Check for updates

RESEARCH ARTICLE

Moderate chronic fetal alcohol exposure causes a motor learning deficit in adult outbred Swiss-Webster mice [version 1; peer review: 1 approved, 1 approved with reservations]

Tyler H. Reekes^{1*}, H. Thomas Vinyard III^{1*}, William Echols^{1*}, Andrew J. Eubank III¹, Michael D. Bouldin¹, William H. Murray¹, Stephen Brewer¹, Blake T. Brown¹, Harold L. Willis Jr¹, Zachary Tabrani¹, Carlita B. Favero², Erin B.D. Clabough¹

¹Hampden-Sydney College, Hampden-Sydney College, Hampden-Sydney, VA, VA 23943, USA

²Department of Biology, Ursinus College, Collegeville, PA, PA 19426, USA

* Equal contributors

V1 First published: 01 Aug 2016, 5:1896
<https://doi.org/10.12688/f1000research.9237.1>
 Latest published: 01 Aug 2016, 5:1896
<https://doi.org/10.12688/f1000research.9237.1>

Abstract

Prenatal ethanol exposure can negatively affect development, causing physical and/or cognitive deficits in the offspring. Behavioral changes are typically characterized during childhood, but they can also persist into adulthood. The extent of Fetal Alcohol Spectrum Disorder (FASD) abnormalities depends upon the amount and manner of ethanol intake, leading to a large variety of animal models. In order to mimic the genetically diverse human condition, we examined an outbred strain of mice exposed to chronic gestational ethanol and characterized subsequent behavioral alterations during adulthood. To detect deficits in cognitive ability and/or motor function, we ran the mice through tests designed to detect either memory/learning ability or motor strength/skill. We tested cognitive responses using the Barnes Maze and the Open Field Aversion Test, and motor skills using Kondziela's Inverted Screen Test and the rotarod. As adults, the FASD mice showed no significant differences on grip strength, open field, or the Barnes maze; however, we found that outbred mice who had experienced moderate prenatal ethanol exposure were slower to learn the rotarod as adults, though they did not differ in overall performance. Our data suggest a specific FASD vulnerability in motor learning ability, and also open the door to further investigation on the effect of ethanol on brain areas involved in motor learning, including the striatum.

Keywords

FASD, Fetal Alcohol Exposure, ethanol, chronic, outbred, Swiss-Webster mice, motor skills

Open Peer Review

Approval Status ? ✓

	1	2
version 1	?	✓
01 Aug 2016	view	view

1. **Derek Hamilton**, University of New Mexico, Albuquerque, USA

2. **Kelly J. Huffman**, University of California, Riverside, Riverside, USA

Any reports and responses or comments on the article can be found at the end of the article.

Corresponding author: Erin B.D. Clabough (eclabough@hsc.edu)

Competing interests: No competing interests were disclosed.

Grant information: A portion of this work was funded by a San Diego Instruments Rotarod Equipment Research Loan Award from the Faculty for Undergraduate Neuroscience to EC.

The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Copyright: © 2016 Reekes TH *et al.* This is an open access article distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited. Data associated with the article are available under the terms of the [Creative Commons Zero "No rights reserved" data waiver](#) (CC0 1.0 Public domain dedication).

How to cite this article: Reekes TH, Vinyard III HT, Echols W *et al.* **Moderate chronic fetal alcohol exposure causes a motor learning deficit in adult outbred Swiss-Webster mice [version 1; peer review: 1 approved, 1 approved with reservations]** F1000Research 2016, 5:1896 <https://doi.org/10.12688/f1000research.9237.1>

First published: 01 Aug 2016, 5:1896 <https://doi.org/10.12688/f1000research.9237.1>

Introduction

Ethanol (EtOH) is a toxin that produces variable detrimental physical and neurobehavioral effects in individuals subjected to ethanol exposure *in utero*, possibly resulting in Fetal Alcohol Spectrum Disorder (FASD). Animal models can replicate the human condition of FASD relatively well (Wilson & Cudd, 2011), but the broad spectrum of human drinking choices made during pregnancy is reflected in the enormous number of different FASD models currently employed by researchers. Even within a single species, differences in acute versus chronic exposure, the timing of the exposure, route of administration, and forced versus voluntary drinking patterns can result in a vast array of possible phenotypes.

