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Effects of Serotonin Content on Pain Sensitivity in The Rat[1]

JOHN TELNER, FRANCO LEPORE[2] AND JEAN-PAUL GUILLEMOT

Département de Psychologie, Université de Montréal, Montréal, Québec, Canada H3C3JC

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TELNER, J. I., F. LEPORE AND J. -P. GUILLEMOT. *Effects of serotonin content on pain sensitivity in the rat.* PHARMAC. BIOCHEM. BEHAV. **10**(5)657–661, 1979.—In this study the role of serotonin in pain sensitivity was investigated. Brain serotonin was elevated via low and high doses of precursor tryptophan and lowered via parachlorophenylalanine or lesions placed in the dorsal raphe nucleus. The effects on pain sensitivity were then assessed using two psychophysical pain testing procedures: (1) minimum shock intensity (threshold) which produced a conditioned escape response; and (2) total activity elicited by highly aversive inescapable shock. The results showed that only a large elevation of serotonin produced a change in escape thresholds in the direction of hypoalgesia. When total activity to a painful inescapable stimulus was evaluated only lowering of serotonin produced an effect, and this change was in the direction of hyperalgesia. The conclusion was made that serotonin does contribute to the mechanism of pain.

Pain Serotonin Tryptophan Parachlorophenylalanine Raphe lesions

CONFLICTING reports have appeared recently concerning a possible involvement of serotonin in pain sensitivity. Thus Tenen [19] Harvey and Lints [5], Fibiger *et al.* [3] as well as Harvey and Yunger [7] have shown that lowering serotonin via pharmacological or surgical means produces a significant heightening of pain sensitivity. On the other hand, Harvey *et al.* [6] as well as Hole and Lorens [8] have shown that lesions placed in the raphe nuclei, which result in an appreciable drop in brain serotonin, have no effect on pain sensitivity.

If serotonin is implicated in pain sensitivity, with a lowering of brain serotonin producing a heightened sensitivity to pain, then it seems plausible that increasing brain serotonin via precursors might produce a decrease in sensitivity to pain. However, most studies examining this possibility have shown negative results [5, 9]. Indirect evidence has demonstrated, however, that serotonin may indeed play a role in analgesia. For example, Samanin and Valzelli [17] produced an augmentation of morphine analgesia by stimulation of the dorsal raphe nucleus, an area rich in serotoninergic terminals. Similarily, Sparkes and Spencer [18] found an increase in morphine analgesia after intraventricular administration of serotonin. More direct evidence was obtained by Wada *et al.* [20] who observed a stoic response to shock in cats administered the precursor, 5-hydroxytryptophan, and by Guillemot *et al.* [4] who found a significant decrease in pain sensitivity in the cat after megadoses of tryptophan.

Part of the problem encountered when attempting to link serotonin to pain may be the aspect of pain examined. Fibiger *et al.* [3] have postulated two different mechanisms through which serotonin could affect the reaction to painful stimuli. The first mechanism involves the modulation or filtering of incoming information from the periphery. According to this model pain detection thresholds would be modified during serotonin manipulations since the filtering mechanism has been altered. The second mechanism involves only the nature of the reaction to the stimulus. Thus brain serotonin content would not affect sensory systems but rather the response parameters to the stimulus. In other words, aversive stimuli would produce a stronger reactivity in an animal with lowered brain serotonin content. Elevation of serotonin level would be expected to produce opposite effects, although, as already stated, available data are rather sparse.

The aim of the present experiment was to examine whether brain serotonin affects response thresholds and the magnitude of the response to aversive stimuli and whether hyperalgesia and/or hypoalgesia are both involved. Lowering of brain serotonin was achieved via parachlorophenylalanine (PCPA) injections and dorsal raphe lesions (DR) [1]. Raising of serotonin was accomplished by injecting either a low or a high dose of the serotonin precursor tryptophan. Thresholds and reactivity to painful electric shocks were evaluated using psychophysical testing procedures.

