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MONOAMINE INVOLVEMENT IN HIPPOCAMPAL SELF-STIMULATION

by

DEREK VAN DER KOOY

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Department of PSYCHOLOGY

The University of British Columbia
2075 Wesbrook Place
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ABSTRACT

The roles of the noradrenergic and serotonergic innervations of the hippocampus were investigated with respect to their involvement in the intracranial self-stimulation of this structure. In the first study, 6-hydroxydopamine-induced lesions of the dorsal tegmental noradrenergic bundle ascending to the forebrain had no effect on hippocampal self-stimulation in rats. In the second study, intragastric administration of para-chlorophenylalanine decreased hippocampal self-stimulation, suggesting the importance of a serotonin input in maintaining this behavior. Identical para-chlorophenylalanine treatments resulted in temporary depletions of brain serotonin which paralleled the changes in hippocampal self-stimulation. The maximal decreases in both the biochemical and behavioral measures occurred at 4 days' post-drug. Interpretations of this deficit in hippocampal self-stimulation in terms of gross sensory and/or motor changes were ruled out, as animals with lateral hypothalamic electrodes showed increases in self-stimulation paralleling the post-drug serotonin changes. An intrasessional analysis of the para-chlorophenylalanine-induced behavioral changes revealed that lateral hypothalamic self-stimulation was facilitated mainly during the first hr of the 2 hr test sessions, whereas the depression in hippocampal self-stimulation occurred primarily in the last hour of the sessions. The differential effects of para-chlorophenylalanine on lateral hypothalamic and hippocampal self-stimulation provide evidence against simple monoamine theories of reinforcement.

A. G. Phillips

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Experimentation on the neurochemical substrates of reinforcement has emphasized monoaminergic mechanism^{17,56}. Until recently most studies of intracranial self-stimulation (ICSS) have employed electrode placements in the medial forebrain bundle. However, in attempts to factor out the involvement of norepinephrine (NE), dopamine (DA) and serotonin (5-HT) in ICSS, many studies now use electrode placements specifically in the cell body or terminal regions of one of these monoamine systems. Some of these studies have been particularly enlightening, as they have demonstrated that manipulation of a particular monoamine system may have effects on ICSS that are specific to the locus of the electrical stimulation. For example, ipsilateral 6-hydroxydopamine (6-OHDA)-induced lesions of the nigro-neostriatal DA system produce a depression of ICSS at sites in a DA terminal area, the caudate nucleus³¹, but leave ICSS of the DA cell body in the substantia nigra relatively unaffected⁸. Somewhat similarly, intragastric administration of parachlorophenylalanine (PCPA), a drug which inhibits the biosynthesis of 5-HT²¹, decreases ICSS in the caudate but increases ICSS in the lateral hypothalamus³².

One area in the brain containing monoamine terminals which has been little studied in relation to ICSS is the hippocampus. ICSS in the hippocampus has been previously demonstrated⁴⁹ and thus motivational functions must be added to the myriad of other behavioral functions in which the hippocampus has been implicated⁴. The unique monoamine innervation of the hippocampus makes it an ideal locus in which to study monoamine involvement in ICSS. Both NE and 5-HT are found in significant amounts in the hippocampus²⁸, however, the DA content of the hippocampus is within the range expected for a precursor

of NE in NE-producing neurons¹³. The NE innervation of the hippocampus appears to arise entirely from the locus coeruleus^{28,35,41}, whereas the 5-HT innervation comes primarily from the median raphe nucleus^{11,20,29}. The 5-HT innervation of the hippocampus overlaps the NE innervation in its terminal distribution²⁸. Moreover, when iontophoretically applied both NE⁴⁴ and 5-HT⁴³ inhibit the firing of hippocampal pyramidal cells. The present experiments, then, were designed to investigate the involvement in hippocampal ICSS of these topographically similar NE and 5-HT innervations.

