EFFECTS OF EARLY LIFE NEGLECT ON COCAINE USE DURING ADOLESCENCE AND SUBSEQUENT EFFECT ON FGF-2 LEVELS IN ADULTHOOD

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by

Vaidehi Niravkumar Patel

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Thesis written by

Vaidehi Niravkumar Patel

Approved by

_____,
Advisor

_____, Chair, Department of Biological Sciences

Accepted by

_____, Dean, Honors College

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Introduction:

Humans, in their early life period are influenced by various factors such as the quality and extent of parental care, intellectual stimulation, nutrition and socioeconomic status. The early postnatal period is very sensitive to the overall development, especially the brain of living beings. Exposure to adverse events such as physical, sexual or emotional abuse during this period can lead to a lasting impact on cognitive and emotional abilities. Having a traumatic childhood increases the risk of developing stress – related psychopathology later in life (McLaughlin, K.A. 2014). Early life adversity (ELA) is believed to affect the development of the brain and is also associated with increased alcohol and drug abuse in adulthood. Childhood neglect is a potent form of ELA and can generate life – long consequences like development of major depressive disorder. Child neglect can be defined as insufficiency in providing a child's basic necessities like inadequate supervision, healthcare, clothing, housing as well as failure to provide other physical, emotional, educational and safety needs. This is becoming an increased concern across the country, predominantly amongst minority groups and families with lower socioeconomic status. Childhood adversity is strongly associated with morbidity and mortality. There is evidence from human studies that ELA can also induce structural and functional changes in the brain affecting hippocampus, amygdala and prefrontal cortex. For example, smaller hippocampal volume is associated with childhood poverty and early life stress or childhood maltreatment. Developmental impairments such as this may be mitigated by early support from caregivers as early maternal support is positively associated with hippocampal

volumes within the same group of children that experienced ELA induced smaller hippocampal volume. Furthermore, similar studies revealed smaller prefrontal cortex and amygdala volumes due to early life stress and childhood maltreatment. In addition to these structural and functional changes, ELA also influences changes in neural circuitry that underlies cognitive control and emotional processing, including threat and reward processing networks. These alterations in neural circuitries are associated with psychological and behavioral adaptations which further leads to increased risk of alcohol consumption and other health – risk behaviors (Duffy et. al., 2018).

Animal studies are important to investigate the correlations between ELA and neurocognitive consequences. Childhood neglect is the most potent form of early life adversity and can be modeled through neonatal maternal separation (MS) in rodents. MS in rodents is a form of extreme early – life stress that can be helpful in understanding the long – term effects of ELA on brain development, behavior and changes in expression of different proteins. Kambali et. al., (2019) showed increased anxiety – like behavior, impaired flexibility in social interactions, and increased reward – seeking behavior in adult rats that underwent neonatal MS. Furthermore, repeated MS leads to increased voluntary alcohol consumption and corticosterone levels which is a risk factor for development of substance use disorder or long – term depression (Odeon and Acosta, 2019). Thompson et. al. (2019) also found that MS in rodents increased alcohol intake in adolescent rats. Finally, MS is believed to induce chronic stress in rodents that leads to long – lasting behavioral change including impairment of spatial memory processes (Krugers et. al., 2016).

Fibroblast Growth Factor – 2 (FGF – 2), also known as basic fibroblast growth factor is a developmental protein expressed in the brain by neurons and glial cells. FGF – 2 is characterized for its roles in cell proliferation, differentiation and growth (Turner et. al., 2012). Previous studies have shown that repeated stimulant drug use increases FGF – 2 expression in different areas of the brain which is associated with behavioral changes. These drug – induced increases in FGF – 2 cell expression may also promote neural plasticity in the brain (Doncheck et. al., 2018). FGF – 2 has been known to play a significant role in learning and memory as well as in several neuropsychiatric disorders including stress and drug addiction (Even-Chen & Barak, 2018). Up or down regulation of FGF – 2 expression in different brain regions will allow us to determine whether FGF – 2 mediates the effects of early life neglect and/or adolescent cocaine use in adulthood.

The exact effects of early life neglect and adolescent exposure to drugs of abuse on FGF – 2 expression in adulthood is unknown. In this project, we propose to use animal models of early life neglect and examine cocaine sensitivity during adolescence using the cocaine conditioned place preference (CPP) paradigm, and subsequently examine the change in basal level of FGF – 2 expression in brain regions of interest as a result of early life neglect and potential interactions with cocaine experience during adolescence. We hypothesize that increased reward – seeking behavior will be demonstrated by the MS rats at adolescence following cocaine conditioning; we expect to see a decrease in the expression of FGF – 2 cell density in the MS rats that will persist through adulthood; an increase in the expression of FGF – 2 cell density will be seen in the animals conditioned

with cocaine at adolescence; finally, the direction of change in the expression of FGF – 2 in rats that were both subjected to MS and conditioned with cocaine is yet unclear.

