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Characterization of the South American Fur Seal (*Arctocephalus australis*) Electrocardiogram

[1]Mariela Dassis, [2]Alejandro Diaz, [1]Carolina De Leon, [1]Diego H. Rodriguez, [3]Edgardo Rodriguez, [4]Alberto Ponce de Leon and [5]Eduardo Castro

[1]Instituto de Investigaciones Marinas y Costeras, Consejo Nacional de Investigaciones Cientificas y Tecnicas (CONICET), Facultad de Ciencias Exactas y Naturales, Universidad Nacional de Mar del Plata (UNMdP), 4046 Rodriguez Pena, Nivel 1 (7600) Mar del Plata, Argentina

[2]Instituto de Investigacion en Ciencias de las Salud, Universidad Nacional del Centro de la Provincia de Buenos Aires, CONICET, Pje. Arroyo Seco, S/N (7000) Tandil, Argentina

[3]Departamento de Sanidad Animal y Medicina Preventiva, Facultad de Ciencias Veterinarias, Universidad Nacional del Centro de la Provincia de Bs As, CONICET-Unidad Ejecutora CIVETAN, Ciencias Veterinarias de Tandil. Pje. Arroyo Seco, S/N 7000 Tandil, Argentina

[4]Departamento de Mamíferos Marinos, Dirección Nacional de Recursos Acuaticos (DINARA), Ministerio de Ganadería, Agricultura y Pesca, 1497 Constituyente, C.P. 11200 Montevideo, Uruguay

[5]Departamento de Fisiopatologia, Facultad de Ciencias Veterinarias, Universidad Nacional del Centro de la Provincia de Bs. As, CONICET-Unidad Ejecutora CIVETAN, Ciencias Veterinarias de Tandil. Pje. Arroyo Seco, S/N 7000 Tandil, Argentina

marieladassis@gmail.com, +549-223-5760682

Abstract: The goal of this study was to characterize the Electrocardiogram (ECG) of anesthetized South American Fur Seals (SAFS). Nineteen wild SAFS females were anesthetized with isofluorane at Isla de Lobos (Uruguay). ECG recording was performed at ventral recumbence following standardized procedures. All animals showed normal sinus rhythm. Amplitude and duration of P and T waves, QRS complex, PR interval (PRi), QT interval (QTi) and ST segment (STs) were determined. QT corrected (QTc) was determined in lead II. P wave polarity was consistent among animals (positive in LI, LII, LIII and AVF and negative in AVL and AVR). T wave polarity was consistent among animals only in LI (positive) but without a constant pattern in the others leads. The PRi (0.128±0.009 sec) was highly similar to the allometric prediction (0.130±0.004 sec) for most of mammalian species. Mean eupneic Heart Rate (HR) was 118.95±18.23 bpm (range = 100-160) and also similar to the allometric prediction (111.58±2.79 bpm). The STs were normal in 17 of the SAFS but showed STs depression in 2 animals. Most of the animals (58.9%) had a negative electrical axis. This study constituted the first detailed description of the SAFS ECG.

Key words: Electrocardiography, pinnipeds, isofluorane anaesthesia, dive physiology, ST segment, Heart Rate (HR)

INTRODUCTION

The mammalian heart has very conservative structural and functional characteristics which contrast with the evolutionary changes recorded in other anatomical structures (Meijler, 1985; Noujaim *et al*., 2004; Meijler and Meijler, 2011). From mouse to whale, all hearts show similar structural and functional characteristics

Corresponding Author: Mariela Dassis, Instituto de Investigaciones Marinas y Costeras, Consejo Nacional de Investigaciones Cientificas y Tecnicas (CONICET), Facultad de Ciencias Exactas y Naturales, Universidad Nacional de Mar del Plata (UNMdP), 4046 Rodriguez Pena, Nivel 1 (7600) Mar del Plata, Argentina, marieladassis@gmail.com, +549-223-5760682

(Noujaim *et al*., 2004; Meijler and Meijler 2011). However, diving mammals are adapted to long interruptions in breathing and have shown an extreme ability to cardiac regulation not observed in their terrestrial counterparts (Davis, 2014; Williams *et al*., 2015). This last includes an interplay between different degrees of tachycardia and bradycardia and a high incidence of cardiac arrhythmias that can be considered as a normal occurrence during deep dives (Williams *et al*., 2015).

