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Electrocardiogram Recordings in Anesthetized Mice using Lead II

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TITLE:
Electrocardiogram Recordings in Anesthetized Mice using Lead II

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KEYWORDS:
electrocardiogram, noninvasive method, anesthesia, tribromoethanol, autonomic nervous system

SUMMARY:
We present an ECG protocol that is technically easy, inexpensive, fast, and affordable in small mice, and can be performed with enhanced sensitivity. We suggest this method as a screening approach for studying pharmacological agents, genetic modifications, and disease models in mice.

Abstract
The electrocardiogram is a valuable tool for evaluating the cardiac conduction system. Animal research has helped generate novel genetic and pharmacological information regarding the electrocardiogram. However, making electrocardiogram measurements in small animals in vivo, such as mice, has been challenging. To this end, we used an electrocardiogram recording method in anesthetized mice with many advantages: it is a technically simple procedure, is inexpensive, has short measuring time, and is affordable, even in young mice. Despite the limitations with using anesthesia, comparisons between control and experimental groups can be performed with enhanced sensitivity. We treated mice with agonists and antagonists of the autonomic nervous system to determine the validity of this protocol and compared our results with previous reports. Our ECG protocol detected increased heart rates and QTc intervals on treatment with atropine, decreased heart rates and QTc intervals after carbachol treatment, and higher heart rates and QTc intervals with isoprenaline but did not note any change in ECG parameters on administration of propranolol. These results are supported by previous reports, confirming the reliability of this ECG protocol. Thus, this method can be used as a screening approach to making ECG measurements that otherwise would not be attempted due to high cost and technical difficulties.

INTRODUCTION:

The electrocardiogram (ECG), a test that measures the electrical activity of one's heartbeat, is a valuable tool for evaluating the cardiac conduction system. The parameters that are measured by an ECG include heart rate, PR interval, QRS duration, and QT interval. In brief, PR interval corresponds to the time that is required for an electrical impulse to travel from the atrial sinus node through the atrioventricular node to the Purkinje fibers; QRS duration is the time for ventricular depolarization to occur through the Purkinje system and ventricular myocardium; and QT interval is the duration of ventricular repolarization.

ECG recordings in mice have helped researchers examine cardiac function and determine the physiological and pathophysiological mechanisms of cardiac phenotypes, such as arrhythmia, atrial fibrillation, and heart failure. Most cardiovascular research has involved studies in genetically engineered mouse models. It is often challenging to obtain meaningful data on ECG recordings from small mice that have been genetically manipulated.

There are several methods for performing ECGs in mice[1]. Studies suggest that ECG recordings in conscious animals are preferred over anesthetized animals when possible since the effects of anesthesia on cardiac function have been well established[2]. Two protocols that record ECG in conscious mice are of note[1]. The ECG radiotelemetry system is the gold standard for continuous long term monitoring of ECG in conscious mice[1, 3]. Despite their strength in being recorded in a conscious state, radiotelemetry-coupled ECG measurements have several limitations, including the high expense for setup and for the implant, its requirement of a highly experienced operator, a stabilization period of over 1 week, its need for large mice (> 20 g), and acquisition of only a single lead of ECG recording[1]. Another system that uses paw-sized conductive electrodes embedded in a platform allows ECG recordings in conscious mice without anesthesia or implants[1, 4]. This non-invasive system is an alternative method in situations in which radiotelemetry systems are unavailable since it has many advantages: no requirement of surgical treatment, no need of anesthesia, low cost per mouse (only the initial setup is expensive), short time for measurement, and affordability of neonates[1, 4]. The main disadvantage of this system is that it is not suited for continuous long term monitoring[1].

Here we introduce another inexpensive, simple, and fast ECG recording method in anesthetized mice and demonstrate its validity and sensitivity by performing an ECG after autonomic blockade/simulation of the cardiac conduction system. We suggest this ECG method for screening the effects of pharmacological agents, genetic modifications, and disease models in mice.

