Effects of electrical stimulus composition on cardiac electrophysiology in a rodent model of electroconvulsive therapy

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ABSTRACT

Background: No electroconvulsive therapy (ECT) study on humans or in animal models has so far examined whether differently composed electrical stimuli exert different cardiac electrophysiological effects at constant electrical dose. The subject is important because cardiac electrophysiological changes may provide indirect information about ECT seizure quality as modulated by stimulus composition.

Materials and Methods: Adult female Wistar rats (n = 20/group) received fixed, moderately suprathreshold (18 mC) electrical stimuli. This stimulus in each of eight groups was formed by varying pulse amplitude, pulse width, pulse frequency, and stimulus duration. The electrocardiogram was recorded, and time and frequency domain variables were examined in 30 s epochs in preictal (30 s before electroconvulsive shock [ECS]), early postictal (starting 15 s after stimulation), and late postictal (5 h after ECS) periods. Alpha for statistical significance was set at P < 0.01 to adjust for multiple hypothesis testing.

Results: Cardiac electrophysiological indices in the eight groups did not differ significantly at baseline. At both early and late postictal time points, almost no analysis yielded statistically significant differences between groups for four time domain variables, including heart rate and standard deviation of R-R intervals, and for six frequency domain variables, including low-frequency power, high-frequency power, and total power.

Conclusions: Cardiac electrophysiological measures may not be helpful to identify differences in seizure quality that are driven by differences in the composition of electrical stimuli at constant, moderately suprathreshold electrical dose. The generalization of this conclusion to threshold electrical doses and to human contexts requires a study.

Key words: Electrical dose, electrocardiogram, electroconvulsive shocks, electroconvulsive therapy, electroencephalogram, heart rate variability, rats

INTRODUCTION

The importance of electroconvulsive therapy (ECT) stimulus dosing in depressed patients receiving ECT is well known; higher doses, relative to the seizure threshold, are associated with faster and better response, especially when unilateral ECT is administered and such higher doses are also associated with a greater cognitive adverse effect burden. However, a given brief-pulse ECT dose can be constituted in different ways by varying pulse amplitude, pulse width, pulse frequency, and stimulus duration. Are all of these identical doses therapeutically equal? It is unlikely. While clinical data on the subject are unavailable to date, in a preclinical model of ECT, it was observed...
that a stimulus with a narrow pulse width in combination with a high pulse frequency was best associated with electroencephalographic (EEG) proxies of seizure efficacy.\textsuperscript{[8]}

The pulse amplitude (mA) is the strength of the current in the administered electrical stimulus. Pulse width (ms) is the duration for which the neurons are stimulated during each pulse. Pulse frequency (Hz) is the number of pulses delivered per second and indirectly represents the recovery time allowed to the neurons between pulses. Stimulus duration(s) is the time for which the stimulus is passed and includes the total number of times the neurons are stimulated by pulses.

Besides the EEG, peak heart rate and rate pressure product have also been suggested as proxies of ECT seizure adequacy.\textsuperscript{[9,10]} It is therefore possible that cardiac electrophysiological indices, obtained through analysis of the electrocardiogram (ECG) recorded during and after ECT, may also provide useful proxies of the ECT seizure adequacy. However, do these cardiac electrophysiological indices differ with variations in the ECT dose composition at constant electrical dose? This is presently an unanswered question.

In a study that is the first of its kind in literature, we sought to ascertain whether a moderately suprathreshold dose in a rodent model of ECT, composed in different ways by varying the electrical elements of the stimulus, was associated with differences in cardiac electrophysiological variables in the early and late postictal periods. We considered that if consistent differences could be identified, this would suggest that variations in ECT stimulus composition could have varying biological effects and hence, possibly, varying effects on the efficacy and adverse effects of the treatment. Thus, our study could generate hypotheses that would drive clinical investigations in the field.

**MATERIALS AND METHODS**

This study was conducted in the Department of Psychopharmacology at the National Institute of Mental Health and Neurosciences (NIMHANS), Bangalore, India. The research protocol was approved by the Institute Animal Ethics Committee at NIMHANS.

