2.6. 12-lead non-invasive mouse electrocardiography (ECG) (Duan lab)

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A. OBJECTIVE

To study electric changes of the heart in an intact mouse.

B. CAUTIONS

- An appropriate anesthetic regimen is pivotal (Kass et al, 1998). Numerous anesthetics have been tested in mice with significant variations in heart rate and blood pressure (Bostick et al, 2011). Isoflurane is increasingly becoming the anesthetic of choice because it is readily available and easy to titrate. Further, isoflurane provides rapid induction and recovery.
- Mouse heart rate is strongly dependent on temperature and the mouse should be kept warm during all phases of the procedure. Induction chamber and operating tables should be monitored with a thermometer to ensure the temperature is kept between 36-38°C.
- While switching between different leads the recording of the ECG tracing should be switched to the monitoring mode. This will aid in differentiating between different leads during analysis and remove any noise from the tracings during switching. Certain heating pads may cause electrical interference during recording and should be switched off during recording.
- Mice with severe muscle disease (such as utrophin/dystrophin double knockout mice and DBA background mdx mice) require extra care during anesthesia because of poor respiratory function. Isoflurane should be carefully titrated to prevent respiratory depression and maintain a respiratory rate above 80 per minute.
- To thoroughly evaluate heart electrical activity, we recommend performing 12-lead ECG assay. Depending on the model used, one may notice abnormal changes in certain leads only.

C. MATERIALS

- PowerLab 4/S data acquisition system (AD Instruments, Colorado Springs, CO, USA).
- Single channel Bio amplifier model ML132 with sub-dermal needle electrodes (AD Instruments).
- ECG lead selector (AD Instruments).

- Chart software v5.5.6 (or higher) with ECG extension module (AD Instruments) .
- Isoflurane portable anesthesia aystem (Summit Medical Equipment, Bend, OR, USA).
- Isoflurane (VetOne, Median, ID, USA).
- Oxygen tank containing 100% oxygen (Airgas National, Charlotte, NC, USA).
- Thermophore heating pad (Medwing, Columbia, SC, USA).
- Heating lamp (Tensor Lighting Company, Boston, MA, USA).
- Vicks digital thermometer (Kaz, Hudson, NY, USA).
- Thermolyne 589 rectal digital pyrometer (Barnstead International, Dubuque, IA, USA).
- Straight serrated fine tip forceps (World Precision Instruments, Sarasota, FL, USA).
- Chart software v5.5.6 or higher with ECG Extension Module (AD Instruments)

D. METHODS

1. Mouse anesthesia. Place the mouse into a clean, empty cage by itself for 5 minutes prior to anesthetizing. Gently transfer the mouse into a pre-warmed induction chamber and anesthetize with 3% isoflurane at an oxygen flow rate of 2 l/min for about 2.5 minutes.
2. Promptly remove the mouse from the induction chamber. Place the mouse onto a pre-warmed recording table with snout inserted into nose cone. Provide maintenance anesthesia of 1-1.5% isoflurane at an oxygen flow rate of 0.5 – 0.6 l/min.
3. Secure mouse limbs to the recording table (Figure 1) using tape placed over the paws. Leave one lower limb unsecured for monitoring the depth of anesthesia with toe pinch.

Figure 1. Mouse ECG recording table.

4. Lubricate the rectal temperature probe with surgical lubricant and gently insert 2-3 mm into the rectum.
5. Insert the sockets of the needle electrodes onto the pins in the ECG lead selector cable.
6. Place the needles subdermally into their corresponding positions on the mouse. To place needles, gently lift the mouse skin with serrated forceps and insert the needle into the resulting skin tent. Limb leads should be placed parallel to the limb running distal to proximal. The chest electrode should always be inserted parallel to the sternum running in the direction of the head to the toe. For the V1 position, the needle should be ~ 1-2 mm to the right of the sternum. For the V2 position, the needle should be placed ~ 1-2 mm to the left of the sternum. V3 should be placed ~ 1-2 mm medially to the left mid-clavicular line. V4 should be placed at the left mid-clavicular line and slight further caudally. V5 should be located at the anterior axillary line slightly further caudally than V4. V6 should be placed at the mid-axillary line slightly further caudally to V5.
7. Record a 1-minute long rhythm strip from the lead II position once the mouse temperature has stabilized between 37-38°C.
8. Record other limb lead sequentially with each lead for about 15-20 seconds.
9. Sequentially record the chest lead tracings for about 15-20 seconds for each lead by moving the needle electrode to each placement listed above.
10. Record a second 1-minute rhythm strip from the lead II position.
11. Immediately turn the maintenance isoflurane to 0 % after the last lead is recorded. Allow the mouse to breath 100% oxygen at 0.5-0.6 l/min while it awakens.
12. Carefully remove all electrodes, the rectal probe and tape restraints.
13. Once the mouse is awake and able to right itself, gently return it to the empty cage.
14. Monitor the mouse carefully for the next 15 minutes before returning it to the original cage.

E. EVALUATION AND INTERPRETATION OF RESULTS

1. Merge each lead tracing into a single signal averaged ECG (SAECG) by utilizing the block averaging function.
2. Identify the P wave, QRS complex and T wave. The P wave is 1st positive deflection of every cycle. The Q wave is the 1st negative deflection following the P wave but it may not always present. The R wave is the 1st positive deflection following P wave and it is usually the largest wave of the cycle. The S wave is the 1st negative deflection immediately after the R wave but it may not always present. T wave is the 1st positive deflection after the S wave.
3. Identify the RR interval, PR interval, QRS interval, and QT interval. The RR interval is the time interval from the one R wave to the next R wave. The PV interval is the time interval from the beginning of the P wave to the beginning

of the Q wave (or R wave if no Q wave is present). The QRS interval is the time interval from the beginning of the Q wave (or R wave if no Q wave is present) to the peak of the S wave. The QT interval is the time interval from the beginning of the Q wave (or R wave if no Q wave is present) to the end of the T wave (the point at which the T wave returns to the isoelectric point).

4. Average all of the "RR Interval" and then take 60000 divided by this number to get "Bpm" (beat per min).
5. Take the square root of the average of all the "RR Interval" and divide the "QT Interval" by this value to get the "QTc Interval" (corrected QT interval).
6. The beginning and ending of the different waves are often ambiguous. For example, from point 1 to point 2 in the P wave may be a positive change but, point 2 to point 3 may be a small negative change. We have tried to account for this by writing the rule such that a point is compared to 4 previous points to ensure that this is not just a momentary dip. However, this may be problematic for small waves because we are unsure how many points to compare.

F. REFERENCES

Bostick B, Yue Y, Duan D (2011) Phenotyping cardiac gene therapy in mice. Methods Mol Biol **709:** 91-104

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