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Effects of Acute Stress and Ethanol Consumption on IL-1β in Female Long Evans Rats: A Pilot Study

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Background: Acute stress elicits many physiological, behavioral, and neurological responses, and many studies have aimed to better our understanding of these responses and their effects. However, a majority of preclinical studies have used male test subjects, despite growing evidence that males and females have different responses to acute and chronic stressors. This study thus aims to evaluate the ability of three different acute stressors to cause a physiological stress response in adult and adolescent female subjects.

Methods: In this preliminary investigation, Female Long Evans rats (N=12) underwent three acute stressors over the course of three consecutive days. In order, these stressors were Swim Stress (SS), Elevated Platform with High Light (EP-HL), and Predator Odor (PO). Plasma samples were collected both prior to and following each stressor, and interleukin 1 beta (IL-1β) levels were measured to assess the subject's physiological response to each stressor. For the two weeks that followed, EtOH self administration was assessed along with final IL-1β levels.

Results: No significant differences were found between the pre and post levels of IL-1β. However, the general trend of our data suggests that IL-1β levels decreased following the stressor for both adult and adolescent subjects. The data also suggests that alcohol decreases levels of IL-1β, and that this impact was greater for adult subjects.

Conclusion: Although this preliminary data offers no conclusive deductions, it does suggest that FS, EP-HL, and PO may not be useful models for eliciting a significant physiological response in female Long Evans rats. However, the need to identify a reliable stressor to use for female subjects remains of great importance for future research aimed at understanding neurobiological correlates of behavior..

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INTRODUCTION

Interleukin-1β (IL-1β) is an inflammatory cytokine that is produced by the central nervous system as part of the body's response to stress, disease, and damage (Srinivasan et al., 2004). IL-1β is a key component in the physiological response to both acute and chronic stress, and various stressors are capable of eliciting the production and expression of IL-1β in both human and animal models. The use of animal models is essential in preliminary research, and the goal of this study was to characterize a model that would allow future research to specifically study the relationships between the immune system's physiological response to acute stress (measured using IL-1β levels) and alcohol (EtOH) consumption in female subjects.

There is a strong, positive correlation between acute stress and depression (Barber et. al., 2014; Wang et. al., 2005), and a relationship between IL-1β and depression has been assessed (Goshen et. al., 2008). Depression is associated with inflammation and neurodegeneration, and both of these pathways involve increases in IL-1 levels (Maes et al., 2012). In humans, increased levels of IL-1β are currently understood to contribute to behaviors that mirror anxious-depressive symptoms (Dantzer et al., 2008). In a recent study, elevated of levels of IL-1 in mice were found to not only be necessary in producing depression, but also that the elevated IL-1 levels were sufficient to induce depression due to its ability to successfully reduce neurogenesis (Goshen et al., 2008).

In addition to being linked to depression, a relationship between IL-1β and alcohol consumption has also been evaluated. Prolonged alcohol consumption has been found to cause inflammation and neurodegeneration in both human and animal models (Crews and Nixon, 2009), and since these pathways involve increases in IL-1 levels (Maes et al., 2012), it may be expected that alcohol consumption would specifically increase IL-1β levels as well. Consistent with this, a significant increase in the levels of IL-1β has been observed in mice that chronically consume EtOH (Lippai et al., 2013). However, research has also shown that EtOH significantly inhibits the production of IL-1β in cultured human macrophages. This EtOH-induced decline in levels IL-1β is believed to be due to EtOH's ability to inhibit activation of caspase-1, an enzyme that cleaves pro- IL-1β and converts it to active IL-1β (Nurmi et al., 2013). The understanding that acute EtOH decreases the immune system's production of inflammatory cytokines is consistent with findings that in humans, excessive acute alcohol consumption (such as that observed in binge drinking) tends to increase the occurrence of bacterial infection (Goral et al., 2008).

Alcohol consumption has been shown to be affected by acute stress in both human and animal models. However, the type and extent of the effect varies based upon both the acute stressor and the subjects. Aspects such as sex and age can have a significant impact on a subject's predisposition to increase their EtOH consumption due

to acute stress. In mice, it was found that predator odor (PO) was able to induce a two phase effect on female subjects, where first the PO stressor significantly reduced EtOH intake the day of the stressor, but then increased EtOH intake the following two days. This same study also found that in male rats, there was only one response of a delayed increase in EtOH consumption (Cozzoli et al., 2014).

When evaluating age, one study found that early life stress has a strong impact on female rats' EtOH intake during their adolescence (Waes et al., 2011), but it remains unknown why adult subjects may be less susceptible to increasing their EtOH consumption following acute stress. When assessing the effects of forced swim on alcohol consumption, it was found that chronic swim stress (SS) over the course of fourteen days was able to significantly increase the sedative and hypothermic effects of EtOH, but that acute stress (one or three days) did not mimic these effects (Boyce-Rustay et al., 2007). In humans, a study focused on alcohol consumption among undergraduate students found that for this age group there is a close relationship between acute stress and single-session alcohol consumption (Magrys and Olmstead, 2015).