The use of inbred mice has decreased variability in a field with a vast variety of models and made it easier to compare data across paradigms, explore voluntary drinking paradigms, and identify specific genetic differences regulating individual sensitivity to ethanol (Mayfield *et al.*, 2008). Genetic variation causes some strains of mice to voluntarily drink more than other strains (Rhodes *et al.*, 2007) — For example, C57BL/6 mice are heavy drinkers, while DBA/2J mice are abstainers (Rhodes *et al.*, 2005). Many FASD studies currently use inbred C57B/6 mice because of the reliability of consumption in this strain (Rhodes *et al.*, 2005).

Inbred mouse strains exposed to chronic gestational ethanol have demonstrated deficits in motor function and learning/memory (Brady *et al.*, 2012; Sanchez Vega *et al.*, 2013). Typically, motor reflexes/coordination and learning/memory skills appear to be less dependent on the timing of alcohol exposure than activity and anxiety-related behavioral changes, which have more narrow windows (Mantha *et al.*, 2013).

However, use of inbred strains do not faithfully replicate the genetic variation that naturally occurs within the human condition, so outbred strains also provide valuable information about FASD. Outbred Wistar rats are commonly used as a FASD rodent model, but use of outbred mouse models is much less common. Previously reported inbred alcohol-induced deficits may not show up in an outbred mouse model—for example, corticothalamic differences were not observed in an outbred mouse FASD model (White *et al.*, 2015). But a recent paper characterized the effect of moderate prenatal ethanol exposure on outbred Swiss Webster mouse neonate behavior (including surface righting, negative geotaxis, cliff aversion, auditory startle, ear twitch, open field traversal, air righting, and eye opening) and found that ethanol-exposed pups achieved some developmental milestones (surface righting, cliff aversion, and open field traversal) at a different rate than non-exposed control pups (Chi *et al.*, 2016).

To examine whether observed deficits in outbred mouse neonates can persist into adulthood, we examined the effect of chronic moderate gestational ethanol exposure on adult neurobehavioral outcomes in the offspring previously studied by Chi & colleagues (2016). We measured both learning/memory and motor skill subsets in male mice in order to better understand the behavioral impact of developmental ethanol exposure in an outbred model.

Materials and methods

Animal models

All experimentation was compliant with the Hampden-Sydney Institutional Animal Care and Use Committee (IACUC). Twenty-four outbred male Swiss Webster mice were obtained from Ursinus College (four litters per treatment of two-four male mice randomly selected from each litter; 24 animals in total) following completion of the early postnatal testing (Chi *et al.*, 2016). All of the dams in the ethanol treatment group were found to have moderate gestational exposure after using the Drinking in the Dark (Boehm *et al.*, 2008) paradigm, consuming an average of $4.41 \text{ g/kg} \pm .43$ standard error (SE) (Table 1). All mice were male and group housed by litter, with a 12-hour light cycle and food and water *ad libitum*. The weights of the mice at 6 months were not significantly different (by t-test, $p=0.43795$; mean EtOH=39.16g, SE=0.68; mean H₂O=39.46g, SE 1.64). All behavioral tests were performed between 4–6 months of age in the following order to reduce the impact that exposure to testing conditions might have on subsequent testing performance: open field, grip strength, Barnes maze, rotarod (a rotating rod). For each measure, scores for all littermates were averaged together before statistical analysis (12 animals in four litters per treatment group) to account for the variability in gestational ethanol exposure that accompanies a voluntary drinking paradigm.

Open field

The open field test was performed using an opaque box constructed of 6 mm plexiglass (56 cm × 56 cm × 51 cm) as a measure of anxiety-like behavior and locomotion. Testing was modeled after Nunes & colleagues (2011). The first 2 minutes of data were analyzed and the field was divided into nine equal sections. Total squares traveled and time spent in the center square were analyzed for relevance using unpaired t-tests.

Barnes maze

The Barnes maze was used to test for cognitive deficits in learning and memory. Litters were brought into the testing room one at a time to run the Barnes maze using a modified version of the

Table 1. The consumption of EtOH (g/kg) during gestation of dams for individual litters 1, 2, 3, 7 (mean = 4.43, SE = 0.43) from embryonic day (E)1.5–E18.5. Litter 1 was delivered before the E18.5 data were collected.