**Animals**

The study was conducted on female Wistar rats, aged about 2–3 months and weighing 150–200 g. The rats were housed four per cage with free access to tap water and standard laboratory diet. The animals were housed and the experimental work was conducted in a disturbance-free environment under ambient conditions of lighting, temperature, and humidity.

The rats were randomly allocated to eight groups of 20 animals per group. Rats in all groups received 18 mC electroconvulsive shock (ECT) stimuli. This stimulus dose was chosen because previous work in our laboratory identified the seizure threshold to lie in the 3–12 mC range\textsuperscript{[11-13]} and because it was our intention that the administered dose be at least moderately suprathreshold so that variations in stimulus composition could have the theoretical opportunity to exert different ECG effects.

The ECS stimulus composition in the eight groups is presented in Box 1. There were two values, each, for pulse amplitude (250 and 500 mA), pulse width (0.6 and 1.2 ms), and pulse frequency (25 and 100 Hz). Stimulus duration ranged from 0.15 to 2.4 s in five steps. Thus, there were different combinations of these stimulus parameters, all of which resulted in a total charge of 18 mC.

**Administration of electroconvulsive shock**

ECT was administered once a day for 5 consecutive days. Each ECS was administered through gel-coated earclip electrodes using the Niviqure constant-current bidirectional brief-pulse ECT instrument (Niviqure Meditech, Bangalore, India). The ECS stimuli were controlled using a computer interface. For each ECS, the motor seizure duration was recorded. In this context, seizure duration was defined to extend from the cessation of the passing of the ECS stimulus to the end of bilaterally symmetrical limb movements.

**Acquisition of the electrocardiogram**

The digitized ECG was recorded using the Niviqure ECG apparatus (Niviqure Meditech, Bangalore, India). This instrument has a sampling rate of 1 kHz. ECG acquisition began about 100 s before the first true/sham ECS and continued for about 5 min. On the day of the first ECS, ECG data were also obtained for about 150 s at an interval of about 5 h after the ECS.

ECG data were obtained in a similar manner on the day of the fifth and last ECS. On ECS days 2–4, ECG was not acquired. This paper presents the analysis of the ECG data obtained on the day of the first ECS; that is, an analysis of the acute effects (on the ECG) of the first ECS in a course. The importance of the first ECT is that clinicians administering ECT often make dose adjustments for future ECT sessions.

**Box 1: Stimulus composition in different electroconvulsive shock arms**

| Group | Pulse amplitude (mA) | Pulse width (ms) | Pulse frequency (Hz) | Stimulus duration (s) |
|-------|----------------------|------------------|----------------------|----------------------|
| 1     | 250                  | 0.6              | 25                   | 2.4                  |
| 2     | 250                  | 0.6              | 100                  | 0.6                  |
| 3     | 500                  | 0.6              | 25                   | 1.2                  |
| 4     | 500                  | 0.6              | 100                  | 0.3                  |
| 5     | 250                  | 1.2              | 25                   | 1.2                  |
| 6     | 250                  | 1.2              | 100                  | 0.3                  |
| 7     | 500                  | 1.2              | 25                   | 0.6                  |
| 8     | 500                  | 1.2              | 100                  | 0.15                 |

*The delivered charge was 18 mC in all treatment groups.
based on results obtained from the first ECT session; hence, EEG, ECG, or other biological effects of the first ECT stimulus could be key drivers of research and practice.

The ECG recordings were acquired from conscious rats that were held in a restrainer that was designed in our laboratory. The restrainer is made of synthetic leather; it contains no metal, and so there is no interference with the collection of digital cardiac electrophysiological data. The rats were suspended above the surface level and in a prone position in their restrainer; nonmetallic rods were used for the purpose.

For the acquisition of the ECG, stainless steel, 24-gauge needle electrodes were inserted subcutaneously into the proximal limb areas. The right lower limb lead was set as the reference lead. Lead II recordings were acquired between the right upper limb and the left lower limb electrodes. Lead II activity was selected for this study because, in pilot experiments, the amplitude of the recordings was found to be highest with this lead.

