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Heart Rate and Electrocardiography Monitoring in Mice

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Abstract

The majority of current cardiovascular research involves studies in genetically engineered mouse models. The measurement of heart rate is central to understanding cardiovascular control under normal conditions, with altered autonomic tone, superimposed stress or disease states, both in wild type mice as well as those with altered genes. Electrocardiography (ECG) is the “gold standard” using either hard wire or telemetry transmission. In addition, heart rate is measured or monitored from the frequency of the arterial pressure pulse or cardiac contraction, or by pulse oximetry. For each of these techniques, discussions of materials and methods, as well as advantages and limitations are covered. However, only the direct ECG monitoring will determine not only the precise heart rates but also whether the cardiac rhythm is normal or not.

Keywords

heart rate monitoring; mice; electrocardiography; ECG

INTRODUCTION

With increasing numbers of transgenic mouse strains the treatment and evaluation of cardiovascular pathophysiology have been explored in more ways than previously possible. The monitoring and controlling of heart rate is central to meaningful interpretation of the results. Under the conscious state the normal murine heart rate ranges from 500–700 beats per minute (bpm) depending on the time of the day, environmental factors as well as activity levels (Kramer et al., 1993; Uechi et al., 1998a; D’Angelo et al., 1997). The monitoring of heart rate is of particular interest since it often reflects the dynamic changes of cardiac function in response to environmental and hemodynamic changes (Appel et al., 1989). We have previously discussed imaging methods such as echocardiography (Gao et al., 2011) and arterial pressure monitoring methods including tail cuff system, implanted fluid filled catheter, Millar solid state micro-pressure transducer tipped catheter and implanted telemetry systems (Zhao et al., 2011). All of these methods allow for the indirect measurement of heart rates once the number of cycles of either arterial pressure waves or cardiac contractions in a given time is calculated (Figure 1). However, since these methods only detect changes in cycle length of successful cardiac contractions and cannot differentiate ectopic beats from sinus beats, the electrocardiogram (ECG) is considered to be the “gold standard” for the monitoring of heart rate.

Additionally, as with arterial pressure and cardiac function, one must consider the effects of anesthesia on heart rate (Table 1). The major techniques that focus on the monitoring of conscious heart rate and that are able to determine the source of the impulse from the heart all revolve around the use of ECG. In this article we will describe the three primary methods that utilize ECG such as non-invasive ECG system, tethered ECG and implanted telemetric ECG monitoring, as well as secondary methods involving the implementation of pulse oximetry and the previously discussed techniques of echocardiography and measurement of arterial pressure, which also provide an indirect assessment of heart rate. All the methods of ECG monitoring allow for the assessment of all electrical activities in the heart in addition to providing information on heart rate, whereas measuring the arterial pressure only assesses those impulses that result in contraction. Through the use of ECG the origin of the electrical activities in the heart can be determined, thus allowing for the monitoring of cardiac rhythm and the determination of heart rate variability, as well as cardiac conduction abnormalities. Materials and methods needed for the monitoring, as well as the advantages and limitations for each of these methods are discussed.

Heart rate variability

This observed time variation from beat to beat is a normal physiological phenomenon, and is the result of changes in sinoatrial (SA) node stimulation. This beat to beat variation in heart rate reflects not only a change in physiologic condition, volume status and cardiac function but also a reflection of the animal's ability to respond to both environmental and physiologic stress such as changes in volume status, arterial pressure and autonomic tone. This beat to beat variation is best described as a change in RR (time interval between consecutive R waves in an ECG tracing; see Figure 2) interval seen on an ECG. Some of the main regulators of this beat to beat variation include both the sympathetic and parasympathetic efferent arms and baroreflex afferent arm of the autonomic reflex system (Kovoor et al., 2001; Uechi et al., 1998a). Body temperature, stress, sleep wake cycle, and animal activity, all affect the autonomic tone of the animal and thus affect beat to beat variability. Furthermore, a decrease in heart rate variability indicates a decrease in the ability of an individual to adapt to stress and has been associated with increased mortality for both myocardial infarction (Bigger et al., 1992; Kleiger et al., 1987) and congestive heart failure (Sandercock and Brodie, 2006) patients. As an example, an increase in heart rate with a decrease in heart rate variability are seen in transgenic mice overexpressing cardiac Gs α and is felt to be a risk factor for the development of cardiomyopathy in these transgenic mice (Uechi et al., 1998a).