	E1.5	E7.5	E11.5	E15.5	E18.5	Individual Gestational Mean
Litter 1	9.73	3.41	3.41	2.56		4.8
Litter 2	6.81	4.5	2.47	5.83	2.87	4.5
Litter 3	6.41	2.74	3.44	2.27	1.27	3.2
Litter 7	3.91	2.36	15.06	3.43	1.34	5.2
Mean	6.71	3.25	6.09	3.52	1.83	4.4
St Error	1.19	0.47	3	0.81	0.45	0.43

shortened paradigm outlined by Attar *et al.* (2013) with a 120w light source suspended 1 meter above the stage in the absence of a noxious noise. During habituation and trial days, mice were allowed 30 seconds to enter into the target hole voluntarily before they were coaxed in. In addition, the escape cage remained on the probe day. Entrance into the correct hole, latency to enter, and the percent of holes explored in the opposite quadrant were assessed, as well as the number of holes explored before and after finding the correct hole. Probe day data were analyzed using unpaired t-tests.

Kondziela Inverted Screen

The Kondziela Inverted Screen test was modeled after Deacon (2013) and measures the overall strength of the mice, as well as their ability to maintain their equilibrium while inverted.

The latency of the mice to fall was tested and an average of their three recordings was taken with an upper limit of 10 minutes per trial. After each trial, the mice were returned to their home cages and allowed to rest approximately 1 hour until the sequential order called for the next test. The mice were randomly selected for the first trial, but the order was maintained for the two subsequent trials. Average latency to fall was analyzed using unpaired t-tests.

Rotarod

The rotarod was chosen to test both motor learning and endurance. The rotarod (San Diego Instruments) is a spinning rod with dividers separating individual testing spaces. It can be set to turn at a certain rotation per minute (RPM) and to increase RPM at a determined rate. When the mice fall off, sensors on the floor stop the clock and record the number of seconds and the precise RPM.

Mice were daily trained on the rotarod over a 3-day period, rested for 1 day, and then exposed to a performance day. All 24 mice were trained to run on the rotarod in a similar manner as Gill & Dietrich (1998) with modifications. Each mouse had a single daily training trial on each of three consecutive days that lasted until they fell off (up to 300 seconds) and animals were not placed on the rod again that day. Animals were rested for one day, followed by a performance assessment as outlined in Nozari *et al.* (2014). Mice started at zero RPM and accelerated by 6 RPM until the mice fell off. Each mouse ran the performance test three times and the average time per mouse was recorded.

The order that the mice were selected to run was randomized, but once that order was established, it remained unchanged. The latency to fall on the performance day and the percent improvement from the first day of training to the final performance day were separately analyzed using unpaired t-tests, and performance over the 3-day training period was assessed by a mixed ANOVA using SPSS.

Results

Open field

In open field testing, the amount of time spent in the open center square was not significantly different between ethanol-exposed and control animals (by t-test, $p=0.2383$; mean EtOH=13.72, SE=8.535; mean H₂O=6.973, SE=2.476; $n=4$ litters; Figure 1A). There was no significant difference in locomotor activity between ethanol-exposed and control mice, as measured by the mean number of squares traversed (by t-test, $p=0.4231$; mean EtOH=34.56, SE=9.691; mean H₂O=37.83, SE=7.781; $n=4$; Figure 1B).

Figure 1. Adult open field performance in Swiss Webster outbred mice exposed to moderate gestational ethanol. (A) There was no significant difference found between ethanol-exposed mice and control mice in the average number of seconds spent in the center square of the open field over a 2 minute period, as a measure of anxiety-like behavior (by t-test, $p=0.2383$; mean EtOH=13.72, SE=8.535; mean H₂O=6.973, SE=2.476; $n=4$ litters). (B) No differences were found in the total number of squares crossed in the open field measure, as a measure of locomotion (by t-test, $p=0.4231$; mean EtOH=34.56, SE=9.691; mean H₂O=37.83, SE=7.781).

Barnes maze

Mice that were exposed to EtOH during development showed no differences on the probe day in total hole exploration (by t-test, $p=0.7777$; mean EtOH=6.96, SE=1.028; mean H₂O=7.67, SE=2.12; $n=4$ litters), number of incorrect hole explorations before finding the target hole (by t-test, $p=0.6009$; mean EtOH=5.79, SE=0.3560; mean H₂O=6.83, SE=1.766; $n=4$), or the number of hole explorations after finding the target (by t-test, $p=0.7352$; mean EtOH=1.17, SE=0.7876; mean H₂O=0.83, SE=0.50; $n=4$; **Figure 2A**). There was also no significant difference found in the percentage of holes explored on the opposite quadrant of the maze (by t-test, $p=0.1272$; mean EtOH=33.33, SE=9.129; mean H₂O=57.50, SE=10.13; $n=4$; **Figure 2B**).