PROTOCOL:

All animal procedures were approved by the local committee for the Care and Use of Laboratory Animals, Kyung Hee University (license number: KHUASP(SE)-18-108) and conformed to the US National Institutes of Health Guide for the Care and Use of Laboratory Animals.

1. Experimental animals

1.1. Keep all mice (39 mice, Balb/c, male, 7–9 weeks old) in a pathogen-free facility as per the guide for the care and use of laboratory animals.

1.2. Maintain the mice on a 12 h light/dark cycle at constant temperature with free access to food and water.

2. Preparation of anesthetics

NOTE: Tribromoethanol is used over ketamine combinations and isoflurane, based on the stability of heart rate and the reproducibility of echocardiography in tribromoethanol-anesthetized mice[1, 5, 6]

2.1. Make a stock solution of 2,2,2-tribromoethanol at a concentration of 1 g per 1 mL tertiary amyl alcohol. Warm at 40–45 °C for 24 h. Store at 4 °C for 12 months.

2.2. For working solution, dilute 0.5 mL of stock solution in 19.5 mL of saline (0.9% NaCl) to 25 mg/mL. Warm at 40–45 °C for 1 h. Store at 4 °C for 1 month.

3. ECG system setup

3.1. Make sure to set up the system such that there is no noise or vibration within 2 m since ECG signals in a mouse are sensitive to the environmental noise and movement.

3.2. Prepare the hardware setup: a data acquisition system, a bio amplifier, and a computer that is installed with an ECG data analysis software.

3.2.1. Connect the data acquisition system to the mains (AC) using the power cable.

3.2.2. Connect the data acquisition system to the computer using a USB cable.

3.2.3. Connect the signal output on the rear panel of the bio amplifier to an analog input on the front panel of the data acquisition system using a cable.

3.2.4. Connect the I^2C output of the data acquisition system to the I^2C input of the bio amplifier using the I^2C cable.

3.2.5. Connect the 3-lead bio amplifier cable to the 6-pin input socket on the front panel of the bio amplifier.

3.2.6. Turn on the data acquisition system using the switch on the back panel.

NOTE: In brief, the signals are amplified through a bio amplifier and recorded using a computerized data acquisition and analysis system with the following channel settings: sampling rate of 2 k/s, range of 20 mV, and low-pass filter setting of 200 Hz.

3.3. Open the analysis software program and set it up for ECG data acquisition.

3.3.1. Go to **Setup | Channel Settings**. Set the **Sample Rate** to 2 k/s. Set the **Range** to 20 mV. Set the **Input Amplifier** to 200 Hz of Low Pass.

3.3.2. Go to **ECG Analysis | ECG Settings**. Choose "Mouse" in the Preset of Detection and Analysis settings.

3.3.3. In the Averaging panel, choose to concatenate N (e.g., 4 beats or 60 s) consecutive cardiac cycles into a single average signal for Averaging View and Table View.

3.3.4. In the QTc panel, select "Bazett" method, which is defined as the heart rate-corrected value of QT interval: $QTc = QT / (RR/100)^{0.5}$, RR interval = 60 / heart rate[7].

4. ECG measurement

4.1. Place a mouse on a precision scale and record its weight.

4.2. Induce anesthesia in the mouse by intraperitoneal (i.p.) injection of a working solution of tribromoethanol (18 mL of working solution per kg body weight (b.w.)).

4.3. Place an anesthetized mouse in supine position. Ensure that the mouse is completely anesthetized (less than 2 min).

4.4. Insert the electrodes with acupuncture needles subcutaneously into the right and left forelimbs and the left hindlimb according to the lead II ECG scheme and fix them with tape (**Figure 1**). Ensure that the depth and position of inserted electrodes are consistent throughout the experiments.

4.5. Connect the other ends of the electrodes by clicking them into the three snap connectors at the other end of the lead wires of the 3-lead bio amplifier cable.

4.6. Inject drugs (i.p.) 3 min after the anesthetics have been delivered (**Figure 2**).

4.7. Begin recording the ECG 10 min after injecting anesthetics. Once the recording is completed, use the ECG data from 12 to 17 min after injection of anesthetics for analysis.