METHOD

Animals

The animals were 72 male albino rats of the Sprague-Dawley strain (Canadian Breeding Farms) and weighed 200 g at reception. They were housed in conventional rat cages,

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[2]Address reprint requests to Franco Lepore, Département de Psychologie, Université de Montréal, C.P. 6128, Montréal, Qué. Canada H3C 3J7. This experiment was supported in part by a National Research Council of Canada grant, Grant No. A8622.

three rats per cage, during the training period. Food (Purina Rat Chow) and water were available ad lib. The room illumination was maintained on a 12 hr light-dark cycle. After the training period the animals were housed one per cage for the remainder of the experiment and food and water availability were identical to that of the training stage.

Apparatus

The pain-testing chamber consisted of a rectangular shuttlebox, 50 cm long × 21 cm wide × 21 cm deep, constructed of transparent Plexiglas. Metal grids (2 mm diameter) with ends joined to a plastic frame composed the floor. Separation between bars was 1.1 cm. Each side of the cage had its floor suspended by springs, attached to the roof of the box, which lowered 1.5 cm whenever an animal was on that particular side of the cage. The floor was divided into two compartments by a hurdle consisting of a plastic cylinder (4.8 cm dia.) located 4 cm above the grid. The cylinder rotated freely about its axis; any attempt by an animal to straddle it to escape the shock was therefore rendered impossible.

The ends of both floors were connected to microswitches, which activated a stepper, a counter, a timer, and one of two shock generators. Scrambled shock was delivered via two Grason Stadler shockers (model E1064gs), one for each side of the chamber. The system was set so that the shock was delivered only to the side of the cage which contained the animal at the start of each trial. During the second part of the experiment, the system was programmed such that the shock was delivered to both sides of the test box simultaneously and was inescapable.

The test chamber was located in a sound insulated room measuring approximately 3×3×2.5 m and illuminated by two 200 watt house lights affixed to the ceiling. The experimenter and the control panel were located in a room adjacent to this room. The animals were continously monitored via a closed circuit television system. Throughout the experiment white noise (90dB) was delivered to the shuttlebox to control for any possible distracting noises which, because of the soundproofing, were generally very low.

Procedure

Training. Training involved the animals learning an escape task. On Day 1, animals were placed in the shuttlebox individually and allowed to explore for five min. Shock (0.3 mA) was then presented on the side containing the animal and remained on for 60 sec. During this shock period the animal could escape by jumping over the hurdle to the other side. Twenty shock presentations with a 60±10 sec inter-trial interval were administered. This procedure was repeated two days later and animals that could not maintain a criterion of 15 out of 20 escapes within 5 sec were eliminated and replaced by other animals. Successful animals were then randomly assigned to one of six treatment groups.

Treatments. Radio-frequency lesions of the dorsal raphe nucleus were produced in 12 animals under sodium pentobarbital (Nembutal; Abbott) anaesthesia (50 mg/kg IP). A David Kopf tungsten cryoprobe, insulated except at the tip (1 mm long by 0.5 mm diameter), was guided stereotaxically into the dorsal raphe (DR) nucleus (A: −6.2 mm from the bregma: L: 0.0 mm; V: 6.5 mm below the surface of the cortex) according to the coordinates of the Pellegrino and Cushman rat brain atlas [15]. During the first 60 sec the electrode was gradually warmed up to 55°C and then maintained at this temperature for 60 sec. A group of 12 sham operated controls received the same surgical treatment except that the electrode was lowered into an area just dorsal to the DR and no current was passed.

All injections were composed of drug and 5 cc of commercial vegetable oil as vehicle and were administered IP. PCPA (Pfizer) in one dose of 400 mg/kg was administered to 12 animals, 72 hr prior to pain testing. Tryptophan (Eastman) was injected in one dose of 100 mg/kg (TRY100), to 12 animals, one hour prior to testing. An additional group of 12 animals received two doses of 800 mg/kg tryptophan (TRY800), 6 hr and then 1 hr prior to testing. Twelve animals served as drug-control group and received 5 cc of the vehicle. The schedule of injection times was partitioned to control for PCPA, TRY100 and TRY800 injection times.

