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Data Article

Data on electroconvulsive seizure in mice, effects of anesthesia on immediate early gene expression

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ABSTRACT

Although electroconvulsive therapy (ECT) is one of the most effective treatments for severe mood and psychotic disorders, the mechanisms underlying its therapeutic effects remain unknown. Electroconvulsive stimulation (ECS), the animal model for ECT, can be used to investigate the potential therapeutic mechanisms of ECT in rodents. ECS produces numerous effects in the brain, such as increasing levels of growth factors, inducing dendritic sprouting, and stimulating neurogenesis. It also induces high-level expression of immediate early genes (IEGs) that have been implicated in the pathogenesis of schizophrenia, such as early growth response 3 (*Egr3*) and activity-regulated cytoskeleton-associated protein (*Arc*), a validated downstream target of *Egr3* [1–3]. However, the effect of isoflurane anesthesia preceding ECS on IEG response in mice has not been well characterized. This article provides immunofluorescent data of the activity responsive

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IEG ARC in the dorsal and ventral dentate gyrus of wildtype (WT) mice following ECS with or without anesthesia, as well as following sham ECS. The data in this article relate to a published article that employed serial ECS in mice to investigate the requirement of *Egr3* in the neurobiological effects of this model of ECT [4]. The ability to study the effects of serial ECS has been limited in mice due to high rates of mortality during seizure. Administration of isoflurane anesthesia prior to ECS significantly reduces rodent mortality, irrespective of the number of times ECS is applied [5]. Since general anesthesia is administered to patients prior to ECT, use of isoflurane prior to ECS also more closely models the clinical use of ECT [6].

Specifications Table

Subject	Neuroscience
Specific subject area	Neurophysiology, Biological Psychiatry
Type of data	Image Graph Figure
How the data were acquired	ARC immunofluorescent images were obtained using a 10x objective with Zeiss AXIOImager M2 epifluorescence microscope in DsRed channel (300ms).
Data format	Raw Analyzed
Description of data collection	A single ECS, versus sham, was administered to anesthetized and non-anesthetized male, WT mice. One hr. after ECS, mice were sacrificed and perfused. Sectioned brain tissue was immunofluorescently labeled with an antibody against the IEG ARC.
Data source location	Department of Basic Medical Sciences, University of Arizona, College of Medicine – Phoenix, USA, and Department of Psychology, Wilfrid Laurier University, Waterloo, ON, N2L 3C5, Canada
Data accessibility	Mendeley Data: https://data.mendeley.com/datasets/98wt47r6jt/1 DOI: 10.17632/98wt47r6jt.2")
Related research article	This data article supports the following original research article: K.T. Meyers, C.C. Damphousse, A.B. Ozols, J.M. Campbell, J.M. Newbern, C. Hu, D.F. Marrone, A.L. Gallitano, Serial electroconvulsive seizure alters dendritic complexity and promotes cellular proliferation in the mouse dentate gyrus; a role for Egr3, Brain Stimul. 16 (2023) 889-900. https://doi.org/10.1016/j.brs.2023.04.022

1. Value of the Data

- These data are useful in understanding the effects of isoflurane anesthesia on the expression of ARC, an activity-dependent IEG, in the hippocampal dentate gyrus following ECS.
- These data can be used to enhance understanding of isoflurane's impact on mouse neural activity as they demonstrate that it does not disrupt the neuronal activity, essential to the ECS procedure.
- Researchers employing ECS in mice may utilize this data when developing experiments that seek to increase the understanding of IEGs.

2. Objective

Electroconvulsive seizure is a powerful model for understanding how the brain responds to electroconvulsive therapy (ECT), one of the most effective treatments for severe mood and psychotic disorders. In humans, this treatment is delivered following anesthesia. The ability to conduct ECS in mice has been limited by the high lethality of the procedure in this species. The objective of this study was to determine whether anesthesia altered brain activity induced by ECS in mice.

3. Data Description

Fig. 1 shows immunofluorescent staining of the immediate early gene, ARC, in the (1A) dorsal and (1B) ventral hippocampus of WT mice following sham ECS and 1 h following a single ECS with, or without, isoflurane anesthesia. Little ARC is detectable in sham-treated mice. ARC expression is evident in the dentate gyrus of the dorsal and ventral hippocampus of both groups of mice that received ECS. Quantification of ARC immunofluorescence in the dentate gyrus of the dorsal hippocampus (1C) and ventral hippocampus (1D) show no significant difference in ARC induction by ECS between mice that were anesthetized with isoflurane and those that were not.

