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# Taming Effects of p-Chlorophenylalanine on the Aggressive Behavior of Septal Rats[1]

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JONES, A. B., J. D. BARCHAS AND B. EICHELMAN. *Taming effects of p-chlorophenylalanine on the aggressive behavior of septal rats.* PHARMAC. BIOCHEM. BEHAV. 4(4) 397–400, 1976. – Septal irritability and shock-induced aggression were suppressed by the administration of p-chlorophenylalanine (PCPA) to septal rats. The levels of septal irritability and shock-induced fighting were significantly lower in septal, PCPA-treated rats than in nontreated septal rats. Since both parameters of septal aggression were reduced by PCPA, and while PCPA has no effect on shock-induced fighting of unlesioned rats under similar parameters, it appears that both forms of aggression may function through a common neural mechanism.

Septum Irritability Fighting Septal syndrome PCPA

LESIONS in the septal area of the rat brain produce a septal rage syndrome characterized by excessive irritability, hyperactivity and increased aggressiveness [1, 3, 5, 9, 13, 20]. Septal lesions increase generalized irritability [5], sensitivity to pain [9,16], and shock-induced aggression [1, 4, 9, 22]. This syndrome declines markedly within 2 to 3 weeks following surgery [5].

Septal irritability is rapidly suppressed by IP injections of para-chlorophenylalanine (PCPA) [7] within 30 min [8]. PCPA does not affect the levels of shock-induced aggression in unlesioned rats of both the Hooded [6] and Sprague-Dawley [11] strains. Consequently, this study was designed to test whether a PCPA-induced attenuation of septal irritability also lowered the level of shock-induced aggression observed in rats with septal lesions, which would suggest that both types of aggression might be of common etiology.

## METHOD

### *Animals*

The animals were 54 naive male albino rats (Sprague-Dawley) from Simonsen Laboratories of Gilroy, California, weighing from 180–200 g at arrival. All animals were housed individually with ad lib access to rat chow and water throughout the experiment.

### *Apparatus*

The testing chambers for shock-induced fighting consisted of two 32 × 25.5 × 30.5 cm blue Plexiglas boxes, each with 1 clear side for viewing. The grid floors were made of stainless steel rods, 0.3 cm in dia. and spaced 1.9 cm apart. Electric shock was delivered by a power source adapted from Belluzzi and Grossman [2]. All footshock was of 2 mA intensity, presented for a duration of 0.4 sec and delivered by a cycling timer every 7.5 sec. During each daily test session each rat pair received 50 footshocks.

### *Behavioral Paradigms*

Irritability was evaluated using the rating scale developed by Brady and Nauta [5]. Ratings were made of all animals for the following 7 behavior components: (a) resistance to capture in the home cage, (b) resistance to handling, (c) muscular tension elicited in reaction to capture and handling, (d) squealing and vocalization elicited by reaction to capture and handling, (e) urination and/or defecation elicited by capture and handling, (f) aggressive reaction elicited by the presentation of forceps in close proximity to the snout, and (g) aggressive reaction elicited by prodding with forceps.

A 4 point rating scale was used for each of the 7 behavior components. The zero point was fixed by the behavior of tame albino rats such as those conventionally employed in laboratory experiments. Ratings of all animals were made on the 3 days prior to the operation and for the 6 consecutive postoperative days.

During shock-induced fighting, an aggressive attack was defined as a directed movement toward the opponent which resulted in contact, including at least 1 additional response of the following: biting, sparring, upright attack posturing, or supine submissive posturing, adopted by the attacked rat. For each day, an attack/shock percentage was

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calculated (the number of attacks divided by the number of shocks administered x 100). This daily percentage was then averaged over the test days to provide preoperative and postoperative mean levels of fighting.

*Surgery*

At the time of surgery, all rats were injected IP with 7.5 mg of atropine methyl nitrate to reduce secretions in airways. This was given 10–15 min before pentobarbital (60 mg/kg, IP). Occasionally the pentobarbital was supplemented briefly with ether during the operative procedure. Lesions were made in the usual stereotaxic manner using cathodal current passed through an insulated stainless steel electrode. The anode was placed in the rat's rectum. Following the lesion, the burr holes were covered with Gelfoam and the incision closed with wound clips. Postoperatively each animal received 1 IM injection of 75,000 units of penicillin.