The relationships between acute stress and alcohol consumption, acute stress and the production of IL-1β, and alcohol consumption and IL-1β levels have been independently investigated using mainly male rat models, but the current study seeks to assess these three variables in one cohesive study using female subjects. We hypothesize that acute stress will increase plasma levels of IL-1β immediately following the stressor, but that alcohol consumption will later decrease IL-1β levels for both adolescent and adult subjects (as compared to subject's baseline levels). Through the use of a within-subject design we intended to assess individual differences and correlations among acute stressors, EtOH consumption and preference, and plasma IL-1β levels. We aim to use this study to help determine which acute stressor is most potent to the female subjects by measuring their physiological immune response via IL-1β. We also seek to use this study to gain a better understanding of the effects of EtOH on IL-1β in female subjects.

There is a strong, positive correlation between acute stress and depression (Barber et. al., 2014; Wang et. al., 2005), and depression currently plagues approximately 350 million people globally, with women being up to 70% more likely to experience depression in their lifetime than men (Holmes, 2015). With this in mind, it becomes essential that this research is conducted using a female rat based model so that we may deepen our understanding of the relationships between depression, acute stress, levels of IL-1β, and EtOH consumption. Then, we may use this knowledge to help us predict and later study how these variables interact in humans.

MATERIALS AND METHODS

Subjects

This study uses female Long Evans rats (N=12; Envigo Laboratories, Indianapolis, IN, USA). 6 rats arrived on post-natal day 21 (PND 21) immediately following their weaning, and the other 6 arrived as adults (PND 60). All rats were group housed in sets of 2/cage (cage size 33.0 cm x 59.7 cm; Nalgene, Rochester, NY, USA) for 1 week as they acclimated to the new lab environment. Each rat was paired with another rat of the same age. Following the week of acclimation, rats were single housed for the remainder of the experiment. All rats were kept within the same colony room, and each rat received minimal handling throughout the course of the experiment. An experimental timeline is provided in Figure 1. All animal care procedures were in agreement with the NIH Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80-23), and this experiment was approved by the University of Dayton Institutional Animal Care and Use Committee (No. 014-03-A1).

Tail Nick Blood Collection

Rats were gently restrained and sharp scissors were used to remove a small "nick" of approximately 0.75 mm from the distal end of the tail at an angle that was perpendicular to the work surface. Caution was used to avoid cutting the tail vertebrae. Two-to-three microhematocrit capillary tubes were then used to collect blood from the tail. The blood was drained from the microhematocrit capillary tubes into centrifuge tubes and placed on ice. Once completed, the samples were centrifuged for 10-20 minutes at a speed of 1,000 x g at 6˚C. The plasma from each sample was then pipetted into a new microcentrifuge tube, and all samples were stored at -80˚C until processing to measure plasma IL-1β.

Swim Stress (SS)

All odd numbered rats were first weighed and then pre-stressor bloods were taken chronologically from each using the tail-nick method. After blood was collected, each rat was placed back into their cage for ten minutes. Next, rats were individually placed in a bucket of room temperature water (24-30˚C) with a depth of approximately 30cm. If need be, additional water was added to ensure that subjects could not touch the bottom of the bucket with their feet or comfortably with their tails. Rats were forced to swim for five minutes, and then they were returned to an empty temporary cage to dry off. Once the rats were fairly dry they were returned to their original home cage. Thirty minutes after

the rats had been removed from the water blood samples were again collected from each using the tail-nick method. Rats were returned to their original cages and placed back into the colony room. The procedure was then repeated using all even numbered rats in chronological order.

Elevated Platform with High Light (EP-HL)

Prior to beginning the experiment, the elevated Plexiglas platforms (18 in x 18 in x 18 in) were set up with two lamps facing directly toward each of them. Once this was done, all odd numbered rats were weighed and then pre-stressor bloods were taken chronologically from each of them using the tail-nick method. After blood was collected, each rat was returned to their original cage and placed in the room where the elevated platform was located for ten minutes (to allow subjects to acclimate to the new environment). The rats were then individually placed on the center of a Plexiglas platform and all lights were turned off except for the lamps that were pointed directly toward the platforms. Rats were left on the platforms for fifteen minutes. If a rat fell off the platform they were picked up and immediately placed back on the center of the platform. After the fifteen minutes had concluded the rats were returned to their home cages for thirty minutes. Following this thirty minutes, blood was collected using the tail nick method. This procedure was then repeated using all even numbered rats in chronological order.

