

# Non-invasive restrained ECG recording in conscious small rodents: a new tool for cardiac electrical activity investigation

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**Abstract** In vivo electrophysiology remains a suitable method to monitor cardiac activity; however, surface electrocardiogram (ECG) monitoring remains complicated in the case of small animals. Sedation has helped to maintain the animal still; however, it is known that anesthetic drugs impair the regulation of the cardiac electrical activity. To circumvent this problem, ECG monitoring using telemetry or restraints has been developed. This study reports a new methodology, based on a restraining system without further sedation, for recording ECGs on small animal models. We investigated its efficacy in Syrian hamsters and in several strains of mice, and we compared these data to those obtained with telemetry devices. We show that this new system can easily be used in animals of different sizes ranging from adult hamsters to newborn mice. When compared to telemetry, this restrained ECG monitoring method shows a very good yield, as 65% of total beats can be used for further analysis. When recorded in the same animals, RR intervals distributions are identical for both techniques. In conclusion, this restrained ECG monitoring technique is a well-suited tool for exploring various aspects of cardiac electrophysiology in a wide variety of small animals including very young mice.

**Keywords** Electrophysiology · Restraints · Electrocardiography · Cardiac phenotype · Mouse · Hamster

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## Introduction

Animal models are invaluable tools to gain fundamental insights into the molecular mechanisms underlying any given pathology. However, the recent explosion in the number of models that can be generated emphasizes the need for simple and noninvasive high throughput screening methodologies to identify animals that will be further investigated. In the case of cardiac diseases, in vivo electrophysiology remains the most suitable method to monitor cardiac activity [4, 9].

In 1968, Goldberg et al. [6] applied electrocardiogram (ECG) technology to unconscious mice from different strains using a six lead XYZ system. Thirty years later, Berul et al. [1, 2] developed the technique and recorded 6-lead and 12-lead ECG using 27-gauge needles as subcutaneous electrodes. Since then, 6-lead surface ECGs are commonly performed on unconscious rodents [1, 2, 10, 16]. However, it is known that anesthetic drugs interfere with the cardiac activity; in particular, depressant effects of the anesthetic agents on autonomic regulation of heart rhythm have been observed [14].

During the past years, the improvement in technology allowed transition between external electrodes and receivers and the development of implantable radiotelemetry devices, making long-term ambulatory ECG recording possible [5, 11–15]. This technique allows the data to be collected from conscious unstressed animals under conditions close to a physiological state of the animals. Moreover, the surgical procedure is relatively noninvasive and is very simple.

In spite of these advantages, the telemetric methodology still suffers from a number of limitations, some of which are linked to the inability for very diseased animals to survive the surgical procedure, whereas another limitation is associated to the mass of the device that requires animals

with a minimal bodyweight. These limitations led scientists to seek more versatile methodologies. One such system, allowing nontraumatizing ECG monitoring, turned out to be limited to a small amount of recorded QRS complexes due to a too important freedom for the animal [3]. Others created more restraining systems, which turned out to be too much traumatizing. Wang et al. [17] recorded ECG from neonate to adult mice, housing them in a perforated restraining tube so that electrodes could be placed on the limbs protruding through the holes. Hamlin et al. [7] developed a bipolar transthoracic ECG monitoring method on guinea pigs, sandwiching animals between copper plates in a padded sling.

This paper reports a new methodology to record ECGs based on a restraining system without further sedation in various conscious small animal models. First, we investigated its efficacy in Syrian hamsters and mice. Second, we compared generated data with those obtained with telemetry devices. We will show here that this restrained ECG monitoring technique is a suitable tool for cardiac electrophysiology in small animals. Being noninvasive and allowing repeated recordings, it makes rapid screening possible.

