Electrocardiogram

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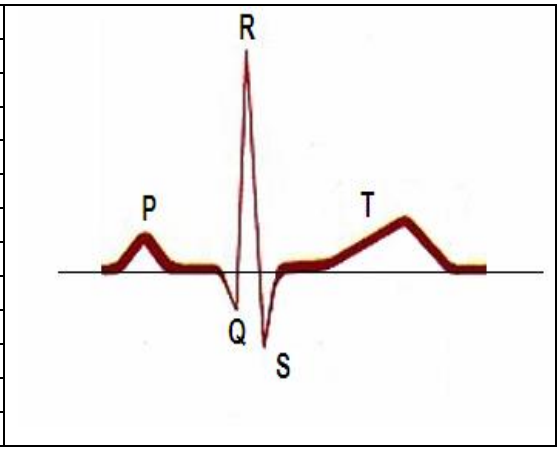
Ref: Daugherty A. JOVE 2009

Summary:

This procedure describes the technique to monitor cardiac markers of electrophysiology in conscious mice using electrocardiogram (ECG). The ECG collects heart function and cardiac conduction data to evaluate cardiac function. Summary of parameters collected by the ECG.

Abbreviation	Parameter
N	Number of signals
HR	Heart rate(bpm)
CV	Coefficent of variability
RR	R waves intervals
PQ	PQ interval (msec)
PR	PR interval (msec)
QRS	QRS interval (msec)
QT	QT interval (msec)
ST	ST interval (msec)
QTc/QTcdisp	QT/HR & QTc dispersion
Ramp/Pamp	Amplitude of specific waves
L/F ratio	Low Frequency:High Frequency



Reagents and Materials:

Reagent/Material	Vendor	Stock Number
For ECG on conscious mice		
ECGenie Instrument	Mouse Specifics	
ECGenie-Lead plates/electrodes	Mouse Specifics	MSI1001
PC equipped with ECGenie	Mouse Specifics	
Cardboard cut outs measuring 6 x 12 cm		
For ECG on anesthetized mice		
Rodent Surgical Monitor Platform	Scintica-Indus	
Nair/Cotton swabs		
4-8 channel analog to digital converter (ADC)	AD Instruments	
PC equipped with LabChart software	AD Instruments	
Optional: electrodes to capture ECG	AD Instruments	
Optional: Pulse oximeter probe	Scintica-Indus	
Isoflurane USP bottles		

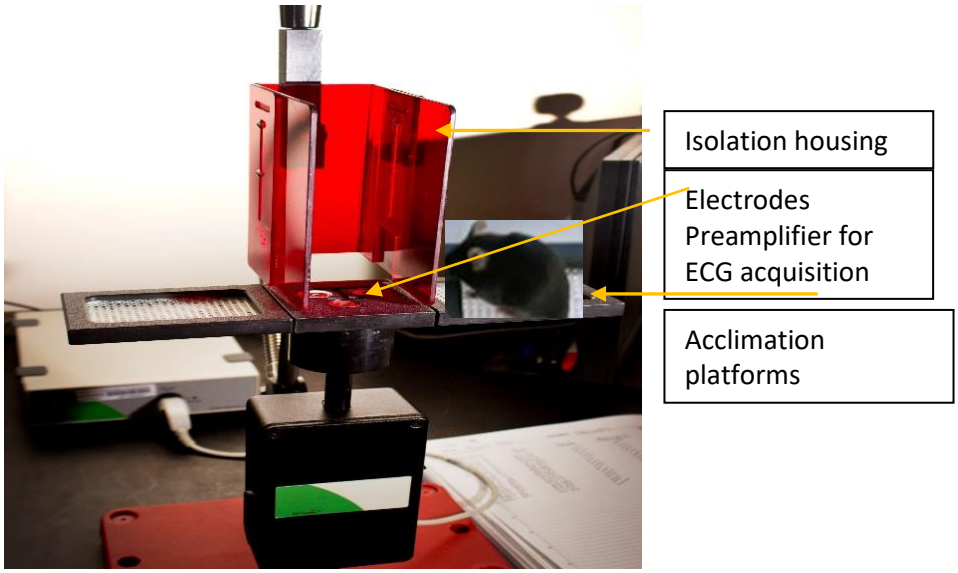
Compressed oxygen tanks		
Charcoal cannisters		
Isoflurane vaporizer/ delivery systems	Kent Scientific	
Anesthesia Induction Chamber		
General use		
Scale	Mettler-Toledo	
Lab coats/gloves/PPE		
70% ethanol/paper towels		
Disinfectant	10% Nolvasan	
Disinfectant Coverage Plus	Steris	

