ORIGINAL RESEARCH

Caffeine consumption disrupts hippocampal long-term potentiation in freely behaving rats

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Abstract

Caffeine, one of the most commonly consumed psychoactive substances in the world, has long been known to alter neurological functions, such as alertness, attention, and memory. Despite caffeine's popularity, systematic investigations of its effects on synaptic plasticity in the brain are still lacking. Here we used a freely behaving rodent model of long-term potentiation (LTP), a frequently studied form of synaptic plasticity, to assess the effects of caffeine consumption on hippocampal plasticity. LTP, which is a persistent increase in the strength of synaptic connections between neurons, is a cellular mechanism widely considered to underlie the processes of learning and memory. A group of 10-week-old Sprague–Dawley rats were administered caffeine (1 g/L) in their drinking water 3 weeks prior to collection of electrophysiological data. Another group of age-matched animals received tap water and served as controls. Stimulating and recording electrodes were chronically implanted in the perforant pathway (PP) and dentate gyrus (DG) region of the hippocampus, respectively, to permit stable electrophysiological recordings of synaptic transmission at this synapse. Population spike amplitude (PSA) measures of LTP induction and duration were acquired in vivo while animals were freely behaving using a well-established electrophysiological recording protocol. Results indicate caffeine-treated rats ($n = 9$) had a significantly ($P < 0.05$) reduced level of LTP induction compared with controls ($n = 10$). More studies are needed to identify the exact mechanism through which caffeine alters LTP induction in this freely behaving model of synaptic plasticity.

Introduction

Caffeine, along with theophylline and theobromine, are natural alkaloid methylxanthines that are normally found together in substances such as coffee, tea, chocolate, energy drinks, and carbonated beverages, such as cola and other soft drinks (Fredholm et al. 1999; Yoshimura 2005; Mitchell et al. 2014; McLellan et al. 2016). Caffeine behaves as a competitive antagonist of adenosine receptors, which are found throughout the brain and body and are thought to regulate the sleep-waking cycle, the stress response, and learning and memory. The presence of caffeine inhibits intracellular enzyme phosphodiesterase, thereby preventing the conversion of cyclic AMP to noncyclic AMP. It is through this transformation that caffeine is believed to exert its influence on the sympathetic nervous system (Nehlig et al. 1992). Additionally, very high and toxic doses of caffeine increase the risk of severe ventricular arrhythmias by increasing the release of calcium from intracellular stores (Thelander et al. 2010; Wolk et al. 2012; Temple et al. 2017).

Nonetheless, caffeine remains one of the most widely consumed psychoactive substances throughout the world (Fredholm et al. 1999; Mitchell et al. 2014; McLellan

et al. 2016). Average caffeine consumption is estimated at about 70–76 mg/person per day worldwide, although in many parts of the western world, including North America, Sweden, and Finland, this number rises to 210 mg/day (Fredholm et al. 1999). In today's popular culture, caffeine is widely viewed as a beneficent substance that can increase alertness and improve mood and performance (McLellan et al. 2016). Thus, an increasing number of people have become highly dependent on the drug, especially in fast-paced societies (Heckman et al. 2010; Cappelletti et al. 2015). Indeed, many studies investigating the effects of caffeine consumption have indicated some benefits, including increased alertness, reduced symptoms of sleep deprivation and delayed onset of neurological disorders such as Alzheimer's disease, Parkinson' disease, and other age-related declines in cognitive performance (Alhaider et al. 2010; Santos et al. 2010; Alhaider and Alkadhi 2015; Panza et al. 2015; Nehlig 2016).

