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Effect of Body Position on Limb Lead Electrocardiographic Findings in Sedated Cynomolgus Macaques (*Macaca fascicularis*)

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Electrocardiograms (ECGs) often are collected from sedated cynomolgus macaques (*Macaca fascicularis*) in drug safety studies to support investigational new drug applications. ECGs are evaluated either manually or electronically, and the quality of the ECG tracing can affect the quality of that evaluation. The body position of the subject sometimes is manipulated to eliminate noise or clarify ECG complex morphology, and typically multiple technicians collect ECG data over time. Both factors—body position and multiple technicians—could affect ECG quality. This study was designed to determine whether body position or multiple technicians affects heart rate, mean electrical axis, or ECG parameters (RR interval, P wave duration, PR interval, QRS duration, QT interval (uncorrected and rate-corrected by using the Bazett [QTcb] and Fridericia [QTcf] formulas), P wave amplitude, R wave amplitude, T wave height, T wave height negative, and ST segment elevation). The results reveal minimal (coefficient of variation [CV] less than 10%) within-animal variation between body positions (ventral, dorsal, right or left lateral), with the exception of P wave amplitude (17.5%), R wave amplitude (23.7%), and ST segment elevation (43%). Minimal variation in ECG parameters (no more than 7%) was detected between technicians, across animals, and across body positions. These findings suggest that neither a change in body position to increase the quality of an ECG tracing nor the use of multiple technicians significantly affect the evaluation of quantitative ECG parameters, especially QTcb (0.1% CV) and QTcf (1.3% CV).

Abbreviations: cb, corrected by using the Bazett equation; cf, corrected by using the Fridericia formula; CV, coefficient of variation; ECG, electrocardiography.

Electrocardiographic (ECG) analysis of nonhuman primates is a component of repeat-dose preclinical drug safety evaluations for new compounds.[1,7] Because ECG analyses are conducted at several points during these studies (for example, before and 1 and 4 wk after dosing), variability between test sessions is possible. Little has been published to date evaluating the variability in ECG parameters of chemically sedated nonhuman primates during the collection of limb lead ECGs using one or multiple positions and different technicians on multiple days.

Reference ranges regarding ECG parameters for awake and sedated cynomolgus macaques have been proposed;[1,5,14] however, position of collection has not been defined for the sedated nonhuman primates. In addition, some authors report that positional changes affect ECG parameters in dogs and recommend that tracings from various positions should not be compared.[10] By comparison, reports on the effect of positional changes in humans have conflicting findings.[8,9,12]

High-quality ECG tracings are essential to analyzing ECG data from nonhuman primates. Multiple variables including plane of sedation, muscle movement or twitching, signal strength (electrode placement), additional background noise (electronic), and animal condition can affect the quality of these tracings.[3,13] Methods to improve signal quality include changing the body position of the animal or the location of subdermal pins and supplementing sedation.

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Evaluation of ECG parameters for drug safety studies typically is performed on an individual animal (that is, each monkey serves as its own control, with comparison with ECGs collected prior to study start) and on a group basis (that is, drug treatment groups are compared with concurrent group controls). Technicians review the digitized waveforms by using a computer program to ensure the appropriateness of the tracing and marking of the waveforms. The current study was undertaken to demonstrate that body position and the use of multiple technicians for ECG collection had no effect on ECG parameters during a limb-lead–only evaluation of chemically sedated cynomolgus macaques. Tracings then could be collected from animals in positions that were procedurally convenient and results could be compared across the study or with published reference ranges for the species involved.

Materials and Methods

Animals. The study population comprised 16 cynomolgus macaques (*Macaca fascicularis*; 8 male and 8 female; age, 1 to 38 mo; weight, 2.5 to 5.1 kg). All macaques were housed individually in one-over-one stainless steel 0.7- m^3 squeeze cages with a 12:12-h light:dark cycle. Animals were fed a commercially available certified primate biscuit diet (2050C, Harlan, Indianapolis, IN). Reverse-osmosis–treated water was provided ad libitum. Fresh fruits and vegetables, commercially available certified treats, and certified cage toys were used as enrichments.

