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Review

# Early caffeine exposure: Transient and long-term consequences on brain excitability

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ABSTRACT

The influence of pre- and postnatal caffeine treatment on brain excitability during development and adulthood is reviewed. Pre- and postnatal exposure to caffeine induces sex- and age-specific long-term neurochemical alterations in the brain and the behavior of rodents. Because adenosine neuromodulation is closely related to the regulation of brain excitability the increased expression in adenosine receptor system due to neonatal caffeine treatment should cause transient and permanent changes in seizure susceptibility. So far, findings have been focused on primarily developmental changes of the brain adenosine modulatory system and have demonstrated that the alterations are not restricted to a single brain region. Neurobehavioral changes and the anticonvulsant effect of early caffeine exposure are dependent on the caffeine dose, developmental stage of exposure and age of testing. Although outcomes of caffeine treatment are still a matter of debate, our review raise questions concerning the impact of early caffeine treatment on regulation of seizure susceptibility during development and adulthood.



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## 1. Introduction

Adenosine is a neuromodulator in the central nervous system, which exerts a depressant effect on neurons (Kostopoulos and Haas, 1988) and a tonic inhibitory effect by reduction of the transmitter release from excitatory terminals and by modulation of intrinsic membrane properties (Chen and Chern, 2011; Nehlig et al., 1992). Considered as an endogenous anticonvulsant, adenosine participates in the seizure arrest and postictal refractoriness (Dragunow, 1990). Therefore, disturbances in adenosine modulatory system could be implicated in the ictogenesis. Experimental and clinical data focused on the crucial role of endogenous adenosine and its receptors in the control of seizure susceptibility contributes to the current understanding of how methylxanthines influence the excitability of the brain. Methylxanthines antagonize the anticonvulsant and neuroprotective effect of endogenous adenosine and possess convulsant and excitotoxic activity (Fredholm et al., 1999; Nehlig et al., 1992). Possible risk

*Abbreviations:* PTZ, pentylenetetrazol; KA, kainic acid; NMDA, N-methyl-D-aspartat; RMA, rhythmic metrazol activity; SW, spike-and-wave; Ads, epileptic afterdischarges.

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factors in methylxanthine-associated seizures are age, previous brain injury or disease and severe pulmonary disease. The identification of the methylxanthines caffeine and theophylline as non-selective adenosine receptor antagonists, together with the knowledge that xanthine-rich beverages such as coffee and tea have a potent reinforcing activity in humans (Fredholm et al., 1999; Griffiths and Woodson, 1988) directed the research on mechanisms underlying the influence of methylxanthines on brain excitability. Caffeine has similar in vitro affinities for adenosine A1, A2A and A2B receptors and much lower affinity for A3 receptors in low doses (Fredholm et al., 2001). The preferential targets for caffeine are adenosine A1 and A2A receptors in the brain, where physiological extracellular levels of adenosine are sufficient to activate them (Ferré, 2010). Although adenosine-related research in epileptic patients is scarce, clinical findings of a proconvulsive role of the methylxanthines are largely based on theophylline (or aminophylline) (Ram et al., 2005). In the neonatal medicine, the methylxanthines, including caffeine, have been used in clinical practice since the 1970s to reduce apnea in premature infants (Comer et al., 2001; Henderson-Smart and Steer, 1999; Schmidt et al., 2006, 2007). Depending on the gestational age at birth, caffeine treatment usually continues days to weeks and is assumed as efficient and a low risk therapy (Hascoet et al., 2000). Clinical data raised the point of consequences of long-term methylxanthine delivery in very preterm infants (Millar and Schmidt, 2004) where the fastest brain growth takes place (Andrews and Fitzgerald, 1997).

There is a lack of well-designed, randomized clinical studies on long-term outcome of methylxanthine's therapy though possible adverse effects might include an impaired growth, lack of neuroprotection during acute hypoxic-ischemic episodes and abnormal behavior (Millar and Schmidt, 2004). Although seizures are a relatively unusual complication of methylxanthine therapy for apnea, clinical and experimental studies suggest certain risk, which could be minimized if predisposing factors as age-related changes in drug metabolism, renal insufficiency and blood–brain barrier immaturity are considered. In experimental animals, repeated exposure to caffeine during peri- and postnatal period has been shown to result in long-lasting neurochemical and behavioral responses that are usually opposite to acute drug effects under normal as well as pathological conditions (Hughes and Beveridge, 1990; Gaytan and Pasaro, 2012; Gaytan et al., 2006; Pan and Chen, 2007; West et al., 1986; Zimmerberg et al., 1991).

