Experimental Study

Electrophysiological Measurement of Rat Atrial Epicardium Using a Novel Stereotaxic Apparatus

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Summary

Flexible, in vivo maneuverable electrophysiology mapping techniques are not available in rat models. A novel cardiac stereotactic electrophysiology epicardial mapping system (CREAMS) allows for various measurements, including: (1) recording unipolar electrograms at multiple sites; (2) positioning of mapped sites and precision testing (Distance between the two “centers” = 297 ± 54 μm, n = 15); (3) evaluation of electrophysiology in an in vivo Sprague-Dawley rat model with high-frequency stimulation (HFS)-induced Atrial fibrillation (AF) at high right atrium (HRA) sites. We found that of the right atrium dispersion of effective refractory period (P < 0.05) and the window of vulnerability (P < 0.01) were significantly increased (P < 0.05) after HRA HFS. CREAMS has the potential for convenient electrophysiology assessment in a rat AF model through stereotaxic and flexible operating manipulation.

Key words: Atrial fibrillation, Electrophysiology mapping, In vivo rat model, Stereotaxic system, Window of vulnerability

Atrial fibrillation (AF) is the most common type of arrhythmia that is accompanied by high mortality and morbidity. Historically, large animals are preferred in AF studies. Rats have not extensively been used in AF studies because the lack of critical cardiac mass was thought to be problematic in inducing AF. Increasing evidence this theory, and rat AF models have become more popular. The isolated atrium is invaluable when studying electrophysiological mechanisms for the deprivation of intact regulatory mechanisms. In these cases, an in vivo rat AF model may be of great potential. When reviewing available in vivo models, most include catheter-based and multiple electrode array-based recording of electrograms and action potentials. However, these techniques have several limitations, including poor flexibility and positioning incompetence of both the catheter and the multiple electrode array, as well as a confined mapping range. Recently, optical mapping has been proven to be a useful method in a rat AF model in vivo, however, optical mapping has not been adequately used in an in vivo AF rat model. Therefore, in the present study, we established cardiac stereotactic electrophysiology epicardial mapping system (CREAMS), which remedies the limitation of popular methods and allows for convenient electrophysiological assessment of AF in an in vivo rat model.

Methods

Animals: In this study, seven- to nine-week-old adult male Sprague-Dawley rats (300-400 g) were used that were supplied by the Model Animal Research Center of the Fourth Military Medical University (Xi’an, China). Rats were anesthetized using urethane (1.4 g/kg, i.p.) and supplementary doses (0.7 g/kg) were given every 50-60 minutes for anesthetic maintenance. First, a tracheal tube was inserted. Next, the right common carotid artery was cannulated, and a cannula filled with heparin (250 U/mL) was inserted for blood pressure (BP) monitoring. To maintain fluid balance, a second cannula was inserted in the right femoral vein for administration of isotonic saline (50 μL/minute). In addition, a cannula was inserted into the trachea after tracheotomy (4 mL/kg at 90-110 strokes/minute). Mouse husbandry, handling, anesthesia, and instrumentation are presented in Supplemental Text.

Electrocardiogram recording and BP measurements: For continuous recording, six surface lead electrocardiograms (ECG) as 25-gauge subcutaneous needle electrodes

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were inserted in each rat limb. BP was recorded with a Taimeng uniflow transducer coupled to a BL-420S biological signal collection system (Chengdu Taimeng Technology Co., Ltd., Chengdu, China). ECG and BP measurements were recorded and analyzed via a BL-420S. ECG signals were amplified (× 5,000-10,000) and filtered (0.1-150 Hz). BP measurements were collected at a frequency of 2000 Hz.

Establishing a novel Cardiac Stereotactic Electrophysiology Mapping System (CREAMS) and measurement of atrial electrophysiology:

Electrode preparation Handmade insulated stainless-steel mixed electrodes were prepared using glass tubes (2 mm diameter, 5 cm long) and three pieces of stainless-steel wire (250 μm diameter, 10 cm long) with Teflon sleeves (Supplemental Figure 1A). One of the electrodes served as a cathode of stimulation. To arrange a current loop, the anode was inserted into the muscle near the inferior detached breastbone. The remaining two electrodes acted as unipolar electrogram, exploring electrodes that consisted of an elastic strand of twisted double stainless-steel wire. The method of handmade electrodes was described by Knollmann, et al. To avoid simultaneous contact with the myocardium, a reference electrode was placed 1 mm proximal from the tip electrode (Supplemental Figure 1B). The reference electrode was hung in the air without connecting the surrounding area, which adopted the floating ground. With such a connection, voltages and current flows were induced by electromagnetic fields or charge accumulation within the conductor rather than by the usual external potential difference of a power source. To minimize tissue damage, electrode surfaces were polished and rounded.

