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An Assessment of Spectral Analysis of Amphetamine-Induced Behavior[1]

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ELLINWOOD, E. H., JR., D. W. MOLTER AND K. A. STAUDERMAN. *An assessment of spectral analysis of amphetamine-induced behavior.* PHARMAC. BIOCHEM. BEHAV. 15(4) 627–631, 1981.—Testing of a new radio frequency capacitance field type transducer and power spectrum analysis system for assessment of rat behavior is described. Power spectrum estimates of amphetamine-induced behavior had an orderly relationship with behavior ratings ranging from inactive to intense stereotypy. The effects of thorazine dose-response blocking on amphetamine-induced behavior were linear. Separation between adjacent doses could not be accomplished with a single frequency, but required differential frequency-time period information.

Spectral analysis Amphetamine behavior Capacitance transducer Chlorpromazine dose-response

PSYCHOACTIVE drugs produce a variety of behavioral changes; yet current automatic activity monitors have quite limited capacity to differentiate them. Current models of activity monitors based on photocell crossing, ultrasonic or capacitance fields, and stabilometers do not adequately distinguish different behaviors, such as locomotion and stereotypy (see [4] for review). Wolthuis, *et al*, [6] using a vertical capacitance field transducer similar to that used by Lát [2], differentiated between amplitudes of rat cage rearings, and were able to demonstrate significant differences between the effects of saline and dextroamphetamine on a measure described as stereotypy. More recently, Marsden and King [3], using a Doppler shift radar, reported that the number of initiations of high velocity and low velocity movements easily distinguished between saline and 2.5 mg/kg of amphetamine, and that behavioral observations indicated that the decrease in stops and initiation of movement were associated with an increase in stereotypy. Despite these technical advances, there have been no reports of instruments that are capable of defining a complex behavioral continuum such as that induced by behavioral stimulants or the dose-response inhibition of these effects by a neuroleptic. For example, thus far only human behavioral raters have been able to differentiate between levels of intensity of behavioral stereotypy. In this report, we will describe a method of data collection and a means of analysis that provide for a moderately effective dose-response curve of chlorpromazine in blocking the effects of a stereotyping dose of dextroamphetamine. In our assessment of the method, we examine aspects of (1) validity, (2) dose-response range, and (3) sensitivity.

METHOD

Male Sprague-Dawley rats used in these experiments weighed approximately 280 g and were individually housed under a 12-hour light/dark cycle. Two groups of animals (N=48, each group) were run in a dose-response study for chlorpromazine inhibition of amphetamine (6 mg/kg) induced behavior. Chlorpromazine, 0.0, 1.5, 3.0, 6.0, and 12.0 mg/kg were administered to the rats one hour prior to the amphetamine injection and testing. Two dose-response studies were accomplished, the second study run 4 months after the first. Groups were counterbalanced over days of testing, times of day, and experimental chambers. Movement was sampled in the transducer for 40, one-second intervals at each period of testing; testing included one pre-injection period and 0, 5, 10, 15, 20, 25, 30, 35, 40, 50, and 60-minute postamphetamine injection. Basic data points reported here were derived from an average of 40 samples for each frequency for each period tested.

Movement was measured in a radio frequency capacitance field type transducer and analysis technique designed in our laboratory. The radio frequency field is generated between two copper plates, 8×12 inches, which reside within a 16×16×16-inch Faraday cage. Rats are placed in a Plexiglas cage 7½ in. long ×3½ in. wide; the cage is affixed to a set of Plexiglas crossbars between the two plates. Movements within the radio frequency field between the two plates are transduced into analog signals that are amplified and monitored on a Grass model 78 polygraph. The 7p511 amplifier's low frequency filter was set at ½ amplitude for 1 Hz in order to partially filter out high voltage slow components that have previously been shown to be non-contributory to the data analysis for amphetamine stereotypy. The signal was subsequently processed through an analog to digital conversion (128/second) and Fast Fourier transform on a Digital Equipment PDP 11-34 computer to provide a spectral estimate of the movement frequency sampled over bands of