When ingested, caffeine is quickly and completely absorbed into the gastrointestinal tract. The nitrogenous double-bond ring structure of caffeine resembles that of adenosine, and studies have found that caffeine nonselectively targets four adenosine receptor subtypes, including A_1, A_{2A}, A_{2B}, and A_3 receptors (Nehlig et al. 1992; Fredholm et al. 1999; Costenla et al. 2010; Dore et al. 2011). As an adenosine receptor antagonist, caffeine tends to increase neuronal excitability which results in heightened arousal and attention (de Mendonça and Ribeiro 2001). The A_1 receptor subtype is the primary target of caffeine and is most prevalent in the hippocampus, a region of the limbic system that has been closely linked to learning, memory, and emotion (Fredholm et al. 1999; Nehlig 1999). Indeed, removal of or damage to the hippocampus has been shown to significantly impair learning (Jarrard 1993; Bear and Malenka 1994; Eichenbaum 2001; Schiller et al. 2015).

A cellular model of learning and memory which has received a lot of attention in the scientific literature is long-term potentiation (LTP) of synaptic transmission. High-frequency or theta-burst stimulation (TBS) of afferent fibers such as the perforant pathway from the entorhinal cortex has been shown to elicit robust and enduring LTP of hippocampal dentate gyrus synapses which may last for days or weeks (Bliss and Collingridge 1993; Malenka and Nicoll 1999; Blaise and Bronzino 2003; Luscher and Malenka 2012; Blaise 2013; Blaise and Hartman 2013; Blaise et al. 2015). Previous studies have examined the effects of caffeine on synaptic plasticity. In one study, caffeine injected directly into the slice chamber was found to enhance LTP in CA1 pyramidal cells synapses in vitro (Martin and Buno 2003). Brief shots of caffeine applied directly to hippocampal slices in vitro resulted in caffeine-LTP in the Schaffer collateral-CA1 pyramidal cell

synapses. The underlying mechanisms that undergirds this form of LTP was reported to be separate from the classical NMDA-dependent form of LTP, since previous history of LTP did not have an effect on the ability of caffeine to induce a new LTP in these same synapses.

Another study showed that caffeine enhanced basal synaptic transmission in CA1 in vitro, but did not alter neither LTP nor paired-pulse facilitation (a form short-term synaptic plasticity) (Lee et al. 1987). This effect was attributed to caffeine-induced presynaptic release of Ca^{2+} from intracellular stores which likely triggered a coincident release of neurotransmitters which are indistinguishable from those released during classical potentiation. This suggests that caffeine-induced enhancement of basal synaptic transmission is not sufficient to induce LTP in hippocampal CA1 pyramidal cells in vitro. Another study found that caffeine facilitated LTP in CA1 in vitro (Grigoryan et al. 2012) by triggering the release of intracellular stores of Ca^{2+} which facilitates neurotransmitter release. Caffeine had a particularly potent influence on enhancing LTP in the CA2 region of the hippocampus since adenosine A1R receptors are particularly more abundant in this region (Simons et al. 2011).

There is also at least one report of chronic caffeine administration reversing stress-induced suppression of LTP (Alzoubi et al. 2013b) by preventing decreases in phosphorylated calcium calmodulin kinase II and brain-derived neurotrophic factors (BDNFs) in CA1 pyramidal cells. Caffeine has also been reported to preclude sleep deprivation-induced deficits in LTP in an anesthetized model of LTP in the dentate gyrus (Alhaider and Alkadhi 2015). In that study, sleep deprivation decreased LTP which was subsequently reversed by chronic caffeine consumption putatively via caffeine-induced enhancements in BDNF levels. Thus, caffeine appears to provide an overall neuroprotective effect to organisms exposed to chronic stressors, including sleep deprivation and chronic stress. This finding is consistent with the popular theory that drinking caffeine enhances performance on mental tasks that require attention and focus.

Despite the popularity and wide consumption of caffeine, its long-term effects on synaptic plasticity in hippocampal circuits involved in learning and memory remain to be elucidated. Notwithstanding the previous in vitro LTP studies described above, even fewer studies have investigated caffeine's effects on a freely behaving animal model of LTP (Alhaider and Alkadhi 2015; McLellan et al. 2016). It is known that LTP studies using in vitro slice or acutely anesthetized preparations may introduce confounding factors such as lack of interconnections to other brain regions and acute anesthesia which is known to block NMDA receptor activation (a necessary component of LTP) (Duffy et al. 1981; Cain

et al. 1992; Watts 2009). To avoid these issues, we systematically investigated the effects of chronic caffeine consumption on LTP in the hippocampal dentate gyrus by recording electrophysiological signals directly from the intact brains of freely behaving rats.