4.8. At the end of the ECG recording session, carefully remove the electrodes.

5. ECG data analysis

5.1. Go to **ECG Analysis | Averaging View** and ensure that the software correctly identifies the start and end of the P wave, QRS complex, and T wave in individual beats. If necessary, manual

correction of these waves and intervals is possible by moving misplaced cursors to the appropriate positions.

NOTE: As depicted in **Figure 3A**, the PR interval spans the onset of the P wave to that of the QRS complex (mostly missing Q wave in a mouse ECG). The QRS duration extends from the onset of the Q wave (primarily an R wave in a mouse ECG) to the end of the S wave. The QT interval comprises the onset of the Q wave (mostly the R wave in a mouse ECG) to the end of the T wave. Note the shorter duration and absence of a Q wave and ST segment in the mouse ECG relative to the human ECG[8].

5.2. Go to **ECG Analysis | Table View** and select the correctly identified ECG data by checking individual beats in the Averaging View window.

NOTE: **Figure 3** shows several examples of actual mouse ECG signals. **Figure 3A** represents a normal wild-type signal that has been correctly identified with regard to P wave, QRS complex, and T wave. Computerized selection of PQRS waves can incur erroneous misplacements, such as in **Figure 3B** a normal wild-type signal that misplaces the onset of the P wave. In **Figure 3C** an ECG signal that misplaces the end of the QRS complex, resulting in an overestimation of QRS duration. In **Figure 3D** an ECG signal that misplaces the end of the QRS complex, resulting in underestimation of the QRS complex due to the ambiguous T wave and **Figure 3E** an ECG signal with an unidentifiable T wave. Without exclusion or manual corrections, PQRS intervals can be over or underestimated. Be sure to select the ECG signals that have been correctly identified and the signals that do not miss the target peaks. Consequently, such cases, including B, C, D, and E (**Figure 3**), are excluded in accurately estimating ECG parameters in general.

5.3. Select the ECG data of interest in Table View, and copy/paste them to a spreadsheet file.

6. Statistical analysis

6.1. Perform the statistical analysis using a statistics program. Analyze the data with the experimental conditions blinded. Perform Student's *t*-test and Mann-Whitney U-test for 2-group comparisons. The numbers in each figure indicate the number of mice that is used for each group. Report the results as mean ± SEM.

6.2. Consider differences with $p < 0.05$ by U-test to be statistically significant: *, $p < 0.05$; **, $p < 0.01$; and ***, $p < 0.005$ versus respective controls.

Representative Results

Pharmacological experiments

To determine whether our noninvasive ECG measurement reflects the influence of autonomic modulation on the cardiac conduction system, normal Balb/c mice were challenged with agonists and antagonists of the autonomic nervous system (ANS). Atropine and carbachol were used to effect parasympathetic autonomic blockade and stimulation, respectively, whereas propranolol

and isoprenaline were administered to elicit sympathetic autonomic blockade and stimulation, respectively[9].

Heart rate increased significantly in atropine- ($p < 0.05$) and isoprenaline-treated mice ($p < 0.05$) and fell with carbachol ($p < 0.005$) as compared with vehicle (vehicle, 391 ± 13 bpm versus atropine, 487 ± 15 bpm versus carbachol, 158 ± 7 bpm; vehicle, 382 ± 14 bpm versus isoprenaline, 548 ± 8 bpm; vehicle, 404 ± 25 bpm versus propranolol, 303 ± 16 bpm) (**Figure 4**). In addition, QTc interval rose in atropine- ($p < 0.05$) and isoprenaline-treated mice ($p < 0.05$) and decreased in carbachol-treated mice ($p < 0.005$) versus vehicle (vehicle, 46.5 ± 0.6 ms versus atropine, 51.1 ± 1.3 ms versus carbachol, 29.4 ± 1.0 ms; vehicle, 41.8 ± 1.2 ms versus isoprenaline, 57.5 ± 3.5 ms) (**Figure 4**). **Figure 5** shows representative Chart Views and Averaging Views for the ECG signals in atropine-, carbachol-, and vehicle-treated mice.