Kondziela Inverted Screen Test

There was no significant difference in grip strength between ethanol-exposed and control mice, as measured by the average latency to fall over the three trials (by t-test, $p=0.855$; mean EtOH=207.396 sec, SE=48.585; mean H₂O=193.39, SE=41.221; $n=4$ litters; **Figure 3**).

Rotarod

By the final performance day, previous ethanol exposure had no effect on the max RPM that mice were able to achieve (by t-test, $p=0.399$; mean EtOH=7.19, SE=0.68; mean H₂O=9.0, SE=1.78; $n=4$ litters; **Figure 4A**), indicating that overall motor performance after training was not affected by the gestational ethanol exposure. However, ethanol-exposed animals showed significantly less improvement between the first day of training and the final rotarod performance day, indicating a deficit in the acquisition of this motor skill (by t-test, $p=0.008$; mean EtOH=30.11% improvement, SE=2.5%; mean H₂O=53.52%, SE=5.5%; **Figure 4B**).

Both saline and ethanol treated mice showed an increase in the latency to fall in succeeding trial days at each age examined,

indicating that both groups of mice were learning how to stay on the rod longer. Analysis by mixed ANOVA showed a significant effect of trial day over the three day training period [$F_{(1,6)}=37.694$; $p=0.01$; **Figure 4C**]. We found a possible effect of gestational ethanol exposure on the max RPM at which the mice were able to run over the course of the three training days ($F_{(2,12)}=4.812$, $p=0.029$; **Figure 4C**), but this result was not significant after Sidak correction.

Dataset 1. Raw data of motor learning deficit in adult FASD mice

<http://dx.doi.org/10.5256/f1000research.9237.d130218>

All raw data supporting the findings described in the paper are provided (Data files 1–5). The readme file contains descriptions of each data file.

Discussion

Our experiment showed that chronic low/moderate gestational ethanol exposure had no effect on some motor measures in adult outbred mice, including grip strength and overall rotarod performance, yet mice exposed to prenatal ethanol did not improve as quickly when learning the rotarod activity. This adult rotarod learning deficits may be due to long-term ethanol induced changes in specific brain areas that are involved in the acquisition of complex motor skills.

A recent meta-analysis of human FASD concluded that prenatal ethanol exposure can result in gross motor deficits, including gait and balance problems (Lucas *et al.*, 2014). Rodent studies have indicated that ethanol exposure, particularly in the third trimester equivalent, can impact cerebellar Purkinje cell development and alter motor behavior (Maier *et al.*, 1999). The cerebellum is traditionally held responsible for the coordination and execution of motor skills, particularly gait and balance. Ethanol-induced cerebellar damage can cause FASD motor skill deficits, and compromised corticocerebellar circuits may result in impaired

Figure 2. Adult Barnes maze performance in Swiss Webster outbred mice exposed to moderate gestational ethanol. (A) There was no significant difference between ethanol-exposed mice and control mice in number of mistakes made before finding the target hole (by t-test, $p=0.6009$; mean EtOH=5.79, SE=0.3560; mean H₂O=6.83, SE=1.766; $n=4$ litters), the number of hole explorations after finding the target (by t-test, $p=0.7352$; mean EtOH=1.17, SE=0.7876; mean H₂O=0.83, SE=0.50), or the total hole explorations on the final probe day (by t-test, $p=0.7777$; mean EtOH=6.96, SE=1.028; mean H₂O=7.67, SE=2.12). (B) When the maze was divided into 4 quadrants for analysis, there was also no difference between the ethanol and control mice in the percent of holes located the opposite quadrant that were explored (by t-test, $p=0.1272$; mean EtOH=33.33, SE=9.129; mean H₂O=57.50, SE=10.13).

Figure 3. Adult inverted screen test performance in Swiss Webster outbred mice exposed to moderate gestational ethanol. There was no significant difference found between ethanol-exposed mice and control mice in the latency to fall from the inverted screen as a measure of grip strength (by t-test, $p=0.855$; mean EtOH=207.396 seconds, SE=48.585; mean H₂O=193.39, SE=41.221; $n=4$ litters).

