ECT Beyond Psychological Disorders: The Anti-Nociceptive Effect of High-Intensity Electrical Brain Stimulation in the Formalin-Pain Model

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Introduction

Electroconvulsive therapy (**ECT**) has been utilized for several decades, particularly in major depressive and psychologically impaired patients. To test if ECT is effective in relieving pain, we utilize local field potential (**LFP**) to show the changes in power in four regions of the rat brain from intracranially implanted electrodes: anterior cingulate cortex (**ACC**), bilateral central amygdala (**CeA**), and the ventral tegmental area (**VTA**). The LFP was recorded in three separate formalin-induced nociceptive conditions: formalin-only (control), ECT post-formalin, and ECT pre-formalin. The multi-ECT shock consisted of three parameters of 50pulse/s, 0.7ms, 2s at 5mA, 20mA, and 50mA delivered three times, 10-15s apart. Power spectrum analysis revealed a mixed effect: ECT-induced inhibition, excitation, or no change. Additionally, formalin behavioral testing was conducted in a freely-moving rats. In the two conditions of formalin-ECT and ECT-formalin, under brief 2% isoflurane, 3% formalin was administered and the parameters of the three ECT stimulations were delivered at 50 pulses/s, 0.7ms, 50mA for 2-seconds, 10-15s apart. Results revealed a significant decrease in pain-score when ECT is administered, specifically between the 30-55min post-formalin mark, without a difference in the sequencing of formalin-ECT or ECT-formalin. Our LFP and behavioral results strikingly demonstrate the analgesic effect that ECT may evoke.

Methods

Behavioral testing:

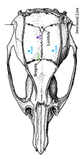
In 12 male Sprague Dawley freely-moving rats (*N* = 12) ranging between 333 and 364g, behavioral testing was conducted. In the control condition (*n* = 6), 50 µl of 3% formalin was injected into the left hind paw. The rat was then immediately transferred to a large clear box to observe specific pain-related behaviors such as "paw down," "paw up," and "paw licking." In one experimental condition (*n* = 3), 50 µl of 3% formalin was injected into the left hind paw and then given three ECT stimulations at 50 pulses/s, 0.7ms, 50mA for 2-seconds, each stimulation given 10-15s apart. During the procedure, the animal was kept under brief 2% isoflurane inhaled anesthesia. In the next experimental condition (*n* = 3), the parameters remained the same, only this time, the set of three ECT stimulations were administered first, immediately followed formalin. Thereafter, in both conditions, the rat was immediately transferred to the clear box to begin behavioral observation. Concluding data analysis, there were no significant differences between the two experimental conditions, so they were merged to the "ECT + Formalin" group (Figure 1).

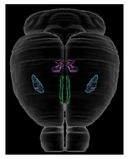
Animals:

32 male Sprague Dawley rats (*N* = 32) with a ranging weight between 318 and 444g were used in this study. The appropriate food and water were available to the animals, and housing consisted of cages in a 12/12h light/dark cycle. The Institutional Animal Care and Use Committees (IACUC) of the University of Texas at Arlington authorized all procedures.

Electrode implantation:

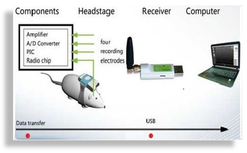
The rat was placed on the stereotaxic frame under 3% isoflurane inhaled anesthesia. Four 0.010-inch electrodes were separately implanted into four regions of brain: right ACC at 0 mm posterior to bregma, 0.70 mm lateral to the right, 3.20 mm deep; right and left central amygdala at 2.04 mm posterior to bregma, 4.00 mm lateral to the right and left, 8.00 mm deep; and right VTA at 4.80 mm posterior to bregma, 0.90 mm lateral to the right, 8.35 mm deep (Paxinos & Watson, 1998). One screw was placed on the upper left region of the skull and another screw on the upper right region of the skull, connecting to a cable as ground and reference. To stabilize the four electrodes and screws onto the skull, dental cement was then used.





Module setup and LFP recording:

The four electrodes and screw cable were connected to a wireless module (designed by SiChuan NeoSource BioTektronics Limited (*http://www.neoscbio.com*)) to receive the LFP signal from the brain. A USB dongle paired with the module was inserted into the computer to transmit the signal from the module to the recording software.