The lesions were made according to coordinates taken from Pellegrino and Cushman [18]. One bilateral placement was used at current parameters of 3 mA for 45 sec. Controls received the identical operative procedure but without the disruption of the dura or the insertion of an electrode.

*Histology*

Upon completion of the study, the animals were perfused by cardiac puncture with 50 cc of normal saline, followed by 50 cc of 10% Formalin. The brains were blocked, embedded in paraffin, and sectioned at 6–8 $\mu$. Every tenth section through the lesion was mounted and stained with cresyl violet.

*Procedure*

On preoperative Days 1 through 3, each animal was removed from its cage and an irritability rating assessed after Brady and Nauta [5], from 8:00 to 10:00 a.m. The rats were then randomly paired and tested for shock-induced aggression in pairs which remained constant throughout the experiment.

On postoperative Days 1 through 6, each animal was again removed from its cage between 8:00 and 10:00 a.m., an irritability rating was assessed, and an IP injection of either 0.9% saline or 300 mg/kg of PCPA methyl ester suspended in 0.9% saline was administered. Eighteen rats which received septal lesions were given PCPA (S-PCPA), 18 rats which received septal lesions were given saline (S), and 18 control rats received sham operations and were given saline (C). The groups were randomly determined. Four hours after the injection, each animal was removed from its cage, an irritability rating was again determined, and the rats were tested for shock-induced aggression. Each pair of rats received a total of 50 footshocks daily.

## RESULTS

*Histology*

The septal lesions damaged large areas of the lateral and medial septal nuclei. They interrupted the precommissural fornix, the hippocampal commissure, and the descending columns of the fornix. They consistently damaged portions of the anterior commissure, the nucleus propius of both the anterior commissure and the *stria terminalis*, the medial parolfactory nuclei, and the diagonal band of Broca. Less consistent damage occurred to the nuclei *accumbens septi* and the paraventricular hypothalamic nucleus. A number of lesions damaged ventral medial portions of the corpus callosum. An illustrative lesion is shown in Fig. 1.

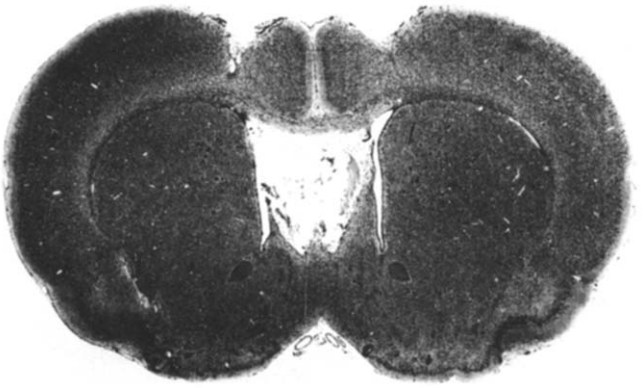

FIG. 1. Photomicrograph demonstrating representative septal damage.

*Prelesion Grouping*

Random selection of groups in this study produced differing baseline levels of aggression. A one way analysis of variance demonstrated significant group differences in irritability ($F(2,51) = 8.63$; $p<0.005$) and shock-induced fighting ($F(2,24) = 4.15$; $p<0.05$). At the beginning of the study, the septal-PCPA group had a greater irritability score than either the sham or septal group. The baseline of the septal group for shock-induced fighting was lower than the control or septal-PCPA group baseline. Consequently, statistical analysis for comparison utilized change from baseline levels ($\Delta$'s) rather than absolute values.

*Irritability Ratings*

Table 1 presents the mean changes in irritability from preoperative baselines for the first 3 days postoperatively and the fourth through sixth days postoperatively. An analysis of variance for repeated measures [23] comparing the change from baseline on Days 1–3 and Days 4–6 ($\Delta$'s) of all 3 groups was significant at the $p<0.001$ level for treatment ($F(2,51) = 32.7$), time ($F(1,51) = 117.4$), and treatment time interaction ($F(2,51) = 37.9$). During the first 3 days postoperatively both the septal ($p<0.001$) and the septal-PCPA ($p<0.001$) groups were significantly more irritable. For the second 3 day comparison, only the septal group remained significantly ($p<0.05$) more irritable.

