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Stability and Reproducibility of Time Structure in Spontaneous Behavior of Male Rats

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KERNAN, W. J. AND P. J. MULLENIX. *Stability and reproducibility of time structure in spontaneous behavior of male rats.* PHARMACOL BIOCHEM BEHAV **39**(3) 747–754, 1991.—The computer pattern recognition system for the study of spontaneous rat behavior has allowed new analytical techniques which expand the definition of experimentally induced changes in behavior. As with any technique, the stability of the measures must be considered when evaluating overall sensitivity. This study evaluates the stability and reproducibility of three behavioral measures: a measure of the number of initiations of specific behavioral acts, a measure of the total time of each act, and a measure of behavioral time structure. Normal statistical parameters are used to evaluate the significance of changes detected using the first two measures, but the third measure utilizes K-functions, the bootstrap and ad hoc criteria to evaluate significance of observed changes. This study compares the stability of results from these three measures as applied to fourteen different groups of control Sprague-Dawley male rats. All three measures provided stable and reproducible results, but the measure of time structure, the K-function analysis, provided the greatest consistency. Behavior, particularly spontaneous behavior, has traditionally been perceived as being intrinsically variable. However, this study shows that the computer pattern recognition system and its analytical techniques provide stable and reproducible values that vary only a few percent.

Spontaneous behavior Time structure K-function Measure stability Sprague-Dawley rats

IN dealing with animal behavior, questions arise as to the reproducibility of measurements made at different times upon different groups. Such questions are most commonly connected with the development of new experimental instruments or analytical techniques. Recently, a computer pattern recognition system for the study of rat behavior was introduced (7), along with a new technique for the analysis of the time structure of spontaneous behavior (8–10). Spontaneous rat behavior consists of discrete behaviors which are simple "descriptive" acts, such as standing, sitting, rearing and walking, that are classified by the computer system (see Behavioral Tests in the Method section). The new time structure analysis utilizes K-functions, a subject of recent statistical development dealing with spatial point processes (2, 16, 17). The K-functions have been shown to be very sensitive to the disruption of behavior due to pharmacological action or toxicological insult. In experiments which include both control and experimental animals observed in pairs, for example, it was demonstrated with K-functions that d-amphetamine at a dose as low as 0.25 mg/kg disrupts the behavior of male Sprague-Dawley rats (11). Also, K-functions were used to detect behavioral abnormalities in an animal model to study the IQ deficits subsequent to central nervous system therapy for childhood acute lymphoblastic leukemia (13). When values of the K-functions for an act, such as stand, or for a pair of joint acts, such as stand-walk, were larger for experimental rats than for controls, it indicated that the experimental treatment caused initiations of that act or that pair of acts to be more clustered in time, while smaller values indicated that the act or pair of acts were more dispersed in time.

The use of functions, instead of normal statistical parameters, complicates the definition of an acceptable measure that determines changes between control and experimental groups. To date, the estimates of the standard deviations of K-functions have been obtained using the computer-intensive technique known as the bootstrap (1, 3–6). The combination of a computer pattern recognition system with K-function analysis and the bootstrap, and especially application of this combination to behavioral science, necessitates study of the stability of the measures, the adequacy of the estimates of uncertainty in the measure, and the reproducibility of the results.

The stability, uncertainty, and reproducibility of these measures can be examined using control data accumulated from multiple studies (9, 11–13). Certain features of experimental design were common to all of these studies. Animals were observed in pairs consisting of one control and one experimental animal. The computer pattern recognition system was used to record and classify individual behaviors. The number of occurrences, total time and time structure of behavior were determined using the same analytical procedures each time. All animals were approximately the same age when tested, and they were all male Sprague-Dawley rats. Using only the control animals, a total of fourteen different groups were accumulated for study, each

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TABLE 1

AVERAGE INITIATIONS AND AVERAGE TOTAL TIME FOR "STAND" FROM 14 GROUPS OF CONTROLS

	Initiations		Total Time		
Data Set	Average	SD	Average	SD	N
1	96.3	35.5	670.6	95.9	20
2	97.6	25.6	628.2	101.7	20
3	119.5	25.6	604.8	67.3	15
4	106.8	20.7	625.9	83.6	15
5	91.3	32.9	650.4	110.7	15
6	111.0	24.0	606.9	93.1	15
7	119.6	19.5	584.9	63.4	15
8	101.3	22.5	607.1	68.4	15
9	122.8	25.7	610.0	33.4	20
10	115.8	31.5	564.0	77.1	20
11	138.0	18.1	568.1	54.9	20
12	106.1	34.2	611.3	119.7	20
13	110.8	31.4	635.0	75.9	20
14	128.9	26.8	592.9	68.7	15
Average (±SD)	105.0 (±30.1)	26.7	611.4 (±29.5)	79.6	

