Fall 2016

The Effects of Caffeine on Prenatal Brain Development

Alyssa J. Van Lenten
Elizabethtown College, vanlentena@etown.edu

The Effects of Caffeine on Prenatal Brain Development

Alyssa J. Van Lenten

FYS100: Gotta Have It: Exploring the Science of Addiction

Dr. Thomas Hagan

November 20, 2016

Abstract

Past studies have determined that pregnant women's consumption of caffeine can be a cause for intrauterine growth restriction as well as other fetal complications. More recently, experiments are revealing that prenatal caffeine exposure can alter the biochemistry in the developing brain of the fetus. Specifically, caffeine has effects on the brain by antagonizing adenosine receptors, inhibiting phosphodiesterase, and releasing calcium stores. However, caffeine's effects can most likely be contributed to its ability to antagonize adenosine receptors.

Caffeine has the potential to slow GABA neuron migration in the developing hippocampus of the fetal brain. One study discussed in this report suggests that caffeine does this by blocking adenosine receptor 2A. While scientists are still conducting research to determine if caffeine's antagonism of AR_{2A} slows cell migration and causes deficits in memory and cognition, there already is a negative correlation between prenatal caffeine exposure and performance on tests based on memory and cognition. Another study discussed in this report tests these abilities in rats, and a third study tests IQ scores for children prenatally exposed to caffeine 5.5 years after birth. The findings overall suggest that a woman should avoid consuming caffeine while pregnant in order to protect the growth and development of the fetus.

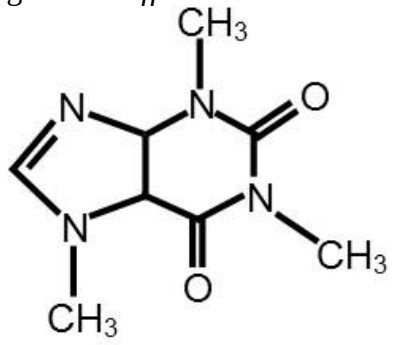
The Effects of Caffeine on Prenatal Brain Development

Caffeine is the most commonly used psychoactive drug throughout the world. In the United States, it is legal for everyone to buy it, and it is the only drug that is readily available in the workplace. All types of people, regardless of age or socioeconomic class, use caffeine. Many cultures also incorporate coffee into their traditions and lifestyles. It is believed that a shepherd in Ethiopia was the first to discover caffeine, in the form of coffee beans (Romano & Russo, 2012, p. 4). Today, the drug is available in many other forms including, but not limited to, coffee, tea, soda, chocolate, caffeine pills, or other pharmaceutical drugs.

Caffeine is a type of methylxanthine which is a chemical compound with a purine base. The chemical name for caffeine is 1,3,7-trimethylxanthine. Other methylxanthines related to caffeine include theophylline (1,3-dimethylxanthine), and theobromine (3,7- dimethylxanthine). Theobromine is commonly found in chocolate, and theobromine and theophylline are both frequently used to treat asthma (Shufer, 2016, para. 26). When ingested, caffeine is actually metabolized in the liver by the enzyme cytochrome P-450 into theobromine, theophylline, and paraxanthine (Ribiero & Sebastiñao, 2010, p. S5). Therefore caffeine is the most potent methylxanthine and has the greatest effects on the central nervous system and the body. These effects include stimulating the central nervous system, increasing alertness, and reducing fatigue. Users most frequently ingest caffeine in the form of coffee in order to “wake up”. Preliminary research might also support that caffeine in moderate doses has the potential to have other long-term health benefits, like aiding in the prevention of Type 2 Diabetes, Alzheimer’s Disease, and Parkinson’s Disease (Romano & Russo, 2012, p. viii**)**. However, caffeine consumption has been known to cause other side effects in adults, such as tremors, nausea, anxiety, insomnia, and gastrointestinal issues (Shufer, 2016, para. 25).