4. Experimental Design, Materials, and Methods

4.1. Mice

Mice were group housed in our AAALAC approved facility on a 14:10 hour light:dark cycle with *ad libitum* access to food and water. For ARC immunofluorescence studies, male C57Bl/6J mice, ages (1.5-2.5 mo.) were obtained from The Jackson Laboratory. Animals were acclimated to the facility for two weeks before experiments.

4.2. Electroconvulsive stimulation (ECS)

Following administration of local anesthetic eye drops, ECS was administered via ocular electrodes using a Ugo Basile instrument (Varese, Italy). Settings for ECS were: pulse frequency 260 Hz, pulse width 0.3 ms, duration 100 ms, and current 80 mA. For ARC immunofluorescent studies, half the cohort received a single ECS without the use of inhalational anesthesia while the other half received a single ECS following isoflurane (5%) anesthesia. For mice receiving isoflurane anesthesia, ECS was administered before mice fully recovered consciousness (2 mins, 30s). Under these conditions, following administration of electrical shock, anesthetized animals displayed behaviors such as lip smacking, arching, stiffening, and running but did not display tonic-clonic movements following delivery of current. Unanesthetized mice exhibited tonic-clonic seizure after current administration. Sham control animals underwent the identical procedure without current administration.

4.3. ARC staining and fluorescence quantification

One hour after administration of ECS, mice were sacrificed via isoflurane overdose and perfused with 1x phosphate buffered saline (PBS) followed by 4% paraformaldehyde (PFA). Brains were post-fixed in PFA for 24 h and then transferred to 30% sucrose at 4°C. Following sucrose