## Materials and methods

**Animals** Wild-type adult Syrian hamsters (strain Rj:AURA, Elevage Janvier, France) with mean body weight of about 120 g were used in initial experiments. Diseased counterparts, cardiomyopathic hamster strain CHF147, bred in the local animal facility (Institut de Myologie, Paris) were also investigated. Murine experiments were carried out on C57/Bl6 mice. Animals were housed in the local facilities and maintained at 20°C with 10:14 h light/dark cycles and free access to food and water was provided. Care of the animals was in compliance with the guidelines. Mice and hamsters were monitored by the restraining technique as well as by telemetry.

**ECG recording** All ECG recording sessions were performed during daytime. Six-lead restrained ECG monitoring of conscious animals (hamsters and mice) were performed with EasyCG® tools system (EMKA technologies, France; Fig. 1). The four sensors of ecgTUNNEL® system platform, one for each paw, were coated with electrocardiographic gel, and an animal translucent size-fitting half-tunnel was set on it. Paws were cleaned with 70° ethanol. Each animal was put inside the tunnel, which was then closed, assuring the animal to be properly restrained. The four wires of the platform were connected to a wireless transmitter and amplifier system (emkaPACK®, EMKA technologies). To minimize the effects of stress, animals were allowed to stay in the restraining system for 5 min



**Fig. 1** Photograph of the six-lead restrained ECG monitoring system formed by a platform with ECG sensors and a size fitting half-tunnel

before starting ECG recordings. Indeed, direct observation of the animals and ECG traces proved that they were calm and that the heart rate was stable. Six leads of ECG were recorded during 30 to 60 min using specific software (iox®, EMKA technologies): three leads were measured (L1, L2, L3), and three were calculated (aVR, aVL, aVF). Single lead of unrestrained ECG was performed on Syrian hamsters and mice using telemetry recording. We used a commercially available data acquisition system composed of an implantable telemetry transmitter (PhysioTel® TA10ETA-F20, Data Science International, USA) and a receiver that is placed under the cage of each animal (PhysioTel® Receiver RPC-1, Data Science International), connected to a data acquisition matrix. Animals were anesthetized with ketamine (75 mg/kg), xylazine (15 mg/kg), and midazolam (0.75 mg/kg) administered intraperitoneally. Animals were shaved on the back and under forepaws. A midline incision was made on the back along the spine. A subcutaneous pocket was made to house the telemetric device that was then suture to the muscle. Cathodal and anodal leads were fitted subcutaneously and sutured on each side of the chest wall. ECGs were recorded with specific software (Dataquest®, Data Science International) 3 weeks after implantation to allow recovery from surgery and adaptation to the implanted device. To compare both methods, five series of 30-min records for each hamster and each system were used for analysis.

**ECG analysis** Dedicated software (Ecg-auto®, EMKA technologies) was used to analyze data collected with both ECG recording methods. If needed, data recorded with the restraining system were submitted to a 50-Hz notch filter with an automatic setting determined by the software. The theoretical amount of beats to record during 1 min was calculated using mean measured heart rate value. The mean, median, and mode of usable beats within a 1-min recording were calculated for each technique using the same animals. Analysis of time series were carried out and allowed us to study RR intervals distribution and determine mean RR intervals for each recording method.

