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# Persistent Neurobehavioral Traits in a Mouse Model of Prenatal Ethanol Exposure

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# ABSTRACT

Fetal Alcohol Spectrum Disorders (FASD) affect an estimated 2% of the population, causing a wide range of symptoms: from inhibited cortical growth to lifelong cognitive and behavioral deficits (Murawski, et al., 2015). These consequences may be avoided or controlled when treatments are administered early; however, early detection of FASD can be extremely difficult in instances where individuals do not display obvious signs and symptoms, such as the craniofacial abnormalities often observed in Fetal Alcohol Syndrome (FAS), and most cases of FASD are not diagnosed until later in childhood (Streissguth, et al., 2004). Our goal is to identify juvenile neurobehavioral abnormalities that persist into adulthood and could be connected to early postnatal markers for FASD. A mouse model of prenatal ethanol exposure was developed using a voluntary drinking paradigm to reduce gestational stress. Neurobehavioral tests conducted postnatally revealed premature performances of certain milestones and abnormal responses to stimulation. This erratic activity was observed during juvenile and adult testing, where the ethanol-exposed mice displayed a lack of cautious behavior in situations that induced anxiety in control mice. Furthermore, ethanol-exposed mice were over-cautious in scenarios in which control mice displayed less anxiety. Ultimately, we observed a persistent failure to accurately gauge and respond to environmental stimuli. This trait is analogous to impaired sensorimotor gating, or the unconscious cognitive ability to filter out irrelevant and attend relevant stimuli from the environment, a distinctive classical symptom of FASD (Schneider, et al., 2013).

# FETAL ALCOHOL SPECTRUM DISORDER (FASD)

FASD is a condition associated with alcohol being consumed during pregnancy, including what could be classified as “binge drinking” behavior, which occurs during approximately 3% of pregnancies (Tan, Denny, Cheal, Sniezek, & Kanny, 2015). The diagnosis of FASD describes the symptoms that result from this prenatal alcohol exposure, and the condition affects an estimated 2% - 5% of the United States population (May, et al., 2009), with the most severe cases being classified as Fetal Alcohol Syndrome (FAS). Some of the difficulty in determining prevalence is due to a significant diagnostic delay, with most individuals being diagnosed as school-age children, typically over the age of 10. This can be problematic for several reasons, including the fact that the most successful therapeutic and pharmacological treatments outcomes occur when administered to young children (Streissguth, et al., 2004). The ultimate effects on one’s life can be devastating, with 50% - 60% of individuals diagnosed with FASD exhibiting poor or disruptive school performance, criminal behavior, incarceration, psychiatric clinic admittance, and inappropriate sexual behavior, and an additional 35% displaying persistent patterns of substance abuse (Streissguth, et al., 2004). While more research is necessary to explore how environmental context or genetic variability factor into these problems, there is an urgency for better diagnostic methods and subsequent treatment interventions to be developed in order to combat such a prevalent and devastating condition.

Individuals across the spectrum of FASD may exhibit birth defects, inhibited growth, craniofacial abnormalities, and lifelong cognitive and behavioral impairments.

Depending on the prenatal stage in which the alcohol exposure occurred, individuals can fail to develop an adequately functioning medial forebrain, smaller hippocampal or cerebellar cortices, or enlarged ventricles reminiscent of hydrocephalus (Murawski, Moore, Thomas, & Riley, 2015). Many gaps remain in our knowledge of FASD; for example, the specifics of the dose-dependency of prenatal alcohol exposure, or exactly how much alcohol exposure is needed at each specific stage of development to induce pathological effects, is not entirely clear. Furthermore, why binge drinking results in FASD for some individuals but not others, what the specific biochemical teratogenic effects of alcohol are, or how prenatal alcohol exposure induces the vast range of FASD symptoms, are just a few of the many remaining questions regarding this disorder that are currently under investigation. It is clear, however, that neurocognitive abilities are significantly altered in several ways. One prominent example is that of sensorimotor gating, or the cognitive ability to unconsciously filter out irrelevant stimuli and attend relevant stimuli. Deficits in this process, such as those observed in FASD, can include being unable to discriminate background stimuli and consequently drowning out important information, or simply being unable to select for important cues from the environment (Schneider, et al., 2013). Given the prevalence of the disorder and its potentially debilitating impairments, it is clear that the latency in diagnosis must be decreased. As such, the goal of these experiments is to identify behavioral markers for FASD that could be used to guide future studies toward developing techniques for earlier diagnosis and subsequent treatment.