Materials and Methods:

Subjects

A total of 64 males and females Sprague Dawley rats were divided into groups of 16 pups and each group was housed with a mother in clear plastic cages from P0 – P21. They were then individually housed in their own plastic cages after being weaned on P21. They were maintained on a 12 – hour light/12 – hour dark cycle (lights on at 0700 hours) and had unlimited access to both water and standard laboratory rat chow. Rats were weighed and handled daily starting from P25. All procedures were approved by the Kent State University Animal Care and Use Committee and the project was supported by a grant from the National Institutes of Health (MD007579).

Drugs

Cocaine HCl was dissolved in sterile 0.9% saline at a concentration of 10 mg/ml and was administered intraperitoneally (i.p.) to animals at a dose of 10 mg/kg. Animals weighing under 100gms were administered with 0.1mL of the solution and those weighing above 100gms were administered with the amount of solution calculated using the following formula: bodyweight/100. For example, an animal weighing 112gms would receive 0.11mL of the cocaine solution.

Early Life Neglect (Maternal Separation)

A total of 64 rats were born to 4 female rats. On P01, all pups were cross fostered to house similar number of males and females with each dam in each cage. Therefore, each dam was housed with a total of 16 pups. Early life adversity was established in 2 out of 4 groups of rats using the model of MS. The pups were subjected to a daily 3 – hour maternal separation at the same time every day from P02 – P16. The dams were removed from their home cages to a clean cage with access to water and standard laboratory rat chow during separation. The pups were left together in their respective home cages and were placed on a heating pad maintained at 110 °F. Additionally, cages containing the dams were placed in a different area of the same room. At the end of the separation period, dams were moved back with the pups into their respective home cages.

Place Preference Apparatus

Behavioral testing and conditioning were conducted using the conditioned place preference (CPP) paradigm. CPP apparatus consisted of three chambers in which two large chambers (13" X 9" X 11.5") were separated by a smaller chamber (6" X 7" X 11.5") (see Fig. 1). One of the larger chambers had wire mesh flooring with white walls and the other had silver – grated flooring with black walls. The smaller chamber in the center had PVC plastic floors. All the chambers consisted of removable trays placed underneath the floors which were raised to 1.5". During conditioning, the rats were isolated to one of the larger chambers using the removable partitioning doors within the chambers. During testing, the

rats were given access to all the three chambers. Time spent in each chamber was recorded using the infrared photobeams located in the larger chambers and the locomotor activity was recorded by the total photobeam breaks made in all three chambers. During all phases of the experiments the lights in larger chambers were turned off and a red light was left on in the room to maintain a semi dark setting.