In a preliminary study it was shown that 6-OHDA induced depletions of hippocampal NE did not significantly affect hippocampal ICSS³⁴. However, in that study animals were run only in 15 min test sessions, and it has recently been suggested that test sessions of longer duration might be more likely to reveal ICSS deficits after monoamine manipulations²⁵. In order to investigate this possibility the first experiment of the present study employed 2 hr test sessions in investigating hippocampal ICSS after NE depletion. The second experiment compared hippocampal ICSS to ICSS in a lateral hypothalamic (LH) control group, after depletions of 5-HT with intragastrically administered PCPA.

METHODS

General procedure:

Subjects were male Wistar rats weighing 280-320 g at the time of surgery. Animals were anesthetized with sodium pentobarbital, placed in a Kopf stereotaxic apparatus, and small diameter bipolar nichrome electrodes (Plastic Products Co. MS 303-0, .005 in.) were chronically implanted according to standard procedures. The coordinates for the hippocampal placements were AP 1.6 mm posterior to bregma, L + 3.0 mm,

and DV 3.8 mm below dura, with the mouthbar 5.0 mm above the interaural line, and for the LH placements AP-3.2 mm, L + 1.5 mm, and DV-8.6 mm, with the mouthbar 4.2 mm below the interaural line. Electrodes were aimed at the lateral part of the dorsal hippocampus in the CA3 zone as previous research^{34,49} has shown this area to be most supportive of ICSS.

Following recovery from surgery, animals were tested for ICSS in 5 identical plexiglass chambers (46 cm x 30 cm x 24 cm). Depression of a small 2.5 cm wide bar activated an AC constant current stimulator which delivered a variable intensity (1-150 μ A) 60 HZ sine wave stimulus of fixed duration (0.2 sec) through a flexible cable, to the chronic electrode assembly. Animals were shaped to the lever for 30 min/day until the response was acquired. After 7 days rats not reaching a criterion of 200 bar-presses/2 hr were rejected from the experiment. Stimulation intensities were individually adjusted to elicit optimal rates. Prior to experimental treatments all animals received 2 hr test sessions, on alternate days, until ICSS rates had stabilized.

Experiment 1: Effects of NE depletion on hippocampal ICSS

Thirteen hippocampal ICSS animals were included in the first experiment. After ICSS responding had stabilized 9 animals were prepared for intracranial injections of 6-OHDA hydrobromide (4 μ g/2 μ l, dosage expressed as the base), and 4 control animals received vehicle injections. The 6-OHDA was dissolved in a 0.15 M saline vehicle which contained 0.2 mg/ml ascorbic acid, and was injected intracerebrally at a rate of 0.4 μ l/min. Lesions were made bilaterally, after acute insertions of a fine 34-gauge cannula into the mesencephalic trajectory of the dorsal tegmental NE bundle. Coordinates were AP + 2.5 mm, L + 1.1 mm, and DV + 3.7 mm from stereotaxic zero (Kopf) with the mouthbar - 4.2 mm

below the interaural line. Testing (2 hr ICSS sessions) was resumed on the second day post-lesion and continued on alternate days until day 16 post-lesion.

At the termination of testing the animals were sacrificed by cervical fracture, their brains removed, dissected on ice, and NE was measured in the ipsilateral hippocampus²⁴.

Experiment 2: Effects of PCPA on brain 5-HT, hippocampal ICSS and LH ICSS.

Biochemical procedure:

Previous studies of the effects of PCPA on brain 5-HT have employed the intraperitoneal route of drug administration^{21,26}. In an attempt to minimize the non-specific peripheral effects of PCPA injections which can be problematic in the interpretation of behavioral experiments⁴⁸, we utilized the intragastric route of drug administration. To determine the effects of intragastric PCPA on brain 5-HT, 36 rats were studied independently of the ICSS portion of the experiment. Twenty-four animals were placed under light ether anesthesia and then intubated with a 400 mg/kg dose of PCPA, as a suspension in H₂O. On each of days 2, 3, 4, 6, 9 and 12 after PCPA treatment, 4 PCPA and 2 control rats were sacrificed by cervical fracture, their brains removed and 5-HT content of the whole brain determined by biochemical assay⁵².

Four additional rats were treated with PCPA in the manner described above, to further determine if the PCPA-induced depletions of 5-HT were evenly distributed in the brain areas to be studied in the behavioral portion of this experiment. On day 4 post-drug, 5-HT content was measured in both the hippocampus and hypothalamus of the 4 experimental rats and compared to values obtained from 4 control animals.