## MOUSE MODEL OF FASD

Swiss Webster mice have been used as successful neurological models for FASD in previous studies, making them an appropriate option for neurobehavioral tests (Chi, Aras, Martin, & Favero, 2016). In addition to exhibiting a docile nature during handling, Swiss Webster mice are an outbred strain. This factor may threaten the consistency of results, but for our purposes, the increased degree of genetic variability will provide a model that is more representative of humans. In recent research performed by this laboratory, timed pregnant dams were given access to an ethanol solution to voluntarily drink *ad libitum* for a limited period each day, in order to best represent human drinking behavior (Chi, Aras, Martin, & Favero, 2016). This method, referred to as Drinking in the Dark, provides the mice with the ethanol solution at their peak wakefulness when they are more likely to drink.

## NEUROBEHAVIORAL TESTS

During previous research conducted in the laboratory, a battery of daily behavioral tests was performed from P1 (post-natal day 1) to P21 on the resulting pups, approximately half of which had undergone the *in-utero* ethanol exposure. These postnatal developmental milestone tests included surface righting, negative geotaxis, cliff aversion, rooting, auditory startle, ear twitch, open field, and air righting (Chi, Aras, Martin, & Favero, 2016). Mice prenatally exposed to ethanol could perform surface righting significantly earlier than the control, but displayed significant delays in cliff

aversion and open field behavioral milestones (Chi, Aras, Martin, & Favero, 2016). Behavioral tests were later conducted on a subset of these mice to examine the persistence of these abnormal traits. Anxiety and motor activity was observed by open field trials, ledge tests were conducted for motor coordination analysis, and a combination of these abilities in addition to sensorimotor gating skills were assessed using Light and Dark Suok tests. These three tests, in addition to an acoustic startle trial, were performed initially on the mice as juveniles (P22-P36) and repeated as fully-matured adults (4 months old).

Encouraged by these previous studies, we have designed and conducted a new set of neurobehavioral tests to identify and follow potential markers for FASD. After developing an adjusted paradigm to increase voluntary drinking by adding saccharine sweetener to the ethanol, a new set of pregnant dams consumed either ethanol or control solutions. The previously mentioned early postnatal developmental milestone tests were performed daily on 8 litters, each containing 6 mice (n=48) from P1 to P21. The juvenile behavioral tests were conducted when the mice reached adolescence, from P22 to P35. To identify a valid and reliable early behavioral FASD marker that could be applicable to humans, we aimed to confirm any behavioral deficits that persist throughout the lifespan of the mouse. As 4 month-old adults, the mice once again underwent the open field, ledge, Light, and Dark Suok tests. The results of our experiments were statistically analyzed in order to compare the results and behaviors of the control mice and ethanol-exposed mice, as well as changes in responses between testing as a juvenile and an adult. The results of the Suok tests proved to be the most

significant, and indicated impaired sensorimotor gating function in mice prenatally exposed to alcohol.

# METHODS

## MICE

A total of 11 litters of Swiss Webster mice were used in these experiments. Litters A, B, and C (*n*=8 control and 10 ethanol-exposed) underwent acoustic startle testing as adults to supplement data collected in previous experiments. The remaining behavioral tests (Ledge, Open Field, Light, and Dark Suok) were performed on Litters #2 - #9 as juveniles and adults, with each litter containing 6 mice (*n*=48). Half of the mice were control and half were prenatally exposed to ethanol (*n* = 24 per group). These litters previously underwent early developmental milestone tests. An acclimation period of at least 1 hour occurred prior to every testing session, and all equipment was cleansed with an ethanol solution in between mice. To avoid any interference with the circadian rhythm of the mice, all procedures were performed during their light cycle (1:30 AM – 1:30 PM).

## PRENATAL ETHANOL EXPOSURE

To best simulate human drinking behavior and reduce stress, the ethanol exposure was entirely voluntary. Experimental Swiss Webster mice were prenatally exposed to ethanol using a modified Drinking in the Dark Paradigm (Boehm III, 2008). At their period of peak wakefulness, 3 hours into their dark cycle, the mice were given 2

hours (4:30-6:30 PM) each day to drink an ethanol solution (Chi, Aras, Martin, & Favero, 2016). Beginning with a concentration of 2.5% ethanol for the first two days, the amount rose to 5% for two days, then 10% for two days, until it reached 20% ethanol for the remainder of the gestation (Allan, Chynoweth, Tyler, & Caldwell, 2003). To promote drinking, a modification to the Drinking in the Dark paradigm was implemented in which both the ethanol and control solutions were sweetened with 0.066% saccharin (Allan, Chynoweth, Tyler, & Caldwell, 2003; Rosenberg, et al., 2010). The testing was conducted under blind conditions, and the treatment status of the subjects was not known to any of the behavioral testers until statistical analyses were conducted.