**Electrocardiogram analysis**

LabChart 8.0 (ADInstruments, Bella Vista, Australia) was used through a graphical user interface for the analysis of cardiac electrophysiological parameters. Each *.nqr file, generated by the Niviqure ECG device, was converted into a *.txt ASCII file and then imported into the LabChart software for analysis. Noise filtering was effected using a notch filter set at 50 Hz. For the frequency domain analyses, the following ranges were auto set in LabChart: very low frequency (LF) at 0.00–0.20 Hz; LF at 0.20–0.75 Hz; and high frequency (HF) at 0.75–2.50 Hz.

Preictal, early postictal, and late postictal recordings, each of 30 s duration, were analyzed using LabChart. The 30 s recording duration was selected to reflect the practicality of identifying early postictal changes; a longer recording duration could risk including time during which the acute effects of the seizure began to wear off.

The preictal period was defined as the 30 s epoch that preceded the ECS stimulus. The early postictal period was defined as the 30 s epoch starting 15 s after the actual (or scheduled) ECS stimulus administration, by which time the motor seizure (in the ECS groups) would have been substantially complete and there would be no electrophysiological noise generated by limb movements. We recognize that the early seconds of this epoch may have included the ECG during the late ictal period. The late postictal period was defined as a 30 s epoch from a recording that was obtained about 5 h after ECS.

**Variables considered for analysis**

Ten cardiac electrophysiological variables were selected for analysis. These are outlined below.

**Time domain variables**

- **Standard deviation of R-R interval (ms)**
  This variable, expressed in ms, is the standard deviation of all R-R intervals in the ECG.

- **Root mean square of successive differences (ms)**
  This variable, expressed in ms, is the square root of the mean of the sum of the square of the differences between adjacent R-R intervals. Higher root mean square of successive differences (RMSSD) values indicate greater parasympathetic activity.

- **pRR10 (%)**
  This variable, expressed as a percentage, is the percentage of differences of >10 ms in adjacent R-R intervals. Higher values of this variable indicate greater parasympathetic activity. The equivalent of pRR10 in humans is the pRR50.

- **Heart rate**
  Heart rate was also included as a time domain variable.

**Frequency domain variables**

- **Total power (in ms²)**
  This variable, expressed in ms², is the power obtained from the entire frequency range of 0.00–2.50 Hz. Total power is sensitive to all influences and hence represents the totality of changes occurring during the selected epoch.

- **Low-frequency power (in ms²)**
  This variable, expressed in ms², is the power obtained in the 0.20–0.75 Hz frequency band. LF power represents both sympathetic and parasympathetic tone.

- **Low frequency (power), normalized units**
  This variable is the LF power expressed in normalized units.

- **High-frequency power (in ms²)**
  This variable, expressed in ms², is the power obtained in the range of 0.75–2.5 Hz frequency band. HF power represents mainly parasympathetic tone.

- **High frequency (power), normalized units**
  This variable is the HF power expressed in normalized units.

- **Low frequency/high frequency ratio**
  LF represents sympathetic and parasympathetic influence and HF represents parasympathetic influence. Therefore, the LF/HF ratio represents sympathovagal balance.

**Statistical analysis**

The data were not normally distributed. Furthermore, variances were observed to be substantially heterogeneous. Therefore, nonparametric procedures such as the Kruskal–Wallis test, the Mann–Whitney test, and the Friedman’s test were performed for between-group and within-group comparisons. For all omnibus statistical inferences, alpha for significance was set
at $P < 0.05$. However, when multiple comparison testing was performed, alpha for significance was more conservatively set at $P < 0.01$.

RESULTS

Despite best efforts, data could not be obtained or used for many rats in the different groups; reasons included recording equipment malfunction, uncertain seizure quality, spinal fracture in treated rats, and ECG noise contamination. Therefore, whereas the original sample comprised 20 rats per group; the final sample for preictal, early postictal, and late postictal recordings comprised 11, 13, 17, 14, 17, 15, 17, and 17 in the groups numbered 1–8 [Box 1], respectively.

