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Reductions in Pain Thresholds and Morphine Analgesia Following Intracerebroventricular Parachlorophenylalanine

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BODNAR, R. J., J. H. KORDOWER, A. RECHES, M. M. WALLACE AND S. FAHN. *Reductions in pain thresholds and morphine analgesia following intracerebroventricular parachlorophenylalanine.* PHARMACOL BIOCHEM BEHAV **21**(1) 79–84, 1984.—The selective decreases in both basal and analgesic pain thresholds following systemic administration of parachlorophenylalanine (PCPA) has been attributed to the inhibition of tryptophan hydroxylase and subsequent depletion of brain serotonin. These effects only occur at high systemic doses which have other general debilitating effects. The present study examined the relationship between PCPA's nociceptive and serotonin-depleting effects following intracerebroventricular (ICV) administration. The first experiment determined that an ICV dose of 3 mg, but not 1 mg, of PCPA significantly decreased jump thresholds at 0.5, 48 and 120 hr after injection. These effects were not due to osmolarity shifts since hypertonic saline injections failed to alter thresholds. The second experiment demonstrated a time-dependent reduction of morphine (5 mg/kg) analgesia as a function of the interval between ICV PCPA and the systemic morphine injection. PCPA reduced morphine analgesia if it was administered 24 hr prior to the opiate and eliminated morphine analgesia if it was administered 48 hr prior to the opiate. Pretreatment with ICV PCPA either 0.5 or 72 hr prior to the opiate failed to alter morphine analgesia. The third and fourth experiments indicated that hippocampal and spinal levels of either serotonin or 5-hydroxyindoleacetic acid were not significantly affected by ICV PCPA pretreatment. These data indicate that the hyperalgesia and morphine analgesia impairments noted following ICV PCPA do not correspond with changes in serotonin from hippocampal or spinal tissue and such effects are discussed in terms of alternative modes of action.

Pain Analgesia Parachlorophenylalanine Morphine Serotonin Rats

SYSTEMIC administration of parachlorophenylalanine (PCPA) has been employed as a tool to alter an organism's responsiveness to painful or noxious stimuli. The initial work of Tenen [39] and others [4, 22, 44] demonstrated that PCPA decreased flinch-jump thresholds between 48 and 72 hr following injection, an effect attributed to the aversive rather than the sensitivity aspects of the shock [17]. Yet the ability of PCPA to reduce pain thresholds depends upon the pain test employed. Hot-plate latencies were not affected by PCPA pretreatment [9] unless the animals were tested during the light cycle 72 hr following the injection [23]. Moreover, while PCPA increased operant escape thresholds elicited by dorsal central gray stimulation, it failed to alter escape thresholds elicited by ventral reticular formation stimulation [28]. Finally, PCPA pretreatment has been shown to increase tail-flick latencies 48 hr following systemic injection [1,4].

The ability of systemic PCPA to attenuate analgesic responses also depends upon the pain test, the species and the test interval employed in a given study. Systemic injections of PCPA attenuated morphine analgesia as measured by the flinch-jump [40] and foot pressure [41] tests if it was administered at 48 or 72 hr, but not at 3 and 24 hr prior to testing. Yet this effect did not occur on the tail-flick or hot-plate tests in rabbits, mice or rats [4, 7, 9, 17]. Systemic PCPA affects other analgesiometric procedures differentially as well. While PCPA decreased the analgesic response elicited by electrical stimulation of the periaqueductal gray and high-frequency electroacupuncture [1,10], it failed to affect the analgesic responses following cold-water swims, 2-deoxy-D-glucose, inescapable foot shock and low-frequency electroacupuncture analgesia [4,10].

The ability of systemic PCPA to decrease both basal nociceptive responses and morphine analgesia has been attributed to its inhibition of tryptophan hydroxylase [29]. An 80–90% depletion of brain serotonin accompanies the hyperalgesia noted 48–72 hr following PCPA pretreatment, while a smaller 20–67% brain serotonin depletion is observed at 3 to 24 hr following injection [29, 39, 40, 41]. These data suggest that systemic PCPA is exerting its effects by interfering with the synthesis of brain serotonin, and have been used to support the hypothesis that serotonin is integrally involved in the modulation of pain perception (see review:

[33]). However, systemic PCPA administration produces other behavioral and biochemical effects as well, including brain catecholamine depletion, tyrosine hydroxylase inhibition, illness, mortality and reactivity to stimuli and novel situations ([6, 8, 14, 26, 32]; also see review: [30]). Therefore, to assess whether PCPA effects upon pain perception are mediated by central serotonergic processes, the present study employed intracerebroventricular (ICV) injections, a technique used previously in determining PCPA effects on food intake [5, 12, 24, 31]. The first experiment examined whether ICV administration of PCPA (1–3 mg) would alter jump thresholds, a test that has reliably displayed the hyperalgesic properties of systemic PCPA [4, 17, 22, 39, 44]. The second experiment analyzed the time course and magnitude of ICV injections of PCPA to suppress morphine analgesia. The third experiment examined whether ICV injections of PCPA depleted brain serotonin in hippocampus, a supraspinal structure rich in serotonin terminals that borders on the lateral ventricle injection site [38]. Since descending serotonergic projections through the dorsolateral funiculus of the spinal cord have been implicated in the mediation of pain perception and analgesia [18, 42, 43], the fourth experiment evaluated whether ICV injections of PCPA depleted spinal serotonin.

EXPERIMENT 1: ICV PCPA AND JUMP THRESHOLDS

Method

Thirty-two male albino Sprague-Dawley rats (300–400 g) were housed individually and maintained on a 12 hr light: 12 hr dark cycle with ad lib access to rat pellets and water. Each animal was anesthetized with sodium thiopenthol (Pentothal: 50 mg/ml sterile water/kg body weight, IP). One stainless steel 22 gauge guide cannula (Plastic Products) was stereotaxically implanted into each rat's brain and aimed so that its tip was positioned 0.3 mm above the left lateral ventricle. With the incisor bar set at +5 mm, coordinates were 0.5 mm anterior to the bregma suture, 1.3 mm lateral to the sagittal suture and 3.6 mm from the top of the skull. The cannula was secured to three stainless steel screws with dental acrylic. Ten days were allowed for recovery from surgery.

Between 4 and 6 hr into the light cycle, all rats were tested for jump thresholds [16] using an ascending method of limits. Electric shocks were delivered through a 30 cm by 24 cm floor composed of 14 grids by a 60-Hz constant current shock generator (BRS/LVE) and grid scrambler (Campden Instruments). The jump threshold was defined as the lowest of two consecutive intensities that elicited simultaneous withdrawal of both hindpaws from the grids. Each trial began with the animal receiving a 300 msec foot shock at a current intensity of 0.1 mA. Subsequent shocks occurred at 10 sec intervals and were increased in equal 0.05 mA steps until the nociceptive threshold was determined. After each trial, the current intensity was reset to 0.1 mA for the next trial until 6 trials were completed. Daily jump thresholds were computed as the mean of these six trials. Stable pre-injection jump thresholds were determined over 4 days.

The thirty-two rats were subdivided into four groups of eight rats matched on the basis of their baseline jump thresholds. A microsyringe (Hamilton Company) was connected to a stainless steel 28 gauge internal cannula (Plastic Products) by PE-50 (Clay-Adams) tubing. When the internal cannula was inserted through the permanently implanted guide cannula, it extended 0.5 mm ventral to the tip. All injections occurred 4–6 hr into the light cycle and were administered in 10 μl solutions infused at a rate of 1 μl every 15 sec. The first three groups received d-1 PCPA methyl ester hydrochloride (Sigma) injections at total ICV doses of 0, 1 and 3 mg. The fourth group received a hypertonic saline (550 mg/ml) injection which served as an osmolarity control for the 3 mg PCPA dose. Jump thresholds were determined 0.5, 24, 48, 72, 96, 120, 144 and 336 hr after injection. The experimenters conducting the jump tests and the microinjections were uninformed of the specific experimental conditions.

After completion of testing, all animals were anesthetized with sodium pentobarbital (100 mg/2 ml normal saline/kg body weight, IP) and perfused through the heart with 0.9% normal saline followed by 10% formalin. Each brain was blocked, sliced in 40 μm sections through the lateral ventricle, mounted and stained with cresyl violet for cell body visualization. Cannula placements of all animals in the present and subsequent experiments were localized by an observer uninformed as to the behavioral results who found that the cannula tips impinged upon the lateral ventricle.

