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SHORT COMMUNICATION

THE EFFECT OF SAMPLING RATE ON THE ANALYSIS OF DIGITAL ELECTROMYOGRAMS FROM VERTEBRATE MUSCLE

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Quantitative electromyography is a widely used, powerful method for determining *in vivo* patterns of muscle activity in diverse animal behaviours. Recent increased capabilities of microcomputers have simplified quantification of the variation in the intensity of recruitment during muscle activity, whereas the times of onset and offset of muscle activity have commonly been determined without computers (reviewed in Basmajian and De Luca, 1985; Loeb and Gans, 1986). Such computer-assisted analyses of the intensity of electromyograms (EMGs) have facilitated correlating movements with muscle activity (Gorniak and Gans, 1980; Weijs and Dantuma, 1981; De Gueldre and De Vree, 1988), determining the stereotypy of motor patterns within and among taxa (Lauder and Shaffer, 1985; Reilly and Lauder, 1989; Wainwright, 1989; Wainwright *et al.* 1989) and clarifying the pattern of recruitment within individual muscles (Carrier, 1989; Hutchinson *et al.* 1989; Jayne *et al.* 1990).

A key step in the computerized analysis of EMGs is the analog to digital (A–D) conversion of the EMG. Mathematically oriented literature has explained how the sampling rate of the A–D conversion affects the results of frequency analysis of a signal (e.g. Bloomfield, 1976). However, there is little practical information on how sampling rate affects certain EMG variables such as number of spikes, the product of spike number times mean amplitude, and rectified integrated area, which are all commonly used by zoologists to assess the intensity of muscle activity (Loeb and Gans, 1986). The purposes of this paper are: (1) to determine how sampling rate affects the EMG variables commonly used by zoologists, (2) to show how a choice of sampling rate may affect statistical conclusions of comparisons across species, and (3) to provide some practical recommendations on appropriate sampling rates for analysis for vertebrate EMGs.

We chose three preparations which represented diverse behaviours and taxa (fish, amphibians and reptiles) in order to increase the likelihood of obtaining EMGs that varied greatly in their intensity, duration and frequency character-

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istics. We examined muscle activity of a pharyngeal jaw muscle (levator posterior) in one fish (*Lepomis gibbosus* Linnaeus) during the crushing of a snail, of a jaw muscle (adductor mandibulae internus) in one salamander (*Ambystoma tigrinum* Green) during transport of prey in terrestrial feeding, and of a hind-limb muscle (red region of the iliofibularis) during walking (at $0.6\,km\,h^{-1}$) in one lizard (*Varanus exanthematicus* Bosc). Hence, the variation among EMG bursts within a behaviour does not contain any variation attributable to differences in preparations. During the experiments, the body temperatures of the fish, salamander and lizard were 21, 21 and 25°C, respectively. EMGs were obtained from bipolar electrodes consisting of 0.051 mm diameter stainless-steel wire with an uninsulated portion of about 0.6 mm. Greater details of methods of electrode construction and implantation can be found elsewhere (fish: Wainwright, 1989; salamander: Reilly and Lauder, 1989; lizard: Jayne *et al.* 1990). We recognize that many factors dealing with amplifying circuitry, electrode construction and electrode placement affect the EMG signal, but we were interested only in examining the effects of the A–D conversion rate on the analysis of these biological signals rather than examining the causes of variation in the EMG signals from different preparations.

