



# The electrocardiogram of anaesthetized southern sea lion (*Otaria flavescens*) females<sup>☆</sup>

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**KEYWORDS**

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**Abstract Objectives:** The goal of this study was to characterize for the first time the electrocardiogram (ECG) of the southern sea lion (SSL) *Otaria flavescens*.

**Animals, materials and methods:** Thirteen wild SSL females were captured at Isla de Lobos (Uruguay) and anaesthetized with isoflurane. Electrocardiographic recording was performed on anaesthetized animals at ventral recumbence following standardized procedures.

**Results:** The ECG recordings showed normal sinus rhythm. Amplitude and duration of P and T waves, QRS complex, PR interval, QT interval and ST segment (STS) were determined for all animals in all leads. QT corrected was determined in lead II. P wave polarity was consistent among animals (positive in LI, LII, LIII and AVF leads and negative in AVL and AVR leads for all animals), but T wave polarity did not present any constant pattern among animals, being either positive, negative or biphasic in different leads and different animals. The PR interval ( $0.15 \pm 0.2$  s) was similar to the allometric prediction for most of mammalian species including humans. The STS were normal in 10 of the SSL but showed STS depression in three of the animals. Almost all animals had a negative electrical axis ( $-30^\circ$  to  $-120^\circ$ ), with one exception that showed a positive electrical axis ( $120^\circ$ ). Mean eupnoeic heart rate was  $104.61 \pm 10.06$  (range = 88–120) beats per minute.

**Conclusions:** This study was the first ECG description for this species, and provides valuable information for cardiac monitoring during anaesthesia.

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**Abbreviations**

AV	atrio-ventricular
bpm	beats per minute
ECG	electrocardiogram
HR	heart rate
LI	Lead I
LII	Lead II
LIII	Lead III
PRi	PR interval
QTi	QT interval
QTc	corrected QT interval
SSL	southern sea lion
STS	ST segment

**Introduction**

Electrocardiographic (ECG) recording has been widely used in cardiac studies of terrestrial mammals. However, its use with marine mammals has been very limited and includes only four seal species,<sup>1–5</sup> one otariid species,<sup>6</sup> two subspecies of manatees<sup>7</sup> and six cetaceans species.<sup>8–13</sup> Because of the logistical challenge of working with marine mammals in the wild, most of these studies have been conducted on captive animals or, in a few studies, used harpoon lead electrodes. The ECG recording in free ranging animals using less invasive techniques has become possible only recently,

with the use of waterproof heart rate (HR)/ECG recorders attached to wild animals.<sup>14–16</sup> Although these instruments are now allowing ECG recordings on more pinnipeds<sup>16</sup> and cetaceans,<sup>14</sup> they use only two leads and do not enable four limb-leads ECG tracings precise enough to determine the normal range of values typically recorded in resting, terrestrial mammals.

The resulting low availability of aquatic mammal's ECG information has pointed out the application of ECG recording as a priority area of research in the cardiac physiology study of these species. In parallel to this scenario, and associated to current conservation issues affecting many worldwide extended populations of marine mammals, the study of their ecology and behaviour through the application of animal-borne technology (i.e., GPS or ARGOS satellite telemetry) have highly increased during the last decades.<sup>17</sup> In consequence, it has also increased the need to anaesthetize wild animals during the procedure of device attachment, which at the same time has prompted a need of reliable information of physiological parameters required for anaesthesia monitoring, such as the ECG range values.

The southern sea lion (SSL), *Otaria flavescens*, is a pinniped species that ranges and breeds throughout the coastal temperate waters of South America. Its annual cycle combines terrestrial periods for breeding and molting with at-sea foraging phases. It was heavily exploited, and despite

receiving legal protection, many populations have never recovered from intense harvesting during the first half of the 20th century.<sup>18,19</sup> Although its life history, ecology and physiology have been broadly studied, there are no previous studies of cardiac physiology and no ECG values have been yet published. In this context, the aim of the present study was to characterize the electrocardiogram of the SSL, determining temporal and amplitude values for all waves, intervals and segments. The study was focused on wild adult females, which constitutes a key component to be studied in decreasing populations. All animals used in the present study were captured in their natural environment, temporarily held in captivity and anaesthetized with isoflurane. The results obtained here represented valuable information for comparative electrophysiology analysis in relation to other aquatic and terrestrial mammals including humans.

