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Serotonergic Influences on EEG Synchronization Induced by Milk Drinking in the Cat

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CERVANTES, M., R. RUELAS AND C. BEYER. *Serotonergic influences on EEG synchronization induced by milk drinking in the cat.* PHARMACOL BIOCHEM BEHAV **18**(6) 851–855, 1983.—Milk drinking elicits electroencephalographic (EEG) synchronization in the parieto-occipital cortex of cats. This EEG change is a reliable correlate of the consummatory phenomena involved in "relaxation" behavior. The effect of varying serotonin brain levels by administering P-chlorophenylalanine (PCPA) or 5-hydroxytriptophan (5HTP) on this response was studied in 25 young cats. Single or repeated injections (4–8 days) of 50, 100 or 150 mg/kg of PCPA resulted in a dose related decrease in the duration of the EEG parieto-occipital synchronization during fixed periods of milk drinking. A single injection of 3, 10 or 30 mg/kg of 5HTP to chronic PCPA treated cats, restored the EEG parieto-occipital synchronization during milk drinking to control values. Moreover, administration of 5HTP to non-treated cats significantly increased the duration of EEG parieto-occipital synchronization during milk drinking. Our results suggest that brain serotonergic neurons are involved in the development of EEG synchronization during milk drinking.

Serotonin EEG synchronization Milk drinking Cats

MILK drinking, grooming and drowsiness are associated in the cat with EEG synchronization in the parietal cerebral cortex [1, 3, 4, 12, 26, 29]. Moreover, EEG parietal synchronization appears in other behavioral conditions induced by stimuli such as perineal tapping, petting and suckling [1,31]. The "positive" nature of such stimuli suggests that EEG parieto-occipital synchronization is an electrophysiological correlate of a group of behavioral responses which have been generically called "relaxation behavior" [1,26]. These behavioral responses share the following characteristics: (a) induction by pleasurable stimuli, (b) quiescence though not necessarily complete stillness (approach maintaining pattern of behavior), (c) synchronization in the parieto-occipital area and (d) inhibition of neuronal firing in the mesencephalic tegmentum (MRF).

There is evidence that EEG synchronization and inhibition of the neuronal discharge of the MRF can be induced by activating brainstem serotonergic mechanisms [18, 19, 23]. Moreover, progesterone may increase parieto-occipital synchronization during milk drinking probably by altering the synthesis or availability of brainstem serotonin [5, 20, 21]. Therefore we considered it interesting to study the effect of some drugs that modify brain serotonin levels on the characteristics of the EEG parieto-occipital synchronization occurring during milk drinking in the cat.

METHOD

Twenty-five young intact cats (9 males, 16 females) were used (weight 0.750–2.0 kg). Ten days before testing, two pairs of cortical electrodes, i.e., stainless steel needles, were chronically implanted over the parieto-occipital cortex and a reference electrode was placed in the frontal sinus. Surgery was performed under pentobarbital anesthesia (30 mg/kg). The cats were kept in individual cages. Meat and water were provided at 15:00 hr and the cats were allowed to eat ad lib until 20:00 hr when the food was withdrawn.

EEG recordings were performed in the morning. The electroencephalogram (EEG) was recorded in a Grass model 7 polygraph. Cats were placed in a soundproof chamber and, after a 15 min adaptation period, a bowl containing 150 ml of milk (50% condensed milk, 50% water) was introduced into the cage. Temperature of the soundproof chamber was maintained at 21°C. The cat was allowed to drink for three one min periods with 3 min intervals. Thus, the experimental data were collected during the 3 min period when milk was available to the cat. The bowl was always introduced and taken out of the cage by the same person. This procedure did not affect the cat. The amount of milk ingested by the cat at each experimental session was measured. The duration of EEG synchronization during the 1 min drinking periods was analyzed as previously described [1, 4, 5]. Values of EEG synchronization were expressed as percentage of the total time of milk drinking, i.e., duration of EEG synchronization × 100/duration of milk drinking.

As previously shown [4] cats reach a relatively constant level of EEG synchronization after several experimental sessions. Once a stable level of EEG synchronization was

