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# Transesophageal atrial burst pacing for atrial fibrillation induction in rats

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**TITLE:**

# Transesophageal Atrial Burst Pacing for Atrial Fibrillation Induction in Rats

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**SUMMARY:**

The present work describes an experimental protocol of transesophageal atrial burst pacing for efficient induction of atrial fibrillation (AF) in rats. The protocol can be used in rats with healthy or remodeled hearts, allowing the study of AF pathophysiology, identification of novel therapeutic targets, and evaluation of new therapeutic strategies.

**ABSTRACT:**

Animal studies have brought important insights into our understanding regarding atrial fibrillation (AF) pathophysiology and therapeutic management. Reentry, one of the main mechanisms involved in AF pathogenesis, requires a certain mass of myocardial tissue in order to occur. Due to the small size of the atria, rodents have long been considered 'resistant' to AF. Although spontaneous AF has been shown to occur in rats, long-term follow-up (up to 50 weeks) is required for the arrhythmia to occur in those models. The present work describes an experimental protocol of transesophageal atrial burst pacing for rapid and efficient induction of AF in rats. The protocol can be successfully used in rats with healthy or remodeled hearts, in the presence of a wide variety of risk factors, allowing the study of AF pathophysiology, identification of novel therapeutic targets, and evaluation of novel prophylactic and/or therapeutic strategies.

**INTRODUCTION:**

Atrial fibrillation (AF) is the most common sustained cardiac arrhythmia encountered in clinical practice and its incidence and prevalence continue to increase dramatically worldwide[1]. This arrhythmia affects up to 4% of the world population according to recent studies[2]. However, given that paroxysmal AF can be asymptomatic and may therefore escape detection, the true prevalence of AF is likely to be much higher than that presented in the literature.

The pathophysiology of AF has been intensely studied. Nevertheless, the underlying mechanisms of this complex arrhythmia remain incompletely elucidated and this reflects in the limited therapeutic options, with questionable efficacy. Animal studies have brought important insights into our understanding regarding AF pathophysiology and therapeutic management. Reentry, one of the main mechanisms involved in AF pathogenesis[3], requires a certain mass of myocardial tissue in order to occur. Thus, large animals have generally been preferred in AF studies, whereas, due to the small size of their atria, rodents have long been considered 'resistant' to AF. However, the use of large animals is hampered mostly by handling difficulties. Meanwhile, although spontaneous AF has been shown to occur in rats[4], long-term follow-up (up to 50 weeks) is required for the arrhythmia to occur in those models[5]. Models that ensure rapid AF occurrence in small rodents have also been developed. Most often, those models use acute electrical stimulation, often in the presence of other favoring conditions, such as concomitant parasympathetic stimulation or asphyxia, to artificially induce AF[6,7]. Although efficient, such models do not allow the evaluation of critical AF-related features, such as the progressive electrical, structural, autonomic, or molecular remodeling of the atria, nor the effects of conventional or non-conventional antiarrhythmic drugs on the atrial substrate or on the risk of ventricular pro-arrhythmia[8,9].

The present work describes an experimental protocol of long-term transesophageal atrial burst pacing for rapid and efficient induction of AF in rats. The protocol is suitable for both acute and long-term studies and can be successfully used in rats with healthy or remodeled hearts, in the presence of a wide variety of risk factors, allowing the study of AF pathophysiology, identification of novel therapeutic targets, and evaluation of novel prophylactic and/or therapeutic strategies.

**PROTOCOL:**

Procedures involving animal subjects were approved by the Ethics Committee of the University of Medicine, Pharmacy, Science and Technology "George Emil Palade" of Târgu Mureș, by the Romanian National Sanitary Veterinary and Food Safety Authority and complied with the International Council for Laboratory Animal Science guidelines (Directive 2010/63/EU).