group containing fifteen or twenty rats. Although treatment of the rats was not the same across all groups (see the Method section for details), all treatments were control procedures selected for minimal impact on behavioral function. In all, the experimental similarities allow comparisons across multiple groups. Such comparisons provide the opportunity to examine the stability and reproducibility of the measures used in these new behavioral techniques.

METHOD

Animals

Pathogen-free Sprague-Dawley male rats were obtained from Charles River Laboratories (Kingston, RI) and allowed to adjust to the Forsyth animal facility for one week before any control treatment procedure began. The number of rats per control group is shown in Table 1. The animals were housed two per cage and given standard Purina Rat Chow and tap water ad lib except just prior to and during behavioral tests. A 12-hour light (0600–1800 hours)/12-hour dark cycle was maintained for these animals. All behavioral tests were conducted in the same facility outside of the animal quarters. The rats were approximately six weeks of age when their behavior was tested.

The fourteen control groups received one of five treatments. Two groups (data sets 1 and 2) were not exposed to any manipulations other than placement in the novel environment for the behavioral test. Six groups (data sets 3 through 8) were administered orally a vehicle containing 0.5% (w/v) methylcellulose, 0.1% (v/v) polysorbate 80 and distilled water, one hour before the behavioral test (12). One group (data set 9) was given a sham exposure to radiation 3–4 weeks prior to the behavioral test, and another group (data set 14) received a similar sham exposure to radiation, but it was preceded by two intraperitoneal saline injections (13). Finally, four groups (data sets 10 through 13) were given a single subcutaneous injection of saline 30 min prior to the behavioral test (11). All of these procedures were designed to be the controls for various experimental treatments. To the extent that the measures examined in this study are stable and reproducible, it can only be in spite of these different control protocols.

Behavioral Tests

Behavior was tested between 0900 and 1300 h each day. The test used for all control groups consisted of placing a pair of rats simultaneously into a divided Plexiglas observation chamber [see (7) for details of the chamber]. The experimental and control rats were separated by a clear partition with small holes, which allowed the rats to see and smell each other while they explored the novel environment. Two video cameras taking a frame per second were used to monitor their spontaneous behavior for a 15-minute period. The video signals were transferred to a MICRO VAX I and a VAX 11/750 for pattern analysis and behavioral classification of the data. The overall system of the cameras, computers, and the computer software has been described elsewhere (7) and hereafter will be referred to as the RAPID system.

The behaviors identified by the RAPID system consisted of five possible major body positions—stand, sit, rear, walk, and lying down—each of which is combined with one of eight possible modifiers—blank (no recognized activity), groom, head turn, turn, look, smell, sniff, and washing face. For operational definitions of these acts, consult Norton (14).

In studying the time structure of the major body positions and modifiers, Norton, Mullenix, and Culver (15) identified certain acts which had similar time structures. Three such sets of acts were noted and arbitrarily labeled "grooming," "exploratory," and "attention." We also have examined the results of using this list of "acts" instead of the usual stand, sit, etc. Previously (9), this list was called the "grouped acts," but this led to confusion for some readers, where "group acts" were confused with calculations per group of rats. For this study, and in the future, we will instead refer to this list as "combined acts":

	Act	Combined Acts
Major Body Position	Stand	Attention
	Sit	Grooming
	Rear	Explore
	Walk	Explore
	Lying down	Grooming
Modifiers	Blank	Attention
	Grooming	Grooming
	Looking	Attention
	Sniffing	Explore
	Washing face	Grooming
	Smelling	Attention
	Head turning	Attention
	Turning	Explore

All calculations presented here are for groups, never for animals considered singly. For these combined acts, when a co-occurring body position and modifier belong to different components of this structure, a full and mutually exclusive list would include "attention-explore," "attention-groom," and "explore-groom." The list for "combined acts," therefore, contains six possibilities.

Data Analysis

In all of the fourteen experiments from which control data were extracted, three measures of spontaneous behavior were

taken. The first was the count of the initiations of any act. The second was a measure of the total time that each act was performed. The third was a measure of the time distribution of the initiation of discrete acts and of sequences of joint acts. The first two measures are similar to those incorporated in a variety of activity studies, but the third is unique to data generated from either time-lapse photographic analysis (8,10) or the RAPID system (9). The third of these measures is the focus in this study, however, results using the first two are presented as well for purposes of comparison.