Behavioral procedure:

Ten animals with hippocampal electrodes and seven animals with lateral hypothalamic electrodes were employed in the second ICSS experiment. After ICSS responding had stabilized all animals were intubated with PCPA (400 mg/kg) in an identical manner to the rats prepared for biochemical analysis in the first portion of this experiment. Two hr ICSS test sessions resumed on the second post-drug day and continued on alternate days until day 10 post-PCPA.

It has been shown that the depressive effect of PCPA on median raphe ICSS is differentially distributed within a 2 hr test session, with the largest effect occurring towards the end of the session²⁵. For this reason measures of bar-press responding during each 30 min period of the test sessions were obtained for all animals. At the completion of the experiment, subjects were asphyxiated with CO₂, their brains rapidly removed and stored in 10% formalin. Brains were frozen, sectioned at 40 μ and sections containing electrode tracts were mounted and stained with Luxol fast blue and counterstained with cresyl violet.

RESULTS

Experiment 1: Effects of NE depletion on hippocampal ICSS

Biochemical assays:

The effect of bilateral mesencephalic injections of 6-OHDA on the levels of NE in the hippocampus ipsilateral to the stimulating electrode are summarized in Table I. The mean level of hippocampal NE in the lesioned group was 3.36% of that in the vehicle injected control group. All 9 lesioned animals had NE levels that were less than 6.0% of the control group level. The assay technique used is sensitive to 2 ng.

ICSS electrode sites:

Histological examination of electrode placements was foregone in favor of the biochemical analysis in the present experiment. However, other animals prepared in the identical manner for inclusion in the second ICSS experiment, were shown to have electrodes terminating in the hippocampal formation. Examples of 2 such placements are shown in Fig. 1.

Behavioral analysis:

The results of this experiment clearly show that the NE innervation of the hippocampus is not necessary for the maintenance of ICSS in this structure. As seen in Fig. 2, ICSS continued at rates comparable to those seen prior to the experimental treatment, both in the group with severe depletions of hippocampal NE induced by 6-OHDA and in the vehicle control group. An analysis of variance indicated an insignificant main effect of groups [$F(1,11) = 1.06, p > .20$].

The present experiment, employing 2 hr test sessions, confirms a preliminary report³⁴ demonstrating a failure to affect short duration sessions (15 min/day) of hippocampal ICSS through NE denervation of the hippocampus. As such, it shows that even the utilization of long duration ICSS test sessions, as a more sensitive measure of behavioral deficits after monoamine manipulations^{8,25}, does not provide evidence for NE involvement in hippocampal ICSS. Alternate substrates of hippocampal ICSS must be considered.

Experiment 2: Effects of PCPA on brain 5-HT, hippocampal ICSS and LH ICSS.

Biochemical analysis:

The present experiment appears to be the first report of brain 5-HT levels after PCPA administered via the intragastric route. Fig. 3 shows the profound effect of intragastric PCPA (400 mg/kg) on whole

TABLE I. Effect of bilateral 6-OHDA injections into dorsal tegmental NE bundle on NE levels in ipsilateral hippocampus.

Group	N	Ipsilateral hippocampal NE ($\mu\text{g/g}$)	% of vehicle control
Vehicle control	4	$.358 \pm .022$	
6-OHDA	9	$.011 \pm .001$	3.3

Data represent means ($\pm$ S.E.M.)

Figure 1

Photomicrographs depicting electrode placements in 2 hippocampal ICSS animals.

Figure 2

Effects of 6-OHDA lesions (—○—) of the dorsal tegmental NE bundle or vehicle control injections (—●—) on hippocampal ICSS. Data represent means ($\pm$ S.E.M.).

brain 5-HT levels. Depletions of 5-HT are greatest, down to 20% of control, on day 4 post-PCPA and recover to baseline levels by day 12 post-PCPA. In Fig. 3, each of the mean 5-HT levels on the days after PCPA is expressed as a percentage of the 5-HT levels of control animals sacrificed on that same day. The mean whole brain 5-HT level for all the control animals ($n = 12$) was $.585 \mu\text{g/gm}$. Statistical analysis of the data was obviated in view of the lack of overlap between days in the biochemistry of the individual animals, either during the depletion or during the recovery of 5-HT levels.