TABLE 1

ALTERATIONS IN JUMP THRESHOLDS (±S.E.M.) FROM BASELINE (BL: MEAN OF 4 DAYS) FOLLOWING ICV INJECTION OF PCPA (1 OR 3 mg), NORMAL SALINE (NS) OR HYPERTONIC SALINE (HS) OVER A POST-INJECTION TIME COURSE

Time Course (hr)		Jump Thresholds (mA)			
		NS	HS	PCPA 1	PCPA 3
BL	Mean	0.471	0.452	0.465	0.466
	SEM	0.022	0.019	0.020	0.022
0.5	Mean	0.456	0.479	0.440	0.401*
	SEM	0.017	0.029	0.011	0.021
24	Mean	0.458	0.466	0.488	0.419
	SEM	0.022	0.036	0.032	0.018
48	Mean	0.482	0.467	0.456	0.404*
	SEM	0.025	0.025	0.018	0.021
72	Mean	0.476	0.468	0.463	0.440
	SEM	0.020	0.025	0.019	0.012
96	Mean	0.481	0.478	0.447	0.432
	SEM	0.031	0.037	0.025	0.028
120	Mean	0.494	0.452	0.456	0.416*
	SEM	0.017	0.028	0.023	0.017*
144	Mean	0.463	0.474	0.456	0.422
	SEM	0.028	0.036	0.019	0.027
288	Mean	0.481	0.450	0.426	0.481
	SEM	0.033	0.040	0.021	0.044

*Denotes a significant difference from both pre-injection BL values and from corresponding NS time point (Scheffe comparisons, $p<0.05$).

Results

Table 1 displays the significant decreases in jump thresholds following ICV administration of the 3 mg, but not the 1 mg dose of PCPA relative to both vehicle controls. While significant differences failed to occur among the groups, $F(3,28)=0.55$, and across testing intervals, $F(11,308)=0.54$, the interaction between groups and testing intervals approached statistical significance, $F(33,308)=3.38$, $p=0.086$. Scheffe comparisons indicated that the four

pre-injection baseline jump thresholds failed to differ significantly among groups, $F(3,28)=0.28$, or across the four days, $F(3,84)=0.14$. Administration of the 3 mg ICV PCPA dose significantly decreased jump thresholds relative to pre-injection thresholds at 0.5 hr, $F=10.84$, $p<0.05$, 48 hr, $F=11.89$, $p<0.05$, and 120 hr, $F=6.32$, $p<0.05$, following injection. In contrast, administration of either the 1 mg ICV PCPA dose, the vehicle injection or the hypertonic saline injection failed to alter jump thresholds from pre-injection baseline values. When comparing post-injection thresholds among groups, rats receiving the 3 mg ICV PCPA dose displayed significantly lower jump thresholds than vehicle-treated rats at 48 hr, $F=4.94$, $p<0.05$, and 120 hr, $F=4.59$, $p<0.05$, following injection. In contrast, jump thresholds of either the 1 mg PCPA group or hypertonic saline group failed to differ from those of the vehicle group over the post-injection time course. That jump thresholds were altered following the 3 mg ICV PCPA dose, but not the hypertonic saline injection suggests that any alterations in osmolarity induced by PCPA could not account for the observed PCPA effects.

EXPERIMENT 2: ICV PCPA AND MORPHINE ANALGESIA

Method

Forty male albino Sprague-Dawley rats (300–400 g) were treated surgically as described in Experiment 1. Based upon their pre-injection baseline jump thresholds, they were divided into five matched groups of eight rats. While the first group received an ICV injection of normal saline (10 μl) 0.5 hr prior to a subcutaneous injection of morphine (5 mg/ml buffered solution/kg body weight), the second, third, fourth and fifth groups received the morphine injection at either 0.5, 24, 48, or 72 hr following an ICV injection of 3 mg of PCPA (3 mg/10 μl normal saline). Jump thresholds of all groups were assessed 30 min following the morphine injections. All injections and tests occurred 4–6 hr into the light cycle and were carried out by experimenters uninformed of the experimental condition. The histological verification of cannula placements was carried out as described in Experiment 1.