Methods

All experimental protocols were performed according to the United States Public Health Service's Guide for the Care and Use of Laboratory Animals and were approved by Trinity College's Institutional Animal Care & Use Committee (IACUC). Experiments were performed on two groups (caffeine-treated and control) of 10–17-week-old male Sprague–Dawley rats (300–550 g). The caffeine-treated group received ad libitum access to 1.0 g/L caffeine in tap water for a minimum of 3 weeks prior to experimentation, whereas the control group received regular tap water (Svenningsson et al. 1999; Alhaider et al. 2010; Costenla et al. 2010). Surgical and recording procedures were performed between 8:30 and 14:00. Details of our stereotaxic surgical procedures have been published in detail elsewhere (Blaise and Bronzino 2000, 2003; Blaise et al. 2008, 2015; Koranda et al. 2011; Blaise 2013; Blaise and Hartman 2013). Briefly, rats were injected with an anesthetic cocktail containing ketamine, xylazine, and acepromazine (25 mg/kg, 2.5 mg/kg, 0.5 mg/kg, respectively). Once all muscle reflexes were lost, each rat was placed in a stereotaxic surgical frame to immobilize the head. An epoxylite-insulated stainless-steel bipolar stimulating electrode was inserted into the perforant pathway (AP: −8.1 mm; LAT: +4.0 mm; relative to the bregma). An epoxylite-insulated tungsten, monopolar recording electrode was placed in the dentate gyrus (AP: −4.0 mm; LAT: +2.5 mm relative to bregma). In addition, a ground electrode and indifferent electrodes consisting of stainless-steel machine screws were positioned equidistantly on the surface of the contralateral parietal cortex. Stimulating and recording electrodes were lowered in small increments while monitoring the evoked responses on a digital oscilloscope. The exact dorsoventral position of the electrodes was determined when the digital oscilloscope indicated a maximum triphasic waveform in the PP-DG response. The electrodes were stabilized using dental cement and the wound was sutured using biodegradable surgical threads.

After ≥5 days of postsurgical recovery, rats were acclimated to noise-minimizing recording chambers for at least 2 h prior to recording. Then, animals were connected to low-noise wires equipped with a commutator that allowed for free movement. Using the DAM50 differential amplifier (World Precision Instruments, Sarasota, FL) and Grass S-88 stimulator (Astro-Med, West Warwick, RI), biphasic square wave pulses (pulse width = 0.25 msec, 50% duty-cycle) were used to evoke the PP-DG response (Blaise and Bronzino 2003; Blaise et al. 2008, 2015; Blaise and Hartman 2013). The responses were amplified (gain = 1000) and bandpass-filtered (0.1 Hz–3 KHz). The signal was recorded using a custom-designed data acquisition analysis software system based on LabVIEW (National Instruments, Austin, TX). The software automatically extracted and quantified the population spike amplitude (PSA) measure from the evoked responses (Fig. 1). Note that the PSA measure was used exclusively as an index for quantifying LTP because PSA exhibits less variability than fEPSP measurements recorded in the dentate gyrus in freely behaving animals. Indeed, the peak fEPSP is often occluded by the superimposed population spike, such that potentiation of the population spike may be observed even in the absence of fEPSP potentiation (Taube and Schwartzkroin 1988; Bliss and Collingridge 1993).