FIGURE LEGENDS:

Figure 1: ECG lead placement. Acupuncture needle electrodes are inserted subcutaneously according to the lead II ECG scheme (right and left forelimbs and the left hindlimb) and are fixed with tape.

Figure 2: Scheme of anesthetic and drug treatments. Three minutes after injection of anesthetics (e.g., tribromoethanol), administer drugs (e.g., atrotpine, carbachol, isoprenaline, and propranolol; i.p.). Ten minutes after the anesthetics have been delivered, start recording the ECG. Collect ECG data from 12–17 min after the injection of anesthetics.

Figure 3: Examples of mouse ECG signals. (**A**) A normal wild-type signal that is correctly identified with regards to the P wave, QRS complex, and T wave. (**B**) A normal wild-type signal that misplaces the onset of the P wave. (**C**) An ECG signal that misplaces the end of the QRS complex. (**D**) An ECG signal that misplaces the end of the QRS complex due to an ambiguous T wave. (**E**) An ECG signal with an unidentifiable T wave.

Figure 4: ECG measurements in mice treated with agonists and antagonists of the autonomic nervous system. (**A**) Administration of atropine (1 mg/kg) increases heart rate and QTc interval. (**B**) Carbachol (0.5 mg/kg) decreases heart rate and QTc interval. (**C**) Isoprenaline (1 mg/kg) increases heart rate and QTc interval. (**D**) Propranolol (1 mg/kg) does not change any ECG parameters. *, $p < 0.05$; ***, $p < 0.005$.

Figure 5: Representative ECG signals of mice treated with agonists and antagonists of the parasympathetic nervous system. (**A**) ECG signals of vehicle-treated mouse acquired from Chart Views and Averaging Views (a data analysis program). (**B**) Signals of atropine-treated mouse. (**C**) Signals of carbachol-treated mouse.

DISCUSSION:

There are several critical steps in the protocol. The surrounding environment should be free from noise and vibration. The ECG electrodes must be inserted under the skin stably and consistently

of which the insertion step requires preliminary experiments until the researcher is technically experienced. Further, the anesthetic should be prepared and stored appropriately and used at the proper dose. Finally, the PQRS waves should be located appropriately in individual ECG beats in the Averaging View window.

Our studies included testing of drugs. However, if pharmacological tests are omitted, the step 4.7 can be modified by beginning the recording 5 min after the injection of anesthetics, and the ECG data can be used from 10 to 15 min. ECG values are relatively stable over 15 min post-anesthesia and have been replicated in the same mouse 6 h after the first measurement[5].

Autonomic blockade and stimulation by drugs elicit differential responses with regards to heart rate. Several protocols have been used in ECG research. Based on telemetered ECG recordings in mice, atropine, isoprenaline, and propranolol did not significantly change heart rate, whereas carbachol significantly decreased it (wild-type, 739 ± 33 bpm; atropine, 726 ± 5 bpm; carbachol, 205 ± 54 bpm; isoprenaline, 722 ± 32 bpm; propranolol, 560 ± 21 bpm)[9]. Based on ECG recordings by the noninvasive system that uses paw-sized conductive electrodes embedded in a platform, atropine and isoprenaline significantly increased heart rate in mice ($p < 0.05$), whereas propranolol did not change it (p = NS) (wild-type, 706 ± 13 bpm; atropine, 727 ± 12 bpm; isoprenaline, 12 ± 2% increase versus control; propranolol, 584 ± 53 bpm)[4, 10]. With this noninvasive ECG system, isoprenaline induced ST segment depression[4].