Pain testing—aversion-escape threshold. All pain testing was carried out during the day hours with animals counterbalanced to control for circadian differences in brain serotonin levels. Shock intensities of 0.05, 0.06, 0.08, 0.10, 0.13, 0.16, 0.20, 0.25, 0.30, 0.40, 0.50. 0.60, 0.80, 1.0, 1.3, 1.6, 2.0, 2.5 and 3.0 mA of 5 sec duration were presented to the side of the shuttlebox containing the animal. Shocks were always presented in ascending order, with no interval between two

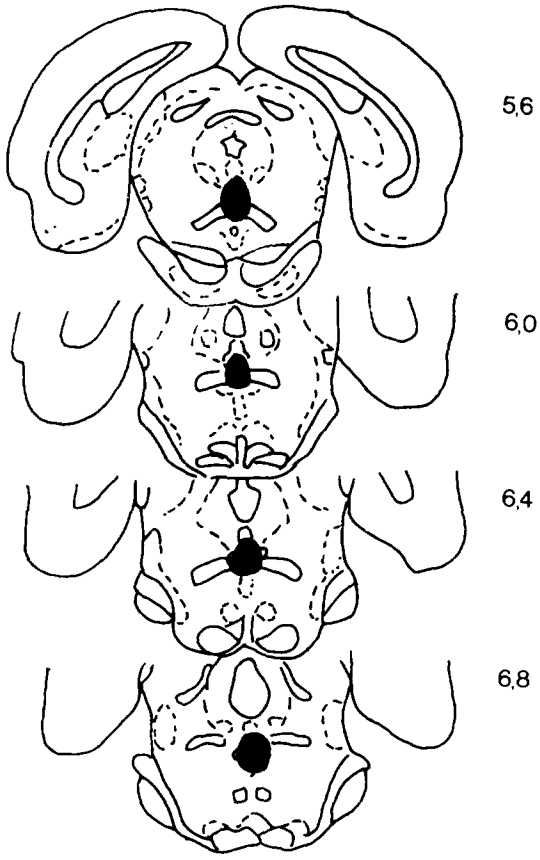

FIG. 1. Schematic representation of a representative dorsal raphe lesion.

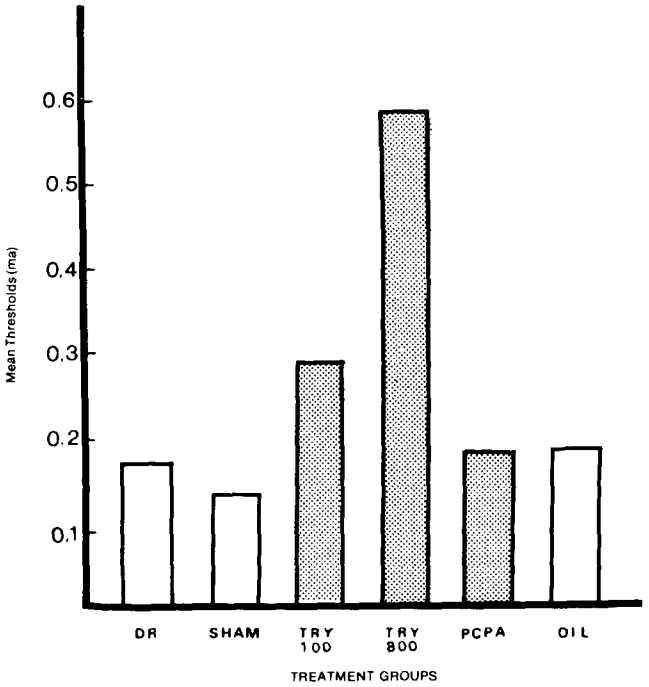

FIG. 2 Mean aversion-escape thresholds for each treatment group.