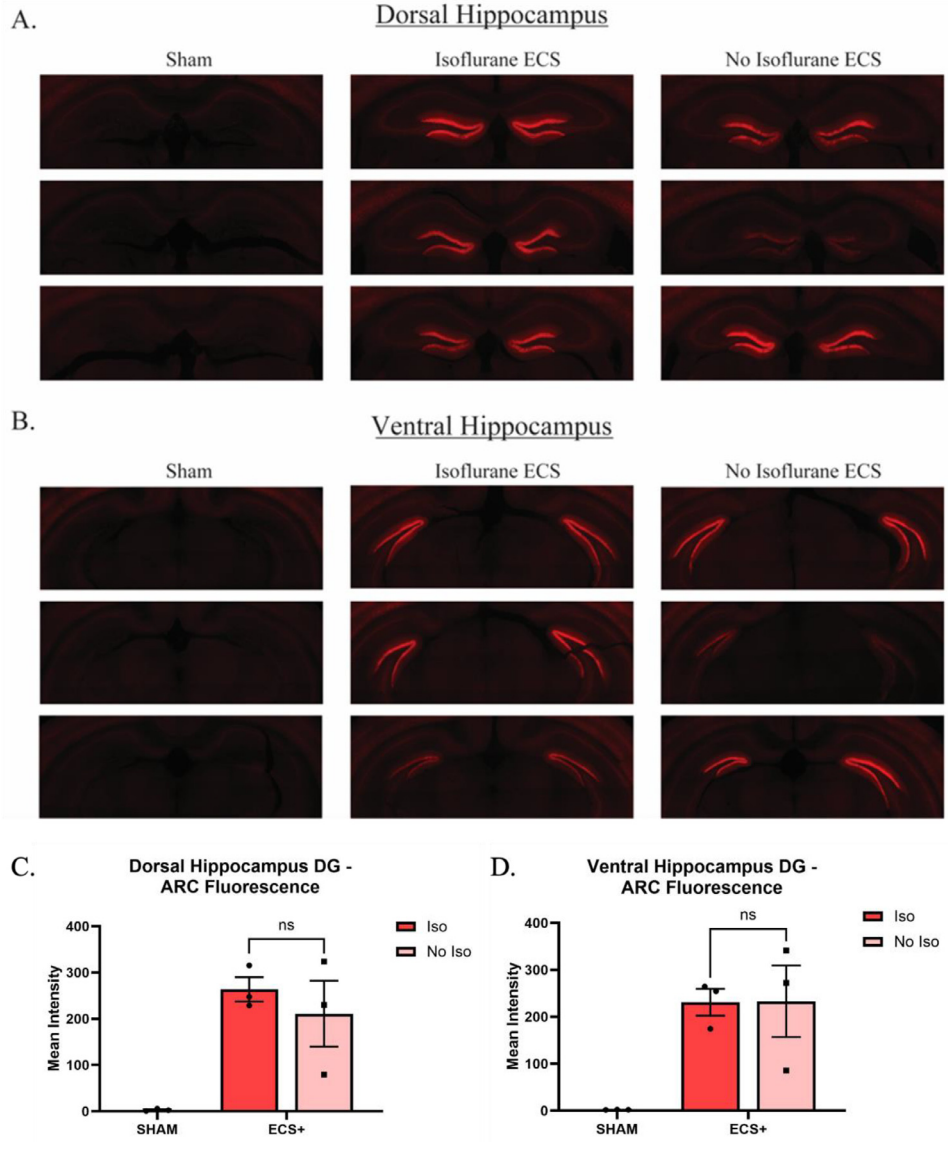

Fig. 1. ARC expression in the hippocampus of mice that were anesthetized with isoflurane followed by sham ECS (Left column), mice anesthetized with isoflurane followed by ECS (Middle column), or mice that did not receive isoflurane prior to ECS (Right column) in the dorsal (A) and ventral (B) hippocampus of WT mice ($n = 3$/group). Images taken on 10X objective with Zeiss AXIOImager M2 epifluorescence microscope in DsRed channel (300ms). Quantification of ARC Fluorescence in sham vs. ECS treated mice in the dorsal hippocampus (C) and ventral hippocampus (D). Fluorescence level following ECS did not differ between mice that received isoflurane pretreatment and those that did not (student's *t*-test $p = 0.53$ (C) $p = 0.98$ (D)). Bars represent mean +/- standard error of the mean; $n = 3$ mice /group.

saturation, brains were sectioned at 40μm on a sliding microtome (American Optical). Free-floating sections were washed in 1x tris-buffered saline (TBS), then blocked in 4% normal donkey serum, diluted in 1x TBS with 0.4% Triton-X 100, overnight at 4°C with constant agitation. Primary antibody, 1:1000 rabbit anti-ARC (Synaptic Systems, Cat. # 156 003), was applied to tissue sections and incubated overnight at 4°C with constant agitation. Following overnight incubation, sections were washed in 1x TBS and then incubated in secondary antibody (1:1000 donkey anti-rabbit, Alexa 568, Invitrogen) overnight at 4°C. Tissue sections were washed in 1x TBS and mounted with Vectashield containing DAPI (Vector Labs).

Images were taken on the 10X objective of a Zeiss AXIOImager M2 epifluorescence microscope in the DsRed channel (300 ms). Images were imported into ImageJ and split into separate color channels. DAPI images were used to draw regions of interest (ROI) around the dentate gyrus (DG) and a region for background fluorescence. The ROIs were superimposed onto the ARC-stained image of the same section to quantify mean intensity of the DG and background fluorescence. Measures of mean intensity in the DG ROI minus the background were calculated individually for the right and left hemispheres. The right and left hemisphere values were then summed. These summed values were entered into GraphPad for analysis and graphing.

4.4. Statistical analysis

Quantification of ARC immunofluorescence was compared between no isoflurane ECS and isoflurane ECS groups by two-tailed student's *t*-test (Graphpad Prism, version 9.5.0).

Limitations

Studies included only male mice.

Ethics Statements

All procedures were approved by the Institutional Animal Care and Use Committee of the University of Arizona and the guide for the Care and Use of Laboratory Animals and carried out in accordance with the National Institutes of Health guide for the care and use of laboratory animals (NIH Publications No. 8023, revised 1978).

Data Availability

Seizure-induced immediate early gene expression with, vs. without, isoflurane anesthesia (Original data) (Mendeley Data).

CRediT Author Statement

A.B. Ozols: Investigation, Formal analysis, Writing – original draft, Writing – review & editing; **K.T. Meyers:** Investigation, Formal analysis, Writing – review & editing; **C.C. Damphousse:** Investigation, Formal analysis, Writing – review & editing; **J.M. Campbell:** Investigation; **R. Khoshaba:** Formal analysis; **S.G. Wallace:** Formal analysis; **C. Hu:** Formal analysis, Writing – review & editing; **D.F. Marrone:** Conceptualization, Formal analysis, Funding acquisition, Supervision, Writing – review & editing; **A.L. Gallitano:** Conceptualization, Formal analysis, Funding acquisition, Supervision, Writing – review & editing.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.