An affordable solution for investigating zebra finch intracranial electroencephalography (iEEG) signals.

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Abstract:

The Zebra finch is a well-studied animal for studying neural mechanisms of speech, and electrophysiology is the primary technique for understanding the song system in them. Most of the studies on zebra finches have focused on intracerebral recordings. However, these methods are only affordable for limited laboratories. Recently, different open-source hardware for acquiring EEG signals has been developed. It's unclear whether these solutions suit zebra finch studies as they have not been evaluated. Electrocorticography signals can provide a preliminary guide for more in-depth inquiries and also aid in understanding the global behavior of the bird's brain, as opposed to the more common localized approach. We provide a detailed protocol for acquiring iEEG data from zebra finches with an open-source device. We implemented stainless steel electrodes on the brain's surface and recorded the brain signals from two recording sites. To validate our method, we ran two different experiments. In the first experiment, we recorded neural activity under various concentrations of isoflurane and extracted the suppression duration to measure anesthesia depth. In the second experiment, we head-fixed the birds and presented them with different auditory stimuli to evaluate event-related potential (ERP). Results showed a significant increase in the suppression duration by increasing the anesthesia depth and evident ERP response to auditory stimuli. These findings indicate that by our methodology, we can successfully collect iEEG signals from awake and anesthetized birds. These findings pave the way for future studies to use iEEG to investigate bird cognition.

Keywords: Zebra finch, animal EEG, open source, Songbird

Introduction:

The investigation of the avian neural system provides a promising avenue for illuminating the underlying neural mechanisms of speech in humans (1). As an exceptional species that can acquire singing via vocal imitation from parents, humans exhibit noteworthy similarities with birdsongs regarding complex sound production and learning. Thus, songbirds have emerged as effective models for neuroscientific inquiries concerning vocal learning paradigms (2). A range of research methods is used in the extensive investigations of the bird's vocal system, including electrophysiology (3), histology (4), genetics (5,6), and behavioral studies (7). The bulk of current knowledge concerning the avian vocal system has been derived from experimental studies conducted on the zebra finch, a widely accepted avian laboratory model species (8). Invasive neural recording in zebra finches has revealed valuable insights into the song system and mechanisms of vocal learning. The ultimate goal in this line of research is to understand the neural mechanism of creating complicated motor sequences and vocal production and translate these findings to the human brain.

Human brain studies are restricted by a limited capacity for intervention due to ethical and technical considerations. These limitations force human research to use noninvasive methods like EEG. Nevertheless, testing these methods on animals is informative in building a solid verification base for their applications. These signals are clinically relevant to consciousness alterations, seizure activity, anesthesia, and sleep states. As an alternative approach, since animals enable invasive practices, iEEG studies have provided more valuable insight into the nature of acquired signals than EEG due to a higher signal-to-noise ratio (9). Animal iEEG studies also have an extensive history that provides complementary data to human research. Specifically, animal EEG models exploring pharmacological agents' impact on the brain represent a crucial translational inquiry area (10).

EEG recording has been conducted to study sleep and migratory behavior in birds (11). Research on birds' sleep patterns, which are essential from an evolutionary perspective, has also been conducted using iEEG. Furthermore, brain activity related to memory consolidation during the learning period of song in juvenile finches can be detected by this method (12). The research literature on working with birds is full of intracranial recordings, in which we can examine the behavior of a neuron during the production or hearing of a song with a very high accuracy. Despite the importance of animal iEEG, avian iEEG has been studied very limited compared to other animals.

IEEG studies in birds have great translational potential. In this concept, reproducing bird songs from iEEG signals can be a suitable model for the brain-computer interface of speech. The advantage of this method is its ease of implementation, which allows it to be performed before other neuronal recording experiments and during behavioral studies. Consequently, the higher cost and energy of conducting neural recording experiments, which make them less feasible, will be evaded.

With the increasing demand for neural data, scientists seek more accessible ways to democratize scientific research (13). To this end, many entities produce tools, programs, and open-source data sets. Given the importance of EEG studies, open-source hardware and software solutions are being introduced in this field. OpenBCI offers an affordable and open-source system designed to capture human EEG signals. However, its use in animal studies is minimal, and to our knowledge, it has not been applied in animal research. In this study, we propose a method for acquiring brain signals via electrodes placed on the cortex using an OpenBCI device.