The time course of 5-HT depletion and recovery reported here for intragastric intubation of 5-HT is very similar to that found after intraperitoneal injections of comparable doses of PCPA^{21,26}. However, the intragastric route of administration of PCPA may be a more appropriate method for depleting 5-HT in behavioral experiments, as peritoneal irritation may result from the intraperitoneal injection of α -methyl-tyrosine, PCPA and other insoluble compounds⁴⁸.

PCPA treatment depletes 5-HT to a comparable degree in the hippocampus and hypothalamus, as shown in Table II. Thus, on day 4 post-PCPA, both of the areas employed as ICSS electrode sites in the behavioral portion of this experiment, show 5-HT levels that are approximately 20% of control.

ICSS electrode sites:

The histology for 5 hippocampal ICSS animals was unavailable. However, the remaining hippocampal ICSS animals and all the LH ICSS animals had electrodes terminating in the hippocampal formation and lateral hypothalamic area, respectively. Examples of 3 hippocampal ICSS placements from the present experiment are shown in Fig. 1 and Fig. 5 (C).

Figure 3

Effects of intragastrically administered PCPA (400 mg/kg) on whole brain 5-HT levels. Data represent means ($\pm$ S.E.M.).

Typically, ICSS placements in the hippocampal formation occur in the anterior portion of the dorsal hippocampus. Most placements are lateral to the dentate gyrus in the CA3 pyramidal zone, with some positive placements extending even farther laterally into the fimbria. Our ICSS placements generally correspond to the previous description of ICSS in the hippocampus⁴⁹.

The histology from 1 representative LH ICSS animal in the present experiment is shown in Fig. 5 (B). All LH ICSS animals had electrodes terminating in this same area of the hypothalamus lateral to the fornix, although some placements were situated more dorsally in the LH.

Behavioral analysis:

As may be seen in Fig. 4, treatment with PCPA produced either a facilitation or depression of ICSS, depending on electrode placement. Hippocampal ICSS animals showed decreased responding for several days after PCPA and LH ICSS animals showed increased responding over the same time period. Both groups returned to baseline rates by day 10 post-PCPA. The time courses for the effects in both groups, peaking at day 4 post-drug, are almost identical to the time course for whole brain 5-HT depletion after intragastric PCPA (Fig. 3).

An analysis of variance performed on the raw data revealed a significant effect of groups [$F(1,15) = 21.49, p < .001$], which reflected, in part; the different baseline ICSS rates in the LH ($\bar{X} = 5678/2hr$) and hippocampal ($\bar{X} = 1083/2hr$) groups. The main effect of PCPA over time was not significant [$F(1,15) = 0.62, p > .20$], as the opposite effects of the drug in the two groups counteracted each other. However, the effect of the groups X PCPA interaction was significant [$F(1,15) = 5.53, p < .05$]. A trend analysis of the groups X PCPA interaction revealed a significant quadratic effect [$F(1,15) = 20.88, p < .001$], reflecting the reciprocal

TABLE II. Effect of intragastrically administered PCPA on 5-HT levels in the hippocampus and hypothalamus 4 days' post-drug.

Group	N	Hippocampus 5-HT ($\mu\text{g/g}$)	Hypothalamus 5-HT ($\mu\text{g/g}$)
Control	4	$0.665 \pm .01$	1.46 ± 0.04
PCPA	4	$0.132 \pm .07$	0.35 ± 0.02
% of control		19.8	24.0

Data represent means ($\pm$ S.E.M.)

Figure 4

Effects of intragastrically administered PCPA (400 mg/kg) on hippocampal (—○—) and LH (—●—) ICSS. Data represent means ($\pm$ S.E.M.).

changes in the rates of the two groups for several days post-PCPA, and then the return to baseline rates by day 10 after the drug in both groups.