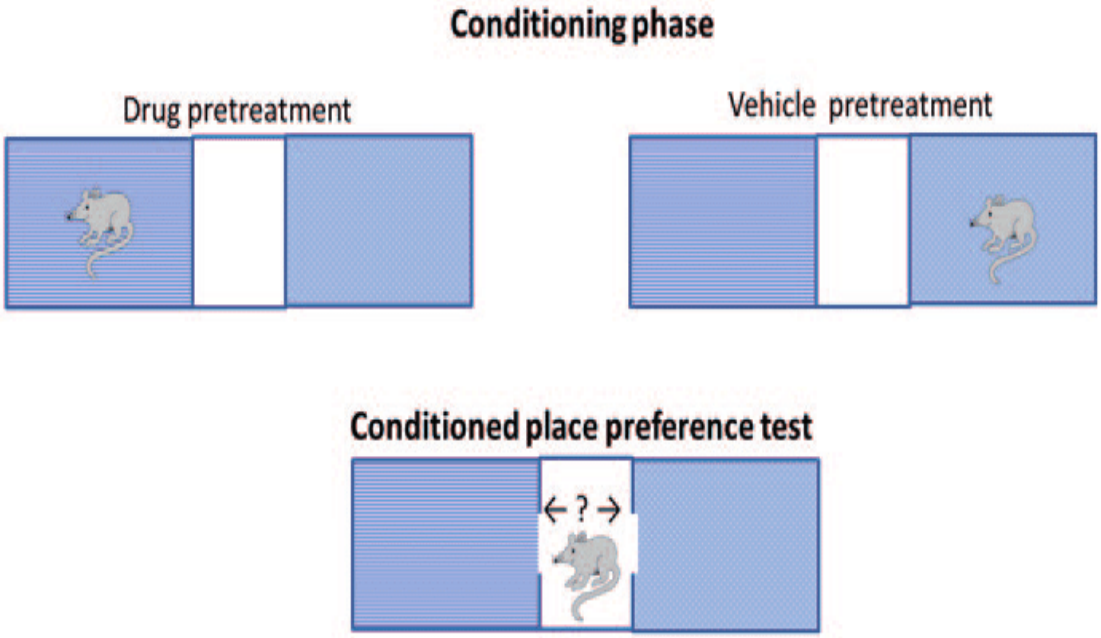


Figure 1: The Place Preference Apparatus Set – Up
The place preference apparatus consists of two large chambers and a small middle chamber. During the conditioning phase, rats are conditioned to associate one of the larger chambers with the drug received and the other chamber with saline. After the end of the conditioning phase, conditioned place preference (CPP) trials take place where the rats are given free access to all three chambers to examine their reward – seeking behavior. If the rats spend more time in the previously drug – paired chamber, a CPP is established. Picture taken from Auber et. al., 2015, p. 333.

Conditioning and Testing

A total of 52 animals, both maternally separated (MS), and control animals underwent the pre – test on P29 where the baseline preferences were determined by placing the rats in the center chamber with the partition doors open and allowing free access to all the chambers for 15 minutes. Time spent in each chamber was recorded during this baseline testing. Overall, the rats showed no bias for either of the large conditioning chambers; however, following the test, a group of 12 MS (6 males, 6 females) and 12 control (6 males, 6 females) rats that exhibited the least bias amongst all of them were selected for further cocaine conditioning and testing. Following baseline testing, the rats were randomly assigned to receive cocaine in either of the larger chambers and saline in the other to follow an unbiased procedure. The rats were conditioned to associate one chamber with cocaine in a counterbalanced way over 8 days from P32 – P39. Saline or cocaine injections were given on alternating days immediately before 20 – minute conditioning sessions during which the rats only had access to their respective chambers. Following 8 days of conditioning, the rats were subjected to a 1 – day break before the CPP trials (P41 – P43). During the tests, the rats were placed in the center chamber with the partition doors open allowing access to all the chambers for 15 minutes. A CPP was determined when rats spent significantly more time in the chamber where they previously received cocaine than the chamber where they received saline. Time spent in each chamber during pretest and test was measured in seconds (s).

Perfusion and Brain Extraction

On P17, a transcardial perfusion was performed on a group of 6 maternally separated and 6 control rats followed by their brain extraction to further process the tissue for immunohistochemistry (IHC) and examine the changes in expression of FGF – 2 cell density (see Table 1). Firstly, the animals were injected i.p. with a solution of 87 mg/kg ketamine and 13 mg/kg xylazine and checked for pain reflex by pinching one of their toes to ensure they were completely anesthetized. Following complete anesthesia, the animals were placed on a wire mesh plate on the top of an empty plastic cage. The animals were then incised through the thorax and further cut along the lateral edges of the rib cage to expose the heart. A syringe with a needle containing 0.1 M phosphate buffered saline (PBS) was inserted into the left ventricle, descending aorta was clamped with a hemostat and a small cut was made in the right atrium to allow the perfusate to exit circulation. The animals were transcardially perfused with PBS until the fluid exiting the rat was clear of blood. Next, the animals were perfused with 4% paraformaldehyde in 0.1 M phosphate buffer (PB) until all the muscle contractions stopped and the animals' extremities were stiff. The bodies were decapitated, and the skin and skull were cut off to extract the brain out of the head using a spatula. Following brain extraction, the brains were stored overnight in 4% paraformaldehyde and then transferred to 30% sucrose in 0.1 M PB. Once the brains were sunk, they were frozen and stored at –80 °C.

Immunohistochemistry

Unfortunately, due to the Covid – 19 crisis leading to shutdown of the university the collected brain tissue were not processed further for IHC. However, IHC will be performed in the future for detection of fibroblast growth factor – 2 immunoreactivity in medial prefrontal cortex, hippocampus and amygdala regions of the brain. The sectioning of the brains will be done using the cryostat, with 10 coronal sections (40 μm thick) of each brain region of interest. These sections of brain will subsequently be imaged under an Olympus microscope and analyzed for immunofluorescence. The regions of interest will be defined and the total number of FGF-2 cells in each region will be quantified to calculate average cell densities per region. Additionally, in early adulthood (P60 – 62) four different groups of adult rats will also be examined using IHC for the expression of FGF – 2 to determine whether the effects of early life neglect persist through adulthood. The four different groups of rats are: a control group that was not subjected to either maternal separation or cocaine conditioning, a group subjected to maternal separation on P2 – 16 that did not receive cocaine conditioning during adolescence from P32 – P39, a group that was not subjected to maternal separation and received cocaine conditioning during adolescence and a group that underwent both maternal separation and cocaine conditioning (see Table 1).