## 1. Transesophageal atrial burst pacing protocol

1.1. Randomize adult male Wistar rats (200–400 g of bodyweight) into two groups: STIM and SHAM.

1.2. Anesthetize the animals.

1.2.1. For induction, use 2.5% isoflurane, 4 L/min, 99.5% $O_2$.

1.2.2. For maintenance, use a mixture of ketamine/medetomidine (75.0/0.5 mg/kg) administered intraperitoneally.

1.2.3. Check the depth of the anesthesia by testing the corneal reflex (5% glucose solution) and

the nociceptive withdrawal reflex. Continue the procedure only after these reflexes are abolished.

1.3. Lay the animal in supine position and place it on a heating pad to maintain body temperature at ~37 °C.

1.4. Attach the three surface ECG electrodes to the rat limbs in a lead II configuration (**Figure 1A**).

1.4.1. Place the negative electrode on the right forelimb.

1.4.2. Place the positive electrode on the left hindlimb.

1.4.3. Place the grounding electrode on the left forelimb.

1.4.4. Secure the electrodes into position using thin elastic bracelet string cords.

1.5. Turn on the surface ECG recording and perform continuous ECG recording throughout the procedure (**Figure 1B**) using a commercial or a locally developed acquisition program[10].

1.6. For electrical stimulation, use a 5–6 F quadripolar catheter connected to a microcontroller-based cardiac pacemaker[10].

1.7. Once the animal is anesthetized, insert the catheter through the oral cavity, into the esophagus.

CAUTION: Be careful not to force the catheter as there is a risk of esophageal perforation.

1.8. Confirm the correct position of the stimulation catheter at the level of the atria as follows.

1.8.1. Apply electrical stimulation at a frequency of 400 stimuli/minute (stimulus duration 6 ms).

1.8.2. Check whether the ECG tracing shows constant capture of the atria (i.e., each electrical stimulus is followed by a narrow QRS complex) (**Figure 2**).

1.9. Determine the diastolic threshold—i.e., the lowest voltage required to obtain atrial capture (generally, between 10 V and 20 V).

NOTE: Perform the following for the animals in the STIM group.

1.10. Once the correct position of the catheter is determined, set the stimulator to a frequency of 4,000 stimuli/minute (stimulus duration 6 ms), at a voltage 3 V above the diastolic threshold (**Figure 3**).

1.11. Apply to each animal 15 successive cycles of stimulation, 20 s each, with a free interval of 5 min between cycles[11]. Depending on the study objectives, repeat the protocol for each rat for 10 days, at a rate of 5 days/week, at the same time on each day.

1.12. Check the effectiveness of the stimulation as follows.

1.12.1. Identify the sinus node recovery time (SNRT), which appears at the end of the rapid pacing as a time interval that is longer than the cycle length recorded during sinus rhythm (**Figure 4A**) and represents the interval of time required for resumption of sinus rhythm after overdrive suppression ends.

NOTE: Overdrive suppression represents the inhibition of sinus node activity by electrically stimulating the heart at a rate higher than the intrinsic rhythm.

1.12.2. Identify the occurrence of the AF episode, which is defined here as the presence of three or more consecutive irregular, supraventricular beats (i.e., irregular ventricular response with narrow QRS complexes), with P-waves absent or replaced by small, distorted "f" waves (**Figure 4B**).

1.13. If the AF episode does not end spontaneously by the time the next stimulation cycle should be performed (i.e., by the end of the five free minutes between cycles), do not apply the next stimulation.

1.13.1. Wait for another 5 min. If the AF episode still continues after those 10 min, end the protocol for that day.

NOTE: If evaluation of the severity of electrically-induced AF is desired, longer ECG monitoring can be performed.

1.14. If severe bradycardia or asystole occurs at the end of the stimulation (i.e., due to electrical stimulation of the vagus nerve), end the protocol. If the electrical activity does not return to normal rapidly, perform external cardiac massage and administer atropine sulfate (0.05 mg/kg) intraperitoneally.

1.15. At the end of the procedure, reverse the anesthesia with atipamezole (1 mg/kg). No other specific animal care is required at the end of the protocol.