Tetanization consisted of theta-burst stimulation (TBS: 10 bursts of 10 pulses at 400 Hz with burst frequency of 5 Hz) at a current of intensity equal to 50% of input/output (I/O) baseline to induce LTP. After tetanization, PSA for the PP-DG signal was calculated as the average of five responses per minute for 15 min. Subsequently, the average of 10 responses was recorded at 30, 60, 120, 180 min, and 24 and 48 h posttetanization. Posttetanization PSA values were compared to baseline for each recorded time point. The data thus obtained were subjected to a two-way repeated-measures analysis of variance (ANOVA) with treatment group as the between group variable and time posttetanization as the within group variable. Percent change from baseline for each time point was compared between groups. Significant main effects ($P < 0.05$) were further analyzed post hoc using the Bonferroni test to assess specific interactions between caffeine treatment and time.

Results

In this study, we compared the effects of chronic caffeine consumption on hippocampal LTP. We observed a significant decrease ($t = 2.7$, $P < 0.05$) in average daily water intake in caffeine-treated rats (41.5 ± 1.4 mL) compared with controls (48.2 ± 2.0 mL) (Fig. 1A). Although there was a trend toward caffeine animals (474.2 ± 12.9 g) weighing less than controls (509.1 ± 11.7 g), statistical analysis revealed no significant ($t = 1.9$, $P > 0.05$) difference in weight (Fig. 1B). Thus, although caffeine-treated rats consumed significantly less water compared with controls, body weight remained consistent between the two groups. Perhaps, caffeine-treated rats compensated for reduction in water intake by increased food intake. But
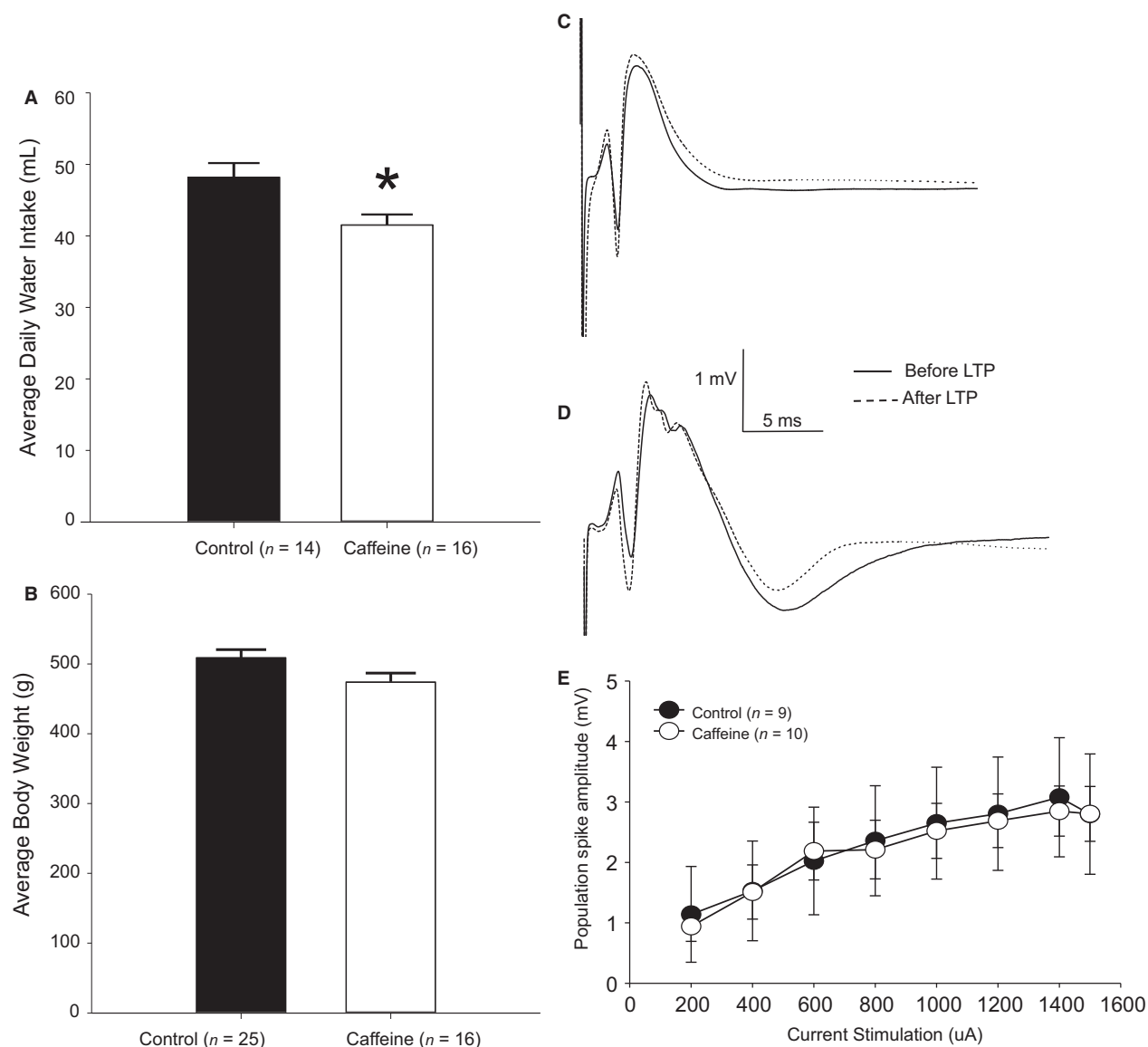
Figure 1. (A) Caffeine-treated rats consumed significantly (*P* < 0.05) less water compared to controls. (B) No significant (*P* > 0.05) differences were observed in average body weight. (C) Representative evoked field potential traces in control and (D) caffeine-treated rats. (E) No significant (*P* > 0.05) differences in the average I/O curves were observed.