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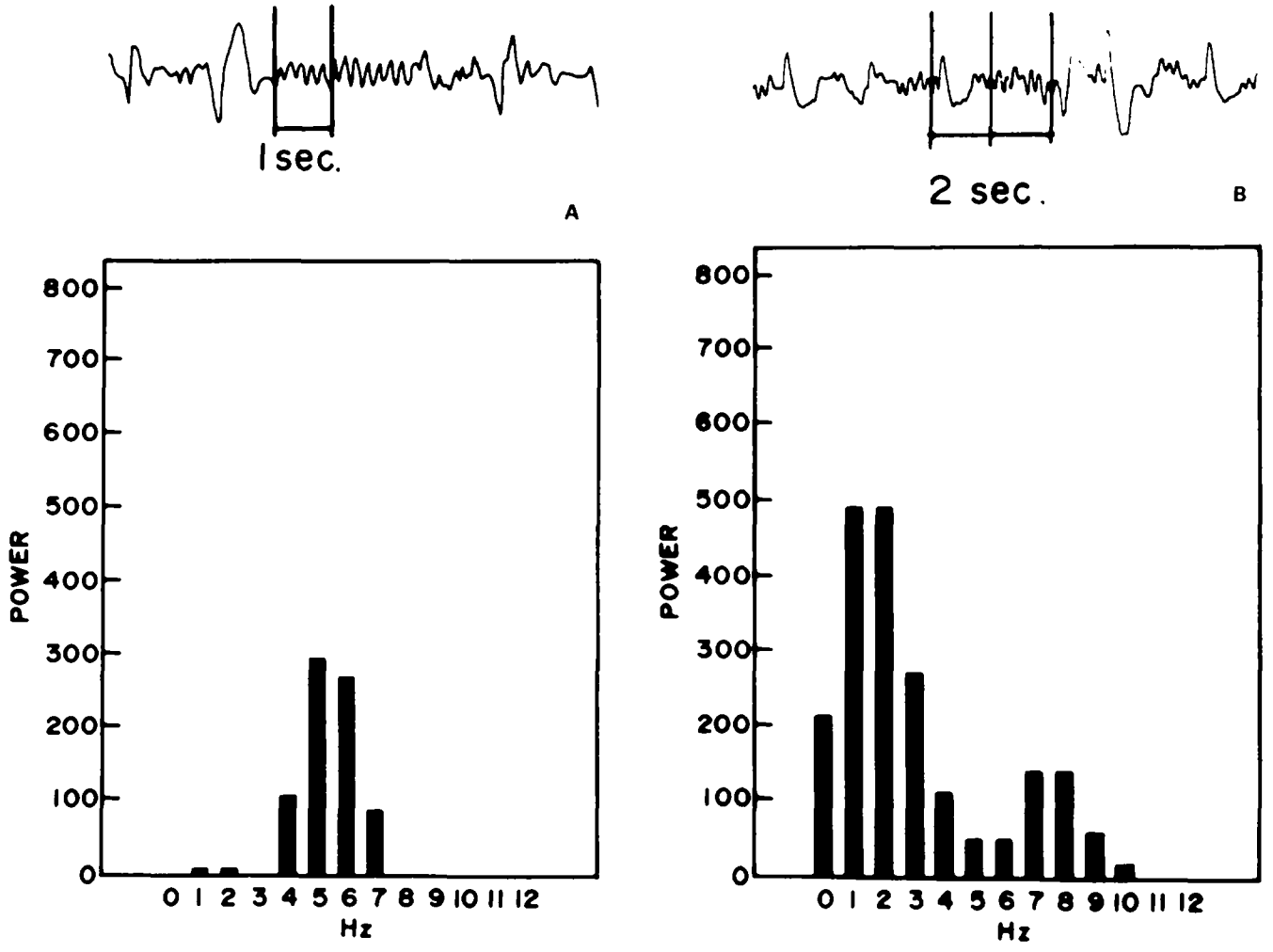


FIG. 1. A. Normal sniffing. B. Head swing and sniffing stereotypy.