Table 1:
Summary of the different groups of animals throughout the experiment used for comparison of the expression of Fibroblast Growth Factor – 2 (FGF – 2) cell density in hippocampus, prefrontal cortex and amygdala

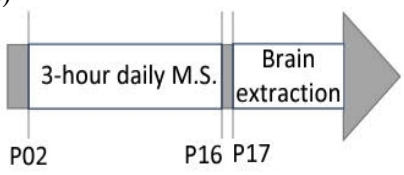
Behavioral Groups				
Group	Maternal Separation (P2 – P16)	Cocaine CPP (P32 – P43)	FGF – 2 Expression Pt. 1 Post MS (P17)	FGF – 2 Expression Pt. 2 (P60 – P62)
Control	-	-	Yes	Yes
MS Only	Yes	-	Yes	Yes
CPP Only	-	Yes	-	Yes
Combined	Yes	Yes	-	Yes

Results:

Timeline of the Experiment

For the first phase of the experiment, a group of newborn rats were subjected to MS from P02 – P16 to examine the effects of early life adversity on the expression of a developmental protein, FGF – 2, in different regions of the brain and cocaine use during adolescence. A small group of MS rats and control rats were perfused on P17, which was one day after the end of maternal separation to extract their brains and examine the effect of maternal separation on FGF – 2 cell expression during childhood (see Fig. 2b). All the rats were weaned and housed in their own separate cages on P22. At adolescence, P32 – P39, a group of MS rats underwent cocaine place conditioning and were tested from P41 – P43 against a group that did not undergo MS. In early adulthood (P60 – P62), rats from different groups were to be sacrificed to examine the changes in expression of FGF – 2 in adulthood (see Fig 2a). The different groups of adult rats that were to be examined are explained in detail in Table 1.

a)



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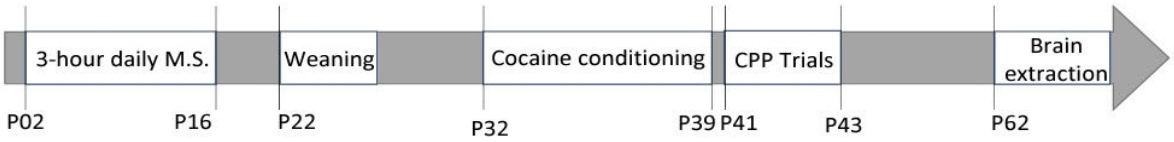


Figure 2: Timeline of the Experiment
A total of 64 animals divided into groups to examine the changes in expression of Fibroblast Growth Factor – 2 (FGF – 2) cell density in medial prefrontal cortex, hippocampus and amygdala. A) Small group of MS and the control animals were sacrificed immediately after maternal separation to examine the effects of maternal separation on FGF – 2 expression in childhood. B) The rest of the animals were divided into groups out of which some of them were conditioned with cocaine at adolescence. These groups of animals were used to examine effects of maternal separation on cocaine preference at adolescence, effects of cocaine conditioning or maternal separation alone on the expression of FGF – 2 in adulthood and combined effects of both maternal separation and cocaine conditioning.

Effect of ELA on baseline level expression of FGF – 2 in different brain regions

We were not able to complete IHC due to the current Covid-19 crisis that led to an essential shutdown of the University. However, we were able to extract the brains of a group of animals in their childhood and preserve the tissue to process it at a later time. We expect that decreased expression of FGF – 2 cell density will be seen in the group of MS rats whose brains were extracted immediately after the end of MS. These changes are likely long – lasting and we expect them to persist through adulthood. We expect to see an increased expression of FGF – 2 cell density in adult rats that were previously conditioned with cocaine at adolescence. However, the consequence of two counteracting effects, MS and cocaine conditioning, on the expression of FGF – 2 in adulthood is still unknown. Brain regions vulnerable to specific changes in FGF – 2 expression also needs to be investigated.

Additionally, we expect to see the FGF – 2 expression in the brain regions of interest under the microscope similar to that represented by the pictures taken during titrations (Fig. 3).