Results

Figure 1 illustrates the time-dependent suppression of morphine analgesia induced by the 3 mg/kg ICV PCPA dose. Significant differences were observed among groups, $F(4,35)=8.82$, $p<0.001$, between conditions, $F(1,35)=185.16$, $p<0.001$, and for the interaction between groups and conditions, $F(4,35)=17.56$, $p<0.001$. Dunnett comparisons revealed that morphine significantly increased jump thresholds over mean baseline values in vehicle-treated rats, and in rats pretreated with ICV PCPA 0.5 hr, 24 hr and 72 hr earlier. Central PCPA injections administered 48 hr prior to morphine eliminated morphine analgesia. In addition, the magnitude of morphine analgesia was significantly smaller in rats treated with PCPA 24 hr and 48 hr before opiate administration relative to vehicle controls.

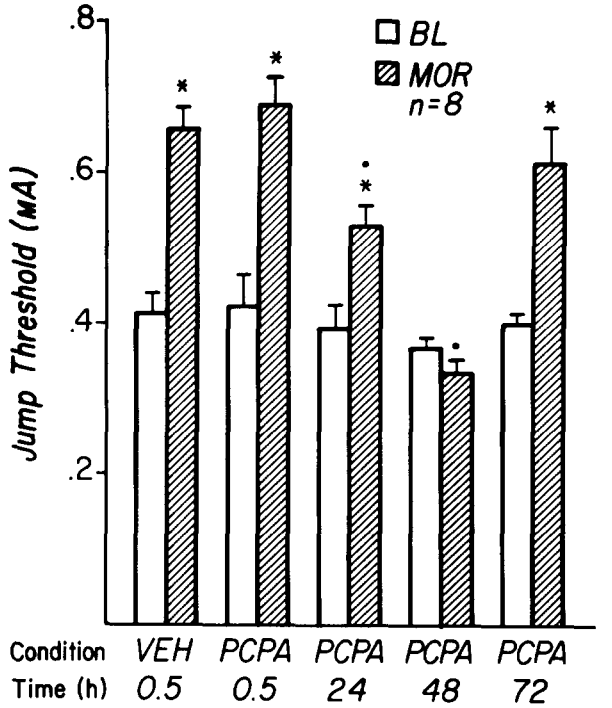

FIG. 1. Mean jump thresholds (±S.E.M.) for five groups of rats receiving a systemic 5 mg/kg dose of morphine (hatched bars) paired with a prior ICV injection of either PCPA (3 mg) or vehicle. While the interval between the vehicle and morphine injections (Group 1) was 0.5 hr, the interval between the PCPA and morphine injections varied across groups: 0.5 hr (Group 2), 24 hr (Group 3), 48 hr (Group 4) and 72 hr (Group 5). The degree of morphine analgesia across groups was assessed by comparing post-morphine thresholds with baseline thresholds (BL: open bars) that were assessed before the ICV and systemic injections. The asterisks denote significant differences from BL; the solid dots denote significant differences from the vehicle/morphine pairing.

EXPERIMENT 3: ICV PCPA AND BRAIN SEROTONIN

Method

Twenty-one male albino Sprague-Dawley rats (300–400 g) were treated surgically as described in Experiment 1. After recovery from surgery, rats were decapitated following one of the following conditions: (a) 3 mg PCPA (10 μl normal saline, ICV) 0.5 hr before decapitation (n=7); (b) 3 mg PCPA 48 hr before decapitation (n=6); (c) vehicle either 0.5 or 48 hr before decapitation (n=8). The brains were quickly removed from the skull and the hippocampi exposed and separated. Hippocampal tissue of each animal was kept at −70°C until assay. Measurements of serotonin and 5-hydroxyindolacetic acid (5-HIAA) were performed by high pressure liquid chromatography (HPLC) with an electrochemical detector [34]. Tissue samples were frozen on dry ice and sonicated (Kontes Microultrasonic Cell Disrupter) in approximately 5 volumes of ice-cold 0.1 M perchloric acid (PCA) containing 10 μ/ml of 0.1 M $NaHSO_3$ and 2 mM Na_2 EDTA. After centrifugation (15,600 g × 5 min), 20 μl of the clear supernatant was directly injected onto the HPLC (C_{18}, ODS column Dupont, LC-4A glassy carbon electrode, Bioanalytical Systems with an applied potential of 0.7 V) and eluted with 0.1 M sodium acetate buffer (pH 4.7 containing 2 mM Na_2 EDTA) and 4% (v/v) methanol. The flow rate was 1.25 ml/min at 35°C. Retention time for serotonin and 5-HIAA was 7.72 and 10.83 min respectively. An internal standard was not used since the assay involved direct injection of the PCA abstract into the HPLC. The HPLC system was calibrated immediately prior to the assays by injections of 5-HT and 5-HIAA. Assays were completed within a few hours. At the end of the assay, standards were reinjected and no deterioration in detected sensitivity was found during the assay period.