## ACOUSTIC STARTLE

Acoustic startle response was measured in the 4-month-old adult mice of Litters A, B, and C to evaluate any deficiencies in sensorimotor gating function. The response of each subject was measured using San Diego Instrument's SR-LAB system and two automated startle chambers. Prior to the initiation of the trial, the weight of each individual was collected. As muscular force of reaction is the primary measurement, it is necessary to control for differences in weight (Geyer & Swerdlow, 1998). Beneath the Plexiglas containment cylinder within a chamber is a calibrated stabilimeter that detects and records the subject's movements (Geyer & Swerdlow, 1998). After acclimating to the testing environment for 1 hour, a single mouse was placed within each chamber and the program initiated an acclimation period of approximately 10 minutes. During the Pulse Alone test, the mice were exposed to 5 consecutive pulses of 117 dB at random

time intervals. This trial accounted for habituation and provided a baseline measure to which later results could be compared. The next test, Prepulse + Pulse (PP), also included a 10-minute acclimation period. The trial consisted of 50 random exposures to no stimulation, 117 dB target pulses, and three different prepulse intensities of 80 dB, 85 dB, and 87 dB immediately followed by the 117dB target pulse. The goal of this experiment is to measure Prepulse Inhibition (PPI), or to what extent a prestimulus inhibits the reaction to the target stimulus (Gulinello). A student's t-test was performed to determine the significance of difference in PPI between control and ethanol-exposed groups. PPI was calculated for each prepulse intensity level using the following formula:

$$\% \text{ PPI} = \frac{Pulse\ Alone - (\text{Prepulse} + \text{Pulse Score})}{Pulse\ Alone}$$

## LEDGE TEST

The ledge test provided a measurement of motor skill and coordination for Litters #2 - #9 at approximately 4 months of age. In this trial, a thin ramp-like structure was placed diagonally between two supporting structures. Soft material was arranged under the ledge to cushion any potential falls (Guyenet, et al., 2010). The mouse was then placed on the lowest side of the decline and observed for a period of 60 seconds. To pass the Ledge Test, the subject must balance and traverse the ledge without falling for the entire 60 second trial period. A student's t-tests was performed to determine significance.

## OPEN FIELD TEST

The Open Field Test (OFT) allowed the explorative, locomotive, and anxiety-related behaviors of Litters #2 – 9 as juveniles (P22-P36) and adults (4 months) to be analyzed (Bailey & Crawley, 2009). The capacity for habituation or sensitization to the arena was also included by repeating the trial on 2 separate days. The mice were placed in the center of a novel environment and visually recorded for a period of 5 minutes. In our experiments, a round chamber with high walls and demarcation lines from Stoelting (item #60106) served as the open field (Bailey & Crawley, 2009). The videos were later manually analyzed for six specific behaviors. Our measurements primarily involved locomotor activity and included: how long the subject stayed within the center circle after initial placement (*Central Circle Duration*), how many times the subject crossed through the center of the round enclosure (*Central Line Crossing*), the number of times the subject reared up on its hind legs without support (*Unsupported Rears*), the number of risk-assessing stretches in which the neck is extended without movement of the limbs (*Stretch Attend Postures*), the amount of time the subject spent grooming or cleaning itself (*Grooming Time*), and how long the subject held a frozen posture (*Freezing Time*) (Bailey & Crawley, 2009). A one-way ANOVA was performed in order to calculate the significance of the difference between the control and ethanol-exposed groups.

## LIGHT AND DARK SUOK

Both Light Suok and Dark Suoks were performed on Litters #2 - #9 as juveniles (P22-36) and again as adults (4 months). The Suok behavioral tests measure exploratory behavior, habituation, anxiety, and sensorimotor gating function. Similar to the OFT, this test provokes anxiety in subjects by providing open areas with little opportunity to hide. As in the ledge test, coordination is measured by placing the mouse on an elevated platform. The Suok is unique in its capacity to measure multiple aspects of behavior (Dow, et al., 2011). In our experiments, we used a plastic tube, approximately 1 meter in length (Figure 1). The ends of the tube were taped off to prevent the mouse from entering and it was elevated by anchoring the ends to short supporting structures. The entire apparatus was closed off to prevent any potential escapes (Dow, et al., 2011). The tube was separated into 3 segments, with each segment containing 10 sub-segments. These markings allowed us to measure the distance traveled by each subject. The 5-minute trial begins with the mouse being placed on the center of the tube (the middle of the second segment). If a subject fell, the tester would promptly return it to the center (Dow, et al., 2011). The Dark Suok differs from the Light Suok in that the room in which the experiment is conducted is completely darkened, with only one half of the tube illuminated by lamps. This separation into a light and dark side provided an additional stressor, as a mouse typically prefers the shadowed area where they can hide, and avoids the brightened exposed region (Dow, et al., 2011).

