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A Frequency Analysis of Behavior Components of the Serotonin Syndrome Produced by p-Chloroamphetamine

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KUTSCHER, C. L. AND B. K. YAMAMOTO. *A frequency analysis of behavior components of the serotonin syndrome produced by p-chloroamphetamine*. PHARMAC. BIOCHEM. BEHAV. 11(6) 611–616, 1979.—A time-sampling frequency analysis was made of criterion behaviors following injection of 2.5–10 mg/kg dosages of p-chloroamphetamine (PCA). Stereotypic behaviors (forepaw treading, circling, head weaving and inching) increased with increasing dosages and normal behaviors (grooming, rearing, and instances of inactivity) decreased. Composite scores of stereotypic behavior were a positive, linear function of PCA dosage. Composite scores of normal behavior showed near maximal inhibition at 5 mg/kg. Splayed hindlimbs is a reliable and sensitive indicator of PCA action, but vocalization, tremors, diarrhea and autonomic signs are not. Preinjection of PCA strongly attenuated the PCA-induced syndrome, as expected, since the preinjection should deplete brain serotonin and reduce the amount released by the second PCA injection.

p-Chloroamphetamine (PCA) Halogenated amphetamine Neurotransmitters Serotonin Stereotypy

BEHAVIORAL stereotypy seen following amphetamine injection includes an increase in general activity, rearing and various types of oral activities—biting, gnawing and licking [11]. These behaviors are believed to be mediated by catecholaminergic neurons. Halogenation of the amphetamine molecule produces a different behavioral syndrome which is apparently mediated by serotonergic neurons [5,12]. Following injection of fenfluramine or p-chloroamphetamine (PCA) an increase in general activity is produced [7] along with splayed hindlimbs, tremor, side-to-side head weaving, lateral forepaw treading, Straub (kink) tail, rigidity of hindlimbs, hyperreactivity and autonomic signs, e.g. salivation, piloerection and ejaculation [1, 2, 5]. The evidence for serotonergic mediation is so strong that this syndrome has been designated the serotonin syndrome and has been proposed as a model for the study of serotonergic mechanisms [5,14].

PCA has both an immediate and a long-term effect on serotonergic neurons. Immediately after injection there is a release of this transmitter accompanied by a decrease in reuptake [9] and an inhibition of monoamine oxidase [3]. As a result, serotonergic receptors are intensely stimulated and the serotonin syndrome behaviors are produced. Over a longer time period, PCA depletes brain levels of serotonin. PCA causes reduced synthesis of tryptophan hydroxylase [8], thus slowing serotonin synthesis. Secondly, PCA has a neurotoxic action on the raphe nucleus [4], a major source of brain serotonin. A single injection of PCA may depress brain serotonin levels for 4 months [10].

In spite of the alleged importance of the serotonin syndrome, a quantitative analysis of the component behaviors has not been done. Some investigators [5,12] have used an all-or-none scoring method determining that the syndrome is present if an arbitrary number of behavioral components were observed, such as forepaw treading, head weaving and splayed hindlimbs. Growdon [1] attempted further quantification by assigning one point for each appearance of 4 criterion behaviors and summed these points to make a composite score. He found that stereotypy peaked at 10 mg/kg PCA with no further significant increase produced by a 20 mg/kg dosage. In the present experiment, we attempted to further extend these observations by: (1) studying stereotypic and normal behaviors which are exclusive, i.e., behaviors which the animal exhibits one at a time; (2) determine the change in frequency of these behaviors as a function of PCA dosage; (3) identify and relate to PCA dosage the appearance or nonappearance of nonexculsive signs, e.g. Straub tail or piloerection, signs which the animal may show simultaneously; (4) determine the effect of previous depletion of brain serotonin by PCA preinjection on this behavior syndrome.

EXPERIMENT 1

In this experiment we determined the nature of the relationship existent between the frequencies of the various behavior components and the PCA dosage.

METHOD

Animals

The 54 naive, female, Long-Evans rats used in this experiment were bred in the animal colony of the Psychology De-

partment of Syracuse University. Rats were 90–140 days old when tested. They were maintained on Purina Chow and tap water.