Comparing conscious vs. sedated heart rate monitoring

Various inhalant or injectable agents are available for anesthesia or sedation in mice, either as pre- and perioperative anesthetic agents or as sedatives during a specific experiment. As described in the articles on echocardiography and arterial pressure monitoring (Gao et al., 2011; Zhao et al., 2011), the effects of anesthesia on cardiac function, blood vessels, and reflex control of the circulation have been well established (Vatner and Braunwald, 1975; Vatner et al., 2002). Therefore, studies in conscious animals are preferred when possible (Kurtz et al., 2005a, b) and if anesthesia must be used, the choice of anesthetic agents is of particular importance since different agents may lead to different degrees of autonomic and heart rate suppression in mice (Table 1) (Erhardt et al., 1984; Odashima et al., 2007; Stypmann, 2007; Yan et al., 2007; Yang et al., 1999). The combination of ketamine/xylazine (50 to 100 mg/kg/2.5 mg/kg) via intraperitoneal (IP) or intramuscular (IM) injection provides approximately 30 min of satisfactory anesthesia. By increasing xylazine from 2.5 mg/kg to 10 mg/kg, the duration of the anesthesia is prolonged to 40 min (Xu et al., 2007). One should take caution in the use of xylazine, since it is a potent respiratory depressant. Therefore, if re-dosing is necessary, ketamine alone (1/2 the original dose) should be used.

However, due to the more pronounced suppression of heart rate, the ketamine/xylazine mixture is less desirable for acute experiments. Tribromoethanol (Avertin®) 2.5%, 290 mg/kg IP, is the preferred agent for acute experiments due to its ability to maintain a more physiologic blood pressure (BP) and heart rate when compared to the ketamine/xylazine cocktail. However, Avertin has a relatively shorter half life and requires frequent re-dosing. Alternatively, pentobarbital (40–85 mg/kg IP) provides a more stable anesthetic duration of 20–60 min, and heart rates are higher under this anesthesia.

Three primary ECG methods for conscious heart rate monitoring in mice

ECG is the surface recording of the electrical currents passing through the heart and represents the electrical activities associated with each heart beat. ECG allows for the visualization of the electrical conditions of the heart. A typical ECG contains P, QRS and T waves representing atrial contraction (P), ventricular contraction (QRS) and ventricular relaxation (T), with the time between each of these waves reflecting the time between each of these components (Figure 2). Since, in general, R waves are not only the most distinct waveform with the highest voltage differential from baseline but also the easiest for an automated system to detect with high fidelity, typically the time between two R waves is considered the time for one beat. The digital heart rate read out is generated by passing the recorded ECG signals through an amplifier and the amplified signal is then analyzed by a cardiometer, where a timing device is started by the first pulse wave and stopped by the next, thus allowing for the calculation of heart rate. Alternatively, the signal is processed by data analysis software. In either case the heart rates are displayed as digital read outs in beats per minute. It is important to note that although the measured electrical activities usually correspond to cardiomyocyte contractions, less electrical activity is seen in cases of extreme shock with poor perfusion pressure.

BASIC PROTOCOL 1

NON-INVASIVE ECG SYSTEM

Due to the size, rapid heart rates and difficulty associated with keeping the mice calm during experiments, the use of surface ECG for conscious monitoring of small animals remains an arduous task. Thus, to facilitate the recording of non-invasive ECG in conscious mice a restraint device is often needed. In general, an ECG records the voltage between a pair of electrodes, the size and direction of deflection obtained reflects the size and direction of the electrical activity in the heart relative to the pair of electrodes. By analyzing the ECG tracings, information about the heart rates and cardiac rhythm is obtained. In mice this is accomplished by using a narrow platform where three paw sized electrodes are embedded and are then connected to an amplifier. The mouse is placed gently on the electrodes where three of the paws are in contact with the electrodes for continuous recording. The electrical signal among these three electrodes is recorded and the resulting ECG is then analyzed on a computer.

Advantages and disadvantages of non-invasive ECG system—This system has the advantages of non-invasively obtaining information not only on heart rates but also heart rate variability, arrhythmia analysis, RR, QT, and PR intervals, as well as conduction abnormalities in conscious animals. Thus it provides information about the phenotype and/or the effects of pharmacologic intervention on both heart rate and arrhythmia risk. Furthermore, the system can be employed to screen a large number of mice for ECG changes related to genetic, pharmacologic or pathologic alterations. The main disadvantage is that such a system is not suited for continuous long term monitoring; therefore, it is better adopted for studies where periodic short term ECG monitoring or screening is needed.

Materials

AnonyMouse® ECG screening system (Mouse Specifics, Inc.)

Gel coated ECG electrodes

Amplifier (HP78901A, Hewlett-Packard, Andover, MA)

Shielded 3 electrode lead set (M1605A Snap, Hewlett-Packard, Andover, MA)

Recording computer

e-Mouse® internet based wave analysis portal

1. Gently place the mice onto the ECG platform and allow them to acclimate for at least 10 minutes.

The platform is capable of testing one mouse at a time, while having two additional spots for the acclimation of two mice.