Electrocardiography is widely used as a method to study cardiac physiology and also as a diagnostic tool for heart diseases in both, human and veterinary medicine. However, its use with aquatic mammals has been very limited and includes only 6 pinnipeds (Murdaugh *et al*., 1961; Van Citters *et al*., 1965; Ponganis *et al*., 1997; Andrews *et al*., 1997; Ponganis and Kooyman, 1999; Falabella *et al*., 1999; Dassis *et al*., 2016), two manatees (Siegal-Willott *et al*., 2006) and seven Cetaceans species (King *et al*., 1953; Senft and Kanwisher, 1960; Meijler and Van der Tweel, 1986; Kastelein and Meijler, 1989; Meijler *et al*., 1992; Williams *et al*., 1993; Ponganis and Kooyman, 1999; Harms *et al*., 2013). In addition, most of these studies have been conducted on captive animals or using harpoon lead electrodes which is manly related to the logistical challenge of working with marine mammals in the wild.

The ECG recording in free ranging animals using less invasive techniques has become possible only recently with the use of water proof heart rate/ECG recorders attached to wild animals (Noren *et al*., 2004; Davis and Williams, 2012; Davis, 2014; Williams *et al*., 2015). Despite this is increasing the number of species studied and highly improving the quality of information obtained these instruments use only two leads and do not enable the precise description of the typical four limb-leads ECG tracing that would help to infer and understand the cardiac bioelectricity and biomechanics subjacent to the extreme cardiac abilities of marine mammals.

The South American Fur Seal (SAFS, *Arctocephalus australis*) is relatively small pinniped species with an insular distribution that extends along the coast of South America from Southern Brazil to Central PerU (Vaz-Ferreira, 1982). The studies that describe in detail the cardiac anatomy of SAFS emphasize that the heart is structurally similar to other mammals with morphological changes that assist in their adaptation to their aquatic environment (Perez *et al*., 2008; Guimaraes *et al*., 2014). Briefly, the SAFS heart is flat, elongated and positioned on the left side of median line in the thoracic cavity between 3rd and 7th ribs (Perez *et al*., 2008). As no previous values of ECG have been reported for this species, the main objective of this study was to characterize the electrocardiogram of anaesthetized SAFS and to determine temporal and amplitude values for all waves, intervals and segments. The information obtained here represented valuable information for comparative electrophysiology analysis in relation to other aquatic and terrestrial mammals, including humans. In this sense, result regarding to heart rate and AV (Atrio-Ventricular) conduction time were discussed in terms of allometric predictions for most of mammalian species.

MATERIALS AND METHODS

This research was performed during May 2013 on Isla de Lobos (35°01'S54°52'W; Uruguay). Animal capture and handling was performed by personnel of the Uruguayan Government (Direccion Nacional de Recursos Acuaticos de Uruguay, DINARA) following local and international ethic regulations for manipulation of wild animals (experimental protocols were approved by Universidad Nacional de Mar de Plata Institutional Animal Care and Use Committee, CICUAL; Resolution 137/15). Fur seals from this study were used to deploy telemetry devices for foraging behaviour studies (Carman *et al*., 2016).

Nineteen wild SAFS females (Table 1) were live captured and held in a corral under veterinary observation for 24 h. Animals were physically restrained using a squeeze cage and anesthetized with isoflurane using a mask and later with an endotracheal tube. 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 first approach. After intubation, these physical signs plus the electronic readings (veterinary monitor Guoteng) were monitored constantly by the anesthetist and recorded at 10 min intervals. Details of animal's manipulation and anaesthesia procedures were similar to those applied by the researchers on a previous study with Southern Sea Lion (SSL) females (Dassis *et al*., 2016).

Table 1: Morphometric measurements of South American fur seals females anesthetized for ECG recording

Animals	Body mass (kg)	Total length (cm)	Curve length (cm)
1	46.0	135.0	137.0
2	46.5	128.0	136.0
3	48.5	126.0	131.0
4	40.0	124.0	130.0
5	37.0	125.0	128.0
6	48.8	133.0	140.0
7	41.4	128.0	133.0
8	34.8	125.0	127.0
9	34.9	120.0	124.0
10	42.1	131.0	133.0
11	39.8	135.0	138.0
12	47.8	133.0	137.0
13	44.2	132.0	136.0
14	37.2	132.0	136.0
15	39.8	136.0	140.0
16	49.9	133.0	135.0
17	40.3	137.0	140.0
18	42.4	133.0	137.0
19	32.8	118.0	119.0

Mean: 41.8, 129.7, 133.5; SD: 5.1, 5.4, 5.8; Coef. var: 12.3, 4.2, 4.3

ECG recordings were performed in ventral recumbence with standard bipolar and unipolar limb leads (LI, LII, LIII, AVR, AVL and AVF) (Bolton, 1975; Detweiler, 1988; Tilley and Tilley, 1992; Kligfield *et al*., 2007). ECG 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 electrode as follows: the left and right forelimb leads were placed 3-5 cm cranial to the pectoral flippers insertion, approximately at the level of the scapular girth. The left and right hind leads were placed 3-5 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 fur seals peel (3.5 cm length, 1 cm opening width). Skin areas of electrode attachment were wetted with alcohol. ECGs were recorded using the portable device RG-401 Plus (Cardiotencia S.R.L, Argentina). The recording speed was 50 mm/sec and the sensitivity set at 1cm = 1mV, using a 45 mm-wide paper. No filter was used during any recording. LII was additionally recorded at 25 mm/sec to determinate Heart Rate (HR).

