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Dorsal Periaqueductal Gray Punishment, Septal Lesions and the Mode of Action of Minor Tranquilizers

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GRAEFF, F. G. AND J. N. P. RAWLINS. *Dorsal periaqueductal gray punishment, septal lesions and the mode of action of minor tranquilizers.* PHARMAC. BIOCHEM. BEHAV. 12(1) 41–45, 1980.—In order to study the role of the septo-hippocampal system and the dorsal periaqueductal gray (DPAG) substance in punished behavior and in the action of minor tranquilizers, two groups of rats were trained to lever press on a continuous reinforcement schedule of food presentation. In one group, every response was subsequently punished by foot-shock delivery; in the other, by brief electrical stimulation of the DPAG of the mesencephalon. In both groups response rates were reduced to less than 10% of pre-punishment rates, but not completely supressed. Response rates did not significantly differ between the two groups, either before or after the introduction of punishment. Septal lesions significantly increased responding in the animals punished by foot-shock, but did not affect responding suppressed by DPAG stimulation. Injection of chlordiazepoxide (5 mg/kg, IP) significantly increased punished responding in both groups of rats, before as well as after the septal lesion. Before the septal lesion was made, responding suppressed by foot-shock was significantly more released by chlordiazepoxide than responding punished by DPAG stimulation. These results suggest that in punishment tests using foot-shock, both a behavioral inhibitory system, including the septo-hippocampal structures and an aversive or punishment system, including the DPAG substance, act together to produce response suppression. Both these systems would be depressed by minor tranquilizers in order to cause their anti-punishment and perhaps their anti-anxiety action as well.

Punishment Foot-shock Dorsal periaqueductal gray stimulation Septal lesions Chlordiazepoxide Anxiety

ANTI-ANXIETY drugs or minor tranquilizers release positively reinforced behavior suppressed by response-contingent foot-shock presentation (punishment), in rats [2, 3, 7, 8, 17]. Also the potency of different minor tranquilizers in such punishment tests is positively correlated with their clinical potency as anxiolytics, making these laboratory tests good predictors of the anti-anxiety action of drugs [2,3].

In the same way as minor tranquilizers, tryptamine receptor blockers [9,11], the inhibitor of 5-hydroxytryptamine (5-HT) synthesis, para-chlorophenylalanine (PCPA) [6,27] or destruction of ascending 5-HT systmes by neurotoxic brain lesions [30] increase foot-shock punished responding in the rat. Since the benzodiazepine minor tranquilizer, ozazepam decreases brain 5-HT turnover at doses that release foot-shock punished responding in this species, the suggestion has been made that the anxiolytic action of benzodiazepines is due to a decrease of 5-HT activity in brain "punishment" systems [32].

Punishment, however, causes different and sometimes incompatible effects, depending on environmental circumstances. It may either stop ongoing behavior, by producing freezing, or induce aversive responses such as escape or avoidance. The electrical stimulation of the median raphe nucleus, site of origin of the mesolimbic 5-HT system [10,22], causes behavioral arrest (freezing), suppressing concurrent lever-pressing in rats. This effect is accompanied by somatic and autonomic changes, that are characteristic of the emotional response of rats exposed to stressful situations they cannot escape. The suppressive effects of median raphe stimulation on responding were attenuated by PCPA, indicating its mediation by 5-HT release [12]. However, no flight behavior or other signs of aversion were observed. On the other hand, brain self-stimulation has been described in rats with electrodes implanted in the median raphe nucleus [23] and in another area rich in 5-HT neurons, the dorsal raphe nucleus. However, in the latter, the rewarding effects are likely to be mediated by adrenergic rather than serotonergic pathways [29]. Therefore, although the mesolimbic 5-HT system may mediate the behaviorally inhibitory emotional responses elecited by threatening stimuli [12], it is unlikely that the ascending 5-HT systems are neural substrates for the aversive effects of punishment. Indeed, 5-HT mechanisms

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seem to inhibit the aversive consequences of electrical stimulation of other brain regions, such as the dorsal periaqueductal gray (DPAG) substance of the mesoncephalon [4, 5, 21, 25, 26, 31]. Skilled escape behavior, maintained by deceases in [18, 19] or termination of [28] DPAG electrical stimulation in the rat, is facilitated by PCPA [18] or 5-HT receptor blockers [28], and inhibited by the precursor of 5-HT synthesis, 5-hydroxytryptophan, or by the presynaptic 5-HT uptake inhibitor, chlorimiparamine [19].