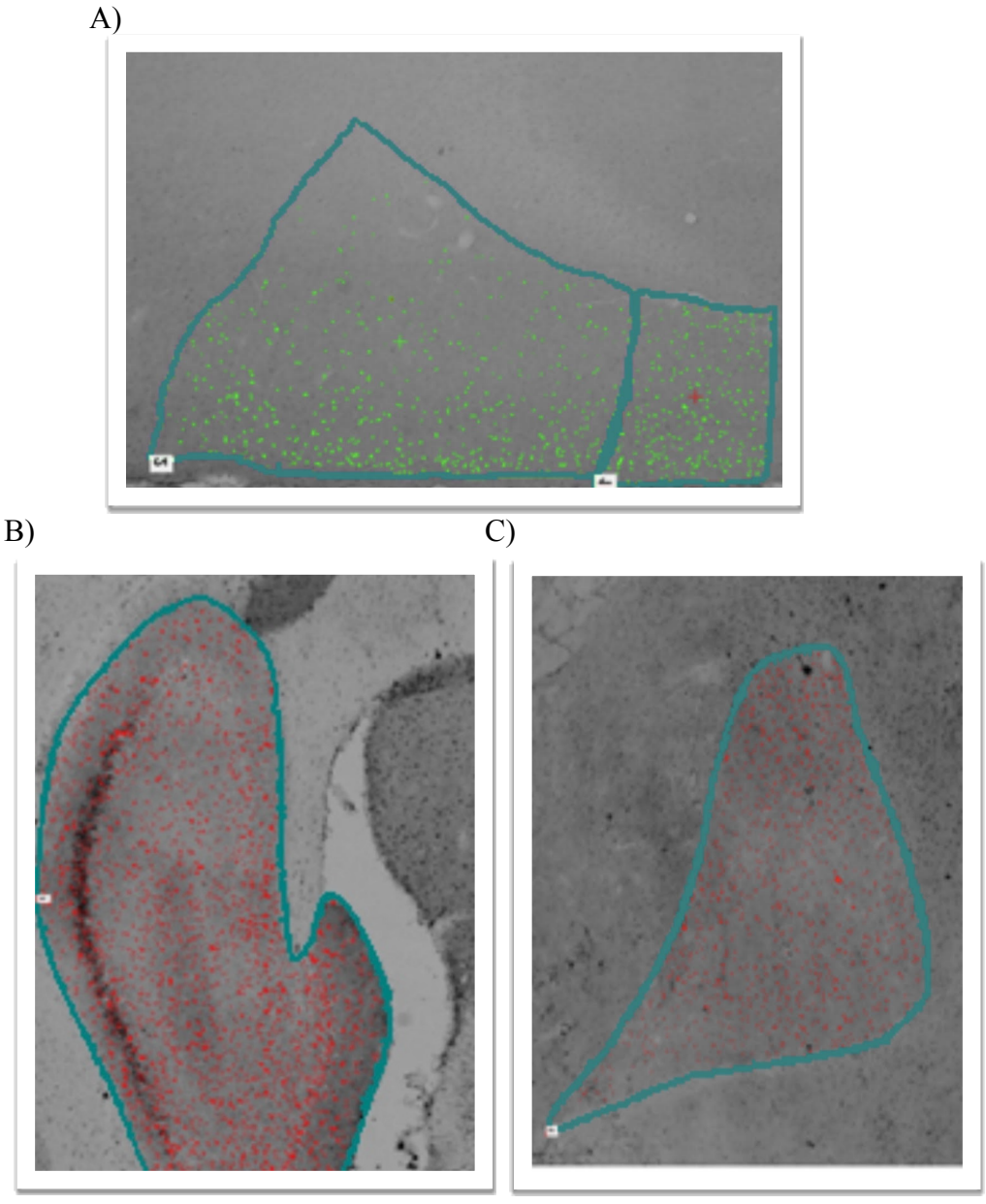


Figure 3: Expression of FGF – 2 in Brain regions.
Representative images of areas of tissue collection and FGF – 2 cell expression in A) prefrontal cortex, B) hippocampus and C) amygdala based on preliminary data.

Effects of ELA on Cocaine – Induced CPP and Learning Behavior at Adolescence

During the second phase of the experiment, we determined the effects of early life neglect on cocaine preference and learning behavior of rats in adolescence. Both groups of the animals, MS and the control were pretested, conditioned with 10 mg/kg cocaine and subjected to 3 – day CPP tests. Time spent in each chamber was measured in seconds (s) (Figure 2A and 2B). During test 1, the MS group of rats demonstrated an impaired CPP as compared to the control group, i.e., the rats that were not subjected to MS. Average time spent by the control group in the previously cocaine – paired chamber was 353.5s and the saline – paired chamber was 260.9s and by the MS group in cocaine – paired and saline – paired chamber was 319.6s and 280.5s, respectively. During test 2, both the control group and the MS group demonstrated a significant CPP and spent relatively more time in the previously cocaine – paired chamber. Average time spent by the control group in cocaine – paired chamber was 401.9s and saline – paired chamber was 238.5s; and average time spent by the MS group in cocaine – paired chamber was 371.4s and saline – paired chamber was 248.9s. During test 3, the MS group demonstrated a significant CPP as compared to the control group. Average time spent by the control group in the cocaine – paired chamber was 365.8s and saline – paired chamber was 284.1s. Average time spent by the MS group in the cocaine – paired chamber was 335.9s and saline – paired chamber was 243.8s (see Fig. 4). Additionally, results from a mixed ANOVA repeated measures that tested 2x2x3 (Group x Chamber x Test) revealed that there was a main effect of Chamber ($F(1,21)=21.71$, $p<0.001$). This suggests that, overall, rats in both the groups showed a preference for previously cocaine – paired chamber over saline – paired chamber across