Apparatus

Rats were group-housed in the animal colony prior to the beginning of the experiment. Seven days before the PCA injection, they were moved within the colony into individual living cages (24×18×18 cm). Colony lights were on for 14 hr per day. Temperature was maintained at 21 ± 2°C and air was humidified during the winter months.

For PCA injection and subsequent behavioral testing, rats were moved from the colony to an isolated test room and were placed individually into a square, wooden, open-field arena, 91 cm on a side. The outside walls of the arena were 30 cm high and were painted with medium gray enamel. The floor was covered with waxed kraft paper which was replaced after each animal was tested. The only illumination was provided by a 15 W light bulb suspended 91 cm above the center of each open field. Three arenas were used to observe 3 rats simultaneously.

Procedure

In pilot studies, rats were injected IP with 10 mg/kg PCA and were observed continuously for 2–3 hr. Emitted behaviors were recorded with an indication of elapsed time since injection. From those data, the following definitions of exclusive behaviors were determined and will be used in the present experiments.

Forepaw treading. Lateral stepping movements of the forepaws with minimal compensatory movement of the hindlimbs causing the rat to pivot alternately to the right and left. Total lateral movement is an arc of 90° or less.

Circling. Pivoting of the animal with forepaw stepping as in forepaw treading, but here the turns are unidirectional and usually move in an arc of more than 360°. Circling is interrupted by other behaviors, but when resumed is usually in the same direction.

Headweaving. Lateral swaying movement of the head with very little net locomotion. Headweaving is often directed toward the walls of the arena.

Inching. Somewhat uncoordinated forward linear locomotion produced by short, rapid steps of the forelegs followed by large, less frequent steps of the hindlegs.

Normal walking. Synchronous, coordinated, linear forward locomotion seen in undrugged rats.

Rearing. Forelegs raised off the floor.

Grooming. While supported on rear legs, rats lick fur and lick forepaws and rub them through fur.

Inactivity. Rat not engaged in any of the behaviors mentioned above. Ray may be asleep or awake, but is inactive. Four legs are in contact with the floor.

The nonexclusive behaviors utilized are splayed hindlimbs, Straub tail, piloerection, vocaliztion, tremor and diarrhea. These categories have been used by others [1, 12, 14] and we attempted to use them in a similar manner.

Seven days prior to PCA injection, rats were removed from group housing, weighed and injected IP with 0.15 M NaCl, (10 ml/kg) to adapt them to handling procedures. No observations were made during the subsequent 7-day period. On the PCA-test day, rats were removed from individual living cages, weighed and injected IP with either 0.15 M NaCl or with PCA (mixed in 0.15 M NaCl) placed immediately into the open-field arena and observed for 60 min. Eleven rats were injected with 0.15 M NaCl (0 mg/kg PCA). Thirteen were injected with 2.5 mg/kg PCA. The 5, 7.5 and 10 mg/kg PCA dosages were each given to 10 rats. Injection concentrations were mixed so that these dosages were given in a standard injection volume of 10 ml/kg.

The exclusive behavior seen at the beginning of each 3-min period was recorded on a checklist producing a record of 20 behavioral responses per animal per 60-min test period. The nonexclusive behaviors were marked on a checklist at the time of first appearance.

Three experienced observers participated in the experiment. They made injections blindly using coded injection bottles and a predetermined injection schedule. Rats were tested from 8 a.m. to 6 p.m. with care taken to evenly apportion the injection dosages over the course of the working day.

RESULTS

Frequencies of each exclusive behavior were summed over the 60-min observation period. The mean 60-min frequencies of these behavior components are shown as a function of the PCA dosage in Fig. 1(a) and 1(b). Data were analyzed on each component behavior separately with a one-way analysis of variance. For every component, except normal walking, behavior frequency varied with PCA dosage ($p<0.01$). The behaviors common in the undrugged rats, rearing, grooming and inactivity (designated here as normal behaviors), are negative functions of the drug dosage. The stereotypic behaviors, headweaving, forepaw treading, circling and inching were not seen in the control group (0 mg/kg), but were reliably emitted within the range of PCA dosages used and were positive functions of the drug dosage. The normal walking measure is problematic since the large apparent differences shown in Fig. 1 (a) are not statistically reliable.