Calculation of act initiations. The number of frames where a specific behavioral act was initiated was totaled for the 15-minute observation period for each rat. In a standard experiment, the mean number of initiations for each act would be determined for the control and experimental groups and the *t*-test would be used to determine significant differences at the $p<0.05$ level. In the present study, comparisons are made only between the averages of the various control groups.

Calculation of total time for each act. The number of frames that a behavior continued, including the initiating frame, was totaled for the 15-min observation period. The mean total time for each act in control and experimental rats was determined in a standard experiment, and significant differences between control and experimental groups were determined using the *t*-test. Again, in the present study, comparisons are made only between the averages for the various control groups.

Calculation of time distribution and time sequence. The time distribution of individual behavioral acts was calculated using an equation described elsewhere (8):

$$K_\alpha(t) = \frac{\tau_\alpha}{(n_\alpha)^2}\left|\sum_{i}\sum_{j\neq i} W_{ij}^{-1}\, It\,(U_{ij}^{\alpha})\right| \qquad (1)$$

In this equation, n_α is the number of initiations of act α, τ_α is the total observational time corrected for the extension of the act α, W_{ij} is an edge correction term, and $I_t\,(U^{\alpha}{}_{ij})$ is 1 (or 0) according to whether the pair (i,j) of initiations of act α occurred (did not occur) within a time separation t. The function $K_\alpha(t)$ evaluated at eight time points (2, 5, 10, 20, 30, 45, 100, and 200 s), is referred to as the time distribution of act α.

In developing the K-functions for the study of the time sequence of the initiations of behavioral acts, a major goal was to minimize the interdependence of the average number of initiations of the act, the average duration of the act, and the K-function corresponding to that act. To achieve this goal, the normalization factor in Eq. 1,

$$\frac{\tau_\alpha}{(n_\alpha)^2},$$

uses an observational time which is corrected to minimize the effects of changes in the average number of initiations and the average duration of act α. As described before (8), the corrected observational time for this act was:

$$\tau_\alpha = (T - t_\alpha) + n_\alpha \qquad (2)$$

The total time of observation was T, the number of α initiations was n_α, and the total time observed for act α was t_α. "Time" was treated as a mathematically discrete variable, and n_α in Eq. 2 allowed the time of initiation to be included in the count for the corrected time.

The W_{ij} edge correction factor was applied because, when act α occurred near the extremes of the observational time, not all ranges of time were available for inclusion in the calculation. The values of t at which K(t) were calculated were relatively small, and hence the weighting factor had little effect.

To assess sequences of acts and their multivariate relationships, another equation described by Kernan and coworkers (8) was applied. This equation provided the K-function for the joint acts α and β:

$$K_{\alpha\beta}(t) = (n_\alpha n_\beta)^{-1}\,\tau_{\alpha\beta}\left\{\sum_{i=1}^{n_\alpha}\sum_{j=1}^{n_\beta} W_{ij}^{-1}\, I_t\,(U_{ij}^{\alpha\beta})\right\} \qquad (3)$$

Each term had a meaning similar to that discussed for Eq. 1. To correct for the extension of each of the two different acts for the sequence analysis, $\tau_{\alpha\beta}$ became:

$$\tau_{\alpha\beta} = (T - t_\alpha - t_\beta) + n_\alpha + n_\beta \qquad (4)$$

In Eq. 3, the $U_{ij}^{\alpha\beta}$ is the separation between the i-th event of act α and the j-th event of act β. The $I_t\,(U_{ij}^{\alpha\beta})$ term is changed to fit the behavioral sequence situation. In order to retain information on possible causal relationships among the acts, the formulation was intentionally asymmetrical in the time relationship. $I_t\,(U_{ij}^{\alpha\beta})$ equaled 1 if event j of act β occurred within a time interval t later in time than event i of act α, and $I_t\,(U_{ij}^{\alpha\beta})$ equaled 0 if event j occurred earlier than event i or if the time separation exceeded t. That is, the sum over j was only for events of act β later than event i of act α.

