Effects of parasympathetic blockade on nonlinear dynamics of heart rate in mice

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Abstract— **The complexity of the heart rate series has been assessed by 3 kinds of nonlinear estimates: correlation dimension (CD), Lyapunov exponents (LE), and approximate entropy (ApEn), in a population of young normal mice before and after IP injection of atropine. The resulting parasympathetic blockade produced an increase in the complexity of the heart rate (RR) series as measured by CD, the first LE and ApEn, and a decrease in heart rate variability, especially in the low frequency component of the RR spectrum. This last feature is a notable difference from the effects of atropine on the human RR spectrum, where it is mainly the high frequency peak which is erased.**

These findings suggest that: 1/ in general, one should not mistake complexity, as measured by these nonlinear estimates, for variability; 2/ the autonomic nervous system of mice, a species of growing interest thanks especially to the recent use of murine transgenic models as tools of cardiovascular exploration, behaves under parasympathetic blockade in a way which is quite different from the human case; 3/ it may be quite hazardous to take without precautions transgenic animals as models in human pathology, since in the control (non transgenic) group, one may encounter very unusual physiological characteristics, as shown on this species.

Keywords— **Nonlinear estimates, heart rate, autonomic nervous system**

I. Introduction

THE autonomic nervous system (ANS) has long been investigated through the variations of heart rate, in man and in various animals. In now classical spectral analysis studies, it has been shown that in man, dog, and other mammals, the parasympathetic branch of the ANS, mediated by acetylcholine (ACh), is reflected on heart rate variability by its high frequency component, whereas the sympathetic branch, mediated by norepinephrine (NE), is reflected mainly by its low frequency component[1]. In the last decade, the growing interest for nonlinear models among physiologists has led to study, as an alternative to spectral analysis parameters, estimates coming from the theory of chaotic dynamical systems. In the absence of an explicit system of equations, it is hardly possible to decide whether or not a physiological time series is chaotic (i.e. coming from a deterministic dynamical system endowed with a strange attractor), although various tests have been proposed to address this question on recorded time series, with varying results. But *assuming* the presence of such an attractor, one may compute estimates of its complexity: dimension of the attractor by correlation dimension (CD), with the help of Grassberger and Procaccia's algorithm[2]; sensitivity to initial conditions and dissipativity: Lyapunov exponents (LE, algorithms by Wolf, Sano and Sawada, Eckmann and Ruelle[3], [4]); and entropy (or approximate entropy, ApEn, as advocated by Pincus[5]). These estimates should indeed be considered as parameters complementary to classical time-domain or spectral measures, which are not to be neglected. We have in this study applied these principles to the analysis of heart rate in a population of mice rather than other laboratory animals because we have developed a model of transgenic mice overexpressing NE $\beta 1$ receptors only in atria, and very little is known of the normal heart rate and ANS in mice. In the first part of the study, only the parasympathetic branch, and only in normal (non transgenic) mice, has been explored, with results presented here.

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II. Materials and methods

Five young female mice, all healthy and born in our husbandry, non-anesthetized and moving freely, underwent a 3-minute recording of their electrocardiogram (ECG) in the husbandry, before and after intraperitoneal injection of a single dose of atropine (100 mg/kg). The ECG was recorded by means of an intraperitoneal transmitter sending an ultrasound signal to a nearby telemeter (Datasciences Inc., St Paul, MN, USA), then digitized at 3 kHz, stored on a personal computer and submitted to R wave detection by a simple threshold crossing, with the help of the Dadisp software (DSP Development Corp., Cambridge, MA, USA). Careful examination of the detected R waves led us to retain only 5 animals out of a primitive number of 8. The RR interval series were subsequently transferred to be analysed on a Sun UNIX workstation.

The 10 (5 control, 5 with atropine) RR series, *not* filtered, *not* resampled at equally spaced points, were uniformly truncated to keep only the first 1000 values (expressed in milliseconds), and submitted to a nonlinear analysis process involving three public domain softwares: the first one (SCOUNT, by Th.-M. Kruel, University of Würzburg, Germany), an implementation of Grassberger and Procaccia's algorithm, allowed us to estimate for each series $log(r)$ vs. $log(C(r))$ and $log(r)$ vs. $\overline{log(C_i(r))}$ plots, necessary to calculate its CD and ApEn; the second one (DLIA, by K. Briggs, University of Adelaide, Australia), used as an implementation of Eckmann and Ruelle's algorithm, gave us an estimation of its LE's; and the third one (SCILAB, by the Scilab group at INRIA) was used for all other calculations and graphical representations, including the ones leading to CD and ApEn.

The first and second statistical moments were also calculated, and a spectral analysis by means of classical FFT performed for each one of the 10 RR series.