These side effects are not demonstrated by all users. Caffeine use also does not display the dangers that other psychoactive drug use does, such as severe impairment of cognitive function, and so caffeine is generally known as a "safe drug". Caffeine has, however, been known to have adverse effects on the fetuses of mothers who consumed caffeine while pregnant. Research has shown a clear connection between prenatal caffeine consumption and low birth weight, intrauterine growth restriction (IUGR), and sometimes miscarriage (Mioranzaa et al., 2014, p. 45**)**. Other effects of caffeine on babies are not clearly associated, and little research on the topic has been done (Mioranzaa et al., 2014, p. 45**)**. Nevertheless, more recent research is just beginning to suggest that caffeine has a deeper effect on the developing brain of the fetus, which in turn can create physiological and psychological problems later in life. Because prenatal caffeine consumption can most likely cause incorrect organization of neurons in the brain, women should avoid drinking coffee while pregnant (Romano & Russo, 2012, p. 16; Galéra et al., 2016, p. 724; Soellner, Grandys, & Nuñez, 2010, para. 47).

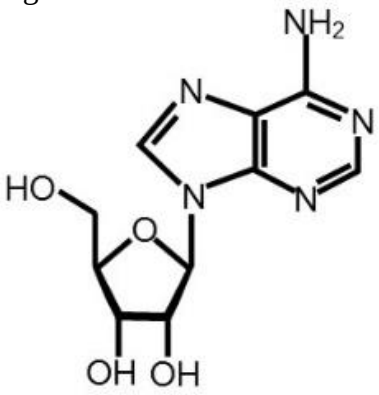
Figure 1: *Caffeine Molecule*



Note: This diagram is adapted from *Caffeine Consumption and Health* by Romano & Russo, 2012, p. 3.

The molecule caffeine is lipophilic and can easily pass through biological membranes such as in the brain and the placenta (Guedes, De Aguiar, Alves-de-Aquiar, & Preedy, 2012, p. 7; Mioranzaa, 2014, p. 45). Therefore, if a pregnant woman consumes caffeine, nothing stops the drug from entering the fetus' body and having a physiological affect. Because of a shortage of cytochrome P-450 in the developing liver, the fetus also has a hard time in the metabolism of caffeine, which means it stays in the body and facilitates its effects for a longer period of time. (Soellner et al., 2010, para. 2)**.**

Figure 2: *Adenosine Molecule*



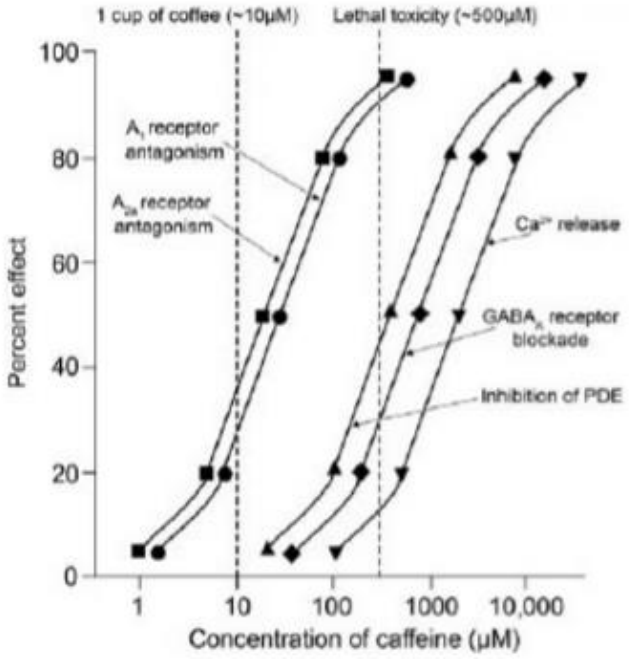
Note: This diagram is adapted from "This is how your brain becomes addicted to caffeine" by Stromberg, 2013.

Because caffeine, as represented in Figure 1, is structurally similar to adenosine, represented in Figure 2, caffeine is able to bind to adenosine receptors. The neurotransmitter adenosine is responsible for monitoring sleep, but it also has several other jobs, which include control of breathing, cognition, and memory. Adenosine, when released, binds to one of its G-protein coupled receptors (IJzerman et al.), A_1, A_{2A}, A_{2B}, or A_3, on another neuron. The response of the postsynaptic cell following this interaction between adenosine neurotransmitter and receptor is what causes a person to feel tired. Caffeine creates alertness by preventing this interaction when it antagonizes the adenosine receptor.