1 to 20 Hz. Only 1 to 10 Hz data are reported, as higher frequencies had very low power. Prior to each experimental day's run, each transducer was calibrated using a saline-filled 30 ml glass bottle pendulum, set to the same arc and excursion. When necessary, sensitivity was adjusted to produce the same standard deflections on the polygraph.

For data analysis, time periods were reduced to three epochs: 0–20 minutes, 20–40 minutes, and 40–60 minutes. The frequencies representing behavior were reduced to 2, 6, and 10 Hz. These frequencies had been noted in previous studes to be relatively independent of each other and related to specific behaviors of interest. The slope and mean intercept for each epoch for each frequency were assessed by growth curve analysis [1] using Statistical Analysis System (SAS-79).

Behavior was rated by a trained technician at each 40-second period in order to make comparisons to the spectral data. A BMDP-77 discriminant function analysis was used to predict ratings from spectral data. Certain aspects of amphetamine-induced behaviors, e.g. locomotion, could not be expressed in the smaller cages used for this experiment, so our behavior rating was modified slightly to accommodate for these changes. The following behavior ratings were scored: (1) Inactive: eyes open, lying or sitting still; very little or no movement; (2) Slow Active: slow periodic sniffing with little or no interim movement; infrequent, slow, lethargic turns; (3) Hyperactive: faster, non-stereotyped sniffing (possibly with much turning, but might be stationary); brief periods of stereotyped activity with intermittent pauses and/or fast grooming or other activity; (4) Slow Stereotypy: broad, slow stereotypy with head swing and some turning; broad S-shaped body swings; (5) Fast Stereotypy: fast, constant stereotyped activity with moderate to large head swing, prominent sniffing, and some turning; (6) Faster Stereotypy: slightly faster (than 5) stereotypy (no turns) with moderate or restricted head swing.

For comparisons of spectral data to more discrete behaviors such as sniffing, licking and various forms of stereotypy, we examined behavior on a second by second basis using a variety of techniques including timed television recordings of behavior and coded pen recordings (representing behavior) superimposed on the polygraph output from the motility chamber. Twenty to thirty episodes of a discrete behavior were compared to their associated one-second sample of analog and spectral data.

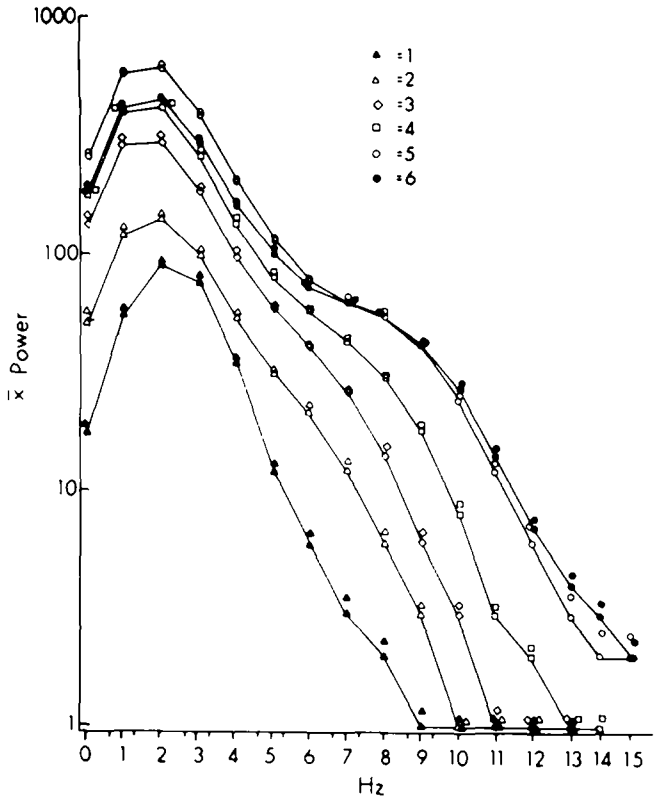


FIG. 2. Power spectrum profiles for behavior ratings in chlorpromazine dose-response study.