Results

Significant differences in hippocampal serotonin levels failed to occur across groups, F(2,18)=1.00: PCPA (3 mg: 0.5 hr pre-decapitation)—0.361 ng/mg tissue (SEM=0.085); PCPA (3 mg: 48 hr pre-decapitation)—0.208 ng/mg tissue (SEM=0.013); vehicle (0.5 and 48 hr pre-decapitation)—0.310 ng/mg tissue (SEM=0.073). Significant differences in hippocampal 5-HIAA levels also failed to occur across groups, F(2,18)=1.38: PCPA (3 mg: 0.5 hr pre-decapitation)—0.096 ng/mg tissue (SEM=0.021); PCPA (3 mg: 48 hr pre-decapitation)—0.109 ng/mg tissue (SEM=0.017); vehicle (0.5 and 48 hr pre-decapitation)—0.140 ng/mg tissue (SEM=0.017). In order to control for any variability caused by the two vehicle subgroups, a further analysis only compared hippocampal 5-HT and 5-HIAA levels in animals treated with either PCPA or vehicle 48 hr prior to sacrifice, and failed to find significant differences between the groups in either hippocampal 5-HT, t(9)=0.80, or 5-HIAA, t(9)=1.00.

EXPERIMENT 4: ICV PCPA AND SPINAL SEROTONIN

Method

Sixteen male albino Sprague-Dawley rats (400–550 g) were treated surgically as described in Experiment 1. After recovery from surgery, eight rats were treated with 3 mg PCPA (10 μl normal saline, ICV) 48 hr before decapitation while eight rats were treated with vehicle in an identical manner. The spinal cords were quickly removed from the vertebral column and the tissue of each animal was kept at −70°C until assay. Serotonin and 5-HIAA measurements were made as described in Experiment 3.

Results

Significant differences in spinal serotonin levels failed to occur, t(14)=0.16, between vehicle-treated, 0.048 ng/mg tissue (SEM=0.006), and PCPA-treated, 0.046 ng/mg tissue (SEM=0.005), rats. Significant differences in spinal 5-HIAA levels also failed to occur, t(14)=0.34, between vehicle-treated, 0.017 ng/mg tissue (SEM=0.004), and PCPA-treated (0.015 ng/mg tissue (SEM=0.002)) rats.

DISCUSSION

Intracerebroventricular PCPA injections produced a dose-dependent decrease in jump thresholds, indicating that animals were hyperreactive to shock at 0.5, 48 and 120 hr following injection, but not at 24, 72, 96 or 144 hr following injection. This appeared to be specific to PCPA's effects rather than secondary changes in cerebrospinal fluid osmolarity since hypertonic saline injections, which were of similar osmolarity to the effective 3 mg PCPA dose, failed to alter jump thresholds. The observed decreases in jump thresholds following ICV PCPA agree in part with effects observed following systemic injections in that both treatments significantly reduce jump thresholds 48 hr following injection [4, 17, 22, 39, 44]. However, while ICV PCPA injections significantly lower jump thresholds 0.5 hr after treatment, systemic injections do not [39]. Moreover, while systemic PCPA injections in rats produce lethargy soon after injection followed later by general debilitation, lack of grooming of the ventral surface of the body, and hyperreactivity, the ICV PCPA injections did not induce any of these side effects. The largest hyperalgesic effect of PCPA was only 15% and appeared only sporadically throughout the post-injection time course. However, it should be noted that a number of manipulations that result in hyperalgesia only produce small decreases in nociceptive threshold. For instance, naloxone, the opiate antagonist has been shown to exert either mild [3, 20, 27] or no [15,21] effects upon normal pain sensitivity. Like PCPA, the occurrence of naloxone hyperalgesia is dependent upon the pain test employed [11] and when effects are observed, they are small in magnitude (see review: [37]). One possibility why hyperalgesic agents like PCPA display such small effects is that they are challenging an overall "floor effect" in the nociceptive threshold; this would have to be confirmed with discrimination studies.

