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Data Article

# Experimental data on developmental elimination of retrosplenial cortex GABAergic interneurons in a mouse model of ethanol exposure during the last trimester of human pregnancy

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Dataset link: Raw data from the quantification of interneuron density in retrosplenial cortex layers of control (air-exposed) and ethanol (EtOH)-exposed mice at the indicated postnatal days (Original data)

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ABSTRACT

It has been previously shown that 40% of murine cortical interneurons are eliminated via apoptosis during the first two weeks of postnatal development [1–3]. Here, we report data on the effect of ethanol exposure on this process in a mouse model of binge-like alcohol exposure during last trimester of human pregnancy (equivalent to the first postnatal week in mice). We used transgenic mice that express the Venus fluorescent protein in GABAergic interneurons under the control of the vesicular GABA transporter promoter (VGAT-Venus mice) [4]. Mice were exposed to air (controls) or ethanol for 4 hr/day on postnatal days 4 to 9 using vapor inhalation chambers [5]. This exposure paradigm produces peak blood ethanol concentrations between 300 and 400 mg/dl. Transcardial perfusions were performed under anesthesia at postnatal days 5, 7, 10 and 30. Cryostat-prepared floating sections were stained with the fluorescent DNA dye, 4′6-diamidino-2-phenylindole (DAPI). We then quantified the density of Venus-positive GABAergic interneurons in layers I, II-IV and V

*Abbreviations:* ANOVA, Analysis of variance; DAPI, 4′,6-diamidino-2-phenylindole; DNA, Deoxyribonucleic acid; GABA, Gamma-aminobutyric acid; VGAT, Vesicular GABA transporter.

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of the retrosplenial cortex, which is part of the limbic memory system [6], and is sensitive to ethanol-induced apoptosis during the first postnatal week in mice [7–11]. The data show that density of interneurons decreases in the retrosplenial cortex layers during the first week of life and that ethanol exposure does not significantly alter this process. These data may be of interest to investigators who are studying the effect of ethanol and other teratogenic agents on developing interneurons in the cerebral cortex.



## Specifications Table

| | |
|---|---|
| Subject | Neuroscience |
| Specific subject area | GABAergic cortical interneuron development in a mouse model of late-pregnancy alcohol exposure |
| Type of data | Image<br>Graphs<br>Tables |
| How data were acquired | Histological images were acquired with a Zeiss Axioplan 2 inverted fluorescent microscope (with a 10X objective) and a Nuance spectral imaging system. Density of Venus-positive GABAergic interneurons was determined using Fiji. Data were collected and analyzed using Excel and GraphPad Prism. |
| Data format | Raw<br>Analyzed |
| Description of data collection | Dams and pups were exposed to air or ethanol in vapor chambers for 4 hr/day on postnatal days 4–9. Brains were collected 18–20 hr after the end of exposure at postnatal days 5, 7 and 10 and also 18–21 days after the end of the exposure paradigm (i.e., postnatal days 27–30). Parasagittal sections were prepared with a cryostat. Fluorescent microscopy images were acquired by an investigator blinded to the experimental conditions. Two independent blinded experimenters outlined the cortical layers I, II-IV, and V and exhaustively counted the number of Venus positive cells in four randomly-selected parasagittal sections containing the ventral retrosplenial cortex. The density of Venus-positive cells was calculated. |
| Data source location | Institution: University of New Mexico Health Sciences Center<br>City/Town/Region: Albuquerque, New Mexico<br>Country: United States of America<br>Latitude and longitude for collected samples/data: 35.0896° N, 106.6180° W |
| Data accessibility | Repository name: Mendeley Data<br>Data identification number: DOI:10.17632/vcd3b286cm.3<br>Direct URL to data: https://data.mendeley.com/datasets/vcd3b286cm/3 |
| Related research article | N/A |

## Value of the Data

- The data shown here provide insight into the effect of one of the most common teratogenic agents (i.e., ethanol) on the survival of inhibitory neurons in the developing cerebral cortex.
- The data complement studies on the effect of a single ethanol exposure (e.g., at postnatal day 7) which causes robust interneuronal apoptosis in the retrosplenial cortex and other brain regions [7–11]. The data shown here indicate that ethanol exposure on postnatal days 4–9 does not increase elimination of interneurons in the retrosplenial cortex, suggesting the development of tolerance to ethanol's effects with repeated exposure.