All animals were Mauritian-origin nonhuman primates, serologically negative for simian immunodeficiency virus, simian retrovirus, simian T-cell leukemia virus, and macacine herpesvirus 1 (formerly known as cercopithecine herpesvirus

1 [CHV1 or BV]). The animals were vaccinated for hepatitis A and measles virus and tested positive for measles antibody. All animals received care in an AAALAC-accredited facility, and all experimental procedures were approved by the institutional animal care and use committee.

Randomization. The 8 male and 8 female animals were allocated randomly by using a commercially available program (Provantis, Instem LifeSciences Data Systems, Stone, Staffordshire, England) into 4 groups (2 male and 2 female macaques per group). The groups were rows in a 4 × 4 latin square that specified the body position order (right lateral, left lateral, ventral, and dorsal recumbency).

Sedation. Monkeys were sedated within their cages by injection of ketamine (40 mg IM; lot no. 6121994, Ketaset, Fort Dodge Animal Health, Fort Dodge, IA). Once the macaques were sedate enough to handle, they were removed from their cages and placed in a recumbent position in a primate restraint chair for ECG collection. They were maintained in a plane of sedation that minimized muscle movement, and additional ketamine was given as needed. Approximately 2 macaques per session received a single additional half-dose of ketamine.

ECG collection. ECGs were collected by different technicians 3 times over an approximate 2-wk period, allowing for a minimum of 2 d between ECG collections, by using subdermal pins (model no. FE3M, Disposable Stainless Steel Needle Electrode, Grass-Telefactor, Braintree, MA) in a limb-lead–only configuration (electrodes placed just proximal to the right and left elbows and stifles). ECGs were collected by using acquisition software (Dataquest OpenART, Data Sciences International, St Paul, MN) and data analysis software (version 3.3.2.2, PONEMAH P3Plus, Data Sciences International, Valley View, OH) previously validated to scientific standards for Good Laboratory Practice compliance. This validation included direct comparison of automated data with hand-read tracings, 100% verification of automated data markings, and positive and negative control experiments. The ECGs were recorded at a frequency of 3 kHz and then were digitized to a signal of 500 Hz. Each monkey was evaluated in each position. Each animal was evaluated in all 4 positions during each session. Collection halted only long enough to accomplish a position change. Three technicians collected ECG tracings from each of the 16 monkeys, according to the randomization schedule (for example, technician 1 on days 1 and 2; technician 2 on days 4 and 5; technician 3 days 8 and 9). Data were collected from male macaques on days 1, 4, and 8 and from females on days 2, 5, and 9.

Efforts were made to collect and evaluate at least 300 cardiac cycles per animal; however, acquired ECG data must have had at least 150 interpretable cardiac cycles to be included in the mean calculations for each body position. The following quantitative parameters were collected: number of hear beats collected (number averaged), heart rate, RR interval, duration of P wave (P width), PR interval, duration of QRS complex, QT interval, P wave amplitude, R wave amplitude, T wave height, T wave height negative, and ST segment elevation. Corrected QT intervals were calculated by software using the Bazett[2] (QTcb) and Fridericia[4] (QTcf) correction formulas. In addition, mean electrical axis was determined manually for each animal in each position on the first day. Six-lead (leads I, II, III, aVR, aVL, and aVF) ECGs were recorded to allow for the determination of mean electrical axis for the first collection and qualitative assessment. Quantitative assessments were performed for lead II only by using digitized calipers provided within the analysis software. Qualitative assessment of each ECG was performed by a veterinarian to assess for arrhythmias and waveform abnormalities.