Protocol:-Conscious mice

1. SET-UP

Equipment preparation

- **a.** Snap a disposable ECG-lead plate onto the preamplifier platform at the top the ECGGenie tower. The lead is three gel coated pads surrounded by a sticking plate that goes on top on the preamplifier.
- **b.** **NOTE:** Lead plate should be replaced when switching from males to females or if proper signals are difficult to obtain; urine and feces will decrease the conductivity of the lead plate.
- **c.** Clean the lead plate with 10% nolvasan between mice of a given sex to remove any urine, feces or odors from previous mice.
- **d.** Turn on the combined amplifier and pre-amplifier.
- **e.** Turn on computer; double-click on ECG acquisition icon to open software.



NOTES:

- Acclimate mice (in their homecage) to the testing room 30min prior the procedure.
- Electrocardiograms were recorded in a dim and quiet analysis room.
- To eliminate circadian influences, record ECG at the same time of day.
- Weight mice prior testing.

ECGGenie software activation:

a. Open the ECG set up file (for default settings).

b. Go to file, click New, a pop-up will appear called New Document, be sure Settings from document "ECGenie_Setup_STARTUP" is selected, click OK. A new document with all default settings should initiate.

2. PROCEDURE

a. After mouse has acclimated to room, place mouse on platform located on either side of the electrode platform for 10 minutes prior to collecting data. You can acclimate up to 5 animals on the platform at a time; 2 on each side of the electrodes and one on the electrodes.

b. Once acclimated to platform, place one mouse between walls of red acrylic housing and on the lead plate; gently prodding mouse with soft end of cotton-tip swab may assist the mouse l in making proper contact with the conducting plates.

c. Place a cardboard cutout on top of the red acrylic housing to create a "ceiling" and prevent rearing. Ensure the cardboard cutout is not touching the mouse.

d. Press "Start"; monitor computer screen for distinct electrocardiograms; approximately 4 seconds of data acquisition provides 20 to 30 good ECG signals in mice sufficient for heart rate and measuring PQRST intervals. If you are having a hard time acquiring a decent signal after about a minute, try lightly blowing on the animal to entice it to freeze in place.

e. Once data collection is complete, toggle the "Start" button to pause data collection; position browser on computer screen and "left click/drag the cursor" to highlight the segment of the ECG to analyze and archive.

f. Click on "File" button top left corner of the screen; click "Save Selection" option. Assign a file name for the recording and choose a directory in which the file is to be saved; make sure file is saved as "chart text file" (and NOT "chart data file").

g. A pop-up menu with quality assurance settings (channels, time, comments, settings, out-of-range settings) will appear; click "OK". If signal includes portions that exceed the bounds of the window, select "Clip out-of-range values" option so that the analysis software is correctly instructed to ignore out-of-range data; data is now ready for analysis by e-Mouse®ECG Software.

h. Resume data collection again by toggling "Start" button.

i. Repeat until data collection is complete; ~12 ECG's can be recorded in ~ 1 hour.

j. Once all testing is complete, wipe down all equipment with coverage plus working solution and allow to dry.