- More broadly, the data should be useful to investigators studying the impact of developmental exposure to ethanol and related agents (e.g., general anesthetics and other central nervous system depressants) on inhibitory neurons in the brain. The data could facilitate the interpretation of functional data.

## 1. Data Description

We studied the effect of exposure to ethanol vapor at postnatal days 4–9 on interneuronal numbers in parasagittal brain sections containing the retrosplenial cortex (Fig 1). Samples were collected at postnatal days 5, 7, and 10, 18–20 hr after the end of alcohol exposure. In addition, samples were collected at postnatal days 27–30 (i.e., 18–21 post-ethanol exposure). Fig 2 shows exemplar images from air- and ethanol-exposed pups obtained at postnatal days 5 and 30. Fig 3 shows the density of Venus-positive interneurons in layers I, II-IV, and V in control and ethanol exposed mice at postnatal days 5, 7, 10 and 27–30. Male and female mice are identified by different symbols. The raw and analyzed dataset can be found on Mendeley (https://data.mendeley.com/datasets/vcd3b286cm/3), including each animal's identification information with the corresponding interneuronal counts and areas (for each of the randomly-selected 4 tissue sections per brain and the average for each mouse) used to calculate interneuronal densities at the different postnatal days. For example, the data from air-exposed postnatal day 5 pups is from 1 female and 1 male pup from litter V313, 1 male pup from litter V317, and 1 male pup from litter V320. When possible, we tested pups from the same litter at different ages (e.g., V313 air-exposed pups were tested at postnatal days 5, 7, 10 and 27).

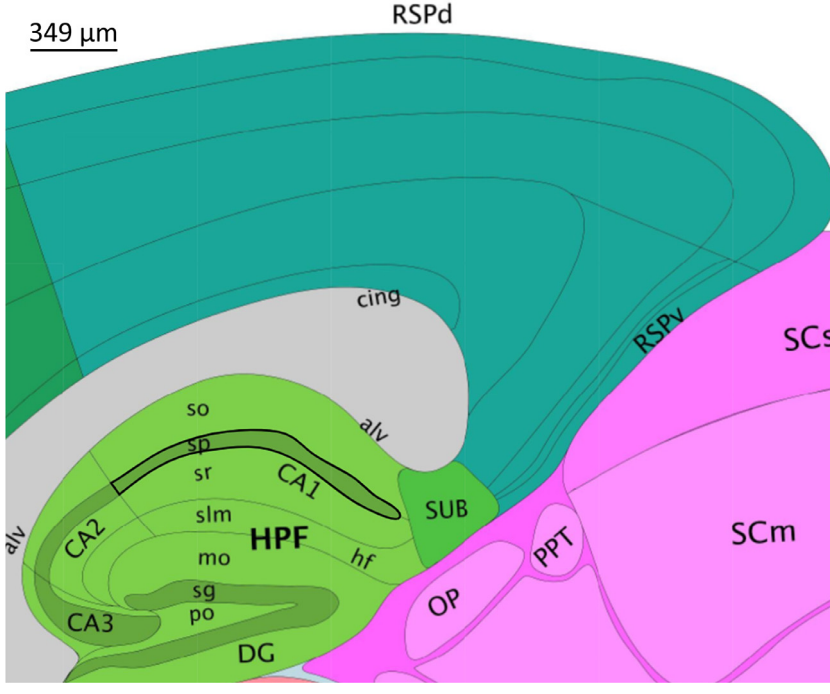


**Fig. 1.** Location of the ventral retrosplenial cortex in the mouse brain. Image from the Allen Brain Sagittal Atlas showing the ventral retrosplenial cortex (RSPv) from a postnatal day 56 mouse (Image 16; ©2015 Allen Institute for Brain Science. Allen Brain Atlas API. Available from: https://atlas.brain-map.org/atlas?atlas=2#atlas=2&structure=81&resolution=3.49&x=8131.591796875&y=1947.5918070475261&zoom=-1&plate=100883888). Image credit: Allen Institute.