## Materials and methods

The study was performed during May 2010 on Isla de Lobos (35°01' S 54°52' W; Uruguay), which is located in the La Plata River estuary and represents a significant portion of the world population for this species. Animal capture and handling was performed at the former sealing facility currently administered by the Uruguayan government (Dirección Nacional de Recursos Acuáticos de Uruguay, DINARA) following local and international ethic regulations for manipulation of wild animals (DINARA permit A/009/2006, Ministerial Resolution 001/1383/2007). Sea lions from this study were also used to deploy telemetry devices for foraging behaviour studies.<sup>20</sup>

Thirteen wild SSL females (body mass = 80.7 ± 27.9 kg; total length = 159.8 ± 17.5 cm) were captured by DINARA staff and held in a corral under veterinary observation for 24 h. Animals were physically restrained using a squeeze cage and anaesthetized with isoflurane using a mask and later with an endotracheal tube.<sup>21</sup> Mask anaesthesia induction lasted 14 ± 7 min (range = 7–43 min) with a constant oxygen flow of 5–10 L/min and 5% of isoflurane. After intubation, anaesthesia was maintained with 3–5 L/min oxygen and an isoflurane concentration of 0.75–1.5%. Total anaesthesia duration ranged between 0.5 and 2 h. Anaesthetized animals breathed constantly and regularly by themselves, but one assisted ventilation with a bag was performed every 5–7 min to expand the lungs, with particular care of not exceeding normal lung inflation. All physical signs, including head movement, jaw tone, palpebral

reflex, eye position, iris appearance, respiratory character, and capillary refill, were monitored from the time of the first approach. Once intubation was established, these physical signs plus the electronic readings (veterinary monitor Guoteng) were monitored constantly by the anaesthetist and recorded at 5 min intervals.

Electrocardiographic recordings were performed in ventral recumbence with standard bipolar and unipolar limb leads (LI, LII, LIII, AVR, AVL and AVF).<sup>22–25</sup> Electrocardiographic recordings were performed 30 min after intubation, when animals reached a stable and profound anaesthetic stage. Limb leads were secured to the lateral aspect of the body wall using clip electrodes as follows: the left and right forelimb leads were placed 5–7 cm cranial to the pectoral flippers insertion, approximately at the level of the scapular girth. The left and right hind leads were placed 5–7 cm cranial to the pelvic flippers insertion, approximately at the level of the pelvic girth.

Clip electrodes were manufactured in an appropriated size to clip sea lions peel (3.5 cm length, 1 cm opening width). Skin areas of electrode attachment were wetted with alcohol. Electrocardiograms were recorded using a portable device.<sup>f</sup> The recording speed was 50 mm/s and the sensitivity set at 1 cm = 1 mV, using a 45 mm-wide paper. No filter was used during any recording. LII was additionally recorded at 25 mm/s to determinate the HR.

Amplitude in millivolts (mV) and duration in seconds (s) of P and T waves, QRS complex, PR interval (PRi), QT interval (QTi), and ST segment (STS) were measured as defined in standard clinical usage.<sup>22,24,26</sup> Each measurement for each wave, complex, interval or segment was performed six times in every lead in each animal. All measurements were made manually using a caliper following standard clinical usage.<sup>22–24,27</sup>

The PRi was measured from the beginning of the P wave to the beginning of the QRS complex and compared to a predicted value estimated from sea lions body mass and the allometric equation proposed by Noujain et al.<sup>28</sup>:

$$\text{Predicted PRi} = 53 \times \text{Body Mass}^{0.24}$$

The QTi was measured from the beginning of the Q wave to the end of the T wave. QT was corrected (QTc) for the HR using Bazett's formula.<sup>29</sup> The STS was measured between the end of the S wave and the beginning of the T wave. Following Bolton<sup>22</sup> and Lightowler,<sup>30</sup> the ST was considered

<sup>f</sup> RG-401 Plus, Cardiotécnica Argentina S.R.L and Bio-desarrollos equipamiento médico.

abnormal when it displayed more than 0.2 mV below the baseline, an occurrence referred to as ST depression. The heart rate in beats per minute (bpm) was estimated from the LII recorded at 25 mm/s (small boxes between consecutive R waves divided by 3,000). The mean electrical axis was determined using the isoelectric method by examining the QRS complexes in each of the six basic leads following a standard procedure.<sup>22,24</sup>

Mean, standard deviation, coefficient of variation and 95% confidence intervals were calculated for each measurement in every lead. The coefficient of variation was calculated as the ratio between the average and the standard deviation, expressed as percentage. Statistic analyses were performed using PROC MEANS y PROC TTEST procedures of the statistic software SAS V9.2.<sup>31</sup>

## Results

Electrocardiographic recordings showed normal sinus rhythm in all animals (Fig. 1). Wave amplitudes were highly consistent for each animal in every lead. P wave polarity was highly consistent among animals: positive in LI, LII, LIII and AVF leads and negative in AVL and AVR leads for all animals. T wave polarity did not present a constant pattern among animals, being either positive, negative or biphasic in different leads and different animals.