In rats that experienced a clearly generalized motor seizure, mean seizure durations ranged from 11 s to 23 s in the different groups. No rat experienced a motor seizure longer than 23 s or briefer than 11 s.

Heart rate

The mean (standard deviation [SD]) (M[SD]) of heart rate data in the different groups is presented in Table 1. There was a substantial, statistically significant decrease in the mean heart rate in the early postictal period. The heart rate returned to baseline levels in the late postictal period. There was no significant difference in heart rate between the eight groups at baseline or at either early or late postictal periods.

Standard deviation of RR interval

The M(SD) of the SD of R-R interval (SDRR) data in the different groups is presented in Table 1. There was a substantial, statistically significant increase in the mean SDRR in the early postictal period. The SDRR returned to baseline levels in the late postictal period. There was no significant difference in SDRR between the eight groups at baseline or at either early or late postictal periods.

Table 1: Mean (standard deviation) time domains electrocardiogram measures in rats receiving eight different compositions of 18 mC electroconvulsive shocks

|     | Group | Heart rate | SDRR | RMSSD | pRR10 |
|-----|-------|------------|------|-------|-------|
|     | Preictal | Early postictal | Late postictal | Preictal | Early postictal | Late postictal | Preictal | Early postictal | Late postictal |
| 1   | 470.55 | 249.38 | 482.33 | 1.78 | 113.47 | 1.03 | 1.61 | 145.01 | 1.16 | 0.00 | 53.41 | 0.00 |
| 2   | (27.89) | (42.18) | (26.68) | (1.25) | (31.64) | (0.40) | (0.96) | (51.95) | (0.32) | (0.00) | (18.36) | (0.00) |
| 3   | 482.55 | 229.12 | 486.97 | 1.69 | 121.46 | 2.05 | 1.79 | 150.38 | 2.24 | (0.00) | 59.54 | (0.00) |
| 4   | (26.04) | (55.93) | (41.70) | (0.58) | (45.91) | (2.25) | (0.62) | (65.93) | (2.70) | (0.00) | (21.90) | (0.00) |
| 5   | 470.76 | 222.65 | 490.05 | 2.19 | 126.69 | 1.55 | 2.52 | 138.32 | 1.77 | (0.00) | 53.63 | (0.00) |
| 6   | (34.03) | (52.17) | (20.84) | (1.82) | (51.18) | (1.16) | (3.11) | (59.92) | (1.30) | (0.00) | (21.75) | (0.00) |
| 7   | 466.35 | 232.10 | 489.05 | 3.56 | 122.41 | 1.33 | 4.02 | 129.25 | 1.42 | 0.21 | 49.16 | 0.00 |
| 8   | (36.97) | (89.86) | (20.84) | (3.40) | (48.49) | (0.81) | (4.24) | (51.63) | (0.75) | (0.68) | (25.13) | (0.00) |
| 9   | 474.71 | 210.73 | 477.06 | 1.93 | 124.14 | 2.21 | 2.05 | 151.77 | 2.48 | 0.00 | 52.94 | 0.11 |
| 10  | (35.61) | (61.29) | (29.80) | (0.72) | (43.35) | (2.86) | (1.08) | (57.12) | (3.42) | (0.00) | (21.24) | (0.45) |
| 11  | 468.96 | 231.87 | 481.37 | 2.25 | 156.41 | 1.92 | 2.46 | 167.59 | 2.28 | 0.00 | 52.17 | 0.10 |
| 12  | (40.59) | (83.96) | (33.91) | (2.50) | (62.74) | (2.64) | (2.67) | (70.55) | (3.11) | (0.00) | (17.64) | (0.41) |
| 13  | 489.11 | 248.46 | 499.86 | 2.53 | 102.77 | 1.17 | 2.09 | 118.32 | 1.26 | 0.00 | 44.95 | 0.00 |
| 14  | (26.25) | (73.51) | (22.80) | (2.23) | (47.19) | (0.77) | (1.09) | (45.32) | (0.50) | (0.00) | (18.47) | (0.00) |
| 15  | 493.24 | 277.57 | 500.59 | 1.40 | 93.89 | 1.11 | 1.59 | 117.62 | 1.18 | 0.00 | 41.27 | 0.00 |
| 16  | (20.75) | (47.82) | (23.16) | (0.43) | (32.70) | (0.37) | (0.50) | (51.02) | (0.23) | (0.00) | (21.05) | (0.00) |