2. Using a cotton tipped stick, gently push the mouse into position where the two front paws and the left rear paw are in contact with the electrodes.
3. Turn on the system and perform the recording as per the manufacturer's manual.
4. While the recording is being completed on the first mouse, place the next mouse on the side platform to allow for acclimation.
5. Analyze the data once the recording is completed using the e-Mouse® internet based wave analysis portal.
6. Calculate the heart rate by either counting number of R waves over time or averaging several RR intervals.
7. Calculate heart rate variability by using the average beat to beat variability, i.e., RR interval variability within the recorded period.
8. Correct the QT by using $QT/\sqrt{(RR/100)}$ (Mitchell et al., 1998).

BASIC PROTOCOL 2

TETHERED ECG SYSTEM

Similar to non-invasive ECG monitoring of mouse heart rate, the tethered system provides information via ECG tracing. Heart rates, conduction abnormalities, and arrhythmias can all be recorded and analyzed. Unlike the non-invasive method of ECG recording, four electrodes are imbedded on the back of the mice where the wires are then tunneled under the skin and exit via a mid scapular incision. The wires are attached to a swivel device via a spring tether and connected to an amplifier where the signal is continuously recorded using an analog or digital recorder.

Advantages and disadvantages of tethered ECG system—The advantage of such a tethered system is that it allows the mouse to have increased freedom of movement and access to food and water without the stress associated with the use of a small restraining cage. Therefore, this system allows for longer duration of recording. Furthermore, since the electrodes are attached to the animal and embedded under the skin, the signal is affected to a lesser extent by mouse movement when compared with the non-invasive method. However, the disadvantages of this system are the need for surgically implanting the electrodes under general anesthesia, as well as the associated surgical morbidity and mortality. Even though the tether is out of the reach of the mouse, the animal should not be left alone during

recording. Therefore, such a system is most suited for periodic recording of intermediate duration.

Materials

ECG (Biotech amplifier, Gould; Tektronix TDS 1002, Oscilloscope, Tektronix)

Insulated wire leads (AWG size 36)

Tether system including a harness (or jacket) (Insteck)

Swivel system (Insteck)

Suture: 6-0 nylon for ECG lead fixation and skin closure

Anesthesia (see Table 1 and step 2)

1 Prepare the implantable ECG wires:

- a. Four strands of insulated wire are tied together as a bundle about 5 cm from the end (make sure the length behind the tie is longer than the length of spring tether) and make the two wires for the forelimbs shorter (2.5 cm) (Figure 3).
- b. Each tip of the wire is then made bare by removing 3 mm of insulation from the wire to increase the contact surface.

Make sure to mark each wire to indicate which tip is for left arm (LA), right arm (RA), left leg (LL) and right leg (RL) leads, and do not forget to distinguish and mark which one of the longer-length wires corresponds to the forelimb leads so that proper recording of the signal is achieved once the whole system is connected to the recording machine.

2 Anesthetize the animal and place the mouse in the prone position.

The choice of anesthetic depends upon the experimental design and the experience of the individual laboratory. Please refer to Table 1 for the effects of each of the anesthetic agents on the heart rate. Different agents may be used for surgery and experiments. In our laboratory, we generally use a mixture of ketamine and xylazine for surgery and perform the experiments under a conscious condition.

3 Make a 1 cm transverse incision in the mid-scapular region of the mouse.

4 Transfix the tied portion of the ECG wires to the underlying muscle with 6-0 nylon suture.

5 Tunnel subcutaneously the four shorter-length wires in the direction of the four limbs respectively so that the bare tips are in good contact with the underlying muscle (Figure 3).

6 Bring out the four longer-length portions of the wires through the previously made skin incision and close with nylon sutures.

7a Pass the wires through the harness and connect to the swivel assembly via a spring tether.

8a Connect the wires to an ECG signal amplifier and a recording system.

Alternative method: Handling the exteriorized ECG wires

Since the tether system is generally employed to house the fluid line and wires together and is more suited to house fluid line for protection, if subcutaneous implantation of ECG wires is the only procedure needed for monitoring, the tether system can be avoided by following the simpler steps below.

- 7b** Coil the exteriorized wires to be housed in a small protective plastic tube which is suture-fixed to the skin around the closed mid-scapular incision site.
- 8b** Whenever ECG recording is needed, place the mouse in a restraint box which has a ceiling hole.
- 9b** Deliver the housing tube through the ceiling hole and uncoil the wires after bringing them out of the housing tube, then properly connect the four designated limb leads to the recording system.
- 10b** Once the recording is complete, re-coil the wires and return them to the housing tube for protection until the next recording.