The PRi ranged from  $0.12 \pm 0.06$  to  $0.18 \pm 0.03$  s, with an overall value of  $0.15 \pm 0.02$  s for all animals. The predicted PRi was highly concordant ( $0.15 \pm 0.01$  s), with minimal differences ranging from 0 (complete concordance between measured and predicted PR intervals) to a maximum of 0.03 s (average difference =  $0.01 \pm 0.01$  s; data of measured and predicted PR intervals for each animal are available in [Supplemental data on-line, Table A](#)).

Amplitude of P, Q, R, S and T waves in LII were  $0.38 \pm 0.05$ ,  $0.05 \pm 0.07$ ,  $0.59 \pm 0.27$ ,  $1.10 \pm 0.28$  and  $0.56 \pm 0.12$  mV, respectively (mean and confidence intervals of amplitude measurements for each lead are available in [Supplemental data on-line, Table B](#)). Duration of P wave, T wave, QRS complex, QT<sub>i</sub> and STS in lead II were  $0.11 \pm 0.01$ ,  $0.11 \pm 0.03$ ,  $0.08 \pm 0.01$ ,  $0.31 \pm 0.02$  and  $0.14 \pm 0.02$  s, respectively (mean and confidence intervals of duration measurements for each lead are available in [Supplemental data on-line, Table C](#)). Corrected QT interval was  $0.41 \pm 0.028$  and the difference with QT<sub>i</sub> was significant ( $t = -0.095$ ,  $p < 0.0001$ ). The STSs were normal (no evident depression) in most of the sea lions studied ( $n = 10$ ), but three exceptions were observed. Animals 1, 5 and 6 showed STS depression in LII and LIII (ST displayed more than 0.2 mV below the baseline; Fig. 2a).

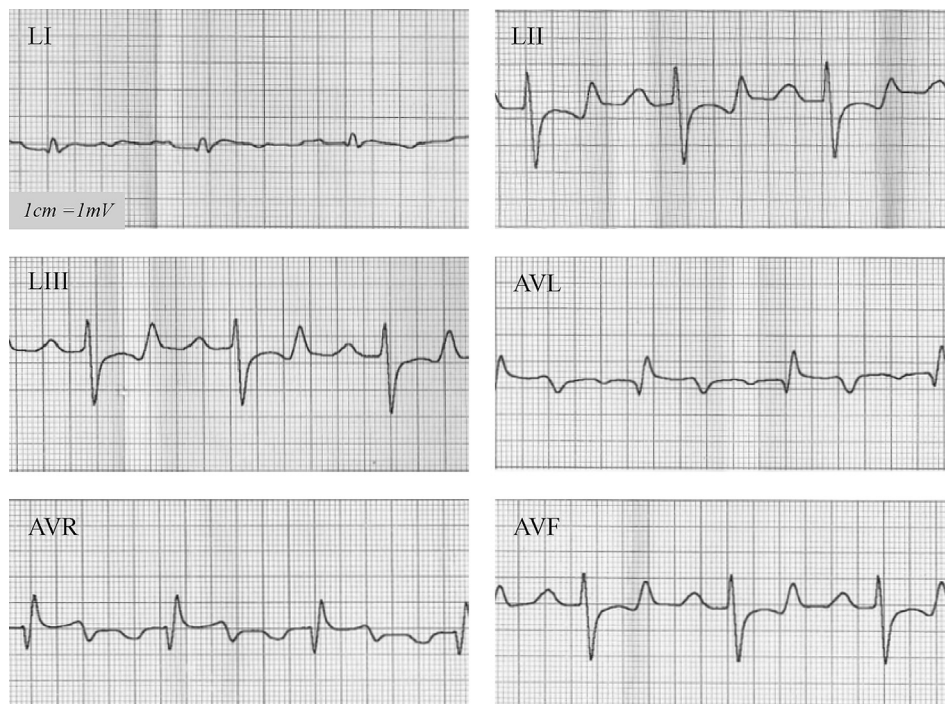
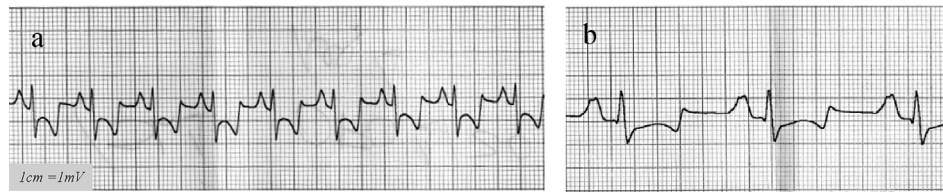


Fig. 1 Typical ECG tracing in anesthetized southern sea lions.