1.16. Analyze the surface ECG tracings and determine the following.

1.16.1. The inducibility of AF which is expressed in percentage (i.e., [number of stimulation cycles followed by AF episodes / total number of stimulation cycles applied] x 100).

1.16.2. The duration of each AF episode.

1.16.3. The presence of ‘persistent’ (i.e., >10 min) AF episodes.

NOTE: Perform the following for the animals in the SHAM group.

1.17. For the rats in the SHAM group, follow steps 1.1 to 1.7 as described above, without applying any electrical stimulation.

1.18. Maintain the catheter into position for 80 min (i.e., the time required for completing the protocol in STIM rats) without applying any electrical stimulation, while continuously recording the surface ECG.

1.19. At the end of the procedure, reverse the anesthesia with atipamezole (1 mg/kg). No other specific animal care is required at the end of the protocol.

1.20. Analyze the surface ECG tracings and determine the parameters described in step 1.16.

**REPRESENTATIVE RESULTS:**

In a proof-of-concept study, 22 adult male Wistar rats (200–400 g) were randomly assigned into two groups: STIM (n = 15) and SHAM (n = 7). All animals were housed individually in polycarbonate cages, in a climate-controlled room (21–22 °C), having free access to water and dry food throughout the study. The transesophageal stimulation protocol described above was applied to all animals for 10 days, 5 days per week. All animals underwent the same protocol, except that the rats in the SHAM group did not receive active electrical stimulation.

As expected, no episodes of AF were induced in the SHAM animals throughout the protocol. Hence, no other parameters (i.e., the duration of AF episodes and the presence of ‘persistent’ AF episodes) could be evaluated in this group.

On the first day of stimulation, 12 (80%) of the 15 STIM animals presented AF episodes (Relative Risk = 3.33, $p < 0.001$ vs. the SHAM group using Fisher’s exact test). In the STIM rats, out of 164 stimulation cycles applied on the first day of stimulation, 42 were followed by AF episodes (median inducibility of 20% [interquartile range of 6.67–72.22] vs. 0% in the SHAM group) (**Figure 5**).

During the 10 days of protocol, AF was efficiently induced in all animals (**Figure 6**). An average of 15.6 ± 8.7 episodes of AF was induced in the STIM animals during the entire duration of the protocol. Of the total number of stimulation cycles applied, 20.05% were followed by AF, and 41 (17.30%) episodes of AF lasted more than 600 s. The average duration of AF episodes lasting less than 600 s is 40.12 s (**Table 1**).

**FIGURE AND TABLE LEGENDS:**

**Figure 1: Surface ECG recording.** (**A**) ECG electrodes positioning—two at the level of the forelimbs and one on the left hindlimb of the animal. (**B**) Surface ECG tracing recorded before

applying electrical stimulation.

**Figure 2: ECG tracing confirming capture of the atria.** The ECG tracing confirms the correct position of the catheter, i.e., a narrow QRS complex is observed after each electrical stimulus at a frequency of 400 stimuli/minute.

**Figure 3: Microcontroller-based cardiac pacemaker settings.** Stimulation parameters are set at a frequency of 4,000 stimuli/minute (ppm: pulses per minute), stimulus duration of 6 ms (WDTh: width), and tension of 11 V (i.e., 3 V above the diastolic threshold).

**Figure 4: ECG tracings confirming the effectiveness of the stimulation protocol.** (**A**) The sinus node recovery time (SNRT). Note that the time interval at the cessation of stimulation (SNRT) is longer than the cycle length recorded during sinus rhythm (RR interval, i.e., the interval between the R-waves of two consecutive QRS complexes, representing the duration of a cardiac cycle). (**B**) The appearance of an atrial fibrillation episode after completion of the atrial electrical stimulation cycle. Note the irregular, narrow QRS complexes, the absence of P waves, and the small, distorted "f" waves.