Both LH and hippocampal animals were tested at near-optimal ICSS rates. For this reason the increase in LH ICSS after PCPA (110% of baseline on day 4 post-drug) was necessarily limited to a small effect, and not one as dramatic as that seen when LH animals are tested at below optimal rates³². However, the increase in LH ICSS on day 4 post-PCPA was observed in all 7 LH animals compared to their individual rates on the day prior to the drug.

The substantially different baseline ICSS rates of the LH and hippocampal groups cannot explain the effects of PCPA seen in the present experiment. As mentioned previously, LH ICSS animals tested at less than optimal rates still show increases in ICSS after PCPA³². In addition, raphe ICSS animals tested at rates comparable to those of the LH group in the present experiment, show a depression in ICSS after PCPA²⁵ (van der Kooy, Fibiger and Phillips, unpublished observations). Further evidence that the differential effects of PCPA are not attributable to rate differences comes from the individual data of some LH and hippocampal animals whose ICSS rates were similar. Fig. 5 presents the behavioral data and histology of a LH animal with lower than average ICSS rates and a hippocampal animal with higher than average ICSS rates. The depressive effect of PCPA on hippocampal ICSS and facilitative effect of PCPA on LH ICSS are clearly evident.

Intrasessional ICSS effects of PCPA:

The effects of PCPA on ICSS are not evenly distributed throughout the 2 hr test sessions. Fig. 6 shows the change in ICSS during each 30 min period within the 2 hr test session on day 4 post-

Figure 5 (A)

Effects of intragastrically administered PCPA (400 mg/kg) on one "high ICSS rate" hippocampal subject (∞) and one "low ICSS rate" LH subject (●).

Figure 5 (B)

Photomicrograph depicting electrode placement of the "high ICSS rate" hippocampal animal.

Figure 5 (C)

Photomicrograph depicting electrode placement of the "low ICSS rate" LH animal.

Figure 6

Intrasessional analysis of ICSS responding on day 4 post-PCPA in LH (open bars) and hippocampal (filled bars) animals. Data represent means ($\pm$ S.E.M.) for each of the four 30 min periods comprising the 2 hr test sessions, expressed as a % of the rates obtained on the day prior to PCPA. * indicates a significant difference from control.

ICSS RATE/30 MIN. (% OF PRE-DRUG)

PCPA, as a percentage of each comparable 30 min period on the baseline day prior to PCPA. In both groups there is a decline in ICSS rates as the session progresses. The analysis of variance performed on the raw data revealed a significant main effect of intrasessional periods [$F(1,15) = 15.70, p < .01$]. A trend analysis on the intrasessional periods showed a significant linear effect [$F(1,15) = 25.24, p < .001$], reflecting the decrease in rates from the first 30 min period through to the last of the four 30 min periods.

There was, however, a substantial difference between groups on day 4 post-PCPA in the manner in which ICSS rates decreased across periods. The LH group showed ICSS rates higher than baseline during the first two 30 min periods, which then decreased to baseline rates over the last two 30 min periods. In contrast, the hippocampal group showed decreased ICSS rates during the first 30 min period, which then decreased even further over the next 3 periods. These differences in responding between the individual 30 min periods on day 4 post-PCPA (the time of maximal 5-HT depletion) and the periods on the day prior to the drug were analyzed for both groups using planned orthogonal comparisons. After PCPA the LH group showed increases in ICSS rates only during the 0-30 min period ($p < .10$) and the 30-60 min period ($p < .05$). The differences from 60-90 min ($p > .25$) and 90-120 min ($p > .25$) were not significant. The hippocampal group showed decreases in ICSS rates during all periods: 0-30 min ($p < .10$), 30-60 ($p < .15$), 60-90 min ($p < .05$), and 90-120 min ($p < .01$).

The intrasessional analysis of the present experiment has shown that PCPA treatment facilitates LH ICSS primarily during the first hour of the sessions and depresses hippocampal ICSS to a greater

extent during the second hour of the sessions. It confirms a previous report demonstrating facilitation of short duration test sessions of LH ICSS using PCPA³². The linear intrasessional decrease in hippocampal ICSS rates after PCPA is similar in form to that reported for median raphe ICSS after PCPA²⁵.