The electrical stimulation of the DPAG in man has been reported to evoke feelings of fear [24]. In addition, the benzodiazepine minor tranquilizer, chlordiazepoxide (CDP) markedly inhibits skilled escape responding in rats maintained by termination of DPAG electrical stimulation [28]. These results suggest that this region, that is part of a larger brain aversive system, comprising the amygdala and peridaqueductal-periventricular areas [4, 5, 21, 25, 26, 31], mediates the driving or motivating component of fear responses and plays a role in the anti-anxiety action of benzodiazepines [28]. On the other hand, several reported results indicate that the septo-hippocampal system, together with its serotonergic and noradrenergic mesencephalic inputs, is also involved in the nervous integration of fear or anxiety and in the action of minor tranquilizers [13]. In particular, like these drugs, certain lesions of the septal area consistently release foot-shock punished responding in the rat [14, 15, 16].

In order to study the interaction between the septo-hippocampal system and the DPAG substance in punished behavior, we made septal lesions in rats trained to press a lever in a conflict situation in which every response was rewarded by food and simultaneously punished by either electric foot-shock or brief electrical stimulation of the DPAG of the mesencephalon. In addition, the effect of CDP on both types of punished behavior was measured, before as well as after the septal lesion.

METHOD

Animals

Male, Sprague-Dawley rats, weighing 400–500 g were housed in individual cages and given food and water ad lib before and until at least 14 days after surgery. Before lever-pressing training, the animals were fed daily for one hour only over several days, until the body weight of each rat was reduced to 80% of its free-feeding weight. This weight level was then maintained constant by giving limited amounts of food (around 16 g) every day following the experimental session (see below).

Surgery

Rats were anaesthetized with 3.5 ml/kg of chlor-nembutal (a mixture of chloral hydrate, ethanol, sodium pentobarbitone, propylene glycol and $MgSO_4$), IP, and operated in a stereotaxic instrument. With the skull horizontal between bregma and lambda, a bipolar electrode made of twisted, stainless steel wires (150 μm in diameter), Diamel-insulated except at the cross-section of the tip was implanted in the dorsal midbrain following the coordinates of König and Klippel's [20] rat brain atlas (anterior 0.2 mm, lateral 0.0 mm, and vertical 0.5 mm). Two monopolar electrodes made of similarly insulated straight wires (250 μm) were lowered into the lateral septal area (0.5 mm anterior, and $\pm$ 0.7 mm lateral to the bregma, 4.5 mm deep from the dura). The electrodes were brought out to an Amphenol plug secured to the skull with stainless steel screws and methylmethacrylate polymer cement.

Apparatus

Brain stimuli were generated by a constant current source made in the laboratory. Square wave electric pulses of 0.5 msec duration and 100 Hz frequency were used. The stimulator current was monitored by means of an oscilloscope.

A Campden Instruments Skinner box (25 $\times$ 20 cm square and 20 cm high), placed inside an insulated chest and provided with fan and an observing screen, was used. During the experimental session, the box was illuminated by a 2 W bulb placed in the ceiling. The rats inside the experimental chamber had their midbrain electrodes connected with the stimulator by means of a slip-ring and a flexible cable protected against bites by a stainless steel wire shield. The grid-floor of the rat chamber was connected to a scrambled Grason-Stadler shock generator. Standard solid-state equipment was used for automatic programming and recording.

A constant current source constructed in the laboratory was used for making the septal lesions (see below).

Procedure

Ten days after the surgery, the rats were placed inside a box (30 $\times$ 30 cm square and 34 cm high), provided with a Plexiglas window and the dorsal midbrain was stimulated through a suspended cable with length compensation that allowed ample movement to the animal. The intensity of the electrical current was gradually increased until either a behavioral change occurred or a ceiling of 500 μA was reached. Eleven rats displaying aversive responses, such as running or jumping [28], at the lower intensities (20–150 μA) were assigned to the brain stimulation (BS) group while the remaining seven constituted the foot-shock (FS) group. Only two of the latter animals showed similar aversive responses during the brain stimulation test, with currents up to 500 μA; in the other five rats, no behavioral changes were produced by the stimulation.

After food-deprivation, rats of both groups were trained to lever-press on a continuous reinforcement (CRF) schedule of food presentation in which every response was immediately followed by the delivery of a 45 mg food pellet. A 10-minute experimental session was conducted every day.

