

The effect of ETS exposure on the BDNF protein

Introduction:

In America, it is common knowledge that smoking cigarettes is bad for one's health; however, it is less well known that secondhand smoke exposure can affect the neurological health of children. According to the Centers for Disease Control and Prevention, in the U.S. 1 in 5 children are at greater risk for mental health problems due to postnatal ETS exposure (Centers for Disease Control and prevention, 2010). Second hand smoke (SHS), also known as environmental tobacco smoke exposure (ETS), is made up of smoke breathed out by a smoker and side stream smoke from the burning cigarette. There are several toxic chemicals that are composed in SHS, such as arsenic, lead, cesium, carbon monoxide, and polyaromatics such as benzene. The toxic chemicals in SHS are known to result in negative health outcomes in the pulmonary and cardiovascular systems.

Different studies have been conducted relating to the negative health outcomes ETS exposure has on certain parts of the brain. Ottens et al. (2010) conducted an experiment that used animal models of ETS exposure, that showed changes in movements of the animal which connected to certain parts of the brain, specifically the cerebellum. The experiment also tests early exposure to ETS during neurodevelopment, which disturbed the wiring of brain circuits and lead to abnormal behavior and deficits. The Cerebellum located at the bottom of the brain, plays a bigger role in motor control than its cognitive functions. These cognitive functions may be attention, language, fear, and pleasure responses. By taking apart the cerebellum of a model rat, Ottens et al. was able

to see movement changes, and circuit wiring that may lead to attention, language, etc deficiencies that can connect back to human brains.

Other experiments like one done by Aguiar et al. also proved to have significant changes in the brain that was exposed by ETS. They were able to associate reduction in cerebella size and function with attention deficit hyperactivity disorder (ADHD). Similarly, research conducted by Bandiera et al., demonstrated that SHS exposure did show a correlation between second hand smoke and children mental health outcomes. However, more research needs to be done to examine different parts of the brain that are affected by Neuro developmental ETS exposure that can bring about other neurological disorders.

Going from there, the frontal lobe is located on the front of the brain containing most of the dopamine- sensitive neurons. This region is connected with attention, short-term memory, planning, motivation and rewards. Research done by Barrett suggests that epidemiological studies also correlated ETS exposure as a risk factor for Alzheimer's (Barrett, 2007). This finding is most likely connected to changes in the frontal lobe rather than the cerebellum. However, much research isn't done on the effects of ETS exposure to the frontal lobe development altering the work of the frontal lobe such as motivation, memory, rewards and etc. Study done by Roth et al. 2009 showed that early life adversity leaves epigenetic marks at the BDNF gene that affected learning, attitude, or mental stability. Thus, this experiment will look at Brain-Derived Neurotropic Factor (BDNF) gene that plays a role in regulating stress response and biology of mood. It will examine the effect of second hand smoke on the BDNF protein that is generated by the BDNF gene.

Experiment:

The purpose of this experiment is to examine the unknown biochemical effects of ETS exposure on the frontal lobe of the brain rather than the cerebellum. It has been discussed before the importance of identifying other significant parts of the brain that can be affected by ETS exposure to study the neurodegenerative diseases and changes. As described by the study of Ottens et al. (2012) the use of proper animal conduct is very important.

1. There will be use of rat models such as Dawley rats taken from Harlan Laboratories.

These rats will be kept under controlled environment so that there won't be any outliers and food/ water will be provided to all of the groups in same amount. There will be a controlled group, another group exposed to ETS 300 (300 ug/m³ TSP) which is compared to ETS exposure in a car. Also, another group with ETS 100 (100 ug/m³ TSP) which is compared to ETS exposure in homes, with a pack a day smoker. Like the experiment of Ottens et al. the rats will be exposed to smoke in Teague TE- 10 smoking system which is a controlled system where side stream cigarette smoking can be conducted. This is measured in TSP which is the amount of smoke that enters the atmosphere from many sources of small particles.

2. At p25 which is a good age to test the brains of these animals, brains will be collected, dissected for the frontal lobe and further processed into 10 µm sagittal sections using the Cryostat. As described in Ottens et al. (2012) these frontal lobe tissue sections will be assessed through the qualitative and quantitative method of immunofluorescence microscopy by looking specifically at BDNF proteins. These sections which are placed onto glass slides will be fixed with paraformaldehyde to preserve the state of the cells.

They will then be assessed using primary antibodies stained first for BDNF proteins then co-stained for neurons that BDNF proteins help promote. Some examples of proliferating and differentiating neuron antibodies are the different forms of Hu, Doublecortin and NeuN. These sections will then be doused with secondary antibodies that will add labels to the primary antibodies so that we may then view them under the microscope. If there is co-localization between the BDNF proteins and the primary antibodies chosen for neurons and synapses, it meant that there was proliferation and differentiation taking place in our frontal lobe sections. This would be the qualitative part of our results. We can easily view the sections after imaging them and see what parts of the frontal lobe actively show our antibodies and at what rates. We can then further analyze the results using quantitative methods such as cell counting and using programs to analyze fluorescence intensity and create excel spreadsheets to study the possible changes between the ETS rats and our controls.

Discussion:

Secondhand smoke is a controversial subject in our society, and while our knowledge of its complete effects both mentally and physically is not completely known, research on this topic is getting closer to a definite explanation. While previous studies have outlined the cerebellum as well as other sections of the brain as possible effected regions, this proposal aims at highlighting the frontal lobe as another effected region by exposure of secondhand smoke. As such BDNF proteins which help with support of neurons as well as neurogenesis was chosen as the focus of the proposal. Since this is a single type of protein, immunofluorescence microscopy was chosen as the method to

analyze its expression in ETS rats versus controls. By using antibodies that stain for BDNF proteins and co-localizing it with antibodies for proliferating and differentiating neurons we should be able to compare the qualitative and quantitative values of the affected BDNF protein with the controlled BDNF protein and see if the protein expression increases or decreases. This will be achieved through cell counting and fluorescence intensity and it should give us an idea of what cellular damages are taking place in the Frontal Lobe.

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