In order to get a single index of PCA action, composite stereotypy scores were calculated for each animal by adding 60-min frequencies of the four stereotypy components (Fig. 1 (c)). A trend analysis performed on these data [6] showed a significant linear component, $F(1/49)=60.66$, $p<0.001$, but no quadratic component. Composite scores and dosages were highly correlated, $r(52)=0.71$, $p<0.001$, calculated with untransformed data.

The relationship of composite scores for grooming, rearing and inactivity to PCA dosage were analyzed with a trend analysis which showed both a linear component, $F(1/49)=37.02$, $p<0.001$, and a quadratic component, $F(1/49)=108.34$, $p<0.001$. Near maximal depression of normal behavior is produced with a 5 mg/kg dosage resulting in a nonlinear dose-response function (Fig. 1(c)). Normal behavior components are highly correlated with the log of the dosages, $r(52)=-0.85$, $p<0.001$, a transformation which tends to attenuate the nonlinearity of the dose-response function at the high dosage end.

Incidence of nonexclusive behaviors is shown in Table 1. Of these measures, splayed hindlimbs and Straub tail were the most useful since they were readily recognizable signs and were rather well related to dosage. Piloerection was marginal in magnitude making identification uncertain. Vocalization, tremors, and diarrhea were infrequent and not reliably related to dosage.

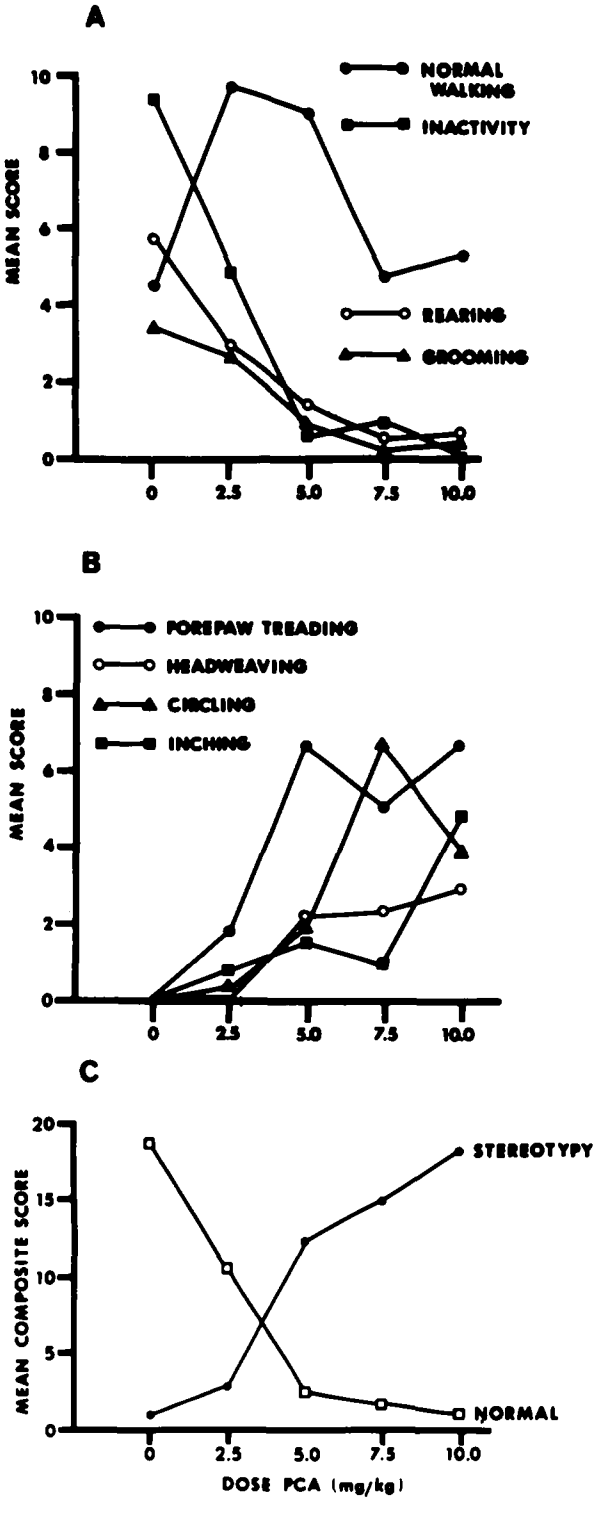

FIG. 1. Frequencies of exclusive behaviors during the 60-min observation period as a function of PCA dosage: (A) Normal behaviors; (B) Stereotypic behaviors; (C) Composite scores for stereotypic behaviors and normal behaviors exclusive of normal walking.

