Impact of adolescent social defeat stress on enduring cognitive effects from early life stress

Introduction:
Exposure to early life stress (ELS) has been associated with many adulthood psychiatric disorders that involve cognitive and social dysfunction such as depression, anxiety, schizophrenia and substance abuse disorders (Miller & Cole, 2012; Read, Perry, Moskowitz, & Connolly, 2001). While the stress occurs during childhood, symptoms such as cognitive and social dysfunction often do not become prominent until adolescence (Teicher, Samson, Polcari, & Andersen, 2009). In our lab, we work to model this early life stress and associated adolescent dysfunction.

One problem our lab has revealed is that rodent models tend to show the expected behavioral and cognitive phenotype during adolescence but not persistently into adulthood. This contradicts the enduring dysfunction observed from adolescence into adulthood in ELS-exposed humans. One possible theory for this disconnect is that humans do not live in a controlled, stress free environment after their initial ELS, unlike our current rat models. Instead, in humans there is often a secondary “hit” of social stress during adolescence, such as bullying or family stress (Fisher et al., 2012). In this study, we aimed to mimic this second “hit” using social defeat stress (SDS) during adolescence. We hypothesized that the animals that undergo both stressors will be more likely to show cognitive and social dysfunction in adulthood.

One region that is likely involved with the delayed effects of ELS is the prefrontal cortex (PFC) (Heidbreder et al., 2000). The PFC is involved in executive function, working memory, emotion regulation, impulse control and decision-making- making it a likely target for the short and long term effects of stress (Arnsten, 2000). Additionally, the PFC is a late developing region- typically reaching maturation in early adulthood (Alexander & Goldman, 1978). This developmental trajectory is consistent with the onset of many of the aforementioned psychiatric conditions that have been shown to be associated with ELS (Paus, Keshavan, & Giedd, 2008). One possible model for our two “hit” hypothesis is that ELS leads to an organizational effect that affects the neurons in the PFC. The secondary “hit” then acts as an activational effect, resulting in the dysfunction that we often see in ELS-exposed patient populations.

The PFC is comprised of a series of inhibitory and excitatory neuronal networks that work in conjunction to mediate all of its functions (Compte, Brunel, Goldman-Rakic, & Wang, 2000). GABAergic interneurons that contain the calcium binding protein parvalbumin (PVB) are one of the major inhibitory classes of interneurons that work to inhibit the excitatory pyramidal cells in the PFC(Lewis & Moghaddam, 2006). PVB expression has been highly implicated in the stress...
Deficits in PVB have been highly implicated in disorders like depression and schizophrenia whose hallmarks include deficits in social behavior and working memory in adolescence through adulthood (Beasley & Reynolds, 1997; Leussis, Freund, Brenhouse, Thompson, & Andersen, 2012). In this study, we hypothesized that two “hits” of stress will therefore result in PVB deficits in the adult PFC.

While there is sufficient evidence that PVB loss is associated with stress and with adulthood psychiatric conditions, the mechanism of PVB loss is still under investigation. Another possible explanation for the social and cognitive deficits associated with ELS is HPA-axis activity. After chronic stress, corticosterone (CORT) levels are reportedly altered following an additional acute stress (Ostrander, Ulrich-Lai, Choi, Richtand, & Herman, 2006). While the mechanism for this change in CORT response is not well understood, one theory is that CORT plays a role in disrupting the HPA-axis negative feedback loop thereby amplifying the stress response (Herman, Ostrander, Mueller, & Figueiredo, 2005). While the effect of CORT in the brain is outside the scope of the present study, this change in CORT levels has been thought to mediate social and cognitive behaviors through the PFC and hippocampus (Ostrander et al., 2006) (Sanchez, Young, Plotsky, & Insel, 2000). The present study therefore examined peripheral CORT levels in adulthood after acute restraint stress. We hypothesized that animals that received both “hits” of stress will show an altered stress response.