Predator Odor (PO)

Two Plexiglas boxes (18 in x 18 in x 18 in) were wrapped with dark colored construction paper. All odd numbered rats were then weighed and pre-stressor bloods were taken chronologically from each using the tail nick method. After blood had been collected, each rat was placed back in their cage and moved to a separate space where the predator odor exposure occurred. The rats were then given ten minutes to acclimate to the room environment. During both the acclimation phase and the stimuli exposure phase, the overhead lights were turned off and small lamps were used to dimly light the room. To prepare the PO stimulus for each rat, a small Kim-wipe was folded into a small square and 150 µL of the PO Red Fox Urine (Pete Rickard's, Cobleskill, NY, USA) was pipetted onto it. This Kim-wipe was then taped 3-4 cm up from the bottom of the prepared cage, in the center of one of the walls. Care was taken to use the same wall for each trial. Next, rats were placed individually into the center of the cages and left there for fifteen minutes. Following the fifteen minute trial each rat was returned to their home cage for thirty minutes. Blood was then again collected using the tail nick method. The procedure was then repeated using all even numbered rats in chronological order.

Ethanol Paradigm

After 1 week of conducting the SS, the EP-HL, and PO stressors, subjects began a two week EtOH self-administration procedure (adolescents: PND 34 - 48 and adults: PND 73 - 87). All rats were provided with ad libitum access to food, and the rats were weighed at the beginning of each drinking day. Subjects remained single housed and were given access to both 20% EtOH and water using a two bottle choice design on Mondays, Wednesdays, and Fridays. All other days the rats were given access to solely water. The rats were given this pattern of access to EtOH because previous research has shown that providing rats with intermittent access to EtOH increases their overall EtOH consumption (Simms et al., 2008). The water and EtOH were each available in graduated drinking tubes (Med Associates), and the position of the drinking tubes were alternated on each drinking day to control for any possible side preferences. EtOH consumption was measured after 30 minutes and then again after 24 hours of access. These measurements were used to calculate EtOH consumption (g/kg) and an EtOH preference ratio (EtOH intake/total fluid consumption) for each rat. Blood was collected using the tail nick method five days after the conclusion of the EtOH paradigm (adolescents: PND 50 and adults: PND 89).
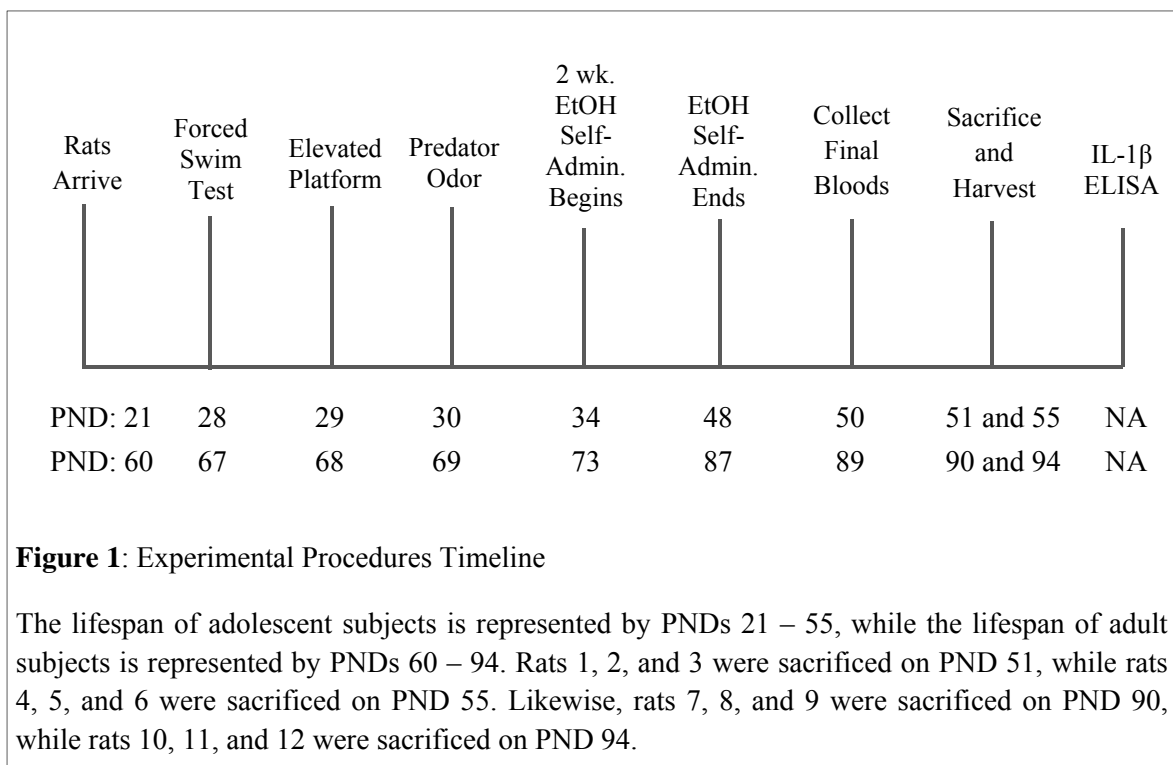
IL-1β ELISA