Cardiac surgery Cardiac surgery involves opening of the chest through a median sternotomy at the fourth intercostal space or of an incision superior to the intercostal at the cardiac point of maximal impulse. For additional exposure of both atria, the bilateral costa inferior was removed and an incision was made across the pericardium. Then, a thread was stitched through the apex cordis and a knot was tied. At both ends of the thread, a 3-5 cm piece was left in place to connect the cardiac repositor. During this process, blood loss and damage to the lungs were limited by the arteriae thoracicae internae. Physiologic saline was used to hydrate the heart.

Introduction and application of CREAMS CREAMS primarily consisted of three parts, including a stereotaxic apparatus (Figures 1A, B, C, and D), a cardiac repositor (Figures 1E and F), and an all-around rotating electrode holder (Figure 2). The stereotaxic apparatus was equipped with a X-Z axis micro-propulsion, which allowed for movement in a horizontal or vertical direction when knobs for the X and Z axis were manipulated (X axis, horizontal direction; Z axis, vertical direction) and two Y axis orbits (Y axis, anterior-posterior direction, Figures 1A and C). This enabled micro-propulsion movement along the Y axis orbits and allowed for coordination at each site along the X, Y, Z axis. This coordination was referred to as the "general positioning coordinate" and was denoted as X, Y, Z. At the end of the vertical fixed rod, an all-around rotating electrode holder with scales was created to plug into the electrode rod and the screw was fastened, which allowed for all-around rotation using a universal joint - a metal sphere (Figure 2). The sphere was able to move in horizontal (X' axis) and vertical (Y' axis) plane. The head of the holding and the local rotatory electrode holder, allowed for self-rotation (Z' axis). This allowed for adjustment of the electrode if necessary. We used an angle of deflection from zero to serve as the scale of X' and Y', which was 180° (from −90° to +90°) (Figures 2A and C). The scale for Z' was 360° (from −180° to +180°) (Figures 2A and C). Local electrode positioning coordinates represented ± X', ± Y', ± Z'.

Rats were connected by the cardiac repositor and were positioned in the middle of CREAMS. The cardiac repositor consisted of a fixator and an elastic reset device. The device can counteract the disturbance from random cardiac displacement by the electrode. Any direction of displacement from pushing of the electrode caused the elastic to reset. When removing the external force on the atria, the heart would recover to the equilibrium position and return to the zero point (Figures 1E and F). This would guarantee the feasibility for repeated positioning on one site. The electrode was secured into the electrode holder and was connected to a biological signal collection system (BL-420S). Rotating the micro-propulsion enabled the electrode to be close to each of the measured sites. Given the cardiac morphological character of the measured site, the rotating electrode holder could be adjusted to let the electrode face the measured site. Unipolar electrograms could be identified from oscillogram tracing by the BL-420S biological signal analysis software (Figure 3). After signal stabilization, each measurement site was confirmed. When operating the all-around rotating electrode holder, attention should be paid to prevent the universal joint from self-rotating. This could be tested through the counterpoint between the surface-marked scale of universal joint and the coordination around the universal joint (Figure 2A). Both the superposition and agonic needed to be guaranteed to meet the criteria for positioning of the plane coordinates of the universal joint and for reading of these plane coordinates (± X', ± Y'), and rotated the local rotatory electrode holder to fine-tune the head direction of the electrode to obtain ± Z' (Figures 2A, C, and D). However, because of the limited curved surface on the sphere, the surface-marked scale may not be superpositioned with the coordination around the universal joint for the spherical surface and may even be angled, especially when the mark scale was close to the margin around the coordinating reading. Therefore, we used a ruler to verify the connecting line (Figure 2A-1) from the point of the mapped site and to its opposite point. Subsequently, the line was used to counterpoint the coordinating reading to guarantee the superposition and agonic with the coordinating reading (Figure 2A). Moreover, for more convenient manipulation of the measuring process at the location, the direction of "X" was often fixed, so that only the directions of Y/Z needed adjustment to achieve the same measure at one site at different times.