Estimates of uncertainty in K(t). The function K(t) was computed for each control group, consisting of 15 to 20 animals in a given experiment. The existence of data from each of 15 to 20 control animals in each experiment allowed the use of the bootstrap technique to estimate the standard deviation at each time point of the K-function for the time distribution of any act or pair of acts. This technique (1, 3–6) uses Monte Carlo methods to generate an estimate of the variance of a statistic based only on the data. A random number generator was used to construct 1,000 simulations of this calculation, each time generating a list of the identification numbers of animals used in the calculation, animals selected from the original control group for that experiment. Obviously one or more animals may be dropped in any one of these simulations, and others would then be included more than once. Standard statistical formulae were then used on the 1,000 simulations to obtain an estimate of the standard deviation of K(t) for the control group in that experiment. The effect of sampling fluctuations upon the estimate of the standard deviation in K(t) was determined by examining changes in that estimate when the 1,000 simulations of the bootstrap were repeated a number of times; care was taken to use a different starting value or "seed" for the random number generator to avoid generating the identical list of animals utilized in each of the 1,000 simulations.

RESULTS

The stability of the initiation, total time and K-function measures of spontaneous behavior can be demonstrated by results from a few representative acts. The results for all behavioral acts, that is for all discrete acts (sit, stand, rear, smell, etc.) considered together, or for all combined acts (attention, explore, etc.) considered together, were quite similar. For the act stand, the average number of initiations and average total time per animal, plus the respective standard deviations, are shown in Table 1 for all 14 control data sets. The number of rats per set is given

TABLE 2

AVERAGE INITIATIONS AND AVERAGE TOTAL TIME FOR "REAR" FROM 14 GROUPS OF CONTROLS

	Initiations		Total Time	
Data Set	Average	SD	Average	SD
1	19.3	11.1	53.3	55.2
2	18.3	7.4	47.3	21.3
3	23.7	9.7	58.1	16.2
4	17.7	6.2	47.4	17.9
5	16.5	9.0	32.7	18.2
6	18.5	6.0	44.8	21.6
7	24.6	11.3	55.1	25.0
8	17.3	6.7	41.9	19.6
9	24.5	10.9	47.7	21.5
10	20.1	9.4	38.8	16.9
11	23.6	7.0	47.6	20.6
12	20.3	9.7	42.0	20.5
13	19.4	9.9	44.5	26.4
14	23.8	11.0	47.8	26.6
Average (±SD)	20.5 (±2.9)	9.0	46.4 (±6.6)	23.4

in Table 1 as well. Tables 2 and 3 present the identical information for the acts rear and smell, respectively.

The K-function was defined and normalized in a manner which minimized the influence of the number of initiations of an act, and the total time spent by the animals in performing that act. Consequently, the K-function measure of spontaneous behavior revealed information about the time relationships of initiations of acts without having the results depend strongly upon the other two measures. However, demonstration of the stability of this new information is not as simple as in the first two measures given in Tables 1–3. It is more complex in that the K-function is evaluated at eight discrete time points and not as a

TABLE 3

AVERAGE INITIATIONS AND AVERAGE TOTAL TIME FOR "SMELL" FROM 14 GROUPS OF CONTROLS

	Initiations		Total Time	
Data Set	Average	SD	Average	SD
1	90.7	30.7	223.1	76.9
2	93.2	25.3	255.6	77.2
3	99.5	22.9	258.0	95.0
4	106.1	21.8	279.4	87.1
5	77.7	23.0	205.4	109.1
6	66.9	14.4	154.9	48.8
7	85.6	24.6	186.2	63.3
8	96.1	23.0	251.2	82.6
9	117.3	21.7	301.0	71.5
10	90.6	28.4	208.9	76.8
11	85.2	21.2	163.6	62.1
12	73.8	26.4	164.4	96.4
13	99.3	20.8	235.7	82.4
14	104.9	16.8	206.9	47.1
Average (±SD)	91.9 (±13.6)	22.9	221.0 (±44.9)	76.9

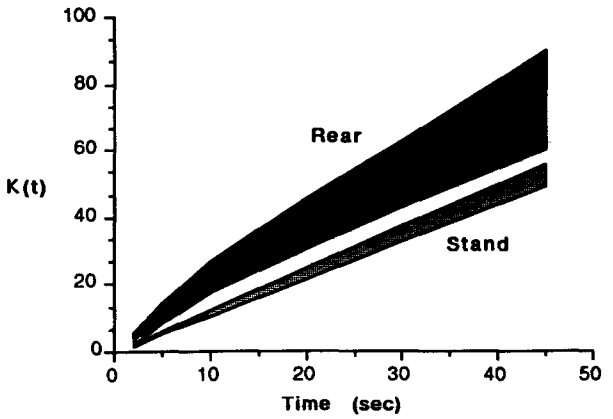

FIG. 1. The range which includes all K-function values for the first six time points for the acts rear and stand from 14 groups of control rats.

continuous function. Moreover, evaluation of a K-function at a 20-second time point, for example, includes all data evaluated at shorter times such as the 10-second time point. The K-function, therefore, is an integrated function, and as such it is a monotonically increasing (or at least a nondecreasing) function.