An enzyme-linked immunosorbent assay (ELISA; R&D Systems, Rat IL-1 beta/IL-1F2 Quantikine ELISA Kit) 96 well plate was used to detect the IL-1β levels in rat plasma both prior to and following each acute stressor (PNDs 28-30 for adolescents, and PNDs 67-69 for adults). To begin this procedure, all plasma samples (that were collected previously using the tail nick method and stored at -80˚C), reagents, and standard dilutions were brought to room temperature. Then 50μL of assay diluent was added to each well. Next 50μL of standard, control, or sample was added to each appropriate well. All samples were run in duplicate (2 wells/sample) while all standards were run in triplicate (3 wells/standard). A cover was then placed over the wells, and the plate was incubated at room temperature for 2 hours. Then each well was aspirated and washed, repeating the process 4 times for a total of 5 washes. 100μL of conjugate was then added to each well, and the plate was covered. Once again the plate was allowed to incubate at room temperature for 2 hours. Following this 2 hour incubation, the plates were again aspirated and washed 5 times. Then 100μL of substrate solution was added to each well, and the plates were covered and allowed to incubate for 30 minutes. During this incubation period the plate was placed in an area where it they was completely protected from light. Finally, 100μL of stop solution was added to each well, and within 30 minutes each plate was read at 450 nm; for this the wavelength correction was set to

540 nm. IL-1β levels were then calculated based on the standard curve using GraphPad Prism 6. Three plates were run in total.

For this study, the IL-1β ELISA was used for the first time by our lab, and as a result three of the data points exhibited IL-1β levels that were too low for detection with the dilution factor used (as compared to the standards). The three stressors that presented these undetectable levels of IL-1β were adolescent EP-HL, adolescent SS, and adult SS. As a result of this we found the dilution factor of 1:0 (pure sample with no dilution) to yield the best results instead of the 1:2 dilution recommended by the kit.

Statistical Analysis

Statistical analysis of the data collected using the IL-1β ELISA kits and the data from the EtOH drinking paradigm was conducted using GraphPad Prism 6 Software. From the ELISA data, the levels of IL-1β prior to and following each stressor were compared using two-tailed t-tests. The data collected during the EtOH paradigm was assessed using a 2-way repeated-measures ANOVA for both EtOH consumption and preference ratio.

Rats Arrive	Forced Swim Test	Elevated Platform	Predator Odor	2 wk. EtOH Self-Admin. Begins	EtOH Self-Admin. Ends	Collect Final Bloods	Sacrifice and Harvest	IL-1β ELISA
PND: 21	28	29	30	34	48	50	51 and 55	NA
PND: 60	67	68	69	73	87	89	90 and 94	NA

Figure 1: Experimental Procedures Timeline

The lifespan of adolescent subjects is represented by PNDs 21 – 55, while the lifespan of adult subjects is represented by PNDs 60 – 94. Rats 1, 2, and 3 were sacrificed on PND 51, while rats 4, 5, and 6 were sacrificed on PND 55. Likewise, rats 7, 8, and 9 were sacrificed on PND 90, while rats 10, 11, and 12 were sacrificed on PND 94.

RESULTS

Acute Stressors Effect on IL- 1β

The three acute stressor of SS, EP-HL, and PO were evaluated for their potential influence on plasma IL-1β levels both prior to and following each stressor. The pre and post IL-1β levels were statistically compared for adolescents and adults using unpaired two-tailed t-tests for all three stressors. No significant difference was observed between the pre-stressor and post stressor values of IL-1β for either adolescent or adult subjects for any of the three stressors (Table 1). However, it is notable that contrary to our hypothesis, there appears to be a general trend where the levels of IL-1β decrease following the acute stressor (Figure 2).

Table 1: Acute Stressors

Statistical results of unpaired two-tailed t-tests for adult EP-HL, adolescent PO, and adult PO. The three additional points of adolescent SS, adult SS, and adolescent EP-HL were not analyzed using unpaired two-tailed t-tests because their data points exhibited IL-1β levels that were too low for detection.

	Pre-Stressor	Post-Stressor	t-Test, p
Adult EP-HL	954.5 ± 319.0	283.0 ± 122.3	n.s., p = 0.1732
Adolescent PO	156.2 ± 81.22	69.18 ± 63.66	n.s., p = 0.5614
Adult PO	709.3 ± 455.7	214.7 ± 64.60	n.s., p = 0.3239

EtOH Paradigm Consumption and Preference

Adolescent and adult subjects were given access to 20% EtOH and water on Mondays, Wednesdays, and Fridays for a total of two weeks. A 2-way repeated measures ANOVA (age x drinking day) showed no significant differences in consumption between these two age groups for the 30 minute time point (Figure 3A). However, there was a significant main effect of age group, such that more EtOH was consumed by adolescent subjects than adult subjects at the 24 hour time point, $(F(1, 10) = 11.02, p = 0.0078)$. Adolescent aged rats (PND 34 - 48) drank an average of 8.56 g/kg (± 0.42) while adult subjects (PND 73 - 87) drank an average of 5.78 g/kg (± 0.40) (Figure 3B). There was also a significant difference in preference ratio at the 30 minute time point based on age group, such that adolescent subjects also show a greater preference than adults, $(F (1, 10)$