Experimental protocol 1 - Electrophysiological study using CREAMS in an in vivo rat model: A total of 12 seven- to nine-week-old adult Male Sprague-Dawley rats
Figure 1. Schematic diagram of cardiac stereotactic electrophysiology epicardial mapping system (CREAMS) and actual pictures. Schematic diagram of the cardiac stereotactic apparatus (A and B) and an actual picture of mapping with CREAMS (C and D). Schematic diagram of cardiac repositor (E and F). A: Coronal plane. B: Vertical plane. Black arrows indicate the direction of movement. Details of the dashed box are highlighted in Figure 4. E: 1. connection point; 2. elastic reset device; 3. fixator; 4. Y-axis slide track with scale. F: Actual picture of cardiac repositor. Direction of arrows indicate the direction of force imposed on the heart. 1. Inverted V track; 2. Y-axis positioning tight screw; 3. Horizontal rotating axis for the vertical arm; 4. Vertical arm with scale; 5. Rotary knob of horizontal movement arm; 6. Vertical movement rotary knob; 7. Vertical fixed rod; 8. All-around rotating electrode holder anchor screw; 9. All-around rotating metal sphere; 10. Sphere center fixed rod; 11. Local rotatory electrode holder; 12. Electrode; 13. Sphere center notch; 14. Metal sphere base; 15. Electrode tight screw; 16. Horizontal arm with scale; 17. Y-axis scale; 18. Slide track device; 19. Local rotatory electrode holder tight screw. Ao indicates aorta; and PT indicates pulmonary trunk.

(300-400 g) were used to finish the measurements of ERP, window of vulnerability (WOV), and HFS in an AF model. We chose rapid firing-induced stimulation as the pattern of HFS (2 x threshold, 20 Hz, duration 2 ms).17)
Figure 2. Schematic diagram of local electrode position and coordinate system (A) and actual pictures (B, C, and D). The position of the electrode can be verified by the coordinates ±X°, ±Y° and ±Z°. An angle of deflection from zero served as the scale for X° and Y°, which was 180° (from −90° to +90°). The black arrows indicate the direction of movement; the scale for Z° was 360° (from −180° to +180°). The solid line indicates the notch of X°-axis and Y°-axis; the dashed line is the suppositive line from the two symmetry points measured with a ruler; the curved arrows indicate the direction of rotation. C: Scale on top of local rotatory electrode holder from plan view. D: Scale on top of a metal sphere base from plan view. 1. actual measurement point; 2. gauge for plane coordinate of the metal sphere; 3. dial for local rotatory electrode holder scale; 4. gauge for local rotatory electrode holder.

Baseline levels of ERP were measured at the six sites prior to HFS at the high right atrium (HRA) site. After 1.5 hours of HRA HFS, ERP was determined at the six sites and after each measurement a 1-minute interval al-
allowed for recovery of atrial electrophysiological stabilization. Because of “the lack of critical cardiac mass”, it would be a challenge to induce AF in healthy rats. HFS at the HRA, leading to atrial electrical abnormality, may help increase the success ratio of AF, thereby allowing for measurement of the WOV.