We conducted two experiments to test the efficacy of our method. In the first experiment, we recorded signals under different concentrations of isoflurane. The suppression duration was used as a proxy for anesthesia depth. After the recovery and adaptation period, we immobilized the awake animals with a homemade fixation method and presented them with different auditory stimuli. We then extracted evoked responses from the raw signals. Our study highlights the usefulness of the open BCI in evaluating the EEG activity of a bird's brain, particularly the zebra finch, for investigating auditory responses.

Methods:

A detailed explanation of the procedure has been provided on protocol.io.

Animal subjects:

We used five male zebra finches (> 60 days old) for experiment one and 2 of them for experiment two. All animals were obtained from the IPM bird garden aviary and kept in a local animal house after surgery. All procedures were done under the guidelines of the Ethics Committee of the Institute for Research in Fundamental Sciences (IPM).

Electrodes and head-post implantation:

Animals were kept under restricted food and water conditions before surgery. Anesthesia was induced using 2% isoflurane, and the animal was secured into the stereotaxic device after induction. We used our designed 3D-printed anesthesia mask compatible with the stereotaxic device to sustain gas delivery to the head-fixed bird. The head feathers were removed, and the incision site was prepared for surgery. After exposing the skull and locating the bifurcation, the coordinates for craniotomy sites were located concerning the bifurcation site. Craniotomy sites were drilled, and after placing the electrodes, antibiotic ointment was applied to the exposed area. The skull was then prepared for cement adhesion, and after applying the cement to the area, the base of the head post was inserted into the forming cement, and then more cement was added to the base of the head post. The cement was allowed to be cured completely before proceeding. Detailed instructions can be found in the protocol section.

Anesthetized recording:

We collected iEEG data from two recording electrodes implanted beneath the skull of the zebra finches during the different depths of anesthesia with isoflurane. After the surgery and electrode implantation, the OpenBCI recording was set up, and the signals were recorded with a 250 Hz sampling rate for about

2 minutes in each of 2.5%, 2%, 1.5%, 1%, 0.6%, and 0.4% of isoflurane. Before analysis, the data were preprocessed to ensure data quality and reliability. Standard preprocessing steps were employed, including a high-pass filter at 1 Hz, a low-pass filter at 80 Hz, and a notch filter at 50 Hz to remove noise, and the signal was re-referenced during recording by placing electrodes on the cerebellum. After that, the data was extracted based on the different levels of isoflurane for further analysis in burst detections.

Awake recording:

Three types of 700 ms audio stimuli were presented with a random period of silence between 2500 ms and 3500 ms as an inter-trial interval. The experiment included two blocks of 300 trials of all audio types that were randomly played for awake finches. The audio types consisted of a 3000 Hz tone, white noise, and a conspecific song. A highly synchronized trigger signal was required during the recording to ensure the signal was annotated with each trial. The audio was played, and the trigger was outputted to the OpenBCI device using a Raspberry Pi 3. An optocoupler circuit was utilized to isolate the OpenBCI device from the Raspberry Pi board to prevent noise induction due to the trigger signal. The task was programmed in C++ and optimized to achieve 1 ms latency to meet the required accuracy.

Dependent variables:

Suppression duration:

Bursts are characterized by short episodes of partial reactivity or temporary spikes in neural firing, followed by a significant decrease or suppression of neural activity. We measured suppression duration in zebra finches under varying levels of isoflurane anesthesia. Suppression duration is a crucial metric for evaluating brain activity and detecting instances of burst suppression, which may indicate fluctuations in brain function or anesthesia-induced alterations in neural activity. Our objective was to explore the neural dynamics and anesthesia effects on zebra finches while administering different levels of

isoflurane. We calculated the suppression duration using a well-established methodology, as explained in previous studies conducted by Westover et al. (14). To perform further statistical analyses, we sorted the dataset based on the isoflurane concentration into three distinct categories: low, medium, and high levels of anesthesia. Specifically, isoflurane below one percent was classified as low-level anesthesia, those above two indicated a high level, and values falling between 1 and 2 were classified as medium levels of anesthesia. Mann-Whitney U test as a nonparametric test for independent samples was utilized to compare experimental conditions over suppression duration.