Surface ECG signals (lead II via limb electrodes) are acquired under isoflurane anesthesia during high-resolution transthoracic echocardiography (TTE) with an ultrasound system[11]. ECG recordings by the TTE suggested that heart rate increased 15 min after the administration of atropine[11]. Similar to our protocol, 6-lead ECG recordings under anesthesia with tribromoethanol using 5-needle electrodes (1 electrode implanted subcutaneously in each limb and 1 placed in the precordial position) that are connected to a data acquisition system with an amplifier set[12]. With this method, using 6-lead ECG, carbachol significantly lowered heart rate ($p < 0.001$) and increased QT interval ($p < 0.001$), but propranolol did not significantly change either parameter (wild-type, 395 ± 65 bpm; carbachol, 177 ± 36 bpm; propranolol, 351 ± 30 bpm)[12]. Another report that made 3-lead ECG measurements under anesthesia with tribromoethanol showed that isoprenaline significantly increased heart rate in wild-type mice ($p < 0.01$) (wild-type, 422 ± 17 bpm; isoprenaline, 503 ± 27 bpm)[13]. [14] Overall, the heart rate is lower in ECG measurements under anesthesia than in those in a conscious mouse. Differences between control and drug-treated groups are well reflected in ECG recordings under anesthesia and by the system that uses paw-sized conductive electrodes embedded in a platform, in a conscious mouse, because changes in heart rate and QT interval are detected on treatment with atropine, carbachol, and isoprenaline but not propranolol alone[10-13]. In contrast, telemetered ECG recordings detect only changes in heart rate by carbachol[9].

This ECG method under anesthesia with tribromoethanol also notes differences in heart rate and QTc interval on administration with atropine, carbachol, and isoprenaline but not propranolol, implying its high sensitivity. Here with autonomic disturbances, we showed changes in heart rate and QTc interval. Further we have published a manuscript with our ECG method that describes a

change in PR interval and another one that addresses changes in QRS duration and QTc interval, partially supporting the sensitivity in all of PQRS waves[15, 16].

The protocol has many advantages comparable to the non-invasive method that allows ECG recording in a conscious mouse with paw-sized electrodes embedded in a platform. However, the major limitation of our protocol is the use of anesthetics such as tribromoethanol. Tribromoethanol is used over ketamine combinations and isoflurane, based on the stability of heart rate and the reproducibility of echocardiography in tribromoethanol-anesthetized mice[1, 5, 6] Although ECG recordings in a conscious animal is preferred to those under anesthesia, variations in sympathetic and parasympathetic tone, and relatively high heart rate sometime make measurements in conscious mice less than ideal for all applications of echocardiography[6].

Overall, despite its limitations (e.g., the use of anesthesia), our ECG method has many advantages: (i) it is a technically simple procedure only requiring stable insertion of ECG electrodes under the skin, (ii) has low experimental costs—the outlay is primarily for the initial hardware setu]; (iii) has short measurement times of less than 20 min per mouse, and can be conducted on young mice (>15 g body weight, in our experience)[16] and even neonates (postnatal days 2–4)[17]. Thus, screening experiments for drugs and various types of mice (e.g., genetically modified, disease models) can be performed quickly and without much cost per mouse, constituting a reliable and sensitive analysis and can be used as an additional supporting data beyond telemetered ECG recordings.

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DISCLOSURES:

No conflicts of interest, financial or otherwise, are declared by the authors.