Formalin model induction:

All rats had a 10-minute baseline LFP recording prior to any procedure. In the control condition (*n* = 10), after baseline, 50 µl 3% formalin was injected to the left hind paw of the rat. Continuously, the LFP recording continued for an additional 60 minutes (***group A***).

ECT model induction:

ECT unit (57800 by Ugo Basile, Italy) was utilized for the stimulation. The LFP signal was recorded for a baseline of 10 minutes. In the first experimental condition (*n* = 12), following the baseline recording, a 50 µl 3% formalin injection was administered followed by a 20-minute LFP recording. Subsequently, the first stimulation parameter was delivered three times at 50 pulses/s, 0.7ms, 5mA for 2-seconds, each stimulation given 10-15s apart. Following the stimulation, LFP was recorded for 10-minutes. This sequence was repeated two more times with the same parameters, although the mA increased to 20mA and then 50mA for the last set of stimulations (***group B***). In the second experimental condition (*n* = 10), the 10-minute LFP baseline recording remained the same. This time, the stimulations were administered immediately after baseline recording. The ECT parameters for the stimulations remained the same as they did for the first experimental condition. 10-minutes after the completion of the stimulations, the formalin injection was administered. The LFP recording continued for 60-minutes (*group C*).

Data analysis:

The raw data of LFP recorded from the module was processed by power spectrum analysis with the custom program of MATLAB. The power was calculated in MATLAB every ten-seconds, we then averaged the power intensity depending on the duration. Finally, the power of each frequency band was normalized by the average power of the baseline. Next, the raw data was imported into Spike2 to analyze the data in power spectrogram and waveform graphs. A mixed-design analysis of variance (ANOVA) was utilized in SPSS to determine statistical significance.

Electrode verification:

To verify that electrode placement was inserted into the correct corresponding brain regions, a Nissl histological staining was conducted post-mortem. Following electrode verification, electrodes not within range were excluded from this study.

ECT significantly suppresses formalin behavioral response

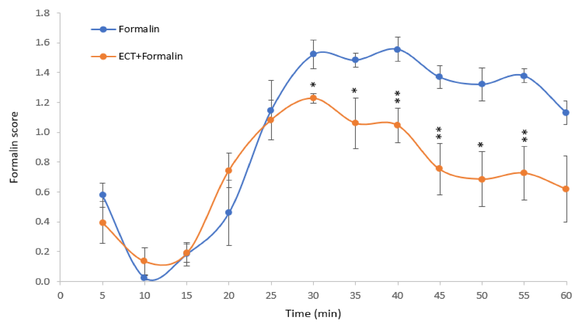


Figure 1. Behavioral testing in the formalin-only group (*n* = 6) versus ECT combined with formalin group (*n* = 6) during each 5-minute test interval. The pain score trend reveals a significant decrease when ECT is administered in comparison to the formalin-only group. Results reveal a significant difference between the 30 to 55min time points between groups. This behavioral testing data demonstrates the analgesic effect ECT may evoke. '*' represents $p < .05$, '**' represents $p < .01$, '***' represents $p < .001$.

Discussion

- Results from the behavioral testing reveal a significant decrease in pain-score when ECT is administered, specifically between the 30 to 55 min post-formalin, without a difference in the sequencing of formalin-ECT or ECT-formalin. This data demonstrates the analgesic role that ECT plays in formalin-induced pain.
- An increase of power was observed post-formalin injection (***group A***).
- Formalin followed by ECT administration (***group B***) revealed a trend of brief inhibition of formalin-induced activity in all four brain regions, whereas ECT administration followed by formalin (*group C*) demonstrates a facilitatory effect. Furthermore, the ECT-induced increase or decrease of power has a short duration of a few seconds to a few minutes, revealing that ECT does not exert long-lasting effects, but rather exhibits a brief effect.
- Due to the ECT administration followed by formalin (*group C*) condition not demonstrating as salient of an inhibitory LFP effect as the formalin followed by ECT administration (***group B***) condition, we lead to the conclusion that administering ECT after a pain-inducing event displays a trend that it may be the most effective.