Results:

Anesthetized recording:

Our analysis revealed distinct changes in suppression duration across different stages of isoflurane anesthesia. As isoflurane was administered, we observed a gradual increase in the suppression duration, indicating an increase in burst suppression events (Fig 2 C, D, E) and reduced brain activity, as evidenced by computed fast Fourier transforms in each of low, medium and high anesthesia for both anterior and posterior electrodes (Fig 2 A, B). Additionally, we statistically compared the suppression durations across ten blocks of 120-second recordings at low, medium, and high anesthesia levels. The comparison of each pair of conditions was statistically significant for both anterior and posterior electrodes. Specifically, in the anterior region, comparisons between low and medium anesthesia yielded a p-value of 0.035 (Figure 2 F), between medium and high anesthesia a p-value of 0.0376 (Figure 2 G), and between low and high anesthesia a p-value of 0.014 (Figure 2 H), demonstrating significant differences in suppression duration.

Furthermore, in the posterior region, comparisons between low and medium anesthesia resulted in a p-value of 0.0073 (Figure 2 I), between medium and high anesthesia a p-value of 0.0173 (Figure 2 J), and

between low and high anesthesia a p-value of 0.0006 (Figure 2 K), highlighting significant disparities in suppression duration.

Awake recording:

ERP results from awake recordings indicated distinct responses to each of the three presented stimuli: a 3000 Hz tone, white noise, and conspecific song, as illustrated in Figure 3. Finches exhibited varying responses to the task conditions, with noticeable differences in the ERP shape within each recording site. As the plots illustrate, differences between posterior and anterior sites are evident. In the anterior electrode, a sharp negative spike around 400 ms after the stimulus onset can be seen in all stimulus types (Figure 3 D, E, F) . This negative component in the anterior electrode is even larger for the 300 Hz tone, followed by another spike at 1000 ms after the onset of the trials (Figure 3 D). This distinct difference in ERP for the 3000 Hz tone can validate the reliability of the recording and raises the question of why this event only appeared in this specific auditory stimulus. Furthermore, ERPs exhibit entirely different shapes in the posterior electrode in response to the three presented stimulus types (Figure 3 A, B, C). Despite the large negative component in response to the auditory stimuli in the anterior site, we can see a positive spike around 300 ms after the stimulus onset. Similar to what we observed in the anterior electrode, the 3000 Hz tone has a distinct shape in the posterior recorded sites. Specifically, a negative response follows immediately rather than a positive response around 300 ms after the stimulus (Figure 3 A). Moreover, after 1000 ms, another positive response appears only in response to the 3000 Hz tone in the posterior site. Notably, compared with all the auditory stimuli, responses to the songbird have lower amplitude.

Discussion:

Songbirds are the primary animal models for studying vocal perception production and learning (15). In the past decades, zebra finch has been utilized as a significant model in these studies, and spike recording was the primary method in these experiments. However, the techniques used to track the activity of a single neuron are expensive (16) and complicated and provide a narrow field of view to capture the brain's overall activity (17). Recently, there has been a growing interest in studying the cognitive processes in birds as they show fascinating abilities in doing high-level mental functions (18). These functions are processed in a distributed manner in the brain, and to study them, we need to have a larger field of view. Our experiment tries to address the problems mentioned above. In summary, we attempt to solve two issues as a newly established lab. first, to reduce the cost of the avian studies by using open-source solutions, and second, to capture the brain’s dynamic state during mental activity by recording intracranial EEG.