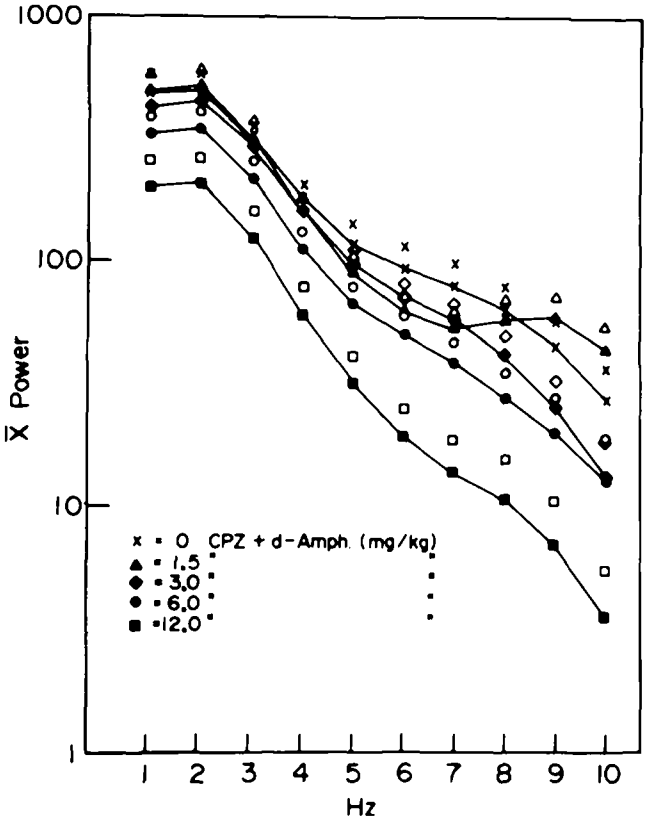


FIG. 3. Power spectrum for CPZ inhibition at 35 min postinjection of amphetamine 6 mg/kg.

RESULTS

Second by second comparison of the behavior description with the power spectrum of the transducer analog output revealed several interesting features of normal behavior and amphetamine-induced behavior. In Fig. 1A, normal sniffing is manifested by a remarkable sinusoidal 5–6 Hz rhythm. Observation across one-second intervals of sniffing reveals that the frequency of this movement was in a reasonably tight band between 5 and 6 Hz. Observation of other behaviors demonstrates licking to be associated with a periodicity of 4, scratching with 10–12, and head swings with 2 per second. Cages were specifically designed to restrict turning and larger body movements (by narrowing the cage width). These movements, although restricted, did periodically occur and resulted in a signal of high voltage admixture of slow and sharp waves. These episodes contributed considerable variance to the data.

The movements of most current experimental interest are the snout, head and neck movements representing exploratory activity and stereotypy induced by dopamine agonists. Amphetamine stereotypy does induce frequency changes in the specific motor acts. As can be noted in Fig. 1B, the sniffing head movement shows both low frequency head-neck movements and a high frequency sniffing snout movement. With the more intense, faster stereotypies, the intrinsic sniffing frequency shifts to a 9–10 Hz. Although the individual signals from a one-second epoch can represent very specific behaviors, the summed average over 40 seconds includes all activity, gross as well as fine motor.

Validation of the frequency changes as a function of behavioral changes was examined in the first study of the dose-response effect of chlorpromazine on amphetamine stereotypy. Figure 2 demonstrates the relationship of frequency changes to a behavior rating accomplished at the time of each 40-second sample. The data represent 561 behavioral observations and associated power spectrums. The faster frequencies show a progressive increase with increasing rating score. The lower frequencies, for example 2 Hz, have an orderly progression up to the 6 level rating, where power drops back. This decrease in 2 Hz power represents the more restricted head swing that is associated with the fastest, most intense sniffing. The combination of these two frequencies, i.e., a decreased 2 Hz and an increased 10 Hz, often discriminates between the rating of 5 and 6 better than does either frequency alone. Understanding the nature of this downward shift in the 2 Hz frequency is important in assessing the dose-response relationships. In order to further test validity, 75% of the data were used as a model set to develop discriminant functions that were subsequently tested on the remaining set. The discriminant functions correctly identified the following: (1) 66% of the human behavior rating, (2) 85% of either the designated rating or an adjacent rating, (3) 83% when stereotypy ratings, i.e., 4, 5, and 6, were lumped and treated as a single group, and (4) 96% of any ratings of stereotypy as differentiated from all other behaviors when 4, 5, and 6 were lumped. Thus, the spectral classification of behavior has a very reasonable range and accuracy in identifying behavior ratings in our scale if the intensity of stereotypy is not considered, i.e., there is an 83%