Intracerebroventricular injections of a 3 mg PCPA dose also time-dependently decreased the analgesic response following systemic administration of a 5 mg/kg dose of morphine. While jump thresholds were increased similarly by morphine in animals treated with ICV injections of either normal saline or PCPA at 0.5 hr before opiate administration, increases in the PCPA-morphine interval significantly reduced morphine analgesia 24 hr later and eliminated morphine analgesia 48 hr later. Yet if the ICV PCPA injection preceded the systemic morphine injection by 72 hr, it failed to affect significantly the opiate-induced increases in jump thresholds. The observed ICV PCPA effects upon morphine analgesia are again similar to, but not identical with systemic PCPA administration. Both treatments fail to affect morphine analgesia shortly after PCPA administration and attenuate the opiate effect 48 hr later. However, systemic, but not ICV, PCPA reduces morphine analgesia 72 hr after PCPA injection [40,41].

The decrease in basal pain thresholds and morphine analgesia following systemic PCPA pretreatment has been attributed to PCPA's ability to inhibit tryptophan hydroxylase, and thereby deplete brain serotonin levels [29, 39, 40, 41]. Such data support the hypothesis that brain serotonin (see review: [33]), and particularly the descending bulbospinal serotonergic pathways [18, 42, 43], are important modulators of pain-inhibitory processes. In the present study, ICV administration of PCPA has been shown to produce similar decreases in basal pain thresholds and morphine analgesia in the absence of corresponding significant depletions in serotonin or 5-HIAA either in hippocampus or in the spinal cord. The data do indicate that PCPA-treated rats display lower, though not significantly lower, levels of 5-HT in the hippocampus. The lateral ventricle injection site with its rich ependymal 5-HT varicosities could allow penetration into other central nervous system sites that may have resulted in significant serotonin depletion. Therefore, the lack of significant, robust effects in either the spinal cord or hippocampus must be viewed with caution until the failure to observe such effects in other 5-HT-rich regions is firmly established.

That PCPA administered by the ICV route can cause behavioral changes in the absence of significant serotonin alterations has also been observed in food intake studies. Hoebel and co-workers [5,24] initially reported that ICV injections of PCPA (2–4 mg) produced a dose-dependent hyperphagia, the time course of which correlated with significant 66–78% depletions of whole brain or forebrain serotonin. These data suggested that serotonin acted to inhibit food intake and paralleled similar findings with serotonin neurotoxins [35,36]. However, Coscina [12] failed to observe serotonin depletion induced by ICV PCPA and found that the PCPA parent amino acid, phenylalanine, produced simi-

lar hyperphagia to that of PCPA following ICV administration. Subsequently, Hoebel [31] was unable to replicate the initial observations of ICV PCPA-induced serotonin depletions, and found that leucine and tryptophan elicited similar hyperphagia to that of PCPA following ICV administration. These studies have led to the suggestion that the observed hyperphagia following PCPA or these amino acids are due to a nonspecific neurotoxicity rather than actions upon specific neural systems.

While the hyperalgesia and morphine analgesia impairments in the absence of serotonin depletion following ICV PCPA correspond with the hyperphagia in the absence of serotonin depletion following ICV PCPA, it does not necessarily follow that the former effects are due to nonspecific neurotoxicity. If nonspecific actions accounted for PCPA and amino acid effects, the expectation would be that both types of manipulations would exert similar changes. While leucine, phenylalanine and tryptophan, like PCPA, each produce hyperphagia [12,31], leucine and phenylalanine, unlike PCPA, each produce analgesia [13] while tryptophan fails to alter pain thresholds [25]. Furthermore, while PCPA decreases morphine analgesia, phenylalanine and leucine display cross-tolerance and synergy with morphine analgesia [2,19]. It would appear from these data that the observed hyperalgesia and morphine analgesia impairments following ICV PCPA are neither the result of brain serotonin depletion nor the result of nonspecific neurotoxicity (also see review: [30]). Rather it may be that central PCPA administration produces such effects by interacting with other presently undetermined neural or peptidergic elements or with other regional serotonin brain areas that have not as yet been assayed.

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