succeeding levels and were terminated when a crossing occurred. The shock intensity that resulted in a crossing was defined as the threshold for that particular series. In all, 11 series were presented, separated by a 10 min (approximate) intertrial interval. The first two series were presented to determine whether treatment had any effect on the escape task. Animals which appeared to react to the shock (e.g. jump, squeal) but did not cross over during high shock (over 1.3 mA) were rejected. Only one animal was eliminated from the testing due to its failure to shuttle. The median of the remaining nine series was defined as the animal's aversion-escape threshold.

Pain testing—reactivity to suprathreshold shock. During this stage of testing the shock was programmed to remain on at all times on both sides of the test box. Testing began 10 min after the last aversion-escape trial had ended for a particular animal. High shock (0.8 mA) was present continuously for 10 min and the number of crossings from one compartment to the other was recorded. The number of jumps on the same side of the cage (defined by any movement of the grid floor which activated the microswitch) was recorded as well.

RESULTS

Histology

The method of Wolf [27] was followed for all histologies. After testing for pain sensitivity, lesioned animals were injected with a fatal dose of sodium pentobarbital and then perfused intracardially with isotonic saline followed by 10% Formalin. The rats were decapitated, the brains were removed and stored in Formalin for one week or more and then placed in a gelatin solution. Frozen sections were cut at 35 μ and every tenth section was conserved and stained with cresyl violet. A typical lesion is shown schematically in Fig. 1.

To determine degree of lesion, histological slides were projected on paper on which the outline of the raphe structures at the appropriate anterior-posterior coordinates were indicated. The borders of the lesion were drawn on the paper, which was then cut along the two borders. The weight of the pieces of paper representing the lesions were compared to the combined weight of the lesioned and unlesioned portions of the raphe representations. Only animals which showed at least 50% damage of the DR nucleus were kept for the statistical analysis. Those showing less than 50% destruction were eliminated and replaced by appropriately lesioned animals. In all, 21 animals were lesioned, of which twelve showed greater than 50% destruction of the DR nucleus and were retained for the statistical analysis.

Analysis of Results

For all measures, a one-way analysis of variance was employed [21] followed by a Duncan New Multiple Range Test [2].

To eliminate extreme scores, the median of the 9 thresholds (aversion-escape data) was defined as the threshold for individual animals. An analysis of these medians showed a significant difference in threshold values among treatment groups, $F(5,66)=8.84$, $p<0.01$. However, a further analysis revealed that only the high tryptophan group (TRY800) had significantly higher thresholds than all other groups ($p<0.01$). The mean thresholds for group medians are shown in Fig. 2.

An analysis of group means for crossings recorded during inescapable shock (reactivity to suprathreshold shock) revealed a significant difference among groups, $F(5,66)=8.21$, $p<0.01$. A further analysis showed that the PCPA group made significantly more crossings than either the sham control, TRY100, oil and TRY800 groups. The lesioned group crossed significantly more than the TRY800 group ($p<0.01$), the oil control ($p<0.05$) and TRY100 groups ($p<0.05$). This is displayed graphically in Fig. 3.