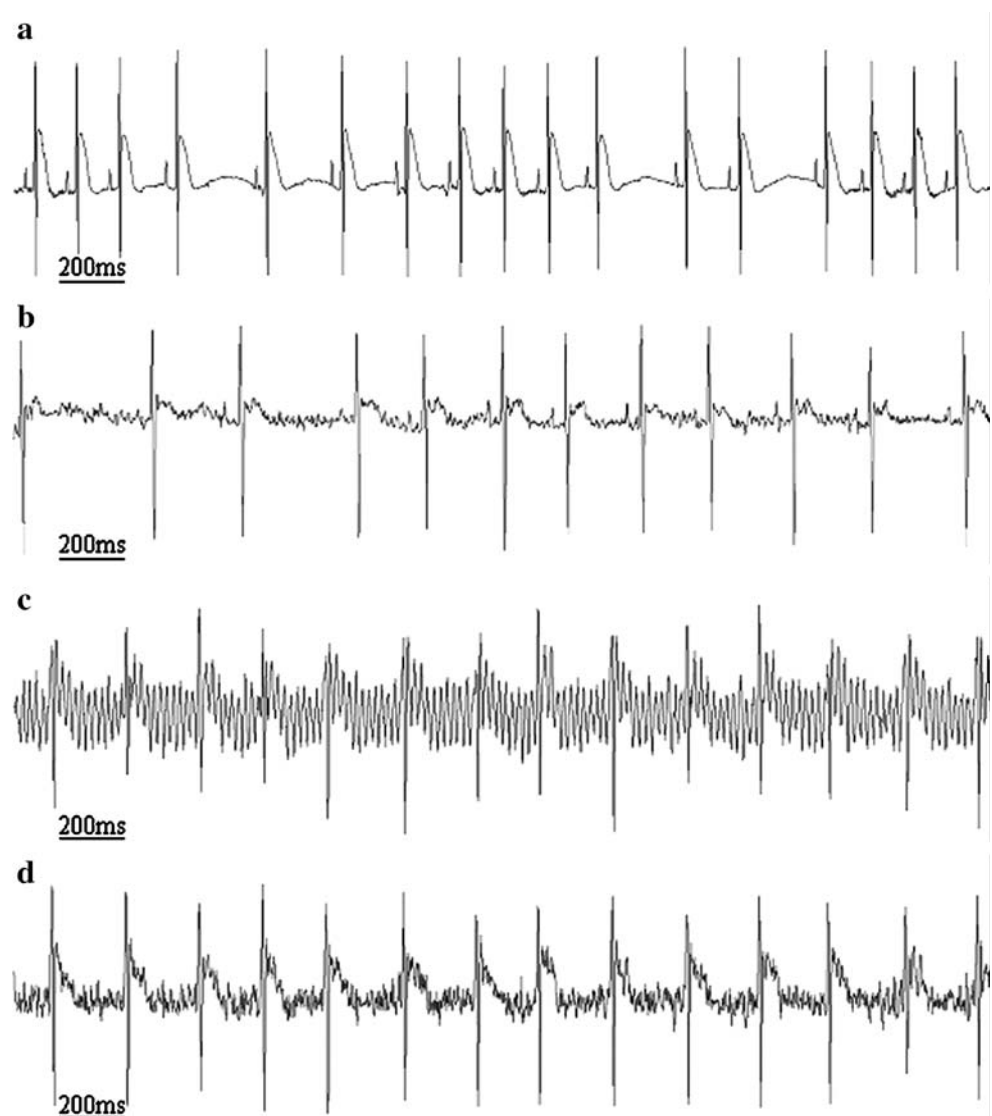
**Statistics** All data are presented as mean±SD. Comparisons between both ECG recording methods were performed using Student's *t*-test.  $P < 0.05$  was considered significantly different.

## Results

**Unrestrained ECG monitoring of conscious animals** Typical ECG recordings are shown on Fig. 2a for a wild-type Syrian hamster. Equivalent recordings were obtained with adult mice. However, in this case, the telemetry device imposes the bodyweight of the animal not to be below 20 g (Fig. 3a).

**Restrained ECG monitoring of conscious animals** Wild-type hamsters were monitored using the restraining system.

**Fig. 2** Typical electrocardiograms from wild-type Syrian hamsters obtained with telemetry and restrained ECG monitoring. **a** Typical ECG trace of a sinus rhythm recorded with a telemetric system (PhysioTel® implant) allows identification of P waves QRS complexes and T waves. **b** Example of low noise unfiltered signal obtained with the restraining tunnel. **c** Unfiltered signal with high noise. **d** Same signal as C after 50-Hz notch filtration