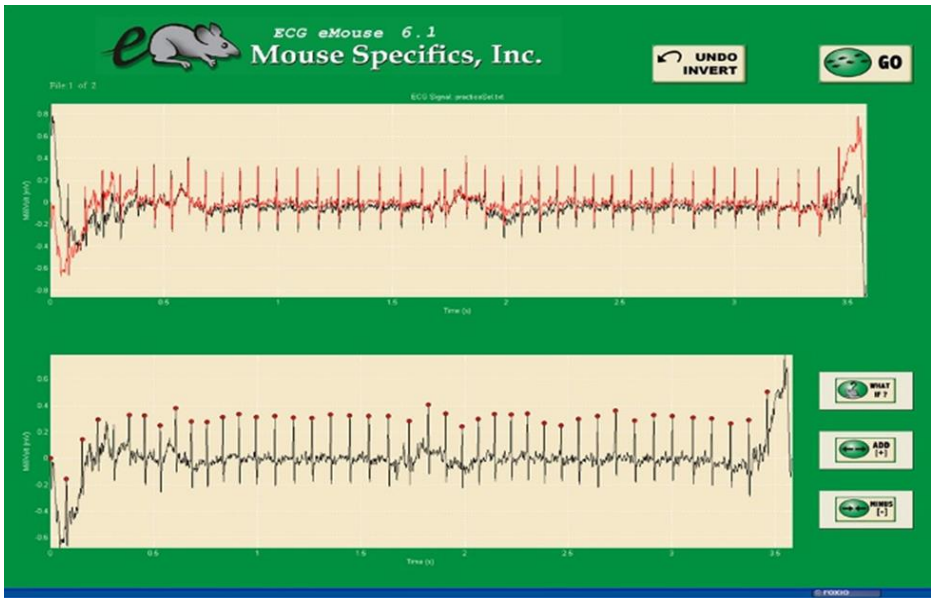
3. CLEAN UP

a. At the end of the procedure, disinfect the instrument and equipment with 10% Nolvasan (do not use alcohol).
b. Clean room and bench top with Coverage Plus.

4. SUPPLEMENTARY INFO: Data analysis

The ECGGenie software uses a peak-detection algorithm to find the peak of R waves and to calculate heart rate. The software plots its interpretation of P, Q, R, S and T for each beat so that heart rate, QRS duration, PQ interval, PR interval, QT interval and ST interval are measured and reported automatically. Noise and motion artifacts are rejected automatically by the software.

a. Open Emouse Analyses icon
b. Select ECG signals
c. Open Directory where and the files to analyze



d. Bottom image shows the correct file for analysis with red dots visible on peak of R waves, if image appears inverted click invert.
e. Click Add, or minus if R waves are not marked with red dots or if too many are marked
- L click to zoom in
- R click to zoom out

f. Use "What if?" button to remove unwanted sections
- L click image (zooms in)
- L click left boundary
- L click right boundary

g. Hit save, a new results folder will be created within the folder with the ECG analyzed data. Then can click quick save or next.