Amplitude in millivolts (mV) and duration in seconds (sec) of P and T waves, QRS complex, PRi, QTi and STs were measured as defined in standard clinical usage (Bolton 1975; Tilley and Tilley 1992; Anonymous, 2010). 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 calliper following standard clinical usage (Bolton 1975; Tilley and Smith, 2008; Detweiler, 1988; Tilley and Tilley, 1992).

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 fur seals Body Mass (BM) and the allometric equation proposed by Noujaim *et al.* (2004). Predicted PRi = $53 \times BM^{0.24}$. HR in bpm (beats per min) was estimated from the LII recorded at 25 mm/sec (small boxes between consecutive R waves divided by 3000). Similar to PRi this value was compared to a predicted value estimated from fur seals BM and the allometric equation proposed by Noujaim *et al.* (2004) predicted HR = $235 \times BM^{-0.2}$.

The QTi was measured from the beginning of the Q wave to the end of the T wave. QTi was corrected (QTc) for heart rate using Bazett's formula (Bazett, 1920). The STs was measured between the end of the S wave and the beginning of the T wave. Following Bolton (1975) the ST was considered abnormal when it displayed more than 0.2 mV below the baseline an occurrence referred to as ST depression. The mean electrical axis was determined using the isoelectric method by examining the QRS complexes in each of the six basic leads following standard procedure (Bolton, 1975; Tilley and Tilley, 1992). Mean ($\bar{x}$), Standard Deviation (SD), Coefficient of Variation (CV) and 95% confidence intervals were calculated for each measurement in every lead. Statistic analyses were performed using PROC MEANS y PROC TTEST procedures of the Statistic Software SAS V9.2 (SAS Software, 2011).

RESULTS AND DISCUSSION

ECG 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 consistent among animals (positive in LI, LII, LIII and AVF leads and negative in AVL and AVR leads for all animals (Table 2, Fig. 1). One animal also presented an unusual notched P wave in LII (Fig. 2). T wave polarity was consistent among animals only in LI (always positive) but did not present any constant pattern in the rest of the leads, being either positive, negative or biphasic (Table 2).

The PRi ranged from 0.111±0.003 to 0.149±0.002 sec in animals 8 and 12, respectively with an overall mean value of 0.128±0.009 sec for all animals (measured PRi; Table 3). The predicted PRi was highly concordant (0.130±0.004 sec) with minimal differences ranging from 0 (complete concordance between measured and predicted PRi) to a maximum of 0.02 sec. (average difference = 0.008±0.006 sec). An inverse correlation was observed between PRi and HR (r^2: 0.64). Mean eupneic HR for anesthetized animals (n = 19) was 118.95±18.23 bpm, ranging from 100-160 bpm (Table 3). Predicted HR (111.58±2.79 bpm) was also similar to the measured value (Table 3) with differences ranging from 5-50 bpm (average difference = 16.34±10.95 bpm).

Amplitude of P-T waves and duration of P, T waves, QRS complex, QT interval and ST segment are summarized in Table 4 and 5, respectively. QTc was 0.365±0.037 and the difference with QTi (0.261±0.028) was significant (t = -17.86, $p<0.0001$). The STs were normal (no evident depression) in most of the fur seals studied (n = 17). However, 2 exceptions were observed. Animals 2 and 15 showed STs depression in LII (Fig. 3). Most of animals (58.9%) had a negative electrical axis ranging between -90 and -120° with the rest of the animals (41.1%) with a positive electrical axis (between 30 and 120°).