BASIC PROTOCOL 3

IMPLANTED ECG TELEMETRY SYSTEM

The ECG radiotelemetry units are the gold standard for monitoring the heart rates in conscious mice. A typical mouse ECG telemetry system contains two electrical ECG leads connected to a radio transmitter. The radio transmitter is placed into a subcutaneous pocket on the back of the mouse. And the leads are implanted with one lead toward the right upper chest and the other near the left lower chest. Upon activation of the transmitter by a magnet the electrical signals are transmitted wirelessly to a nearby receiver attached to an amplifier and computer system for data acquisition, storage and analysis. Through such a system, the heart rate variability in different transgenic animals is recorded and analyzed as exemplified by the decreased heart rate variability seen in transgenic mice overexpressing *G α* (Figure 4) (Uechi et al., 1998a).

Advantages and disadvantages of implanted telemetry system—With the use of an implanted telemetry device, a continuous conscious recording of the mouse heart rate is possible. This provides a method for long term monitoring of the mice in their natural living environment that is often needed to detect circadian variations, as well as a means to monitor arrhythmia frequencies and to determine if arrhythmias are the cause of death in transgenic mice. Recently, the telemetry systems that allow simultaneous monitoring of ECG and arterial pressures in mice are in development. The disadvantages include high cost as well as surgically related morbidity and mortality. Since only two electrodes are present, only a single lead of ECG recording is obtainable. Furthermore, a 5–7 days recovery period is often needed after implantation for stabilization of the heart rates (Lorenz, 2002). Therefore, the implanted telemetry system is most suited for studies where long term monitoring of mouse heart rate and cardiac rhythm in their natural living environment is needed.

Materials

Telemetry transmitter device (ETA F-10 or EA-F20) (Data Sciences International, MN)

Suture: 7-0 silk for telemetry wire fixation, 5-0 nylon for transmitter fixation to skin

Anesthesia (see Table 1 and step 1)

- 1.** Anesthetize and place the mouse in the supine position, then make a 1cm mid-scapular transverse incision on the back of the mouse.

The choice of anesthetic depends upon the experimental design and the experience of the individual laboratory. Please refer to Table 1 for the effects of each of the anesthetic agents on heart rate. Different agents may be used for surgery and experiment. In our laboratory we generally use a mixture of ketamine and xylazine for surgery and perform the experiments under conscious condition.

2. Create a subcutaneous pocket by using a pair of round tip scissors undermining the skin on the left lower back to create a pocket large enough to accommodate the transmitter device.
3. Insert the transmitter device into the pocket with the side of the device containing the predrilled suture holes facing up and transfix it to the overlying skin with 5-0 nylon sutures (Figure 5).
4. Remove 5 mm of silicon insulation from the tip of both wires to increase the contact surface.
5. Make an extra small skin cut in the right shoulder area and tunnel the negative wire (white) subcutaneously toward the skin cut and through the cut opening transfix the bare wire tip to the underlying muscle with a 7-0 silk suture.
6. Turn over the animal, tunnel the positive wire (red) subcutaneously and transfix the tip to the abdominal muscle through a small skin cut just caudal to the left rib cage in a similar fashion. The correct lead length is managed by coiling the wires around the device.
7. Close all incisions and cut openings with nylon sutures with the knots buried beneath the skin.
8. Power on the battery in the transmitter using a magnet.

Verify the functioning of the transmitter by powering up an AM radio and bringing it close to the device and by listening to a high-pitched tone corresponding with the cardiac cycle.
9. Return the animal to the cage and allow to recover.
10. Place the signal reception plate under the animal cage and connect the cables to the data acquisition system for recording, storage and analysis (Figure 6).

BASIC PROTOCOL 4

PULSE OXIMETRY

The use of pulse oximetry has been a long accepted method for the monitoring of mammalian heart rates and blood oxygen saturation. In 1991, Iyriboz et al. validated the accuracy of pulse oximetry for the monitoring of heart rates both at rest and during exercise (Iyriboz et al., 1991). Since that time many different versions of the pulse oximeter have been developed for use in small laboratory animals including mice. In general, pulse oximetry uses the inherent differences in the level of absorption of red light (600–750 nm) and infrared light (850–1000 nm) between deoxy- and oxyhemoglobin. By transmitting these two different wavelengths of light through a translucent part of the animal and monitoring the received light by a photodiode, the ratio of oxy- and deoxyhemoglobin are determined. Furthermore, since the signal intensity increases with arterial vessel expansion associated with the heart beat, the pulse rate is obtained simultaneously and recorded on a computer for data analysis.

