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Time Course Analysis of Para-Chlorophenylalanine Induced Suppression of Self-Stimulation Behavior

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GRATTON, A. *Time course analysis of para-chlorophenylalanine induced suppression of self-stimulation behavior.* PHARMAC. BIOCHEM. BEHAV. 17(4) 597–602, 1982.—The hypothesis of a serotonergic basis of reward rests partly on data showing that serotonin (5-HT) depletion by para-chlorophenylalanine (p-CPA) causes depression of self-stimulation (SS) rates. These data do not clearly demonstrate a time course relationship between 5-HT depletion and SS suppression. The present study shows that SS behavior has fully recovered from p-CPA induced suppression when 5-HT levels are still maximally depleted. The data reveal that the effects of electrical brain SS on p-CPA induced reductions of 5-HT cannot explain the temporal dissociation between 5-HT depletion and SS suppression. As a whole the data suggest that midbrain 5-HT neurons are not critically involved in SS behavior.

Self-stimulation behavior Serotonin para-Chlorophenylalanine Medial raphé nucleus Time course

SEVERAL studies have suggested that serotonergic (5-HT) neurons mediate brain stimulation reward. The hypothesis of a 5-HT basis of reward rests mainly on the fact that high self-stimulation (SS) rates can be obtained with electrodes located in the medial (MR) and dorsal (DR) raphé nuclei [19, 20, 21], a region known to contain high concentrations of 5-HT perikarya [2,9]. Furthermore, pharmacological data seem to indicate, although inconsistently, that SS behavior can be either enhanced or inhibited by drugs that alter 5-HT function. The most widely used drug to test 5-HT's role in SS is para-chlorophenylalanine (p-CPA). This drug is a potent tryptophan hydroxylase inhibitor and has been shown to reduce brain 5-HT levels maximally 72 hours after its administration [12]. Two independent series of studies performed by Miliaressis [16,17] and Van der Kooy [26,27] suggest that p-CPA specifically suppresses DR and MR SS two days after its injection, while SS of either the ventral tegmental area (VTA) [16,17] or the lateral hypothalamus (LH) [26] remain comparatively unaffected by this treatment. The differential sensitivity to 5-HT reduction between so called catecholaminergic (VTA, LH) and 5-HT SS sites led these authors to speculate that 5-HT neurons might also subserve SS behavior. Although several other studies have obtained midbrain raphé SS suppression after p-CPA, 5-HT's involvement in reward was usually dismissed on the basis that maximal SS inhibition did not correlate with the time of maximal 5-HT depletion [8, 15, 22]. Characteristically these studies showed moderate to high suppression of SS, 12 to 24 hours after p-CPA, at which time 5-HT levels are only minimally reduced and a partial to complete recovery of SS behavior 2 to 3 days after p-CPA when 5-HT levels are at their lowest. These results, however can probably be partially explained by the relatively short SS sessions used in these studies (30 to 45 minutes). In fact it has been shown that p-CPA induced suppression of SS is more clearly seen when rats are permitted to self-stimulate continuously for at least 2 hours [16, 17, 27]. Thus it is possible that studies using shorter SS sessions failed to observe the full inhibitory effect of p-CPA on SS behavior. Nonetheless, even in studies using long SS sessions a causal relationship between p-CPA induced suppression of SS behavior and 5-HT depletion was still difficult to assess either because the exact time of recovery from SS suppression was not established [16,17] or because 5-HT depletion and SS suppression time courses still seemed to be out of synchrony [27]. One possible explanation for the differential time courses of SS suppression and 5-HT depletion after p-CPA might be found in increased levels of 5-HT caused by electrical brain stimulation. It has been demonstrated that although p-CPA nearly completely inhibits 5-HT production in the forebrain, by contrast the hindbrain may retain enough tryptophan hydroxylase to synthesize moderate amounts of 5-HT [1,10]. When this is the case, it may be argued that increased neuronal activity resulting from electrical brain stimulation might in some way enhance 5-HT synthesis; hence shortening the time course of p-CPA induced depletion of 5-HT and consequently of SS suppression.