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Fig. 1

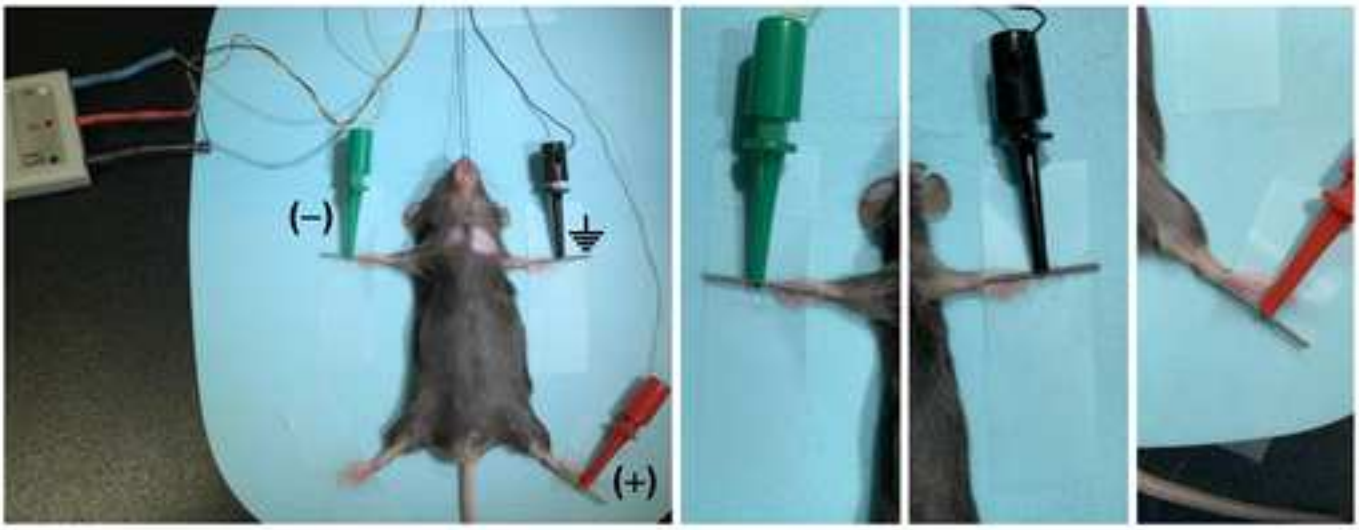

Fig. 2

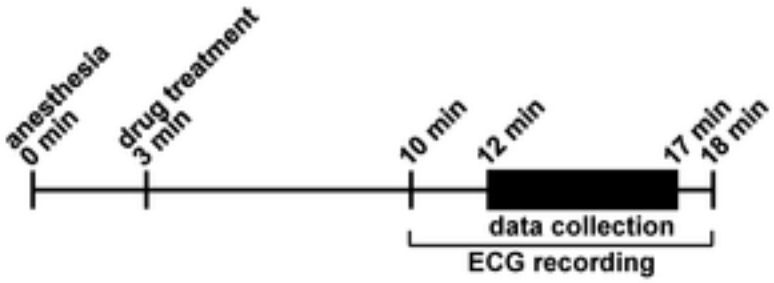

Fig. 3