Programmed stimulation: To produce a desired sequence of rectangular impulses for atrial pacing (at 2 × threshold), a programmable electrical stimulator (SEN-7203, Nihon Kohden, Tokyo, Japan) was employed. Special equipped current isolators (SS-201J, Nihon Kohden, Tokyo, Japan) was employed. Special equipped current isolators (SS-201J, Nihon Kohden, Tokyo, Japan) were used to determine the amplitude of the impulses in milliamperes. At all six atrial sites (HRA, right atrium [RA], low right atrium [LRA], left atrial appendage [LAA], left atrium [LA], and the junction of left atrium and left pulmonary vein [JLA-LPV]), ERPs were determined by using eight consecutive stimuli (S1–S1 = 150 ms) followed by a premature stimulus (S1–S2). S1–S2 intervals started at 100 ms and were reduced by decrements of 10 ms followed by decrements of 1 ms when approaching ERP. ERP values were obtained by measurements in triplicate after which the mean ERPs were calculated. ERP dispersion was calculated offline as the coefficient of variation (SD/mean) of ERPs at all six sites recorded (HRA, RA, LRA, LAA, LA, and JLA-LPV). In addition, we adopted a bursting stimulus to induce AF. In brief, a 30-Hz burst pacing (pulse width 2 ms, 4 × threshold current) was applied for 3 seconds. AF was defined as a rapid (> 800 beats/minute) irregular atrial rhythm that lasted for at least 1 ms.7 In addition, the WOV of RA was chosen for quantitative measurement of the vulnerability of AF, which was defined as the difference between the longest and shortest S1–S2 interval at which AF was induced.

Experimental protocol 2 - Testing the feasibility of positioning using the new cardiac stereotactic system: A total of 10 seven- to nine-week-old adult Male Sprague-Dawley rats (300–400 g) were employed in Group 1. CREAMS was used to record unipolar electrograms and to measure ERPs at baseline level at six different sites. Corresponding coordinates were noted at the six sites and all six sites were relocated with regard to coordinates and unipolar electrograms, and ERPs were recorded. Five consecutive unipolar electrograms were selected and the average of maximum and minimum peak value, amplitude, area under curve, the maximum ascending velocity of voltage (dMax/dt), and the minimum descending velocity of voltage (dMin/dt) were obtained. Comparison of the graphic parameters of the unipolar electrograms allowed for examination of the accuracy of positioning. Data were collected and normalized by using the self-contained measuring software in BL-420s.

To further evaluate the precision of CREAMS, we adopted a bipolar-concentric circular electrode (TM33 CCINS, WPI, USA) using two measures (Supplemental Figure 3). We adopted the approach that temporarily increased the DC output through the electrode to allow for local myocardium denaturation and randomly selected 15 sites in three rats to compare the denatured site of two measures with the same coordinates. After testing, rats were intracardially perfused with 300 mL PBS, then hearts were excised and stained with 2, 3, 5-triphenyltetrazolium (TTC) to delineate the detection sites in the outside surface of both the atrium and the ventricle.24,25 Hearts were post-fixed in paraformaldehyde overnight and dehydrated in a 30% sucrose solution. Then, hearts were sectioned (40 μm), images were taken using a Leica microscope, and the detection sites were visualized by light microscopy (AHBT3; Olympus, Tokyo, Japan). Statistical analysis: Data are expressed as the mean ± standard error of the mean. All data satisfied statistical criteria for normal distribution. Mean values were compared by paired student t-test using SPSS 20.0 software (IBM, New York, NY, USA). Statistical significance was defined as P < 0.05 (two tailed).
Results

In total, 25 out of 30 rats survived the end of the study. The mortality rate was 20%. Two animals died when the right common carotid artery was cannulated, which was presumably caused from massive hemorrhage due to surgical complications. Three animals died within 15 minute of urethane injection, which may likely be due from an overdose of anesthesia. Throughout the measurements, the BP remained stable and heart failure was not observed in the 25 rats that survived.

In vivo electrophysiological measurements in rats: ERP Figure 4 shows the measuring process of ERP at six sites. When compared to baseline level (dERP = 0.147 ± 0.034), dERP was significantly increased by HRA HFS (dERP = 0.219 ± 0.065, \( P < 0.05 \)), indicating that HFS caused electrophysiological remodeling by increasing dERP (Figures 5A, B), thereby causing an increase in atrial heterogeneity to provide a substrate for reentry of AF vulnerability that allowed for convenient measuring of WOV.