TABLE 1

INCIDENCE OF NONEXCLUSIVE SIGNS AS A FUNCTION OF PCA DOSAGE

	PCA Dose (mg/kg)				
Sign	0	2.5	5.0	7.5	10.0
Splayed hindlimbs	0/11*	5/14	6/10	8 ± 10	10/10
Straub Tail	0/11	0/14	4/10	3/10	7/10
Piloerection	0/11	2/14	5/10	3/10	4/10
Vocalization	0/11	0/14	0/10	0/10	2/10
Tremors	0/11	0/14	1/10	2/10	2/10
Diarrhea	0/11	2/14	0/10	0/10	0/10

*0 out of 11 animals showed trait during the 60-min observation period.

EXPERIMENT 2

In this experiment we studied the effect of one PCA injection on the behavioral action of a second given either 2 or 4 days later.

METHOD

Rats were 40, naive, Long-Evans females, 85–120 days old, bred in the colony of the Psychology Department of Syracuse University. Colony conditions were the same as described previously. Seven days prior to PCA injection and behavioral testing, rats were moved from group housing into individual cages. Rats were assigned to one of 4 groups designated by type of IP preinjection given: (1) 11 rats injected with 0.15 M NaCl, 4 days before PCA test; (2) 9 rats injected with 10 mg/kg PCA, 4 days before test; (3) 10 rats injected with 0.15 M NaCl, 2 days before test; (4) 10 rats injected with 10 mg/kg PCA, 2 days before test. Rats were observed in the individual living cages during the hr following injection to verify that PCA-injected rats showed stereotypy [1, 12, 14] and that saline-injected rats did not. No other behavioral observations were made during this time period.

On the PCA test day, all rats were weighed, injected with 10 mg/kg PCA IP, 10 ml/kg, and immediately placed into the arenas for a 60-min observation period. Behavioral testing was the same as described in Experiment 1 except that one experienced observer was used and all observations were made between 8 a.m. and 11 a.m. The observer did not know the type of preinjection.

RESULTS

Frequencies of each exclusive behavior component were analyzed with a separate 2×2 analysis of variance (preinjection solution × day of injection). For each behavioral component, scores differed as a function of type of preinjection, PCA or saline ($p<0.001$ in all cases), but not as a function of delay between the injections (2 or 4 days). No significant interactions were found. Based on these findings, data from the 2- and 4-day injection groups were combined. The resulting means of behavior frequencies are shown in Table 2. The effect of PCA preinjection on subsequent PCA injection is distinct. It attenuates the frequency of stereotypic behavior and increases the frequency of normal behaviors.

