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Research report

Chronic caffeine ingestion causes microglia activation, but not proliferation in the healthy brain

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ABSTRACT

Caffeine is the most popular psychoactive drug in the world which contributes to behavioral and metabolic changes when ingested. Within the central nervous system (CNS), caffeine has a high affinity for A1 and A2a adenosine receptors. Serving as an antagonist, caffeine affects the ability of adenosine to bind to these receptors. Caffeine has been shown to alter neuronal functioning through increasing spontaneous firing. However, the effects of caffeine on non-neuronal cells in the CNS have not been studied extensively. Microglia are one phenotype of non-neuronal glia within the CNS. Acting as phagocytes, they contribute to the immune defense system of the brain and express A1 and A2a adenosine receptors. Caffeine, therefore, may affect microglia. In order to test this hypothesis, CD-1 mice were randomly placed into one of three groups: control, low caffeine (0.3 g/L water) and high caffeine (1.0 g/L water) and were allowed to drink freely for 30 days. Following 30 days, brain sections were stained to reveal microglia. Morphological reconstructions and density measurements were examined in cortical and subcortical areas including the primary sensory cortex, primary motor cortex and striatum. Results indicate that microglial density throughout the brain is decreased in the caffeine groups as compared to the control. Caffeine also impacted microglia morphology shortening process length and decreasing branching. These results suggest that chronic caffeine ingestion has a systemic impact on microglia density and their activation.

1. Introduction

The psychostimulant, caffeine is the most widely used psychoactive drug in the world (Julien et al., 2010). Caffeine is found in coffee, tea, soft drinks, chocolate as well as several over-the-counter pain relievers. According to the Food and Drug Administration (FDA), the average American adult consumes 300 mg/day and the average teenager consumes 100 mg/day (Somogyi, 2010). Once consumed, caffeine produces a variety of behavioral effects including increased attention, reduced fatigue and enhanced motor activity (reviewed by Smith, 2002). Caffeine readily crosses the blood brain barrier (McCall et al., 1982). Caffeine's mechanism of action involves blocking adenosine receptors, thus reducing adenosine transmission throughout the brain (reviewed by Fisone et al., 2004). Caffeine also does not display stimulant effects on mice lacking the adenosine receptor A2a (Ledent et al., 1997). Adenosine is a purine nucleoside produced extracellularly through the breakdown of adenosine triphosphate (ATP). Furthermore, while not traditionally classified as neurotransmitter, there are numerous receptors specific for adenosine found throughout the central nervous system. These g-protein coupled receptors include A1, A2a, A2b and A3 receptors. Caffeine binds primarily to A1 and A2a receptors serving as an antagonist to those receptors (reviewed by Fisone et al., 2004). Xanthines, such as caffeine, function as an antagonist to adenosine, which prevents the suppression of neuronal activity (Daly and Fredholm, 1998; Garrett and Griffiths, 1997). Adenosine is also involved in neuroprotection following brain injury, and is

Abbreviations: 3D, three-dimensional; ANOVA, analysis of variance; DAB, 3,3′-Diaminobenzidine; IBA-1, ionized calcium-binding adapter molecule 1; M1, primary motor cortex; NA, numericalaperture; P, postnatal day; PBS, phosphate buffered saline; S1, primary somatosensory cortex.

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known to play a role in central immune responses (reviewed by Cunha, 2005; Wardas, 2002; Li et al., 2013; Gyoneva et al., 2009; Wollmer et al., 2001). However, although the behavioral effects of caffeine, as well as its action on neurons, have been well studied, the effects of caffeine on the brain's immune responses have yet to be fully investigated. Microglia are one type of glial cell and serve as the immune system of the central nervous system (Gehrmann et al., 1995). Primarily acting as phagocytes, microglia engulf and destroy dangerous or foreign material in the brain. Microglia transition between two states: activated and surveillant. When microglia are in the surveillant state, they have extended and heavily branched processes which survey their surroundings. When in contact with infectious or otherwise dangerous material, they enter an activated state during which their processes retract and engage in phagocytosis; engulfing and eliminating harmful debris (Ling and Wong, 1993; Gehrmann et al., 1995). Numerous studies have shown that microglia response is regulated, in part, by adenosine (Li et al., 2013; Gyoneva et al., 2009; Wollmer et al., 2001). For instance, A2a receptor stimulation during brain inflammation causes microglia to retract their processes and take on the characteristic ambeloid shape indicating its activation (Orr et al., 2009). In addition, stimulation of A1 and A2 receptors promote microglia proliferation whereas A1 receptor blockade reduces proliferation (Gebicke-Haerter et al., 1996). More recently, it was confirmed that microglia contain A1 receptors and that stimulation of these receptors using adenosine agonists inhibits microglia activation (Luongo et al., 2014).

While microglia has been most associated with immune response, there is evidence that microglia play an important role in the active maintenance of the brain. Recent studies have revealed that microglia are capable of removing weak synapses (Bialas and Stevens, 2013; reviewed by Siskova and Tremblay, 2013) as well as weak, but still viable, neurons (Brown and Neher, 2014). Within the healthy brain, microglia regulates synaptic activity and aides in the reorganization of neuronal circuits (Tremblay et al., 2011). In this resepect we should reconsider the role of microglia, and examine how microglia function, and how that function can be altered, within the healthy brain.