The recording period lasted 30 min and could be extended to 60 min. Repeated recordings could be performed on the same animals. As animals were not sedated, noise, due to muscular activity, was sometimes important and required the signal to be filtered. Nevertheless, raw ECG data could be used without further signal treatment when noise was low (Fig. 2b). When necessary, analysis software allowed removing the noise. Once 50-Hz filtered, ECGs were of excellent quality for analysis (Fig. 2c,d). Similar recordings have been carried out on CHF147 cardiomyopathic hamsters. Diseased hamsters withstood recording from 30 to 60 min, and repeats. Collected data were of the same quality (data not shown). A similar approach was used with adult and neonate (6 g) mice (Fig. 3b,c). The recordings were of the same quality as those obtained with adult hamsters. Animals withstood recordings up to 1 h long, and we were able to perform repeats.

**Fig. 3** Typical electrocardiograms from C57/Bl6 mice obtained with telemetry and restrained ECG monitoring. **a** ECG trace recorded on an adult mouse using telemetric system based on a PhysioTel® implant. **b** Representative ECG strip from an adult mouse obtained with the ecgTUNNEL® system. **c** Sample of restrained ECG trace recorded on a young mouse (6 g) demonstrating the feasibility of recording electrocardiographic activity on low weight animals

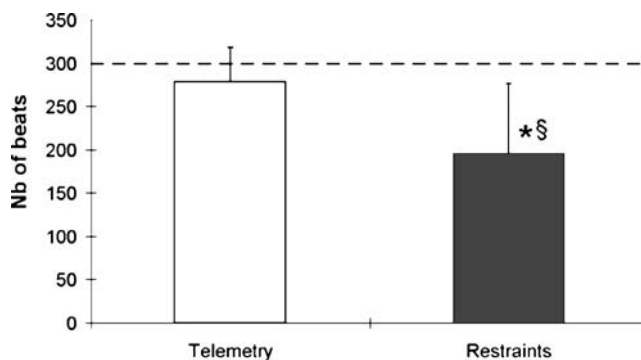


*Comparative analysis of restrained vs unrestrained ECG recordings* ECGs from the same wild-type Syrian hamsters were monitored with both techniques. Noise filtering was necessary for some data sets recorded with the restraining method, but never with those obtained from the radiotelemetry system. QRS complexes were then identified with the same software setup. Based on surface ECG recordings and manual numbering, we observed that the Syrian hamster has a stable heart rate with a mean value around 300 bpm (5 Hz), and we took this value as the normal value to be compared with. The corresponding mean RR interval is 200 ms.

Using the implanted telemetry system, we found an average count of  $279 \pm 39$  beats that were recorded per minute. This number corresponds to 93% of the expected value, but statistical analysis proved that the two values are not significantly different ( $P=0.123$ ). The mean RR interval measured on consecutive beats was found to be  $212 \pm 26$  ms, again not significantly different from the expected value of 200 ms. Note that if the recording is performed 2 weeks, instead of 3, after implantation, the yield drops to 65% (data not shown), indicating the importance of the recovery period.

When the restraining tunnel is used, only  $196 \pm 81$  recorded beats could be counted during 1 min. This amount of beats is significantly different from the expected value

( $P=0.005$ ) and from the unrestrained value ( $P=0.017$ ). Hence, only 65% of the beats from the data recorded with the restraining system can be used for further analysis (Fig. 4). Similar results have been obtained for mice (data not shown). Nevertheless, mean RR interval could be measured from consecutive identified beats and was about  $232 \pm 49$  ms. Statistical analysis using Student's *t*-test



**Fig. 4** Amount of beats recorded during a 1-min recording with telemetric and restrained ECG monitoring system for Syrian hamsters. *Open square* data obtained from telemetry. *Filled square* data obtained from restraining tunnel. *Broken lines* expected amount of beats per minute calculated with observed heart rate. \* $P=0.005$  when compared to theoretical amount of beats/min. § $P=0.017$  when compared with telemetry