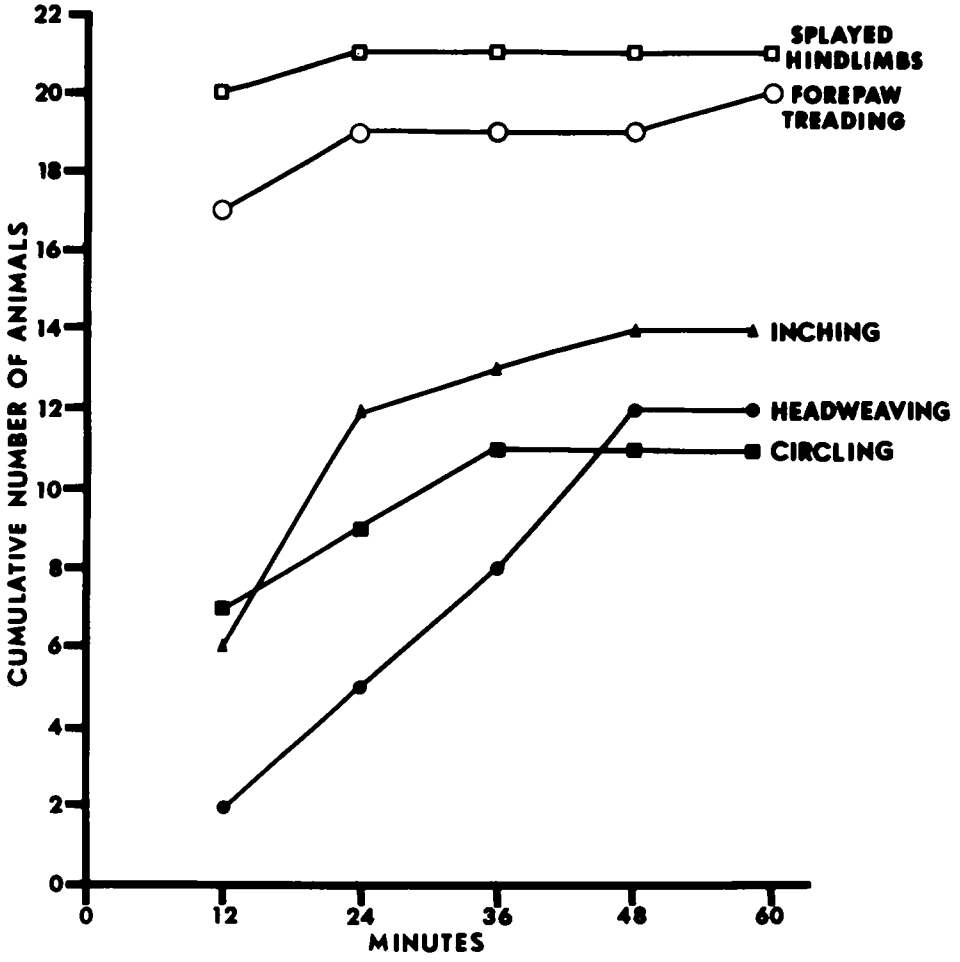

FIG. 2. Cumulative frequency of rats showing various stereotypic behaviors during the hour after receiving 10 mg/kg PCA dosage either 2 or 4 days after a saline preinjection.

TABLE 2

FREQUENCY OF BEHAVIORS INDUCED BY PCA IN PREINJECTED ANIMALS

Behavior	Saline Preinjection	10 mg/kg PCA Preinjection
Forepaw Treading	9.8 ± 1.0*	1.8 ± 0.7
Circling	2.7 ± 0.7	0.1 ± 0.7
Headweaving	1.8 ± 0.5	0.5 ± 0.2
Inching	1.6 ± 0.4	0.2 ± 0.2
Normal Walking	0.5 ± 0.2	4.8 ± 1.0
Inactivity	0 ± 0	5.5 ± 1.4
Rearing	0.4 ± 0.2	2.1 ± 0.5
Grooming	0.5 ± 0.5	1.7 ± 0.4

*Mean ± SE.

For rats preinjected with saline, 21 out of 21 displayed splayed limbs while 12 out of 19 PCA-injected rats showed the phenomenon. Straub tail, diarrhea, piloerection, vocalization and tremor occurred rarely and were not reliably influenced by type of preinjection.

Total duration of behavioral symptoms cannot be determined from data from the present experiment since many component behaviors were still being emitted at the termination of the 60-min observation period. These data can provide information, however, concerning the latency of onset of the behaviors. For each animal it was determined at which 12-min time interval each of the stereotypic behaviors first appeared. For each group, cumulative curves were constructed indicating how many of the group had shown the behavior component by each 12-min segment of the observation period (Fig. 2). This analysis was done only for the saline-preinjected group, since stereotypic behaviors were greatly reduced in the PCA-preinjected group. Splayed limbs and forepaw treading are clearly the most ubiquitous signs of the syndrome and they appear with very short latencies (within 12 min for most animals). Headweaving and inching were seen in about half the animals and seemed to require more than 12 min for full development. Failure to observe any of these behaviors is not conclusive evidence that they did not appear in an animal. It is possible that our 3-min time-sampling techniques failed to detect the appearance of a behavior, especially if it was emitted at a low rate.