The stability and reproducibility of the K-function is demonstrated in Fig. 1, which presents the range of K-functions for the first six time points (2, 5, 10, 20, 30, and 45 s) for the acts stand and rear from the 14 control data sets. Although this measure is not evaluated as a continuous function, the graphs are drawn, for convenience sake only, as if the measure was continuous. At any one time point, when larger K values are shown for one act over another, it means that that particular act is more "clustered" in time while the other act is correspondingly more "dispersed" in time. The difference in magnitude for the acts stand and rear in Fig. 1 shows that rear is more clustered in time than is the act stand. The relative independence of the K-function measure from the number of initiations and total time measures is indicated by the fact that the behavior with the largest number of initiations or total time was not necessarily the behavior with the highest range of K-functions. Stand had an average number of initiations per animal of approximately 112

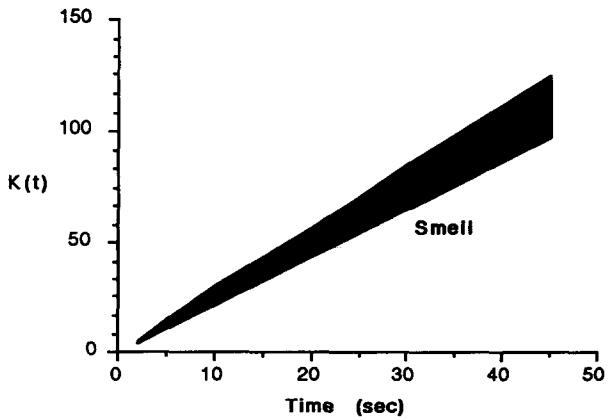

FIG. 2. The range which includes all K-function values for the first six time points for the act smell from 14 groups of control rats.

TABLE 4

REANALYSIS OF DATA SET 10

	Stand		Rear		Smell	
Replication:	1	2	1	2	1	2
Time						
2	0.083	0.082	0.833	0.828	0.232	0.224
5	0.177	0.175	1.645	1.644	0.633	0.611
10	0.368	0.365	2.923	2.990	1.252	1.218
20	0.755	0.745	4.289	4.462	2.249	2.176
30	1.086	1.069	5.490	5.765	3.006	2.892
45	1.595	1.571	7.203	7.665	4.198	4.035
100	2.930	2.838	14.046	15.277	7.088	6.924
200	4.773	4.521	26.683	28.515	10.909	11.062

Bootstrap estimation of standard deviation for eight time points for "stand," "rear," and "smell"

(the range equaled 91 to 138), while rear had an average of approximately 21 (the range equaled 17 to 25). The average total time for stand was approximately 611 (the range equaled 564 to 671), whereas the average total time for rear was approximately 46 (the range equaled 33 to 58). The K-function for the more frequently occurring stand had a smaller area encompassing the results from all 14 data sets. Furthermore, the range of values for the K-function for a given act is appreciably smaller than the range for the number of initiations or total time for the same act. The modifier acts had similar time distributions as is shown for the act smell in Fig. 2.

The stability of the estimate of the standard deviation for the K-function is demonstrated by close examination of results from control data set ten. These data were reanalyzed with a different starting point for the random number generator. Such a reanalysis never changes the value of the K-function at any time point, since that is based upon the actual data from the experiment. However, potential for variation does exist in the estimate of the standard deviation taken from the bootstrap. Replications of the estimates of the standard deviation for the K-function at each of the eight time points for the acts stand, rear, and smell are shown in Table 4. As can be seen in this table, the estimate of the standard deviation for the act stand changed at most by 6%, and for the first six time points the estimate varied by less than 2%. Estimates for the act smell varied by less than 4%. Those for the act rear were most affected, changing at one time point by almost 9%. Overall, however, the estimate of the standard deviation provided by the bootstrap technique was uncertain by only a few percent. Thus the bootstrap provides an adequate estimate of the standard deviation for the K-function measure of spontaneous behavior.