The A_{2A} receptor has the highest affinity for caffeine and is located on GABAergic neurons (Goldstein, 2001, p. 213), which synthesize, store, and release the neurotransmitter gamma-aminobutyric acid (GABA). GABA is usually an inhibitory neurotransmitter but also plays an important role in the migration of neurons in the developing brain (Goldstein, 2001, p. 49; Luhmann, Fukuda, & Kilb, 2015, p. 7). By binding to the A_{2A} receptor on a GABAergic neuron, adenosine creates an action potential in the GABAergic neuron and facilitates a release of GABA. When GABA is released, it binds to GABA receptors on the postsynaptic neuron and inhibits an action potential. Caffeine blocks adenosine receptors, inhibiting an action potential; thus, the neuron does not release GABA. Because GABA is an inhibitory neurotransmitter, its release typically inhibits dopamine. Therefore when GABA release is inhibited, dopamine is released in large quantities. (Goldstein, 2001, p. 213). This is how caffeine plays into the

dopamine hypothesis and becomes addictive in the adult human brain. Specifically, caffeine works in two areas of the brain: the prefrontal cortex and the hippocampus, which are responsible for cognition and memory.

Figure 3: *The Circulation Concentration of Caffeine Required to Activate Various Molecular Targets in relation to human caffeine consumption*



Note: This graph is adapted with permission from Bertil. B Fredholm (1979) as cited in *Neuropathology of Drug Addictions and Substance Misuse Volume 3: General Processes and Mechanisms, Prescription Medications, Caffeine and Areca, Polydrug Misuse, Emerging Addictions and Non-Drug Addictions* by Preedy, (2016), p. 756.

In addition to blocking adenosine receptors, caffeine is responsible for releasing calcium from ryanodine stores and inhibiting phosphodiesterase activity (Goitia, 2016, p. 527; Guedes et al., 2012, p. 6-7). Phosphodiesterase (PDE) is an enzyme that breaks cyclic adenosine monophosphate (cAMP), which is an important molecule in regulating many cellular functions, into adenosine monophosphate (AMP). By causing a high amount of cAMP in the cells, caffeine could potentially hinder growth and development of fetal neurons (Roman & Russo, 2012, p. 16).

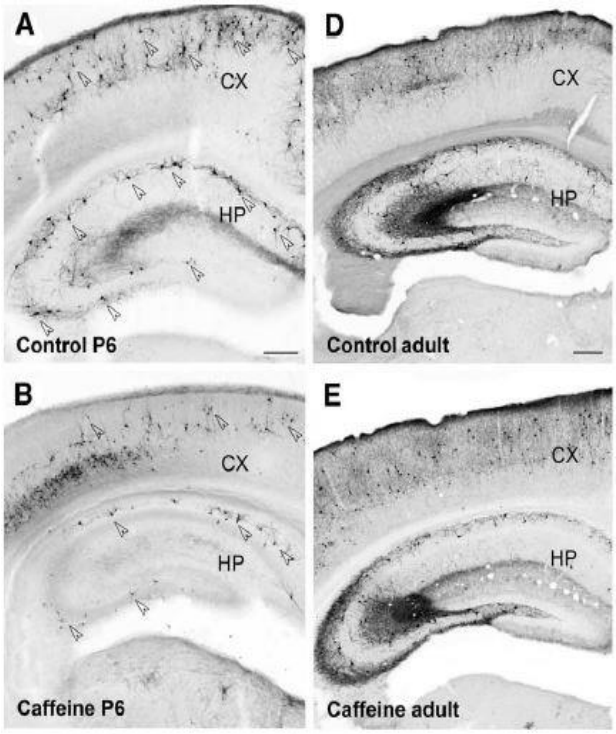
Caffeine can have effects on calcium stores and PDE, but it is much more likely that caffeine's effects are due to antagonism of adenosine receptors (Preedy, 2016, p. 794).

Figure 3 displays how different amounts of caffeine create different effects at different intensities. Ten micro molar (μM) of caffeine is about one cup of coffee, and 500 micro molar is

a lethal amount of caffeine (Preedy, 2016, p. 794). Based on the scale in Figure 3, adenosine receptors would be about 95 percent blocked by 500 micro molar, while PDE would only be about 40 percent inhibited. At 200 micro molar, intracellular calcium release is only just beginning. In fetal cells, chronic exposure to caffeine could enhance these effects because of a longer presence of caffeine (Soellner et al., 2010, para. 2), but antagonism of adenosine receptors is still more likely (Preedy, 2016, p. 794).