Statistics. The objectives of the statistical analysis were to determine statistically significant mean differences in ECG parameters were present among the 4 positions (dorsal, ventral, and right and left lateral) and to evaluate technician-to-technician variability. Mean electrical axis was calculated manually because results were available only for technician 1; therefore, analyses were performed separately for this parameter. Repeated-measures ANOVA methods appropriate for a latin square design were used to evaluate mean differences between positions for the ECG parameters. If there was no significant difference between the means for both sexes ($P > 0.05$), position means were compared by averaging across sex. The Tukey multiple comparison t test procedure[6] was used for all possible pairwise comparisons of mean differences between positions. For all parameters, 2-sided t tests were calculated at the 5% significance level. Analyses were performed by using SAS version 9.1.2 (SAS, Cary, NC).[11]

Variance components methods were used to quantitate technician and position differences. The differences were expressed first as variances and then as coefficients of variation (%CV), calculated as 100% × (1 SD / mean), where SD is the square root of the variance, and mean is the overall mean for each parameter. The analysis was performed for all parameters except mean electrical axis, for which data for only 1 d were available. For all remaining parameters, an ANOVA model with random factors for technicians, positions, and animals was used. Because of the hierarchical nature of these derivations, it was possible that once within- and between-animal variation was derived, there was no variation 'left over' to attribute to position-to-position and technician-to-technician variation. In those cases, the precision estimates were set to zero.

Results

Tables 1 and 2 summarize the results of analyses to assess the effect of body position on ECG parameters of male and female macaques. For all analyses, because there were no significant mean differences between male and female monkeys ($P \geq 0.05$), results for each parameter were combined for male and female macaques and the combined means for the 4 positions were compared. There were no significant mean differences among position for mean electrical axis, QRS, QT, QTcb, and QTcf. Low %CV (heart rate, PR, P wave width, and RR interval) were associated with small biologically irrelevant but statistically significant changes. Differences in amplitude are expected in change of position (P wave amplitude, R wave amplitude, and ST segment elevation). Because intraanimal variability was relatively low for most ECG parameters, the ANOVA model was sensitive to small changes among position means.

Table 3 summarizes the between-technician and between-position variability estimates, calculated as variance estimates and reported as %CV values for all ECG parameters. This analysis was not performed for mean electrical axis because data were

Table 1. Position-associated differences in mean electrical axis (in degrees) of electrocardiograms performed by technician 1

Position	Minimum	Maximum	Mean	1 SD
Dorsal	−30.0	130.0	54.8	40.5
Left lateral	−10.0	155.0	55.4	40.9
Right lateral	−30.0	132.0	51.3	45.2
Ventral	−30.0	110.0	38.9	34.8

A total of 16 observations were included in each assessment.
No significant differences were observed among positions ($P = 0.10$)

Table 2. Position-associated differences in parameters of electrocardiograms performed by technicians 1,2, and 3