The purpose of the present experiment is to establish the time at which SS behavior recovers from p-CPA induced suppression and to measure 5-HT levels at the time of recovery. Self-stimulation suppression and recovery time

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courses for VTA, LH and MR SS were compared to see if they showed differential sensitivity to 5-HT reduction. Secondly, the effects of electrical brain SS on p-CPA induced reduction of 5-HT levels was examined as a possible explanation for the temporal dissociation between 5-HT depletion and SS suppression time courses.

METHOD

Animals and Surgery

Male Sprague-Dawley rats (300–350 grams) were anesthetized with sodium pentobarbital (Nembutal 50 mg/kg) and stereotaxically implanted with stainless steel bipolar electrodes. With the incisor bar adjusted to maintain the skull in a horizontal position, electrodes were aimed at either the MR nucleus (from bregma; A.P.: −7.6, Lat.: 0, Vert.: 7.8 below the cranium), the VTA (A.P.: −6.0, Lat.: 0.4, Vert.: −8.3) or the LH (A.P.: −3.0, Lat.: 1.7, Vert.: −8.8).

Behavioral Procedure

A week after surgery each rat was shaped to bar press on a continuous reinforcement schedule for either 250 msec trains of 100 Hz or 500 msec trains of 60 Hz sine wave current stimulation. Once the rats had acquired a stable pattern of responding, rate-intensity functions were determined. Current intensities were systematically increased and decreased in 2 or 5 μamp increments to determine the maximal response rate of each rat. The rats' behavioral output ceilings were judged to be stable when they reached their maximal response rate at the same current intensity for 5 consecutive days. Thus a value of 100% corresponds to the rats' maximal response rate. Although it was assumed that p-CPA would suppress SS, several previous studies had reported an enhancement of SS. In order to detect both suppression and facilitation of SS, current intensities were adjusted so the rats would lever press at approximately 75% of their maximal response rate. Rats were then permitted to self-stimulate until they maintained stable baseline response rates for a 180 minute session on 2 consecutive days. Baseline behavior was considered stable when response rates at the end of the session deviated no more than 20% from response rates at the beginning of the session. Immediately after the second stable baseline session rats received by gastric intubation 400 mg/kg of dl-p-CPA methyl ester (Sigma) as a neutral aqueous suspension. Rats were subsequently tested daily for 180 minutes 1, 2, 3 and 5 days after p-CPA. At the end of the experiment, all rats were given a lethal dose of sodium pentobarbital and perfused transcardially with saline (0.9%) followed by a 10% solution of formalin. After a minimum storage of 7 days in 10% Formalin, brain tissue was sliced in 28 μm sections and stained with thionin. Exact position of electrode tips were compared to König and Klippel's *Stereotaxic Atlas* [13].

Biochemical Assays

Immediately after the third post-drug SS session, 5 MR rats were decapitated. Their brains were quickly removed and dissected in the following manner. The pineal gland and cerebellum were discarded and the hindbrain and the forebrain were separated by an oblique cut extending from the anterior inter-penduncular fossa to the superior colliculi. Serotonin was assayed for hindbrain and forebrain tissue by using the Barchas, Erdelyi and Angwin procedure [3]. Serotonin levels of rats which had self-stimulated following p-CPA administration were compared to 5-HT levels of rats that received either saline (n=5) or p-CPA alone (n=5) 3 days prior to decapitation.