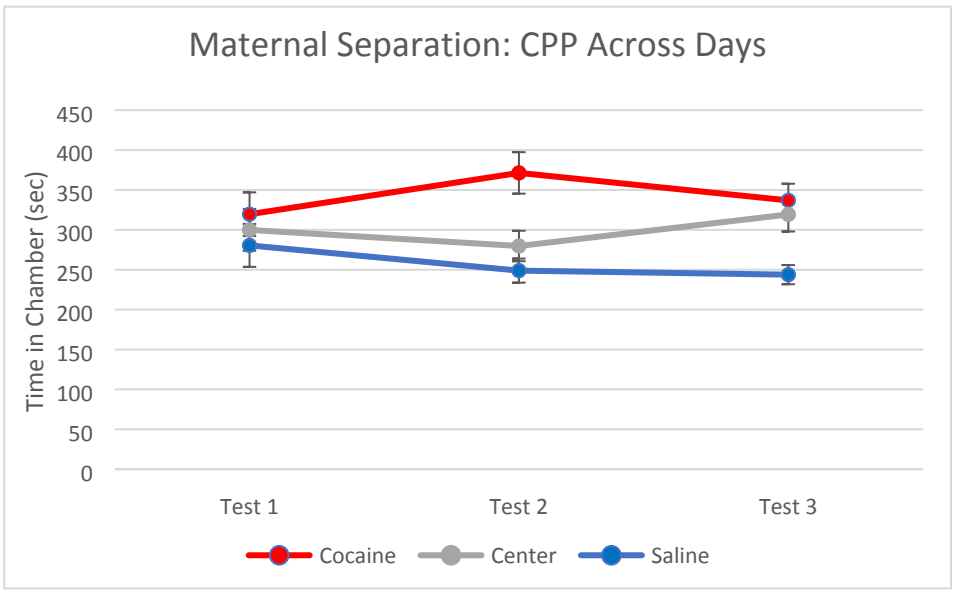
tests. Furthermore, a Test x Chamber interaction ($F(2,20)=4.26$, $p=0.028$) demonstrated an effect on the test day on time in chamber as MS group showed no CPP on first day of the trial but a significant CPP on the second and third day of the trial. *Post hoc* analyses were also done to calculate p-values and paired t-tests were used as a secondary means to analyze the mean differences between time in cocaine chamber and time in saline chamber (see Table 2).

Table 2:

Summary of the p – values and paired t-tests from *post hoc* analysis for the CPP trials

	Control	**Maternal Separation**	**Overall**
Test 1	p=0.014* t(11) = 2.90	p=0.43 t(11) = 0.82	p=0.03* t(23) = 2.31
Test 2	p<0.001* t(10) = 5.25	p=0.008* t(11) = 3.22	p<0.001* t(22) = 5.77
Test 3	p=0.084 t(11) = 1.90	p=0.005* t(11) = 3.51	p=0.002* t(23) = 3.54

A)



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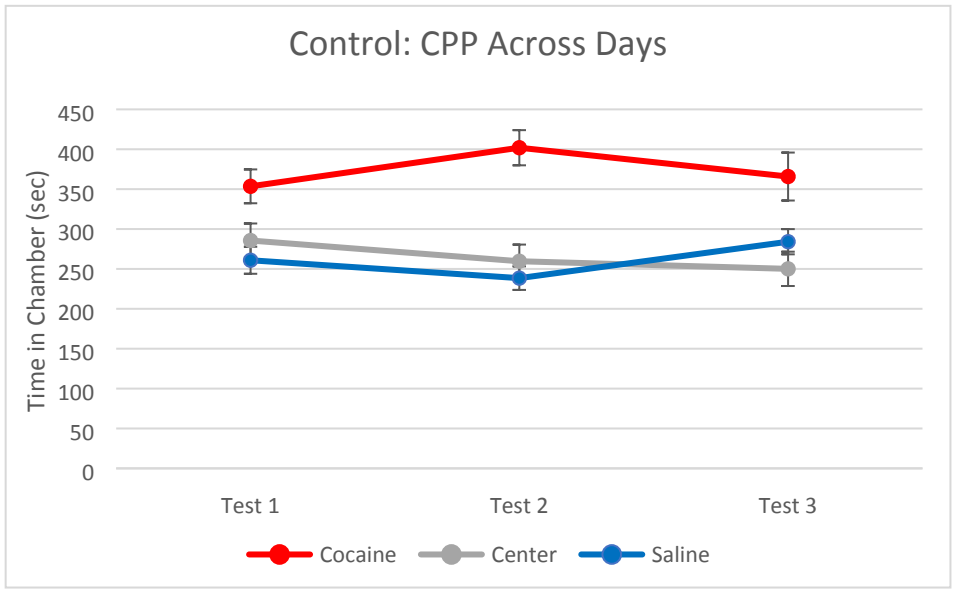