AF induction and WOV changes of RA Figure 5 shows the recording of AF by HRA HFS. For obtaining WOV results, we used HRA HFS to allow for easier assessment of WOV. Figures 6a and b show the WOV of RA measurements. Supplemental Figure 2 shows the full process of WOV of RA measurement. When compared with baseline levels, a significant increase in WOV of RA after HFS was observed (0.4 ± 0.2 versus 5.1 ± 0.9, \( P < 0.01 \), \( n = 10 \), Figure 6C)

Comparing the morphology of unipolar electrograms and ERP at the same site measured by CREAMS: Representative coordinates of the six rats are presented in Table I. After obtaining the coordinates at all six sites, these sites were repeatedly relocated using these coordinates to test the positioning veracity of CREAMS. In Group 1, corresponding coordinates were used to repeatedly determine the ERP at six sites. No significant differences were observed in ERP between the first measurement group (baseline) and the repeated measurement group (\( P > 0.05 \)) (Table I). For quantification of the congruence of the wave shapes that were repeatedly measured from unipolar electrograms, the graphic parameters were determined as described above. Table II and shows that at these indexes no significant differences were observed between baseline status and relocated electrograms (\( P > 0.05 \)).

Comparing the precision between two measures using a denatured myocardium method: For the purpose of comparing the precision easily, we chose the ventricle point as an example (Figure 7). The image shows that in both measures the denatured “center” could be clearly ob-
Figure 5. Atrial fibrillation (AF) in high right atrium (HRA) by bursting pacing. After high-frequency stimulation (HFS)-HRA for 1.5 hours, AF was successfully induced by bursting pacing in HRA (30Hz, pulse width 2 ms, 4 thresholds current). From II leads, the R-R interval was obviously irregular, representing the onset of AF. The black box represents a magnified image of the dashed black box.

Figure 6. Unipolar electrogram recording for the determination of the window of vulnerability (WOV) at right atrium (RA) with the cardiac stereotactic electrophysiology epicardial mapping system after high-frequency stimulation (HFS). Prior to HFS, Atrial fibrillation (AF) was hard to induce. After 2 hours of HFS, AF was induced by S1S2. The longest S1S2 = 68 ms (A) and the shortest S1S2 = 65 ms (B) indicated that the WOV at RA equals a difference of 3 ms. (C) Comparison between WOV of RA before and after high right atrium (HRA) HFS. Using the Wilcoxon test, a significant increase in WOV of RA was observed as compared with the baseline (0.4 ± 0.2 versus 5.1 ± 0.9, P < 0.01, n = 10).

served. By measuring the distance between the two “centers” (DTC), the precision between the two measures could be evaluated. Supplemental Table presents the distance at 15 points that were randomly selected in three rats (DTC = 297 ± 54 μm).
### Table 1. Coordinates and ERP at Six Atrial Sites at Baseline and When Relocated