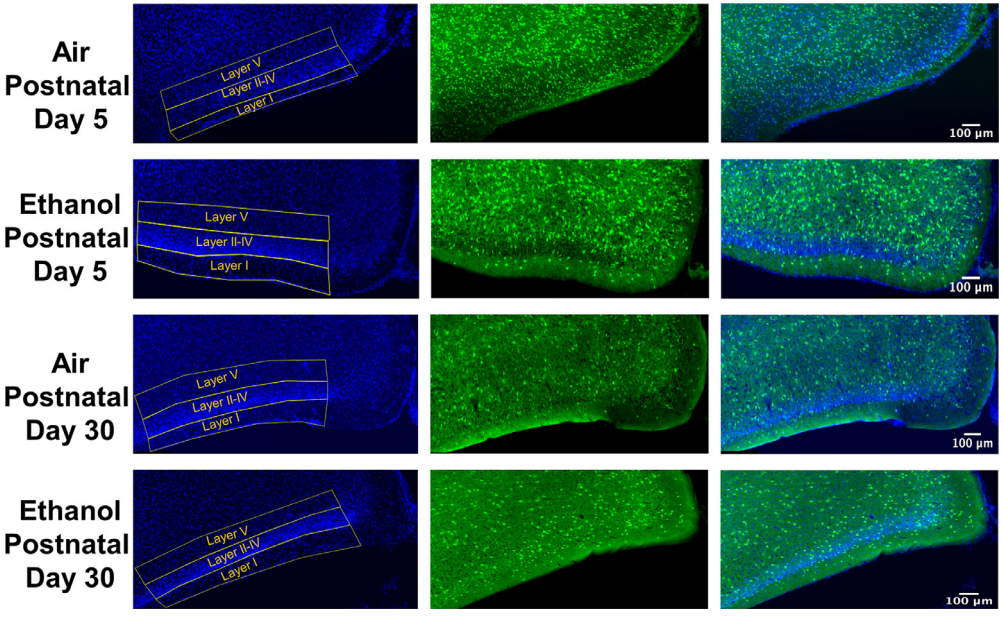


**Fig. 2.** Sample images from ventral retrosplenial cortex of control (air-exposed) and ethanol exposed VGAT-Venus mice at postnatal days 5 and 30. The left panels show DAPI nuclear stain images with labeled layers I, II-IV, and V. The middle panels show GABAergic interneurons labeled with Venus fluorescent protein. The right panels show merged images. Images were obtained with a 10X objective (scale bars = 100 µm).

## 2. Experimental Design, Materials and Methods

We used transgenic mice that express the Venus fluorescent protein in GABAergic interneurons under the control of the vesicular GABA transporter promoter (VGAT-Venus mice) [4]. These mice were generously provided by Dr. Yanagawa at the Department of Genetic and Behavioral Neuroscience at the Gunma University Graduate School of Medicine in Maebashi, Japan. Mice were maintained as heterozygous at 22 °C on a reverse 12-hr light/dark cycle (lights on at 8 pm) with standard chow and water available ad libitum. Wild type C57BL/6NHsd (Envigo, Indianapolis, IN) or VGAT-Venus heterozygous female mice were paired with C57BL/6 or VGAT-Venus heterozygous males, respectively. At postnatal day 2, pups were screened using a "miner's lamp" (Biological Laboratory Equipment Maintenance and Service LTD, Budapest, Hungary) by illuminating the skull with 460–495 nm wavelength light and observing yellow fluorescence emitted by the brain at 520–550 nm.