= 16.65, p = P = 0.0022). Adolescent aged rats had an average preference of 60.53% (± 7.35%) at the 30 minute time point, meaning that EtOH accounted for 60.53% of their total-volume consumption. Adult rats, on the other hand, had an average preference of only 46.30% (± 6.65%) (Figure 3C) at the 30 minute time point. No significant difference in preference was observed between age groups at the 24 hour time point (Figure 3D).

The 2-way ANOVA also showed a significant difference among drinking day for the 24 hour time point, such that subjects consumed significantly more EtOH on day 1 as compared to subsequent days 4 and 5 (F (5, 50) = 5.541, p = 0.0004) (Figure 3B). No significant difference in drinking day was observed at the 30 minute time point (Figure 3A). Likewise, no differences in preference were found based on drinking day for either the 30 minute or 24 hour time points (Figure 3C and 3D).

Effects of EtOH Consumption on IL-1β

The levels of IL-1β before sacrifice (collected 5 days following the conclusion of the EtOH paradigm; adolescents PDN: 50 and adults PND: 89) were compared using an unpaired two-tailed t-test. No significant difference was observed between adolescents and adults in these levels of IL-1β (Figure 4). However, although it was not statistically analyzed, it is notable that these post-alcohol-consumption levels of IL-1β are lower than the original baseline levels (Figure 2) for both adolescent and adult subjects.
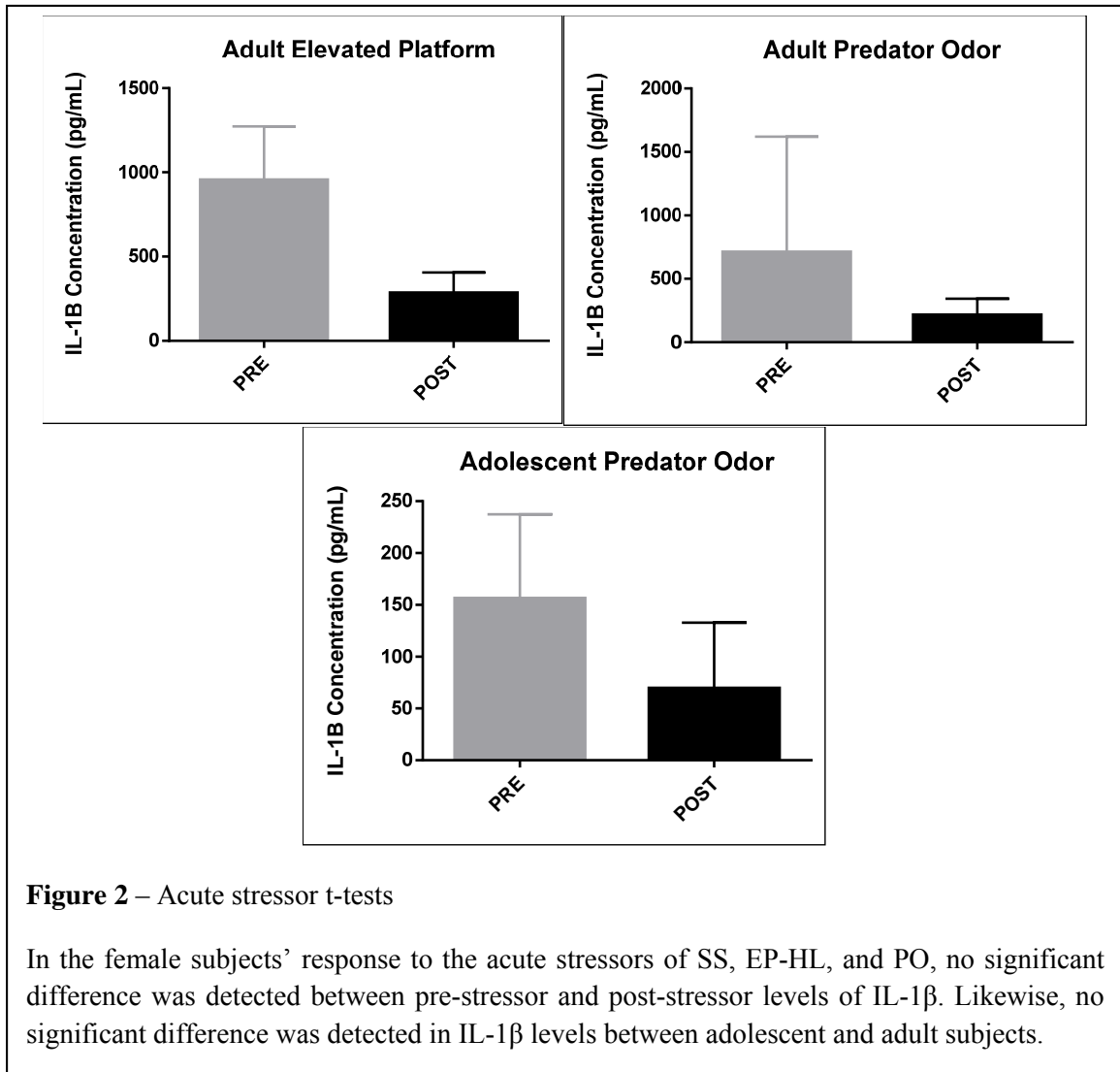
Figure 2 – Acute stressor t-tests