|   | GPC | LEPC | ERP  |
|---|-----|------|------|
|   | X (mm) | Y (mm) | Z (mm) | ± X° | ± Y° | ± Z° | ERPb (ms) | ERPr (ms) |
|1  | HRA  | 7.3  | 20.1 | 35.3 | −9° | 10° | −20° | 54.1 | 53.5 |
|   | RA   | 8.4  | 20.1 | 36.4 | −9° | 6° | −30° | 62.5 | 62.5 |
|   | LRA  | 9.8  | 36.6 | 35.6 | 10° | 5° | 47° | 54.3 | 53.4 |
|   | LAA  | −7.3 | 22.6 | 37.0 | 22° | 10° | 40° | 40.7 | 40.7 |
|   | LA   | −8.1 | 22.0 | 38.2 | −10° | 18° | 52° | 40.4 | 39.2 |
|   | JLA-LPV | −8.9 | 36.8 | 38.3 | 7° | 0° | −20° | 55.2 | 55.9 |
|2  | HRA  | 8.8  | 35.7 | 47.9 | 10° | −15° | −20° | 59.2 | 58.8 |
|   | RA   | 9.6  | 42.7 | 48.3 | 10° | −15° | −15° | 62.5 | 62.5 |
|   | LRA  | 10.8 | 55.7 | 49.4 | 10° | −15° | 45° | 55.4 | 54.6 |
|   | LAA  | −10.4| 34.2 | 46.2 | 10° | −15° | 43° | 49.4 | 48.4 |
|   | LA   | −7.3 | 34.2 | 45.6 | 10° | −15° | 10° | 44.0 | 43.0 |
|   | JLA-LPV | −9.0 | 37.2 | 46.0 | −10° | −15° | 10° | 44.0 | 44.0 |
|3  | HRA  | 6.3  | 32.3 | 38.6 | −20° | −5° | −30° | 50.1 | 49.7 |
|   | RA   | 7.2  | 45.3 | 39.1 | −20° | −5° | −20° | 59.1 | 58.7 |
|   | LRA  | 8.3  | 52.3 | 40.1 | −20° | −5° | 40° | 52.5 | 51.7 |
|   | LAA  | −11.2| 30.8 | 37.0 | −20° | −5° | 50° | 41.2 | 41.0 |
|   | LA   | −9.8 | 30.8 | 36.6 | 40° | 20° | 40° | 49.1 | 48.5 |
|   | JLA-LPV | −10.5| 33.1 | 37.0 | −25° | 20° | 30° | 44.3 | 44.0 |
|4  | HRA  | 3.5  | −41.4| 44.5 | 15° | −5° | −40° | 48.2 | 47.5 |
|   | RA   | 4.3  | −30.4| 48.2 | 15° | −5° | −10° | 61.6 | 61.5 |
|   | LRA  | 5.5  | −21.4| 46.0 | 15° | −5° | 60° | 51.3 | 50.8 |
|   | LAA  | −15.1| −42.9| 43.3 | 15° | −5° | 60° | 42.9 | 42.6 |
|   | LA   | −12.5| −42.9| 43.2 | 30° | −5° | 55° | 53.1 | 53.8 |
|   | JLA-LPV | −14.8| −40.5| 43.6 | −30° | −5° | 35° | 42.4 | 42.4 |
|5  | HRA  | 9.4  | −29.3| 55.6 | −25° | −20° | −35° | 56.5 | 55.7 |
|   | RA   | 10.5 | −24.3| 55.1 | −25° | −20° | 0   | 66.3 | 66.8 |
|   | LRA  | 11.4 | −9.3 | 57.1 | −25° | −20° | 65° | 55.9 | 54.7 |
|   | LAA  | −11.8| −30.8| 52.0 | −25° | −20° | 60° | 50.8 | 50.4 |
|   | LA   | −10.1| −30.8| 51.0 | 10° | 15° | 50° | 38.9 | 38.8 |
|   | JLA-LPV | −10.8| −26.8| 51.4 | −20° | 15° | 20° | 39.0 | 37.4 |
|6  | HRA  | 2.1  | −7.4 | 40.7 | −5° | 30° | −50° | 44.6 | 42.7 |
|   | RA   | 3.0  | 5.0  | 41.4 | −5° | 30° | 5° | 60.8 | 60.9 |
|   | LRA  | 4.1  | 12.6 | 42.2 | −5° | 30° | 70° | 64.0 | 61.1 |
|   | LAA  | −18.8| −8.9 | 39.1 | −5° | 30° | 35° | 52.9 | 51.6 |
|   | LA   | −17.2| −8.9 | 38.7 | 10° | 10° | 28° | 37.1 | 36.1 |
|   | JLA-LPV | −17.2| −5.4 | 39.1 | 10° | −5° | 15° | 47.2 | 46.9 |

* Values are given as the mean ± standard deviation (SD). No significant differences were found between the baseline and relocated groups. ERPb indicates ERP at baseline; ERPr, ERP when relocated; HRA, high right atrium; LA, left atrium; LRA, low right atrium; LAA, left atrial appendage; RA, right atrium; and JLA-LPV, junction of left atrium and left pulmonary vein.

### Discussion

**Main findings:** In this study, we established a novel cardiac stereotactic system that allows for the recording of atrial unipolar electrograms in a flexible and stereotaxic manner. This study also allowed for a convenient assessment of electrophysiological properties in the rat atrium in vivo.