This paper presents a detailed protocol for recording iEEG from an anesthetized and awake zebra finch. Previous experiments on birds' EEG under isoflurane anesthesia showed similar signal patterns to those of mammals (19). This pattern is entirely detectable by visually inspecting a bird's EEG signal under surgical anesthesia, characterized by brief periods of burst activity and silent suppressed intervals between them (20). We used suppression duration as an indicator of the anesthesia's depth in our experiment to test the efficiency of our method. The results show that this index is significantly higher in the high concentrations of gas than the low concentrations, indicating that our method successfully captures the brain's dynamic. Although we chose this experiment to show the validity of our method as Anesthesia produces a characteristic feature of brain dynamics that has been previously observed, there are some scientifically valuable facts in showing this phenomenon. The emergence of burst suppression in this species under anesthesia shows an evolutionarily conserved arousal mechanism. These findings can open future studies to understand a shared mechanism behind producing a conscious state in the brain. Comparative Studies related to conscious states in other animals can create insight into the serious questions about the evolutionary aspect of consciousness (21), and studies on birds' cognition regarding their high cognitive ability are in this category.

Our ultimate aim in developing this method is to use it in studies related to the cognitive process in birds, particularly those connected to vocal perception and production. We conducted a second experiment with a head-fixed animal exposed to different auditory stimuli. Our results show presenting these stimuli changes the electric activity in the iEEG signal (Fig 3). Event-related potentials (ERPs) were plotted in response to three different auditory stimuli: a 3000 Hz tone, white noise, and songbirds for both anterior and posterior regions. We observed a clear response to all auditory stimuli at both sites. The distinct shapes of ERP components across stimuli and brain regions demonstrate the applicability of our method. This method can become more robust and worthy of investigation by conducting further experiments in these areas using more complex designs, such as incorporating various auditory or other sensory stimuli and employing high-level analyses like examining connectivity between regions. By validating the existence of responses through this straightforward and affordable protocol, a wide range of ideas can be explored more easily and quickly.

This finding is valuable since the literature on bird EEG is slim despite the growing number of researchers in avian cognition and the demand for physiological measurements during distributed brain functions. Our method offers a way to record chronic data from animals, as they can carry implanted materials for their lives. This recording approach is helpful when we want to capture the changes in brain function during aging and maturation (e.g., changes in vocal perception during the transition from the juvenile stage to adulthood) (22). Given that the main noninvasive current method in BCI uses EEG signals to control effectors in humans, we think the zebra finch iEEG can be an appropriate model for the vocal prosthesis. Previous studies that have utilized the biomechanical model of the birds' vocal organs and fed them by neural signals gained tremendous success (23). Future studies can improve this method fed those models and create a strong basic model for vocal prosthesis controlled by EEG signals.

Finally, The wireless communication protocol employed by OpenBCI presents an exciting opportunity for developing small-scale devices capable of chronic recording in freely behaving animals, especially in flying birds. Thanks to its open-source nature, research groups can modify these devices to improve their reliability for animal studies (24). While OpenBCI has proven useful in human studies (24), its application in animal studies has been limited until now. This study paves the way for future research endeavors to leverage this technology to collect a broader range of signals, including LFP and spikes. Birds are increasingly used in neuroscience and cognitive studies; however, the EEG signal has not been used extensively in this line of research. Our findings can trigger future studies to use EEG in bird cognitive studies.

References:

1. Zhang Y, Zhou L, Wang S, Meng W. Analogies of human speech and bird song: From vocal learning behavior to its neural basis. Front Psychol [Internet]. 2023 Feb 22 [cited 2024 Mar 14];14. Available from: https://www.frontiersin.org/journals/psychology/articles/10.3389/fpsyg.2023.1100969/full

2. Doupe AJ, Kuhl PK. Birdsong and human speech: common themes and mechanisms. Annu Rev Neurosci. 1999;22:567–631.

3. Lewicki MS. Intracellular Characterization of Song-Specific Neurons in the Zebra Finch Auditory Forebrain. J Neurosci. 1996 Sep 15;16(18):5854–63.

4. Farries MA, Perkel DJ. A telencephalic nucleus essential for song learning contains neurons with physiological characteristics of both striatum and globus pallidus. J Neurosci. 2002 May 1;22(9):3776–87.

5. Gordon RL, Ravignani A, Hyland Bruno J, Robinson CM, Scartozzi A, Embalabala R, et al. Linking the genomic signatures of human beat synchronization and learned song in birds. Philos Trans R Soc Lond B Biol Sci. 2021 Oct 11;376(1835):20200329.