The entirety of the trial was visually recorded, and data was obtained from subsequent manual video analysis. In our study, we measured the length of time before the subject moved away from the initial placement location (*Latency to Leave Central Zone*), how many segments out of the three did the subject visit over the course of the trial (*Proportion of Apparatus Visited*), the total amount of time spent in locomotion (*Time Mobile*) and time spent not in locomotion (*Time Immobile*), the total distance the subject traveled (*Distance Traveled*), the amount of full pauses (*Stops*), the number of upright exploratory rears performed without leaning against something (*Vertical Rears*), how many times the subject reared with the support of a wall within the enclosure (*Wall Leans*), directed exploratory behaviors such as the number of times the subject looked underneath the suspended tube (*Head Dips*) or on either side of it (*Side Looks*), how often the subject performed risk-assessment stretches (*Stretch Attend Postures*), and the number of times the subject nearly or completely slipped and fell from the apparatus (*Missteps and Falls*).

Approximately 192 videos were carefully observed to accurately measure behavior. To prevent issues with inter-rater reliability, only experienced individuals scored the behavior, and each trait was assigned to one individual, instead of spread out over many. This prevented the issues that would arise with some individuals scoring the same behavior differently. We performed extensive statistical analyses on the data we have collected, including a comparison of means between Light and Dark Suok, as well as comparison of the juvenile and adult results, in addition to the control and ethanol exposure comparisons. The significance of the results was determined through

an initial one-way ANOVA, and a subsequent 2-way t-test statistical analysis was performed for the purposes of representing the results.

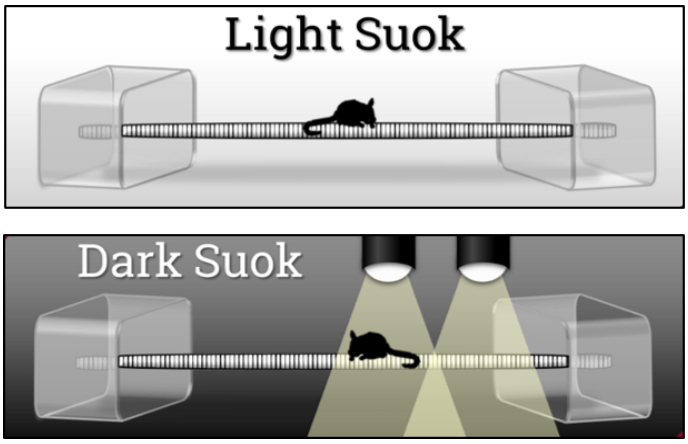


**Figure 1. Depiction of Light Suok and Dark Suok apparatus**. The subject is placed upon the center of the elevated tube and its behaviors are observed over a period of 5 minutes. The Light Suok is designed to induce an anxious response in mice, while the Dark Suok should be less anxiety-inducing.

# RESULTS

## ACOUSTIC STARTLE

The data obtained from the Acoustic Startle test performed on Litters A - C yielded no significant results regarding pre-pulse inhibition between control and treatment groups (Table 1).

## LEDGE TEST

In both the juvenile and adult trials for Litters #2-9, the Ledge Test for motor coordination provided statistically insignificant results when treatment groups were compared to control (Table 1).