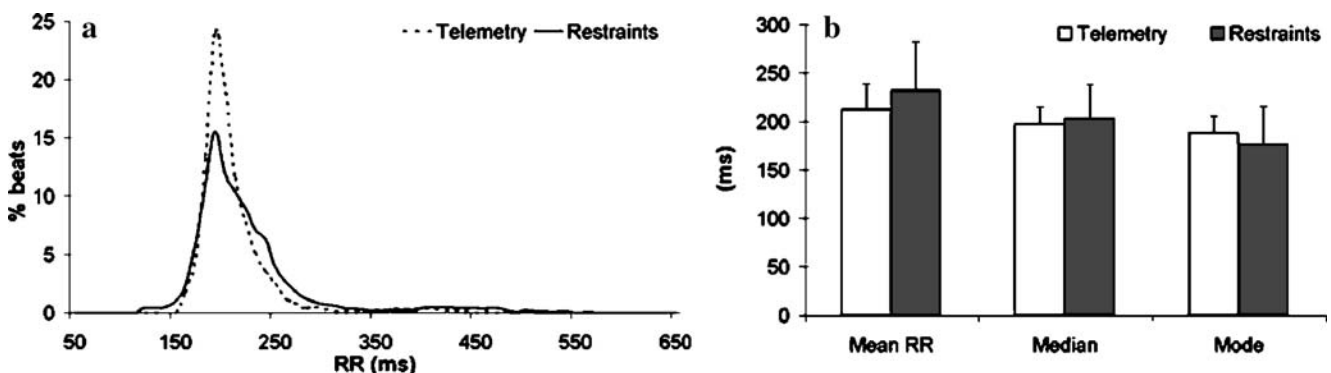
showed no significant difference in RR intervals between both recording methods ( $P>0.265$ ).

We further investigated the distribution of RR intervals observed for both telemetry and restrained ECG recording. An example of these distributions is presented on Fig. 5a. Each distribution was further analyzed using descriptive statistics. In the case of the telemetry experiments, the mean RR interval was  $212\pm 26$  ms, the median was  $197\pm 18$  ms, and the mode was  $187\pm 17$  ms. In the case of the restrained ECG, these values were  $232\pm 49$  ms,  $203\pm 35$  ms, and  $176\pm 39$  ms, respectively. The fact that these three values are not identical indicates that the distribution is not Gaussian, which explains the shape of the curves presented on Fig. 5a, and this is true for both methods. To compare the two distributions, comparisons were performed pairwise for each parameter (Fig. 5b). No statistical difference could be detected when such comparison was performed ( $P=0.265$  for mean RR,  $P=0.627$  for medians, and  $P=0.442$  for modes), which allowed us to conclude that the two systems of recording generates identical distributions of RR intervals.

## Discussion

As animal models and, in particular, transgenic models of cardiovascular diseases have become increasingly useful tools for cardiovascular physiology studies, the need to monitor the electrical activity of the heart in small laboratory animals has gained new interest [8, 18]. However, recording such data in small animals remains challenging due to the size but also to the high heart rate. Since Goldberg's [6] description of the ECG in the anesthetized mouse, this technique has incredibly evolved. ECG monitoring on anesthetized rodents is now routinely