DISCUSSION

This experiment shows that when a time-sampling

analysis is made of the frequency of exclusive behavior components of the PCA-induced serotonin syndrome, the frequencies are dose-dependent, with the exception of the normal walking category. When composite stereotypy scores are formed in order to best assess the PCA action, a linear dose response function was observed within the range of the PCA concentrations used here. The use of time sampling permits determination of the level of PCA action rather than the usual binary determination of the syndrome being present or absent. For the nonexclusive behaviors, we found splayed hindlimbs to be the best indicator of PCA action since this sign developed with short latency in most animals given a 10 mg/kg dosage. For animals given the various dosages, the incidence of the sign was approximately dose-related. Straub tail related rather well to dosage level, but piloerection, vocalization, tremors, diarrhea and other autonomic signs did not.

The only other attempt to quantify the action of PCA dosage on criterion behaviors was made by Growdon [1] who arbitrarily assigned one point to each of the following: rigid posture, tremor, myoclonus and autonomic signs (diarrhea, piloerection, lacrimation, salivation and ejaculation). We did not measure rigidity in order to avoid interferring with ongoing behavior. In our hands the other criterion signs used by Growdon were not frequent, reliable or distinct. Growdon [2] states that splayed hindlimbs, forepaw treading and circling, behaviors he did not utilize as criterion behaviors, are highly characteristic of PCA injection, an interpretation which our data support. He states that tremor and myoclonus are more characteristic of the syndrome produced by 5-hydroxytryptophan injections into rats pretreated with 5, 7-dihydroxytryptamine than of the syndrome produced by PCA. These considerations suggest that Growdon did not use the most sensitive and reliable indicators of PCA action.

Sloviter, Drust and Conner [12] scored the syndrome as present or absent based on the presence of three criteria: forepaw treading, splayed hindlimbs and headweaving. These choices are strongly supported by data in the present experiment. All are distinct signs with short latency (less than 12 min). The first two appeared in almost all animals given a 10 mg/kg dosage (Fig. 2). Headweaving is not so ubiquitous,as indicated by the time-sampling technique, but was easy to identify, unlike the autonomic signs.

Trulson and Jacobs [14] rated the serotonin syndrome as present if 4 out of 6 criterion behaviors were seen. They used forepaw treading, splayed hindlimbs and headweaving, as did Sloviter, Drust and Conner [12], plus Straub tail, rigidity and tremor. Data from the present experiment suggest that the first 4 are defensible criteria. The first two are, perhaps, the most sensitive and easy to determine. Tremor seems problematic in view of its low frequency of appearance.

Among the normal behaviors, the determination and interpretation of walking behavior was difficult. It was expected that PCA injection would produce an increase in general activity [13] and that this increment might be displayed as increased walking. Perhaps the high variability of the data and the lack of statistical significance results from our failing to distinguish qualitative differences in types of walking. Compared to the control group, PCA-injected rats tend to walk at a more uneven pace in a perserverative manner usually closely oriented to the walls of the arena. They often darted from corner to corner. Walking of normal rats is punctuated with frequent bouts of rearing and grooming which are infrequent in PCA-treated rats. It is clear that the PCA-injected animal tends seldom to engage in sleeping or resting (behaviors rated as inactivity), but is almost continuously engaged in some activity. The inhibition of normal behavior is a sensitive indicator of PCA action (Fig. 1–C), although it is not a specific action of PCA as are the stereotypic behaviors [5].

The ability of the PCA preinjection to attenuate the behaviors of the serotonin syndrome fits well with the prevailing notion that the stereotypy results from the release of brain serotonin [5,12]. A 5 mg/kg PCA injection can produce a 60% depletion in brain serotonin [13] which may endure for months [10]. It is likely, therefore, that our 10 mg/kg PCA preinjection produced a significant brain serotonin depletion resulting in greatly attenuated release of serotonin when the second injection was administered. A similar interpretation was given to the finding that preinjection of p-chlorophenylalanine (PCPA) attenuated stereotypy produced by PCA injection [1]. The frequency of every stereotypic behavior component was significantly altered by the PCA preinjection (Table 2) as expected if these behaviors are mediated by serotonergic neurons and functionally are part of the same syndrome. The PCA preinjection should produce only a transient change in catecholamine levels which should dissipate within one day following injection [9]

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