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Effects of Chronic Phencyclidine on Fixed-Ratio Responding: No Relation to Neurotransmitter Receptor Binding in Rat Cerebral Cortex[1]

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SCHWARTZ, R. D., J. M. MOERSCHBAECHER, D. M. THOMPSON AND K. J. KELLAR. *Effects of chronic phencyclidine on fixed-ratio responding: No relation to neurotransmitter receptor binding in rat cerebral cortex.* PHARMAC. BIOCHEM. BEHAV. **16**(4) 647–652, 1982.—The effects of chronic phencyclidine (3.2 mg/kg for 25 days) on responding maintained under a fixed-ratio 30 schedule of food presentation were studied in rats. Initially phencyclidine produced large decreases in the overall rate of responding. This decrease was due primarily to long pauses in responding and secondarily to a decrease in local rates of responding. Although tolerance developed to the rate-decreasing effects of phencyclidine in each subject, the extent and pattern of its development differed among the subjects. After the chronic drug regimen, the rats were sacrificed. Ligand binding to muscarinic cholinergic, opiate, adrenergic, and serotonergic receptors in cortex was then compared to that in rats which received saline with operant training, phencyclidine alone, or saline alone. Neither operant behavior alone, phencyclidine alone, nor the interaction of phencyclidine and operant behavior was found to alter binding to these receptors. The results indicate that behavioral tolerance develops to phencyclidine, but it is not accompanied by changes in binding to the receptors studied.

Fixed-ratio schedule Phencyclidine Behavioral tolerance Receptor binding Lever press Rats

THE acute effects of phencyclidine on schedule-controlled behavior have been studied in several animal species [2]. In comparison, however, relatively little is known concerning the effects of chronic phencyclidine. For example, Chait and Balster [3] investigated the acute and chronic effects of phencyclidine in squirrel monkeys responding under a chain fixed-interval 4-min fixed-ratio 15 schedule of food presentation. Using an individualized chronic drug regimen, they found that in both components the rate-decreasing effects of phencyclidine were gradually attenuated with repeated administration. In addition, when the dose-effect curve (0.01–0.6 mg/kg) was redetermined following chronic administration, there was a two-fold shift to the right. Tolerance to the behavioral effects of phencyclidine has also been reported in rats responding under variable-interval (VI) and fixed-interval (FI) schedules of water presentation [8,17]. In both of these studies tolerance was found to develop when the drug was administered either before or after the experimental session. While these data may indicate the importance of pharmacological variables in the development of tolerance to phencyclidine, behavioral variables have also been reported to influence its development [17]. Woolverton and Balster [17] found that in rats injected before the experimental session tolerance was more complete than in rats injected after the session.

The neurochemical mechanisms by which behavioral tolerance to phencyclidine develops are unknown. However Pearl and Seiden [9] have suggested that behavioral tolerance to amphetamine and methylphenidate is accompanied by changes in central catecholaminergic neurotransmitter activity. It has been shown that operant behavior modulates the metabolism of brain catecholamines [1, 5, 13]. For example, Emmett-Oglesby *et al.* [5] found an increase in central dopamine and norepinephrine metabolism in rats responding under a VI 30-sec schedule of water presentation. They concluded that a critical variable was the presentation of stimuli associated with the reinforcer. Such interactions between behavior and central neurotransmitter activity have been proposed as a determinant of specific drug-behavior interactions [13]. Similarly, it has also been proposed that activation of a receptor system following administration of a drug may depend upon the ongoing behavior [9]. Phencyclidine has been shown to produce multiple effects on neurotransmitter systems. It is reported to inhibit uptake of cate-

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cholamines and serotonin from synaptosomal preparations [14], and to interact *in vitro* with muscarinic and opiate receptors in whole rat brain [15]. The development of behavioral tolerance might, therefore, reflect specific changes in receptor systems resulting from drug-behavior interactions. The purpose of the present study was to investigate the effects of chronic phencyclidine administration in rats responding under a fixed-ratio schedule of food presentation and to determine if the drug-behavior interaction would be reflected in changes in receptor binding in the brain.