As microglia contain A1 and A2a receptors, we believe caffeine can exert an effect on these cells. Indeed, it was found that caffeine suppresses the proinflammatory response that occurs during prolonged activation of microglia (Lee et al., 2013; Ruiz-Medina et al., 2013; Kang et al., 2012; Brothers et al., 2010) and therefore may be neuroprotective in neurodegenerative diseases such as Parkinson's disease and multiple sclerosis (Yadav et al., 2012, Tsutsui et al., 2004). Caffeine also increases microglia reactivity when treated with 3,4-methylenedioxy-*N*-methylamphetamine (MDMA) (Khairnar et al., 2010). However, the aforementioned studies examined caffeine's effect on mircoglia following brain insult. The purpose of the current study is to better understand how caffeine affects microglia in the healthy brain.

2. Methods

2.1. Animals and materials

Female CD-1 mice ($n = 21$, Charles River Laboratories) were randomly divided into three groups: low caffeine, which received 0.3 g/L of caffeine dissolved in tap water, high caffeine, which received 1.0 g/L of caffeine dissolved in tap water, and control, which received only tap water ($n = 7$ per group). Females were used as caffeine can cause dramatic effects in females including altering estrogen levels (Schliep et al., 2012) and affecting reproductive outcomes (Klonoff-Cohen et al., 2001). However, it should be noted males receive a larger therapeutic benefit of caffeine in regard to Parkinson's disease (Palacios et al., 2012) and amyotrophic lateral sclerosis (ALS) (Seevaratnam et al., 2009). Dosages were determined based on previous literature (Rieg et al., 2007; Li et al., 2008). All animals were postnatal day (P)30. There was no significant difference in the initial weight of the control, low dose and high dose groups (13.04 ± 0.20 g, 13.27 ± 0.31 g and 13.06 ± 0.32 g, respectively; ANOVA, $p > 0.05$). Mice were handled in accordance with Queens College, CUNY IACUC (Protocol #100, approved July 3, 2012) and NIH rules governing the ethical and responsible treatment of animals for biomedical research.

2.2. Experimental procedure

Mice were housed individually in standard plastic cages and allowed to drink and eat freely for 30 days. The weight of the mice was measured every week and the volume of liquid consumed was recorded daily (Fig, 1A and B). Daily caffeine intake was also calculated (Fig. 1C).

2.3. Immunohistochemistry

At the end of the 30 day treatment period mice were anesthetized with an intraperitoneal injection of Euthasol (Virbac AH,

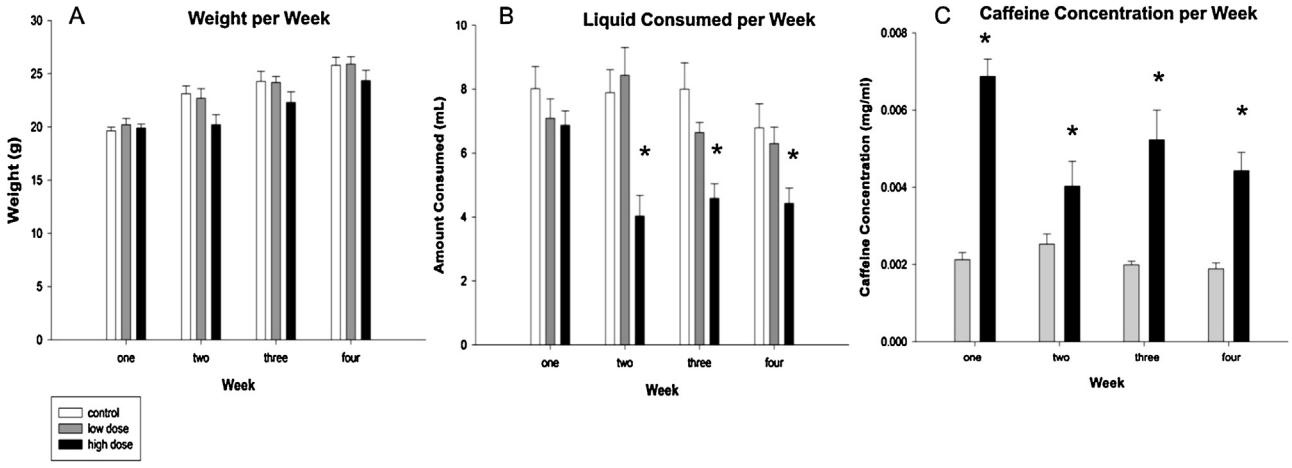

Fig. 1. Effects of caffeine on drinking behavior. (A) Weight of mice in grams per week. (B) Total liquid consumed, in milliliters, per week. (C) Concentration of caffeine ingested in the low and high dose group per week. Asterisks denote significant differences between groups ($p < 0.05$). Plots represent population means and error bars represent one standard error of the mean.