This is the first study of its kind to investigate the effects that a secondary “hit” of stress has on adult social behavior. In this study, we used a combination of maternal separation ELS and adolescent maternal social defeat stress (SDS) in rats. Their social and cognitive behavior was examined and correlated with PVB loss and CORT activation after an acute stress.

Methods:

Animals:
The animals were obtained and treated in a similar manner to the animals described previously by our laboratory[16]. Pregnant female multiparous Sprague-Dawley rats (250–275 g) were obtained from Charles River Laboratories (Wilmington, MA) on day 15 of gestation. Rats were housed with food and water available ad libitum in constant temperature and humidity conditions on a 12 h light/dark cycle (light period 0700–1900). This experiment was conducted in accordance with the 1996 Guide for the Care and Use of Laboratory Animals (NIH) and was approved by the Institutional Animal Care and Use Committee at Northeastern University.

Early Life Stress- Maternal Separation:
The early life stress paradigm was modeled as we previously described (Holland, Ganguly, Potter, Chartoff, & Brenhouse, 2014). The day of birth was designated as postnatal day 0 (P0). At P1, litters were randomly assigned to either a maternal separation group (ELS group) or control group (CON group). Pups in the ELS group were isolated for 4 h per day between P2 and P20, and kept in a thermoneutral environment of 36 °C with a circulating water bath until they could regulate their own temperatures. Pups in the CON group were not disturbed after P2, except for weekly changes in cage bedding. Rats were weaned on P21, and group-housed with same-sex littermates with two rats per cage.

Social Defeat Stress:
Once the subject rats reached adolescence (P40), half of the ELS group and half of the CON group were subject to a secondary stressor in the form of maternal aggression social defeat stress (SDS group). The SDS paradigm was adapted from that used by Bourke and colleagues (Bourke & Neigh, 2012). In this paradigm, the adolescent was placed in the home cage of a lactating female Sprague-Dawley rat whose pups had been removed from the cage. The subject rat and the lactating female were allowed to interact freely for five minutes, or until the subject displayed three submissive poses. The subject and the lactating female were then separated by a wire mesh screen for thirty minutes. This paradigm was repeated for four days. The adolescents that were not exposed to SDS were not disturbed in their cages.

Cognitive Assessment:
Once the rats reached adulthood (P100), they were subjected to a learning-memory test called win-shift. This test was performed as we previously described in our lab (Holland et al., 2014). The subject rats were food-deprived to 85% of their free-feeding weight and habituated to an eight-radial-arm maze for 10 min/day for two days. After each habituation session, rats were returned to their home cages and given fruit punch flavored sucrose pellets. Once habituated, each rat learned the task, which was comprised for two parts: a training phase and test phase. During the training phase, four of the eight arms were blocked, and the remaining four were baited with a reward (sucrose pellet). During the test phase, all eight arms were accessible, but only the arms that were previously blocked contained a reward. Training and test phases were separated by a 5-min delay, until subjects reached a criterion of retrieving all four rewards in five or fewer choices (i.e., one or fewer errors) for 2 consecutive days. After the criterion was reached, daily tests were given with increasing delays of 5, 30, 180 min introduced between training and testing phases.

Social Place Preference: Once the rats had completed the win-shift protocol and had been eating normal portions of food, they were tested using a social place preference (SPP) paradigm that was adapted from the traditional conditioned place preference paradigm (Carboni & Vacca, 2003). Rats were habituated to an empty open field for five minutes. After five minutes, the rat was removed. Two wire cages were placed upside down in opposite corners of the arena. One of
the cages contained a conspecific, the other cage remained empty. The subject rat was then placed back into the arena and the movements were tracked using Noldus EthoVision software and later scored based on time spent in each quadrant of the arena.