METHOD

Subjects

Twenty, experimentally naive, male Sprague-Dawley rats (approximately 280 grams) were maintained at 80% (±10 grams) of their free-feeding body weight throughout the study. Their diet consisted of 45-mg food pellets (Bioserve, Biomix #T101) and Purina Rat Chow. The animals were individually housed in a temperature-controlled room with a 9 hr light-15 hr dark cycle. Water was continuously available in the home cage.

Apparatus

Two identical chambers (Lehigh Valley Electronics, Model 313204), each measuring 23.5 cm × 30 cm × 26.5 cm, were used. A lever was mounted on the side wall of each chamber, 5 cm above the grid floor. A downward force of 0.3 N was required to activate the lever. Three pilot lamps (#1820) were located 5 cm above the lever. The lamps were mounted 2 cm apart (center-to-center) and each was covered with a green glass lens. A food cup was located 9 cm to the left of the lever and a houselight was mounted 22 cm above the food cup. The chamber was housed in a larger insulated shell equipped with a blower for ventilation. Events were scheduled and recorded by means of solid-state circuitry, counters, running-time meters and a cumulative recorder.

Experimental Design

The 20 subjects were arbitrarily divided into 4 groups: (1) phencyclidine-treated rats in which responding was maintained under a fixed-ratio 30 (FR 30) schedule of food presentation (N=4), (2) saline-treated rats in which responding was maintained under the same schedule (N=4), (3) phencyclidine-treated rats with no operant training (N=6) and (4) saline-treated rats with no operant training (N=6). The diets, housing conditions, etc., were identical for all subjects.

Behavioral Procedure

Between 7 and 15 sessions were required to initially magazine train the subjects, shape the lever-press response and to establish an FR 30 performance. Responding was allowed to stabilize under the FR 30 schedule for 25 additional sessions. The reinforced response began a 3-sec feeder cycle during which the stimuli over the levers were off and the houselight was on; responses during the feeder cycle had no programmed consequences. Each session terminated after 75 food presentations or 1 hr, whichever occurred first. If less than 75 reinforcers were obtained during the session the balance was fed in the home cage. Sessions were conducted daily.

Drug Testing

Phencyclidine hydrochloride or saline was injected IP, in a volume of 2 ml/kg body weight, 10 min prior to the start of the session. A dose of 3.2 mg/kg was chosen for chronic administration. In previous studies (unpublished) we have found this dose to produce substantial rate-decreasing effects in rats responding under FR schedules of food presentation. Initially all subjects were injected with saline for 10 days. On the eleventh day either the chronic phencyclidine administration was begun (Groups 1 and 3) or saline injections continued (groups 2 and 4) for an additional 25 days.

Receptor Binding Assays

Twenty-four hours after the last injection the animals were sacrificed by decapitation and the brains removed, dissected and frozen at −80°C until assayed. The cerebral cortex from each rat was homogenized with a Brinkmann Polytron in 50 mM tris-HCl buffer, pH 7.4. The homogenate was washed twice in fresh buffer with centrifugation at 49,000 g for 10 min. The final pellet was resuspended in the appropriate buffer as described below. Aliquots of homogenate equivalent to 8 mg of tissue were assayed for alpha- and beta-adrenergic, serotonin (5-HT) and opiate receptors. Aliquots equivalent to 1 mg of tissue were assayed for muscarinic cholinergic receptors.