## 2. Clinical findings

Methylxanthines, which stimulate breathing efforts, are among the most often prescribed drugs in neonatal medicine (Millar and Schmidt, 2004). Apnea may occur repeatedly in preterm babies. The mechanism underlying the methylxanthine activity may include increased chemoreceptor responsiveness, enhanced respiratory muscle performance and generalized central nervous system excitation. Although caffeine has effects in the treatment of apnea similar to theophylline (Steer and Henderson-Smart, 2000) it has potential advantages due to the larger therapeutic index, more reliable enteral absorption and longer half-life, which allows once-daily administration (Blanchard and Aranda, 1992). At present, clinical data considering the consequence of prophylactic methylxanthine treatment for apnea on long-term development are limited (Henderson-Smart and Steer, 1999; Schmidt et al., 2007).

Future clinical studies focused on the methylxanthine treatment in preterm infants at higher risk of apnea should include examination of developmental changes in brain excitability and seizure susceptibility in particular.

## 3. Experimental findings

### 3.1. Neurochemical and behavioral changes as a consequence of postnatal repeated caffeine exposures

Growing body of evidence supports the idea that repeated exposure to caffeine during development induces long-lasting neurochemical and behavioral changes (Table 1). Due to the high variability in experimental design related to the dose, age period and duration of caffeine treatment as well as the age of testing, there are discrepancies hampering drawing of general conclusions. The exposure of rats to low doses of caffeine during gestation and lactation was reported to provoke different sex-specific changes of DNA and RNA content, proteins, Zn levels, cholesterol content, saturated fatty acids in individual brain areas; a decrease in the total brain weight, the cerebellum and the body weights, a delay in incisor eruption and eye opening (Nakamoto and Shaye, 1984; West et al., 1986; Yazdani et al., 1988, 2004; Zimmerberg et al., 1991). Some of these changes are transient, others are long-lasting or permanent. Perinatal caffeine exposure also induces a number of changes in neurotransmitter and receptor systems, including modifications in the cerebral levels of catecholamines, tyrosine, tryptophan, serotonin, 5-hydroxyindole acetic acid, cyclic nucleotides, minor effects on development of adenosine $A_1$, $A_{2A}$ and $GABA_A$ receptors in the rat brain, delayed migration and insertion of GABA neurons into the hippocampal circuitry during the first postnatal week, down-regulation of several mGluR/PLC transduction pathway components accompanied by hypoactivity or increased activity during the development of animals (Ådén et al., 2000; Holloway, 1982; Hughes and Beveridge, 1990; León et al., 2005; Lombardelli et al., 1984; Nakamoto et al., 1991; Nehlig et al., 1992; Silva et al., 2013). The postnatal stage between 2 and 10 days, when the brain growth spurt starts (Dobbing and Sands, 1979), seems to be a crucial period for the modulatory effect of the repeated caffeine treatment, associated with a decrease in the cerebellum weight, an increase in the saturated and monounsaturated fatty acids, a dose-dependent decrease in myelin concentration and modifications in dendritic morphology of the pyramidal cells of the prefrontal cortex (Fuller et al., 1982; Juárez-Méndez et al., 2006; Yazdani et al., 2004). Some of these effects were transient (Fuller et al., 1982) other persisted after puberty (Juárez-Méndez et al., 2006; Hughes and Beveridge, 1990; Nakamoto and Shaye, 1984; Nakamoto et al., 1991; Zimmerberg et al., 1991).

The behavioral consequences are usually opposite to acute caffeine effects under normal as well as pathological conditions. The outcome of the behavioral responses as a result of pre- or postnatal caffeine exposure in rats is dose- and age-dependent, what could be a reason for the contradictory results. Thus, though the mode of delivery and the period of the caffeine exposure were comparable, Sobotka et al. (1979) reported that adolescent male rats were hyperactive, whereas Concannon et al. (1983) found the hypoactive offsprings. Caffeine exposure in utero increased the activity level of pups and altered the effect of an acute caffeine challenge (Holloway, 1982). Furthermore, while Guillet (1990) reported that neonatal caffeine exposure at doses of 15–20 mg/kg/day increased the mobility of 12-day-old rats, whereas doses of 1 or 9 mg/kg/day administered at the same age exert a depressant effect on motor behavior at two weeks (Table 1). Therefore, the age of testing appears to be crucial for the direction of behavioral changes probably due to an altered ontogeny of adenosine receptors. The 18-day-old rats are the most sensible to the effect of acute caffeine on locomotor activity in neonatally caffeine-exposed rat (Guillet, 1990). The correlation between the age-related alterations in the receptor binding and behavioral responses confirmed that the crucial period of 15–18 postnatal days can be influenced by neonatal caffeine exposure (Guillet and Kellog, 1991). Early caffeine