An appreciation of the stability and reproducibility of the K-function values can be gained from review of results shown in Tables 5–8. The K-function values ($\pm$ SD) for the 20-second time point for the acts stand, rear, and smell are listed in Table 5 for all fourteen control data sets. The average of these 14 val-

TABLE 5

K-FUNCTIONS AT THE 20 S TIME POINT FOR "STAND," "REAR," AND "SMELL" FROM 14 GROUPS OF CONTROLS

	Stand		Rear		Smell	
Data Set	K	SD	K	SD	K	SD
1	22.13	0.393	44.08	3.506	56.01	2.981
2	24.46	1.503	39.46	4.054	54.40	2.543
3	22.75	0.438	30.65	2.111	52.45	1.633
4	23.40	0.290	36.26	2.622	50.11	1.443
5	24.77	1.129	45.73	5.451	57.88	2.685
6	22.46	0.686	32.78	2.378	55.76	2.586
7	21.98	0.581	40.06	3.344	54.24	2.668
8	24.94	1.336	37.64	4.985	55.76	2.468
9	22.87	0.534	35.46	2.913	48.57	1.179
10	23.78	0.755	41.03	4.289	53.75	2.249
11	21.80	0.242	32.00	2.091	51.66	1.355
12	22.95	0.497	38.27	3.870	56.78	2.700
13	23.11	0.765	36.10	3.652	50.60	1.200
14	22.20	0.387	31.42	2.279	44.20	0.708
Average	23.11	0.681	37.21	3.396	53.01	2.028
SD	1.03	(0.388)	4.62	(1.065)	3.72	(0.740)

TABLE 6

K-FUNCTIONS AT EIGHT TIME POINTS FOR "STAND" FROM 14 GROUPS OF CONTROLS

	Time Points (s)							
Data Set	≤2	≤5	≤10	≤20	≤30	≤45	≤100	≤200
1	1.85	5.19	10.88	22.13	33.25	49.65	106.7	193.3
2	1.95	5.75	12.05	24.46	36.67	54.68	118.5	217.6
3	1.83	5.28	11.20	22.75	34.13	51.11	111.5	212.7
4	1.93	5.47	11.61	23.40	34.82	51.61	109.3	205.2
5	1.87	5.69	12.17	24.77	37.02	54.60	113.9	203.0
6	1.64	5.23	11.07	22.46	33.72	50.55	111.1	209.4
7	1.68	5.08	10.76	21.98	33.15	49.91	109.6	213.3
8	1.86	5.78	12.24	24.94	37.48	55.63	118.6	219.4
9	1.78	5.22	11.24	22.87	34.40	51.33	110.5	210.3
10	1.80	5.49	11.70	23.78	35.62	53.12	114.1	214.3
11	1.59	4.98	10.69	21.80	32.72	48.84	107.1	209.1
12	1.63	5.21	11.18	22.95	34.32	50.99	108.7	204.0
13	1.74	5.28	11.31	23.11	34.85	52.24	114.2	214.2
14	1.64	5.10	10.96	22.20	33.35	49.67	107.4	208.3
Average	1.77	5.34	11.36	23.11	34.68	51.71	111.5	209.6
SD	0.12	0.26	0.51	1.03	1.51	2.10	3.9	6.7

ues, along with the associated standard deviation, are also shown. The obvious stability is not an artifact of choosing the 20-second time point data for purposes of illustration. On the contrary, stability is reiterated by the data in Table 6 which show the K-function values at all eight time points for the 14 control data sets for the behavior stand. Likewise, the data from the 14 control data sets for all eight time points are given in Tables 7 for the sequence stand . . . walk and Table 8 for the combined act attention.

DISCUSSION

This study of 14 different control data sets demonstrated the stability achieved with initiation, total time and K-function measures of spontaneous behavior. The K-function analysis yielded results which were the most stable and reproducible, a fact readily appreciated when the percent fluctuation in the average result is compared for the initiation, total time and K-function measures. Percent fluctuation is determined from each average result and its associated average standard deviation. For exam-