**Table 1. Acoustic Startle and Ledge Test Outcomes Yielded Results That Were Not Significant.** Pre-Pulse Inhibition (PPI) was calculated using the results of Prepulse + Pulse (PP) trials. Each trial consisted of 50 randomized auditory stimulations. In addition to no stimulation, subjects were also exposed to 117 dB target pulses that were preceded by three possible prepulse intensities: 80 dB, 85dB, and 87dB. The resulting PPI percentage indicates to what extent a prestimulus inhibited the subjects' reaction to the upcoming target stimulus. All three prepulse intensities inhibited the response to the upcoming pulse by approximately 50% in both control and prenatal ethanol-exposed mice. Also represented are the results of the Ledge Test, in which between 20-40% of both control and ethanol-exposed mice fell from the ledge during the trial period. A t-test was performed to determine statistical significance, as indicated by *p*-values.

| | Control | EtOH-Exposed | *P*-Value |
|---|---|---|---|
| Average PPI: 80dB (%) | 43.22% | 42.50% | 0.968415 |
| Average PPI: 85dB (%) | 42.96% | 44.48% | 0.935422 |
| Average PPI: 87dB (%) | 42.14% | 46.66% | 0.828272 |
| Ledge Test: Falls (%) | 21% | 38% | 0.245 |

## OPEN FIELD TEST

Six behaviors were analyzed over a five-minute period during the OFT: Central Circle Duration, Central Line Crossing, Unsupported Rears, Stretch Attend Postures, Grooming Time, and Freezing Time. Ethanol-exposed adult mice froze (37.17 seconds) for a substantially longer amount of time than the control group (10 seconds; Figure 2 A; $p$=0.016). Additionally, the adults prenatally exposed to alcohol spent significantly more time in the central circle (45.7 seconds) compared to control adults (7.1 seconds; Figure 2 B; $p$=0.030). The remaining parameters did not yield significant differences.

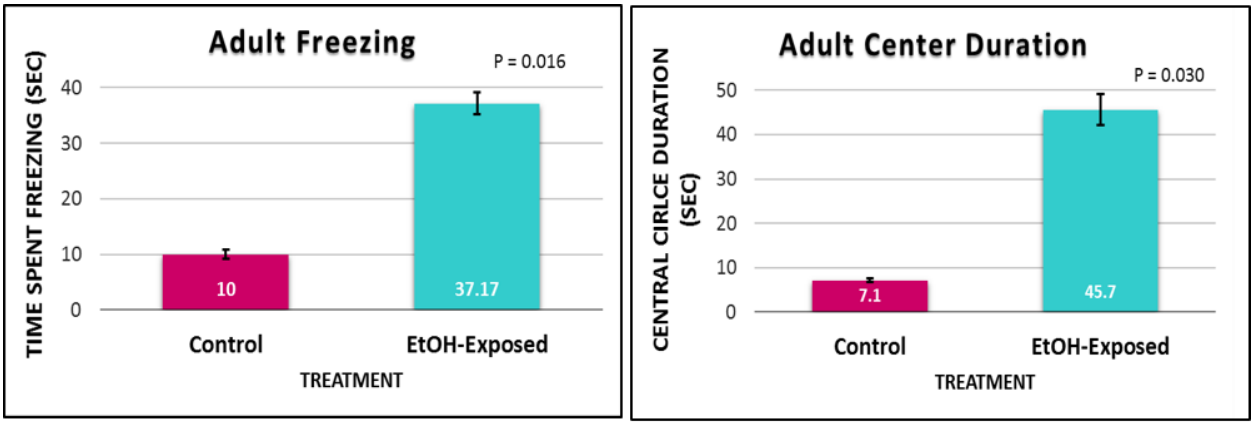


**Figure 2. Quantitative analysis of Open Field Test results indicate a lack of panic response in adult ethanol-exposed mice.** Adult ethanol-exposed mice froze significantly more often than control ($p$=0.016). Ethanol-exposed mice also casually explored the arena for a significantly longer amount of time ($p$=0.030). Statistical analysis was performed using a one-way ANOVA.

## SUOK

During the five-minute duration of both the Light and Dark Suok, twelve behaviors were recorded: Latency to Leave Central Zone, Proportion of Apparatus Visited, Time Mobile/Time Immobile, Distance Traveled, Stops, Vertical Rears, Wall Leans, Head Dips, Side Looks, Stretch Attend Postures, and Missteps or Falls. Several behaviors revealed statistically significant distinctions between control and ethanol-exposed mice.