Parameter	Position	Mean	SD	Minimum	Maximum
Heart rate	Dorsal	167.7	22.3	114.8	209.0
(bpm)	Left lateral	171.5	23.3	124.8	221.3
	Right lateral	168.3	20.1	124.0	217.7
	Ventral	179.8	24.5	116.2	225.0
PR[a]	Dorsal	89.3	10.8	71.6	124.1
(ms)	Left lateral	85.2	8.1	66.3	98.7
	Right lateral	85.1	8.9	68.2	109.6
	Ventral	85.4	9.3	64.7	106.9
P amplitude[a]	Dorsal	0.113	0.025	0.055	0.171
(mV)	Left lateral	0.123	0.034	0.008	0.180
	Right lateral	0.100	0.029	0.038	0.158
	Ventral	0.134	0.026	0.081	0.185
P width[a]	Dorsal	50.3	6.5	42.7	66.9
(ms)	Left lateral	44.7	4.0	35.5	54.8
	Right lateral	43.5	4.1	36.5	57.0
	Ventral	48.7	6.3	39.2	65.5
QRS	Dorsal	36.5	5.5	24.3	47.3
(ms)	Left lateral	36.0	4.9	21.5	43.9
	Right lateral	36.1	4.9	20.9	44.9
	Ventral	36.6	4.2	27.6	49.3
QT	Dorsal	201.8	30.2	134.9	277.6
(ms)	Left lateral	200.5	26.2	144.6	274.8
	Right lateral	197.6	25.0	140.4	268.6
	Ventral	192.9	28.9	141.5	281.5
QTcb	Dorsal	333.5	33.2	249.7	383.7
(ms)	Left lateral	335.6	27.9	276.5	396.1
	Right lateral	328.3	28.1	267.5	385.7
	Ventral	330.2	31.1	262.6	402.4
QTcf	Dorsal	282.0	32.2	203.4	344.5
(ms)	Left lateral	282.5	26.9	223.4	350.7
	Right lateral	277.1	26.8	215.7	342.0
	Ventral	275.9	30.3	213.9	351.1
RR[a]	Dorsal	364.8	50.0	289.6	523.5
(ms)	Left lateral	356.9	51.6	271.2	481.3
	Right lateral	362.1	44.4	275.6	485.0
	Ventral	340.6	50.1	266.8	516.9
ST elevation[a]	Dorsal	0.021	0.013	0.000	0.058
(mV)	Left lateral	0.018	0.013	0.001	0.071
	Right lateral	0.012	0.009	0.000	0.042
	Ventral	0.029	0.014	0.001	0.074
R wave amplitude[a]	Dorsal	0.693	0.231	0.340	1.155
(mV)	Left lateral	0.887	0.346	0.134	1.547
	Right lateral	0.758	0.332	0.150	1.390
	Ventral	0.873	0.356	0.295	1.782

A total of 48 observations were included in each assessment. Because no parameter demonstrated sex-associated differences, data for both sexes were combined.

[a]Position exerted a significant ($P \leq 0.05$) effect on the following parameters: PR (dorsal [D] > left lateral [L], right lateral [R], ventral [V]), P-H (V > D, L, R; D, L > R), P width (D > L, R; V > L, R), RR (D, L, R > V), ST-E (V > D, L, R; D, L > R), and R-H (L, V > D; L, V > R).

Table 3. Summary of intertechnician and interposition variability

Parameter	Variance component	Coefficient of variation (%)
Heart rate	Between-technicians	5.2
(bpm)	Between-positions	3.0
	Within-animals	7.9
PR	Between-technicians	1.4
(ms)	Between-positions	2.2
	Within-animals	5.9
P width	Between-technicians	1.8
(ms)	Between-positions	6.8
	Within-animals	8.2
QRS	Between-technicians	0.4
(ms)	Between-positions	0.0
	Within-animals	9.2
QT	Between-technicians	3.1
(ms)	Between-positions	1.4
	Within-animals	9.7
QTcb	Between-technicians	0.1
(ms)	Between-positions	0.0
	Within-animals	6.9
QTcf	Between-technicians	1.3
(ms)	Between-positions	0.5
	Within-animals	7.7
RR	Between-technicians	5.4
(ms)	Between-positions	2.8
	Within-animals	8.4
R amplitude	Between-technicians	1.2
(mV)	Between-positions	11.1
	Within-animals	23.7
ST elevation	Between-technicians	7.2
(mV)	Between-positions	35.7
	Within-animals	43.0
P amplitude	Between-technicians	3.1
(mV)	Between-positions	12.1
	Within-animals	17.5

A total of 192 observations were included in each assessment. Between-animal variation was omitted from this table; it was expected that interanimal differences would exist.

available for only 1 d. Using heart rate as an example, variation among technicians was 5% (after removing the effects of different animals and positions), and variation in responses among the 4 positions was 3% (after removing the effects of different animals). The within-animal variation estimate of 8% reflects the average variability of each animal's individual responses. Between-animal variation estimates were calculated but are not reported, since they are expected to be high and are not of scientific interest.

In general, between-technician differences were low, ranging from 0.1% to 7%. Differences among body positions were within 12% for all parameters except ST segment elevation (36% CV). The % CV for QRS and QTcb are reported as 0.0%. In these analyses, there was more variation within and among the animals than there was among the positions, and a variance estimate could not be calculated. Within-animal variability estimates were less than 10% for all parameters except P wave amplitude (18% CV), R wave amplitude (24% CV) and ST segment elevation (43% CV). These results suggest that in general, differences among technicians and positions were relatively small.