Figure 4. Adult rotarod performance in Swiss Webster outbred mice exposed to moderate gestational ethanol. (A) There was no significant difference between ethanol-exposed mice and control mice in the average max RPM attained on the final performance day (5) (by t-test, $p=0.399$; mean EtOH=7.19, SE=0.68; mean H₂O=9.0, SE=1.78; $n=4$ litters). (B) On average, control mice improved more than ethanol-exposed mice over the course of the rotarod testing, as measured by the percent change in RPM reached from the first training day (1) to the last performance day (5) (by t-test, $p=0.008$, EtOH mean=30.11%, SE=2.5%; H₂O mean=53.52%, SE=5.5%). (C) We found a possible effect of gestational ethanol exposure on the max RPM at which the mice were able to run over the course of the three training days ($F_{(2,12)}=4.812$, $p=0.029$), but this result was not significant after Sidak correction.

visuospatial abilities or other more cognitive processes. However, in rodent models, cerebellar Purkinje cells seem particularly vulnerable to degeneration when exposed to alcohol during the critical period that occurs during the early postnatal period (human third trimester equivalent) (Jaatinen & Rintala, 2008), whereas our mouse model was only exposed during the first and second trimester equivalents.

The striatum (caudate/putamen) also plays a central role in the acquisition of long term motor skills, and remains of particular interest to alcohol researchers because of its central role in addictive behavior. In rodents, moderate gestational ethanol exposure can alter medium spiny neuron dendritic branching in the striatum pups (Rice *et al.*, 2012), and studies show that developmental ethanol exposure can alter brain-derived neurotrophic factor (BDNF) expression in adult offspring, which can, in turn, impact propensity to drink and lay the groundwork for future addictive behavior, regulated by the striatum (Davis, 2008). BDNF is enriched in the striatum, delivered predominately via corticostriatal afferents, and BDNF polymorphisms have been linked to decreased synaptic plasticity involved in learning both complex and simple learning motor tasks (Cárdenas-Morales *et al.*, 2014). It is possible that BDNF alterations in the striatum could impact synaptic plasticity or neurogenesis and could cause our observed complex motor learning changes.

Our experiment showed that chronic low/moderate gestational ethanol exposure had no effect on open field behavior or Barnes maze performance in outbred adult mice. Our results are in line with some previous studies that have not detected deficits that persist into adulthood for open field, learning/memory tasks, nor locomotor activity in inbred mouse models after chronic gestational ethanol exposure (Boehm *et al.*, 2008; Downing *et al.*, 2009), but it is also possible that our outbred strain introduces enough genetic variability to mask learning or open field phenotypes that have been previously found in inbred strains.

However, these ethanol-exposed offspring previously exhibited significantly altered timing to achieve surface righting, cliff aversion, and open field traversal developmental milestones as neonates (Chi *et al.*, 2016), so our results may also indicate some sort of recovery or compensatory response by the central nervous system to the effects of early ethanol exposure has occurred. In most rodent FASD models, motor deficits are visible early in life and have disappeared by adulthood (Patten *et al.*, 2014).

The value of non-inbred FASD models is currently being explored in other ways: High Alcohol Preferring mice and Low Alcohol Preferring mice have been developed by interbreeding eight inbred

strains that display these drinking tendencies (Bice *et al.*, 2011). Testing has shown differences in open field behavior between High and Low Alcohol Preferring mice in the absence of alcohol exposure (Can *et al.*, 2012). Further behavioral characterization of these animals using an FASD model would provide useful motor behavior information, while decreasing the genetic variance present.

Future ethanol research should pay particular attention to adult phenotypes in order to document the persistence of these changes in FASD models, and to investigate compensatory mechanisms that may allow ethanol-exposed juveniles to overcome these deficits by adulthood. Neuroanatomical analysis should be performed at both juvenile and adult time points to detect possible apoptosis in brain areas involved in motor function. Apart from cell death, both cerebellar and striatal mechanisms that govern the execution of complex motor tasks should be examined, as well as possible long-lasting changes in synaptic plasticity or neurogenesis that may occur following developmental ethanol exposure.

Data availability

F1000Research: Dataset 1. Raw data of motor learning deficit in adult FASD mice, [10.5256/f1000research.9237.d130218](https://doi.org/10.5256/f1000research.9237.d130218) (Reekes *et al.*, 2016).

Author contributions

EC and all other authors conceived the study, designed the experiments, prepared all manuscript drafts, and have agreed to the final content. SB, MB, WE, TR, BB, and HW carried out the open field and Barnes maze experiments. TV, AE, ZT, and WM carried out the motor testing. All authors were involved in the revision of the draft manuscript. CF performed the gestational ethanol exposure and acted as a consultant.

Competing interests

No competing interests were disclosed.

Grant information

A portion of this work was funded by a San Diego Instruments Rotarod Equipment Research Loan Award from the Faculty for Undergraduate Neuroscience to EC.

The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Acknowledgements

The authors would like to thank John Reekes for logistical assistance and Jennie Jenkins for laboratory support.