SDRR – Standard deviation of all R-R intervals; RMSSD – Square root of the mean of the sum of the square of the differences between; pRR10 – Percentage of differences of $> 10$ ms in adjacent R-R intervals

Root mean square of successive differences

The M(SD) of the RMSSD data in the different groups is presented in Table 1. There was a substantial, statistically significant increase in the mean RMSSD in the early postictal period. The RMSSD returned to baseline levels in the late postictal period. There was no significant difference in RMSSD between the eight groups at baseline or at either early or late postictal periods.

pRR10

The M(SD) of the pRR10 data in the different groups is presented in Table 1. There was a substantial, statistically significant increase in the mean pRR10 in the early postictal period. The pRR10 returned to baseline levels in the late postictal period. There was no significant difference in pRR10 between the eight groups at baseline or at either early or late postictal periods.

Total power

The M(SD) of the total power data in the different groups is presented in Table 2. There was a substantial, statistically significant increase in the mean total power in the early postictal period. The total power values returned to baseline levels in the late postictal period. There was no significant difference in total power between the eight groups at baseline or at either early or late postictal periods.

Low-frequency power

The M(SD) of the LF power data in the different groups is presented in Table 2. There was a substantial, statistically...
significant increase in the mean LF power in the early postictal period. The LF power values returned to baseline levels in the late postictal period. There was no significant difference in LF power between the eight groups at baseline or at either early or late postictal periods.

**High-frequency power**
The M(SD) of the HF power data in the different groups is presented in Table 2. There was a substantial, statistically significant increase in the mean HF power in the early postictal period. The HF power values returned to baseline levels in the late postictal period. There was no significant difference in HF power between the eight groups at baseline or at either early or late postictal periods.

**Low frequency (power), normalized units**
The M(SD) of the LFnu power data in the different groups is presented in Table 3. There was no significant change in mean LFnu power across the three assessment points. There was no significant difference in LFnu power between the eight groups at baseline or at either early or late postictal periods.

**High frequency (power), normalized units**
The M(SD) of the HFnu power data in the different groups is presented in Table 3. There was no significant change in mean HFnu power across the three assessment points. There was no significant difference in HFnu power between the eight groups at baseline or at either early or late postictal periods.

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**Table 2: Mean (standard deviation) frequency domain electrocardiogram measures in rats receiving eight different compositions of 18 mC electroconvulsive shocks**