All EMGs were amplified 10 000 times using Grass P511J a.c. preamplifiers with a 60 Hz notch filter and half-amplitude low and half-amplitude high filter settings of 100 Hz and 3 kHz, respectively. For the lizard, a supplemental r.c. filter with a cut-off frequency of 50 Hz was also used to minimize further low-frequency motion artefacts caused by the greater movements of the lizard on the treadmill compared to those of the feeding fish and salamander. All amplified, filtered analog EMGs were stored on magnetic tape using a Bell & Howell 4020A FM tape recorder operated at a speed of $19.05\,cm\,s^{-1}$ (bandwidth=d.c. to 5 kHz). For the analog to digital conversion, EMGs were played at a tape speed of $1.1\,cm\,s^{-1}$ into a 12-bit Keithley analog-to-digital converter operating at a sampling rate of 2 kHz. Consequently, an effective sampling rate of 32 kHz was attained. The digital files of the EMGs were analyzed with customized computer programs which counted the numbers of spikes, calculated the product of spike number times the mean of the absolute values of spike amplitude and determined the rectified integrated area of EMGs over 10 ms bins. Before calculating these three variables, the noise (less than 5 % of the maximum signal amplitude) of the baseline was removed by converting all digital voltages below the noise threshold to zero. We used the spike-counting algorithm of Beach *et al.* (1982) which detects positive and negative 'peak' voltages by detecting a change in the sign of the slope of the lines joining successive pairs of voltages. For example, for a noise level of ±0.5 and the series of voltages of 0, 0.3, 0, 1, 2, 3, 2, 2, 5, 0, −1 and 0, three spikes would be counted with peak voltages of 3, 5 and −1, respectively, and the 'mean amplitude' of the counted spikes would be 3. To avoid potential complications of integrating circuits, we also used a computer program to determine the rectified integrated area of the EMGs by calculating the area enclosed by the absolute values of the digital voltages (exceeding noise threshold) and the baseline. To obtain different

sampling rates, the computer program regularly skipped various numbers of points in the original digital file (created at 32 kHz) so that EMGs were sampled at rates of 1, 2, 4, 8, 16 and 32 kHz (=kilosamples s^{-1}). For each burst of activity, we measured the total per burst (=BURST) and maximum per 10 ms bin (=MAX) within a burst of: (1) SPIKES=numbers of spikes, (2) SPIKE×AMP=spike number times the mean amplitude (mV) and (3) AREA=rectified integrated area (mV×ms).

We analyzed these six EMG variables for each of five bursts of muscle activity from the three different behaviours. The PC+ version of the SPSS statistical package was used for all statistical analyses. Our primary analysis for the effect of sampling rate on EMGs was a two-way nested analysis of variance with sampling rate (fixed effect) and animals (random effect) as crossed factors. Burst number was nested within each animal and crossed with sampling rate. Following the guidelines given in Zar (1984), the *F*-test for the significance of the sampling rate effect involved dividing the mean squares (MS) of the sampling rate by MS for the rate × burst interaction term, whereas MS animal was divided by MS burst within animal to test for differences among animals.

As summarized in Table 1, the two-way analyses of variance (ANOVAs) detected widespread effects of sampling rates on EMG variables. For all variables there was a significant animal × sampling rate interaction term, which indicates that the effect of sampling rate depends on the nature of the signal obtained from each preparation. The analog copies of EMGs shown in Fig. 1A–C show that the signals of the three preparations varied greatly in their intensity, duration and heterogeneity of activity within a burst. Furthermore, a fast Fourier transform

Table 1. *Summary of two-way ANOVAs testing the significance of effects of sampling rate and different animals (preparation) on electromyographic variables (indicated in all capital letters)*

	F-tests of significance of effect		
Variable	Rate (5,12)	Animal (2,20)	Animal×rate (10,20)
BURST SPIKES	113.1**	48.3**	37.8**
BURST SPIKE×AMP	100.9**	14.6**	10.5**
BURST AREA	0.5 NS	1.8 NS	7.0**
MAX SPIKES	48.9**	6.1*	5.6**
MAX SPIKE×AMP	30.6**	67.8**	13.7**
MAX AREA	3.3*	80.4**	4.3*

BURST refers to the total quantity per burst of activity and MAX refers to the maximum value observed for a single bin (10 ms) within a burst.

SPIKES, number of spikes exceeding the noise voltage.

SPIKE×AMP, number of spikes times their mean amplitude (mV).

AREA, rectified integrated area (mV×ms).

Degrees of freedom for each *F*-value are indicated parenthetically.

NS, * and ** indicate $P>0.05$, $P<0.05$ and $P\leq 0.001$, respectively.