The assay methods for alpha- and beta-adrenergic, serotonin-1 (5-HT-1), muscarinic cholinergic, and serotonin-2 (5-HT-2) receptor binding have been previously reported by Kellar *et al.* [6,7]. The assay method for opiate receptor binding was similar to the procedure reported by Pert and Snyder [10]. Briefly, the incubation buffer, time, and temperature for each receptor assay were as follows: alpha-1-adrenergic receptor—50 mM tris-HCl, pH 7.6, at 25°C for 15 min; alpha-2-adrenergic receptor—50 mM tris-HCl, pH 7.6, at 25°C for 30 min; beta-adrenergic receptor—50 mM tris-HCl containing 0.1% ascorbic acid, pH 7.9, at 23°C for 15 min; 5-HT-1 receptor—50 mM tris-HCl containing 0.1 % ascorbic acid, 10 μM pargyline and 4 mM $CaCl_2$, pH 7.4, at 37°C for 10 min; 5-HT-2 receptor—50 mM tris-HCl containing 0.1% ascorbic acid and 4 mM $CaCl_2$, pH 7.4, at 37°C for 15 min; opiate receptor—50 mM tris-HCl, pH 7.6 at 37°C for 15 min; and muscarinic cholinergic receptor—40 mM Na_2HPO_4 and 10 mM KH_2PO_4, pH 7.4, at 37°C for 60 min. The ^{3}H-ligands used were: ^{3}H-WB4101 (alpha-1 receptor), ^{3}H-clonidine (alpha-2 receptor), ^{3}H-dihydroalprenolol (^{3}H-DHA, beta receptor), ^{3}H-5-HT (5-HT-1 receptor), ^{3}H-spiperone (5-HT-2 receptor), ^{3}H-naltrexone (opiate receptor), and ^{3}H-quinuclidinylbenzilate (^{3}H-QNB, muscarinic cholinergic receptor). Binding assays were carried out in sextuplicate with half the tubes containing saturating concentrations of the following non-radioactive drugs to define non-specific binding: 100 μM norepinephrine (NE), 10 μM NE, 20 μM propranolol, 20 μM 5-HT, 1 μM lysergic acid diethylamide (LSD), 10 μM naloxone, and 1 μM atropine, respectively. Specific binding was defined as total binding (binding in the absence of non-radioactive drug) minus non-specific binding. Specific binding was between 60–90%.

Incubations were terminated by the addition of cold buffer to the tubes and by rapid filtration over Whatman GF/B glass fiber filters. The filters were washed with three aliquots of cold buffer and placed in scintillation vials with scintillation fluid and counted in a Searle Mark III Scintillation counter at a counting efficiency of 40%.

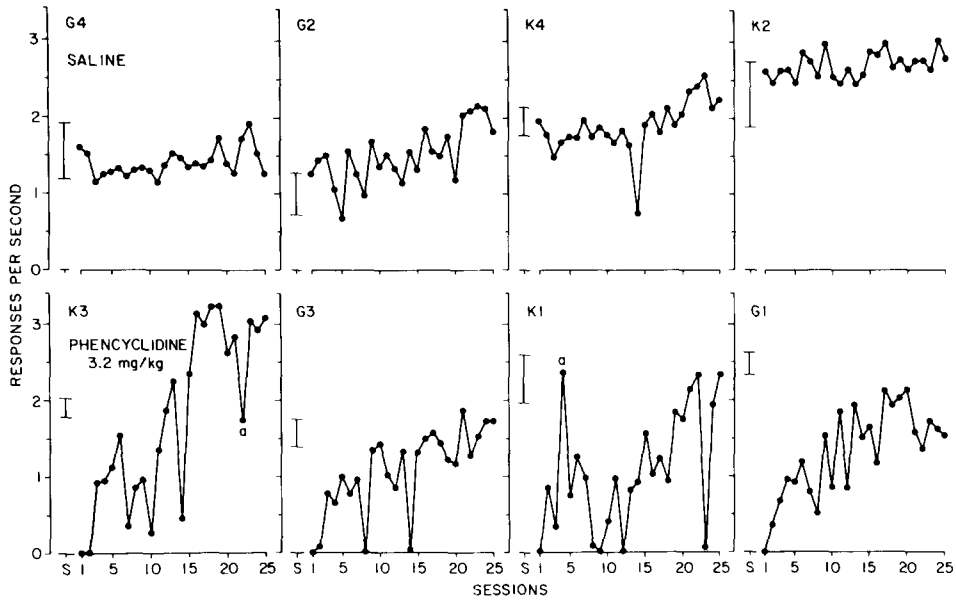

FIG. 1. Effects of repeated administration of either saline (top panels) or phencyclidine (lower panels) on the overall rate of responding for each subject under the fixed-ratio 30 schedule of food presentation. The vertical lines at S indicate the range of 7 consecutive saline sessions which preceded the chronic administration. Points (a) indicate sessions for which the IP injection was suspect.