The number of times the mouse looked over to the side during the Light Suok, considered a directed exploratory behavior (El Shawa, Abbott, & Huffman, 2013), was significantly decreased in juvenile ethanol-exposed mice (17 side looks) compared to control juveniles (22 side looks; $p$=0.004). The ethanol-exposed mice also paused (4 stops) more frequently during the adult Dark Suok than the control did (3 stops;

*p*=0.022). Notably, control mice explored on average a larger portion of the apparatus (100% traveled) during the juvenile Dark Suok compared to the ethanol-exposed (67% traveled; Figure 3 A; *p*=0.0028). Wall leanings, a vertical exploratory behavior, were performed less frequently on average by ethanol-exposed subjects (1 lean) compared to control (3 leans) during both Light (*p*=0.007) and Dark Suok (*p*=0.048) tests conducted during adulthood (Figure 3 B). The time it takes for a subject to move to one side of the apparatus after the initial placement in the center of the arena was dramatically increased in the ethanol-exposed adult mice (73 seconds) compared to the control (8 seconds; Figure. 3 C-D; *p*=0.006) during the Light Suok. Matching this trend is the finding that the total distance traveled by the ethanol-exposed mice (393 cm) during the juvenile Dark Suok was significantly decreased compared to the control mice (592 cm; Figure. 3 E-F; *p*=0.011). The remainder of the parameters were not statistically significant between control and ethanol-exposed mice, Light and Dark conditions, or juvenile and adult results.