Caffeine's blocking of the adenosine receptors also has the potential to prevent GABA cell migration in the developing fetal brain (Galéra et al., 2016, p. 724). The human central nervous system, making up the brain and the spinal cord, forms through a neural plate which rolls into a neural tube. This tube contains three layers: the sub-ventricular zone, which is next to the ventricle in the neural tube; the cortical plate; and the sub-pial zone. New cells proliferate in the sub-ventricular zone and then migrate through the cortical plate to a new location where they differentiate into different kinds of neurons (Luhmann et al. 2015, p. 1). Figure 4 displays migrating neurons (identified by the white arrows) tagged with green fluorescent protein (GFP) in coronal sections of mice brains at postnatal day 6 (P6) (A and B) and in adulthood (D and E). At P6,

Figure 4: *Caffeine exposure during the embryonic and early postnatal period impairs the migration of hippocampal GABA neuron subpopulations in mouse brain*



Note: From "Adenosine receptor antagonists including caffeine alter fetal brain developmet in mice" by Silva et al., (2013), p. 3.

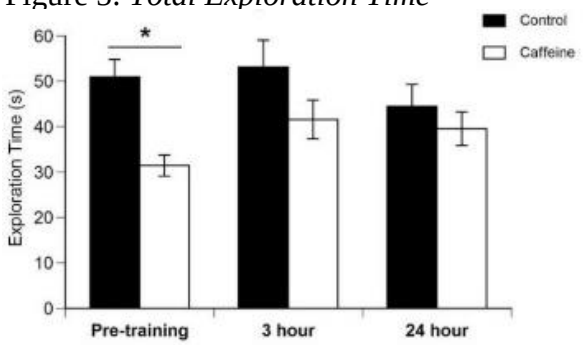
13 GFP-tagged migrating neurons are identified for the control, whereas 8 GFP-tagged migrating neurons are identified for the caffeine-treated brain. In the adult coronal sections, there are not significant differences (Silva et al., 2013, p. 2). Silva et al. (2013) also performed an experiment substituting caffeine with another AR_{2A} antagonist, SCH58261, causing a 56 percent decrease in the speed of migrating cells. Because caffeine is correlated with slow migration of GABA neurons, this information led scientists to believe that AR_{A2} antagonism of caffeine is likely to control the migration of GABA neurons (p. 2-3).

GABA neurons are important in the hippocampus, and because the GABA neurons did not complete migration perfectly, the hippocampus might be organized oddly as it continued to develop (Galéra, 2015, p. 724). The hippocampus is responsible for memory and cognition, and so disorganization could cause issues in these operations, according to different experiments with animals and humans.

Soellner et al. conducted experiments with rats in 2010 to study the effects of prenatal exposure to caffeine on cognition and memory in adulthood. Pregnant female rats were chronically exposed to tap water containing caffeine. Once the rats were born and reached adulthood, two separate neurobehavioral tests were conducted that support impairment of cognition and memory by prenatal caffeine exposure. The first test was the Novel Object Recognition test. During pre-training, rats were given the opportunity to explore two identical objects (A, A). During the test, rats were given three minutes to explore one of the original objects (A) and one new object (B) 3 hours after pre-training and three minutes to explore one of the original objects (A) and another new object (C) 24 hours after pre-training. Figure 5 displays the results of this test. The pre-training results were calculated by adding the times for both objects (A+A). The results for the test at 3 hours were calculated by adding the time explored of

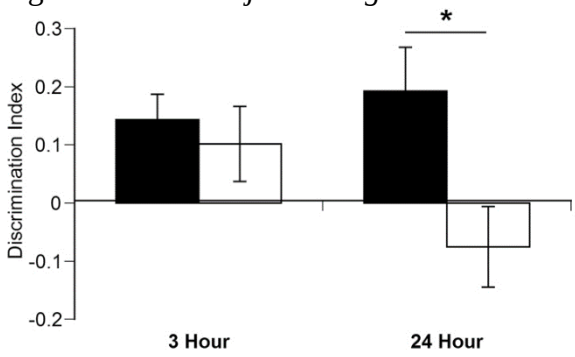
the original object plus the first novel object (A+B), and the results for the test at 24 hours were calculated by adding the time explored of the original object plus the second novel object (A+C). Figure 5 shows that on average, the rats prenatally exposed to caffeine explored less overall for each phase of the test (para. 10).