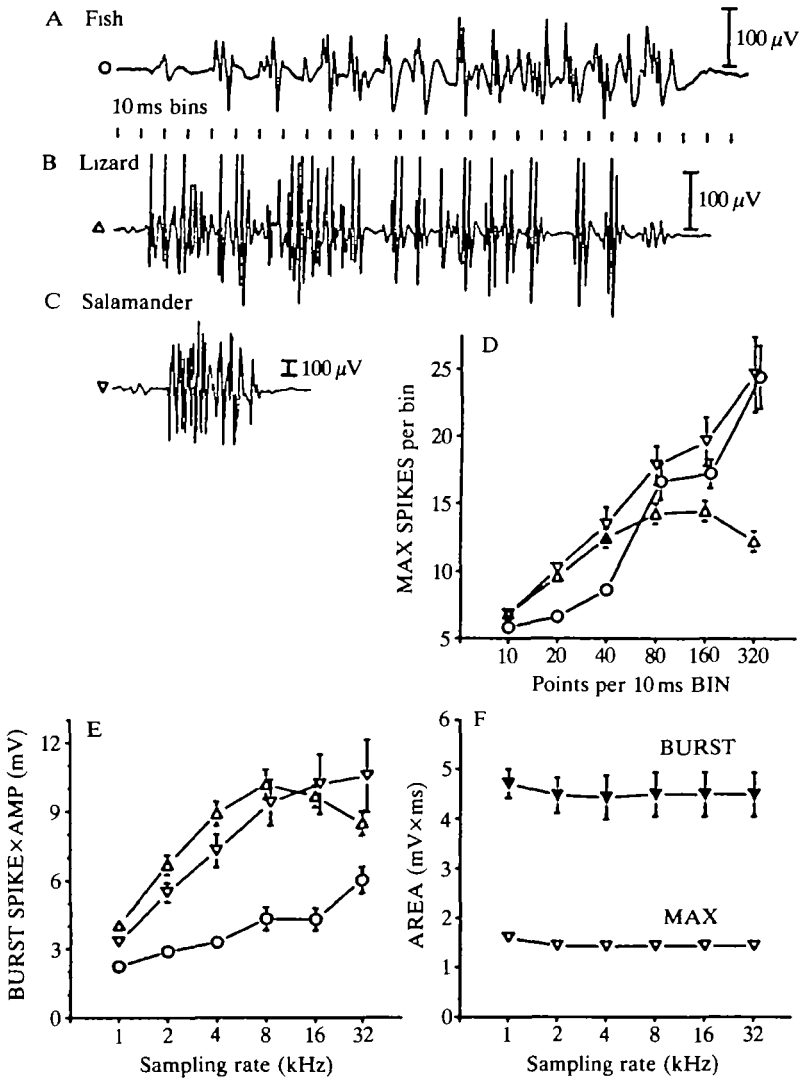

Fig. 1. (A–C) Analog traces of EMGs shown with identical time scales. The vertical bar to the right of each EMG indicates the voltage scale of 100 μV. (A) Levator posterior muscle of the fish, *Lepomis gibbosus*. (B) Red region of the iliofibularis muscle of the lizard, *Varanus exanthematicus*. (C) Adductor mandibulae internus of the salamander, *Ambystoma tigrinum*. (D–E) Mean values (±S.E., N=5 bursts) of EMG variables *versus* sampling rate. Data from the fish (○), lizard (△) and salamander (▽) are shown. (F) Mean (±S.E., N=5 bursts) rectified integrated areas (filled symbols=total per burst and hollow symbols=maximum per 10 ms bin within a burst) *versus* sampling rate for EMGs from the salamander preparation.

(Fig. 2) analyzing the frequencies of the EMGs shown in Fig. 1 reveals that the rank of the preparations from lowest to highest median frequency is: (1) fish, (2) lizard and (3) salamander. Presumably, these differences in the signal frequencies