DISCUSSION

Comparison of experimental treatments:

The present experiments demonstrate that decreasing the 5-HT input to the hippocampus suppresses hippocampal ICSS, whereas decreasing the NE input has no effect on ICSS in this structure. However, in so studying monoamine involvement in hippocampal ICSS, different methods were used in depriving the hippocampus of its 5-HT and NE input. A permanent 6-OHDA-induced lesion of NE neurons is very different from a temporary PCPA-induced inhibition of synthesis in 5-HT neurons. Nevertheless, it is difficult to argue that the failure to affect hippocampal ICSS after 6-OHDA-induced lesions reflects a less severe neurochemical manipulation compared to that induced by PCPA treatment. Lesions induced by 6-OHDA decreased NE levels in the hippocampus to 3.3% of control levels, whereas a dose of PCPA that proved behaviorally effective in decreasing hippocampal ICSS resulted in a maximal depletion of 5-HT to only 20% of control levels. Another possible explanation for the failure to affect ICSS after 6-OHDA lesions is the development of post-synaptic supersensitivity which, in combination with the few surviving catecholamine afferents, may have been sufficient to sustain ICSS in the hippocampus. At present, however, there are no data available to indicate that behavioral compensation can be accomplished by

denervation supersensitivity in telencephalic NE sensitive neurons.

In fact, recent data showing that neurotoxic lesions identical to those used in this experiment, profoundly influence morphine analgesia³⁸ would appear to argue against such a compensatory role.

The intragastric intubation of PCPA is not without its problems when utilized as a tool for investigating behavioral effects of reduced 5-HT levels. The administration of PCPA also results in the fall of NE levels^{2,53} and may result in changes in adrenal function⁵⁵, although it is unlikely that these changes mediate the effects on ICSS observed in the present experiment. The increase in LH ICSS and the decrease in hippocampal rates, as well as the decrease seen in caudate ICSS rates³², all follow the time course of 5-HT depletion after PCPA, peaking at day 4 post-drug. Brain NE levels, on the other hand, are reduced for a shorter time span and appear to return to pre-drug baseline before day 2 post-PCPA^{2,53}. PCPA effects on adrenal functioning also show a very short time course, with the increased corticosterone levels returning to control values over several hours post-drug⁴⁸.

Nature of the changes in ICSS after PCPA:

The increase in LH ICSS and the decrease in hippocampal ICSS after PCPA have been shown here to follow the time course of brain 5-HT depletion produced by the drug. For this reason it is likely that the ICSS changes are mediated at some level by the changes in 5-HT concentrations. It is always possible that PCPA may affect some as yet uninvestigated hippocampal transmitter, such as histamine³, over a time course similar to 5-HT and thus mediate the changes in ICSS. However, at present no evidence exists to support this or other similar possibilities. Furthermore, the differential effects of PCPA on hippocampal

and LH ICSS cannot be due to differential effects of the drug on 5-HT levels at the 2 electrode sites. 5-HT appears to be approximately equally depleted in the two areas after PCPA, whether measured biochemically (Table II) or by histochemical fluorescence¹.

The opposite effects of PCPA on LH and hippocampal ICSS have implications for neuroanatomical perspectives on ICSS. Prior to the present study, hippocampal ICSS in general could have been conceived of as a simple facilitation, by way of the fimbria and fornix³⁰, of "more primary" reinforcement systems in the lateral hypothalamus. However, the opposite effects of PCPA administration on LH and hippocampal ICSS suggests at least partial independence of the two ICSS systems. This suggestion is supported by evidence from a lesion study differentiating fornical and LH ICSS⁷.

The depression of hippocampal ICSS cannot be due to any gross sensory and/or motor changes produced by PCPA, as LH ICSS was not depressed after the drug. However, several possible explanations of the results, other than one hypothesizing PCPA-induced modifications of the reinforcing values of the brain stimuli, do exist. Complex interactions²³ between PCPA and different behaviors elicited by the stimulation at LH as compared to hippocampal sites might explain the ICSS effects observed. For instance, PCPA produces changes in activity^{16,19} and aggressive behavior²⁷. If brain stimulation elicits more activity, for example, at LH sites as compared to hippocampal sites, then conceivably the PCPA-induced activity changes might differentially interact with the brain stimulation-induced activity at the 2 sites to produce the observed effects on bar-press responding. However, at present it is not clear how any of these interactions would produce opposite effects on

ICSS in the 2 groups.

