Video Article

Programmed Electrical Stimulation in Mice

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Abstract

Genetically-modified mice have emerged as a preferable animal model to study the molecular mechanisms underlying conduction abnormalities, atrial and ventricular arrhythmias, and sudden cardiac death.[1] Intracardiac pacing studies can be performed in mice using a 1.1F octapolar catheter inserted into the jugular vein, and advanced into the right atrium and ventricle. Here, we illustrate the steps involved in performing programmed electrical stimulation in mice. Surface ECG and intracardiac electrograms are recorded simultaneously in the atria, atrioventricular junction, and ventricular myocardium, whereas intracardiac pacing of the atrium is performed using an external stimulator. Thus, programmed electrical stimulation in mice provides unique opportunities to explore molecular mechanisms underlying conduction defects and cardiac arrhythmias.

Protocol

Part 1. Surgical preparation

1. In case programmed electrical stimulation experiments are performed as survival surgeries in mice sterile conditions will be necessary. However, the most common type of experiment is terminal in nature, for which regular clear surgical techniques suffice.
2. The mouse is anesthetized using 2% isoflurane in 0.5 L/min 100% O_2.
3. Hair clippers are used to shave the fur from the neckline to mid chest level.
4. The anesthetized mouse is placed in a supine position, with its limbs taped onto ECG electrodes incorporated in a heating board (Indus Instruments, Houston, TX). And the surgical area is disinfected with 10% Povidone Iodine. A recommended system includes a rectal temperature probe connected to a heating pad controlled by a thermoanalyzer system, such that the body temperature is maintained at 37.0 °C ± 1.0 °C.

Part 2. Cannulation and insertion of EP catheter into right atrium and ventricle

1. After confirming by a toe-pinch that the mouse is fully anesthetized, an 1/2 inch incision is made to the right of the midline with the caudal terminus at the level of the clavicle.
2. Subcutaneous tissue, salivary glands, and lymphatic tissues are separated by means of blunt dissection to visualize the right jugular vein.
3. The proximal end of the vein is tied with a 6-0 suture. Pulling gently on this suture will keep the internal jugular vein straight while inserting the catheter. Another suture is placed under the vein at the distal end of the visualized segment. This suture will be tied around the catheter once the catheter is placed optimally within the heart, to ensure hemostasis and maintain the catheter in the desired position (**Figure 1**).
4. The computer-based data acquisition is now started to record surface ECG and 4 leads of intracardiac electrograms simultaneously (i.e., IOX-2 acquisition software, Emka Technologies, VA, USA). For intracardiac electrogram recording, electrodes on the catheter are connected to an external stimulator in "recording" mode (i.e., model STG3008, MultiChannel Systems, Reutlingen, Germany).
5. Using micro-scissors, a small incision is made in the longitudinal direction of the vein, and the 1.1F octapolar catheter (EPR-800, Millar Instruments, Houston, TX) is advanced through the vein into the right atrium. Gently pulling on the proximal suture will help keep the internal jugular vein straight and will allow easier passage of the catheter into the right atrium and ventricle. Proper catheter position is verified by visualization of the waveforms of the 4 intracardiac electrograms at the level of the apex of the right ventricle, the base of the right ventricle, the atrioventricular node, and the right atrium, respectively (**Figure 2**).
6. The distal suture is now tied off to secure the catheter position and prevent possible bleeding.

Part 3. Programmed Electrical Stimulation

1. In the case of selective atrial pacing, the electrode pair located inside the atrium is switched from "recoding" mode to "stimulation" mode, while the other electrode pairs remain in "recording" mode.
2. To determine whether a mouse has an increased vulnerability to atrial arrhythmias, programmed electrical stimulation of the right atrium is performed. First, the atrial pacing threshold is determined by applying 2-ms current pulses (at least 50) at different basic cycle lengths (BCL) to test for consistency of stimulus capture. The BCL of the initial pulse train is slightly lower than the intrinsic BCL, and is decreased by 10 ms (for example, 100 ms, 90 ms, 80 ms, and 70 ms). The typical current amplitude required for stimulus capture is 100-200 μA.
3. The sinus node recovery time (SNRT) is measured after applying a 15-s atrial pacing train at a BCL of 100 ms. SNRT is defined as the interval between the last stimulus in the pacing train and the onset of first spontaneous sinus beat.
4. The atrial effective refractory period (AERP) is determined by applying series of atrial pacing trains at a fixed BCLs (i.e., 100 ms) with a coupled shorter S2 premature stimulus. The S1-S2 interval is progressively reduced by 2-ms in each pacing train from 70ms to 20ms. The AERP is defined as the longest S1-S2 coupling interval for atria that failed to generate a propagated beat with S2 (S1 being the regular train pulse, and S2 the premature stimulus). Between each stimulation protocol, there was a recovery period of at least 30 seconds.
5. The effective refractory period of the atrioventricular (AV) node (AVNERP) is determined by applying series of atrial pacing trains at BCL of 100 ms with a coupled S2 premature stimulus. The S1-S2 interval is progressively reduced by 2 ms each pacing train from 70 ms to 20 ms. The AVNERP is defined as the longest S1-S2 coupling interval at which the premature stimulation delivered to the atrium is followed by a His potential but not by a QRS complex. Between each stimulation protocol, there was a recovery period of at least 30 seconds.