since we did not explicitly measure food intake we cannot discount other physiological factors which may underlie these results. Even so, our findings of no significant difference in body weight are consistent with previous reports of similar lean body mass, total body fat, bone weight, and length in caffeine-treated adult rats compared with controls (Kwak et al. 2017).

Evoked field potential traces recorded in control (Fig. 1C) and caffeine-treated animals (Fig. 1D) were quantified by measuring the amplitude of the population spike and were used to establish a baseline input/output

(I/O) excitability response curve for each animal prior to tetanization. As stimulation current intensity was gradually increased, both groups demonstrated a dose-dependent increase in excitability as measured by the amplitude of the population spike (PSA) which plateaued around 1200 μA (Fig. 1E). However, no significant differences ($F = 0.21$, $df = 18$, $P > 0.05$) in baseline excitability as measured by the I/O curves were observed between caffeine-treated and control rats. After establishing a stable baseline, application of a 5-Hz theta-burst stimulation (TBS) protocol (10 bursts of 10 pulses delivered at
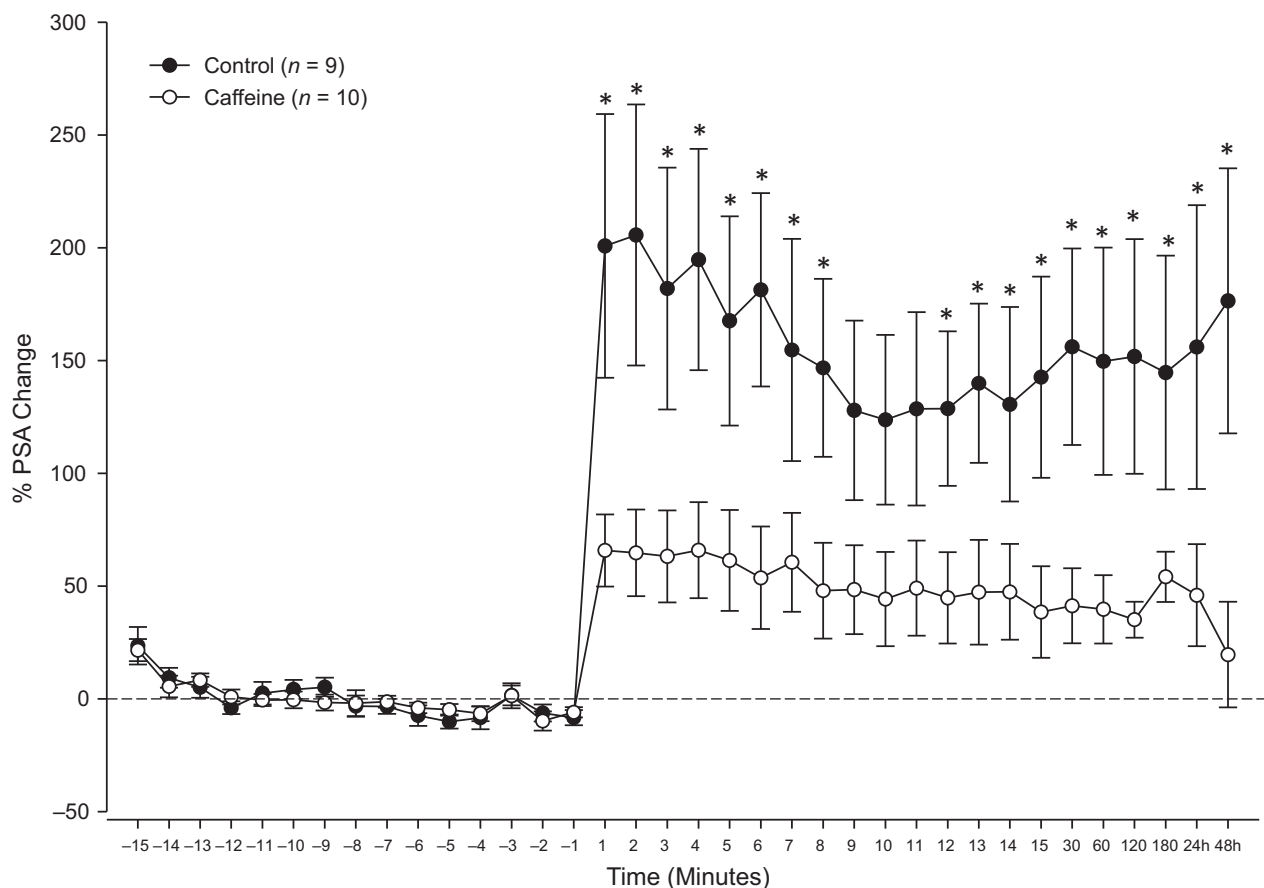
400 Hz with burst rate of 5 Hz) was used to induce LTP. As illustrated in Figure 2, LTP was reliably and robustly induced and maintained up to at least 24 h in both caffeine-treated and control animals ($F = 12.70$, $df = 35$, $P < 0.05$). However, LTP was