The present study provides the first detailed description of the anaesthetized SAFS electrocardiogram. The ECG pattern resembled, in its essential details, the ECG tracing of terrestrial and marine mammals (Meijler and

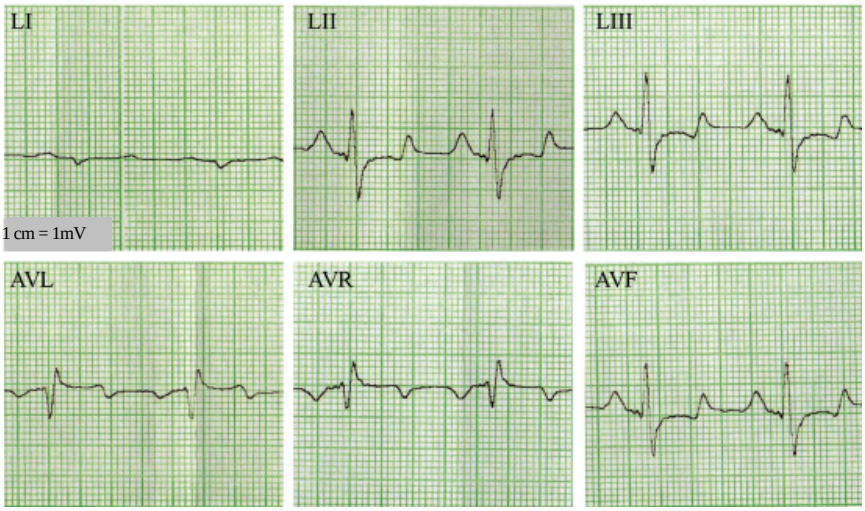

Fig. 1: Typical ECG tracing in one anesthetized South American fur seals female

Table 2: Polarities of P (left) and T (right) waves in ECG recordings of South American fur seals females

	P wave polarity						T wave polarity					
Animals	LI	LII	LIII	AVR	AVL	AVF	LI	LII	LIII	AVR	AVL	AVF
1	+	+	+	-	-	+	nd	+	+	-	-	+
2	+	+	+	-	-	+	+	+	+	-	-	+
3	+	+	+	-	-	nd	+	±	±	±	±	nd
4	+	+	+	-	-	+	+	±	±	±	±	±
5	+	+	+	-	-	+	nd	-	-	+	+	-
6	+	+	+	-	-	+	+	+	+	-	-	+
7	+	+	+	-	-	+	nd	+	+	-	-	+
8	+	+	+	-	-	+	+	+	+	-	±	+
9	+	+	+	-	-	+	+	+	+	-	-	+
10	+	+	+	-	-	+	+	+	+	-	-	+
11	+	+	+	-	-	+	+	+	±	-	±	+
12	+	+	+	-	-	+	+	+	+	-	-	+
13	nd	+	+	-	-	+	+	±	±	-	+	±
14	+	+	+	-	-	+	nd	±	±	±	±	±
15	+	+	+	-	-	+	+	±	-	±	+	-
16	+	+	+	-	-	+	+	+	+	-	-	+
17	+	+	+	-	-	+	+	+	+	-	±	+
18	+	+	+	-	-	+	+	+	+	-	-	+
19	+	+	+	-	-	+	+	+	+	-	-	+

ND = No Data, (±) = Isolectric deflection

Table 3: Heart Rate (HR in bpm) and PR interval of each lead (PRi in sec) in South American fur seals ECGs (N = 19), respectively, compared to predicted HR and PRi according to body mass and Noujaim *et al.* (2004) equations

Animals	Body mass (kg)	LI	LII	LIII	AVR	AVL	AVF	Measured PRI (mean±SD)	Predicted (PRI)	Measured (HR)	Predicted (HR)
1	46.0	nd	0.12	0.12	0.11	0.12	0.12	0.114±0.003	0.133	160	109.3
2	46.5	0.11	0.13	0.13	0.13	0.11	0.13	0.122±0.009	0.133	140	109.0
3	48.5	0.13	0.12	0.12	0.13	0.13	nd	0.125±0.005	0.135	120	108.1
4	40.0	nd	0.14	0.13	0.13	0.12	0.13	0.130±0.005	0.128	120	112.4
5	37.0	nd	0.13	0.12	0.13	0.13	0.12	0.126±0.005	0.126	120	114.1
6	48.8	0.12	0.12	0.12	0.12	0.12	0.12	0.120±0.004	0.135	120	108.0
7	41.4	nd	0.13	0.12	0.13	0.12	0.12	0.124±0.002	0.130	140	111.6
8	34.8	0.11	0.11	0.11	0.12	0.11	0.11	0.111±0.003	0.124	140	115.5
9	34.9	0.14	0.14	0.14	0.14	0.14	0.14	0.141±0.003	0.124	100	115.5
10	42.1	0.13	0.13	0.13	0.14	0.13	0.13	0.131±0.004	0.130	120	111.2
11	39.8	0.15	0.12	0.12	0.13	0.12	0.13	0.130±0.010	0.128	100	112.5
12	47.8	nd	0.15	0.15	0.15	0.15	0.15	0.149±0.002	0.134	100	108.4
13	44.2	nd	0.13	0.12	0.13	0.13	0.13	0.127±0.003	0.132	100	110.1