270 6. Xiao L, Merullo DP, Koch TMI, Cao M, Co M, Kulkarni A, et al. Expression of FoxP2 in the basal
271 ganglia regulates vocal motor sequences in the adult songbird. Nat Commun. 2021 May 11;12(1):2617.

272 7. Brazas ML, Shimizu T. Significance of visual cues in choice behavior in the female zebra finch
273 (Taeniopygia guttata castanotis). Anim Cogn. 2002 Jun;5(2):91–5.

274 8. Mello CV. The Zebra Finch, Taeniopygia guttata: An Avian Model for Investigating the
275 Neurobiological Basis of Vocal Learning. Cold Spring Harb Protoc. 2014 Oct 23;2014(12):1237–42.

276 9. Parvizi J, Kastner S. Human Intracranial EEG: Promises and Limitations. Nat Neurosci. 2018
277 Apr;21(4):474–83.

278 10. Drinkenburg WHIM, Ruigt GSF, Ahnaou A. Pharmaco-EEG Studies in Animals: An Overview of
279 Contemporary Translational Applications. Neuropsychobiology. 2015;72(3–4):151–64.

280 11. Rattenborg NC, Voirin B, Cruz SM, Tisdale R, Dell'Omo G, Lipp HP, et al. Evidence that birds sleep
281 in mid-flight. Nat Commun. 2016 Aug 3;7(1):12468.

282 12. Shank SS, Margoliash D. Sleep and sensorimotor integration during early vocal learning in a
283 songbird. Nature. 2009 Mar 5;458(7234):73–7.

284 13. White SR, Amarante LM, Kravitz AV, Laubach M. The Future Is Open: Open-Source Tools for
285 Behavioral Neuroscience Research. eNeuro. 2019 Aug 9;6(4):ENEURO.0223-19.2019.

286 14. Brandon Westover M, Shafi MM, Ching S, Chemali JJ, Purdon PL, Cash SS, et al. Real-time
287 segmentation of burst suppression patterns in critical care EEG monitoring. J Neurosci Methods. 2013
288 Sep 30;219(1):131–41.

15. Sakata JT, Woolley SC, Fay RR, Popper AN, editors. The Neuroethology of Birdsong [Internet]. Cham: Springer International Publishing; 2020 [cited 2024 May 19]. (Springer Handbook of Auditory Research; vol. 71). Available from: http://link.springer.com/10.1007/978-3-030-34683-6

16. Cheng N, Murari K. OSERR: an open-source standalone electrophysiology recording system for rodents. Sci Rep. 2020 Oct 12;10:16996.

17. Sejnowski TJ, Churchland PS, Movshon JA. Putting big data to good use in neuroscience. Nat Neurosci. 2014 Nov;17(11):1440–1.

18. Emery NJ. Cognitive ornithology: the evolution of avian intelligence. Philos Trans R Soc Lond B Biol Sci. 2006 Jan 29;361(1465):23–43.

19. McIlhone AE, Beausoleil NJ, Kells NJ, Johnson CB, Mellor DJ. Effects of halothane on the electroencephalogram of the chicken. Vet Med Sci. 2018 Jan 12;4(2):98–105.

20. Effects of barbiturates and ether on spontaneous electrical activity of dog brain - PubMed [Internet]. [cited 2024 Mar 10]. Available from: https://pubmed.ncbi.nlm.nih.gov/18114367/

21. Irwin LN, Chittka L, Jablonka E, Mallatt J. Editorial: Comparative animal consciousness. Front Syst Neurosci [Internet]. 2022 Oct 19 [cited 2024 Mar 11];16. Available from: https://www.frontiersin.org/articles/10.3389/fnsys.2022.998421

22. Funabiki Y, Konishi M. Long Memory in Song Learning by Zebra Finches. J Neurosci. 2003 Jul 30;23(17):6928–35.

23. Arneodo EM, Chen S, Brown DE, Gilja V, Gentner TQ. Neurally driven synthesis of learned, complex vocalizations. Curr Biol. 2021 Aug 9;31(15):3419-3425.e5.