6. Inducibility of atrial arrhythmias, including atrial fibrillation (AF), may be tested using the burst pacing protocol described by Verheule *et al.*[2] A series of 2 second bursts is applied to determine inducibility of atrial arrhythmias. The first 2 second burst has a cycle length (CL) of 40 ms, and each successive 2 second burst has a CL of 2-ms shorter than the previous burst, until the final CL of 20 ms. In the absence of an arrhythmogenic substrate, the heart will resume sinus rhythm immediately following the pacing protocol. In the case of atrial flutter, there will be a regular pattern of fast A waves seen on the atrial electrogram, whereas the frequency of the ventricular response is typically slower, as seen on the ventricular electrogram. In case of atrial fibrillation, the surface ECG will show irregular RR intervals in the absence of P waves. Additionally, the atrial electrogram will reveal rapid and irregular A waves, whereas the ventricular electrogram will reveal irregular and slower ventricular waves (**Figure 3**). Arrhythmia induction protocols are typically performed in triplicate, and an arrhythmia is considered present if it can be evoked in at least 2 out of 3 trials.[3,4].

Part 4. Removal of Catheter

1. After all pacing protocols are finished, data acquisition is stopped. The suture at the distal end of the catheter is gently cut off, in order to release the catheter.
2. In case of terminal EP studies, the knot is gently loosened to release the catheter.
3. At the conclusion of the study, the mouse will be humanely euthanized while under isoflurane using cervical dislocation.

Representative Results

Surface ECG and intracardiac electrograms are recorded simultaneously throughout the *in vivo* electrophysiology study, and are reviewed in detail following the completion of all protocols. The baseline electrophysiological parameters include PR interval, PQ interval, QRS duration, QT intervals, and QRS morphology. These parameters can be measured manually, or automatically using the data acquisition software IOX-2 or ECG-AUTO (Emka Technologies, VA, USA).

The SNRT, AERP, and AVNERP provide information regarding the sinus node "pacemaker" function, atrial, and AV nodal conduction properties, respectively. Examples of pacing-induced episodes of atrial fibrillation can be found in the paper by Chelu *et al.*[4]

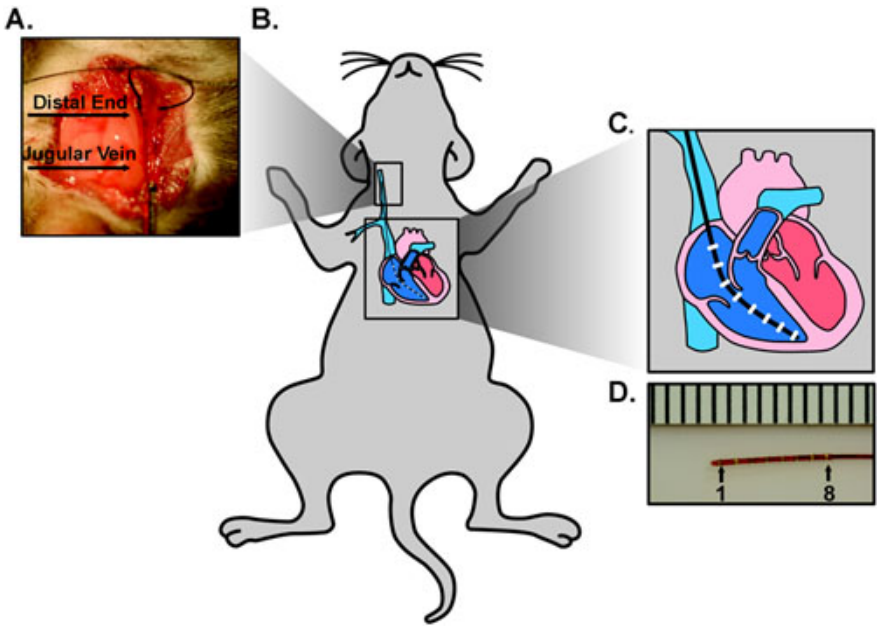

Figure 1. **Illustration of intracardiac catheterization for electrophysiology studies in mice. A.** Right internal jugular vein is isolated and cannulated. The distal end of vein is tied off once proper catheter position is achieved. **B**. The mouse is placed in a supine position for this procedure. **C.** Cartoon depicting the intracardiac position of catheter. Pair of electrodes are positioned at the level of the apex of the right ventricle, the base of the right ventricle, the atrioventricular node, and the right atrium, respectively. **D**. Close-up of the 1.1F octapolar catheter. Figure modified from Mathur *et al*. with permission of Circ Arrhtyhm Electrophys [5].