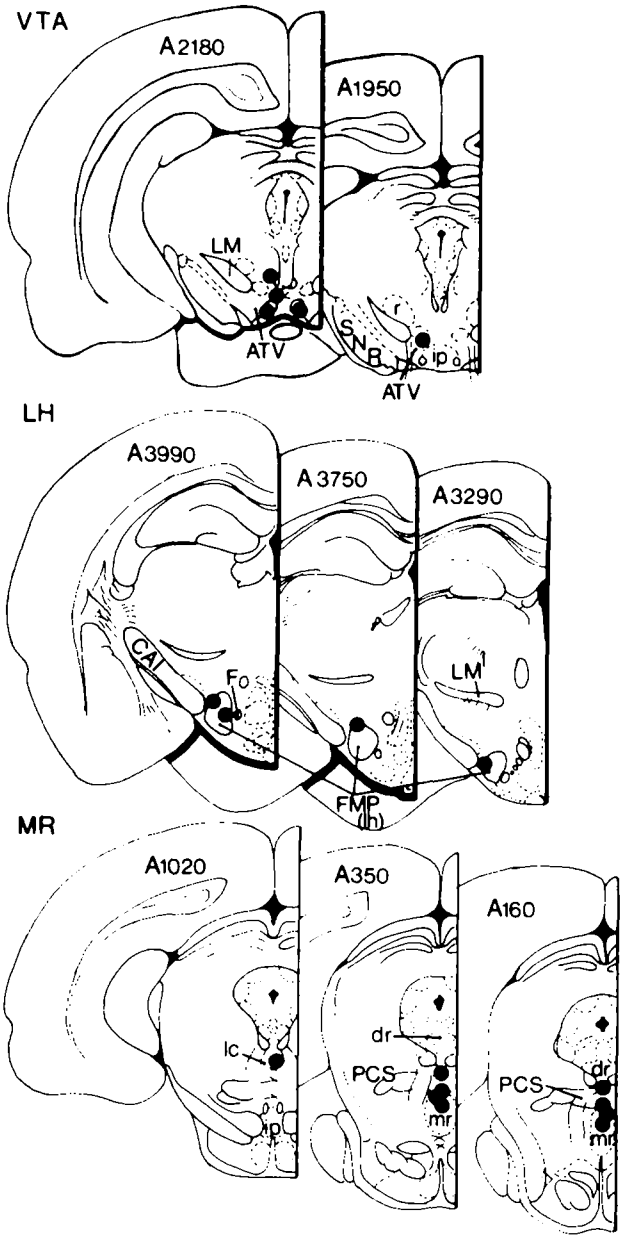

FIG. 1. Electrode placements at the level of the ventral tegmental area (VTA), the lateral hypothalamus (LH) and the medial raphé nucleus (MR). Points represent electrode tips. Abbreviations: ATV: Ventral tegmental area of Tsai, CAI: Internal capsule, dr: Dorsal raphé nucleus, F: fornix, FMP: Medial forebrain bundle, ip: Interpeduncular nucleus, lc: Nucleus linearis caudalis, LM: Medial lemniscus, mr: Medial raphé nucleus, PCS: Superior cerebellar peduncle, r: Red nucleus, SNR: Substantia nigra (reticulata). Drawings taken from König and Klippel [13].