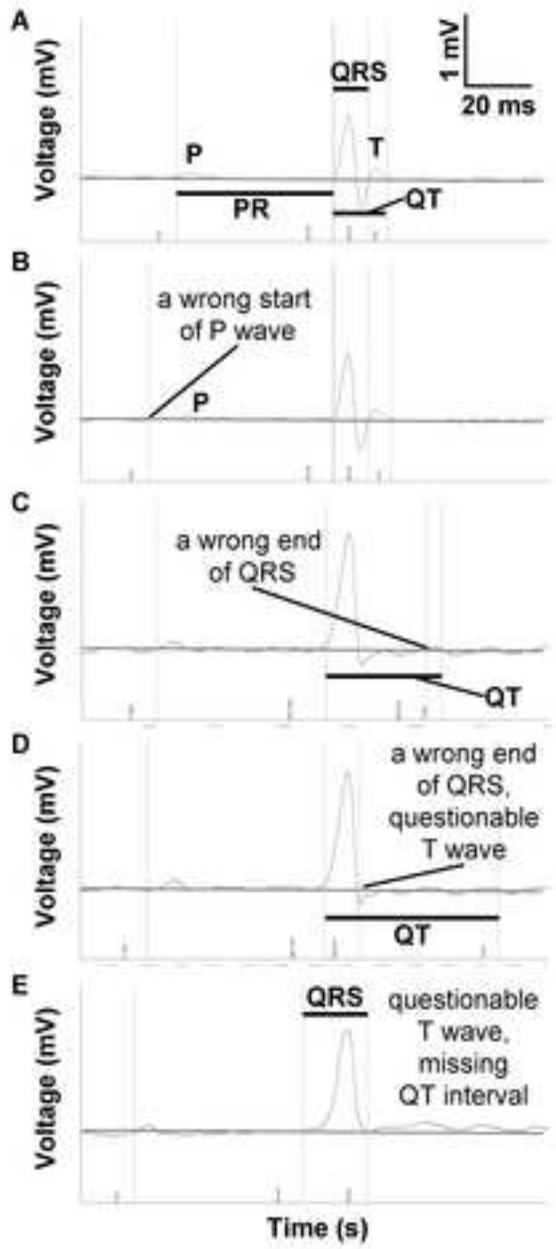

Figure 4

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Fig. 4

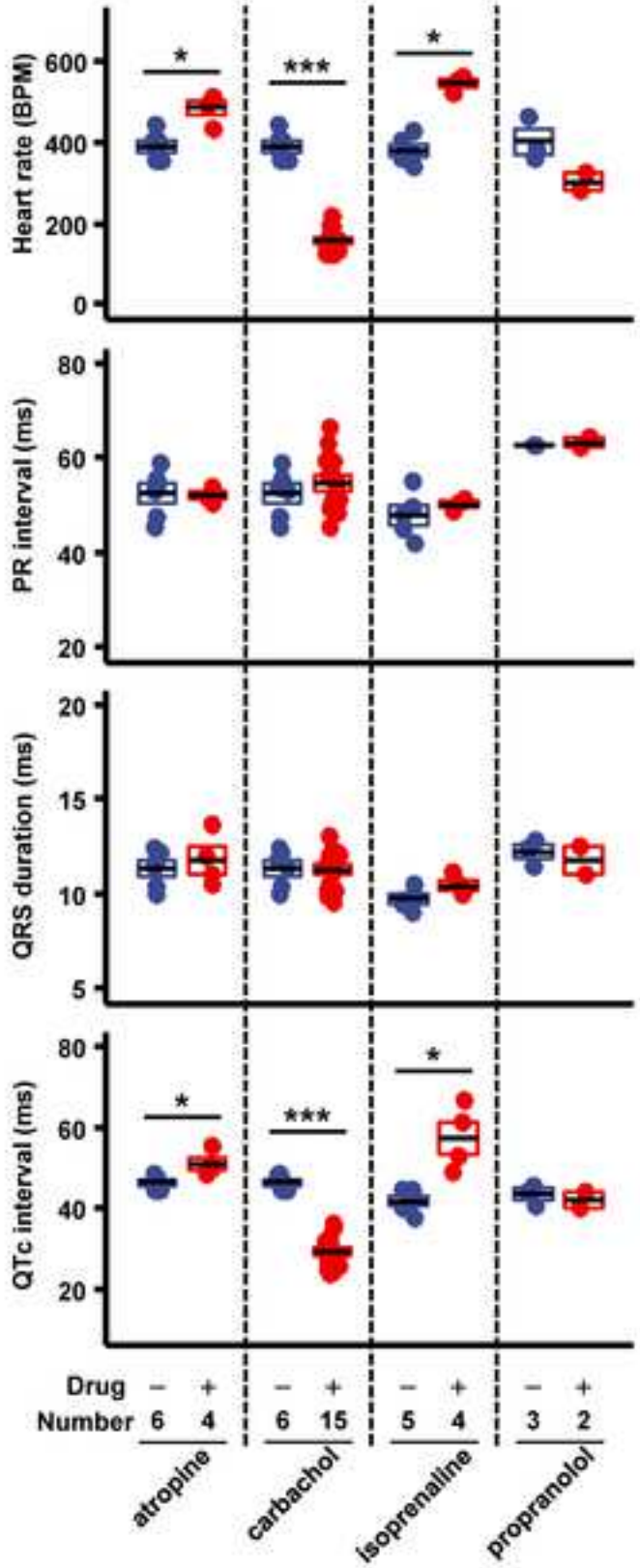
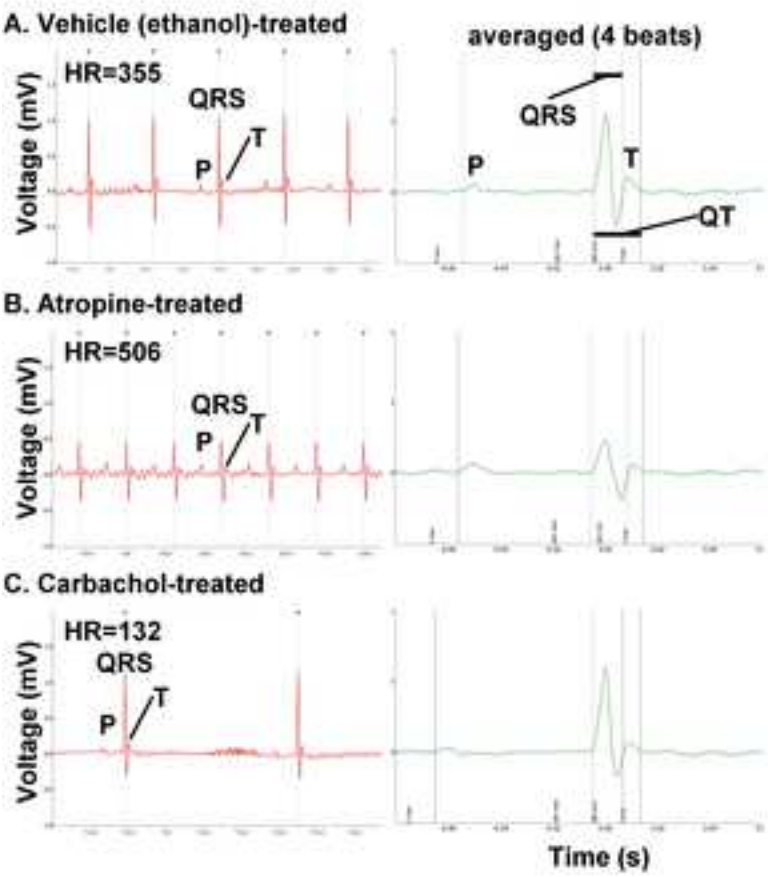