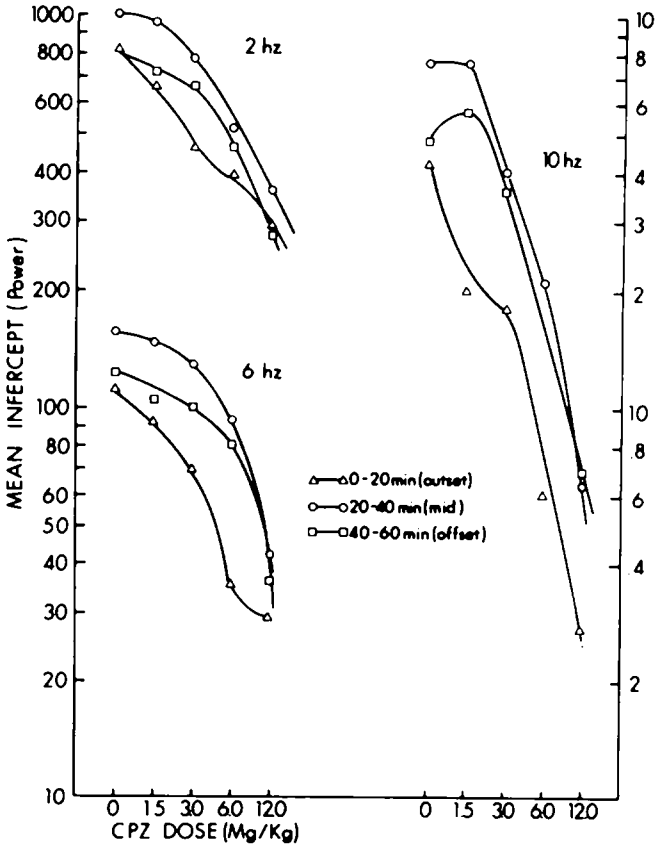


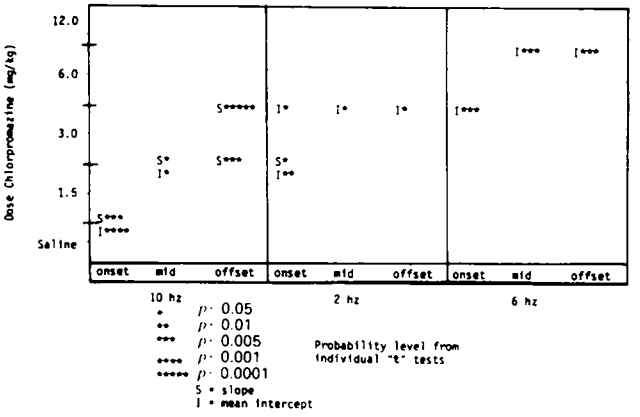
FIG. 4. Chlorpromazine dose-response inhibition of 2, 6, 10 Hz activity for different epochs.

identification of behaviors (1) Inactive, (2) Slow Active, (3) Hyperactive, and (4) Stereotypy.

Chlorpromazine pretreatment dose-response effects on amphetamine-induced frequency changes 35 minutes post-injection of amphetamine are shown in Fig. 3. There appears to be a reasonably linear relationship across doses, with inhibition of power in all frequencies except for the lowest dose, 1.5 mg/kg. At the 1.5 mg/kg dose, there is a very characteristic potentiation of higher frequencies that is associated with more intense stereotypy. Since this potentiation of the higher frequencies was *not* noted in the onset epoch, but was even more pronounced in the offset epoch, one could question whether this low dose potentiation at 35 minutes was secondary to chlorpromazine's presynaptic activation of dopamine turnover. In order to examine whether the potentiation was a spurious result of this particular experiment, we ran another chlorpromazine dose-response study five months later. The results of these two studies were combined in order to increase our N sufficiently to assess the multiple variables by use of discriminant function analysis and multivariate growth curve analysis.