**Fig. 2** (a) ECG of animal 1 in LII, showing an example of the ST segment depression observed in three of the animals of the present study. (b) ECG of animal 11 in LII, showing the atypical notched P wave morphology.

Almost all animals ( $n = 12$ ) had a negative electrical axis ranging between  $-30^\circ$  and  $-120^\circ$ , with one exception (animal 11) with a positive electrical axis of  $120^\circ$  (electrical axis data for each animal are available in [Supplemental data on-line, Table D](#)). This animal also presented an unusual notched P wave ([Fig. 2b](#)), with the highest amplitude of all records obtained ( $0.43$  mV at LII), higher than the average obtained for all animals ( $0.38 \pm 0.05$  mV). The mean eupnoeic HR for anaesthetized animals ( $n = 13$ ) was  $104.61 \pm 10.06$  bpm, ranging from 88 to 120 bpm (HR data for each animal are available in [Supplemental data on-line, Table D](#)).

## Discussion

This study constitutes the first—and detailed—description of the anaesthetized SSL electrocardiogram. It resembled, in its essential details, that of man and other mammals. All animals showed normal sinus rhythm. All the waves of the ECG were detectable and could be easily separated. The SSL ECG characteristically displayed a distinctly defined STS and QT<sub>i</sub>, with a T wave clearly differentiated from the QRS complex. No polarity inversion was registered in any lead.

All animals showed P wave low voltage in LI, suggesting that atrial cardiac vector activation runs perpendicular to LI. We observed a consistent pattern of atrial depolarization for all leads in all ECGs. The only exception was the animal 11, which showed a notched P wave and the highest amplitude among all animals. This P wave morphology might be considered normal in the horse, where the right atrium depolarization precedes the left atrium and causes a biphasic P wave owing to the large size of the atria. However, in other species like the dog, a biphasic P wave might be associated with left atrial enlargement.<sup>22</sup> Although we cannot determine if sea lion 11 presented left atrium enlargement, the additional finding of a mean positive electrical axis ( $+120^\circ$ ), different from that of the other animals which had a left cranial orientation of the mean electrical axis ( $-30^\circ$  to

$-120^\circ$ ), might support this hypothesis. Similar to our results, Siegal-Willott et al.<sup>7</sup> reported an occasionally a biphasic P wave in the manatee, although its causes were not established.

The PR<sub>i</sub> represents the total amount of time required for both atrial depolarization and the delay in the atrio-ventricular (AV) node.<sup>22</sup> The PR<sub>i</sub> of sea lions was almost invariable ( $0.15 \pm 0.02$  s; coefficient of variation = 10.9%), longer than the southern elephant seal ( $0.012 \pm 0.02$  s<sup>5</sup>) and shorter than the horse ( $0.28 \pm 0.12$  s<sup>30</sup>). In the latter, the PR<sub>i</sub> is usually long, variable and functionally prolonged by vagal influence. Sea lions' PR<sub>i</sub> resulted concordant with the predicted PR<sub>i</sub> ( $0.15 \pm 0.01$  s) derived from the Noujaim's allometric equation,<sup>28</sup> which was established with the largest set of published ECGs (33 species, including humans). This equation has an exponent within the range of scaling factors that describe the relation between body mass and many biological processes such as metabolic rate, life span and HR.<sup>28,32</sup> Because it uses optimal heart function as its basis, the good correlation between measured and predicted PR intervals suggests that SSLs have a normal PR<sub>i</sub> in necessary of efficient cardiac function similar to other mammals including humans.

Owing to its role as an indicator of the AV conduction time, the PR<sub>i</sub> can be considered as a valuable indicator of cardiac function efficiency. It is known that atrial contraction contributes to approximately 20% of the end diastolic volume after rapid ventricular filling.<sup>23</sup> This requires adequate AV propagation of the electrical impulse that will trigger the ventricular contraction at the appropriate time, leading to a correct diastolic ventricular filling. Following this reasoning, and in concordance with the predicted PR<sub>i</sub> ( $0.15 \pm 0.01$  s) derived from the Noujaim's allometric equation,<sup>28</sup> it is probably that AV conduction time in the SSL works at an adequate timing to allow an efficient cardiac output.