**Figure 5: Inducibility of atrial fibrillation (AF) on the first day of stimulation in the STIM (n = 15) and SHAM (n = 7) groups.** Data are expressed as median and interquartile range.

**Figure 6: Mean daily inducibility of atrial fibrillation during the 10 days of the stimulation protocol in the STIM rats.**

**Table 1: Temporal parameters of electrically-induced 'persistent' and 'non-persistent' atrial fibrillation episodes in the STIM group.**

**DISCUSSION:**

The present paper describes an experimental protocol of long-term transesophageal atrial burst pacing for rapid and efficient induction of AF in rats, suitable for both acute and long-term AF studies. The 10-day stimulation protocol described herein has been successfully used to develop a 'secondary spontaneous AF model' (i.e., a model in which, following a period of AF induction by electrical stimulation, AF develops spontaneously)[10]. However, the duration of the protocol can vary depending on the exact purpose of the study.

Other parameters, such as the size of the stimulation catheter, can also be adjusted, depending on the size of the animals. However, care should be taken to avoid the use of excessively large catheters, as they may cause pressure on the trachea and impede normal breathing. For 200–400 g rats, 5–6 F catheters cause negligible pressure on the trachea and allow the protocol to be implemented without the need for endotracheal intubation.

A key step of the protocol is the correct positioning of the stimulation catheter inside the esophagus, at the level of the atria (step 1.7). This step should only be performed after careful check of the depth of the anesthesia, as lack of effective anesthesia increases the risk of cardio-

respiratory arrest during the following steps of the protocol. Monitoring the electrical activity of the heart using surface ECG generally provides sufficient data to confirm that the catheter is correctly positioned inside the esophagus (i.e., stimulation at a frequency higher than the intrinsic heart rate illustrates the overdrive suppression phenomenon on the sinus node and each stimulus is followed by a narrow QRS complex). However, performing esophageal electrocardiogram recordings could be used to further confirm the correct position of the stimulation catheter.

Considering the normal baseline heart rate of Wistar rats[12], it is important to perform the initial stimulation at a frequency higher than the rats' own heart rate (i.e., >400 stimuli/minute), to ensure constant capture of the atria (step 1.8). During this step, stimulation frequency should be adapted to each animal's baseline heart rate. In the presence of a correct stimulation rate, lack of constant atrial capture could be due to either incorrect positioning of the catheter or to stimulation at a voltage below the diastolic threshold (step 1.9). Both scenarios can result in inefficient stimulation and protocol failure. Given that variations in body temperature can promote cardiac arrhythmias[13], attention should be given to maintaining constant body temperature (37 °C) during the entire procedure.

The technique described herein also has a number of limitations. Given the anatomical proximity of the vagus nerve to the esophagus, concomitant electrical stimulation of the vagus nerve can occur during the protocol, increasing the risk of cardio-respiratory arrest. In addition, one should keep in mind that parasympathetic stimulation is likely to also contribute to AF occurrence in this model and that other models may be more adequate for studies aiming to evaluate and/or manipulate the autonomic nervous system.

Animal models continue to play an important role in unraveling the pathophysiological mechanisms that underlie AF and in improving therapeutic strategies. An ideal animal AF model should be fast and easy to recreate, reproducible, and should mimic as much as possible the pathology observed in humans[14]. In rodents, most AF models consist in acute AF induction, most commonly in the presence of other favoring factors, in addition to electrical stimulation of the atria[6,15]. However, such models cannot assess the role of progressive atrial remodeling in AF pathophysiology, cannot test the long-term effects of various antiarrhythmic drugs, and cannot assess the ventricular proarrhythmic risk associated with chronic antiarrhythmic treatment[8,9]. In other studies[16], a single stimulation protocol was applied to chronically remodeled, AF-prone atria. Although this strategy overcomes some of these disadvantages, it does not take into account the impact of the AF *per se* on atrial proarrhythmic remodeling and on future AF occurrence[8,9]. Meanwhile, prolonged (e.g., 10 days) application of the transesophageal atrial pacing protocol described above induces progressive atrial proarrhythmic remodeling and creates the atrial environment required for the spontaneous occurrence of AF, after the stimulation protocols are completed[10].