Figure 4: Average CPP of the MS and the Control groups during testing across 3 days. A) All rats from the MS group expressed a CPP for the previously cocaine-paired chamber over the previously saline-paired chamber during all three days of the trial. Impaired CPP demonstrated during the first day of the trial. B) All rats from the control group expressed a CPP for the previously cocaine-paired chamber over the previously saline-paired chamber during all three days of the trial. Impaired CPP demonstrated during the third day of the trial.

In summary, the group of rats that underwent neonatal MS demonstrated a significant CPP on the second and third day of the trial. *Post hoc* analyses confirmed that MS group spent significantly more time in the previously cocaine – paired chamber than the saline – paired chamber during the last two days of the trial as compared to the first day (see Table 2; Fig. 4a). Significance is defined as $p<0.05$.

The rats that were not subjected to neonatal maternal separation demonstrated a significant CPP on the first and second day of the trial. *Post hoc* analyses confirmed that the control group spent significantly more time in the previously cocaine – paired chamber than the saline – paired chamber during the first two days of the trial as compared to the third day (see Table 2; Fig. 4b). Overall, both groups of the animals demonstrated a CPP for the previously cocaine – paired chamber across the 3 days of trial (see Fig. 3). Thus, our findings suggest that ELA does not lead to increased reward – seeking behavior in adolescence when compared against the control group. However, these results do indicate impaired learning and memory in the animals that underwent neonatal MS.

Discussion:

We investigated the effects of the early life adversity using the model of MS on cocaine use during adolescence and changes in baseline level expression of a developmental protein, FGF – 2, in different regions of the brain during childhood and adulthood. Equal number of males and females underwent MS and were compared with the same number of males and females that did not undergo MS. We observed that both the control and the MS group showed a CPP for the previously cocaine – paired chamber. To our surprise, the MS group did not show increased reward – seeking behavior at adolescence, contrary to our original prediction. However, we did observe a delay by one day in significant CPP in the MS group during testing which indicates impaired learning and memory in the rats that faced early life adversity. We were able to extract brains of animals from the MS group and the control group at infancy before the animals were weaned. Once the University opens up, we will be able to process our preserved tissue employing the technique of IHC. We expect to see decreased expression of FGF – 2 cell density in their childhood following MS. Additionally, we were not able to extract brains from the animals in adulthood after being conditioned and tested at adolescence, but we had expected to see an increase in the FGF – 2 cell density in the brain regions of animals that were previously conditioned with cocaine.

Our finding that ELA does not result in increased reward – seeking behavior is consistent with one of the similar previous studies done with nicotine (Delavari et. al., 2016), but not consistent with the some of the other previous investigations. The amount

and quality of maternal care is one of the most important environmental influences for offspring during the early postnatal period. Maternal care in rodents is most prominently expressed by licking and grooming, therefore, repeated disruption in this care for extended periods of time leads to lasting effects on brain development and other stress – related disorders later in life (Krugers et. al., 2017). Thus, MS is a major stressor for the newborn pups and can also lead to increased alcohol consumption (Thompson et. al., 2019) and increased reward – seeking behavior that persist through adulthood (Kambali et. al., 2019). Our results necessitate optimization of early life stress and supplementing additional early – life stressors in the future studies for more effective results. Alterations in the duration of daily maternal separation, extending the time period of maternal separation, possibly until weaning or increasing the frequency of separation in 24 hours can be more stressful for both the pups and the dams. Additional modifications include less nesting material in the cage, placing the dams and pups in different rooms to socially isolate the pups and reduce dam – pup interactions (Rice et. al., 2008), reducing the temperature of heating pad and eventually stop using the heating pad after a few days during separation or separating the pups daily at random times to avoid habituation of being separated at the same time. One or a combination of these modifications can be employed in future replication studies to induce chronic stress in the animals for a more severe outcome.