III. Results

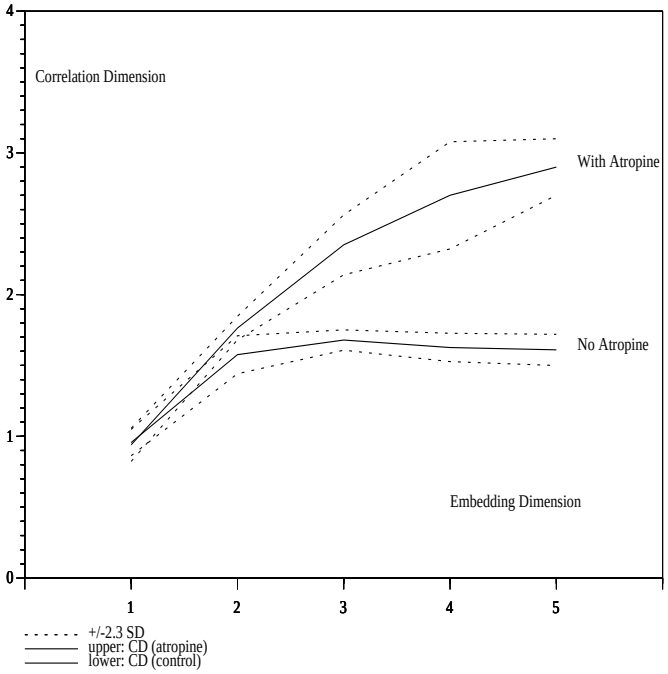

Figure 1. *Correlation dimensions of a series of 1000 RR in one mouse before and after IP injection of atropine (animal number 3). On this figure, dotted lines indicate +/- 2.3 times the standard deviation (95% confidence interval) of the slope (CD), calculated by linear regression on 10 points in the linear zone of the $log(r)$ vs. $log(C(r))$ plot, and assuming normality of the error.*

We calculated correlation dimension (CD) and Lyapunov exponents (LE) for embedding dimensions ranging from 1 to 5. CD's are given in Tables 1 and 2. An illustration of CD contrasts in one animal is presented on Figure 1.

Table 1. *CD for control recordings (before injection).*

	CD for embedding dimension:				
	1	2	3	4	5
Animal 1	0.81	1.36	1.42	1.07	1.22
Animal 2	0.97	1.89	2.41	2.32	2.70
Animal 3	0.96	1.58	1.68	1.63	1.61
Animal 4	1.01	1.84	2.27	2.24	2.21
Animal 5	0.91	1.61	1.99	2.01	1.98

Table 2. *CD for recordings after atropine injection.*

	CD for embedding dimension:				
	1	2	3	4	5
Animal 1	0.81	1.77	2.24	2.07	1.92
Animal 2	0.90	1.78	2.37	2.44	2.76
Animal 3	0.94	1.76	2.36	2.70	2.90
Animal 4	0.95	1.71	2.45	2.78	2.82
Animal 5	0.83	1.58	1.99	2.07	1.98

Wilcoxon's matched-pairs signed rank 2-sided test gave a $p < 0.1$ in dimensions 3, 4 and 5. Assuming normality, a 2-sided Student's test gave $p < 0.07$ in dimensions 4 and 5, non significant in dimension 3; the limited number of animals, resulting from a rigorous selection of the recordings, did not allow us to improve these results (p cannot be under 0.1 for $n = 5$ at Wilcoxon's test).

LE's were calculated in dimensions 3 to 5; only the results in dimension 3 are presented in Table 3, but the tendency to increase the first LE with atropine injection persisted in dimensions 4 and 5, and the sum of all LE's was always negative (an argument in favour of dissipativity).

Table 3. *Lyapunov exponents in dimension 3.*

	Control:			Atropine:		
	λ_1	λ_2	λ_3	λ_1	λ_2	λ_3
Animal 1	0.84	-0.08	-0.96	0.95	-0.04	-0.93
Animal 2	0.79	0.03	-0.94	0.88	-0.03	-0.98
Animal 3	0.90	0.00	-0.90	1.10	-0.04	-1.22
Animal 4	0.93	0.01	-0.97	1.09	-0.05	-1.16
Animal 5	0.85	-0.06	-1.29	0.85	-0.13	-1.12

Again, at Wilcoxon's test, $p < 0.1$ (all differences < 0) for the first LE. ApEn values ($m = 2$ and r equal to 0.05 times the standard deviation of each series) were the following (control / *atropine*): 0.03 / *0.10*; 0.18 / *1.00*; 0.08 / *0.45*; 0.19 / *1.57*; 0.96 / *0.65*; at Wilcoxon's test: $p < 0.1$.

IV. Discussion

Atropine had a marked effect on these nonlinear estimates (CD, 1st LE and ApEn), suggesting an increase in complexity of the underlying dynamics; but the overall heart rate variability, as measured by standard deviation of the RR series (in milliseconds) was constantly decreased by atropine injection: 9.4 / *6.5*; 10.9 / *4.2*; 11.7 / *6.9*; 11.9 / *1.7*; 3.9 / *2.7*, as may also be evidenced on spectral representations (not shown) by a global erasing of the spectrum, more visible in its lower part which carries most of the variability. Mean RR (110–160 ms) was not significantly changed by atropine. These actions of atropine are quite different from the human case, where mean RR is decreased, and the variability is erased mainly in the high frequency component. It is possible that mice, unlike humans, have a permanent leading sympathetic tone, with a vagal correction, rather than the contrary. But, independently of this discussion on the particular species under study, a more general conclusion may be drawn from these results: it is not true that increasing the variability of a time series will result in an increase of its complexity. Indeed, we have shown that the opposite may happen.

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