The experimental model described herein can therefore be efficiently used not only for assessing acute AF induction, but also for creating a model of (secondary) spontaneous AF. This model therefore brings a number of major advantages, creating the premises for a better

understanding of the mechanisms involved in the occurrence and maintenance of AF, as well as for identifying and testing new therapeutic strategies.

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**DISCLOSURES:**

The authors have no conflicts of interest.

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Figure 1

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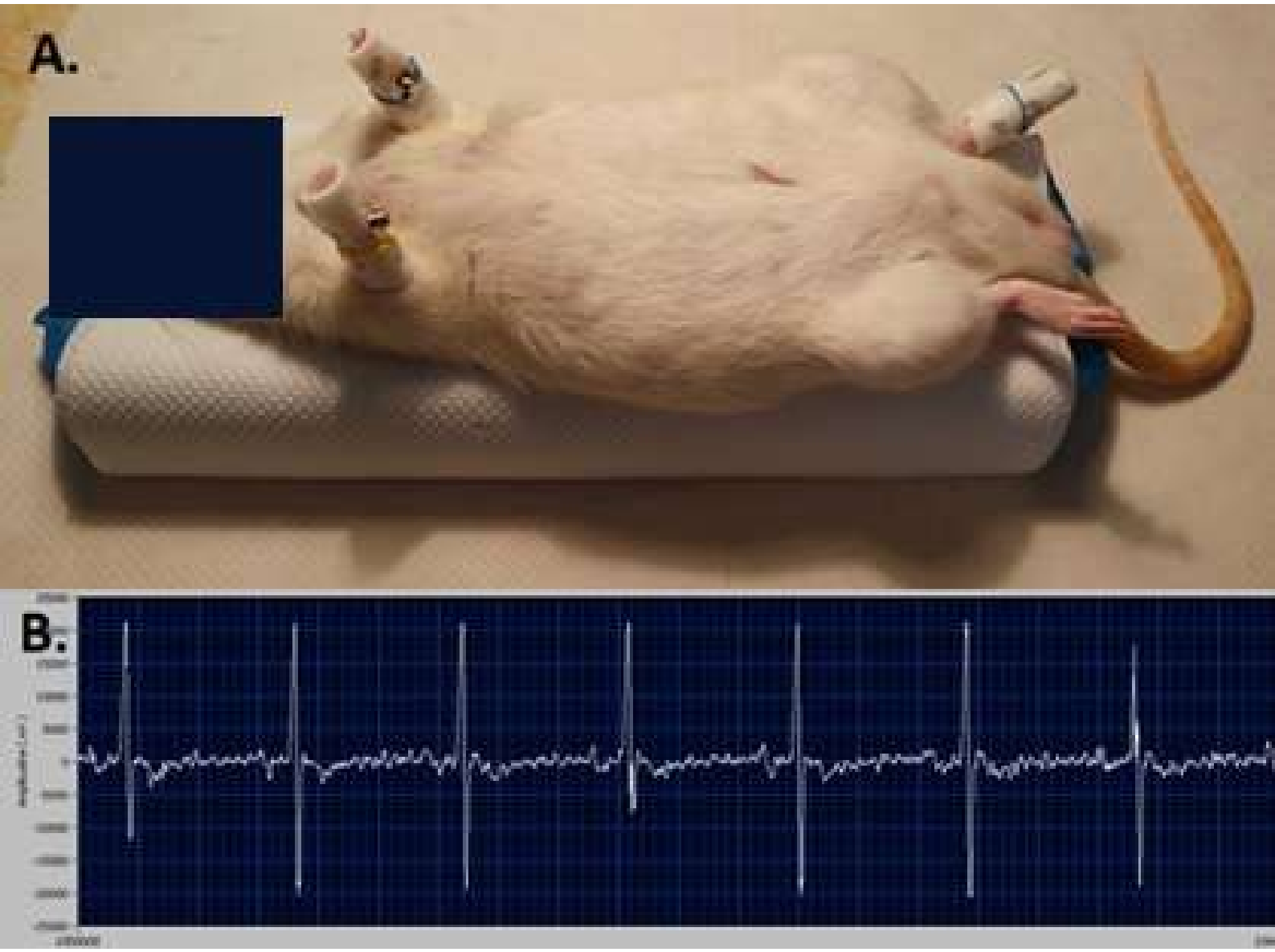

