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Quantitative Analysis of Physostigmine-Induced Changes in Behavior[1]

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GONZALEZ, L. P. *Quantitative analysis of physostigmine-induced changes in behavior.* PHARMACOL BIOCHEM BEHAV **21**(4) 551–554, 1984.—This paper reports the use of an RF capacitance field transducer and spectral analysis to examine the effects of the cholinesterase inhibitor physostigmine on the behavior of unrestrained rats. The technique is shown to permit simultaneous quantification of changes in a variety of motor behaviors. Physostigmine induced a dose-related increase in high-frequency movements and reduced low-frequency movements, but had no effect upon habituation of five to ten Hz movements which occurred with continuous exposure to the testing apparatus.

Physostigmine Cholinergic Tremor Locomotor activity Exploratory behavior Habituation Motility Spectral analysis

CHOLINOMIMETICS induce high frequency muscle tremor and a decrease in gross locomotor activity [1, 8, 14, 22] which can be blocked by the administration of muscarinic cholinergic receptor blockers [22]. The degree of tremor induced by such pharmacological manipulations is typically estimated either by observational ratings of tremor intensity [1, 15, 22], by attaching various types of strain gauges or pressure transducers to the animal [3, 17, 21], or by direct recording of EMG activity [16]. Observational techniques are of necessity limited in their sensitivity to fine differences in tremor intensity, and EMG recordings do not distinguish between complex behavioral patterns involving many independent muscle groups. Other techniques require various degrees of behavioral restraint [3, 17, 21] and thus may interfere with the measurement of other behavioral variables.

Although a variety of automatic activity monitors are commercially available for the quantification of motor activity, these devices are limited in their ability to differentiate various types of motor behavior (see [20] for review). Most capacitance-field devices, stabilimeters, and photocell crossing devices will not distinguish between such behaviors as head-bobbing stereotypy and gross locomotor activity, and few such devices can measure the fine movements associated with muscle tremor. Recently, however, the use of a radio frequency (RF) capacitance field device has been reported [7, 11, 12] for the quantification of amphetamine-induced behaviors in rats. This device is capable of distinguishing not only between gross locomotor activity and stereotyped movements, but is also able to distinguish between such similar behaviors as normal and stereotyped sniffing, and is sensitive enough to monitor respiratory movements and muscle tremor as well. These distinctions are made possible by examining the frequency components of which complex behaviors are composed.

The research reported in this paper extends the application of the spectral analysis of motility data to the quantification of effects on a wide range of motor behaviors produced by systemic administration of physostigmine.

METHOD

Subjects

The subjects for these experiments were 48 male, Sprague-Dawley rats, 60 to 90 days old, and weighing 200 to 250 g. Animals were housed in individual cages with free access to food and water, and were maintained for at least seven days under the same conditions of environment, diet, and daily handling before any experimental treatment.

Apparatus

Measurements of motor behavior were performed in a Stoelting activity monitor, modified to permit the quantification of repetitive behaviors [7]. The motility monitor consists of a pair of parallel copper plates (20.0×30.0 cm) connected to a Stoelting movement sensoring module. The plates are housed in a 40.0×40.0×40.0 cm Faraday cage to eliminate the influence of external electrical fields. An animal is placed in the center of a radio frequency capacitance field generated between the plates such that the movement of the animal

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disrupts the field. Based upon this disruption, the motility monitor produces an analog signal with a frequency of oscillation equal to the frequency of occurrence of movements within the field of the monitor. This signal is then amplified with a Grass 7P511 amplifier. In order to record the low amplitude, high frequency tremor induced by physostigmine, the required amplification used in this study was approximately six times higher than that used previously to monitor stimulant-induced motility [7, 11, 12]; and, the low-frequency filter of the amplifier was set with a 1/2 amplitude cutoff at three Hertz (Hz) to reduce the high amplitude contribution of large whole-body movements.

Quantification of movement was accomplished by spectral analysis of the frequency components of the amplified analog output of the motility monitor. The resulting amplitude-frequency distribution has been shown [7] to accurately depict the occurrence of specific repetitive movements (sniffing, licking, head bobbing, etc.).