RESULTS

Histology

Figure 1 shows electrode placements for 5 VTA, 4 LH,

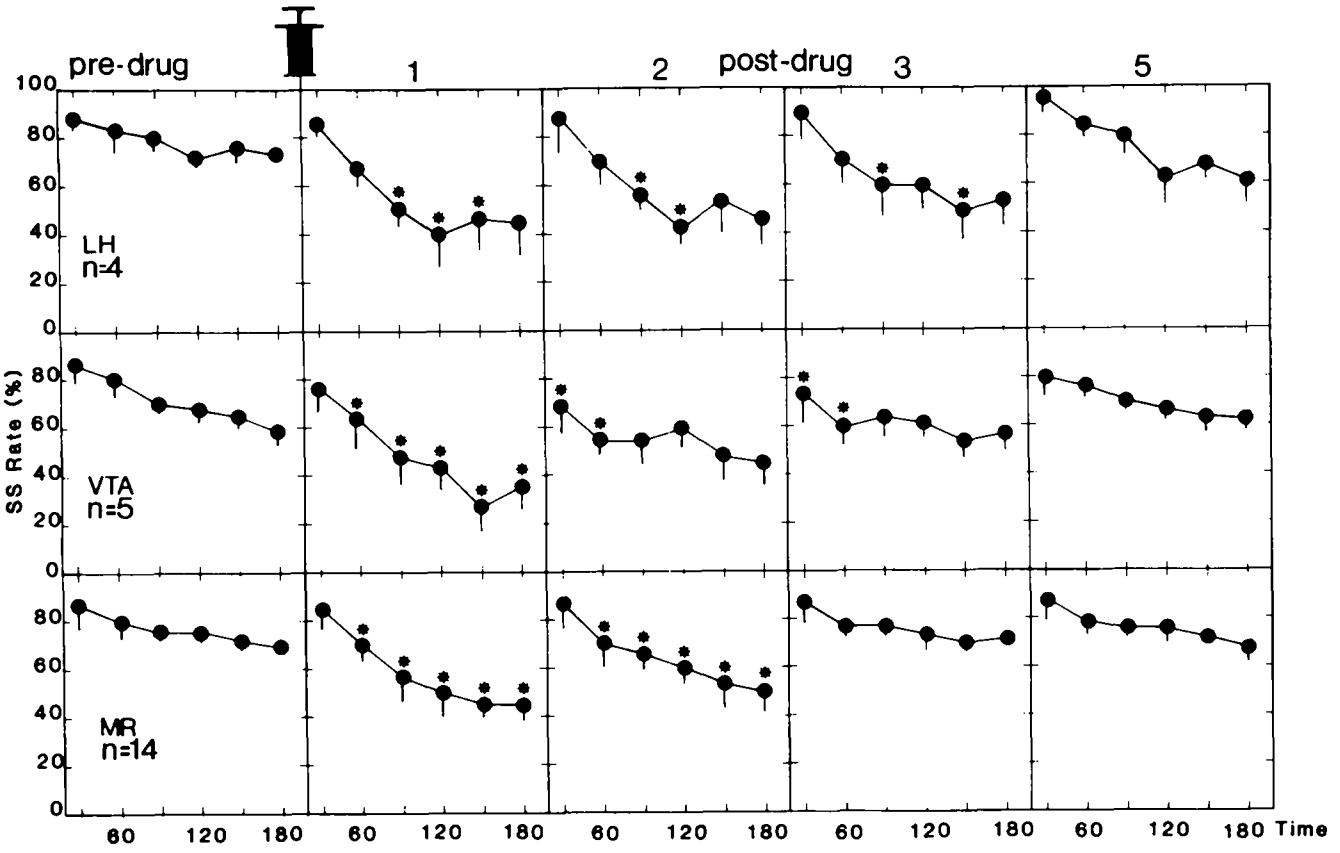

FIG. 2. Comparative effects of para-chlorophenylalanine (400 mg/kg IG) on self-stimulation of the lateral hypothalamus, the ventral tegmental area and the medial raphé nucleus 1, 2, 3 and 5 days after injection. Ordinate: mean lever pressing rate (±S.E.M.) expressed as a percentage of maximal response rate. Abscissa: time of self-stimulation sessions on successive pre and post-drug days. Asterisks represent significant differences between corresponding 30 minute blocks of pre- and post-drug days (L.S.D., $\alpha=0.05$).

and 9 MR rats used in the experiment. Except for 1 MR electrode which fell in the nucleus linearis caudalis, all electrode tips were found to be in or very close to target structures. Because of processing for biochemical analysis after post-drug day 3, the histology for 5 MR rats could not be determined and hence post-drug day 5 shows the average SS rates for only 9 MR rats.

Behavioral Data

Figure 2 shows the effects of p-CPA on SS in the LH, the VTA and the MR nucleus. To facilitate statistical analysis of the data, the two pre-drug sessions for each rat were averaged. One way analysis of variance with repeated measures over SS rates ($\alpha=0.05$) were performed for each 30 minute block of the sessions. Subsequent least significant difference (LSD) post-hoc tests showed that in comparison to pre-drug sessions p-CPA significantly ($\alpha=0.05$) suppressed MR SS 24 and 48 hours after injection whereas VTA and LH SS showed significant suppression on post-drug day 1, 2 and 3. Three days after p-CPA, SS rates in the MR had returned to baseline levels whereas SS rates in the VTA and LH rats showed partial recovery. Five days after p-CPA, SS rates for VTA and LH rats had returned to normal. On post-drug days 1 and 2, rats usually had normal SS rates at the beginning of the session, onset of suppression usually occurring 60 to 90 minutes after the start of the session. Towards the end (last hour) of the first and second post-drug sessions, the rats characteristically went to sleep, intermittently returning to the lever for 5 to 10 minutes of low to moderate SS. However, on post-drug days 2 and 3, VTA rats seemed to show significant reductions of SS rates at the beginning of the session, but maintained a comparatively steady SS rate throughout the rest of the session.