Figure 5: *Total Exploration Time*



Note: This graph is obtained from "Chronic Prenatal Caffeine Exposure Impairs Novel Object Recognition and Radial Arm Maze Behavior in Adult Rats" by Soellner et al., 2010.

Figure 6: *Novel Object Recognition*

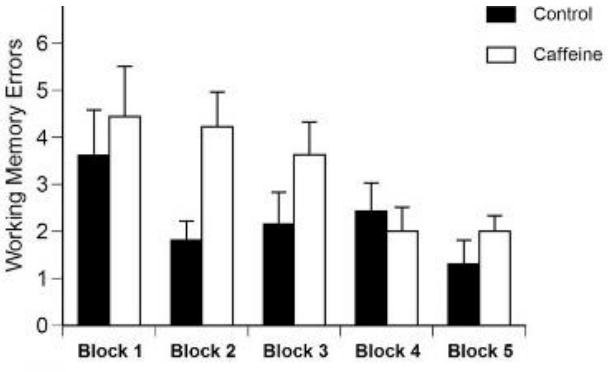


Note: This graph is obtained from "Chronic Prenatal Caffeine Exposure Impairs Novel Object Recognition and Radial Arm Maze Behavior in Adult Rats" by Soellner et al., 2010.

Figure 6 displays whether or not the rats spent more time exploring the novel object or the known object. The discrimination index was calculated by subtracting the time exploring the original object from time exploring the novel object and then dividing that by total exploration time [(B-A)/(B+A)]. Hence, a positive discrimination index implies that the rat spent more time exploring the new object rather than the old object. In theory, the rats should explore the new object more than the old object because they would already be familiar with the old object. Graph 2 shows that the rats prenatally exposed to caffeine explored less overall and explored the old object more than the new object 24 hours after pre-training (para. 10)

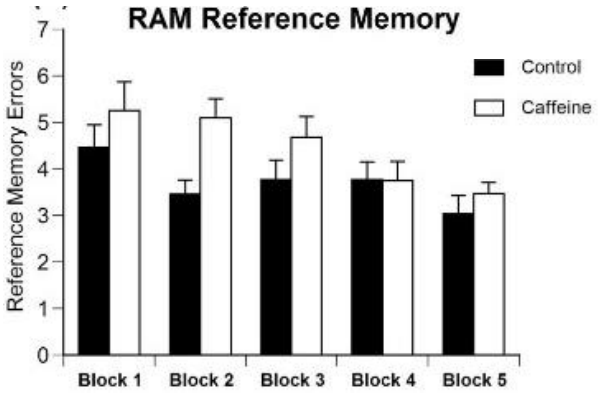
The second test, the Radial Arm Maze test, assesses spatial navigation for the hippocampus and prefrontal cortex. The maze consisted of a platform with eight radial arms. A treat was placed at the end of the same four arms for each of ten trials, and a rat was given ten minutes per trial to find all of them. An error in working memory was counted when a rat went down an arm which it had already eaten the treat from, and an error in reference memory was counted when a rat went down an arm that contained no treat. Figures 7 and 8 show that rats prenatally exposed to caffeine executed more errors than the control rats, especially regarding working memory. These consistent results support that prenatal exposure to caffeine in rats affects their cognition and memory after birth (para. 11, 12).

Figure 7: *Radial Arm Maze: Working Memory*



Note: This graph is obtained from "Chronic Prenatal Caffeine Exposure Impairs Novel Object Recognition and Radial Arm Maze Behavior in Adult Rats" by Soellner et al., 2010.

Figure 8: *Radial Arm Maze: Reference Memory*



Note: This graph is obtained from "Chronic Prenatal Caffeine Exposure Impairs Novel Object Recognition and Radial Arm Maze Behavior in Adult Rats" by Soellner et al., 2010.