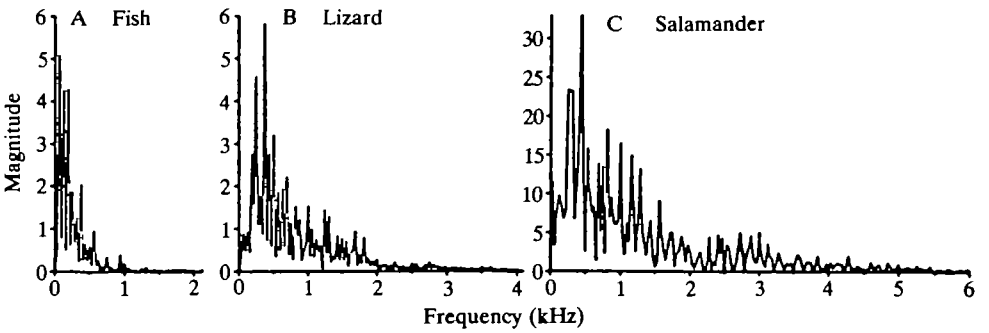

Fig. 2. Averaged fast Fourier transforms (FFT) showing the frequency components of the EMGs illustrated in Fig. 1A–C, (A) fish (B) lizard and (C) salamander. FFTs were performed using 1024 point intervals within each digital file that was created with a 32 kHz sampling rate. Although each analysis gave magnitudes for frequencies ranging from 0 to 16 kHz, values are illustrated only over the range of frequencies where the magnitude exceeds 1% of the maximum value. Median frequencies of the EMGs shown in A–C are 188, 625 and 875 Hz, respectively.

are the primary cause for the significant sample rate × preparation interaction term.

Interestingly, in the two-way ANOVA on BURST AREA, there were no significant main effects of either sampling rate or preparation, whereas variables involving spike counts had highly significant differences attributable to both sampling rate and preparation (Table 1). The nature of the effect of sampling rate on these variables is illustrated in Fig. 1. For both the fish and salamander, EMG variables involving spike counts (Fig. 1D,E) increased or remained the same (fish, Fig. 1E 8–16 kHz) with each successive increase in sampling rate. In contrast, spike count variables for the lizard EMGs generally attained a maximum at the 8 kHz sampling rate. Variables indicating maximal activity per bin generally showed changes with sampling rate similar to those of burst total variables (Table 1, Fig. 1F). The different patterns of the lines shown in Fig. 1D–F further clarify why the two-way interaction terms were highly significant, and close examination also reveals that the rank order of the preparations based on a single EMG variable is not constant across all sampling rates.

Because an increasingly common goal of quantitative EMG studies is to compare motor patterns among taxa, it is instructive to examine the differences among the three preparations, assuming that the variation in EMGs among preparations could represent an extreme example of the variation in the motor pattern of a homologous muscle when comparing a single behaviour among widely divergent taxa. Hence, we performed one-way ANOVAs and range tests separately on each EMG variable at each sampling rate to determine whether sampling rate could influence the outcome of such inter-taxa comparisons. As Table 2 clearly shows, sampling rate can affect comparisons of EMG variables among taxa. For example, using BURST SPIKE×AMP one may have concluded that: (1)

Table 2. *Summary of one-way ANOVAs and range tests (Tukey's procedure) performed separately for each sampling rate (kHz) to detect differences among taxa (preparations)*

EMG variable	Sample rate	BURST variables: Taxon pair fish, sal	BURST variables: Taxon pair fish, liz	BURST variables: Taxon pair sal, liz	MAX variables: Taxon pair fish, sal	MAX variables: Taxon pair fish, liz	MAX variables: Taxon pair sal, liz
SPIKES							
	1	*	NS	*	NS	NS	NS
	2	*	*	*	*	*	NS
	4	*	*	*	*	*	NS
	8	*	NS	*	NS	NS	NS
	16	*	*	*	NS	NS	*
	32	*	NS	*	*	*	NS
SPIKE×AMP							
	1	NS	*	NS	*	NS	*
	2	*	*	NS	*	*	*
	4	*	*	NS	*	*	*
	8	*	*	NS	*	*	*
	16	*	*	NS	*	NS	*
	32	*	NS	NS	*	NS	*
AREA							
	1	NS	NS	NS	*	NS	*
	2	NS	NS	NS	*	NS	*
	4	NS	NS	NS	*	*	*
	8	NS	NS	NS	*	*	*
	16	NS	NS	NS	*	*	*
	32	NS	NS	NS	*	*	*

* and NS, respectively, indicate significant ($P<0.05$) or nonsignificant differences for each pair of taxa.

sal and liz are abbreviations for salamander and lizard.

the fish and the lizard differed significantly (1 kHz), (2) the fish differed from both the salamander and the lizard (2–16 kHz), or (3) the fish and the salamander were different (32 kHz).

The patterns of differences among preparations across all six sampling rates formed two groups (Table 2). First, for all four variables involving spike counts, conclusions regarding inter-preparation differences continued to change as sampling rate increased from 1 to 32 kHz. Second, the two variables involving rectified integrated area did not change over the entire range of sampling rates that we used. For BURST AREA, regardless of the sampling rate, conclusions regarding differences among preparations did not change. For MAX AREA, conclusions about inter-taxa differences remained unchanged as sampling rate increased from 4 to 32 kHz. This suggests that some intermediate sampling rate is adequate to quantify rectified integrated area for signals similar to the EMGs we analyzed,

whereas variables involving spike counts do not approach some asymptote over the range of sampling rates that we examined.