Figure captions:

Figure 1: Graphical abstract of two experiments. In experiment one, we used different concentrations of isoflurane to produce different depths of anesthesia. The finch was head-fixed in experiment two, and iEEG data were recorded from implanted electrodes.

Figure 2: FFT analysis of isoflurane levels, suppression duration comparison over conditions, and a sample of different isoflurane level oscillation A,B) Shows FFT analysis under low, medium, and high levels of isoflurane anesthesia. The plot illustrates a decrease in power across different frequency ranges as isoflurane levels increase, observed at both posterior (A) and Frontal (B) sites. C) 20-second sample of oscillation under low isoflurane anesthesia, showing a higher amplitude frequency range. D) 20-second sample under medium isoflurane anesthesia. Visual comparison with plot C indicates a decrease in frequency power and initiation of burst suppression with higher isoflurane levels. E) Illustration of brain signals under high levels of isoflurane anesthesia, displaying burst suppression pattern and reduced brain activity as observed in FFT plots (A,B) . F) Comparison of suppression duration between low and medium levels of isoflurane for the posterior site . G) Suppression duration comparison between medium and high levels of isoflurane for the posterior site . H) Similar comparison as in F, but between low and high levels of isoflurane for the posterior site. I) Comparison of suppression duration between low and medium levels of isoflurane for the fontal site. J) Similar comparison as in I, but between medium and high levels of isoflurane. K) Comparison of suppression duration between low and high levels of isoflurane for frontal site

Figure 3: ERP of a 3000 Hz tone, white noise tones, and songbird stimuli for both posterior and frontal electrodes. A and B depict the ERP of 3000 Hz tones for frontal and posterior electrodes, respectively. These two brain sites exhibit distinct responses to 3000 Hz tones, with a secondary late response occurring approximately one second after stimulus presentation. C and D represent the responses to white noise tones for frontal and posterior sites, respectively. Similar to the 3000 Hz tone ERPs, these

two plots show distinct responses to the stimuli. However, the late response observed in the 3000 Hz tone ERP does not appear in the white noise ERP. E and F display the ERP plots for the songbird stimuli recorded from the frontal (E) and posterior (F) electrodes. As depicted, the songbird stimuli elicit different effects on each recorded site.