Data Analysis

The behavioral data were analyzed in terms of the overall response rate (responses/sec, excluding the feeder cycle). The range of response rates for the last 7 saline sessions prior to either the start of drug administration (Group 1) or continued saline treatment (Group 2) served as the control. For each subject, an injection was considered to have an effect to the extent that the data point fell outside of that animal's control range of variability. Within-session changes in responding were monitored by a cumulative recorder. Receptor binding data were statistically analyzed using Duncan's Multiple Range Test ($p<0.05$).

RESULTS

Behavior

The effects of repeated administration of either phencyclidine (3.2 mg/kg) or saline on overall rate of responding are shown for each subject in Groups 1 and 2 in Fig. 1. The range of control rates for the preceding 7 saline sessions (S) is shown at the left for each subject. In each of the phencyclidine-treated rats (lower panels) responding was virtually eliminated during the first drug session. During subsequent sessions the rate-decreasing effects of phencyclidine gradually diminished in each subject. There were, however, individual variations in each subject's response to the chronic drug administration. For example, while subjects G3 and K1 developed complete tolerance to the rate-decreasing effects of the drug (i.e., response rates returned to within the control range), subject G1 exhibited only partial tolerance to phencyclidine. Though the rate-decreasing effects of the drug were attenuated in this subject, overall response rate did not return to the control range. The effects of chronic phencyclidine in subject K3 were biphasic. Initially, phencyclidine produced substantial decreases in response rate (sessions 1–10). Response rate then returned to the control range (sessions 11–15) and then increased and remained at rates greater than control (sessions 16–25). During the chronic phencyclidine administration, each of the subjects showed multiple perturbations in response rate. For example, in subject K3, response rates decreased in sessions 7, 10 and 14. Similar perturbations occurred in the other subjects.

The effects of chronic saline administration on overall response rate are shown for each subject in the upper panels of Fig. 1. Generally, saline had little or no rate-decreasing effects in these subjects. Three of the subjects (G2, K4, K2) did, however, show a slight upward drift in rate of responding. In G2 the drift was gradual, beginning with session 2. In K4, rates tended to increase toward the end of the chronic administration (session 20). Finally, K2 showed intermittent increases in response rates throughout the chronic study.

Selected cumulative response records for saline and phencyclidine sessions are shown for subject G3 in Fig. 2. The saline record shown approximates the mean of the last seven saline sessions that preceded chronic drug administration. Phencyclidine (3.2 mg/kg) virtually eliminated responding during the first session. In the second session there was a long initial pause followed by sporadic periods of responding at low local rates. Considerably less pausing occurred during the third session and local rates tended to increase as the session progressed. This general attenuation of the disruptive effects of phencyclidine continued through session 5. While the overall rate remained lower than the saline control (see Fig. 1), there were large fluctuations in the local rate of responding within the session. Such fluctuations are evident in the