significantly reduced ($F = 3.47$, $df = 35$, $P < 0.05$) in caffeine-treated rats compared with controls at all the times tested, except at 9–11 min and 48 h posttetanization. Early LTP which consists of the first 15 min immediately following TBS reached a peak of only $65.7 \pm 16.0\%$ in caffeine-treated rats compared to $200.8 \pm 58.4\%$ in controls. While late LTP (24 h post-TBS) persisted in both groups, it dropped to $45.9 \pm 22.6\%$ in caffeine-treated rats compared to $155.9 \pm 62.9\%$ in controls ($P < 0.05$).

Discussion

Three weeks prior to acquisition of electrophysiological recordings, caffeine was administered (1.0 g/L) to male rats (10–17 weeks old) through drinking water. While it had no significant ($P > 0.05$) effect on baseline excitability as indicated by the I/O curves (Fig. 1E), caffeine consumption significantly ($P < 0.05$) reduced LTP (Fig. 2). These results may reflect the upregulation of A_1R following caffeine consumption. Indeed, this is consistent with previous reports linking caffeine to enhanced A_1R expression which may act as a compensatory response that persists even after the cessation of caffeine treatment (Fredholm 1995; Fredholm et al. 1999). However, one previous study found a significant downregulation of A_1R following caffeine exposure in pregnant rats and their fetuses (Leon et al. 2002). However, this discrepancy between that study and the present one may be attributable to gender differences and/or pregnancy (Turgeon et al. 2016).