Following six days of apparently stable responding under CRF, punishment was gradually introduced. In the FS group, electric shocks of 0.5 sec duration were applied to the rat's feet immediately following each lever-press, whereas in the BS group, response-contingent brain stimuli consisting of a 0.2 sec train of square-wave pulses were applied to the dorsal midbrain. In both groups, the stimulus intensity was adjusted over several sessions, until response output was reduced to less than 10% of unpunished response rates, but not altogether abolished. Eventually, intensities of 0.1 to 1 mA foot-shock and 80–400 μA brain stimulation were used.

In a subsequent phase, all rats were injected with a control saline solution (1 ml/kg) and three days later with 5 mg/kg of CDP (chlordiazapoxide HC1, Roche), IP, 1 hr before the experimental sessions. The animals were run for five consecutive days following the drug injection. After the fifth

session, a bilateral septal lesion was made under light ether anaesthesia. A current of 1 mA was passed for 20 sec using each septal electrode in turn as an anode. From the next day on, six further sessions were conducted under the same stimulus conditions. On the fifth day after the lesion, CDP (5 mg/kg) was again injected.

Analysis of Results

The total number of lever presses in each experimental session (10 min) was recorded in a digital counter. The data were subjected to an analysis of variance on a ICL 1906A computer, using the GENSTAT library. The significance levels of specific comparisons in significant interactions were determined by using the appropriate standard error for the interaction to derive t values.

Histology

Rats were sacrificed under deep pentobarbital anaesthesia and their brain removed after perfusion through the heart with saline followed by 10% Formalin solution. The brains were embedded in celloidin and frontal plane sections, 30 μm thick, were made and stained with cresyl violet.

Electrode placements were localized and septal lesions reconstructed in diagrams from König and Klippel's [20] atlas.

RESULTS

Effect of Septal Lesions on Punished Responding

As shown in Fig. 1 the FS group did not differ significantly from the BS group with respect to the number of responses per session emitted during the period of unpunished CRF ($F=0.08$, $df=1$ and 16, $p>0.05$). The introduction of punishment markedly reduced responding in both groups to approximately the same extent, so that no significant difference in punished responding between the two groups occurred ($t=0.58$, $df=70$, $p>0.05$). The septal lesion, however, clearly released punished responses in the FS group ($t=7.13$, $df=70$, $p<0.005$), but did not significantly affect the BS group ($t=0.19$, $df=70$, $p>0.05$). Thus, after the lesion, response rates were higher in the FS group than in the BS group ($t=4.6$, $df=70$, $p<0.005$).

Effect of CDP on Punished Responding Before and After the Septal Lesion

The injection of saline caused negligible effects on punished responding in both groups. Response differences after saline were 3.6 ± 2.8 (mean $\pm$ SEM calculated as specified in Fig. 2) for the FS group and -0.8 ± 2.9 for the BS group, respectively. The effects of CDP on punished behavior are shown in Fig. 2. CDP produced increased responding compared to saline in the FS group, before ($t=5.82$, $df=32$, $p<0.005$) as well as after ($t=3.55$, $df=32$, $p<0.005$) the septal lesion. Similar comparisons for the BS group also yielded significant differences both before ($t=3.30$, $df=32$, $p<0.005$) and after ($t=3.02$, $df=32$, $p<0.005$) the lesion. The increases in punished responding caused by CDP were significantly larger in the FS compared to the BS group, before the septal lesion was made ($t=3.41$, $df=32$, $p<0.005$). After the lesion, however, the difference between the groups was no longer significant ($t=1.57$, $df=32$, $p>0.05$). The septal lesion did not significantly affect the response to CDP in the BS group ($t=0.28$, $df=32$, $p>0.05$), but in the FS group, the