Biochemical Data

Figure 3 shows that all 5 self-stimulating rats used for the biochemical analysis of 5-HT levels had, by post-drug day 3, fully recovered from p-CPA induced suppression of SS and that the significant suppression of SS followed the same time course observed for the 9 other MR rats. Figure 4 shows nanogram levels of 5-HT per gram of forebrain and hindbrain tissue 3 days after either saline, p-CPA alone or p-CPA + SS. Student's t-test comparisons between saline and p-CPA alone conditions showed a significant decrease of 5-HT levels in both the forebrain, $t(8)=19.90$, $p<0.01$, and hindbrain, $t(8)=13.18$, $p<0.01$.

Compared to control levels, 5-HT levels in rats which had

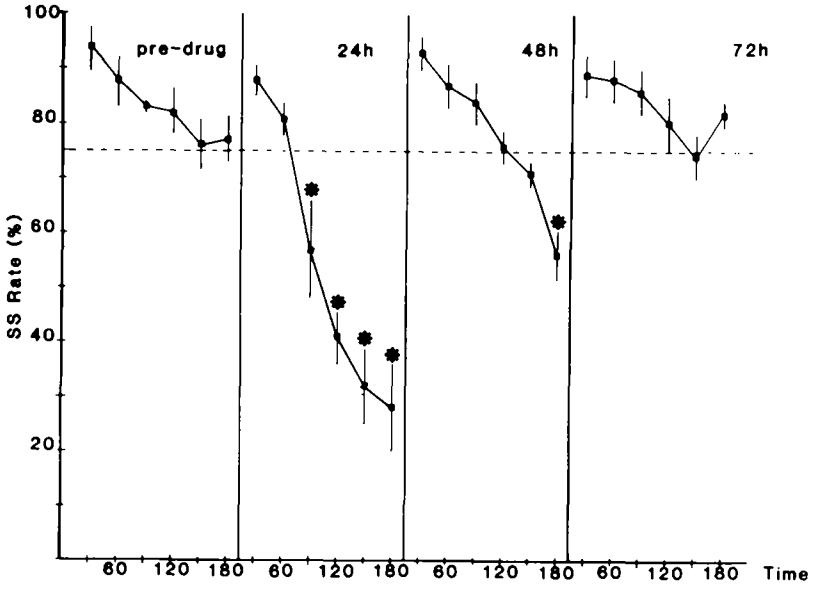

FIG. 3. Effect of para-chlorophenylalanine (400 mg/kg IG) on medial raphé self-stimulation of rats used for analysis of brain serotonin levels. Ordinate: mean lever pressing rate (±S.E.M.) expressed as a percentage of maximal response rate. Abscissa: time of self-stimulation sessions on successive pre and post-drug days. Asterisks represent significant differences between corresponding 30 minute blocks of pre- and post-drug days (L.S.D., $\alpha=0.05$).