Of course, data from an experiment with rats cannot necessarily apply directly to humans. However, Galéra et al. (2016) led an observational study on prenatal caffeine exposure and IQ at 5.5 years in human children. The study asked pregnant women to report caffeine consumption throughout pregnancy. 91% percent of mothers consumed caffeine during pregnancy, and 12.3 % of mothers consumed a high amount daily (greater than or equal to 200 milligrams per day) (p. 722). Then,

at 5.5 years of age, the children's full IQ, verbal IQ, and performance IQ were tested by professional psychologists using the Wechsler Preschool and Primary Scale of Intelligence Third Edition (p. 721). Table 1 displays the average IQ scores of these tests compared to the amount of caffeine consumed daily by the mothers while pregnant. Clearly, the IQ scores decrease as caffeine intake increases. The results for full IQ and performance IQ were statistically significant, which means there is a negative association between these IQ scores and prenatal caffeine exposure (p. 722).

Table 1: *Children's IQ Scores distributed by Mothers' Caffeine Consumption*

	Reported Caffeine Consumption of Mothers		
IQ Tests for Children	0-100 mg/day	100-290 mg/day	≥ 200 mg/day
Average Full IQ	103.7	102.7	100.5
Average Verbal IQ	106.9	106.5	105.1
Average Performance IQ	100.2	98.6	96.3

Note: This tabled is adapted from information in "Prenatal caffeine exposure and child IQ at age 5.5 years: the EDEN mother-child cohort" by Galéra et al., 1016, p. 723.

These scientific studies suggest that prenatal caffeine exposure somehow affects the hippocampus and/or the prefrontal cortex, which are the areas of the brain involved in thinking and memory. The experiments by Soellner et al. (2010) show that rats prenatally exposed to caffeine had a more difficult time recognizing new objects and remembering where they could find treats (para. 38). The report by Galéra (2016) showed that children tend to have lower IQs after being prenatally exposed to caffeine (p. 722). Researchers hypothesize that prenatal caffeine exposure can alter the organization of the developing hippocampus, possibly by delaying GABA cell migration. The photomicrographs by Silva et al. (2013) display fewer GFP-tagged migrating GABA neurons after prenatal caffeine exposure in mice, which helps to further support this argument (p. 3).

Scientists are still investigating the exact process of how caffeine delays GABA cell migration. It is likely that this occurs through antagonism of adenosine receptor AR_{2A}. Silva et al. (2013) explored this topic by replacing caffeine with AR_{2A} antagonist SCH58261. This chemical also slowed the process of cell migration. Thus, the study concluded that migration of GABA cells is possibly controlled by AR_{2A}, and because caffeine also has an effect on AR_{2A}, it is likely that caffeine can alter migration as well (p. 2-3).

Caffeine does exhibit other effects on the cell, including release of intracellular calcium stores and inhibition of PDE. Because PDE plays an important role in regulation many cellular functions, these implications could be huge (Goitia, 2016, p. 527; Guedes et al., 2012, p. 6-7). However, due to the amount of caffeine that is required to produce these effects, it is much more likely that caffeine' ability to antagonist AR_{2A} is what causes the delay in GABA neuron migration.

The photomicrographs of the coronal sections of the brains at P6 and full adult suggest that caffeine exposure does not affect fetus and adult brains similarly. Studies show that adults are able to metabolize and eliminate caffeine much more quickly. This is due to a larger amount of functional cytochrome P-450 in the adult liver compared to the developing liver of the fetus (Soellner et al., 2010, para. 2)**.** Also, an adult brain is very different than a fetal brain. Neural connections have already been made, and cells are already differentiated. In the fetal brain, the effects are going to be more extensive because these processes are occurring.

The effects of hippocampal disorganization by caffeine are subtle, but important. The IQ scores in children age 5.5 prenatally exposed to caffeine were not dropping severely, and the results on the rats prenatally exposed to caffeine eventually were not so extreme. These results are statistically significant, but caffeine is not causing severe mental handicaps. This means that

women might be causing slight detriments in the brains of their unborn children without fully understanding that this is possible. On the other hand, caffeine is already shown to cause low birth weight, IUGR, and miscarriage (Mioranzaa et al., 2014, p. 45**)**. These effects are more serious and more prominent, but effects on the developing brain should not be disregarded.

While there are a few studies on the effects of prenatal caffeine exposure in the developing brain, not enough research has been done to come to any specific and concrete conclusions. More information is necessary to determine a direct causation instead of a correlation. Studies in the future will hopefully clarify if it is safe for pregnant women to consume caffeine. In the mean time, it is suggested that a pregnant woman should avoid consuming caffeine to protect the health and development of the fetus.