Advantages and disadvantages of pulse oximetry—The advantage of such a system is that it is non-invasive and allows for the conscious monitoring of mouse heart rate while simultaneously monitoring the blood oxygen saturation. By using the neck clip, the mouse has limited amount of freedom in movement, yet the heart rate is monitored in a relatively non-stressed state. However, the disadvantages of such a system is that it does not allow for long term monitoring and free movement, with the latter usually leading to distortion of the transmitting signal. Furthermore, the measured hemoglobin oxygen saturation may not always reflect accurately the respiratory sufficiency as the measured oxygen saturation reflects only the percent hemoglobin saturation and not necessarily the amount of delivered oxygen. In cases of severe anemia the actual amount of oxygen delivered is low despite a high percent saturation. Additionally, carboxyhemoglobin and methemoglobin all lead to falsely elevated oxygen saturation.

Materials

MouseOx® pulse oximeter with collar, throat clips and thigh sensors (Starr Life Sciences Corp.)

MouseOx® software (Starr Life Sciences Corp.)

STARR-Link™ analog output module (Starr Life Sciences Corp.)

1. Prepare the animals by training them with desired clip at least for 1 hour prior to the scheduled experiment (3–6 hours are preferred).
2. Select the correct sensor. Extra-small sized sensors are used on unshaven mouse with body weight lower than 15 g or shaved mouse with body weight lower than 20 g. For mice of greater body weight, use a small size sensor.
3. Remove hair from the clip sensor site to allow for improved signal acquisition, preferentially remove hair several hours prior to the start of the experiment.

With pulse oximeter, the experiments are conducted under either conscious or sedated condition. Usually white CollarClip™ is used in both conditions with animals in a prone position, while black ThroatClip™ and Thigh sensor are used only on anesthetized mice in the supine position.

4. Place the CollarClip™ right behind the ears and center the clip handle. For neonates, the clip is placed on the head across the ears.

If the mouse struggles, hold the tail and let its front legs hold on to the cage, then attach the sensor. If necessary, animals may be lightly anesthetized to help placement of the sensor. It is important to make sure that the clip is in the correct position with the tines squeezed together to ensure consistent signal quality.

5. Send the mouse back to the cage with just enough length of wire so that the animal will not chew on the loose cable.
6. Place the animal on a non-reflective surface and use a light-blocking cloth covering on the cage to avoid light reflection or artificial light.
7. Connect the other end of the wires to the MouseOx® before starting the software. Then follow the menu to start recording.
8. Go to the MouseOx® software main menu, select “Monitor Subject” which will ask whether the animal is anesthetized or awake.
9. Push the “Start/Reset” button to initiate data recording after the animal is stable for at least 15min.

If the signal doesn't come up quickly, check if the sensor is in the "ON" position. If the sensor is removed from the animal during recording, the "Start/Reset" button has to be clicked again when the sensor is put back on the animal.

10. Perform the recordings. The recording window displays heart rate waveforms on the left panel and Pulse Pleth on the right upper panel. Below the Pulse Pleth figure, there is a digital display showing the real time reading and cursor pointed reading.

If pulse distention shows low values ($<5\mu\text{m}$), it may be due to vessel contraction or low blood volume, etc.

If the experiment requires controlling a relatively stable heart rate, a "Set Alarm" button (on the right panel) is used to remind the investigator if the heart rate is above or below the limit set for the experiment.

11. Save the MouseOx® data results, these are saved as a ".txt" file accessible by Microsoft Excel for further analysis. On the last column, the listed "error codes" provide information on unstable pulse or lost pulse/signal. The manufacturer's manual shows the listed description of all the codes.

COMMENTARY

Background Information

The monitoring of mouse heart rate is central to mouse phenotyping and determination of mouse cardiovascular pathophysiology. Different genetically altered mouse models have been shown to have either markedly higher or lower baseline heart rates (Table 2) (D'Angelo et al., 1997; Peter et al., 2007; Uechi et al., 1998b) making the determination of baseline heart rate an important variable for phenotyping. An added dimension to differences in heart rates is the differences in heart rate variability. Heart rate variation reflects not only cardiac function but also the animal's ability to respond to physiologic and environmental stress such as change in volume status, arterial pressure and autonomic tone. For the monitoring of cardiovascular function and physiology various methods have been developed and described. Here we discuss three major ECG methods as well as other secondary methods such as pulse oximetry, echocardiography and arterial pressure wave form analysis for determination of the heart rate in mice (see below).

It has been over a century since William Einthoven first described the use of surface electrodes to record cardiac electrical activity and generating the first ECG (Rivera-Ruiz et al., 2008). Since that time ECG has been used to monitor the cardiac electrical activity within different mammalian models. Since R waves are the most distinct wave forms, they are the easiest for an automated system to detect with high fidelity. The amplified ECG signals are analyzed by either cardiometer or commercially available software where heart rate is determined by calculating the time between two consecutive R waves.

In general, all three primary methods involve the analysis of ECG function by way of using the surface electrodes to monitor the electrical activity in the heart. By observing this electrical activity the heart rate is calculated with the assumption that each of the observed electrical pulses results in a cardiac contraction cycle. However, in cases of extreme shock and hypo-perfusion pulse, less electrical activity is observed. The major advantage of ECG is its ability to assess all electrical activities within the heart, whereas measuring arterial pressure only assesses those impulses that result in contraction. ECG monitoring allows for the identification of arrhythmia and arrhythmogenic electrical activities. By determining the

origin of cardiac electrical activity, those heart beats that originate in the SA node can be identified and this information is needed for the determination of heart rate variability.

Non-invasive ECG system—Uses a narrow platform embedded with three paw sized electrodes where contact is maintained between three paws of the mouse and the electrodes. The electrical signal among these three electrodes is recorded and the resulting ECG is used for the analysis of heart rates.

Tethered ECG system—This system imbeds four electrodes on the back of the mice that are tunneled under the skin toward four limbs and exited via a mid scapular incision. These wires are connected to an amplifier and the obtained ECG signals are then used for heart rate analysis.

Radiotelemetry ECG system—This system is the gold standard for conscious monitoring of heart rates. A typical mouse radiotelemetry system for ECG monitoring contains two electrical ECG leads connected to a radio transmitter. Once they are implanted the ECG signal is transmitted wirelessly to receiving and recording units for heart rate analysis via commercially available software. For detailed background information for each of the individual ECG methods as well as pulse oximetry, please refer to the Basic Protocols.

Secondary methods for the monitoring of heart rate in mice—Echocardiography, tail cuff arterial pressure monitoring system, implanted fluid filled catheter, Millar solid state micro-pressure transducer tipped catheter and implanted telemetry system all can be used for the determination of heart rate through either a computer analysis software or a cardiometer, whereby the heart rate is determined by measuring the time required for each of the cycles of either arterial pressure waves or cardiac contractions, in this way a heart rate tracing is generated (Figure 1).

Echocardiography: In terms of echocardiography, the heart rate is determined either by analyzing the M-mode images or using the simultaneous ECG recordings. Since distance per time is a known variable, by marking either the systolic or diastolic peak of the M-mode images, or the R wave peak on the ECG distance between the peaks are calculated by the image analysis software. These data points are then imported into data analysis software such as Microsoft Excel whereby the time between the beats are calculated. Then by averaging 3–4 consecutive beats, average heart rates are determined.

Along with arterial pressure monitoring: In the case of implanted fluid-filled catheters, Millar solid state micro-pressure transducer tipped catheter and implanted telemetry system, the heart rate is calculated via analysis of the pressure wave signals by the recording system and displayed as an instantaneous read outs on the computer screen. In brief, the recorded pressure wave signals are first passed through an amplifier, and the amplified signals are then processed through a cardiometer where a timer is started by the first pulse wave and stopped by the next wave, after which the heart rate is calculated and displayed in beats per minute. An additional method for heart rate monitoring is the tail cuff system, in which the heart rate is calculated via the attached pulse oximetry detailed below.

Since these methods are only able to detect changes in cycle length and are unable to exclude ectopic heart beats that do not originate from the SA node, the ECG is considered to be superior for the monitoring of heart rate variability.

Critical Parameters and Troubleshooting

Non-invasive ECG system

1. It is a necessary step to acclimate the animals by keeping them on the platform for at least 10 minutes before experiments.
2. It is important to use the correctly sized electrode pads for the size of the mouse. Place the electrode pads with a spacing of 2.5 – 3 cm. Spacing that is too large or too small may cause a loss of signal with animal movement.
3. It is best to avoid bright lights in the room and reduce nearby motion and noise during the experiments as any of these factors may lead to agitation of the mouse thus leading to poor data quality.

Tethered ECG system

1. For the implanted ECG tether, embedding the leads within the muscles (thus keeping the leads in good contact position) results in a more stable signal. A suitable way is to loop the bare wire tips and bury them between the muscle fibers. Fixation sutures are used to securely hold the wire tips in place.
2. Prior to recording it is important to establish connection of the properly designated lead wires to the recording system. Therefore, confusion will be minimized by marking each of the wires with any available means (e.g. knotting, painting, etc.) to indicate which tip is for left arm (LA), right arm (RA), left leg (LL) and right leg (RL) leads during implantation.

Implanted ECG telemetry system—The critical parameters are the same as those of the tether system. Proper fixation of the ECG leads will help to obtain a stable signal as well as lessen the pain associated with ECG wire tip movement within the muscles.

Pulse oximetry—The most common problem encountered with the system is poor signal acquisition. The following will help minimize this potential complication:

1. It is important to use the right size sensor and make sure the clip is well attached.
2. Shaving the sensor site will help improve signal quality, especially on mice with dark fur, in which case shaving is required.
3. For the thigh clip, it should be applied to the upper thigh, as close to the pelvis as possible to ensure good signal acquisition.
4. It is important to ensure that the animals maintain a physiological temperature during recording. Utilize an external heating device (i.e. heating pad or lamp) under or over the cage if needed.
5. If all else fails and signal quality continues to remain poor, the sensor application site should be re-adjusted and press “Start/Reset” button to restart the recording.

Anticipated results

The anticipated result for all three primary methods of obtaining and recording mouse ECG is the recording of the ECG tracings (Figures 2 and 6) to be used for data analysis by ECG analysis software. Whereas the anticipated result for echocardiography is the recording of echocardiographic images, often accompanied by ECG tracings (for examples please refer to our previous paper on echocardiography in Gao et al., 2011) and the anticipated result for methods involving invasive monitoring of arterial pressure is the recorded arterial pressure wave forms (Figure 1), the anticipated result from the heart rate monitoring systems is an

analog or digital read out of the heart rate. The effects of physiologic and experimental conditions, sedation, volume status, time of day, age, gender, activity and temperature on the measured heart rate has been well established. Therefore, there is often some amount of disagreement for the measured heart rate obtained from different laboratories. However, as a general reference some normal conscious heart rate values for a few of the more commonly used strains of mice can be found in Table 3. In an ideal experiment, arterial pressure should also be monitored. With abnormal blood pressure or unexpected deviation in either heart rate or respiratory rate one should consider repeating the experiment.

Considerations and Experimental Design—The common aspect between the two non-invasive methods (pulse oximetry and non-invasive ECG monitoring) is that both of them share the advantage of being non-invasive while both are more suited for short term monitoring. Although pulse oximetry provides additional information in terms of oxygen saturation, the proper interpretation of this information must be taken into account for hemoglobin concentration and the possible presence of carboxyhemoglobin and methemoglobin. An important advantage of ECG systems over indirect measurements of heart rate is the ECG's ability to determine which heart beat originated from the SA node vs. ectopic heart beats. This is of particular importance since this allows for the exclusion of ectopic beats, thus allowing for the calculation and comparison of heart rate variability. Non-invasive ECG monitoring provides information on rhythm in addition to rate. This allows for the measurements of QT, RR and PR intervals. At a basic level this allows for the detection of conduction abnormalities and the presence of arrhythmias. Furthermore, since RR interval represents the time between two consecutive beats, the variation of RR intervals represents heart rate variability, where a decrease in this variability has been shown to be associated with increased mortality after myocardial infarction (Bigger et al., 1992; Kleiger et al., 1987) and congestive heart failure (Sandercock and Brodie, 2006) in patients with myocardial infarction (Bigger et al., 1992; Kleiger et al., 1987) and heart failure (Sandercock and Brodie, 2006). There are currently two major methods used for the comparison of heart rate variability: A time domain method, where the heart rate variability is compared over a given time period (usually 24 hours), versus a frequency domain method where the frequencies of the heart rates are compared, most commonly this is done through discrete Fourier transformation. Aside from heart rate variability, the heart rate corrected prolongation in QT has also been associated with an increase in cardiac arrhythmia and in cardiac mortality in the elderly (de Bruyne et al., 1999) as well as the general population (Montanez et al., 2004). Animal models that are capable of detecting drug associated QT prolongation may prove to be useful for the safety screening of pharmacologic compounds. In mouse models, one accepted formula for the heart rate associated QT correction is $QT_c = QT/\sqrt{(RR/100)}$ (Mitchell et al., 1998). In non-invasive ECG systems, the mouse must maintain continuous contact with the platform for recording, therefore animal movement has the potential to disrupt this contact and long term continuous ECG monitoring is not possible.

If longer term continuous monitoring is desired, a tether allows for the monitoring of free moving mice for an extended period of time under the direct supervision of the investigator. In addition, the monitoring of heart rate changes associated with exercise is possible with this system. The data are analyzed and graphically illustrated to reflect heart rate changes and heart rate variability over the entire study period. However, if long term monitoring of circadian variability is needed, the implanted system should be used. This is considered the gold standard for the monitoring of heart rate in mice as it allows for obtaining the most physiological values. Furthermore, telemetry systems capable of simultaneous recording of arterial pressure and ECG are now in development.

In brief, any particular method selected for monitoring heart rate in mice should depend on the experimental purpose, design and duration. The methods and materials discussed in this article provide a practical blueprint for utilization of the chosen technique.

Time considerations

Non-invasive ECG system—Expect acclimation time of at least 10 minutes and approximately 10–15 minutes to complete the recording procedure.

Tethered ECG system—Expect approximately 5–10 minutes to prepare and anesthetize the animal. While awaiting the anesthesia to take effect the ECG wires can be prepared. The implantation of the wires can be completed in approximately 10–20 minutes. Once the procedure is complete, allow the animal to recover over night prior to recording. Actual time requirement depends upon the skill of the surgeon. In general, it takes approximately 2 minutes to connect the animal to the recording system. If the harness and swivel is to be used for recording, an additional 5 minutes of time should be added for connecting the animal to the recording system.

Implanted ECG telemetry system—Expect approximately 5–10 minutes to prepare and anesthetize the mouse. The implantation of the telemetry unit takes approximately 5 to 20 minutes to complete. Actual time requirement depends upon the skill of the surgeon. Once the procedure is complete allow the animal to recover for 3–5 days prior to recording.

Pulse oxymetry—The mouse should be trained for at least 1 hour prior to recording (3–6 hours preferred). Signals are obtained instantaneously. Allow the animal to stabilize for at least 15 minutes prior to recording. Total preparation time prior to training/recording is 5 minutes.

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Figure 1. Calculation of heart rate (HR) (panels C and D) by counting the number of aortic pressure waves (AOP) (panels A and B) in a sedated mouse. The arrows represent the injections of phenylephrine (PE) to increase pressure and sodium nitroprusside (SNP) to decrease pressure. The arterial baroreceptor reflex reduces heart rate with pressure elevation and decreases heart rate when pressure falls.

Figure 2.

Sample of telemetry ECG simultaneous recording of three mice. A typical mouse ECG contains a P wave, followed by the QRS complex and the T wave. The distance between two consecutive R waves is termed RR interval and represents the time it takes for one beat, and the PR interval represents the time for electrical conduction from the atria to the ventricle. The + on the ECG represents the computer identified R wave.

Figure 3.

An illustration of tethered ECG insertion and recording of mouse heart rate. The ECG leads are labeled and subcutaneously tunneled onto the back of the mice in positions of the four limbs. Mice are housed individually during the recording process with the leads connected to the tether system for data acquisition.

Figure 4.

Circadian variation of heart rate in transgenic mice with cardiac specific overexpression of *Gsa* (TG) and wild-type mice. Heart rates were obtained from ECGs recorded using a telemetric system in conscious unrestrained mice. The inset shows the difference in heart rate variability over a 1-hour period in one TG and one wild-type control mouse. Figure and legend used and modified with permission (Uechi et al., 1998).

Figure 5.
An illustration of the telemetry unit with the anchoring sutures in place. In general 2–3 anchoring sutures are used to fix the telemetry unit to the chest wall of the mice.

ECG recording using a telemetric system from a free-ranging transgenic mouse (top)
and a wild-type control mouse (bottom)

Figure 6.

Sample of ECG recording using a telemetric system for a free-ranging transgenic mouse overexpressing $G\alpha$ (top) and a wild-type control mouse (bottom). The telemetric implant is shown in the abdominal cavity of the mouse. The recorded heart rates were higher in the transgenic mice. The darker lines at the right of the ECG tracing shown are the compressed ECG tracing printed at a slow paper speed. Figure and legend used and modified with permission (Uechi et al., 1998).

Table 1

Heart rate (HR) in conscious and anesthetized mice

Anesthesia	None	Avertin	Ketamine/Xylazine	Pentobarbital	Isoflurane
HR (bpm)	580±18	411±17	159±11.8	377±11	457±17
Data resource	Uechi et al., 1998	Yan et al., 2007	Erhardt et al., 1984	Yang et al., 1999	Stypmann, 2007

Table 2

Baseline Heart Rates of Transgenic Mice

Overexpressed Gene	Transgenic	Wild Type	Data Source
β_1 -AR	507 $\pm$ 18	423 $\pm$ 20	Peter et al. 2007
β_2 -AR	551 $\pm$ 51	423 $\pm$ 20	Peter et al. 2007
Gsa	736 $\pm$ 15	580 $\pm$ 18	Uechi et al. 1998
Gaq-25	427 $\pm$ 9	690 $\pm$ 13	D'Angelo et al. 1997

Baseline heart rate in beats per minute in transgenic mice overexpressing β_1 -, β_2 -adrenergic receptors (AR) (conducted under Avertin anesthesia), Gsa, or Gaq (conducted in conscious mice) are compared to wild type littermates.

Table 3

Conscious heart rate (HR) in three strains of mice

Strain	C57/B6SJL	FVB	129Sv/J
HR (bpm)	588±14	617±26	522±11
MAP (mmHg)	103±1	99±2	102±8
Data resource	Uechi et al., 1998	Lin et al., 2010	Gross et al., 2003

All values are mean±SE. (MAP=mean arterial pressure; HR=heart rate)