The problem discussed in the preceding paragraph is a familiar one to researchers studying neurochemical-behavioral relationships. It is the problem of specifying cause-effect mechanisms based on correlations between a neurochemical variable and a behavioral variable. In analyzing the present data two possible explanatory mechanisms exist. First, as mentioned above, PCPA-induced changes in the excitability of certain sensory and/or motor mechanisms (for example, locomotor activity) may interact with the behavior elicited differentially at the 2 electrode sites to mediate the ICSS changes observed. Alternatively, the depletion of 5-HT levels after PCPA may directly and differentially alter the reinforcing value of the brain stimulation at the hippocampal and LH sites. These changes in the reinforcing value of the stimulation might then mediate the other behavioral changes observed (again for example, locomotor activity); the reinforcement changes might exist independently of the activity changes; or finally, they might actually be the same change if theories of reinforcement suggesting isomorphism between reinforcement and the activity in neural pathways underlying species-specific behaviors, are correct^{18,50}. The only means of differentiating between these complex interactive explanations is through a more detailed analysis of the behavioral, neurophysiological and neurochemical changes that occur after PCPA. As the description of the changes induced by PCPA at each level becomes more precise, the relationship between the effects at the neurochemical level and those at the behavioral level (and thus the nature of the ICSS effects) should be more easily specifiable.

In the present experiment, the intrasessional analysis of

the PCPA effects on LH and hippocampus ICSS (Fig. 6) may provide some initial data in the more detailed behavioral characterization of post-drug ICSS changes. At the time of maximal 5-HT depletion (4 days' post-PCPA), the increase in LH ICSS occurs primarily during the first hour of the 2 hr session, whereas the decrease in hippocampus ICSS is maximal during the last hour of the session. A gradual decrease in median raphe ICSS within test sessions after PCPA treatment has been attributed to the progressive disappearance of remaining brain 5-HT, which had been partially maintaining ICSS until the electrical stimulation depleted 5-HT levels even further²⁵. However, there is no independent evidence that electrical stimulation further depletes 5-HT levels in neurons previously depleted by PCPA administration. In addition, it is difficult to relate this further depletion after electrical stimulation to the increase in LH ICSS that occurs during the initial portion of the test sessions. Nevertheless, similar attempts in the future to correlate more discrete behavioral with more subtle biochemical changes after PCPA treatment offer the only solution to characterizing the nature of the ICSS changes observed here.

Theories of monoamine involvement in ICSS:

Theories of ICSS emphasizing one monoamine system as the neural basis of reinforcement, if not doomed in principle, have received little or no support in recent studies. The discovery that ICSS could be elicited from sites corresponding to the NE cell bodies in the locus coeruleus^{15,40} provided initial support for the role of NE in ICSS^{14,17,46}. However, the "NE theory" has not been supported in recent tests of this theory employing specific lesions of central NE pathways. For example, locus coeruleus ICSS survives 6-OHDA-induced

lesions severely depleting forebrain⁹ or whole brain NE¹², and ICSS in more anterior brain areas survives lesions of the locus coeruleus^{10,22}. In addition, the present results and those of a preliminary study³⁴ revealed that ICSS in telencephalic areas is not affected by nearly complete denervation of the NE input to those areas. Furthermore, recent studies have suggested the involvement of other non-catecholaminergic systems in ICSS elicited from the area of the locus coeruleus^{42,51}.