References

Galéra, C., Bernard, J.Y., van der Waerden, J., Bouvard, M., Lioret, S., Forhan, A., De Agostini, M., Melchior, M. & Heude, B. (2016). Prenatal caffeine exposure and child IQ at age 5.5 years: the EDEN mother-child cohort. *Biological Psychiatry*, *80,* 720-726. doi:10.1016/j.biopsych.2015.08.034

Goitia, B., Rivero-Echeto, M. C., Weisstaub, N. V., Gingrich, J. A., Garcia-Rill, E., Bisagno, V., & Urbano, F. J. (2016). Modulation of GABA release from the thalamic reticular nucleus by cocaine and caffeine: role of serotonin receptors. *Journal of Neurochemistry*, 136(3), 526-535. doi:10.1111/jnc.13398

Goldstein, A. *Addiction: From Biology to Drug Policy.* New York: Oxford, 2001. Print.

Guedes, R.C.A., De Aguiar, M.J.L., Alves-de-Aquiar, C.R.R., & Preedy, V.R. (Ed.). (2012). *Caffeine: Chemistry, Analysis, Function, and Effects.* Retrieved from http://pubs.rsc.org/en/content/chapter/bk9781849733670-00003/978-1-84973-475-2#!divabstract

IJzerman, A.P., Fredholm, B.B., Jacobson, K.A., Linden, J., Müeller, C.E., Frenguelli, B.G., Schwabe, U., Stiles, G.L., Hills, R., & Klotz, K. Adenosine receptors. Retrieved from http://www.guidetopharmacology.org/GRAC/FamilyDisplayForward?familyId=3.

Luhmann, H. J., Fukuda, A., & Kilb, W. (2015). Control of cortical neuronal migration by glutamate and GABA. *Frontiers In Cellular Neuroscience*, 9(4), 91-15. doi:10.3389/fncel.2015.00004

Mioranzaa, S., Nunes, F., Marques, D.M., Fioreze, G.T., Rocha, A.S., Botton, P.H.S., Costa, M.S., & Porciúncula, L. O. (2014). Prenatal caffeine intake differently affects synaptic

proteins during fetal brain development. *International Journal of Developmental Neuroscience, 36, 45-52.* doi:10.1016/j.ijdevneu.2014.04.006

Preedy, V.R. (Ed). (2016). *Neuropathology of drug addictions and substance misuse volume 3: General processes and mechanisms, prescription medications, caffeine and areca, polydrug misuse, emerging addictions, and non-drug addictions.* Amsterdam: Alsevier Academic Press. Retrieved from http://scribd.com

Ribiero, J.A. & Sebastiñao, A.M. (2010). Caffeine and Adenosine. *Journal of Alzheimer's Disease, 20,* S3-S15. doi:10.3233/JAD-2010-1379

Romano, F.D. & Russo, P.F. (2012). *Caffeine Consumption and Health.* Nova Science Publishers, Inc. Retrieved from https://ebscohost.com

Shufer, V. (2016). A North American Plant Source of Caffeine. *Herbalgram*, (109), 42-47. Retrieved from https://www.ebscohost.com

Silva, C.G., Métin, C., Fazeli, W., Machado, N.J., Darmopil, S., Launay, P., Ghestem, A., Nesa, E. B., Szabò, E., Baqi, Y., Müller, C.E., Tomé, A.R., Ivanov, A., Isbrandt, D., Zilberter, Y., Cunha, R.A., Esclapez, M. & Bernard, C. (2013). Adenosine receptor antagonist including caffeine alter fetal brain development in mice. *Science Translational Medicine, 5*(197). doi:10.1126/scitranslmed.3006258

Skuse, D. H. & Baker, K. D. (2003). Brain: Neurodevelopmental Genetics. *Encyclopedia of the human genome*. Hoboken: Wiley. Retrieved from http://search.credoreference.com/content/entry/wileyhg/brain_neurodevelopmental_genetics/0

Soellner, D.E., Grandys, T., Nuñez, J.L. (2010). Chronic prenatal caffeine exposure impairs novel object recognition and radial arm maze behaviors in adult rats. *Behavioural Brain Research, 205,* 191-199. doi:10.1016/j.bbr.2009.08.012

Stromberg, J. (2013, August 9). This is how your brain becomes addicted to caffeine. *Smithsonian Magazine.* Retrieved from http://www.smithsonianmag.com/