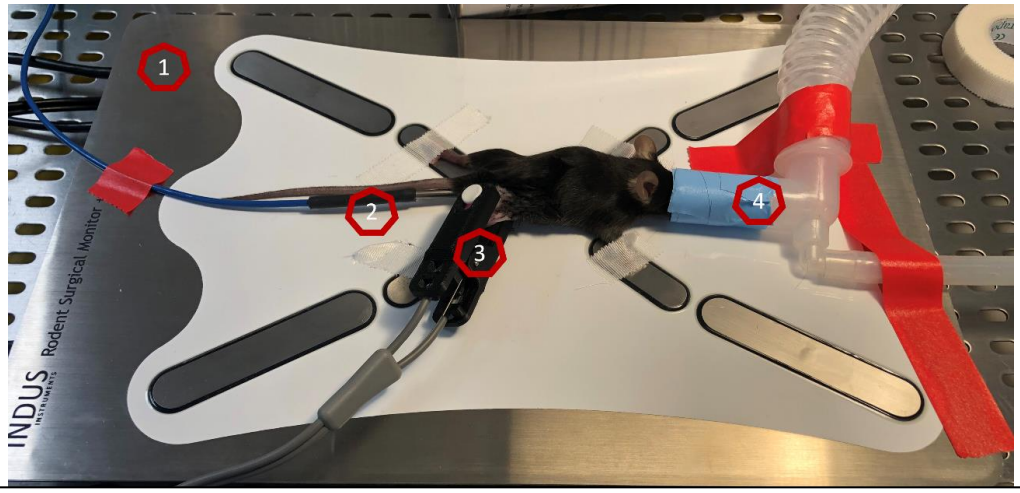
Protocol:-Anesthetized mice

1. SET-UP

a. Bring mice from vivarium to testing laboratory and acclimate for 30 minutes.
b. Make sure the rectal probe, pulse oximeter and ADC are connected to the Rodent Surgical Monitor (RSM).
c. Turn on the RSM, tablet and laptop.
d. Open the RSMonitor app on the tablet. Click the SETTINGS button on the lower right corner and enter an Experiment Name and Animal Identifier. Click on ECG and verify the electrodes are set for a mouse in a caudal, prone position. Click on Pulse Oximeter and verify the sensor type is selected as thigh and the heart rate range is 240 to 960 bpm. Click OK to save changes to the settings.
e. Open LabScribe on the laptop. Under the Settings menu choose ECG Pulse Ox. Press Preview to see live data.

2. PROCEDURE

a. Place mouse in induction chamber and anesthetize with 5% isoflurane. When mouse is ready, switch isoflurane flow to nose cone and set at 2.5%.
b. Transfer mouse to the warmed Rodent Surgical Monitor (RSM) in a prone position with the nose in the cone.
c. Apply Nair with a cotton swab to the right hindlimb and remove fur with a damp paper towel after 30 seconds.
d. Apply electrode cream to each foot and use surgical tape to secure each foot to an electrode on the RSM.
NOTE: Alternatively insert needle electrodes subcutaneously into the limbs using Lead Configuration I: Left Arm – Right Arm (LA-RA) and/or Lead Configuration II: Left Leg – Right Arm (LL-RA).
e. Position the rectal probe and monitor the temperature on the RSM (should be about 37^{o}C).
f. Attach the pulse oximeter to the right thigh and cover with a dark cloth.
g. Adjust the position of the feet and pulse oximeter until a consistent ECG and pulse plethysmograph are observed on the RSMonitor app. The ECG and pulse oximeter filters can be adjusted under SETTINGS if the signals are unclear.
h. Press RECORD on the RSMonitor app and in the main window on LabScribe (will stop recording after 2 minutes). Press STOP RECORDING on the RSMonitor after 2 minutes.
i. In the RSMonitor app, click SETTINGS and enter the Animal Identifier for the next mouse. In LabScribe, save the data with an appropriate name and extension (*.iwxdata). Open a new file to collect data for the next mouse.



Mouse in caudal, prone position on Rodent Surgical Monitor (RSM).
1: Heated RSM; 2: Rectal temperature probe; 3: pulse oximeter thigh clip; 4: nose cone.

3. CLEAN UP

- **a.** Remove the pulse oximeter, rectal probe and surgical tape and return the mouse to its cage for observation. Anesthetize the next mouse as needed.
- **b.** After each mouse, clean the RSM, nose cone and induction box with 70% ethanol.
- **c.** Return mouse to its homecage/vivarium.

4. SUPPLEMENTARY INFO: Data analysis

Oxygen Saturation

- **a.** Open the RSMonitor app on the tablet and click FILE PLAYBACK on the lower right corner. Select the file for the mouse to be analyzed and click Export. Click Browse and select ExportedFiles as the destination folder. Make sure only the SpO2 box is checked and click Export.
- **b.** Plug the tablet into the laptop with the Display Unit Power Cable. On the laptop, use the File Explorer to navigate to Galaxy Tab S7>Tablet>RodentMonitor>ExportedFiles and move the .csv file to the laptop.
- **c.** Open the file in Excel and average SpO2 over 1 minute of data collection (=AVERAGE(B120011:B360011).

ECG Analysis

- **a.** Review the tracing and note any abnormal findings. Take images for upload where required.
- **b.** Analyse all or a selection of the tracing (approximately 100 beats minimum).
- **c.** Open LabChart software, select Mouse preset and QTc = Bazett.
- **d.** The software will automatically mark the P, QRS and T waves of each beat (Beats to average = 1) and calculate the interval and amplitude data. The average of all beats will be determined for each parameter.
- **e.** Click the Export button at the bottom of the data table and save the file (*.csv) to the laptop.