**The preponderance of CREAMS mapping compared to the classic method:**

**Flexibility** Catheter-based recording has been the leading technique for electrophysiology mapping in rat atria and allows the catheter to easily reach the RA through the venous system. However, because of anatomical limitations, the LA and PV cannot easily be accessed, which results in incomplete electrophysiological information for the assessment of AF.4,7,10,16 Another multi-electrode array-based approach was very popular in AF studies because of its high-resolution mapping results. However, this method has been more frequently used in isolated hearts because of the difficulty to fix multi-electrode arrays to in vivo rat LA and PV where space is limited.6) CREAMS will not be restricted by the limitation of anatomy. Firstly, the specially designed all-around rotating electrode holder and stereotaxic arms allow CREAMS to freely slide on the surface of the atrium and JLA-LPV. Secondly, target sites
Table II. Comparison of Unipolar Electrograms Before and After Relocating at the Six Sites

| Site     | Baseline | Relocated | I.RA | RA     | LA     | JLA-LPV |
|----------|----------|-----------|------|--------|--------|---------|
| HRA      | 3.9 ± 2.9| 2.4 ± 2.2 | 3.8 ± 2.9| 3.6 ± 2.2| 3.6 ± 2.7| 3.7 ± 2.2|
| RA       | 3.4 ± 2.2| 2.0 ± 1.0 | 3.4 ± 2.2| 3.4 ± 2.2| 3.6 ± 2.2| 3.7 ± 2.2|
| LRA      | 2.3 ± 1.0| 2.4 ± 1.0 | 3.2 ± 0.6| 2.3 ± 1.0| 2.4 ± 1.0| 3.6 ± 2.2|
| LAA      | 3.8 ± 1.5| 2.2 ± 0.3 | 2.4 ± 0.7| 3.7 ± 2.2| 2.4 ± 0.3| 3.8 ± 2.7|
| LA       | 3.1 ± 0.6| 0.4 ± 0.3 | 3.1 ± 0.6| 3.1 ± 0.6| 0.4 ± 0.3| 3.1 ± 0.8|
| JLA-LPV  | 2.9 ± 0.9| 0.7 ± 0.1 | 3.0 ± 0.3| 2.9 ± 0.3| 0.7 ± 0.1| 3.1 ± 0.8|

Max Peak (mV) 3.9 ± 2.9 2.4 ± 2.2 3.8 ± 2.9 3.6 ± 2.2 3.6 ± 2.7 3.7 ± 2.2
Min Peak (mV) −2.5 ± 2.0 −3.4 ± 2.2 −2.2 ± 0.6 −2.4 ± 1.9 −3.4 ± 2.2 −2.4 ± 1.9
Amplitude (mV) 6.3 ± 4.8 6.9 ± 3.9 5.0 ± 1.6 6.3 ± 4.5 7.0 ± 4.1 6.3 ± 4.5
Area under curve (mV·second) 0.09 ± 0.03 0.08 ± 0.05 0.07 ± 0.03 0.08 ± 0.02 0.09 ± 0.03 0.08 ± 0.02
dMax/dt (mV/ms) 1.6 ± 1.5 2.0 ± 0.8 1.7 ± 0.7 1.3 ± 0.5 1.6 ± 0.9 1.1 ± 0.7

dMin/dt (mV/ms) −0.98 ± 0.77 −1.6 ± 0.9 −0.5 ± 0.4 −0.9 ± 0.4 −0.9 ± 0.4 −0.9 ± 0.4

* Values are given as the mean ± SD. No significant differences were found between the baseline and relocated groups. HRA indicates high right atrium; LA, left atrium; LAA, left atrial appendage; RA, right atrium; JLA-LPV, junction of left atrium and left pulmonary vein; dMax/dt, the maximum ascending velocity of voltage; and dMin/dt, the minimum descending velocity of voltage.

Stereotactic function The stereotactic function of CREAMS is an ability that the current three leading mapping techniques are not equipped with. Therefore, this ability endowed the system with a great advantage. Firstly, when the design of the experiment requires repeated measure at one site, which can either not be achieved or the electrode cannot easily be fixed, such as in case of the LPV, stereotactic function seems to be more practical in order to reset the electrode to its original position with these positioning coordinates (Tables I and II). Secondly, when the position was obtained, the electrode was retrieved from the heart, which reduced friction between the metal tip of the electrode and heart tissue to prevent physical damage. Thirdly, these special abilities may benefit studies that focus on catheter ablation. Catheter ablation has often been applied in large animals, but only few studies have reported catheter ablation in rats. The reason may be that the smaller size of a rat atrium may be difficult to operate. However, the stereotactic function of CREAMS allows for accurate positioning of the catheter in a rat atrium, and with the positioning information, we can guide the ablation catheter exactly to any site. In particular, the method could be used at sites that are hard to find, such as ganglionated plexi, PV, and LA. Therefore, an AF in vivo rat model would benefit from CREAMS. In our study, we have completed testing of the precision of stereotactic function, and observed that the average distance was 297 ± 54 μm. Due to the close distance between the two measures, the electrophysiological properties were very similar and resemble ERP testing, as presented in Table II. Due to continuous beating of the heart, slight movement cannot be avoided when performing electrophysiological mapping. Finally, the method is a promising approach to ablate the heart, which may help us finish epicardial ablation in rats with CREAMS.