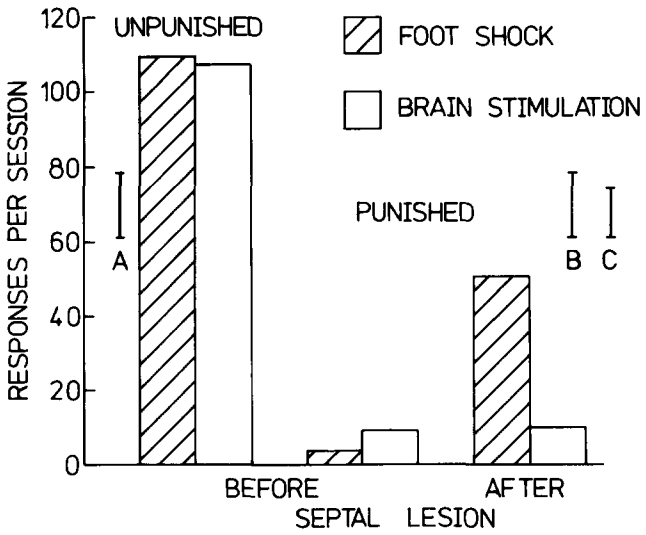

FIG. 1. Total number of responses in 10-minute daily session emitted by the rats punished by either foot-shock (n=7) or dorsal periaqueductal gray stimulation (n=11) on a continuous reinforcement schedule of food presentation: (left) during the six days before punishment was introduced. In the punishment phase, during four days before (center) and four days after (right) septal lesions. Columns represent means. Bar A represents 2 SED for comparison of unpunished response means. Bars B and C represent 2 SED for comparison of means of punished responses between and within punishment groups, respectively.

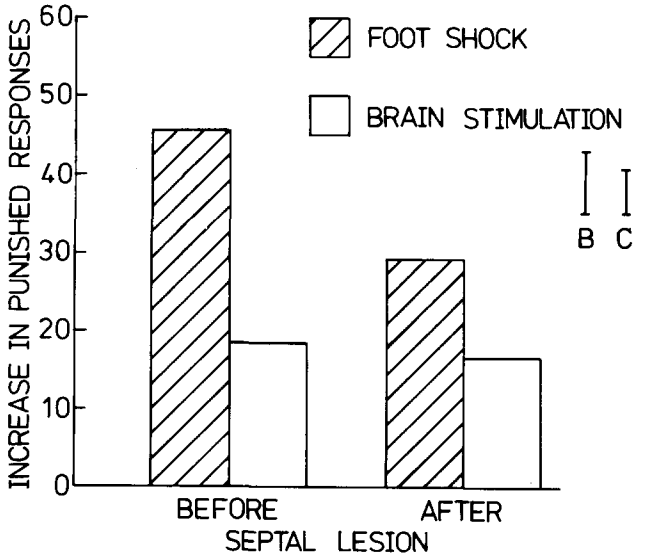

FIG. 2. Effect of chlordiazepoxide (5 mg/kg) on responding punished by either foot-shock or dorsal periaqueductal gray stimulation, before and after septal lesions. The increases in punished responses were calculated for each rat as the difference between the total number of responses in the drug session and the average of the responses emitted in the day before and in the day after the drug injection. Columns represent means. Bars B and C represent 2 SED for comparison of response means between and within punishment groups, respectively.

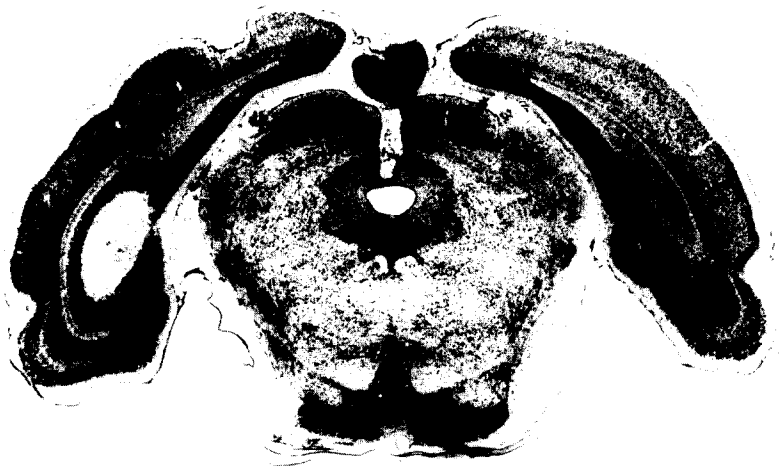

FIG. 3. Histological section showing a stimulating electrode tip lying in the dorsal periaqueductal gray substance of the midbrain, just above the aqueductus of Sylvius.

effect of the drug after the lesion was smaller than before ($t=2.27$, $df=32$, $p<0.05$), probably due to differences in pre-drug baseline response rates.

Localization of Brain Electrodes and Septal Lesions

The tips of the midbrain electrodes of the animals in the BS group were localized inside the DPAG substance or adjoining tectum of the mesencephalon, as reported before [28]. A typical example is illustrated in Fig. 3.