Figure 1

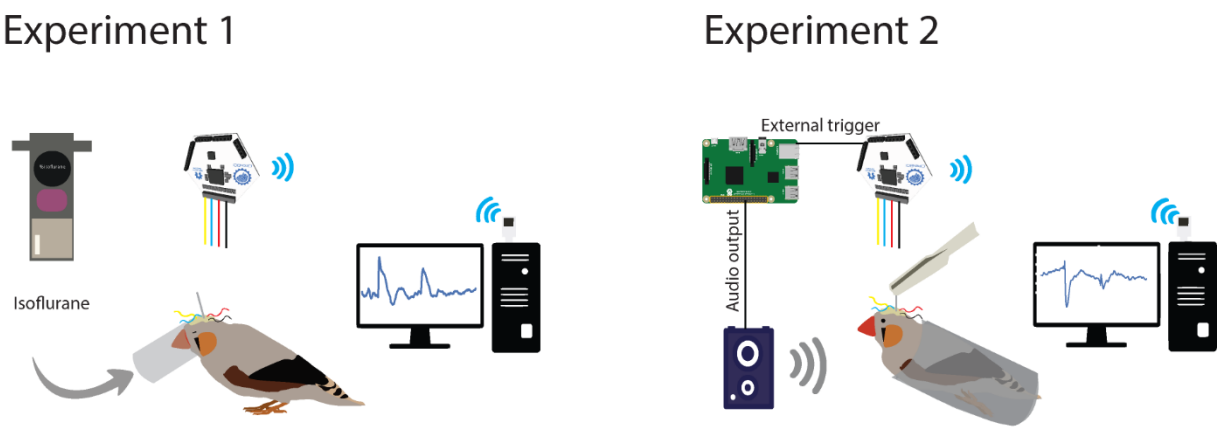

Figure 2

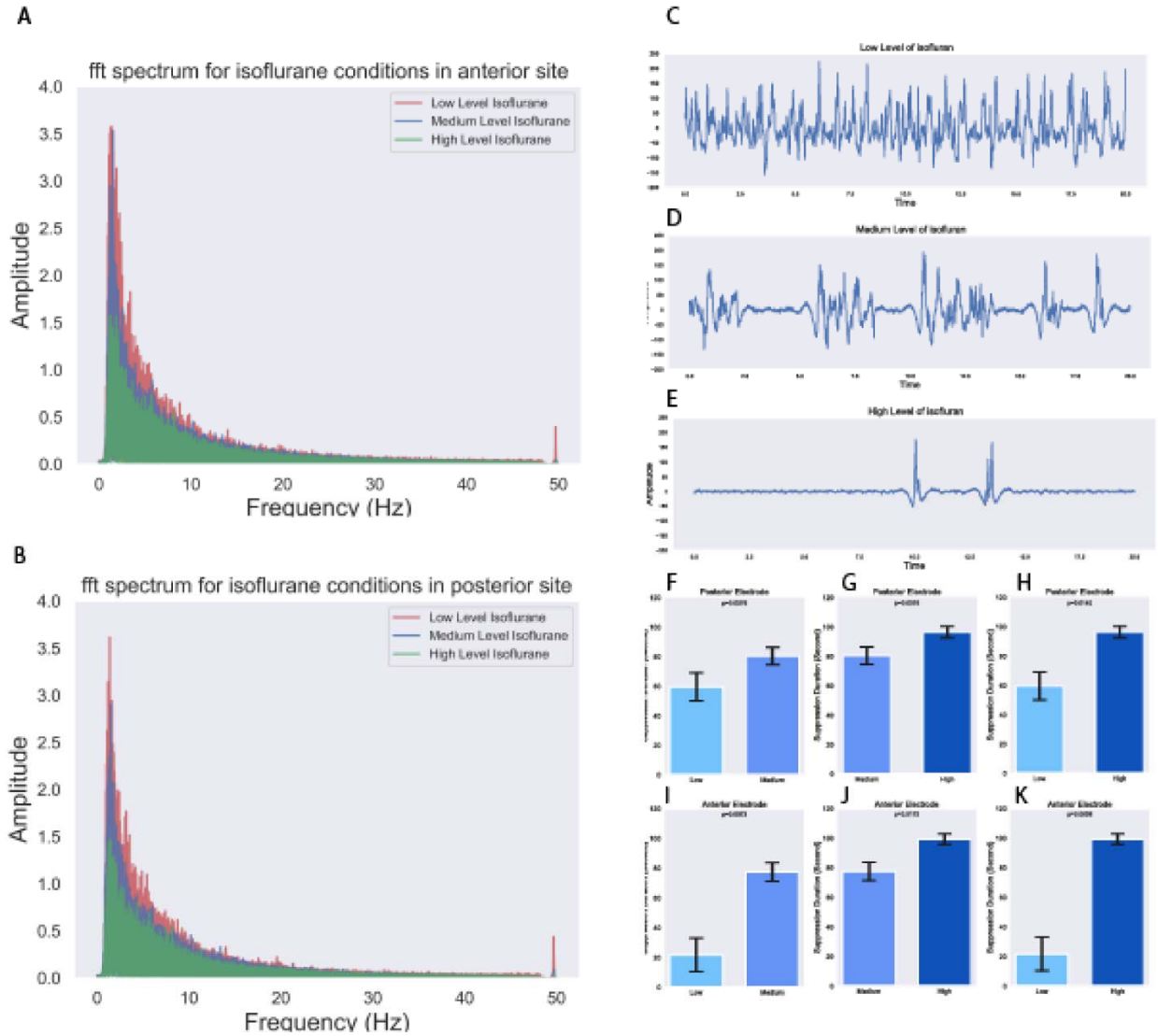
A
fft spectrum for isoflurane conditions in anterior site
Low Level Isoflurane
Medium Level Isoflurane
High Level Isoflurane
Amplitude
Frequency (Hz)
B
fft spectrum for isoflurane conditions in posterior site
Low Level Isoflurane
Medium Level Isoflurane
High Level Isoflurane