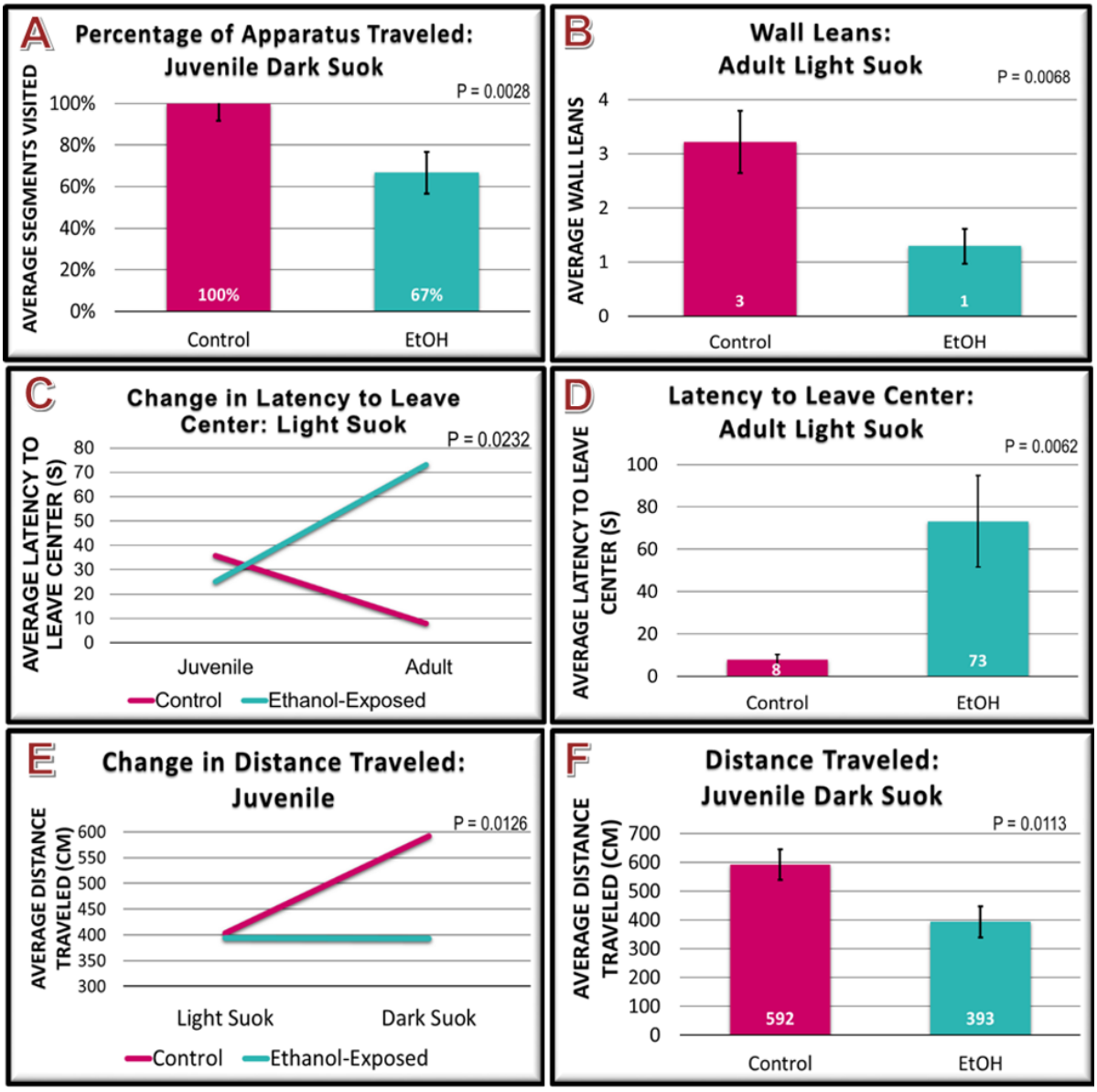


**Figure 3. Quantitative analysis Light and Dark Suok Tests performed on juvenile and adult mice indicate abnormal responses to environment in adult ethanol-exposed mice.** (A) The percentage of the length of the apparatus traveled by the control and ethanol-exposed mice ($p$=0.0028). (B) The amount of wall leans performed by ethanol-exposed and control mice during the adult Light Suok ($p$=0.0068). (C) Statistically significant change ($p$=0.0232) in how long a subject spent in the placement point before moving to one of the sides during a Light Suok. The line traces the change between juvenile and adult results. (D) In relation to the adjacent chart, this depicts the statistically significant difference between the control and ethanol-exposed latency to hide as adults ($p$=0.0062). (E) Quantification of how the amount of distance traveled along the apparatus varies for juveniles depending on the Light or Dark condition of the test ($p$=0.0126). (F) In relation to the adjacent chart, this depicts the difference in distance traveled (cm) between juvenile control and ethanol-exposed subjects during the Dark Suok ($p$=0.0113). Error bars represent standard error. Statistical analysis was performed using a one-way ANOVA and two-tailed t-tests. ($n$=48)

# DISCUSSION

## ACOUSTIC STARTLE, LEDGE, AND OPEN FIELD TESTS

The neurobehavioral tests conducted yielded interesting results and provided future directions that may be explored. While the results of the Ledge test and Acoustic startle proved insignificant, the Open Field test did yield some interesting results. The behaviors we analyzed during the Open Field test mainly serve as a measurement of locomotor activity, anxiety, and exploration (Walsh & Cummins, 1976). As adults, the mice exhibited a peculiar set of related behaviors. When a control mouse was placed in the center of the Open Field apparatus, it would often run out of the middle towards the edges after a few moments. As our specific set up involved a rounded enclosure, rather than a square one that provides corners to hide in, this attempt at evasion would quickly be realized as ineffective. Attempting to find another angle that would conceal them better, the control mice would soon run across the field to the other side, crossing the central line in the process. This back-and-forth retreat continued for the duration of the five-minute trial, as evidenced by the frequency at which they cross the field. In contrast, the ethanol-exposed adults would not flee after a few moments in the center of an open field, as would be expected of a typical control mouse (Walsh & Cummins, 1976). Rather, they idled along, casually exploring the area in which they were placed. Thus, the ethanol-exposed adults spent significantly more time in the center of the field, and dashed across it significantly less often than the control mice, indicating a higher level of exploration than control (Walsh & Cummins, 1976).

Mice and other rodents are positively thigmotaxic, and will seek out enclosed spaces and tend to stay near vertical walls (Lamprea, Cardenas, Setem, & Morato, 2008). Mice should also want to avoid open areas that could increase the risk of predation (Walsh & Cummins, 1976). When placed in a setting like the Open Field apparatus, in which there is no way of avoiding exposure, a typical mouse will display anxious and non-exploratory behaviors. This was the behavior seen in our control mice, but the ethanol-exposed mice acted in precisely the opposite manner. Instead of showing panic when placed in a potentially dangerous environment, these mice acted unconcerned and displayed more exploratory behavior than the control mice.

## Light and Dark Suok

The Light and Dark Suok tests yielded particularly fascinating results, and we were particularly interested in the success of the Suok tests, as these assays are capable of measuring multiple neurological behaviors and functions, including motor coordination and sensorimotor gating. The Light Suok test consists of a long, thin pipe on which the mice must remain balanced, while the entire apparatus is illuminated. With nowhere to hide, and with the additional component of sustaining balance in a novel, elevated environment, these mice would be expected to display cautious and anxious behaviors. In addition to being positively thigmotaxic, mice are also negatively phototaxic, and will avoid direct lighting and attempt to seek out shade or darkness (Johnson, et al., 2010). Given these behavioral traits in mice, we would expect that placement in the Light Suok would result in an anxious response, in which the mice

would immediately attempt to escape the center and hide in one of the two corners. The Dark Suok Test was unique, in that this test provided the mice with an option of concealing themselves in the shaded portions of the apparatus. A control mouse would be expected to display less anxious, less cautious, and more exploratory behavior in the area they should instinctively feel safe in. Under the cover of darkness, the mice should feel less anxious compared to the Light Suok and more comfortable exploring the area.