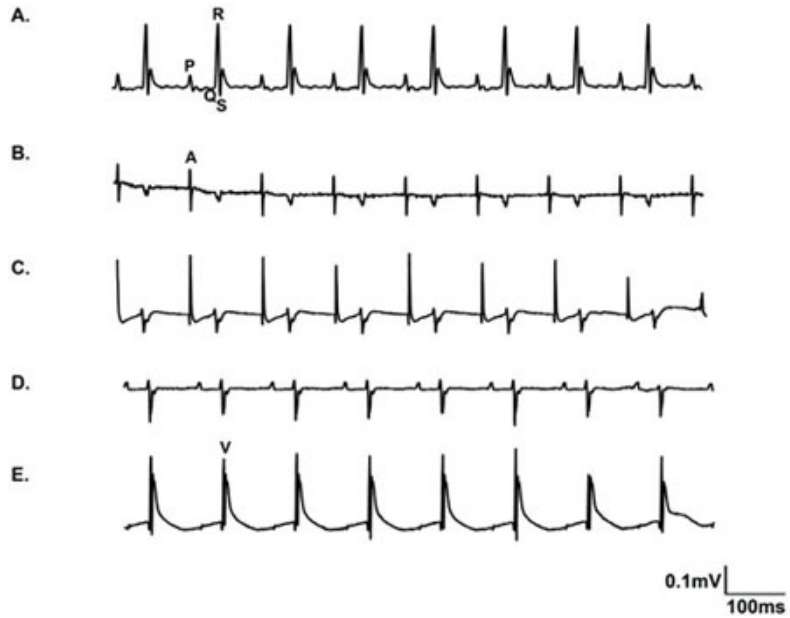

Figure 2. Representative Surface ECG and Intracardiac Electrograms in a Mouse. (A) Surface ECG in lead II configuration showing regular sinus rhythm with a frequency of 540 beats per minute. **(B-E)** Bipolar intracardiac electrogram recordings at the level of the right atrium (**B**), atrioventricular node (**C**), and base of the right ventricle (**D**), and the apex of the right ventricle (**E**), respectively. Note that the intracardiac A-wave in panel B corresponds to the P-wave on the surface ECG. The V-wave on the ventricular electrogram corresponds to the QRS wave on the surface ECG.

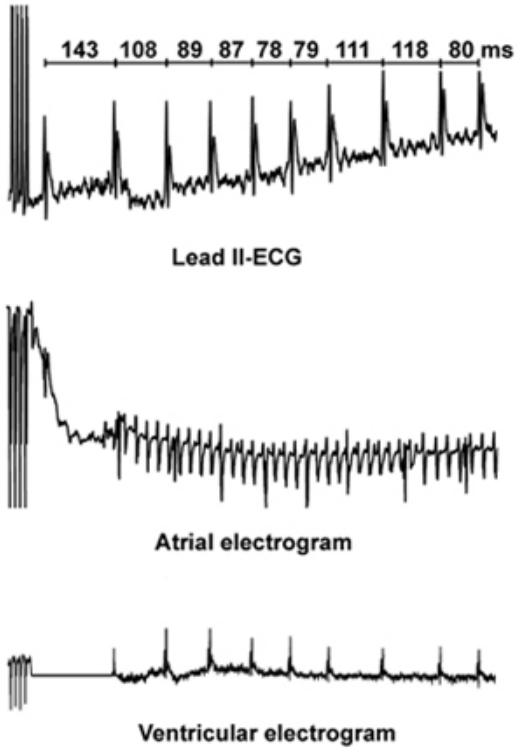

Figure 3. Representative surface ECG and intracardiac electrograms in a mouse that developed atrial fibrillation after atrial burst pacing.

Discussion

During cardiac catheterization, extensive bleeding during catheterization could increase the heart rate due to hypovolemia. In this case, an intraperitoneal injection of sterile saline (0.3 1.0 mL) could normalize filling pressure and reduce hemodynamic stress in the mouse.

Exposure to isoflurane longer than 2 hours, or higher concentrations of isoflurane (>2 %) could suppress cardiac and respiratory functions in the mouse. Therefore, it is recommended that all studies be completed in less than 2 hours. Moreover, it is essential that the body temperature is always maintained within the normal range 37.0 ± 1.0°C. Both hypothermia and hyperthermia will affect the heart rhythm and the potential presence of an arrhythmogenic substrate.

Each experiment should begin by determining atrial capture thresholds. In the case of atrial pacing, atrial threshold, SNRT, AERP, and AVNERP should be determined to assess whether the conduction properties of the sinus node, AV node, and atrial tissue are normal.

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