Immunohistochemistry and blood analysis: The immunohistochemistry protocol was performed as previously described in order to visualize and quantify PVB expression in the PFC (Holland et al., 2014). Once the adult subjects completed their social and cognitive assessments, they were subject to an acute restraint stress. The animals were then immediately anesthetized and transcardially perfused. 40-µm frozen sections were incubated with a monoclonal mouse antibody raised against PVB (1:10,000; Sigma) and then with biotinylated anti-mouse secondary serum (1:500; Sigma) and streptavidin (1:4,000; Invitrogen, Camarillo, California). The antigen-antiserum complex was detected by incubation in diaminobenzidine and nickel sulfate in presence of hydrogen peroxide. All steps were preceded and followed by washes in phosphate-buffered saline–Triton X-100. Sections were mounted on gelatin-coated slides, dehydrated, clarified, and coverslipped with Permount (Thermo Fisher Scientific, Inc., Waltham, Massachusetts). Stereo Investigator Image Analysis System (MBF BioScience, Williston, Vermont) was used to estimate the density of PVB cells. An ELISA kit was used to measure levels of CORT in the blood serum.

Results:
Cognitive Behavior:
Figure 1 shows that only rats that underwent both ELS and SDS showed significant impairment on the win-shift working memory task (q-value). A two-way ANOVA revealed a significant interaction of rearing condition and adolescent stress ($F_{(1,24)} = 4.403$, $p = 0.0466$).

![Figure 1: Cognitive performance on win-shift task is deficient in maternally stressed adults who underwent adolescent social stress. *p<.05 difference from controls.](image)
Social Behavior:
Figure 2 shows that there was no significant difference between subjects that underwent stress and controls in the social place preference task. There was an insignificant trend showing that subjects that underwent SDS spent more time with the conspecific.

![Figure 2: Early life and adolescent stress insignificant with regards to social place preference.](image)

Discussion:
These results do support the hypothesis that in order to see significant adulthood cognitive dysfunction, there needs to be two “hits” of stress. We showed that neither stressor alone was enough to drive the adulthood cognitive dysfunction in the win-shift task. This result has high implications clinically in terms of interventions after ELS in order to prevent the activational effect of further adolescent stress.

While the social place preference task did not yield significant results, the number of animals tested in each group was extremely low (n=4). We hypothesize that with more animals, the trend observed with SDS animals spending more time in the social zone would become significant. This could have great implications looking at the coping strategies post-stress. One possible explanation is that animals that are stressed in adolescence end up seeking social comfort in adulthood in order to cope with the stress. Further data will help shape this hypothesis.

Due to the developmental nature of this project, there are still several analyses left to pursue. One key analysis will be examining the levels of PVB in the PFC using immunohistochemistry. In preliminary experiments, we were not able to see significant enduring changes in PVB using western blots. The hope is that by using immunohistochemistry, we will be able to see if there
are subtle changes in PVB that could be mediating cognitive and social deficits we see both in this study and clinically in individuals who have undergone ELS.

Additionally, we collected blood right after an acute restraint stress and we will run an exploratory analysis investigating whether the stress response is altered in subjects that have undergone stress. An altered stress response is an alternative avenue that we are pursing in order to investigate the mechanism that drives the psychiatric dysfunction that is seen clinically in individuals who have undergone ELS.

One major weakness of this study is that we only examined the effect of stress in males. Future studies will examine sex differences both behaviorally and mechanistically. We see in clinical populations that anxiety and depression disorders are much more common in women while substance use disorders and schizophrenia are much more common in males (Eaton et al., 2012). Males were examined first here in order to follow-up previous findings in adult ELS-exposed males. However by looking only in males, we are definitely missing a key part of the picture. Future studies will examine sex differences using this two “hit” model of stress.

Conclusions:
In this study, we were able to conclusively show that in order to see adulthood cognitive dysfunction, the subjects had to undergo both ELS and SDS. The implications of this study should inform future ELS studies that are examining adulthood dysfunction since its results align with current clinical outcomes of ELS. Additionally, it shows that ELS is not enough to explain the cognitive deficits seen clinically. Our hope is that this study will help inform treatment and intervention post-ELS clinically in order to help reduce the number of individuals who are inflicted with psychiatric illness after ELS.

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References:


