Continuous Observation on Heart-Disease-Model Mice Using Biomagnetic Measurement System

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Abstract. Magnetocardiography (MCG) is a non-invasive method that can contribute to elucidating heart disease mechanisms and the verification of pharmacological effects. The object of our study is to show the potential of MCG for such study in mice. By using the developed MCG system, which adopts a single channel superconducting quantum interference device (SQUID) magnetometer with the spatial resolution of 500 μm, we continuously measured MCGs for 2 heart-disease-model mice with a high incidence of cardiac infarction from 7-weeks-old to death. An abnormal MCG appeared 1 or 2 weeks before death. The abnormal MCG changes indicate that the damaged place in the ventricles was different for each individual. In addition, we have developed a method to obtain MCGs for newborn mice in particular because they are small and frail. The MCGs of newborn mice were similar to those of adult mice. This study proved the potential of MCG for detecting abnormal cardiac excitation at the early stage of cardiac infarction and monitoring the progress of heart disease in detail from infancy to old age in mice.

1. Introduction
Currently, heart disease is the major cause of death in humans in many countries. In Japan, it is the second most common cause of death. On the other hand, a large variety of heart-disease-model mice has been generated by gene modification or by administering drugs, and these models can be used to study disease development and treatment. Studies involving these mice mainly use invasive anatomical methods. It is noticed that the non-invasive methods permit continuous observation of the condition of the heart in the same one mouse. Therefore, non-invasive methods are suitable for studying disease occurrence, disease progression, and the recovery process with medicine. Besides invasive methods, the non-invasive methods should also be considered for elucidating heart disease mechanisms and the verification of pharmacological effects.

Magnetocardiography (MCG) is a non-invasive method having the best temporal resolution among non-invasive methods, such as fMRI and PET. Further, the spatial resolution is better than that of...
electrocardiography (ECG). Consequently, MCG is already used to diagnose human heart diseases. To show that MCG has potential even in studies that use mice, we have already developed a system for obtaining MCGs specifically for mice. By using the developed MCG system with a spatial resolution of 500 μm, healthy mice were subjected to MCG and using the MCG data obtained, we were able to draw a magnetic contour map. The magnetic contour map represents the condition and position of the cardiac excitation. In this study, we applied it to disease-model mice in order to show that MCG could detect heart disease in mice. We continuously measured MCGs for the heart-disease-model mice with a high incidence of cardiac infarction to observe abnormal excitation of the cardiac muscle at the early stage of the infarction. An abnormal MCG appeared 1 or 2 weeks before death. The magnetic contour map was of great use for detecting the abnormality.

Heart disease in foetuses and infants is also a serious problem. Therefore, we also attempted continuous MCG measurement for an infant mouse aged from 1 to 5 weeks. It was found that the waveform and magnetic contour map for the newborn mouse were similar to those for the adult mice.

2. Biomagnetic measurement system

The biomagnetic measurement system, shown in Figure 1, comprises a single channel superconducting quantum interference device (SQUID) magnetometer and a non-magnetic measurement table. The system is installed in a magnetically shielded box, which is placed in an electromagnetically shielded room.

![Figure 1. Biomagnetic measurement system.](image1)

![Figure 2. Internal configuration of the dewar.](image2)

A low-Tc direct coupled dc SQUID magnetometer using Nb/AlOx/Nb Josephson junctions is adopted to suppress magnetic flux noise. The minimum distance between the pickup coil and the outside of the dewar tail (lift-off distance) is reduced to 700 μm (Figure 2). The minimum spatial resolution of the SQUID magnetometer is 500 μm. The magnetic field sensitivity is 1 pT/Hz^{1/2} at 10 Hz, and 0.75 pT/Hz^{1/2} in the frequency range between 100 Hz and 10 kHz. The MCG data were stored in a computer by 4 kHz sampling through a band pass filter of 0.08–1000 Hz and a notch filter at a power line frequency of 50 Hz. Incidentally, the frequency of the mouse heartbeat is approximately 10 Hz. The ECG in the lead II configuration (a negative electrode placed on the right forefoot, a positive electrode placed on the left rear foot, and an earth electrode placed on the left forefoot) was obtained simultaneously. The obtained MCGs were averaged 400 times by aligning the signals at the time point of the R wave peak of the ECG. A silicon tube for anaesthetic gas supply was introduced from the outside of the electromagnetically shielded room. The gas is jetted into the mouse’s nose during MCG measurement. The body temperature of the mouse decreases under anaesthesia; therefore, a handmade waterbed was placed on the non-magnetic measurement table to keep the body temperature constant.

3. Measurements and results
3.1. MCG measurement of the heart-disease-model mouse

To observe the onset of cardiac infarction, we continuously measured MCGs for 2 heart-disease-model mice. The model mice were (NZW × BXSB) F1 type with a high incidence of cardiac infarction. The incidence of cardiac infarction at age 4 months is 20% and 50% at age 6 months. The measurement for these mice commenced at age 7 weeks. We used a NZW/N-type mouse as the control.

Anaesthesia was administrated with isoflurane (1.5–2.0%), O2 gas, and N2O gas using the tube. The mice were placed in the supine position on the waterbed (Figure 3). Figure 4 shows the measurement points based on the xiphoid process. There were 16 measurement points (4 × 4 grid points shown as black dots in Figure 4) on the thorax of the mice. The measurement points were set at 4 mm intervals. MCG measurement was performed for 1 minute at each point by moving the table.

Figure 3. Measurement set-up.  
Figure 4. Measurement points.

Figure 5 is an example of an averaged MCG of a control mouse aged 9 weeks. Figure 6 shows the magnetic contour maps at the peak time of the S wave; the left column shows the results for a control mouse aged 7, 9, and 13 weeks and the right column shows the results for heart-disease-model mouse A at 7, 9, and 11 weeks. For the control mouse, the magnetic contour map pattern is maintained up to 13 weeks of age. By contrast, a distinct change in the magnetic contour map appeared at age 11 weeks in the case of heart-disease-model mouse A. This mouse died when it was 13 weeks old. Incidentally, a healthy mouse survives for more than 1 year. That is, we obtained evidence of abnormal excitation of the heart muscle 2 weeks before death. However, the magnetic contour map pattern at the peak time of the R and T waves is maintained at 11 weeks of age.

Figure 5. Example of averaged MCG waveforms of a control mouse aged 9 weeks.
Figure 6. Magnetic contour maps at the S wave for the control mouse and heart-disease-model mouse A. The dimensions of the maps are $12 \times 12$ mm. The contour line interval is 5 pT. The white and black areas indicate the positive and negative components of the magnetic field, respectively.

Figure 7 shows the magnetic contour maps at the peak time of the R and T waves for heart-disease-model mouse B. The dimensions of the maps are $12 \times 12$ mm. The contour line interval is 5 pT. The white and black areas indicate the positive and negative components of the magnetic field, respectively.

Figure 7. Magnetic contour maps at the R and T waves for heart-disease-model mouse B. The dimensions of the maps are $12 \times 12$ mm. The contour line interval is 5 pT. The white and black areas indicate the positive and negative components of the magnetic field, respectively.

3.2. MCG measurement of the newborn mouse

Heart disease in foetuses and infants is also a serious problem. However, there are some problems with performing MCG on infant mice. A newborn mouse is too small (approximately 20 mm long when it is 1-week-old) and frail; therefore, better methods of determining the measurement area, administering
anaesthesia, and performing simultaneous ECG are required. To prove that MCG measurement is possible for newborn mice, we attempted MCG measurement for a NZW/N-type healthy mouse. There were 25 measurement points (5 × 5 grid points) on the thorax of the mouse. These points were set at 1 mm intervals. At each point, the MCG measurement was performed for 1 minute. Anaesthesia was administered with O₂ gas and N₂O gas using the tube. The mouse was placed in the supine position on the waterbed (Figure 8). We continuously measured MCGs for the mouse from 1 to 5 weeks of age.

Figure 8. Measurement set-up for a 1-week-old mouse.

Figure 9 is the averaged MCG waveforms of a 1-week-old mouse. The QRS complex and T wave are visible. These waves, including the amplitude, are similar to the corresponding waves in a healthy adult mouse, as shown in Figure 5. Figure 10 shows the magnetic contour maps at the peak time of the R, S, and T waves alongside those of an adult mouse. It is noticed that the cover area on the thorax is relatively the same, considering the size of the heart, although the actual size of the map is different. Afterwards, we continued MCGs for the mouse every week until it was 5-weeks-old by changing the anaesthetizing ratio and measurement points according to body size. The waveforms and magnetic contour maps for the newborn mouse were maintained up to 5 weeks of age.

Figure 9. Averaged MCG waveforms at 1-week-old
Figure 10. Magnetic contour maps at the R, S, and T waves for a 1-week-old mouse and an adult mouse. The dimensions of the maps are $4 \times 4$ mm and $12 \times 12$ mm, respectively. The contour line interval is 5 pT. The white and black areas indicate the positive and negative components of the magnetic field, respectively.

4. Conclusion
We obtained continuous MCGs for the heart-disease-model mice. An abnormal MCG was appeared 1 or 2 weeks before death. The abnormal MCG changes indicate that the damaged place in the ventricles was different for each individual. In addition, we have developed a method to obtain MCGs that is specific for newborn mice because they are too small and frail for conventional MCG. It was found that the waveforms and magnetic contour maps obtained for a newborn mouse are similar to those of a healthy adult mouse. Therefore, we succeeded in obtaining MCGs for mice from birth until old age. This study proved the potential of MCG for detecting abnormal cardiac excitation at the early stage of cardiac infarction and monitoring the progress of heart disease in detail from infancy to old age in mice.

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