TABLE 7

K-FUNCTIONS AT EIGHT TIME POINTS FOR THE SEQUENCE "STAND-WALK" FROM 14 GROUPS OF CONTROLS

	Time Points (s)							
Data Set	≤2	≤5	≤10	≤20	≤30	≤45	≤100	≤200
1	2.8	6.9	11.9	22.1	31.7	45.2	87.6	136.6
2	3.2	7.9	13.4	24.8	35.6	50.7	96.7	148.7
3	2.9	7.1	12.3	22.7	32.6	46.5	92.3	148.1
4	3.0	7.4	12.6	23.2	32.8	46.2	87.2	138.1
5	3.2	8.0	13.8	25.9	37.0	51.5	93.8	139.1
6	2.9	7.1	12.3	22.9	32.9	47.3	92.4	144.5
7	2.9	6.8	11.9	21.9	31.6	45.5	89.8	148.0
8	3.3	8.1	14.1	26.3	37.7	53.2	99.7	152.6
9	2.8	7.1	12.2	22.5	32.4	46.4	90.2	147.8
10	3.1	7.4	13.1	24.4	35.2	50.3	97.2	154.8
11	2.7	6.5	11.5	21.5	31.0	44.5	88.2	146.9
12	3.0	7.1	12.6	23.5	33.7	48.0	91.1	141.9
13	2.8	7.0	12.1	22.8	32.8	46.8	90.0	140.1
14	2.7	6.4	11.3	21.0	30.3	43.6	86.9	146.9
Average	3.0	7.2	12.5	23.3	33.4	47.6	91.7	145.3
SD	0.2	0.5	0.8	1.6	2.2	2.8	4.0	5.5

TABLE 8

K-FUNCTIONS AT EIGHT TIME POINTS FOR THE COMBINED ACT "ATTENTION" FOR 14 GROUPS OF CONTROLS

	Time Points (s)							
Data Set	≤2	≤5	≤10	≤20	≤30	≤45	≤100	≤200
1	1.78	5.11	10.85	22.04	32.93	49.10	107.1	205.8
2	1.82	5.50	11.61	23.69	35.44	52.97	115.3	222.2
3	1.64	5.11	11.06	22.63	34.04	50.96	110.8	215.5
4	1.80	5.38	11.43	23.22	34.59	51.35	111.3	211.6
5	1.81	5.52	11.82	24.04	36.09	53.90	114.4	211.9
6	1.59	5.19	11.10	22.46	33.55	50.04	109.1	212.2
7	1.59	5.06	10.78	22.13	33.19	49.90	109.1	213.6
8	1.80	5.59	11.92	24.51	36.77	54.61	116.5	220.9
9	1.66	5.18	11.13	22.67	34.09	51.03	110.0	211.4
10	1.68	5.39	11.58	23.58	35.32	52.68	112.6	211.8
11	1.50	4.90	10.62	21.66	32.71	48.96	107.1	210.2
12	1.66	5.18	11.22	22.82	34.21	50.85	109.2	209.7
13	1.60	5.17	11.11	22.77	34.38	51.55	113.3	220.2
14	1.61	5.06	10.86	22.17	33.27	49.80	108.4	212.5
Average	1.68	5.24	11.22	22.89	34.33	51.26	111.0	213.5
SD	0.10	0.20	0.40	0.82	1.21	1.73	3.0	4.6

ple as shown in Table 1, initiations of stand fluctuated by 25.4% (26.7/105.0 × 100) across the 14 data sets, and stand total times fluctuated by 13% (79.6/611.4 × 100). Using the 20-second time point as an example (see Table 5), K-functions for stand fluctuated by only 2.9% (0.681/23.11 × 100). For the behavior rear (see Tables 2 and 5, respectively), the fluctuation was 43.9% (9.0/20.5 × 100) in the initiation measure, 50.4% (23.4/46.4 × 100) in the total time measure, and 9.1% (3.396/37.21 × 100) in the K-function. For the behavior smell (see Tables 3 and 5, respectively) initiations, total times and K-functions across the 14 data sets fluctuated by 24.9% (22.9/91.9 × 100), 34.8% (76.9/221.0 × 100), and 3.8% (2.028/53.01 × 100), respectively. The K-function analysis obviously provided the most stable result, and in turn the best potential for low-level sensitivity.