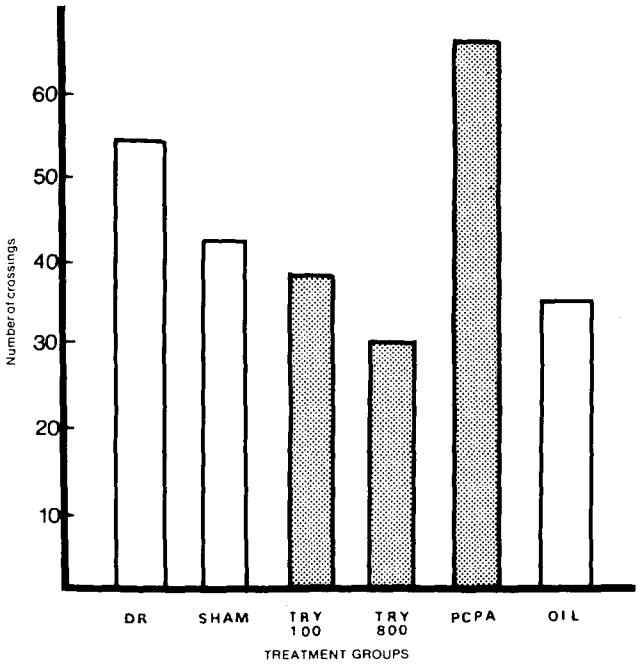

FIG. 3 Mean number of crossings for each treatment group.

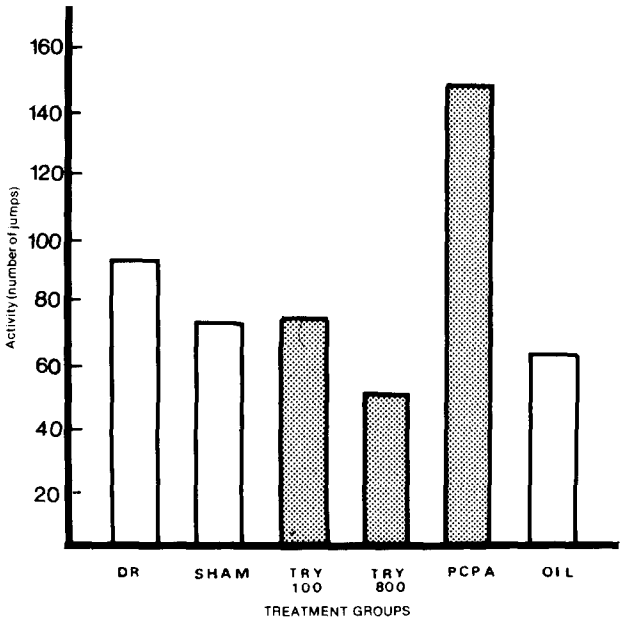

FIG. 4 Mean activity level (number of jumps) for each treatment group.

An analysis of the total number of jumps (combined crossings and on site jumps) revealed a significant difference among groups, $F(5,66)=4.91$, $p<0.01$. A further analysis showed that the PCPA group jumped more freguently than either the TRY100, sham vehicle, TRY800 groups ($p<0.01$) and the lesioned group ($p<0.05$).This is shown graphically in Fig. 4.

DISCUSSION

The main finding of the analysis of the aversion-escape thresholds was that only the high tryptophan group (TRY800) displayed significantly altered pain thresholds and in the direction of hypoalgesia. Raising serotonin content with small amounts of tryptophan or lowering serotonin quantity either pharmacologically with PCPA or surgically via lesions in the dorsal raphé nucleus did not significantly alter pain sensitivity as measured by the aversion-escape test. (It must be pointed out that since our laboratory is not equipped to carry out brain assays, changes in brain serotonin levels can only be assumed to have taken place. This assumption, however, rests on fairly solid grounds since dosage levels and electrode lesion coordinates were based on studies which had in fact carried out such assays under similar experimental conditions [1,14]).

The few published studies that do examine the effects of precursor loading strategies using tryptophan [9] or 5-hydroxytryptophan [5] show no change in pain thresholds and thus contradict our findings. However, a number of differences can be observed when comparing our experiment with the studies demonstrating negative findings: these involve the type of precursor used, the dosage, and the form of pain assessment procedure employed. For example, Harvey and Lints [5] demonstrated that injection of 5HTP into animals lesioned in the medial forebrain bundle will raise pain thresholds to that of controls. However, they could not produce hypoalgesia by loading these animals with doses of 5HTP sufficient to significantly raise serotonin to above that of controls. These researchers used 105 mg/kg 5HTP and the flinch-jump pain assessment procedure, while we used two doses of 800 mg/kg tryptophan and the aversion-detection test. Moir and Eccleston [14] have documented the problems associated with using 5HTP, the most important being that it produces an abnormal distribution of serotonin in the brain, and is taken up nonspecifically and metabolized abnormally. Furthermore Johnson *et al.* [10] have demonstrated that 5HTP decarboxylation occurs in catecholamine neurons with a depleting action on these amines.