Safety of CREAMS Being equipped with micro-propulsion, CREAMS can finely adjust the electrode in high spatial resolution (minimal precision 0.1 mm), which is more effective for delicate surgery in rats. By fine controlling, we could find an optimum position where the recording signal was stable. Limited contact between the electrode and atrial tissues may reduce atrial damage by the electrode.

Assessment of electrophysiology in an in vivo rat model: The stability of CREAMS and manipulating capabilities The atrium is located at the bottom of the heart and connects the aorta and pulmonary vessels. Thus, when extra force is applied to the atrium, the heart will slightly move because of the pull on the vessels and cardiac repository. We tested this by the stability of the unipolar recording and the morphology of the electrogram of the mapping site. Figure 3 shows that all six sites were very stable in recording the electrogram. In fact, the main factors influencing the unstable electrode touching the atrium were not...
the heart beats themselves, but the vertical up-and-down movement caused by artificial ventilation. To solve this issue, the electrode was adjusted obliquely so that it would touch the atrial surface. By creating an angle with the respiratory plane, the electrode slides less during the up-and-down movement, thereby stabilizing the mapping.

**AF recognition** AF could be identified by the morphology of the unipolar electrograms, which showed significant shortening of the cycle length of the atrial impulse, the constant transformation of the electrograms activation sequence, and the heterogeneous morphology of the unipolar electrograms including slow and abnormal depolarization, and heterogeneity of the amplitude, "riding sign".\(^{16}\) The repolarization phase was terminated early following depolarization in a different level of repolarization and the disappearance of the diastolic time interval between consecutive electrograms (Figure 5).

**ERP and WOV** ERP and WOV were important indicators for evaluating AF. Using multiple sites of ERP, the dERP could be calculated to demonstrate the mechanism of AF occurrence and to demonstrate the vulnerability of AF.\(^{19-22}\)

**The limitation of CREAMS:** Although promising, CREAMS also has several limitations. Firstly, CREAMS could not achieve simultaneous recordings and high-density mapping for atrial electrophysiology, which go against the high-demand evaluation of AF electrophysiology in rat models. Thus, in the future, combining with CREAMS and multiple electrode arrays or optical mapping may be more significant in more comprehensive electrophysiological mapping. Secondly, due to the continuously beating heart, the electrode may slightly slide on the myocardial surface. However, by employing the precision test, we could accept slight movement for DTC of just about 300 μm.

**Clinical application of CREAMS:** During cardiac surgery, CREAMS will be more suitable for epicardium ablation. As we know, complex arrhythmia may originate from the epicardium. If the position information of the cardiac surface was collected in advance, we could depict a “cardiac atlas”, which is very meaningful for ablation as one could avoid several important sites, such as important vessels, get access to sites that are not easy to get to, such as the pulmonary vein and the back of heart, and precisely locate some specific sites such as the ganglia plexus. In addition, CREAMS, if equipped with an automatic control system, could serve as an autonomic manipulator to contribute to robotic ablation for arrhythmia. We could input the coordinators of the target sites and CREAMS would take the electrode to that specific site, which would make ablation more convenient.

**Conclusions**

In this study, we established a cardiac stereotactic epicardium mapping system. The novel approach described in this study may serve as a practical tool to benefit studies that focus on the electrophysiology in an in vivo rat model.

**Disclosures**

Conflicts of interest: All authors listed have contributed sufficiently to the project to be included as authors and all those who are qualified to be authors are listed in the author byline. To the best of our knowledge, no conflict of interest, financial, or other, exists.
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Supplemental Files

Supplemental Text
Supplemental Table
Supplemental Figures 1-3
Please see Supplemental Files; https://doi.org/10.1536/ihj.18-215