| Group | Total power | LF power | HF power |
|-------|-------------|----------|----------|
|       | Preictal    | Early postictal | Late postictal |
|       | Early postictal | Late postictal | Preictal | Early postictal | Late postictal | Preictal | Early postictal | Late postictal |
| 1     | 4.46 (7.56) | 25,811.13 (24,980.67) | 1.41 (1.18) | 0.89 (1.86) | 3283.89 (3412.90) | 0.36 (0.42) | 2.02 (3.94) | 17,742.5 (19,058.33) | 0.58 (0.43) |
| 2     | 2.78 (1.99) | 26,291.7 (30,321.64) | 8.02 (20.80) | 0.42 (0.45) | 2904.63 (3986.41) | 1.06 (2.68) | 1.37 (0.99) | 17,260.41 (21,678.66) | 5.28 (15.09) |
| 3     | 19.75 (40.76) | 23,085.62 (16,846.87) | 4.06 (7.50) | 4.36 (8.31) | 2814.78 (2660.71) | 0.71 (1.31) | 9.03 (23.05) | 13,980.69 (11,488.07) | 2.28 (5.07) |
| 4     | 18.81 (40.51) | 24,719.22 (25,761.75) | 1.95 (2.44) | 2.91 (5.45) | 3497.13 (3722.58) | 0.45 (0.88) | 11.17 (27.95) | 13,402.81 (13,945.58) | 0.80 (0.82) |
| 5     | 5.95 (6.06) | 24,107.66 (23,004.82) | 10.94 (28.49) | 0.90 (1.05) | 2840.06 (4066.36) | 3.02 (7.84) | 3.01 (3.53) | 13,546.27 (11,688.05) | 5.11 (14.63) |
| 6     | 11.90 (34.67) | 55,248.33 (60,197.11) | 6.71 (20.48) | 1.91 (5.81) | 6916.20 (7918.45) | 1.20 (3.71) | 8.10 (26.87) | 34,174.19 (39,830.79) | 3.81 (11.97) |
| 7     | 10.93 (15.20) | 20,969.19 (21,039.38) | 1.38 (2.02) | 2.35 (3.67) | 2682.54 (3959.12) | 0.33 (0.69) | 4.49 (6.91) | 12,336.28 (11,244.30) | 0.54 (0.61) |
| 8     | 5.01 (10.83) | 14,473.46 (21,360.54) | 1.26 (1.23) | 0.92 (2.46) | 2008.28 (3670.75) | 0.25 (0.35) | 2.80 (6.62) | 8595.63 (13,291.26) | 0.56 (0.44) |

**Table 3: Mean (standard deviation) frequency domain electrocardiogram measures in rats receiving eight different compositions of 18 mC electroconvulsive shocks**

| Group | LF/HF ratio | LF normalized units (LFnu) | HF normalized units (HFnu) |
|-------|-------------|---------------------------|---------------------------|
|       | Preictal    | Early postictal | Late postictal | Preictal | Early postictal | Late postictal | Preictal | Early postictal | Late postictal |
| 1     | 0.36 (0.21) | 0.23 (0.22) | 0.57 (0.63) | 21.77 (10.74) | 14.07 (8.82) | 26.90 (19.59) | 64.83 (10.95) | 71.90 (14.86) | 59.47 (14.15) |
| 2     | 0.34 (0.29) | 0.19 (0.18) | 0.37 (0.28) | 19.82 (12.68) | 12.39 (8.64) | 21.67 (13.93) | 67.45 (12.16) | 73.51 (12.67) | 64.56 (11.44) |
| 3     | 0.59 (0.64) | 0.24 (0.15) | 0.51 (0.40) | 27.56 (19.63) | 15.37 (8.32) | 24.94 (14.83) | 60.70 (14.78) | 68.86 (8.37) | 56.99 (13.30) |
| 4     | 0.32 (0.18) | 0.35 (0.44) | 0.45 (0.41) | 20.63 (8.71) | 17.60 (15.09) | 24.03 (16.95) | 68.18 (10.04) | 64.69 (13.60) | 63.17 (11.98) |
| 5     | 0.35 (0.22) | 0.18 (0.12) | 0.52 (0.50) | 21.38 (12.34) | 11.68 (6.61) | 26.54 (16.74) | 66.04 (9.17) | 68.97 (10.27) | 62.36 (13.09) |
| 6     | 0.36 (0.28) | 0.20 (0.07) | 0.37 (0.39) | 21.34 (12.01) | 13.59 (3.80) | 20.23 (14.36) | 65.25 (11.89) | 70.61 (7.56) | 65.88 (13.22) |
| 7     | 0.44 (0.42) | 0.19 (0.13) | 0.39 (0.35) | 24.32 (15.98) | 12.78 (7.63) | 21.14 (12.76) | 67.37 (14.47) | 71.28 (10.48) | 62.98 (12.19) |
| 8     | 0.29 (0.21) | 0.20 (0.16) | 0.40 (0.25) | 17.71 (9.93) | 12.54 (7.90) | 23.55 (10.12) | 68.81 (12.81) | 68.99 (11.35) | 63.11 (10.07) |

**DISCUSSION**

We studied four time domain variables and six frequency domain variables in the ECG of rats that received 18 mC ECS formulated in eight different ways [Box 1]. We did not use a control group of rats treated with sham ECS because it was not our purpose to determine whether ECS changes with ECS differ from sham treatment; we have already established this.[14] Rather, we sought to examine whether differences exist in the cardiac electrophysiological responses to the same ECS dose constituted in different ways. If specific cardiac electrophysiological changes with ECS can be identified as markers of good-quality seizures, then the ECS dose compositions that are associated with
these cardiac electrophysiological changes could be studied further in preclinical and clinical situations.