Hole and Marsden [9] administered tryptophan and observed no effect on pain sensitivity in the rat. However the highest dosage used in their study was 200 mg/kg while our consisted of two doses of 800 mg/kg. The dose-response curves for tryptophan [14] show that the lower dose used by Hole and Marsden [9] does not produce nearly as large an increase in brain 5HT and 5HIAA as do the two 800 mg/kg dosages. Thus it is possible that our high tryptophan animals had brain serotonin levels that were much higher than those of Hole and Marsden. This may account for the hypoalgesic effect observed in our animals.

Our findings with high tryptophan concur with Wada *et al.*[20] as well as with Guillemot *et al.* [4] in our laboratory who found significant decreases in pain sensitivity in cats after two daily injections of 800 mg/kg tryptophan for 3 days. Lastly, our results seem to be supported by Harvey *et al.* [6] who demonstrated that rats are more sensitive to aversive stimuli during the night hours than during the day hours. This finding may support our hypothesis that serotonin is involved in hypoalgesia since it has been shown that rats show significant increases in brain serotonin during the day as compared to the night hours [16].

One criticism that can be made about our high levels of tryptophan is that they may be simply sedating our animals and/or producing ataxia. If this were the case, then these animals would not be able to make the appropriate escape response to the other side of the shuttlebox at the first perception of pain. However, the results of the second pain test, response to suprathreshold inescapable shock, casts doubt on this hypothesis, since the high tryptophan group did not display significantly lower number of crossings or activity counts when compared to the vehicle control group. This shows that tryptophan loading even at high dosages did not make animals less active or less mobile than controls. On the other hand, high levels of tryptophan may produce not only high levels of serotonin but also of metabolites which may have behavioral effects of their own. This however, is a problem with most psychopharmacological studies using systemic injections and we have no way of assessing the importance of this parameter here.

The results of the second experiment, where the animal's reactivity to inescapable high shock was examined showed that lowering brain serotonin level via the use of PCPA produced a significant increase in reactivity as measured by number of crossings and activity level. Lowering brain serotonin level via dorsal raphe lesions produced a greater reactivity in these surgical animals when compared to both tryptophan groups as well as to the vehicle control group. It should be noted that although raphe lesions and PCPA both deplete the brain of serotonin, PCPA is more potent as it lowers serotonin content in the entire brain whereas raphe lesions only deplete the striatum. This may account for the slight difference in results between these two groups.

These results are in agreement with those of Fibiger *et al.* [3] who used a pain assessment procedure similar to ours. They found that PCPA lowered pain thresholds only when the response to highly aversive shock in an inescapable shock paradigm was measured. PCPA had no effect on pain sensitivity when aversion-detection thresholds were assessed. Our findings also concur with those of Tenen [19] and Lints and Harvey [11] who found hyperalgesia to foot shock in rats only at high levels of stimulation after significant lowering of brain serotonin.

Finally our results are in agreement with those showing that tryptophan-poor diets produce lower shock response thresholds in rats, and that thresholds can be returned to normal values when tryptophan supplements are given [12, 13].

In summary, previous studies as well as the results of this experiment implicate serotonin in the pain process. Our results show that when serotonin level is elevated, sensory threshold is modified in the direction of hypoalgesia. When serotonin level is lowered, sensitivity to painful stimuli is not altered, but the response parameters to peripheral painful stimulation is magnified indicating that the effect is in the direction of hyperalgesia.

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