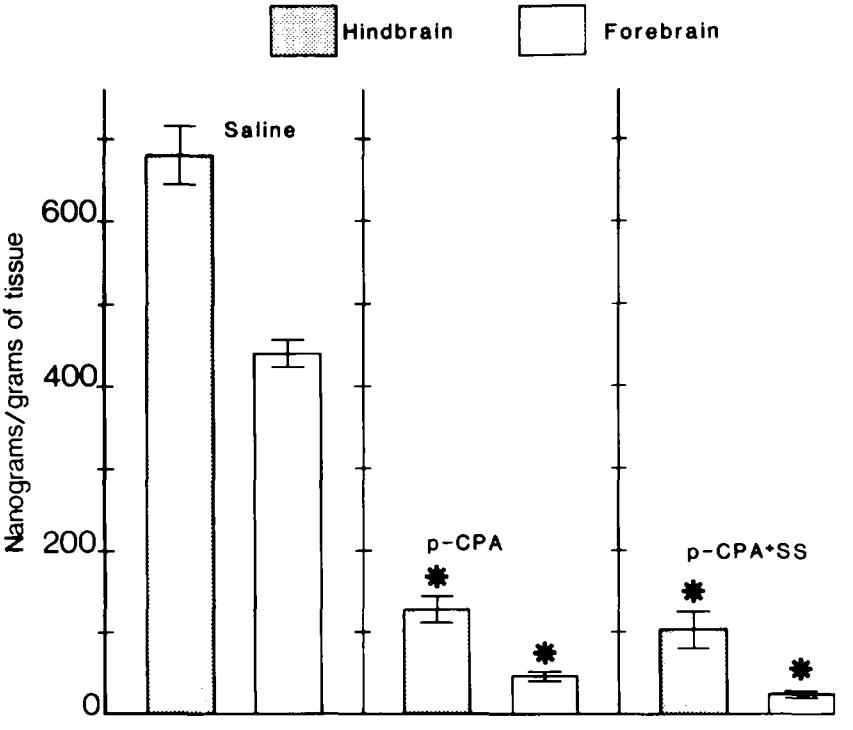

FIG. 4. Mean nanogram levels of serotonin (±S.E.M.) per gram of forebrain and hindbrain tissue three days after either saline (n=5), 400 mg/kg of p-CPA (n=5) or in rats which self-stimulated 3 hours per day for three consecutive days after p-CPA (n=5). Asterisks indicate significantly lower serotonin levels when compared to saline condition (t-test, $\alpha=0.01$).

self-stimulated after p-CPA showed the same significant reduction in both the hindbrain, $t(8)=12.58$, $p<0.01$, and the forebrain, $t(8)=20.99$, $p<0.01$.

DISCUSSION

As shown by previous studies, SS behavior can consistently and reliably be obtained in the MR nucleus region [19,20]. In the present study, SS rates in the MR were comparable to more rostral SS sites, with up to 8,500 lever presses per hour being observed in some MR rats. Several reports have suggested that SS behavior in the midline region of the mesencephalon might have a 5-HT substrate on the basis that p-CPA suppressed MR and DR SS whereas it had no significant inhibitory effects on SS of "catecholaminergic" structures such as the LH and the VTA [16, 17, 26, 27]. Although several studies had obtained DR and MR SS suppression after tryptophan hydroxylase inhibition by p-CPA [8, 15, 22], the hypothesis of a 5-HT mediation of reward had been dismissed on the grounds that SS suppression time course did not match the 5-HT depletion time course established by Koe and Weissman's original study [12]. However the relatively short SS sessions used in these experiments is at least partly responsible for these discrepencies. As suggested earlier [16,17], suppression of MR SS was much more salient when long SS sessions were used. Present results show that SS rates are relatively normal during the first 30 to 60 minutes of the post-drug sessions and that onset of any pronounced suppression became apparent only about 90 minutes after the start of the session. Thus it is possible that previous studies which used comparatively shorter SS sessions, failed to appreciate the full inhibitory effect of p-CPA on SS behavior.

However, even with long SS sessions, the present results still show a temporal dissociation between p-CPA induced suppression of MR SS and depletion of 5-HT and thus are not in agreement with similar studies which have suggested a 5-HT depletion dependant inhibition of raphe SS [16, 17, 27]. In fact, MR SS rates were maximally reduced 24 hours after p-CPA when 5-HT levels are only marginally reduced, while SS behavior returned to baseline rates 3 days post-drug, when 5-HT levels are reported to be at their lowest [12]. Accordingly, biochemical data from the present study indicate that p-CPA produced approximately a 5 to 10 fold reduction (18% and 11% of control in hindbrain & forebrain respectively) in brain 5-HT, 3 days after its injection. Moreover the 5-HT levels found after p-CPA in the present study are in agreement with other studies that have found different amounts of 5-HT in the forebrain and hindbrain after p-CPA [1,10]. In addition present biochemical data show that electrical stimulation of the MR region in no way enhanced 5-HT levels and thus cannot account for the lack of temporal correlation between 5-HT depletion and suppression of SS. In fact 5-HT levels of rats that self-stimulated after p-CPA administration were lower (15% and 6% of control in hindbrain and forebrain respectively) than p-CPA alone rats, suggesting that electrical stimulation increased turnover of available 5-HT.