Changes in rectified integrated areas with sampling rate were more conspicuous for EMGs with higher frequency. For example, values of BURST AREA for the salamander muscle at 2 kHz averaged 5.6 % less than those values determined at 1 kHz (Fig. 1F), although no overall significant effect of sampling rate was detected in the two-way ANOVA on this variable (Table 1). When activity within a burst is heterogeneous (Fig. 1A–C), the single bin with maximal activity will often have a higher median frequency than that calculated for all the bins comprising the burst. Consequently, one would expect that measures of maximal activity per bin are more strongly affected by sampling rate than variables describing whole-burst activity. The values of MAX AREA for the salamander muscle averaged a 10.8 % decrease when using a 2 kHz compared to a 1 kHz sampling rate (Fig. 1F), and for MAX AREA there was also a significant overall main effect of sampling rate in the two-way ANOVA (Table 1). For the fish EMG with low median frequency (Figs 1A, 2A), changes in area variables were less than 1 % comparing 2 kHz values to those from 4 to 32 kHz sampling rates. Supplemental observations on other vertebrate EMGs with very high frequency revealed that 1 and 2 kHz sampling rate consistently overestimated rectified integrated area (from 20 to 5 %). For all the EMGs of the vertebrates that we have examined, values of rectified integrated area at 16 and 32 kHz changed less than 1 % compared to those obtained at an 8 kHz rate. Therefore, an 8 kHz rate appears adequate for quantifying rectified integrated area for a wide variety of vertebrate EMGs, and in some cases 2–4 kHz is sufficient. Furthermore, care should be taken to have a high enough sampling rate so that the rectified area of the most intense portion of an EMG is not differentially overestimated.

In contrast to variables describing rectified area, those involving spike counts varied substantially with sampling rate. Plotting the maximum number of spikes per (10 ms) bin (MAX SPIKES) *versus* the number of data points per bin (Fig. 1D) helps to clarify which sampling rates may be inadequate for counting numbers of spikes. For the EMGs that we analyzed, the ratio of points per bin to MAX SPIKES was less than 2 at the 1 and 2 kHz sampling rates, and this would appear not to give a meaningful result. This ratio of points to spikes counted per bin exceeds 4 at an 8 kHz rate, and this would seem to provide a reasonable reconstruction of major spikes.

All the EMG variables that we have examined are commonly used in zoological studies of vertebrate muscle function (references cited previously). Variables indicating maximal activity per bin have been used by themselves or as a scale to calculate the relative activity of other bins within a burst (Jayne *et al.* 1990; De Gueldre and De Vree, 1988). A very low sampling rate (1 kHz) often adversely affects such measures of maximal activity per bin more than activity per burst. The effect of the sampling rate on EMG variables describing the intensity of muscle activity depends on the frequency properties of the signal, and it can result in misleading conclusions regarding differences in motor pattern among preparations

(taxa). Although we did not analyze mammalian and avian EMGs, the tremendous differences among the frequency spectra of EMGs from our three preparations (Fig. 2) suggest that our findings may be widely applicable among many groups of vertebrates. For studies using intramuscular fine-wire bipolar electrodes, sampling rates less than 2 kHz are unlikely to give accurate quantification of the EMG. Clearly, useful insights into muscle recruitment can be gained from determining the numbers of spikes in an EMG, but one faces the rather subjective evaluation of what sampling rate is best for determining these quantities. Rectifying and integrating the EMG before it reaches the storage device complicates the detection of low-frequency artefacts, but recording a bipolar signal and then calculating rectified integrated area avoids this drawback (Loeb and Gans, 1986). Given the signal-dependent effects of sampling rate, the most conservative approach for choosing sampling rate would involve a preliminary analysis for the preparation one is studying. However, a good choice for many quantitative EMG studies in comparisons of vertebrate behaviour would be recording unrectified EMGs, performing a digital conversion at a rate of 4–8 kHz, and then calculating the rectified integrated area from the digital file in order to quantify the intensity of muscle activity.

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