At postnatal days 4–9, the pups with their dams were exposed to air or ethanol for 4 hr/day (approximately 10 am-2 pm) using custom-built vapor chambers, as we previously described [5]. The ethanol concentration in the vapor chamber was 8–9 g/dl, as determined with a breathalyzer (Intoximeters, St. Louis, MO). This paradigm produces pup blood ethanol concentrations between 300 and 400 mg/dl. On the morning of postnatal days 5, 7, 10 and 30, mice underwent transcardial perfusion, fixation, cryoprotection, and embedding, as previously described [12]. On postnatal days 5, 7 and 10, mice were perfused approximately 18–20 hr after the end of the 4 hr ethanol exposure. After the end of exposure on postnatal day 9, some pups were left undisturbed until postnatal days 27–30, when cardiac perfusions were performed. Brains were stored at −80 °C and allowed to equilibrate for 24 hr at −20 °C prior to the preparation of 50 µm parasagittal slices with a cryostat (Microm Model HM 505E, Waldorf, Germany). Floating sections were stored at −20 °C in 0.05 M phosphate buffer (pH 7.4), 25% glycerol and 25% ethylene glycol. Four randomly-selected sections that contained the ventral retrosplenial cortex (Fig 1) were

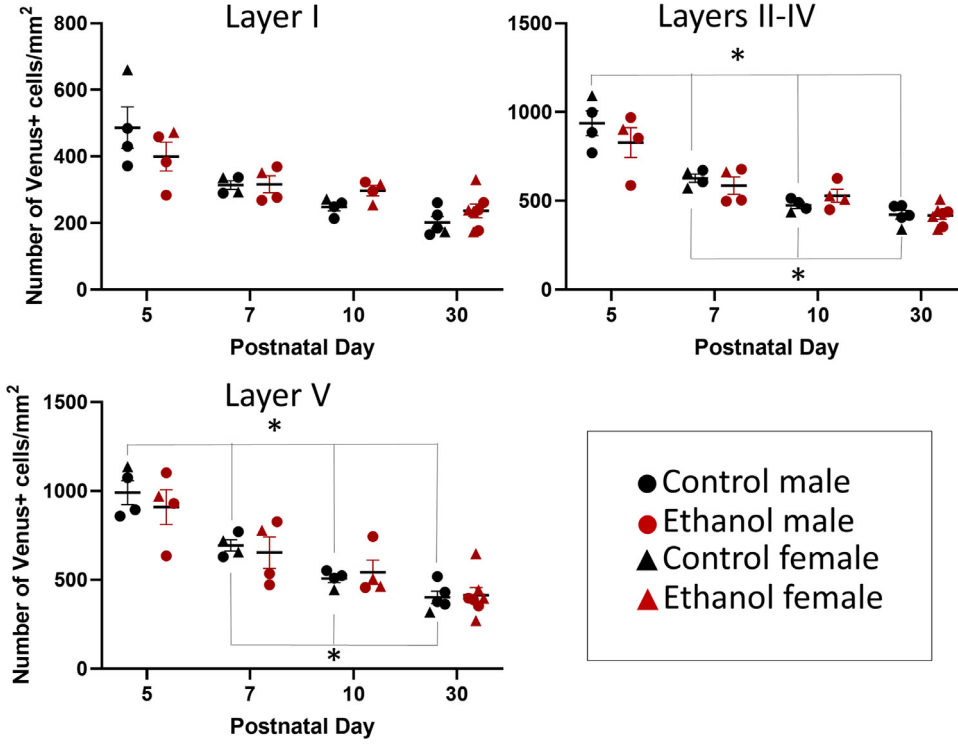


**Fig. 3.** Developmental profile of interneuronal density in the ventral retrosplenial cortex of air- and ethanol-treated VGAT-Venus mice. Shown is the density of Venus+ cells in layers I, II-IV, and V at the indicated postnatal days. Male and female mice were grouped together at each postnatal day and are labeled as shown in the lower right panel. Each point represents the average of four randomly-selected sections from a single mouse brain. For more details see https://data.mendeley.com/datasets/vcd3b286cm/3. The data were analyzed using a mixed-effect model two-way ANOVA with Geisser-Greenhouse correction. Layer I: Age: F (1.229, 7.373) = 25.77; Geisser-Greenhouse's epsilon = 0.409; $p = 0.0009$. Exposure: F (1, 10) = 0.02591; $p = 0.87$. Age x Exposure: F (3, 18) = 3.419; $p = 0.039$; Šídák's multiple comparisons test for exposure was >0.19 in at all ages. Šídák's multiple comparisons test for age >0.05 in both treatment groups. Layer II-IV: Age: F (1.749, 10.49) = 45.51; Geisser-Greenhouse's epsilon = 0.58; $p < 0.0001$. Exposure: F (1, 10) = 0.6287; $p = 0.44$. Age x Exposure: F (3, 18) = 1.196; $p = 0.33$. * $p < 0.05$ by Šídák's multiple comparisons test for postnatal day 5 vs postnatal days 7, 10, and 30 and postnatal day 7 vs. postnatal days 10 and 30 in control group only; no significant differences were detected in ethanol group. Layer V: Age: F (2.095, 19.55) = 31.51) = 45.51; Geisser-Greenhouse's epsilon = 0.69; $p < 0.0001$. Exposure: F (1, 28) = 0.1945; $p = 0.66$. Age x Exposure: F (3, 28) = 0.3696; $p = 0.77$. * $p < 0.05$ by Šídák's multiple comparisons test for postnatal day 5 vs postnatal days 7, 10, and 30 and postnatal day 7 vs. postnatal days 10 and 30 in control group only; no significant differences were detected in ethanol group.