Although overall brain monoamine levels give a fairly good indication of the state of the 5-HT storage pools after p-CPA, the observed residual 5-HT may reflect the neuron's ability to synthesize functionally significant amounts of serotonin despite the p-CPA treatment. The possibility that newly synthesized 5-HT might be sufficient to maintain SS behavior cannot be ruled out from the present data. Consequently it may be that recovery from p-CPA induced suppression of SS behavior is more directly linked to the return of normal tryptophan hydroxylase activity than to normal 5-HT levels. However, given that tryptophan hydroxylase is the rate limiting step in serotonin production and that its decrease in activity after p-CPA follows a time course similar to 5-HT levels, one would expect that the suppression of serotonin synthesis and the changes in SS behavior should still have matching time courses. Nonetheless, being that tryptophan hydroxylase activity after p-CPA was not measured in the present study, the possibility that the return of normal SS behavior is dependent on a yet unknown threshold rate of synthesis cannot be dismissed at this time.

Finally, contrary to previous reports [16, 17, 26, 27] no differential sensitivity to p-CPA induced reduction of 5-HT could be found between MR SS and VTA and LH SS. Although recovery from suppression was comparatively longer in VTA and LH rats than in MR rats, maximum inhibition of SS in all three groups of rats occurred 24 hours after p-CPA and the general configuration of daily suppression and recovery curves were similar for all groups. However, the suppression of LH and VTA SS obtained in this study after p-CPA is not consistent with other studies which have shown either facilitation of LH [5, 18, 26] and VTA SS [17] or no effect on both VTA [16,25] and LH SS [4,25]. The absence of any effect or the enhancing action of p-CPA on VTA and LH SS reported in these studies are difficult to reconcile with the present data, especially in view of the fact that some of these experiments [16, 17, 26] utilized testing paradigms (i.e., long SS sessions) similar to the one used in the present study. It may be that self-stimulation testing on post-drug days 1 and 2 in some way precluded the expression of the excitatory effect on post-drug day 3 reported by others. However unlikely, this cannot be ruled out and would represent a complex interaction of p-CPA with non-serotonergic mechanisms involved in post-drug days 1 and 2 self-stimulation. Whatever the explanation for the difference between, on the one hand the present study and others that fail to show any facilitation of self-stimulation after p-CPA [11, 23, 24] and on the other, the four studies that do indicate such an effect [5, 17, 18, 26], it does not appear that serotonin can be the transmitter in the system directly activated by the rewarding stimulation.

Although the time course of p-CPA's action on SS does not correspond with its action on 5-HT levels, it is evident that this drug does have inhibitory properties on SS behavior at least 24 hours after its injection. This raises the possibility that p-CPA might act on SS behavior through another neurochemical mechanism. It has already been shown that p-CPA causes a slight (10 to 20%) transitory reduction in brain norepinephrine (NE) 6 to 24 hours after its injection [12]. Based on this effect several authors have suggested that suppression of SS behavior by p-CPA is more directly related to its NE action and in doing so have proposed that SS behavior obtained from the midline central gray region is mediated by fibers of the dorsal NE bundle (DNB) [15,22]. Although this hypothesis reflects the anatomical proximity of NE fibers to SS sites in this region, it is doubtful that such small reductions in brain NE can cause such a large decrease in SS rates. Studies involving lesions of the locus coeruleus [6,14] or the DNB [6,7] clearly indicate that SS behavior is fairly resistant to more dramatic reductions of brain NE. However the possibility that small reductions of NE levels might cause a generalized decrease in activity or a greater susceptibility to fatigue cannot be dismissed. Thus it is plausible that because

of fatigue, the rats could not sustain the physical effort necessary to maintain high lever pressing rates for long periods of time. The fact that p-CPA uniformly suppressed SS in the three structures tested could lead us to speculate that this might be the case.

In conclusion, the present data strongly suggest that 5-HT is not critically involved in MR SS behavior. Although the present study does not give any indication as to what neurochemical system subserves SS behavior in this region of the midbrain, it does suggest that p-CPA's inhibitory action on SS behavior might be more closely linked to its acute nonspecific effects and that this drug might not be ideally suited for behavioral studies.

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