For the measurement of motility, animals were placed in Plexiglas chambers, 19.0×13.0×8.0 cm, which were then positioned within the movement sensor of the motility monitor. Analog-to-digital conversion of the transduced signal was performed with a Rockwell AIM-65 microcomputer and subsequent analyses were performed on a DEC PDP-11/34 mini-computer.

Procedures

Subjects were randomly divided into one of four treatment groups. Animals were placed within the motility monitor and a 40-second sample of motility was obtained. Animals then received intraperitoneal injections of either saline, 0.15 mg/kg, 0.30 mg/kg, or 0.60 mg/kg physostigmine salicylate, as appropriate to the group designation of a subject. Motility was again monitored for 40-second periods, ten and 20 minutes after injection.

A Fast Fourier Transform (FFT) was used to obtain power spectra for each one-second segment of motility data, and the spectra were averaged across the 40 seconds of each sampling period. Following a log transformation of the mean power spectra, an analysis of variance with repeated measures was used to determine the significance of group differences at the various sampling periods and at various movement frequencies, with Duncan's multiple range test used for individual post-hoc group comparisons.

RESULTS

Analysis of variance with repeated measures indicated that each of the main effects were significant (drug, $F(3,44)=3.08$, $p<0.05$; time, $F(2,88)=15.9$, $p<0.0001$; and movement frequency, $F(29,1276)=1707.14$, $p<0.0001$). Interactions between drug group and movement frequency ($F(87,1276)=1.71$, $p<0.0001$), time and frequency ($F(58,2552)=16.41$, $p<0.0001$), and group by time by frequency ($F(174,2552)=3.87$, $p<0.0001$) were also significant.

Post-hoc between-group comparisons, collapsed across time period and movement frequency, indicated that saline animals did not differ significantly ($p>0.05$) from animals receiving 0.15 mg/kg physostigmine, but both of these groups were significantly different ($p<0.05$) from animals receiving either 0.3 or 0.6 mg/kg physostigmine, and animals receiving 0.3 mg/kg physostigmine were also significantly different ($p<0.05$) from those receiving 0.6 mg/kg physostigmine.

Comparisons of the significance of changes in log power from pre-injection to 20 min post-injection were made at each movement frequency for each drug group, using Duncan's multiple range test. Saline-injected animals (Figs. 1 and 5) exhibited a significant decrease in movement frequencies between 5 and 15 Hz ($p<0.001$). Each of the other groups (Figs. 2–5) also showed decreases in approximately this same frequency range. These decreases in log power were significant at frequencies between 5 and 12 Hz in animals receiving 0.15 mg/kg physostigmine, and between 5 and 10 Hz in animals receiving either 0.3 mg/kg or 0.6 mg/kg physostigmine.

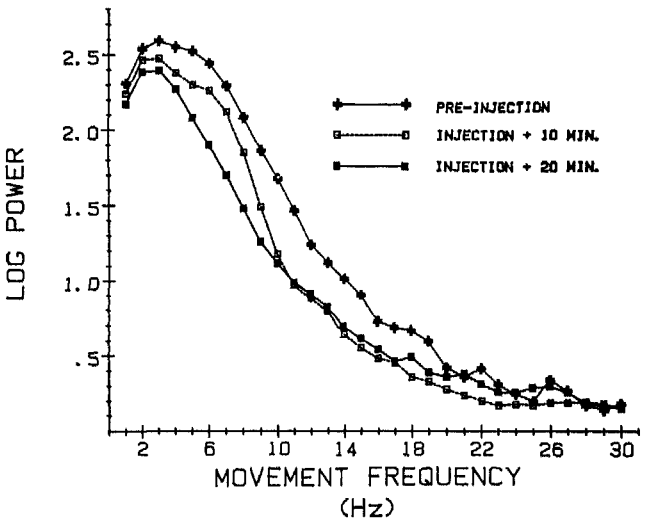

FIG. 1. Motility following saline treatment. The mean power spectra are shown for subjects receiving a systemic saline injection (1 cc/kg). Spectra were computed from data obtained before, 10 minutes after, and twenty minutes after injection. In this figure as in the three which follow (Figs. 2–4), arbitrary log power units are represented on the Y-axis and movement frequency on the X-axis; the standard error of the means presented in these figures ranged from ±0.04 to ±0.19.