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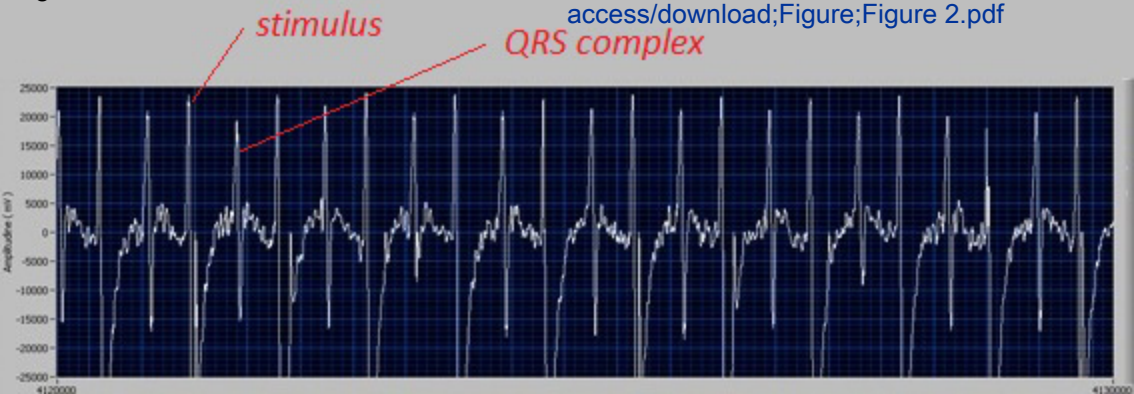

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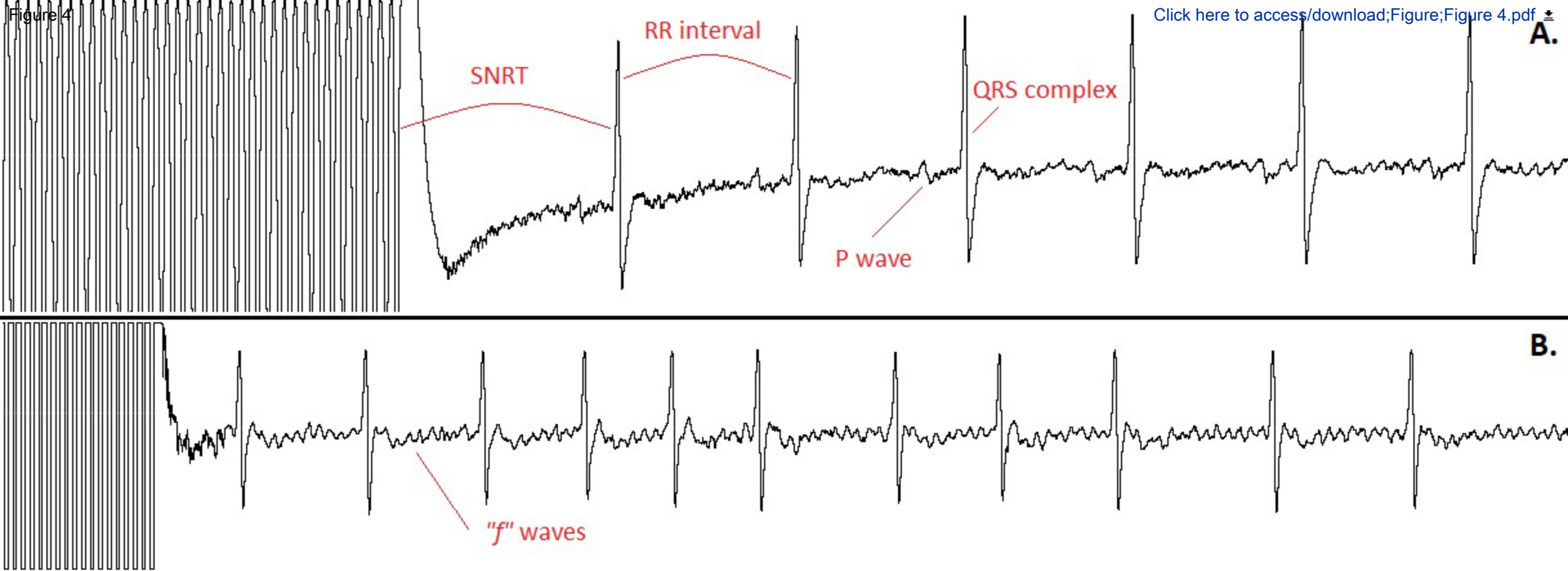