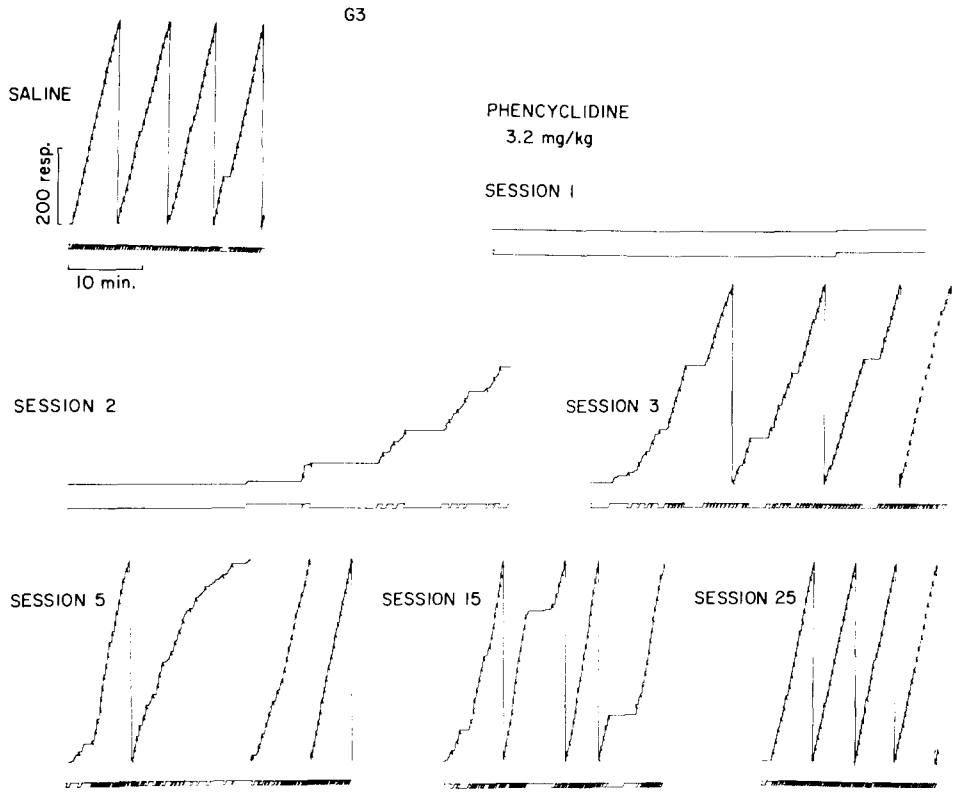

FIG. 2. Cumulative records for subject G3 showing the pattern of responding under the fixed-ratio 30 schedule during a representative saline session and during selected sessions of the chronic administration which were preceded by injections of phencyclidine (3.2 mg/kg). The response pen stepped upward with each response and was deflected downward each time food was presented. The delivery of each reinforcer deflected the event pen, which remained down until a response was made.

cumulative record for session 5. By session 15, local rates had stabilized and pausing occurred infrequently. These short pauses became less frequent during subsequent sessions and by session 25 the pattern of responding was comparable to the saline control. The pattern of responding and rate of tolerance development for subjects K1 and G1 were similar to that of G3 although subject G1 never became completely tolerant (cumulative records not shown).

The effects of chronic phencyclidine on the responding of subject K3 are shown in Fig. 3. In general, the pattern of tolerance development in this subject differed from the pattern seen in subject G3. Few responses were made during the first phencyclidine session. The local rate of responding during session 3 tended to increase as the session progressed. The longer pauses in responding generally occurred toward the beginning of the session. By session 5 the frequency of pausing was reduced and in most subsequent sessions, pausing was no longer evident. During sessions 5–15, local rates of responding continued to increase within each session. By session 15 the overall rate of responding exceeded the control range (see Fig. 1). This was primarily due to a continued increase in local rates of responding as is evidenced in the cumulative records for sessions 20 and 25.

Receptor Binding

No significant differences in receptor binding were found among any of the treatment groups studied (Table 1).

DISCUSSION

The initial decreases in response rate obtained in the present study are consistent with previous reports of the acute effects of high doses of phencyclidine on responding in squirrel monkeys (0.3–1 mg/kg, [3]), rats (5.6–7.5 mg/kg, [12]), and mice (10–30 mg/kg, [16]) under FR schedules of food presentation. With repeated administration of phencyclidine (3.2 mg/kg), the rate-decreasing effects gradually diminished, indicating the development of tolerance. Similar results have been reported in squirrel monkeys responding under a chain FI-FR schedule [3] and in rats responding under interval schedules [8,17]. The general time-course for the development of tolerance to the rate-decreasing effects of phencyclidine in the present study was also comparable to that previously reported in rats [17]. However, in the present study, the initial recovery in response rate was more rapid than that reported by Woolverton and Balster [17]. This is