Table 3: Continue

Animals	Body mass (kg)	LI	LII	LIII	AVR	AVL	AVF	Measured PRI (mean±SD)	Predicted (PRI)	Measured (HR)	Predicted (HR)
14	37.2	nd	0.14	0.14	0.14	0.14	0.14	0.137±0.002	0.126	100	114.0
15	39.8	nd	0.14	0.13	0.14	0.12	0.13	0.133±0.006	0.128	100	112.5
16	49.9	0.13	0.12	0.13	0.14	0.12	0.12	0.127±0.008	0.135	120	107.5
17	40.3	0.14	0.14	0.13	0.14	0.13	0.14	0.137±0.006	0.129	100	112.2
18	42.4	nd	0.13	0.13	0.14	0.13	0.13	0.134±0.002	0.130	120	111.1
19	32.8	0.11	0.12	0.12	0.12	0.12	0.12	0.117±0.003	0.122	140	116.9

Mean: 41.8, 0.128, 0.130, 118.95, 111.58; SD: 5.1, 0.009, 0.004, 18.23, 2.79; Coef. var: 12.3, 7.2, 3.0, 15.3, 2.50; ND = No Data

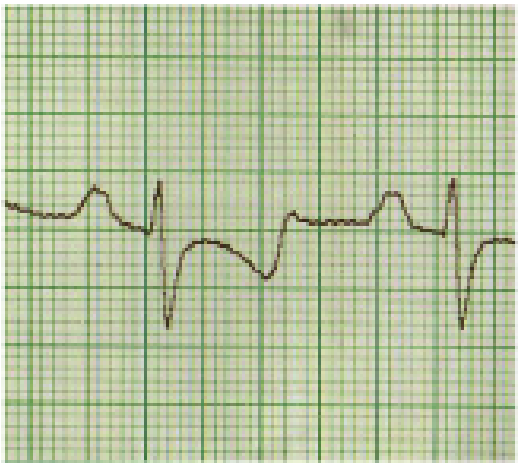

Fig. 2: ECG in LII showing a typical notched P wave morphology detected in one animal from this study

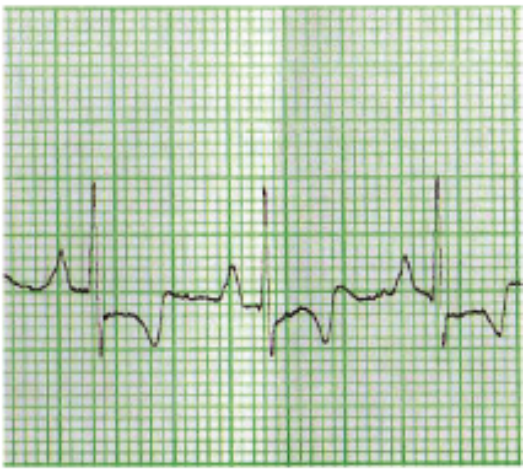

Fig. 3: ECG in LII, showing an example of the ST segment depression observed in two of the animals studied

Meijler, 2011). All the waves, intervals and segments of the ECG were identified and could be easily separated. All animals revealed normal sinus rhythm with no detectable arrhythmias. The SAFS ECG characteristically displayed distinctly defined ST segment and QT interval 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 this finding suggest that atrial cardiac vector activation runs perpendicular to LI. We observed a consistent pattern of atrial depolarization for all leads in all ECGs but 1 animal presented an unusual notched P wave. According the species under study this unusual P wave morphology could be considered a normal finding (Hamlin *et al*., 1970) or suggest an atrial enlargement (due to delayed interatrial depolarization) (Bolton, 1975; Tilley and Smith, 2008). Similar to our results, previous reports described an occasionally biphasic P wave in elephant seals, harbour seals, California Sea lions (Hamlin *et al*., 1972) and manatees (Siegal-Willot *et al*., 2006), although, their causes were not established. In this sense, studies of macroscopic anatomy on the size and structure of the atria in SAFS are scarce and show controversial data. Perez *et al*. (2008) reported that both atria had few pectinate muscles and that the right atrium was much smaller than the left, based on 12 specimens (Perez *et al*., 2008). By Guimaraes *et al*. (2014) studied the macroscopic characteristics of both atria in 24 SAFS specimens showing that the right atrium was larger than the left atrium.