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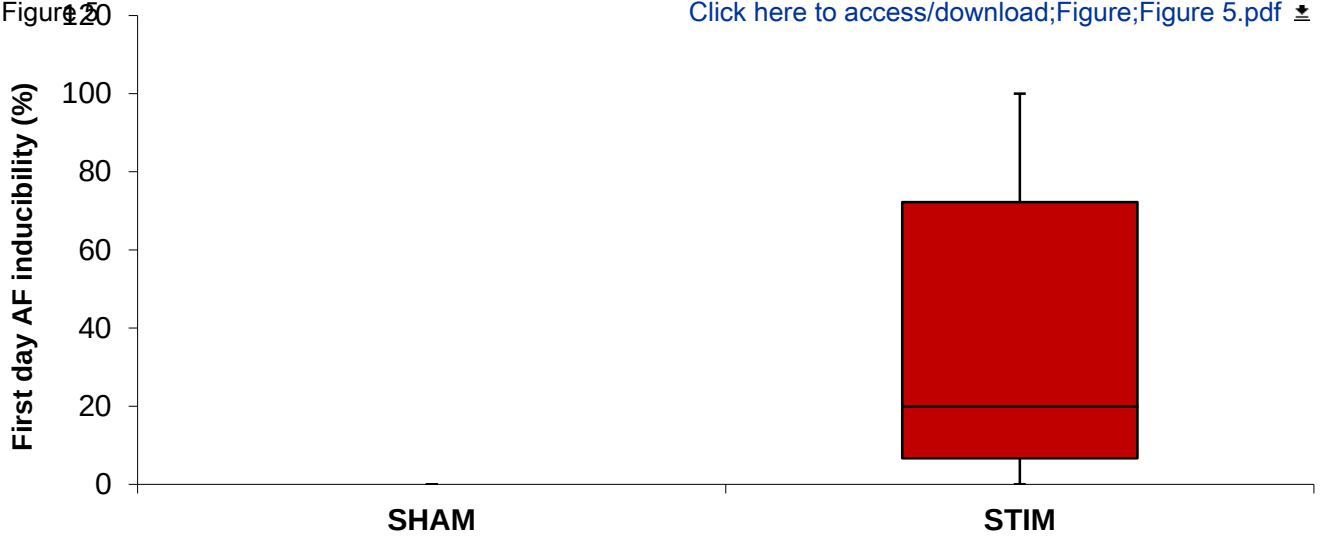