incubated for 20 min with 600 nM DAPI (Sigma-Aldrich, St. Louis, MO), washed with phosphate-buffered saline, mounted on Superfrost Plus microscope slides (VWR, Radnor, PA) with the use of Fluoromount G mounting media (Southern Biotech, Birmingham, AL) and covered with a glass coverslip.

Images were acquired with the Nuance spectral imaging system (Caliper Life Sciences/PerkinElmer, Hopkinton, MA) at the University of New Mexico Health Sciences Center Fluorescence Microscopy Shared Resource. This system includes a Pan-NEOFLUAR 10X/0.30 objective on an Axioplan 2 inverted microscope (Zeiss, Peabody, MA). Flat-field correction was used to yield uniform illumination during image acquisition. A spectral library was generated for Venus and DAPI fluorescence by subtracting the autofluorescence. Experimental images were then un-mixed using the computed single-labeled spectrums to obtain composite images. Images were stitched together by the Grid/Collection stitching plugin located in Fiji [13]. Using

the polygon selection tool in Fiji, two independent researchers separately outlined the retrosplenial cortical layers I, II-IV, and V using DAPI fluorescence as a guide (Fig 2) and Venus positive cells were exhaustively counted with the multipoint selection tool. Additionally, the area of each cortical layer was calculated and used to calculate the density of neurons per $mm^2$. The interneuronal density in each of the 4 sections/animal was averaged and this was considered the unit of determination. Data independently obtained by two investigators were averaged (https://data.mendeley.com/datasets/vcd3b286cm/3). The investigators were blinded to which treatment the animal received.

We performed statistical analyses using Prism 9 (GraphPad Software, San Diego, CA). In total, we used 35 animals for these experiments. Data are presented as mean ± SEM in all cases. With the exception of the layer V ethanol group, all data passed the Shapiro-Wilk normality test. The data were analyzed using a mixed-effect model two-way ANOVA with Geisser-Greenhouse correction.

## Ethics Statement

All the procedures involving animals comply with ARRIVE guidelines and were approved by the Institutional Animal Care and Use Committee of the University of New Mexico Health Sciences Center (Protocol #18–200,802-HSC). Additionally, all procedures followed the National Institutes of Health guide for the care and use of Laboratory animals (NIH Publications No. 8023, revised 1978). The sex of animals is indicated, as well as the influence of sex on the data collected in the study (Fig 3 and https://data.mendeley.com/datasets/vcd3b286cm/3).

## CRediT Author Statement

**Megan Barber:** Formal Analysis, Investigation, Data Curation, Writing – original draft, Visualization; **Casey D. McDonald:** Formal Analysis; **Glenna J. Chavez:** Formal Analysis, Resources; **Clark W. Bird:** Methodology; **C. Fernando Valenzuela:** Conceptualization, Methodology, Writing – review & editing, Supervision, Funding acquisition.

## Declaration of Competing Interest

All author declares that there are no known competing financial interest nor personal relationships they have or could be perceived to have influenced the work reported in this paper.

## Data Availability

Raw data from the quantification of interneuron density in retrosplenial cortex layers of control (air-exposed) and ethanol (EtOH)-exposed mice at the indicated postnatal days (Original data) (Mendeley Data).

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