In this study the measured HR was concordant with predicted HR (118 vs. 111 bpm, respectively) and showed a correlation with body mass (mean 41.8±5.1 kg). Resting HR is known to scale inversely to body mass in terrestrial mammals, for example, HR changes from 600 bpm in the conscious mouse (Gehrmann *et al*. 2000) to 30 bpm in the humpback whale (Meijler *et al*. 1992). This scaling of the HR with the body mass is also observed in marine mammals. Taking as an example it is observed that large marine mammals as beluga whale (mean 1136 kg; King *et al*. 1953) show an average HR of 16 bpm, however, pinnipeds with smaller body mass have higher HR with averages of 104-118 bmp (80 and 50 kg for Southern Sea Lions (Dassis *et al*., 2016) and SAFS, respectively. Moreover, specifically in pinnipeds, both apneic and eupneic HR declined with increasing body mass (Castellini and Zenteno-Savin, 1997).

The PRi reflects the slowed conduction through the AV node (Bolton, 1975; Tilley and Smith, 2008). The changes in the mammalian heart that did take place were mostly adjustments in order to compensate body size

Table 4: Amplitude measurements (mV) of each lead recorded in South American fur seals ECGs (N = 19)

					CL 95% for mean	
Wave/Lead	n	Mean	SD	Coef. var.	Lower	Upper
P						
LI	5	0.0866	0.0083	21.4921	0.0635	0.1097
LII	19	0.3209	0.0112	15.1628	0.2974	0.3443
LIII	19	0.2794	0.0123	19.2535	0.2535	0.3053
AVR	19	0.1952	0.0068	15.2235	0.1809	0.2095
AVL	19	0.1201	0.0069	24.8832	0.1057	0.1345
AVF	18	0.3018	0.0120	16.8862	0.2764	0.3271
Q						
LI	5	0.0751	0.0155	45.984	0.0322	0.1180
LII	16	0.1051	0.0139	52.8778	0.0755	0.1347
LIII	12	0.0956	0.0134	48.6949	0.066	0.1252
AVR	18	0.4012	0.0400	42.3136	0.3168	0.4856
AVL	18	0.3632	0.0289	33.7039	0.3023	0.4241
AVF	12	0.1085	0.0147	46.8518	0.0762	0.1407
R						
LI	5	0.1570	0.0183	26.0597	0.1062	0.2077
LII	19	0.7381	0.0521	30.7461	0.6288	0.8475
LIII	19	0.7077	0.0506	31.1935	0.6013	0.8141
AVR	19	0.3824	0.0340	38.7034	0.3111	0.4537
AVL	19	0.3362	0.0235	30.4852	0.2868	0.3856
AVF	18	0.7462	0.0547	31.1218	0.6307	0.8616
S						
LI	5	0.1058	0.0265	55.9257	0.0323	0.1793
LII	19	0.7263	0.0567	34.0429	0.6071	0.8455
LIII	19	0.7003	0.0529	32.9042	0.5892	0.8113
AVR	4	0.3644	0.1002	55.0001	0.0455	0.6832
AVL	1	0.3306	.	.	.	.
AVF	18	0.7073	0.0577	34.6004	0.5856	0.8290
T						
LI	5	0.1337	0.0197	33.0301	0.0789	0.1885
LII	19	0.3876	0.0255	28.7226	0.3339	0.4412
LIII	19	0.3498	0.0278	34.6481	0.2914	0.4082
AVR	19	0.2373	0.0144	26.4039	0.2071	0.2676
AVL	19	0.1656	0.0140	36.753	0.1363	0.1949
AVF	18	0.3665	0.0248	28.7071	0.3142	0.4188

Table 5: Duration measurements (sec) of each lead recorded in South American fur seals ECGs (N = 19)

					CL 95% for mean	
Measurement/lead	n	Mean	SD	Coef. var.	Lower	Upper
P wave						
LI	11	0.0696	0.0020	9.33660	0.0652	0.0740
LII	19	0.0834	0.0013	6.87250	0.0807	0.0862
LIII	19	0.0805	0.0017	9.08260	0.0770	0.0840
AVR	19	0.0791	0.0015	8.28960	0.0759	0.0823
AVL	19	0.0708	0.0017	10.4749	0.0672	0.0743
AVF	18	0.0803	0.0013	6.82960	0.0776	0.0830
QRS complex						
LI	11	0.0594	0.0052	29.2309	0.0477	0.0710
LII	19	0.0751	0.0033	18.8747	0.0683	0.0819
LIII	19	0.0735	0.0029	16.9774	0.0675	0.0795
AVR	19	0.0721	0.0021	12.4156	0.0678	0.0764
AVL	19	0.0679	0.0021	13.4217	0.0636	0.0723
AVF	18	0.0761	0.0021	11.8220	0.0716	0.0805
PR interval						
LI	10	0.1282	0.0044	10.8282	0.1182	0.1381
LII	19	0.1288	0.0024	7.99490	0.1239	0.1338
LIII	19	0.1269	0.0020	6.97970	0.1227	0.1312
AVR	19	0.1320	0.0024	7.98480	0.1269	0.1371
AVL	19	0.1252	0.0023	8.17660	0.1203	0.1301
AVF	18	0.1284	0.0023	7.61090	0.1235	0.1332
QT interval						
LI	10	0.2392	0.0079	10.5098	0.2212	0.2572
LII	19	0.2592	0.0061	10.1760	0.2465	0.2719
LIII	19	0.2592	0.0054	9.07980	0.2479	0.2705
AVR	19	0.2553	0.0055	9.33480	0.2438	0.2668