Discussion

Position changes within an institution during the course of a study or differences in collection positions across institution can occur. In addition, previously reported reference ranges in sedated cynomolgus macaques do not define the collection position.[1,5,14] In dogs, differences in position can change the lead II ECG parameters collected, namely R wave amplitude and mean electrical axis, leading to a recommendation that tracings obtained from animals in different positions should not be compared and that position-specific reference ranges are needed.[12]

In general, the results of the present study demonstrate that there was minimal variation in ECG data (less than 10%) within animals during the same test session at different body positions (ventral, dorsal, right or left lateral) including QTcb (6.9% CV) and QTcf (7.7% CV) and with the exception of P wave amplitude (18% CV), R wave amplitude (24% CV), and ST segment elevation (43% %CV). Data on amplitude parameters, including P wave amplitude, R wave amplitude, and ST segment elevation, should be assessed carefully because of the potential for nondrug-related changes. Substantiating data such as anatomic pathology should be used to determine whether amplitude changes are incidental or drug-related. Minimal variation (less than 10%) was detected across animals with respect to body position on different test session days and different technicians conducting data collections (in particular, QTcb, 0.1% CV across technicians; QTcf, 1.3% CV across technicians). In contrast to dogs, statistically significant variations in mean electrical axis were not detected across positions for macaques. This difference between species may be related to the relative heart size compared with the space in the chest cavity. Because of this variation in outcome, we propose that comparison across position is more appropriate in cynomolgus macaques than in dogs (with the exception discussed earlier).

Ketamine, in macaques, has been administered in doses ranging from 10 to 20 mg/kg.[1,5] In the present study, we administered 40 mg to each macaque, the commonly accepted dosage at our institution for nonhuman primates with weights similar to those of the animals we used here. The goal of sedation for ECG collection should be to minimize background noise caused by muscle movement while maintaining consistent heart rates across time. Ketamine has been shown to have little effect on ECG parameters, with the exception of heart rate (causes increases in heart rate during the first 20 min after administration).[1,5,9] Because collection times were limited to 300 beats (approximately 2 min), heart rate variability due to ketamine administration was assumed to be minimal. Therefore, given the convenience of administration for studies involving large numbers of subjects, the required amount of sedation, and the potential to administer ketamine to animals with body weights not in the computer-dosing systems (that is Provantis), per-animal dosages were implemented at our institution.

Sample size in studies using nonhuman primates can be limiting due to cost and availability of subjects, and if the collection method is reliable, the estimate of variation should be small, therefore the sample size for the current study was selected to mimic the recovery phase of preclinical drug safety studies at our institution. Variation estimates are considered to be 'pure' estimates that describe the variation only in the factor of interest (for example, technicians and positions), where other sources of variation in the ANOVA model have been removed (for example, interanimal and within-animal differences). This analysis differs from the ANOVA in that it describes the average variability observed in responses among the 3 days and 4 positions, without applying any statistical significance. When generated by different days and positions for ECG collection, variation analysis gives a measure of how far responses diverge (on average) from the overall mean. Although increasing the number of subjects to better estimate the variance can be considered, the results obtained showed high reproducibility (generally less than 10% CV); therefore, increasing animal numbers would not have increased the sensitivity of this analysis.

High-quality ECG tracings with consistent marking of ECG intervals are of the utmost importance to support accurate evaluation. High-quality tracings using limb lead collection can be difficult to obtain,[15] and complications can include low-amplitude T waves and signal noise. The use of different technicians for multiple-day collection or changes in subject body position during collection can be used to improve signal quality without significant related variation in the data obtained from cynomolgus macaques, particularly for QT intervals. These findings suggest that changing subject body position to improve the quality of the ECG tracing and using published reference ranges likely will not significantly affect the evaluation of quantitative ECG parameters in cynomolgus macaques.

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