Fig. 5



Name of Material/Equipment	Company	Catalog Number	Comments/Description
2,2,2-tribromoethanol	Sigma-Aldrich	T48402-25G	anesthetics, Avertin
Animal	Japan SLC, Inc., Shizuoka, Japan		Balb/c mice, male, aged 7-9
Atropine	Sigma-Aldrich	A0123	parasympathetic antagonist
BioAmp	AD Instruments, Bella Vista, Australia	ML132	bio amplifier
Carbachol	Sigma-Aldrich	C4382	parasympathetic agonist
Electrodes with acupuncture needles	DongBang Acupuncture Inc., Sungnam, Korea	DB106	0.20 x 15 mm
Isoprenaline	Sigma-Aldrich	I2760	sympathetic agonist
LabChart 8	AD Instruments, Bella Vista, Australia		data analysis software
Mouse food	LabDiet, St. Louis, MO, USA	5L79	Mouse diet
PowerLab 2/28	AD Instruments, Bella Vista, Australia		data acquisition system
Propranolol	Sigma-Aldrich	P0884	sympathetic antagonist
SPSS Statistics program	SPSS	SPSS 25.0	statistics program

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Editor-in-Chief

Dear Editor,

On behalf of my coauthors, I am submitting the enclosed manuscript, entitled "Method for electrocardiogram recordings in anesthetized mice using lead II," for consideration by JoVE as a Video Produced by JoVE.

We are very thankful for the efforts of the editor and the reviewer's comments. We have tried to respond to the reviewer's comments, incorporate any necessary components, and have made a great amount of changes in our manuscript as you will find in the "Revised Manuscript". We believe that the changes have made our manuscript more acceptable to the journal.

In brief, as you would find in the manuscript, we introduced an electrocardiogram recording method using lead II. This electrocardiogram measurement has many advantages: it is a technically simple procedure, is inexpensive, has short measuring time, and is affordable even in small mice. We validated this protocol in mice treated with agonists and antagonists of the autonomic nervous system, confirming the reliability and sensitivity of this ECG protocol. We believe that despite the limitation of using anesthetics, this method can be used as a simple and fast screening approach to making ECG measurements.

All authors have read and approved the submission of the manuscript and have declared that they have no competing interests. This manuscript has not been published and is not being considered for publication elsewhere, in whole or in part, in any language, except as an abstract. We hope that our article is suitable for publication in JoVE.

Sincerely,

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