The control sample performed as expected during both juvenile and adult assays. During the Light Suok analyses, these mice would rapidly seek out the corners to obtain what little cover is possible in that condition. During the Dark Suok, they performed less cautious and anxious behavior, and even displayed some indications of exploratory behavior and inquisitiveness. The responses of the ethanol-exposed mice, however, were in complete contrast to those of the control. The frequency at which behaviors such as wall leaning or side looks were performed was statistically significantly different between control and ethanol-exposed mice, indicating that the prenatal ethanol exposure may have influenced the anxiety-associated behaviors mice tend to perform in an exposed environment, such as the Light Suok. In the Dark Suok, a condition intended to be less anxiety-provoking relative to the Light Suok, ethanol-exposed mice appeared hesitant to explore the arena, unlike the control who explored the entire length of the tube on average.

It was important to consider possible differences in ability of the mice to adapt to the two different contexts. When juvenile control mice were introduced to the Dark

Suok, there was a significant increase in the amount of distance travelled compared to the Light Suok. This is most likely because, in the Light Suok condition, control mice rapidly seek out the safest possible location. In this case, that would be either of the two available and covered corners. Once in the corner, they may be too cautious to come back out and risk placing themselves in a wide-open area. In the Dark Suok condition, a good deal of the area is darkened and therefore less dangerous than a lightened area where the mice may be seen. In this context, the control mice feel more free to cautiously explore. In stark contrast to this intuitive adaptive response, the ethanol-exposed mice barely alter their behavior between the Light and Dark Suok. They do not adapt to their novel surroundings.

During the Light Suok, the ethanol-exposed mice would not immediately seek out cover, as indicate by the long latency to leave the center. To analyze this relationship between responses over age, the average difference between control juvenile and adult responses was compared to the average difference between ethanol-exposed juvenile and adult responses. Interestingly, the control and ethanol-exposed mice expressed very similar responses as juveniles, and it was not until they had reached adulthood that this stark difference is observed. This is not entirely unexpected. As is observed in clinical cases of mild FASD, individuals may not display blatant symptoms until later in life (Streissguth, et al., 2004). It is possible that this trend we observed may be related to the pathology of FASD; however, more research will be needed to further investigate this.

## PRENATAL ETHANOL EXPOSURE MAY IMPAIR SENSORIMOTOR GATING IN MICE

It is important to keep in mind the potential flaws inherent in our experimental design. Using different individuals to analyze the videos of the mouse behaviors may have resulted in unintentional subjectivity. To correct for this, all videos were re-analyzed by a select group of experienced individuals assigned to one or two behaviors. This allowed the results to remain consistent in terms of scoring. Additionally, due to a limited supply of subjects, each mouse underwent testing at all three stages: postnatal, juvenile, and adult. This may have introduced some issues regarding habituation and sensitization among the mice, who had already undergone these test months previously. Upon the arrival of a new strain of mice, repetitions of these experiments will be carefully revised to prevent any such confounds from re-appearing, and this strategy is already underway.

These results reveal a consistent trend in which the control mice are behaving in the expected way, by displaying anxious and cautious behavior in an environment that deliberately provokes such a response. The ethanol-exposed mice, however, frequently act in the opposite manner. They exhibit behaviors one would expect from a subject feeling safe and completely unaware or unconcerned with potential danger. Instead of hiding, the ethanol-exposed mice prefer to sit out in the open light. In the wild, that sort of behavior would drastically increase the risk of predation. The ethanol-exposed mice appear to no longer be responding to positive thigmotaxic or negative phototaxic

survival instincts. This could have serious implications for human applicability, as reduced awareness of and appropriate response to ones' surroundings could prove deadly for an animal and devastating for a human.

In all three tests, the ethanol-exposed sample consistently demonstrated inappropriate anxiety responses to their environments. This trait could be analogous to the sensorimotor gating we observe in individuals with FASD. In both cases, environmental stimuli are not being filtered or interpreted correctly. Further studies will need to be performed to confirm this tentative conclusion. Our results suggest that early neurobehavioral markers in mice, such as cliff aversion or surface righting, may be correlated with the emergence of other neurobehavioral impairments, which may be indicative of sensorimotor gating. Finally, to further support these findings, we must explore the strength of the association between abnormal early postnatal behaviors and the unusual behaviors observed throughout the mouse's life, in order for us to more conclusively identify and characterize a persistent early neurobehavioral trait that will be applicable to diagnosing and treating patients early.

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