In order to pare down further the variables used for analysis, we selected three frequencies at three epochs. Frequencies 2, 6, and 10 Hz had been shown by previous analysis [5] to be independent of each other. They also represent the slow and fast head movements associated with stereotypy (2 Hz and 10 Hz) as well as the exploratory sniffing behaviors represented by 6 Hz. The time course was

TABLE 1



reduced to three epochs: 0–20 minutes (onset), 20–40 minutes (mid or peak effect period), and 40–60 minutes (offset). Growth curve analysis [1] was used to assess these three periods. Variables examined were the slope and mean intercept for each epoch for each of the three frequencies. In Fig. 4, the mean intercept for each Hz at each epoch is shown. These dose-response curves are all significant within each epoch and frequency combination ($p<0.0001$, Likelihood Ratio Criteria). Using a BMDP stepwise discriminant function analysis with a jackknife procedure, individual rats could be sorted into correct dose groups 48% of the time, where 20% would have been the random chance level.

Because multivariate analyses, i.e., Roy's largest root, Likelihood Ratio Criteria, and discriminant function analysis, had all shown highly significant dose-response effects for the time period frequency analysis, we proceeded to examine the individual frequency-epochs for significant differences between adjacent doses. One would expect that certain time frequency-epochs would be more predictive of high dose differences and others more predictive of low dose differences. Table 1 outlines the frequency-epoch, slope, and intercepts that were significant between adjacent doses. These comparisons have been provided for descriptive purposes only. The frequency-epoch differences between adjacent doses must be considered in light of multiple t-test analysis. However, reasonable significance ($p<0.005$ or less) is found for at least one variable for all adjacent dose comparisons. Ten Hz during the onset epoch is reduced by even the 1.5 mg/kg dose of Thorazine. Other frequency-epochs are more predictive of differences between adjacent dose levels, although 2 Hz seems to contribute little beyond that for 6 and 10 Hz alone.

In Fig. 4, a tendency toward potentiation of high frequencies that was noted in the original examination of the 1.5 dose Thorazine is found in the offset epoch where one might expect a potentiated dopamine turnover to be reflected. In contrast, the onset effect of 1.5 mg/kg chlorpromazine dose effect shows considerable inhibition of 10 Hz in keeping with an initial postsynaptic blockade. The lack of 10 Hz inhibition during the offset epoch for the 1.5 mg/kg dose may represent the level providing for blockade of amphetamine's inhibition of dopamine turnover, allowing the releasing properties of amphetamine to act longer at this level, and overcoming a

relatively weak chlorpromazine postsynaptic blockade. The difference between onset effects and offset effects may provide an estimate of the relative presynaptic and postsynaptic neuroleptic effects, but this requires further study.

DISCUSSION

The dose-response curves do demonstrate that the spectral analysis method of measuring motility is capable of providing for considerable sensitivity over a wide range of behavioral alterations. The dose-response levels reflect behaviors ranging from the most intense stereotypy to behavioral inactivation. Although the dose-response effect is relatively robust, distinguishing between adjacent dose levels will require further analysis of the individual frequency time variable. Judging from the data found with chlorpromazine, it would seem possible, if armed with knowledge of the dose-response curve for a given neuroleptic, to design experiments that would reflect more sensitive changes of behavior.

The generalizability of the spectral method to the assessment of behaviors induced by other drugs has yet to be completely explored. However, apomorphine, methylphenidate, cocaine, and physostigmine, as a few examples, show dose-response effects (unpublished results). The method, in its current developmental stage, still has problems, including (1) the need to reduce the variability induced by turning in the cages; (2) the need to reduce the computation time; and (3) the lack of any precise, moment to moment behavioral descriptors. Current development of the system includes changes in equipment design as well as alternative assessment of procedures, including pattern recognition.

In neuropharmacology, there is considerable need for sensitive, yet reliable behavioral methods that are capable of responding to the dose-response range of various neurobiological variables. The method described above shows promise as one method that answers those needs.

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