most widely used psychostimulant and its consumption is associated with lower risk of depression and better cognitive function (Lara 2010). Caffeine demonstrates dose-dependent effects (Smit and Rogers 2000; Haskell et al. 2005) where moderate doses produce positive mood effects and improve psychological symptoms while high doses of caffeine are associated with negative effects such as anxiety and tension (Kaplan et al. 1997; Broderick and Benjamin 2004). Moreover, consistent with previous reports, our data showed caffeine at the 50 mg/kg dose had an anxiolytic effect and antidepressant effects in social defeat mice. In addition, in contrast to other studies, caffeine at 100 mg/kg dose improved the social avoidance in social defeat stress in mice. The more beneficial effects observed with a lower dose of caffeine compared to higher dose could probably be ascribed to the adverse effects associated with high caffeine doses (Kaplan et al. 1997). Animal doses of caffeine can be translated to human equivalent doses through the following formula (Reagan-Shaw et al. 2008; Yin et al. 2015): human equivalent dose (mg/kg) = mouse dose (mg/kg) × (mouse Km factor/human Km factor), where Km factor equals 3 for a mouse and the Km factor equals 37 for a human. Hence, the dose of caffeine in an average person of 75 kg bodyweight equivalent to the doses used in our study is: is 50 mg/kg (or 100 mg/kg) × 3/37 × 75 = 304 (or 608) mg per day which is equivalent to consumption of two cups (or four cups) of brewed coffee (each cup contains 100–200 mg of caffeine). Therefore, chronic moderate caffeine consumption could promote stress resilience and positive psychological effects.

In conclusion, we showed that caffeine could alter and modify the cognitive and gene-related effects of sensory contact model induced stress in mice. In our study, SCM induced long lasting deleterious effects on adolescent Swiss albino male mice social behavior, anxiety- and memory-related behaviors as well as hippocampal and frontal cortex

gene expression of Na/K ATPase isoforms. 50 mg/kg dose of caffeine was able to ameliorate the anxiogenic state elicited by social defeat as it significantly increased time spent in the center of open field arena. Caffeine treatment at both doses failed to restore both distance traveled and immobility time in OF to normal values. Moreover, 50 mg/kg dose of caffeine increased preference index of the treated mice in NOR test compared to defeated untreated group although this was statistically insignificant, while higher dose of caffeine managed to bring it to normal levels. These data provide evidence that caffeine could be a relevant therapy to alleviate depression-associated cognitive disorders (Ribeiro and Sebastiao 2010; Espinosa et al. 2013; Machado et al. 2017). SCM-induced mnesic, social and other reported behavioral deficits might be related to alterations in Na/K ATPase isoforms gene expression. These effects were altered by chronic caffeine treatment, possibly through epigenetic-related mechanisms. However, further studies are needed to decipher such mechanisms.

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Compliance with Ethical Standards

Conflict of interest M. K. Ibrahim, M. Kamal, R. Tikamdas, R. Nouh, J. Tian and Moustafa Sayed declare that there is no conflict of interest.

Human and Animal Rights and Informed Consent Accordance with theGuidelines for Animal Experiments of Faculty of Pharmacy, the British University in Egypt and approved by the Ethical Committee of Faculty of Pharmacy, the BritishUniversity of Egypt with the ethical approval number is: EX-1906.

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