Table 5: Continue

Measurement/lead	n	Mean	SD	Coef. var.	CL 95% for mean	
					Lower	Upper
AVL	19	0.2529	0.0052	8.94010	0.2420	0.2638
AVF	18	0.2601	0.0056	9.13090	0.2483	0.2719
ST segment						
LI	10	0.1026	0.0081	24.8763	0.0843	0.1208
LII	19	0.1092	0.0053	21.3549	0.0979	0.1204
LIII	19	0.1168	0.006	22.4080	0.1042	0.1294
AVR	19	0.1152	0.0068	25.8436	0.1008	0.1295
AVL	19	0.1245	0.0054	18.7608	0.1133	0.1358
AVF	18	0.1149	0.0054	19.9820	0.1035	0.1264
T wave						
LI	10	0.0761	0.0066	27.4915	0.0612	0.0911
LII	19	0.0860	0.0053	27.0298	0.0748	0.0972
LIII	19	0.0839	0.0052	26.8936	0.0730	0.0948
AVR	19	0.0837	0.0053	27.5180	0.0726	0.0948
AVL	19	0.0719	0.0043	25.8494	0.0630	0.0809
AVF	18	0.0839	0.0050	25.3966	0.0733	0.0945

variations. The scaling of AV conduction velocity (PRi) compensates for size differences, resulting in the strong similarity and optimal haemodynamic function of the heart (Meijler and Meijler, 2011). The PRi in mammals indeed vary for instance from about 0.04 sec in mice to about 0.40 sec in elephants and whales (Noujaim *et al*., 2004). In SAFS, the PRi showed low variability (7.2%) and similar to other mammals it decreased with the increase in HR. Its duration was comparable with Southern Elephant Seals (Falabella *et al.* 1999) but shorter than in other aquatic mammals as manatees (Siegal-Willott *et al.* 2006), California gray whales (Ponganis and Kooyman, 1999), Southern Sea Lions (Dassis *et al*., 2016) and other terrestrial mammals as the horse (0.28±0.12 sec). In the latter, the PRi is usually long, variable and functionally prolonged by vagal influence.

The measured PRi in SAFS resulted concordant with the predicted PRi (0.13±0.004 sec) derived from the Noujaim's allometric equation (Noujaim *et al*., 2004) which assumes optimal heart functioning and was established with the largest set of published ECGs (33 species, including humans). As we previously proposed for the Southern Sea Lion (Dassis *et al*., 2016), the good correlation between measured and predicted PRi might indicate and adequate AV conduction time to allow an efficient ventricular filling and a consequent efficient cardiac output. An optimal coordinated function between atrial and ventricular contraction is essential in any mammal species but even more, so, in marine mammals that must precisely adjust cardiac function during breath-hold diving in order to maximize dive duration and foraging success (Scholander, 1940; Butler and Jones, 1982, 1997; Davis, 2014). Despite, these adjustments have been traditionally associated with the dive response (cessation of breathing accompanied by a bradycardia a reduction in cardiac output and a peripheral vasoconstriction (Scholander, 1940; Butler and Jones, 1982, 1997; Davis and Kanatous, 1999; Davis and Williams, 2012), recent investigations using high resolution ECG recorders attached to weddell seals and bottlenose dolphins have suggested an even more extreme ability for cardiac regulation (Davis, 2014; Williams *et al*., 2015). This last include the interaction between the bradycardia associated to the dive response and the tachycardia of the exercise response (with one or the other predominating depending on the exercise level) and a significant occurrence of cardiac anomalies such us arrhythmias and ectopic beats, attributed to the interplay between sympathetic and parasympathetic drivers during dive (Williams *et al*., 2015). Such marked cardiac variability has changed the common view of a stereotypic dive response and have pointed out the need of further detailed studies in marine mammals cardiac biomechanics and bioelectricity.