References

- Attar A, Liu T, Chan WT, *et al.*: **A shortened Barnes maze protocol reveals memory deficits at 4-months of age in the triple-transgenic mouse model of Alzheimer's disease.** *PLoS One*. 2013; **8**(11): e80355.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Bice PJ, Lai D, Zhang L, *et al.*: **Fine mapping quantitative trait loci that influence alcohol preference behavior in the High and Low Alcohol Preferring (HAP and LAP) mice.** *Behav Genet*. 2011; **41**(4): 565–570.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Boehm SL 2nd, Moore EM, Walsh CD, *et al.*: **Using drinking in the dark to model prenatal binge-like exposure to ethanol in C57BL/6J mice.** *Dev Psychobiol*. 2008; **50**(6): 566–578.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Brady ML, Allan AM, Caldwell KK: **A limited access mouse model of prenatal alcohol exposure that produces long-lasting deficits in hippocampal-dependent learning and memory.** *Alcohol Clin Exp Res*. 2012; **36**(3): 457–466.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Can A, Grahame NJ, Gould TD: **Affect-related behaviors in mice selectively bred for high and low voluntary alcohol consumption.** *Behav Genet*. 2012; **42**(2): 313–322.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Cárdenas-Morales L, Grön G, Sim EJ, *et al.*: **Neural activation in humans during a simple motor task differs between BDNF polymorphisms.** *PLoS One*. 2014; **9**(5): e96722.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Chi P, Aras R, Martin K, *et al.*: **Using Swiss Webster mice to model Fetal Alcohol Spectrum Disorders (FASD): An analysis of multilevel time-to-event data through mixed-effects Cox proportional hazards models.** *Behav Brain Res*. 2016; **305**: 1–7.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Davis MI: **Ethanol-BDNF interactions: still more questions than answers.** *Pharmacol Ther*. 2008; **118**(1): 36–57.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Deacon RM: **Measuring the strength of mice.** *J Vis Exp*. 2013; (76): e2610–e2610.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Downing C, Balderrama-Durbin C, Hayes J, *et al.*: **No effect of prenatal alcohol exposure on activity in three inbred strains of mice.** *Alcohol Alcohol*. 2009; **44**(1): 25–33.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Gill K, Deitrich RA: **Acute tolerance to the ataxic effects of ethanol in short-sleep (SS) and long-sleep (LS) mice.** *Psychopharmacology (Berl)*. 1998; **136**(1): 91–98.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Jaatinen P, Rintala J: **Mechanisms of ethanol-induced degeneration in the developing, mature, and aging cerebellum.** *Cerebellum*. 2008; **7**(3): 332–347.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Lucas BR, Latimer J, Pinto RZ, *et al.*: **Gross motor deficits in children prenatally exposed to alcohol: a meta-analysis.** *Pediatrics*. 2014; **134**(1): e192–209.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Maier SE, Miller JA, Blackwell JM, *et al.*: **Fetal alcohol exposure and temporal vulnerability: regional differences in cell loss as a function of the timing of binge-like alcohol exposure during brain development.** *Alcohol Clin Exp Res*. 1999; **23**(4): 726–734.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Mantha K, Kleiber M, Singh S, *et al.*: **Neurodevelopmental timing of ethanol exposure may contribute to observed heterogeneity of behavioral deficits in a mouse model of fetal alcohol spectrum disorder (FASD).** *J Behav Brain Sci*. 2013; **3**(1): 85–99.
[Publisher Full Text](#)
- Mayfield RD, Harris RA, Schuckit MA: **Genetic factors influencing alcohol dependence.** *Br J Pharmacol*. 2008; **154**(2): 275–287.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Nozari M, Shabani M, Hadadi M, *et al.*: **Enriched environment prevents cognitive and motor deficits associated with postnatal MK-801 treatment.** *Psychopharmacology (Berl)*. 2014; **231**(22): 4361–4370.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Nunes F, Ferreira-Rosa K, Pereira Mdos S, *et al.*: **Acute administration of vinpocetine, a phosphodiesterase type 1 inhibitor, ameliorates hyperactivity in a mice model of fetal alcohol spectrum disorder.** *Drug Alcohol Depend*. 2011; **119**(1–2): 81–87.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Patten AR, Fontaine CJ, Christie BR: **A comparison of the different animal models of fetal alcohol spectrum disorders and their use in studying complex behaviors.** *Front Pediatr*. 2014; **2**: 93.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Rice JP, Suggs LE, Lusk AV, *et al.*: **Effects of exposure to moderate levels of ethanol during prenatal brain development on dendritic length, branching, and spine density in the nucleus accumbens and dorsal striatum of adult rats.** *Alcohol*. 2012; **46**(6): 577–584.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Reekes TH, Vinyard HT III, Echols W, *et al.*: **Dataset 1 in: Moderate Chronic Fetal Alcohol Exposure Causes a Motor Learning Deficit in Adult Outbred Swiss-Webster Mice.** *F1000Research*. 2016.
[Data Source](#)
- Rhodes JS, Best K, Belknap JK, *et al.*: **Evaluation of a simple model of ethanol drinking to intoxication in C57BL/6J mice.** *Physiol Behav*. 2005; **84**(1): 53–63.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Rhodes JS, Ford MM, Yu CH, *et al.*: **Mouse inbred strain differences in ethanol drinking to intoxication.** *Genes Brain Behav*. 2007; **6**(1): 1–18.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Sanchez Vega MC, Chong S, Burne TH: **Early gestational exposure to moderate concentrations of ethanol alters adult behaviour in C57BL/6J mice.** *Behav Brain Res*. 2013; **252**: 326–333.
[PubMed Abstract](#) | [Publisher Full Text](#)
- White SA, Weber JN, Howard CD, *et al.*: **Effects of binge ethanol exposure during first-trimester equivalent on corticothalamic neurons in Swiss Webster outbred mice.** *Neuroreport*. 2015; **26**(18): 1083–1088.
[PubMed Abstract](#)
- Wilson SE, Cudd TA: **Focus on: the use of animal models for the study of fetal alcohol spectrum disorders.** *Alcohol Res Health*. 2011; **34**(1): 92–8.
[PubMed Abstract](#) | [Free Full Text](#)