Theories of reinforcement emphasizing the action of an inhibitory or aversive 5-HT system^{39,56} have also not been completely consistent with current research. Studies employing LH ICSS electrode placements generally show increases in ICSS after PCPA treatment^{5,32,36} (the present results). Similarly, the neurotoxic lesion of 5-HT neurons by intraventricular 5,6-dihydroxytryptamine increases LH ICSS³⁷, and the administration of a 5-HT precursor, 5-hydroxytryptophan, depresses LH ICSS⁶. However, the utilization of ICSS electrode placements specifically in 5-HT cell body or terminal areas gives very different results after 5-HT manipulations. ICSS in forebrain areas innervated by 5-HT neurons, such as the caudate³² and the hippocampus (the present experiment), is suppressed after PCPA treatment. The effects of PCPA on ICSS elicited from sites corresponding to the 5-HT cell bodies appear to depend on electrode placement. Increases in dorsal raphe ICSS⁴⁷ and decreases in median raphe ICSS²⁵ have been reported after PCPA. Recent data from our laboratory (van der Kooy, Fibiger and Phillips, unpublished observations) have shown a small suppression of raphe ICSS that is a function of electrode placement, duration of test session and baseline rate of responding.

The differential effects on hippocampal and LH ICSS in the present experiments, as well as the literature cited above, clearly show that the effects of PCPA on ICSS are dependent on electrode placement. Conceptually similar effects on ICSS occur after the manipulation of central DA systems. 6-OHDA-induced lesions of the nigrostriatal bundle depress ICSS in one area, the caudate³², and leave relatively unaffected ICSS in another area, the substantia nigra⁸. Neither of these placement dependent series of effects (after either DA or 5-HT manipulations) can be explained by theories of intracranial reinforcement that emphasize one transmitter system which "codes" reinforcement functions in the brain.

Although theoretically parsimonious, the idea that a single neurotransmitter system subserves reinforcement functions in the brain now seems simplistic. As the present experiments show, a certain neurochemical manipulation may have opposite effect on ICSS depending on electrode site. Moreover, ICSS can be elicited from very diverse brain areas, including primary sensory systems in the olfactory bulb³³ and final motor pathways in the trigeminal nucleus⁵¹. Therefore, one reason for the correlation between ICSS electrode sites and catecholamine neurons¹⁷ is that both exist almost everywhere in the brain. Future attempts to understand the structural organization of reinforcement functions in the brain will have to deal with the ubiquitous nature of ICSS. A single neurochemical reinforcement system should not be expected in the brain, given the variety of neural systems activated by sensory stimuli even in a simple operant reinforcement situation involving bar-press responding. One suspects that the problem of ICSS anatomical organization is not one of simply "more

data needs"⁵⁴, but one of better conceptualization of reinforcement functions.

One approach to the results of the present experiment is through the question of necessary versus sufficient neurochemical substrates of hippocampal ICSS. The results suggest that the NE innervation of the hippocampus is not necessary for hippocampal ICSS, but that the 5-HT input is necessary for the normal maintenance of this behavior. This is not to say that the NE input may not be sufficient to maintain hippocampal ICSS in the absence of other afferents to the hippocampus. However, the NE input may not even be a sufficient condition for normal hippocampal ICSS, as the NE afferents failed to maintain hippocampal ICSS, in the present study, after the PCPA-induced reduction in 5-HT input. It may also be the case that two or more identified inputs to a certain brain structure are necessary for the maintenance of ICSS in that area. For example; depriving the caudate of either its 5-HT³² or DA³¹ inputs depresses caudate ICSS. Further data on the anatomy of hippocampal ICSS might be profitably gained from a study of the necessity of individual efferent fiber systems in the maintenance of hippocampal ICSS.

In questioning the importance of a locus coeruleus-hippocampal NE reinforcement pathway⁴⁵ and pointing to the necessity of the 5-HT input for hippocampal ICSS, the present experiments should not be construed as evidence for a new "5-HT theory" of reinforcement. First, the depressive effect of PCPA administration on ICSS behavior in the present study is specific to the hippocampus, with opposite results occurring in the LH. In addition, the complete neural system necessary for hippocampal ICSS, including intrinsic and efferent hippocampal

pathways, has not been outlined. It is doubtful that the necessary anatomical substrates of hippocampal ICSS end pre-synaptically in 5-HT afferent terminals. Finally, it has not been unequivocally demonstrated that the deficit in hippocampal ICSS after PCPA is actually the result of a decrease in the positively reinforcing value of the brain stimulation. The nature of this deficit in ICSS is probably the most interesting problem for future experimentation.

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