Amplitude
Frequency (Hz)
C
Low Level of Isoflurane
D
Medium Level of Isoflurane
E
High Level of Isoflurane
F
G
H
I
J
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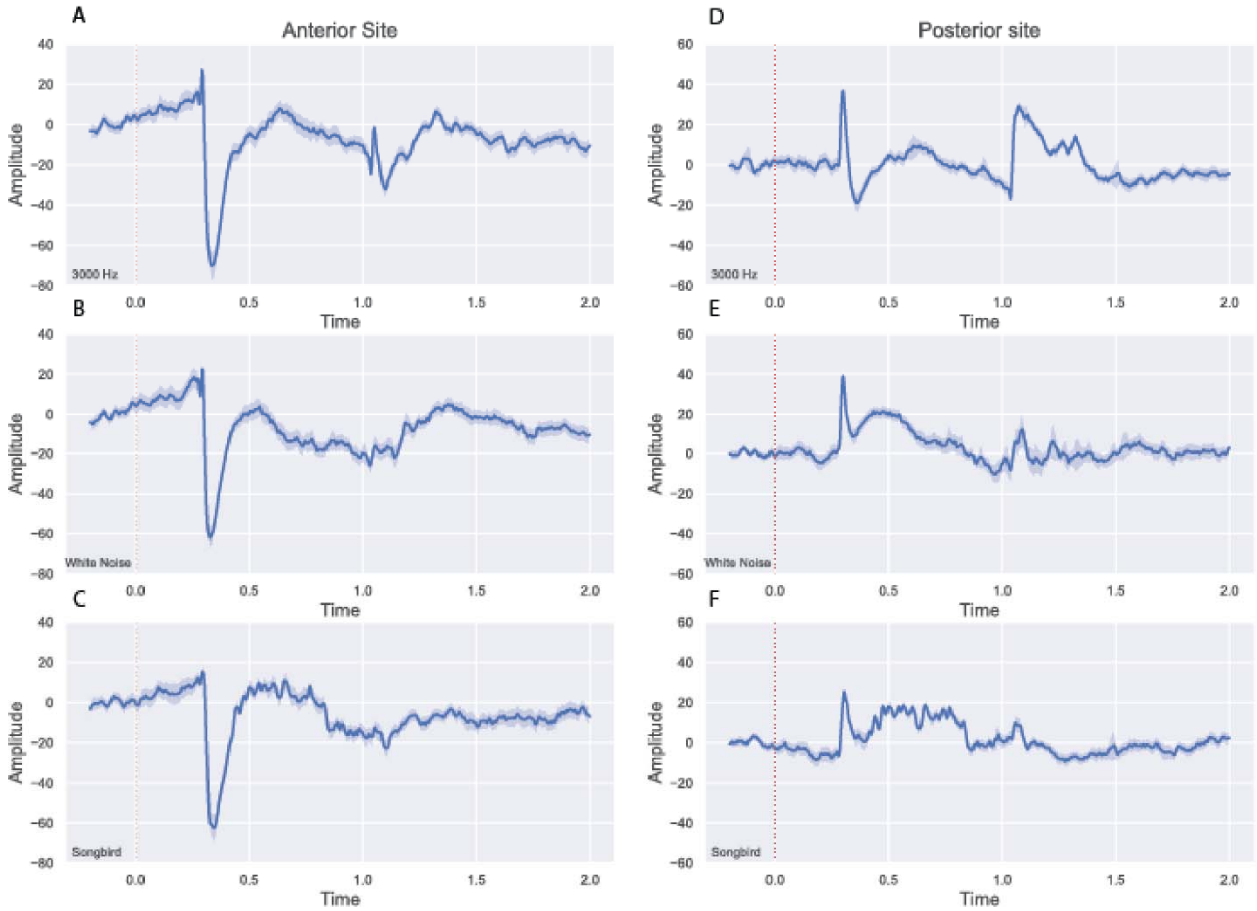
Figure 3


A
Anterior Site
D
Posterior site
B
E
C
F
Amplitude
Time
3000 Hz
White Noise
Songbird

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