Results

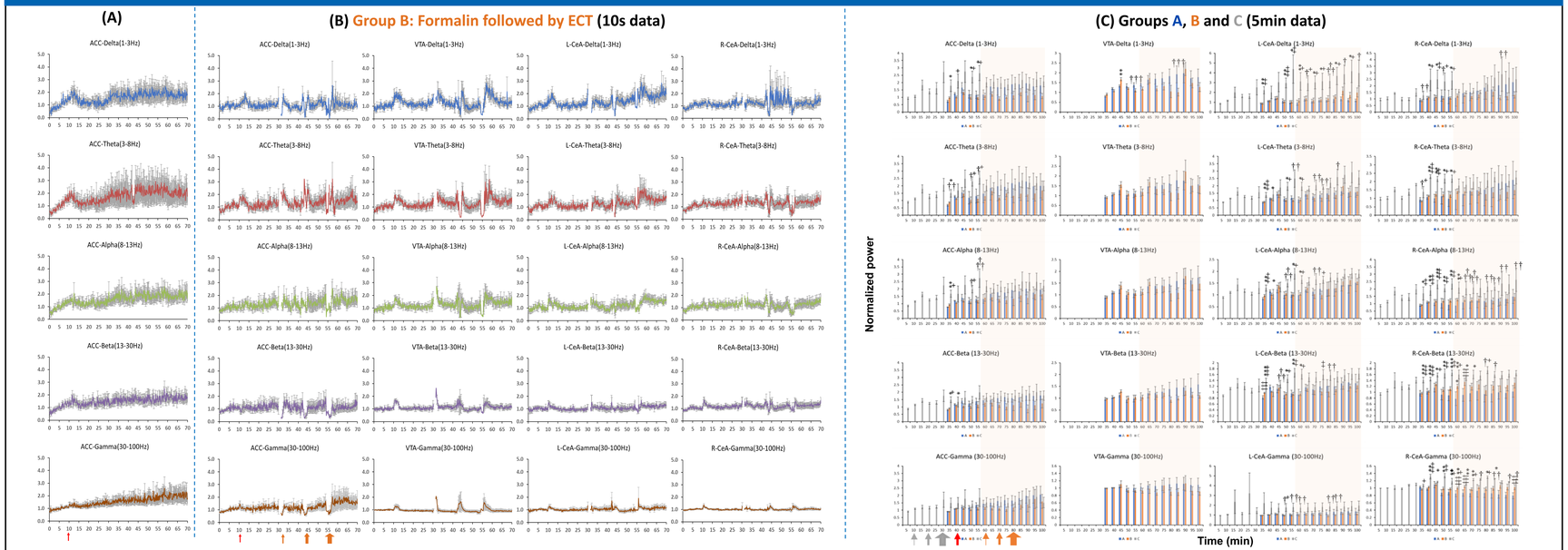


Figure 2. (A) Representative formalin-only power spectrum analysis trace. (B) Power spectrum analysis every 10 seconds for formalin followed by ECT administration (***group B***) in the ACC, VTA, left central amygdala (L-CeA), and right central amygdala (R-CeA). (C) Power spectrum analysis every 5-minutes in all 3 groups: **formalin-only**, **formalin followed by ECT administration**, **ECT administration followed by formalin administration** (groups A – blue bar, B – orange bar, and C – gray bar, respectively) in the ACC (*n* = 5, group A; *n* = 6, group B; *n* = 8, group C), VTA (*n* = 5, group A; *n* = 5, group B; *n* = 0, group C), left CeA (*n* = 4, group A; *n* = 6, group B; *n* = 7, group C), and right CeA (*n* = 3, group A; *n* = 9, group B; *n* = 3, group C). Note: red arrows indicate formalin injection whereas orange and grey arrows indicate ECT administration. The thickness of the arrows represents each ECT stimulation intensity, starting with the thin arrows depicting the weakest stimulation. '*' indicates significant difference between groups A and B, or group A and C, depending on the location, '+' indicates significant differences between groups B and C. '*' indicates $p < .05$, '**' indicates $p < .01$, '***' indicates $p < .001$, and is the same for the symbol '+.'

References

1.Paxinos, G., & Watson, C. (1997). The rat brain, in stereotaxic coordinates. (6th edition) San Diego: Academic Press.
2.Wang, Zhen, and Yuan B. Peng. "Multi-Region Local Field Potential Signatures in Response to the Formalin-Induced Inflammatory Stimulus in Male Rats." Brain Research, vol. 1778, 2022, p. 147779., https://doi.org/10.1016/j.brainres.2022.147779.

Conclusion

The behavioral and LFP results demonstrate that ECT evokes an analgesic and anti-nociceptive effect.

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