Although the K-function is more stable than either the initiation or total time measures of spontaneous behavior, investigators are unaccustomed to presentations of data for functions. When presented, K-function data are usually in a form similar to that in Table 9, which shows an example of K-functions for the time sequence stand . . . walk affected by 0.5 mg/kg amphetamine in rats (11). First, K-functions for the control and experimental animals for all eight time points are listed. Second, the difference at each time point (control value minus the exposed value) is given along with the bootstrap estimate of the standard deviation in this difference. Finally, the ratio between the difference and the estimated standard deviation in this difference is shown so that the significance of the difference can be appreciated. As in this example, a significant change in time structure of behavior occurs when the ratio exceeds 2.0 at three or more adjacent time points all with the same positive or negative sign, criteria established in a prior ad hoc test (8, 10, 11). Significance of the effect is emphasized when the K-function values for an experimental group fall outside of a range set by multiple control data sets. The K-function values for the amphetamine-treated rats in this example were lower than any of those found in the 14 control data sets for the equivalent time points through 45 seconds. The stability which is achieved with the K-function is an improvement much needed to create confidence in results generated with measures of spontaneous behavior.

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TABLE 9

K-FUNCTIONS AT EIGHT TIME POINTS FOR THE SEQUENCE "STAND-WALK" FROM CONTROLS AND AMPHETAMINE-TREATED (0.5 mg/kg) RATS

	Time Points (s)							
	≤2	≤5	≤10	≤20	≤30	≤45	≤100	≤200
Control	2.7	6.5	11.5	21.5	31.0	44.5	88.2	146.9
Exposed	2.4	6.1	10.7	20.3	29.6	43.1	88.0	151.7
Difference	0.255	0.412	0.765	1.216	1.363	1.334	0.243	−4.796
SD of Difference	0.079	0.155	0.269	0.398	0.534	0.746	1.444	2.835
Difference/SD	3.23	2.66	2.84	3.06	2.55	1.79	0.17	−1.69

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REFERENCES

1. Diaconis, P.; Efron, B. Computer intensive methods in statistics. Sci. Am. 248:116–130; 1983.
2. Diggle, P. J. Statistical analysis of spatial point patterns. New York: Academic Press; 1983.
3. Efron, B. Bootstrap methods: Another look at the jackknife. Ann. Stat. 7:1–26; 1979.
4. Efron, B. Computers and the theory of statistics: Thinking the unthinkable. Soc. Indust. Appl. Math. Rev. 21:460–480; 1979.
5. Efron, B. The jackknife, the bootstrap and other resampling plans. Philadelphia: Soc. Indust. Appl. Math., Monograph #38; 1982.
6. Freedman, D. A.; Peters, S. C. Bootstrapping a regression equation: Some empirical results. J. Am. Stat. Assoc. 79:97–106; 1984.
7. Kernan, W. J.; Mullenix, P. J.; Hopper, D. L. Pattern recognition of rat behavior. Pharmacol. Biochem. Behav. 27:557–564; 1987.
8. Kernan, W. J.; Mullenix, P. J.; Kent, R.; Hopper, D. L.; Cressie, N. A. C. Analysis of the time distribution and time sequence of behavioral acts. Int. J. Neurosci. 43:35–51; 1988.
9. Kernan, W. J.; Mullenix, P. J.; Hopper, D. L. Time structure analysis of behavioral acts using a computer pattern recognition system. Pharmacol. Biochem. Behav. 34:863–869; 1989.
10. Mullenix, P. J.; Kernan, W. J. Extension of the analysis of the time structure of behavioral acts. Int. J. Neurosci. 44:251–262; 1989.
11. Mullenix, P. J.; Kernan, W. J.; Tassinari, M. S.; Schunior, A. Generation of dose-response data using activity measures. J. Am. Coll. Toxicol. 8:185–197; 1989.
12. Mullenix, P. J.; Tassinari, M. S.; Schunior, A.; Kernan, W. J. No change in spontaneous behavior of rats after acute oral doses of aspartame, phenylalanine and tyrosine. Fundam. Appl. Toxicol. 16: 495–505; 1991.
13. Mullenix, P. J.; Kernan, W. J.; Tassinari, M. S.; Schunior, A.; Waber, D. P.; Howes, A.; Tarbell, N. J. An animal model to study toxicity of CNS therapy for childhood acute lymphoblastic leukemia. Effects on behavior. Cancer Res. 50:6461–6465; 1990.
14. Norton, S. Amphetamine as a model for hyperactivity in the rat. Physiol. Behav. 11:181–186; 1973.
15. Norton, S.; Mullenix, P. J.; Culver, B. Comparison of the structure of hyperactive behavior in rats after brain damage from x-irradiation, carbon monoxide and pallidal lesions. Brain Res. 116:49–67; 1976.
16. Ripley, B. D. The second-order analysis of stationary point processes. J. Appl. Probabil. 13:255–266; 1976.
17. Ripley, B. D. Spatial statistics. New York: John Wiley and Sons; 1981.