Open Peer Review

Current Peer Review Status: ? ✓

Version 1

Reviewer Report 28 September 2016

<https://doi.org/10.5256/f1000research.9941.r16659>

© 2016 Huffman K. This is an open access peer review report distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Kelly J. Huffman

Department of Psychology, University of California, Riverside, Riverside, CA, USA

This study examines the long-term behavior effects of prenatal ethanol exposure in an outbred murine stock. The authors discuss a very important idea that the sole use of inbred mice to study FASD is problematic due to the lack of genetic variation. If scientists attempt to model a human condition like FASD, it is ultimately more informative to use a genetic model that is more similar to humans, who are by nature an outbred stock.

Issues: The NIH has stated that scientist should no longer neglect female mice as subjects in studies like this and if the authors do not wish to include females in their analyses (if they did it would strengthen the paper) then they should give justification in the text for the sole use of males. Sex could be used a potential covariate, as is time of measurement. The 4-6 month age time period may cause some differences in the data. For example, a 6-month old mouse has had 50% more time for neural plasticity than mice that are 4 months old. I would not require redoing the experiments but perhaps including age as a covariate to determine whether it plays a role in the effects would be a wise idea. There are two reports left out of the discussion that should be mentioned as they employ use of an outbred stock of mice (CD1) in a prenatal alcohol exposure model. They should be included in the discussion (El Shawa *et al.*, 2013 and Abbott *et al.*, 2016).

In summary, the title is appropriate for the article and the abstract represents a suitable summary of the work. The design, methods and analysis of the results from the study are appropriate and explained well. I think that the conclusions drawn by the authors are sensible, balanced and justified. All the data provided is in a usable format/ structure, and enough information has been provided so that the experiment can be replicated.

Overall this is a good paper.

Competing Interests: No competing interests were disclosed.

I confirm that I have read this submission and believe that I have an appropriate level of

expertise to confirm that it is of an acceptable scientific standard.

Reviewer Report 16 August 2016

<https://doi.org/10.5256/f1000research.9941.r15345>

© 2016 Hamilton D. This is an open access peer review report distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Derek Hamilton

Department of Psychology, University of New Mexico, Albuquerque, NM, USA

This paper is motivated in part by the discrepancy between genetic variation in humans that is absent in many animal models of FASD that involve assessments in inbred strains. The authors examined learning/memory and motor strength/skill in adult outbred (Swiss-Webster) mice exposed to gestational alcohol exposure (first two trimester equivalent). Exposed mice showed no statistically significant deficits in grip strength, open field exploration, or in the Barnes Maze. Exposed mice were slower to learn the rotorod, but did not differ in overall performance. The authors conclude that motor learning abilities are affected by moderate gestational ethanol exposure in the absence of conspicuous effects on motor performance. The strengths of the study are 1) the use of multiple behavioral and cognitive tasks within subjects, 2) measurements taken during adulthood to address persistence of effects, 3) the use of an established voluntary drinking paradigm, and 4) potential for motivating more research on genetic variability and ethanol exposure. Addressing several points outlined below would strengthen the paper and its impact on the field.