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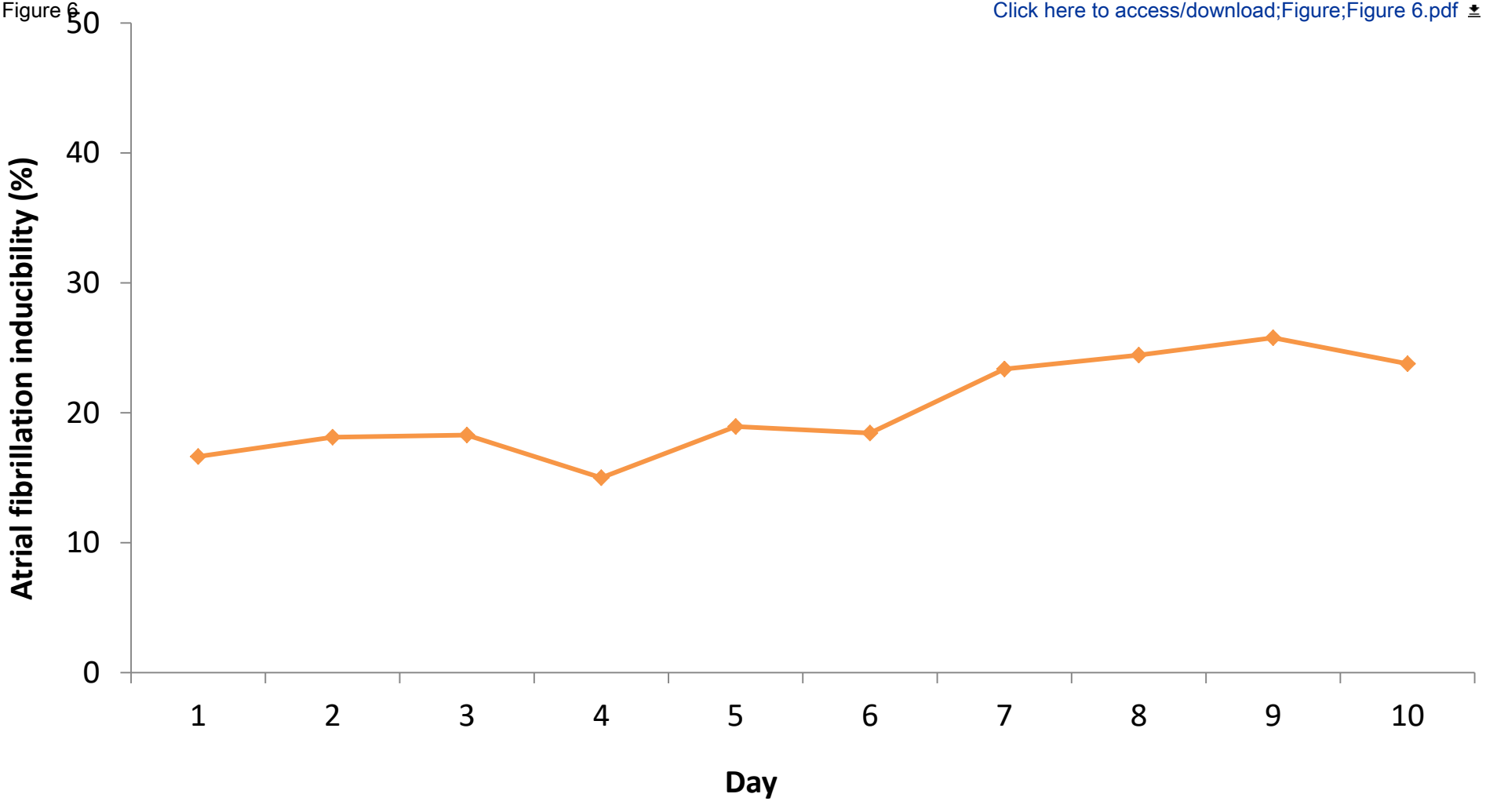

Table 1

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| Electrically-induced atrial fibrillation episodes (n = 237) | Number (%) | Mean duration (seconds) |
|---|---|---|
| duration ≥600 seconds | 41 (17.30%) | - |
| duration < 600 seconds | 196 (82.70%) | 40.12 |

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