Our finding of decreased LTP in caffeine-treated rats contrasts with a previous study indicating caffeine exposure did not significantly alter LTP in the DG of

Figure 2. LTP was reliably and robustly induced and maintained up to at least 24 h in both caffeine-treated and control animals. LTP was significantly lower ($P < 0.05$) in caffeine-treated rats compared with controls at all the times tested, except at 9–11 min and 48 h posttetanization.

anesthetized animals compared with controls (Alhaider and Alkadhi 2015). This discrepancy may likely be due to the difference in preparation (anesthetized vs. freely behaving) and/or type of stimulation (multiple high-frequency stimulation vs. single theta-burst stimulation). In the latter study, rats were systematically stimulated with multiple sequences of high-frequency stimulation pulses. In contrast, this study uses the more physiologically relevant theta-burst stimulation protocol.

NMDA receptor-dependent LTP has two main components. First, repeated stimulation has an additive effect, driving synapses toward stable potentiation (Cain et al. 1992; Lante et al. 2006). The second component is a system in which potentiated synapses are undergirded by protein-dependent structural changes (Luscher and Malenka 2012). A wide array of proteins and enzymatic mechanisms underlie these structural changes in the synapse, including BDNFs and antioxidant enzymes, such as superoxide dismutase (SOD) (Poo 2001; Noschang et al. 2009; Viggiano et al. 2012; Sallaberry et al. 2013; Mioranzza et al. 2014). SOD is an antioxidant enzyme that catalyzes the conversion of superoxide into hydrogen peroxide, which is then neutralized by catalase (Alzoubi et al. 2013a). Likewise, chronic caffeine consumption has been shown to increase SOD levels in the hippocampus (Noschang et al. 2009; Abreu et al. 2011). SOD plays a vital role in hippocampal LTP as previous studies have indicated its extinction alters LTP (Thiels et al. 2000; Kamsler and Segal 2003; Viggiano et al. 2012; Pomierny-Chamiolo et al. 2013). Therefore, it is likely that our finding of reduced LTP in caffeine-treated rats is the result of elevated levels of SOD in these animals.

On the other hand, it is important to highlight the possibility that chronic caffeine consumption may have decreased LTP by disrupting the normal sleep-waking cycle in the caffeine-treated rats in this study. Presumably, caffeine induces wakefulness through the blockade of A_{2A} adenosine receptors which are critically involved in the promotion of sleep (Huang et al. 2005; Lazarus et al. 2011). Unlimited access to caffeine may have disrupted the sleep-waking cycle in these animals, which in turn may have triggered a synaptic response similar to what has been observed in animals exposed to stress. Indeed, sleep deprivation or lack of sleep, which are both considered physiological stressors, has been shown to impair LTP and, thus, may have contributed to the caffeine-induced reduction in LTP observed in this study (Diamond and Rose 1994; Shors and Dryver 1994; Lovallo et al. 2006). In conclusion, this study examined the effects of chronic caffeine consumption on synaptic plasticity in the hippocampus in freely behaving rats. Our results indicate that caffeine has an inhibitory effect on LTP at the PP-DG synapse which suggests that adenosine receptors

may be upregulated to compensate for caffeine's blockade of adenosine. Another possibility is caffeine-induced elevation of SOD may downregulate hippocampal LTP. A third possibility is linked to caffeine-induced downregulation of BDNFs which may disrupt synaptic plasticity in hippocampal neuronal networks. More studies are needed to identify the exact mechanism responsible for caffeine-induced reduction in LTP in freely behaving animals as seen in this study.

Conflict of Interest

None declared.

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