The contractions of ventricles are preceded by their electrical activation. The QRS complex represents the ventricular depolarization (left ventricle, interventricular septum and right ventricle) (Tilley and Smith, 2008). This activation (QRS complex) is linked by a varying time interval. This interval variation depends on cardiac mass or body mass and controls the optimisation of cardiac output under all circumstances (Meijler and Meijler, 2011). In this way, even in marine mammals, the direct relationship between body mass and QRS duration seems to be maintained. The duration of the QRS complex of the SAFS (0.070 sec) was lower than that described in other pinnipeds and marine mammals with higher body mass (for example, 0.080 sec in SSL (Dassis *et al*., 2016); 0.080-0.110 sec in manatees (Siegal-Willott *et al*., 2006) and 0.208 sec in California gray whale (Ponganis and Kooyman, 1999). The ventricular activation has been classified in type A mammals (human, monkey dog, cat,

rat) with three fronts of depolarization waves and type B mammals (cow, pig, horse, sheep etc.,) with two wave fronts. This classification of ventricular activation requires the characterization of the anatomy of the cardiac conduction system. However, Siegal-Willott *et al.* (2006) 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. To date, the conduction system within the SAFS heart has not yet been determined and we cannot make similar assumptions. However, mean electrical axes reflected left and cranial orientation of the mean QRS vector in most of the SAFS studied, similar to Northern elephant seals, Southern elephant seals, harbour seals, pig and horses (Hamlin *et al.*, 1972). Based on these similarities we suggest that SAFS may have a type B ventricular activation, however, further research is needed to elucidate this issue.

The STs reflects the end of ventricular depolarization and precedes ventricular repolarization. In accordance with previous reports in SSL (Dassis *et al.*, 2016) most of the SAFS studied showed normal STs, only two animals showed pattern of STs depression. Elevation and depression of STs in human and veterinary electrocardiography represent several disorders such as myocardial ischemia and infarction, pulmonary embolism, early repolarization, electrolyte disorders, left ventricular hypertrophy, conduction abnormalities, valvular diseases, pericardial diseases and cardiomyopathies (Tilley and Smith 2008; Hanna and Glancy, 2011, 2015). The identification of morphology of ST-segment may help veterinarians to detect some abnormalities from the observation of ECGs. Nonetheless, there was not enough evidence in this study to suggest these pathologies were present in the SAFS with ST depression. In addition, T waves did not exhibit abnormalities which would is also suggesting a normal ventricular 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 (Bolton, 1975). Until more data is available, the STs depression could be considered an incidental finding in the SAFS ECG.

The QTi represents the time required to achieve ventricular depolarization and the time required to complete the repolarization processes (Tilley and Smith, 2008). The QTi has been a focus of attention in veterinary cardiology in the last years. Several conditions, congenital and acquired can have a direct effect on ventricular repolarization (Finley *et al.*, 2003; Agudelo *et al.*, 2011). The prolongation of the QTi interval has clinical importance in the inhalation anaesthesia (Baillard *et al.*, 2000; Da Silva *et al.*, 2002; Yildirim *et al.*, 2004). Several studies have shown that volatile anaesthetics prolonged the QT of the ECG during inhalational induction of anaesthesia (Batcvarow and Malik, 2000; Yildirim *et al.*, 2004). On the other hand, long-QT syndrome is similarly characterized by long QT-interval and an increased risk of sudden cardiac death (Finley *et al.*, 2003). The length of QTi in SAFS was lower than that reported in other pinnipeds (Hamlin *et al.*, 1972; Dassis *et al.*, 2016), cetaceans (as dolphins and whales; Ponganis and Kooyman, 1999) and other aquatic mammals (Siegal-Willot *et al.*, 2006). Considering that there are no previous reports of reference values of QTi specifically in SAFS it would be imprudent to make inferences about the potential effects of isoflouoran on the QTi in SAFS. However, the values of QTi and QTc obtained in this study constitute the first approximation to the reference intervals of QTi for the SAFS a value that must be compared with future electrocardiographic studies in non-sedated resting SAFS.

CONCLUSION

This study constitutes the first detailed description of the anesthetized SAFS electrocardiogram which in general resembled the typical ECG tracing of terrestrial and marine mammals and with no evident arrhythmia in any animal. All the waves, intervals and segments of the ECG were easily identified and characteristically displayed a distinctly defined STs and QTi with a T wave clearly differentiated from the QRS complex. An ST depression and a notched P wave were observed in few animals but both might be considered as normal findings in the SAFS ECG. Results obtained here represented novel and useful information for comparative electrophysiology analysis and have provided valuable information for health assessment and cardiac monitoring during anaesthesia in SAFS.

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