performed, even if it is known that anesthesia impairs cardiac electric activity. Different methods of restrained ECG monitoring have been developed to eliminate the need to use anesthesia. In this study, we report a new technique to perform restrained ECG monitoring in conscious small rodents and show its versatility. This system could be used with healthy wild-type animals of different sizes ranging from 120 g (hamsters) to as low as 6 g (neonate mice) and could successfully monitor ECG from animals with heart rates up to 800 bpm. Limited to a simple tunnel, this method is less traumatizing than many of the methods developed by others. Wang et al. [17] recorded ECG from mice, housing them in a perforated restraining tube so that electrodes could be placed on the limbs protruding through holes. However, this technique could cause injuries, particularly when applied to neonates or severely diseased animals. Hamlin et al. [7] have developed a bipolar transthoracic ECG monitoring method on guinea pigs. They sandwiched animals between copper plates in a padded sling. This restraining technique might impair breathing and would hardly be transferred to diseased animals. The system that we have described presents none of these limitations. Most animals got used to the tunnel very rapidly as evidenced by visual observation of the animals through the transparent tunnel: they calm down within a few minutes and, furthermore, they withstand up to 1 h of recording. Such length of recording allowed collecting important amounts of data when compared with other restrained ECG monitoring methods. For instance, Chu et al. [3] developed a noninvasive technique for obtaining ECG in conscious mice, where animals were placed on a platform embedded with paw-sized ECG electrodes connected with an amplifier. This system was not traumatizing, but the amount of data collected was very low due to important animal freedom. Indeed, they had to discard data collected when mice were sited or when paws



**Fig. 5** Comparison of RR intervals distribution from both ECG recording methods for the same Syrian hamsters. **a** The diagram displays mean RR interval, median, and mode calculated from data recorded with unrestrained and restrained ECG monitoring. *Open*

*square* data obtained from telemetry. *Filled square* data obtained from restraining tunnel. **b** The graph displays the percentage of RR intervals according to the duration of these intervals. *Broken lines* data obtained from telemetry. *Solid line* data obtained from restraining tunnel

were not in contact with three electrodes. Only data from continuous recording of 20–30 beats could be used for analysis.

During the past decade, miniaturization of ECG transmitters permitted the development of implantable radiotelemetry devices and allowed long-term ECG monitoring on unrestrained animals. Unlike our noninvasive method, ECG monitoring using telemetry requires a surgical procedure that can only be considered in animals above a size limit close to 20 g for small transmitters. After the implantation of the telemetry transmitter and before data recording, the animals must be allowed to recover and adapt. We showed that after a recovery of 2 weeks, around 65% of beats could be used for further analysis. This yield goes up to 100% after 3 weeks, demonstrating the need of a period of recovery and adaptation before the experimental use of the instrumented animals. Similar observations have been made by others [12]. After this period of adaptation, the length of the protocol and the life of the device's battery are the only factors that limit the length of the recording period.

When compared to telemetry, the restrained ECG monitoring method shows a rather good yield, as two thirds of the total beats can be used for further analysis. This loss of data can be explained by the existence of periods of noise, during which no cardiac signal can be recorded. These periods of noise are explained by the degree of freedom that the restraining tunnel leaves to the animal. Inside the tunnel, the animal is not compressed and may continue to have spontaneous activity, although with a limited amplitude in its movements. Three paws must be in contact with the sensors for a signal to be recorded. As a consequence of paws movements, we lose the signal during 35% of the recording period. In spite of this restriction, we showed that, when recorded on the same animals, the distribution of RR intervals is identical for both recording techniques.

This restrained ECG monitoring technique presents significant advantage when one compares it to those that are currently available. First, it is very versatile, allowing recording sessions long enough to collect a large amount of ECG traces. Second, once 50-Hz interferences are filtered, the signal can easily be used for beat-to-beat analysis with the limitation that a fine analysis of the ECG trace is frequently difficult, as P and T waves cannot always be distinguished. Third, repeated recordings can be performed, making rapid screening for phenotype identification possible and allowing easy detection of gross abnormalities in cardiac rhythm. Finally, unlike ECG using telemetry, this restrained ECG recording technique allows the screening of several animals in a short time and at low expense. Despite these advantages, this restrained ECG recording method allows neither continuous long time studies nor ECG

recording during activity and exercise, as it would be possible with telemetry. Therefore, we stress the point that this technique may mainly be used as a screening technique for heart rhythm investigation.

In conclusion, our work shows that this restrained ECG monitoring technique is a well-suited tool for exploring various aspects of cardiac electrical activity in small animals. It is well adapted to beat-to-beat analysis and is compatible with the needs for phenotypic and pharmacological studies.

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