Regardless of the minimal differences in CPP demonstrated by the two different groups of rats, another interpretation of our results is that the delay in exhibition of a significant CPP for the previously cocaine – paired chamber by the MS group indicates impaired learning and memory compared to the control group. This finding is consistent

with a similar previous study, Delavari et. al. (2016), except that they used the nicotine induced place preference. That same study demonstrated that the rats that underwent neonatal MS exhibited performance deficits in spatial learning at adolescence. A complementary effect of MS on learning performance maybe by drug exposure at adolescence. In another study, it has been shown that a single 24 – hour MS at post – natal day 3 showed impaired spatial learning ability until adulthood (Oitzl et. al., 2001). Therefore, ELA can have a long – lasting impact on learning and memory that can persist through adulthood. Additionally, ELA due to MS is associated with impaired hippocampal and prefrontal functions as well as weakened spatial memory processes (Krugers et. al., 2017). These findings are also consistent with previous human studies that revealed a decrease in the volumes of hippocampus, prefrontal cortex and amygdala following childhood stress. The reduction in the sizes of these brain regions due to childhood stress is associated with decreased spatial memory and behavior problems later in life (Krugers et. al., 2017). This behavior may also be associated with the changes in FGF – 2 cell expression in the different regions of the brain due to early life stress. Learning and memory in hippocampus depends on FGF – 2 functioning (Stevens, et. al., 2012), therefore, it would be interesting to look at the role of FGF – 2 in learning and memory in different regions of the brain. It has been previously shown that administration of FGF – 2 facilitates long – term memory in developing rats (Graham & Richardson, 2010). Future experiments can be done to verify mechanisms underlying learning and memory following ELA using a non – drug learning paradigm and compare it with a drug – learning paradigm.

Our predictions of a decrease in FGF – 2 cell expression in MS rats and an increase in FGF – 2 cell expression in the rats that were conditioned with cocaine at adolescence is consistent with previous studies. Chronic early life stress has been reported to reduce FGF – 2 expression in brain regions like prefrontal cortex which is long – lasting and persist through adulthood. Embryonic stress is also believed to reduce the expression of FGF – 2 in hippocampus (Turner et. al., 2012). Even-Chen & Barak (2018) demonstrated that short term exposure to drugs of abuse lead to an increase in the expression of FGF – 2. On the contrary, long term exposure to drugs of abuse cause region specific downregulation of FGF – 2 expression in some brain regions. Another study, Doncheck et. al., (2018), also showed that stimulant drug use increases FGF – 2 expression in reward and learning – related brain regions like prefrontal cortex and this increase is reversed by extinction learning.

However, it is difficult to predict the direction of change in FGF – 2 cell expression in the group of rats that underwent both neonatal maternal separation and were conditioned with cocaine. It would be interesting to know the counteracting effects of ELA and cocaine on the expression of FGF – 2 cell density in brain regions of interest (medial prefrontal cortex, hippocampus and amygdala). If we were able to get through the end of the experiment, our data would have demonstrated for the first time whether there is a decrease, increase or neutralization in FGF – 2 cell expression with two counteracting effects. This finding would have opened up doors for future research concerning changes in FGF – 2 cell expression due to counteracting effects through adulthood and its impact on stress – related behavior like PTSD, depression, etc. Turner et. al., (2008) suggested that after

administering FGF – 2 in depressed adult rats and executing multiple tests of depression – like behavior, FGF – 2 proved to have antidepressant properties and could be an endogenous antidepressant. Therefore, depending on the direction of change in FGF – 2, it could be used to develop a treatment for depression or people that faced a traumatic situation in the past. If administration of cocaine increases the overall FGF – 2 cell expression in adults that faced ELA and helps resist stress – like disorders, small amounts of cocaine could possibly be used as a medical treatment to enhance the release of FGF – 2 in the brain which can further act like an induced natural antidepressant. Overall, if there are any significant changes in the baseline levels of FGF – 2 expression persisting through adulthood, further studies can be done to determine whether these changes are reproducible and if these changes impact the offspring's phenotype or behavior.

Some of the findings of repeated social defeat stress in rats are similar to some of the observations in human postmortem brain from a depressed individual (Turner, et. al., 2012). The same study also reported a decrease in FGF – 2 expression in hippocampus due to prenatal stress. Reduction in the expression of FGF – 2 in the reward and learning region impacts their spatial memory. However, it is still unclear whether FGF – 2 plays a casual role in the regulation of cocaine addiction – related behaviors. Future investigations can be done to determine whether FGF – 2 will serve as a marker for risk of cocaine sensitivity following early life stress and its subsequent effect on learning and memory. This can be investigated by directly manipulating the FGF system. FGF – 2 cell density can also be a predictor of other behavioral responses in the rats like PTSD, fear, etc. For example, FGF – 2 in normal rats is predictive of low anxiety and a low fear phenotype. Interpretation of

FGF – 2 expression in MS rats remains unclear. By determining the exact role of FGF – 2 and its impact on the phenotype or behavioral consequences, clinical treatments for cocaine addiction as well as for stress – like disorders following ELA can be established. Drug abuse as a result of ELA can also be prevented by determining whether FGF – 2 serves as a marker for risk or resilience.

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