In the female subjects' response to the acute stressors of SS, EP-HL, and PO, no significant difference was detected between pre-stressor and post-stressor levels of IL-1β. Likewise, no significant difference was detected in IL-1β levels between adolescent and adult subjects.

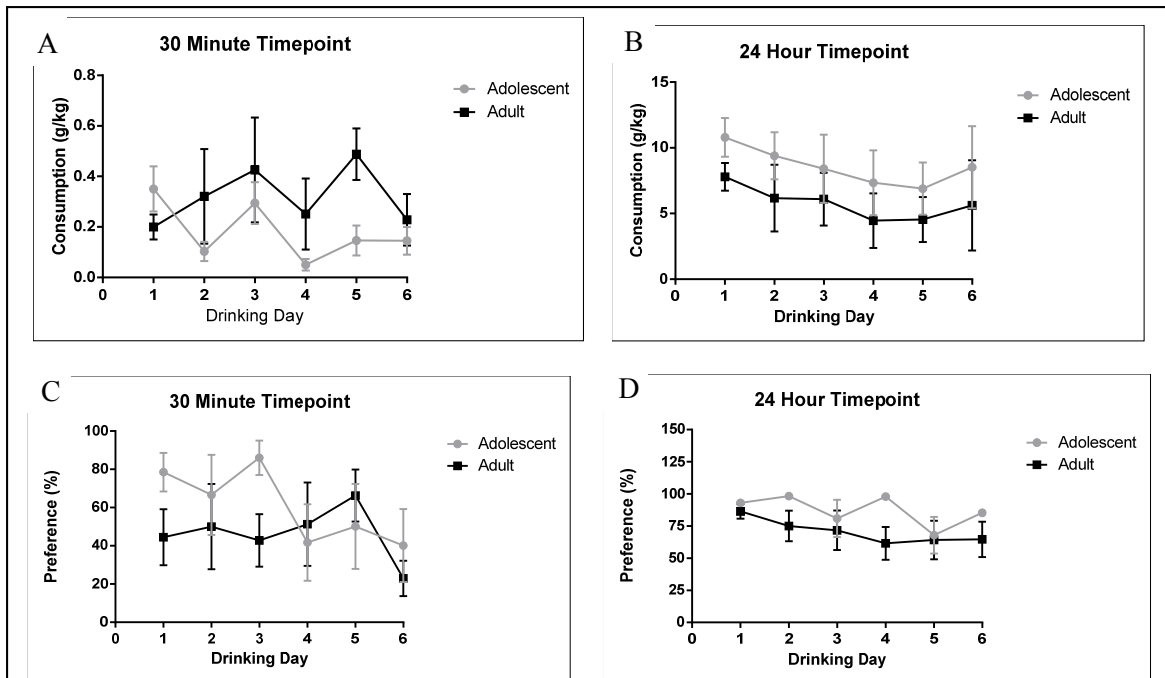**Figure 3** – Intermittent access 2-bottle choice design of 20% EtOH drinking paradigm