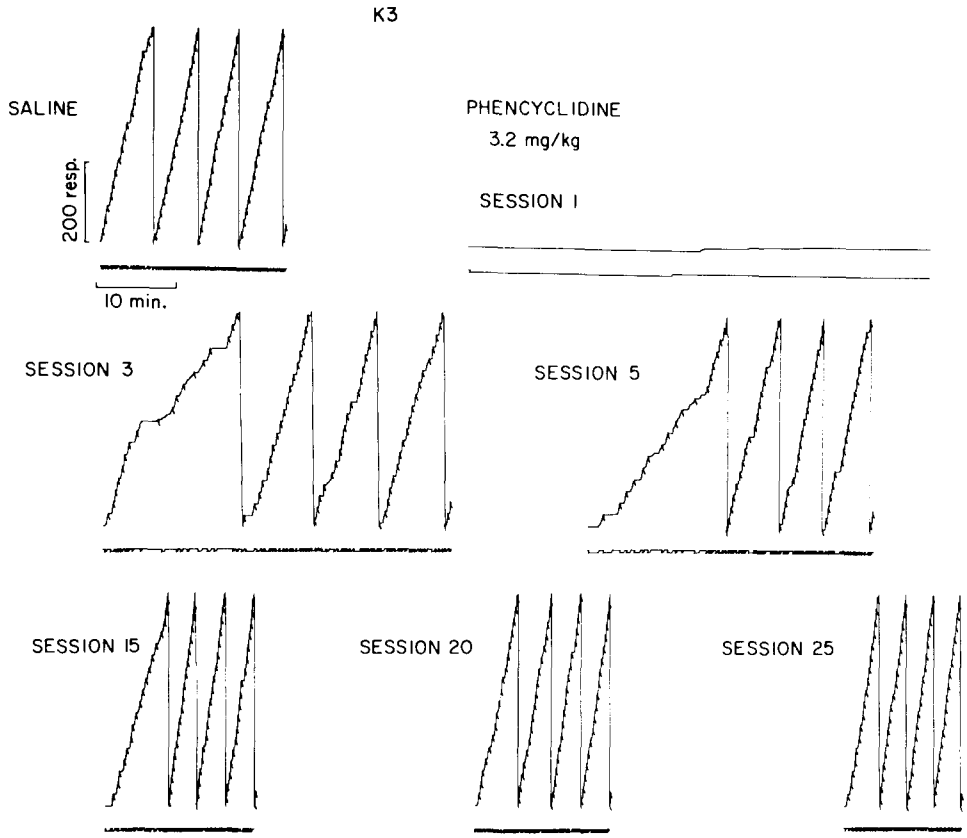

FIG. 3. Cumulative records for subject K3 showing the pattern of responding under the fixed-ratio 30 schedule during a representative saline session and during selected sessions of the chronic administration which were preceded by injections of phencyclidine (3.2 mg/kg). The recording details are the same as in Fig. 2.

TABLE 1

RECEPTOR BINDING IN CEREBRAL CORTEX

	Specific Binding (pmol/g tissue)*			
	Operant Behavior		Without Operant Behavior	
Receptor	Phencyclidine (Group 1)	Saline (Group 2)	Phencyclidine (Group 3)	Saline (Group 4)
Beta-adrenergic (4.1 nM ^{3}H-DHA)	7.5 ± 0.4	7.1 ± 0.4	8.2 ± 0.5	8.4 ± 0.8
Alpha-1-adrenergic (0.5 nM ^{3}H-WB4101)	4.2 ± 0.4	4.1 ± 0.2	4.3 ± 0.1	4.2 ± 0.3 (3)
Alpha-2-adrenergic (2.2 nM ^{3}H-clonidine)	4.7 ± 0.3	4.6 ± 0.1	5.0 ± 0.2	4.1 ± 0.6
Serotonin-1 (4.7 nM ^{3}H-5-HT)	5.4 ± 0.5	5.9 ± 1.3	5.4 ± 2.0	5.8 ± 1.0
Serotonin-2 (1.1 nM ^{3}H-spiperone)	9.6 ± 3.0	9.3 ± 2.0	8.6 ± 1.0	9.0 ± 1.6
Muscarinic (0.2 nM ^{3}H-QNB)	22.4 ± 3.4	23.0 ± 1.0	24.9 ± 2.0	28.9 ± 6.0
Opiate (1.8 nM ^{3}H-naltrexone)	7.7 ± 0.4	7.1 ± 0.2	7.2 ± 0.4 (2)	6.7 ± 0.5 (2)