Animals receiving 0.6 mg/kg physostigmine also showed significantly ($p<0.001$) less activity in the 1 to 4 Hz range as well. Although movements in this frequency range tended to decrease with time in the other groups, this effect was only significant in the animals receiving the highest dose of physostigmine (0.6 mg/kg).

In addition, subjects receiving physostigmine in doses of 0.3 mg/kg or 0.6 mg/kg exhibited significantly ($p<0.01$) more high frequency movements (>18 Hz) at the 20 min observation period, when compared to pre-injection measurements or to saline controls.

DISCUSSION

The data reported here demonstrate that spectral analysis of motility is a useful method by which to quantify the effects of physostigmine on motor behavior. Physostigmine produced a dose-related increase in high-frequency (>18 Hz) movement, indicative of the muscle tremor induced by this drug; and, it produced a decrease in the low-frequency (<5 Hz) movements which have previously been suggested to reflect gross whole-body movements [7].

An additional observation is that all of the groups examined showed significant decreases in 5 to 10 Hz movements with time. These movements frequencies have previously been suggested to reflect the occurrance of sniffing, grooming, and exploratory behaviors [7]. Spectral analysis of motility thus also provides a method by which to quantify the

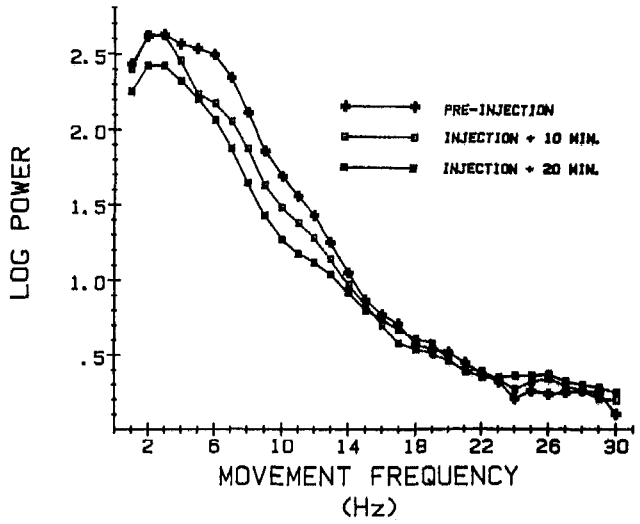

FIG. 2. Motility following treatment with 0.15 mg/kg physostigmine. The mean power spectra are shown for subjects receiving a systemic physostigmine (0.15 mg/kg) injection. Spectra were computed from data obtained before, 10 minutes after, and twenty minutes after injection.

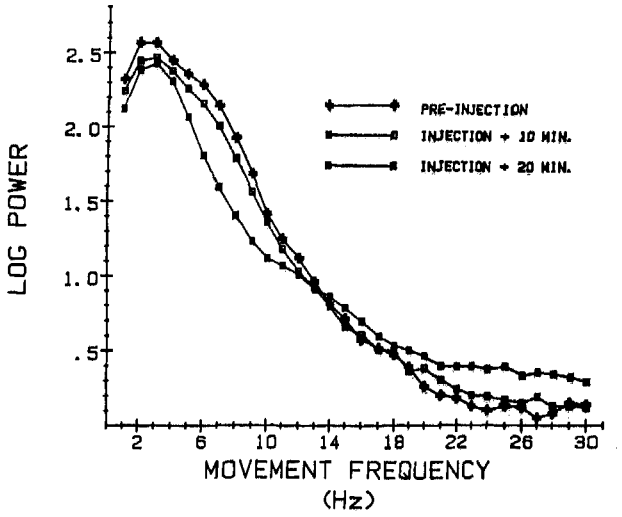

FIG. 3. Motility following treatment with 0.30 mg/kg physostigmine. The mean power spectra are shown for subjects receiving a systemic physostigmine (0.30 mg/kg) injection. Spectra were computed from data obtained before, 10 minutes after, and twenty minutes after injection.