(A) At the 30 minute time point no difference in consumption was observed on the basis of either age or drinking day. (B) At the 24 hour time point adolescent aged rats consumed more EtOH than adult aged rats. Drinking day 1 also showed greater overall consumption than subsequent days 4 and 5 (C) At the 30 minute time point adolescent rats showed a greater preference for EtOH than adult rats. (D) At the 24 hour time point no difference in preference was observed on the basis of either age or drinking day.
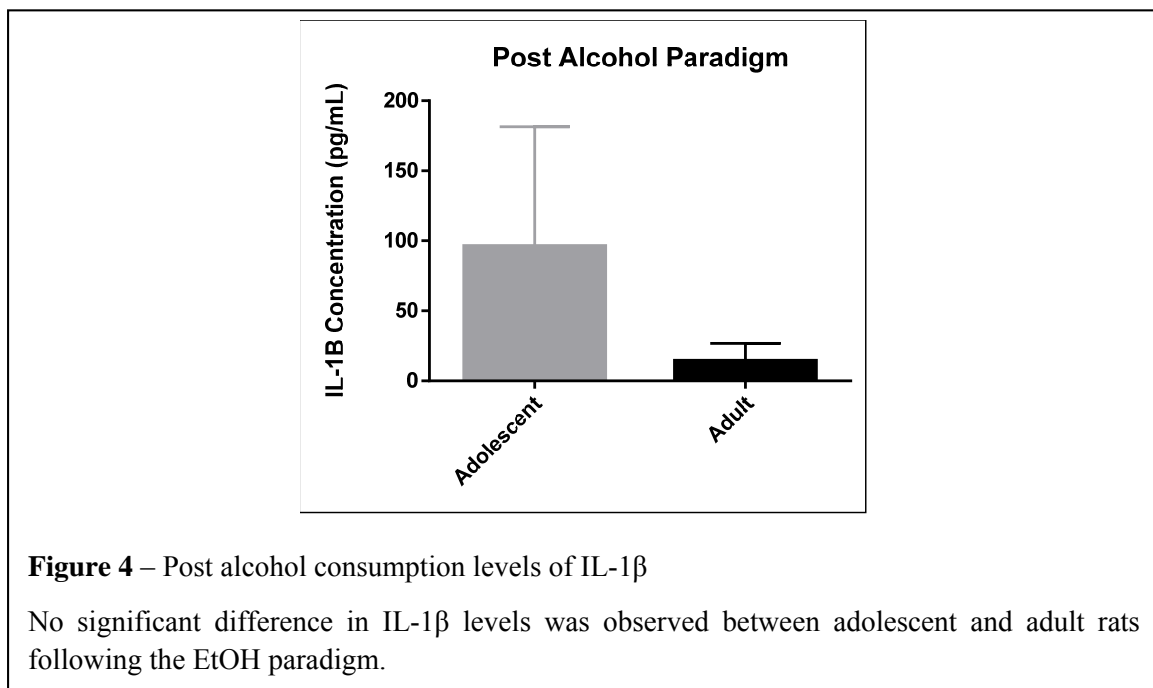

Figure 4 – Post alcohol consumption levels of IL-1β

No significant difference in IL-1β levels was observed between adolescent and adult rats following the EtOH paradigm.

DISCUSSION

Not all acute stressors alter the production of IL-1β in animal models, and research continues to try to determine exactly which stressors are most effective so that they may be reliably used in future experimentation. This study employed the use of the three acute stressors of SS, EP-HL, and PO because they have previously shown significant physiological responses in stress system activation within rat subjects (Price et al., 2002; Degroot et al., 2004; Hegab and Wei, 2014; Apfelbach et al., 2005; File et. al., 1993). The hypothesis of this study was that the three acute stressors would individually increase the plasma levels of IL-1β immediately following the stressor, but that EtOH consumption would later decrease these IL-1β levels for both adolescent and adult subjects (as compared to their baseline IL-1β levels). This study was solely designed to serve as a preliminary investigation on the effects of SS, EP-HL, PO, and EtOH consumption on IL-1β. A small N value of only 12 subjects was used, and no proper control group was utilized for this preliminary investigation. For these reasons, this particular data set cannot be extrapolated very far. However, through the use of a within-subject design this investigation is able to compare pre and post levels of IL-1β. This study's main findings were that for both adolescent and adult subjects, plasma levels of IL-1β were not significantly impacted by the acute stressors or by the subject's consumption of EtOH. However, contrary to our predictions, the general trend of our data suggests that IL-1β levels decreased following the stressor for both adult and adolescent subjects. In addition, and in line with our predictions, the data also suggests that alcohol decreases levels of IL-1β, and that this impact was greater for adult subjects.

IL-1β is an important component in the immune system's response to stress, disease, and damage (Srinivasan et al., 2004). There is a strong positive correlation between acute stress and depression (Barber et. al., 2014; Wang et. al., 2005), and in both human and animal models IL-1β has been shown to increase anxious depressive symptoms (Dantzer et al., 2008; Goshen et al., 2008). In addition, IL-1β levels are also impacted by EtOH consumption, such that as EtOH consumption increases, the levels of active IL-1β decline (Nurmi et al., 2013).

Although the three acute stressors implemented in this study have been successful in implementing stress system activation in other studies (Price et al., 2002; Degroot et al., 2004; Hegab and Wei, 2014; Apfelbach et al., 2005; File et. al., 1993), they have not yet shown to be effective on female subjects when using IL-1β levels as the physiological marker of stress response. It was decided that only these three stressors would be used for this preliminary study so that they could be assessed on consecutive days without the concern that the acute stress may turn into chronic stress.