*Shock-induced Aggression*

Table 2 presents the mean changes in the incidence of shock-elicited fighting between a 3 day baseline and postoperative mean levels of Days 1–3 and Days 4–6. An analysis of variance for repeated measures (again, comparing the change from baseline on Days 1–3 and Days 4–6 ($\Delta$'s) of these groups) was significant for treatment ($F(2,24) = 7.71$; $p<0.005$), time ($F(1,24) = 4.92$; $p<0.05$), and treatment time interaction ($F(2,24) = 4.12$; $p<0.05$). The control group (sham-operated) showed a significant

TABLE 1

MEAN DIFFERENCES IN IRRITABILITY RATINGS FOR EACH GROUP FOR THREE-DAY PREOPERATIVE AVERAGES VERSUS THREE-DAY POSTOPERATIVE AVERAGES

| Group (N) | Prelesion | Postlesion Days 1-3 | Δ | Postlesion Days 4-6 | Δ |
|---|---|---|---|---|---|
| Sham (18) | 1.02 | 1.64 | +0.62* | 1.02 | +0 |
| Septal-PCPA (18) | 1.74 | 3.33 | +1.59† | 1.28 | −0.46 |
| Septal (18) | 0.98 | 8.97 | +7.99† | 2.61 | +1.63* |

Note—Probabilities for matched pairs $t$-test, two-tailed.
*$p<0.05$.
†$p<0.001$.

TABLE 2

MEAN DIFFERENCES IN SHOCK-INDUCED ATTACK SCORES FOLLOWING LESIONS

| Group (N) | Prelesion Attacks per 50 Shocks | % | Postlesion Days 1-3 Attacks per 50 Shocks | % | Difference in % Attacks | Postlesion Days 4-6 Attacks per 50 Shocks | % | Difference in % Attacks |
|---|---|---|---|---|---|---|---|---|
| Sham (9) | 24.1 | 48.2 | 17.3 | 34.6 | −13.6† | 24.4 | 48.8 | +0.6 |
| Septal-PCPA (9) | 22.8 | 45.6 | 21.7 | 43.4 | −2.2 | 21.2 | 42.5 | −3.1 |
| Septal (9) | 14.5 | 29.0 | 23.2 | 46.4 | +17.4* | 25.3 | 50.6 | +21.6* |

Note—Probabilities for matched pairs $t$-test, two-tailed.
*$p<0.05$.
†$p<0.001$.

($p<0.001$) decrease in shock-induced fighting (characteristic of certain strains [10]) over the first 3 postoperative days. This decrease was no longer significant in the second 3 day period. The septal group, conversely, demonstrated a significant increase in both periods ($p<0.05$). The septal-PCPA group did not differ from baseline levels of shock-induced aggression in either period.

## DISCUSSION

These experiments confirm the previous reports [3, 5, 19] that septal lesions induce hyperirritability and increase shock-induced aggression in the rat [1, 4, 9, 22]. PCPA significantly suppressed not only the hyperirritability as indicated by other experiments [7,8], but also suppressed the increase in attacks during shock-induced fighting.

The simplest explanation of these data is that the increase in attacks during shock-induced aggression is concomitant with the hyperirritability exhibited in the septal syndrome, both aspects of which may be conjointly suppressed with PCPA. This is supported by the finding that PCPA administered to normal rats in a similar paradigm has no effect on the level of shock-induced aggression [6,11]. This explanation is further corroborated by the observation that as the septal syndrome attenuates over time, the increase in shock-induced fighting also diminishes toward control levels [1]. A similar parallel appears to exist with muricidal behavior induced by septal lesions in rats, since there is an increase in the mouse killing behavior up to 10 days after lesioning [21] but not after 15 days [12,17]. These data also imply a strong correlation of predatory aggression with the duration of the septal syndrome. These data appear to support the contention that various categories of aggression elicited by septal lesions on the rat are related by a common neural mechanism.

The serotonin-depleting effect of PCPA, a tryptophan hydroxylase inhibitor [15], may not be the direct mechanism for the behavioral effects reported above, since this action of PCPA requires several hours to days to produce a measurable change. The rapidity of onset of the attenuation of irritability in the septal syndrome (30 min, [8]) suggests changes which might be occurring at the synapse and nerve terminal. One possible mechanism is suggested by Knapp and Mandell [14] who have shown that PCPA decreases the synaptic uptake of tryptophan, a serotonin precursor. If PCPA also interferes with the reuptake of serotonin, it may initially leave more functional serotonin available. This could conceivably compensate for the damage to serotonergic terminals in the septum and aid in a restoration of more normal behavior. Such a hypothesis implicates a septal-serotonergic system which when functional, inhibits several forms of rodent aggression.

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