The coordinated function of atrial and ventricular contraction is important in any mammal, but even more so in marine mammals that must precisely adjust cardiac function during breath-hold diving.<sup>33–36</sup> This adjustment is part of the

pulmonary and cardiovascular changes associated not only with the dive response, but also with its interaction with the exercise response. The dive response is associated with the cessation of breathing accompanied by a bradycardia, a reduction in cardiac output and a peripheral vasoconstriction that maintains central arterial blood pressure while maintaining blood flow primarily to the heart, lungs, and brain.<sup>33–35</sup> In contrast, the exercise response is associated with hyperventilation, tachycardia, cardiac output increase and peripheral vasodilatation to active muscles.<sup>16,36,37</sup> Although the latter is the response typically exhibited by terrestrial mammals and birds that do not undergo in apnea during exercise, it appears to be integrated with the dive response in different phases of the dive.<sup>16,36–38</sup>

The QRS complex represents the ventricular activation, which has been classified in type A mammals (humans, monkeys, dogs, cats, rats), with three fronts of depolarization waves, and type B mammals (horses, cows, pigs, sheep and goats), with two wave fronts.<sup>30,39</sup> A reliable classification of ventricular activation requires the description of cardiac anatomy and the characterization of the conduction system, which is not yet available in most marine mammals. However, Siegal-Willott et al.<sup>7</sup> hypothesized that manatees have a depolarization similar to that of the horse and thus manatees should be classified as type B. This inference was based on the only existing anatomical description and the predominantly positive deflection in LI. In the case of SSLs, the conduction system has not been yet characterized and there is no heart anatomical description, so we cannot make similar assumptions. However, mean electrical axes reflected left and cranial orientation of the mean QRS vector in most of the SSL studied ( $n = 12$ ), similar to northern elephant seals, southern elephant seals, harbour seals, pigs and horses.<sup>40</sup> These similarities suggest that SSL might be classified as having a type B activation, but further investigation is needed to elucidate this aspect.

The STS represents the end of ventricular activation, immediately before ventricle repolarization. Most of the SSL studied showed normal STS, but three exceptions were observed, showing clear patterns of STS depression. Although it is known that STS depression in humans and dogs may result from several pathologies, including left ventricular hypertrophy, myocardial hypoxia or electrolyte disturbance,<sup>22</sup> there was not enough evidence in this study to suggest these pathologies were present in the SSL with ST depression. In addition, T waves did not exhibit any polarity inversion within animals, which also suggests a normal mechanism

of ventricle repolarization. The importance of this result relies in that reversal of T wave polarity is most often considered abnormal and might indicate similar pathologies as those suggested by the STS depression.<sup>22</sup> Until further data are available, the STS depression might be considered as a normal occasional finding in the SSL ECG.

The QT<sub>i</sub> includes both ventricular depolarization and repolarization. Because ventricular depolarization is short and constant, QT<sub>i</sub> is used as a measurement of ventricular repolarization. The prolongation of the QT<sub>i</sub> has clinical importance in the inhalation anaesthesia.<sup>41–43</sup> Several studies have shown that volatile anaesthetics prolonged the QT of the ECG during inhalational induction of anaesthesia.<sup>43,44</sup> On the other hand, long-QT syndrome, is similarly characterized by abnormal QT-interval prolongation on the ECG, accompanied by an increased risk of sudden death usually owing to serious cardiac arrhythmias like ventricular fibrillation.<sup>44</sup> Considering that no previous value of QT<sub>i</sub> or QT<sub>c</sub> has been reported for this species, no inference of potential abnormalities or isoflurane effects can be made for the animals in this study. However, average QT<sub>i</sub> and QT<sub>c</sub> values obtained in this study constitute a first approximation to the normal QT<sub>i</sub> for the SSL, a value that must be compared with future ECG in awake resting SSL.

## Conclusions

This study presents the first ECG description for the SSL and provides valuable information for cardiac monitoring during anaesthesia. All the waves of the ECG were detectable and could be easily separated. The SSL electrocardiogram characteristically displayed a distinctly defined STS and QT<sub>i</sub>, with a T wave clearly differentiated from the QRS complex. The results obtained are novel and useful information for comparative electrophysiology analysis in relation to other aquatic and terrestrial mammals including humans.

## Conflicts of interest statement

The authors do not have any conflicts of interest to disclose.

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### Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.jvc.2015.09.003>.

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