The relationship between IL-1β levels and the forced swim stressor has been assessed in rat and mice models, and it was found that in rats (male and female) chronic

forced swim (15 minutes daily for 21 days) had no significant effect on either the expression of IL-1β or on the percentage of IL-1β neurons present in the hippocampus (Badowska-Szalewska et al., 2013). In another study, it was similarly determined that forced swim had no significant effect on limbic IL-1β levels (Plata-Salaman et al., 2000). These are consistent with our findings which likewise show that acute swim stress does not significantly impact plasma IL-1β levels. Conversely, another study found that when a large dose of IL-1β was administered in male mice, subjects spent significantly more time floating in the forced swim test (Dunn and Swiergiel, 2005), which is understood to be a sign of depression. However, subjects of this study were also were less active and consumed less food after the IL-1β was administered, and so these changes may have also contributed to the increased floating during forced swim (Dunn and Swiergiel, 2005). Overall, it appears that forced swim stress does not lead to increased levels of IL-1β, but that administering a large dose of IL-1β prior to the swim stress does lead to depressive behavior.

Elevated Platforms are commonly used to induce stress in rat subjects (Degroot et al., 2004) and when employing this stressor with the addition of high light, one study found that high light - open field (HL-OF) was able to significantly increase the levels of IL-1β in the hippocampus of both adult (PND360) and juvenile (PND28) male and female rats. In addition, this study also found that HL-OF increased the percentage of IL-1β neurons in the hippocampus of the adult subjects (Badowska-Szalewska et al., 2013). However, these findings are inconsistent with those of this study which used only female subjects, and further research should assess if this difference is due to differences that are seen between male and female subjects.

Exposure to a predator odor elicits defensive strategies within a prey species that help the species to recognize and defend against predation (Ferrero et al., 2011; Apfelbach et al., 2005). The endocrine system is known to be greatly affected by predator odors, and research has identified that predator odor activates the hypothalamic-pituitary-adrenal axis and increases the production of corticosterone and adrenocorticotropic stress hormones (Hegab and Wei, 2014; Apfelbach et al., 2005; File et. al., 1993). Previous research has not assessed the impact of PO on IL-1β levels, and so this study's findings that PO does not significantly increase the levels of IL-1β in female Long Evans rats will need to be confirmed in a follow up study. In addition, this aspect of the study should be repeated using other odors and also using male rats to assess if there is a significant difference in the production of IL-1β in male and female rats based on the odor stimulus.

In the EtOH paradigm subjects are given access to both EtOH and water, and the location of each is alternated to account for any possible side preferences. Studies may choose to provide subjects with access to EtOH every day, and different concentrations of EtOH may also be used, but this study employed the use of intermittent access to 20% EtOH since this has been found to have the highest preference ratio in rat subjects

(Simms et al., 2008). Other studies that have employed the EtOH paradigm have observed that female rats show a greater self-administration EtOH preference than males (Juárez and Barrios de Tomasi, 1999). Research has also found that EtOH consumption leads to a decline in levels IL-1β, and this is believed to be due to ethanol's ability to inhibit activation of caspase-1 (Nurmi et al., 2013) an enzyme that inhibits the conversion of pro- IL-1β into active IL-1β. This understanding that EtOH decreases immune function (Goral et al., 2008) is consistent with the findings of this study, which likewise notes the trend of decreased IL-1β levels following the EtOH paradigm.

Many other studies do not incorporate the use of both adolescent and adult subjects. Adolescence characterizes a crucial period of physiological and behavioral development, especially those related to stress and anxiety, for both humans and rodents (Doremus-Fitzwater et al., 2010; Spear, 2009). During this time rats and humans are more susceptible to the deleterious effects of stress. When assessing the age of subjects one study found more elevated levels of IL-1β in the amygdala and hippocampus of aged rats (as compared to juvenile rats) (Badowska-Szalewska et al., 2009). However, contrary to this, another study found that early life acute stress has a strong impact on female rats' EtOH intake during their adolescence (Waes et al., 2011) but it remains unknown why adult rats appear to be less impacted by acute stress. Thus it was important for this study to include the use of both adolescent and adult subjects to gain a comprehensive understanding of the effects of each acute stressor on these two age groups. In this study, the only significant differences that were observed based on age were that at the 30 minute time point adolescent rats showed a greater preference for EtOH than adult rats, and at the 24 hour time point adolescent aged rats consumed more EtOH than adult aged rats.

This study was designed and executed to serve as a preliminary investigation on the effects of SS, EP-HL, PO, and EtOH consumption on IL-1β in adolescent and adult female rats. As a preliminary study, no conclusive deductions may be made based on this data set alone. It is thus required that future research reevaluates these parameters using a larger cohort and a proper control group so that a more reliable assessment may be made. It remains imperative that deeper understandings are gained on the relationships between acute stress, alcohol consumption, and levels of IL-1β because these factors are known to play an important role in depression (Barber et. al., 2014; Wang et. al., 2005; Dantzer et al., 2008; Caldwell et al., 2002; Alati et al., 2005). In addition, it is also essential that female subjects are included in these studies because women remain up to 70% more likely than men to experience depression within their lifetime (Holmes, 2015).

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