Expressed with the help of an example, if the combination of narrow pulse width and high pulse frequency are found to be associated with ECG indices that are proxies of good seizure quality (as demonstrated by Sudha et al.\textsuperscript{[13]} in the context of the EEG), then narrow pulse width and high pulse frequency stimuli can be considered for further study in clinical contexts. To date, there is no study that has examined the therapeutic or adverse effect impact of the same electrical dose constituted in different ways.

**Summary of the findings**
We found that, for all 10 ECG indices, there were no significant differences between groups at baseline. For all time domain variables [heart rate, SDRR, RMSSD, and pRR10; Table 1] and for (only) three frequency domain variables [total power, LF power, and HF power; Table 2], there was a robust early postictal response in the ECS groups, but there was no difference in the early postictal response between the eight different ECS groups. The eight groups also did not differ significantly in the late postictal recordings for any variable. In effect, at fixed electrical charge, differences in the ECS stimulus dose composition cannot be identified through a study of cardiac electrophysiological measures.

**Related studies in literature**
Some animal\textsuperscript{[15,16]} and human\textsuperscript{[17,18]} studies have examined cardiac electrophysiological changes with ECS/ECT. Different studies examined different cardiac electrophysiological measures at different times with reference to the seizure, and none were designed in a way that allows comparisons with our study. Furthermore, no study examined (electrical) dose-dependent effects of ECS, let alone the effects of stimulus dose composition on cardiac electrophysiological parameters, as we did.

**Implications of the findings**
Our hope was to identify easily ascertained, peripheral markers of the quality of the ECS seizure because, although the EEG is a more meaningful marker for central seizure quality, EEG acquisition and interpretation can be associated with practical difficulties. However, although we found that ECS was associated with robust response on most cardiac electrophysiological indices [Tables 1-3], we were disappointed that the responses did not separate across different stimulus dose compositions. A reasonable conclusion is that cardiac electrophysiological changes may not be a useful guide to the quality of the central seizure in the context of ECT in an animal model, if not in clinical contexts, as well; the latter will, of course, require study.

**Limitations**
We conducted this study in female rats because of the instructions of the Institutional Animal Ethics Committee. We acknowledge that estrus could affect autonomic nervous system regulation and hence cardiac electrophysiology. However, we expected that estrus would be harmonized in the study animals because they were housed together. Confirmation of our findings in male rats may be worth considering.

We conducted our study on conscious rats, immobilized as described. Immobilization may have affected autonomic nervous system functioning and hence the cardiac electrophysiological response to ECS. Confirmation of our findings in anesthetized rats may be worth considering. However, anesthesia would have its own effects on the ECG.

A higher sampling rate than the 1 Hz rate that we employed would have been desirable because the rodent heart rate is very rapid; however, a higher sampling rate would merely accommodate a more fine-grained analysis and would not alter the broad outcomes that we studied.

The 18 mC charge that we administered to the eight groups of rats may have resulted in a ceiling cardiac electrophysiological response and hence the absence of differences across groups. Perhaps, a lower dose, such as 10 mC, might have shown statistical separation between groups. However, with the lower dose, some animals may not have experienced a seizure or the dose may not have been modestly suprathreshold as is conventionally desirable for better clinical outcomes.

**CONCLUSIONS**
Cardiac electrophysiological parameters do not help separate seizures elicited by modestly suprathreshold ECS stimuli that have the same electrical charge but different electrical compositions.

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**Conflicts of interest**
There are no conflicts of interest.

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