*Values are mean ± S.E.M. from 4 rats unless noted in ( ), each assayed in triplicate.

probably due to the different schedules used in each study. Under an FR schedule, the rate of reinforcement is dependent upon the rate of responding, while under an FI schedule, response rate may vary considerably without affecting the rate of reinforcement. In the Woolverton and Balster [17] study, rate of reinforcement returned to control levels before response rate.

The cumulative records for subjects G3 and K3 show that although tolerance developed in both subjects, the pattern of development differed between animals. Subject G3 (Fig. 2) exhibited more pausing than subject K3 (Fig. 3). In subject K3, response rate exceeded the control rate after session 15. This large increase in response rate is much greater than the drift in response rates which occurred in three of the control subjects. These variations in both the extent and the manner in which tolerance developed in the different subjects are consistent with reports that there are substantial individual differences in terms of phencyclidine's acute behavioral effects among animals [2]. The perturbations in response rate seen during chronic phencyclidine administration are also consistent with such reports. Despite these differences in rate and pattern of responding among the subjects, tolerance developed in each animal.

That the disruptive effects of phencyclidine diminished with chronic administration in each of the subjects is consistent with the hypothesis that the development of behavioral tolerance depends upon the drug's effect on rate of reinforcement [11]. Initially, phencyclidine produced substantial decreases in response rate under the FR schedule resulting in a decreased rate of reinforcement. As predicted from Schuster, Dockens and Woods' hypothesis [11], behavioral tolerance developed to this effect. Recently, Woolverton and Balster [17] have suggested that pharmacological variables may also play a role in the development of tolerance to the behavioral effects of phencyclidine. The mechanism(s) for this pharmacological effect is, however, unclear. It has been reported that phencyclidine alters presynaptic uptake of norepinephrine, dopamine and serotonin [14] and interacts *in vitro* with muscarinic and opiate receptors [15]. The chronic administration of drugs that alter neurotransmission either presynaptically or postsynaptically may lead to adaptations of the receptors involved [4]. In the present study, however, there was no evidence that either chronic phencyclidine alone (Group 3) or a phencyclidine-behavior interaction (Group 1) affected muscarinic cholinergic or opiate (presumably mu and kappa) receptors in cerebral cortex. Furthermore, no changes were found in cortical adrenergic or serotonergic receptor binding although *in vitro* phencyclidine competitively inhibits the uptake of catecholamines and serotonin into synaptosomal preparations [14]. The present data are based upon chronic phencyclidine treatment; however, a single injection of phencyclidine (5.6 mg/kg) also failed to alter the binding of any of the receptors studied (unpublished observations).

Seiden *et. al.* [13] reported that environmental events may affect catecholamine metabolism and distribution in rat brain. One might also expect an interaction among operant behavior, drugs, and neurotransmission processes such as receptor binding. However,the present data do not support this. Neither operant behavior itself nor the interaction of phencyclidine-induced changes in operant behavior had an effect on the binding of muscarinic cholinergic, opiate, adrenergic or serotonergic receptors in rat cerebral cortex. These results do not, however, preclude the possibility that interactions between phencyclidine and operant behavior may be reflected neurochemically in either different anatomical areas of the CNS, different receptor systems, different neurotransmission parameters or under different schedules of reinforcement.

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