For analyses the litter was used as the unit of analysis. Litter effects are commonly addressed by limiting the number of animals from a given litter used in a particular study. In this report, unequal numbers (2-4 animals) from 4 litters were used. Thus, the n for each group was 4. Considering this, some effects that are numerically noteworthy but not statistically significant (e.g., open field, time in center, Fig 1A; Barnes maze exploration of holes in the opposite quadrant, Fig 2B; final rotorod performance, Fig 4A) could be underpowered. Estimates of effect sizes and power calculations should be provided. This issue takes on added importance given that one of the principal conclusions concerns the lack of motor performance deficits following prenatal alcohol exposure.

An alternative analysis could also be considered. Though litters are commonly used as the unit of analysis in developmental studies with proper justification and design, averaging data from multiple animals in a given litter also eliminates potentially important individual variation. An analysis treating animals as nested within litters within groups (Lazic and Essioux, 2013) represents an alternative that could yield different statistical outcomes.

The relative sensitivity of the tests and measures should be considered. More fine-grained behavioral assessments may be more sensitive to ethanol exposure. Further, different motor behaviors that are distinguished by effector systems and neural circuitry (e.g., orofacial movements, skilled reaching; see e.g., Hamilton, 2014) will likely yield distinct results, which

should be considered in integrating the present results and conclusions with the extant literature.

One of the primary motivations noted in the paper is the issue of using inbred vs. outbred strains in the study of fetal alcohol effects. The present study does not directly compare outbred and inbred strains statistically. The potentially large variation in parameters of ethanol exposure models and behavioral methods make drawing conclusions from qualitative comparisons across studies difficult. The authors should consider providing some recommendations for how to more fully address the issue of genetic variation and fetal alcohol effects.

The authors note that inbred mouse strains display deficits in motor function and learning/memory. It is important to contextualize this discussion with reference to critical variables including the timing, dose, duration, and route of ethanol administration. Further, an expanded treatment of the motor effects following prenatal alcohol exposure would help place the current findings in the broader landscape of the literature.

The authors should provide estimates of blood ethanol concentrations associated with the level of drinking, and clarify what they consider to be moderate ethanol exposure.

The raw data files contain some discrepancies or omissions that should be corrected. Raw data 1 (open field) contains litter numbers that do not correspond to same mouse numbers as provided in other files (e.g., .Raw data 3). Raw data 2 (Barnes maze) only includes litter numbers but not individual mouse id values.

MINOR ISSUES

Abstract : "Mice who" should be "mice that"

The introduction should provide more information and citations to relevant articles regarding various ethanol exposure paradigms.

The second sentence of the third introduction paragraph is a bit vague.

As written the second sentence of the fifth introduction paragraph reads as if the use of male mice is critical to advancing understanding of the behavioral consequences of ethanol exposure.

For the open field please include the lighting parameters.

The methods for the Barnes maze indicate that the task was conducted with a light source but no auditory stimulus. This is inconsistent with the methods cited (Attar et al. 2013).

Page 7. In the sentence beginning "In rodents, moderate gestational ethanol exposure can alter medium spiny neuron dendritic branching in the striatum pups (Rice et al., 2012), ..." The word "pups" should be removed.

References

1. Lazic SE, Essioux L: Improving basic and translational science by accounting for litter-to-litter variation in animal models. *BMC Neurosci.* 2013; **14**: 37 [PubMed Abstract](#) | [Publisher Full Text](#)
2. Hamilton DA: The importance of measurement precision and behavioral homologies in evaluating the behavioral consequences of fetal-ethanol exposure: commentary on Williams and colleagues (. *Alcohol Clin Exp Res.* 2014; **38** (1): 40-3 [PubMed Abstract](#) | [Publisher Full Text](#)

Competing Interests: No competing interests were disclosed.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

The benefits of publishing with F1000Research:

- Your article is published within days, with no editorial bias
- You can publish traditional articles, null/negative results, case reports, data notes and more
- The peer review process is transparent and collaborative
- Your article is indexed in PubMed after passing peer review
- Dedicated customer support at every stage

For pre-submission enquiries, contact research@f1000.com

F1000Research