The septal lesions did not differ systematically between the two groups. Major damage including both lateral and medial septal areas was produced in eight rats, similar to the larger lesion illustrated in Fig. 4. In the ten other rats, the dorso-lateral septum was bilaterally destroyed, but the medial nucleus was variably affected. In three of these animals the lesions of the lateral nuclei were asymmetrical, as in the smallest lesion shown in Fig. 4. Nevertheless, even in this animal, foot-shock punished responding was clearly released by the lesion. Antero-posteriorly, the lesions extended from the coordinates A 9410–A 8620 μm to the coordinates A 7190–A 6670 μm of the König and Klippel's [20] atlas.

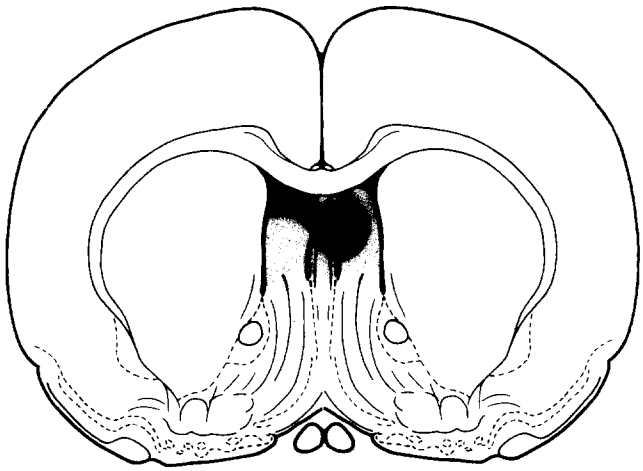

FIG. 4. Graphical reconstructions of the smallest (dark area) and the largest (hatched area) septal lesion made on a diagram (coordinate A7890 μm) of König and Klippel's [20] atlas.

DISCUSSION

These results show that the septal lesion disinhibited lever-pressing behavior punished by foot-shock, but did not increase comparable response rates punished by DPAG electrical stimulation. Since similar septal lesions [14] are supposed to increase responding punished by foot-shock because they impair "behavioral inhibition" mechanisms in the brain [13,14], these mechanisms do not seem to play any major role in the suppression of responding produced by DPAG stimulation. Therefore, in this punishment task, activation of the brain aversive system [5,25] alone seems to determine response withdrawal.

The observed facilitatory effects of CDP on responding punished by DPAG electrical stimulation could be due to a drug-induced decrease in aversiveness of the punishing brain stimulation, since it has been shown that CDP selectively inhibits responding to escape electric stimulation in this area of the brain [28]. Unpublished observations made by one of us (F. G. Graeff with S. Morato de Carvalho) also suggest that this anti-aversive action of CDP extends to other minor tranquilizers. It was verified that pentobarbital as well as CDP markedly increased operant responding of rats in the punished component of a Geller and Seifter [7] type of conflict test in which every response was simultaneously reinforced by water presentation and punished by brief electrical stimulation of the DPAG, as in the present study. Also, like the septal lesions of this study, two 5-HT antagonist drugs, methysergide and cyproheptadine that supposedly impair the same behavioral inhibition system including the septum and hippocampus [12, 13, 14], failed to increase responding punished by DPAG stimulation at the same doses that usually release foot-shock punished responding [11].

The aversive system depressed by minor tranquilizers seem also to operate in conflict tests using foot-shock, since

we observed marked increases in responding punished by foot-shock when CDP was administered, even after septal lesions had already substantially released the same behavior. On the other hand, CDP enhanced punished responding significantly more in rats punished by foot-shock than in those punished by DPAG stimulation. This result indicates that part of the facilitatory action of minor tranquilizers in foot-shock punishment task is due to the impairment of the behavioral inhibition system that includes the septum and hippocampus [13,14]. Thus, it is proposed that, in punishment tests using foot-shock, both the behavioral inhibition system and the brain aversive system discussed above act synergistically to produce response suppression. Brain lesions and anti-5-HT drugs that release punished behavior would affect only the behavioral inhibition system, while minor tranquilizers would impair both systems in order to cause their facilitatory effect on punished behavior. Using active and passive avoidance (punishment) as well as shock-elicited aggression tests, in rats, Blanchard *et al.* [1] have similarly suggested that the fear response has both an avoidance and an immobility component, only the latter being impaired by hippocampal lesions.

In so far as the behavioral effects of minor tranquilizers in animal conflict tests may be related to their clinical anti-anxiety action [2,3], the present results support the view that a depression of the brain aversive system that includes the DPAG substance may be involved in the anti-anxiety action of minor tranquilizers [28].

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