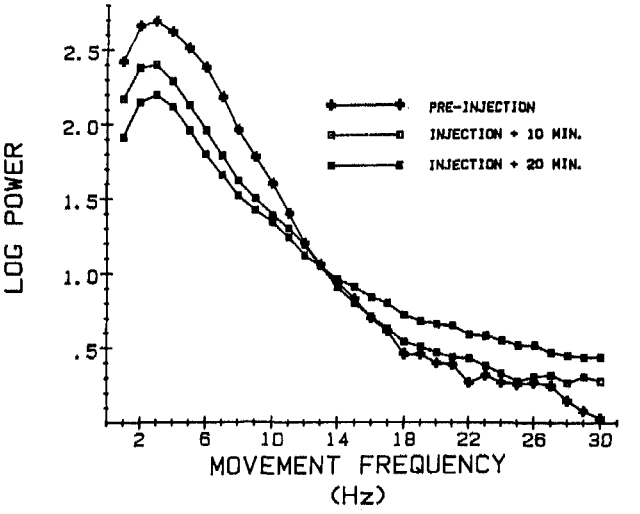

FIG. 4. Motility following treatment with 0.60 mg/kg physostigmine. The mean power spectra are shown for subjects receiving a systemic physostigmine (0.60 mg/kg) injection. Spectra were computed from data obtained before, 10 minutes after, and twenty minutes after injection.

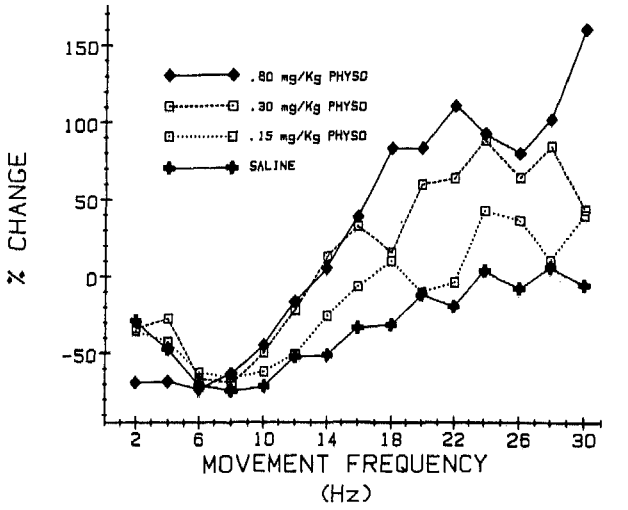

FIG. 5. Effects of saline or physostigmine on the percent change in power from pre-injection to 20 min post-injection at various movement frequencies.

habituation of motor behaviors occurring upon exposure to a novel environment.

Interestingly, physostigmine did not alter the habituation of these moderate-frequency behaviors during the period of observation in this study. Carlton [4,5] has suggested that cholinergic systems may be involved in the suppression of non-reinforced behavioral responses. While cholinergic drugs have sometimes been reported to alter response habituation [2, 4, 6, 9], others have observed no such effects [6, 13, 19, 23, 24] and cholinergic agonists and antagonists do not appear to alter general mechanisms of response suppression [6, 10, 18]. According to the hypothesis of cholinergic mediation of response suppression, the cholinomimetic physostigmine would be expected to facilitate habituation. This result was not obtained in the present study.

This report represents an additional demonstration of the use of the spectral analysis of motility in a neuropharmacological investigation, and extends the application of this technique to the measurement of response habituation. The major advantage of this technique lies in the ability of this procedure to quantitatively evaluate experimental effects simultaneously on multiple behaviors. In the present study, physostigmine induced high-frequency movements and reduced low-frequency activity, but had no effect upon the habituation of moderate frequency movements.

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