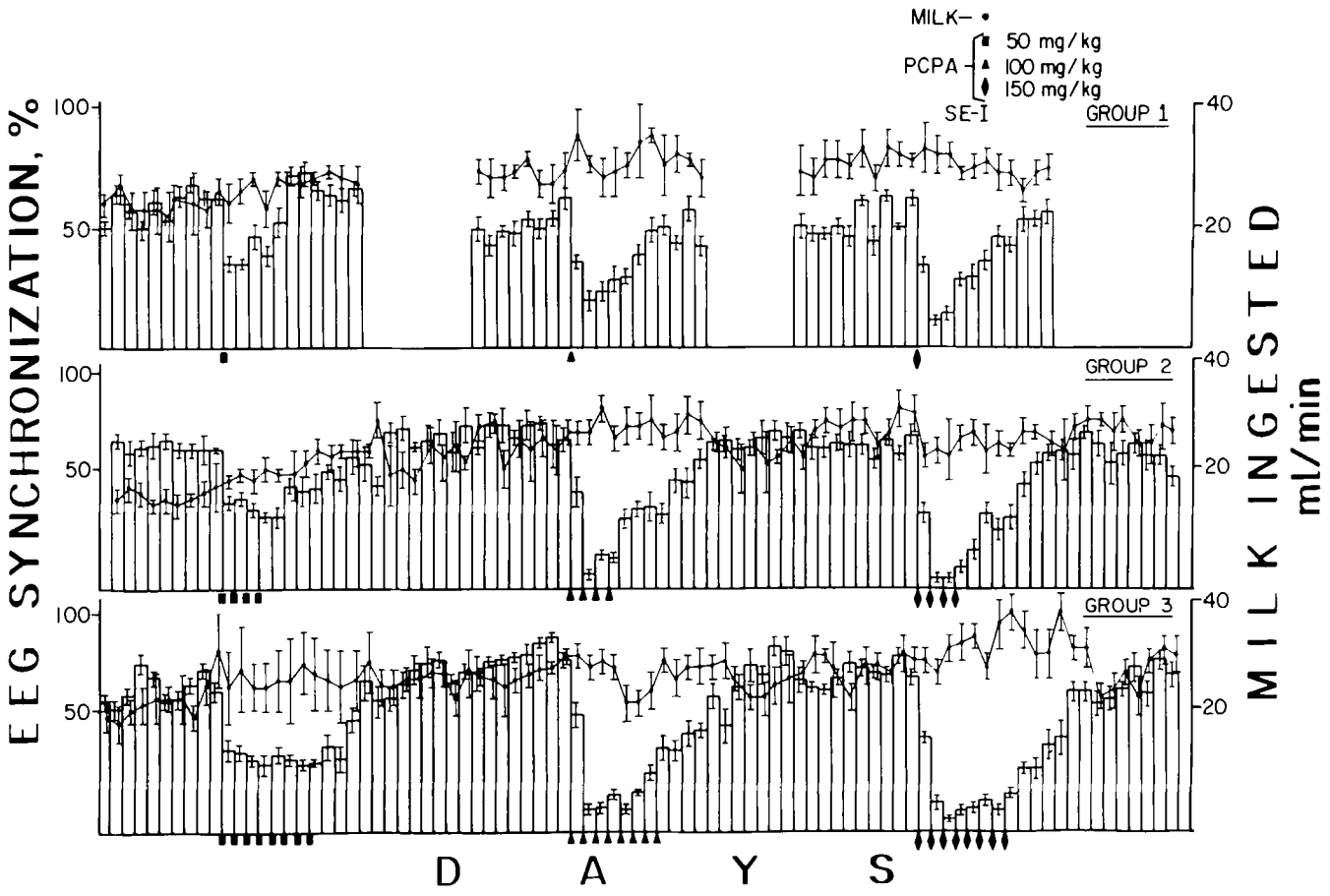

FIG. 1. Effect of PCPA administration on parieto-occipital EEG synchronization during milk drinking. Bars represent the mean average daily percent of EEG synchronization for 5 cats. The connecting line represents their average daily consumption of milk during the test period. Cats in group 1 received a single injection of PCPA; those in groups 2 and 3 received multiple injections. Dosages are indicated in the figure.

achieved (Figs. 1 and 2) the cats were allotted to the following treatment groups.

Group 1

The effect of single IP injections of PCPA on the level of EEG parietal synchronization was tested in five cats. Each cat was treated with three dosages of PCPA (50 mg/kg, first injection; 100 mg/kg, second injection; 150 mg/kg, third injection). Injections were given at two week intervals (see Fig. 1). This period allowed recovery of control preinjection EEG synchronization values.

Group 2

The effect of series of four daily IP injections of PCPA on the level of EEG parietal synchronization was tested in five cats. Each cat was treated with three dosages of PCPA (50 mg/kg/day, first series; 100 mg/kg/day, second series; 150 mg/kg/day, third series). A three week period was interposed between each series of injections. This period allowed recovery of control preinjection EEG synchronization values.

Group 3

The effect of series of eight daily IP injections of PCPA on the level of EEG parietal synchronization was tested in five cats. Each cat was treated with three dosages of PCPA (50 mg/kg/day, first series; 100 mg/kg/day, second series; 150 mg/kg/day, third series). Each series of injections were given at three week intervals. This period allowed recovery of control preinjection EEG synchronization values.

Group 4

Ten cats received single injections of 3, 10 and 30 mg/kg of 5-hydroxytryptophan (5HTP). Each cat received the three dose levels of 5HTP with a 10 day interval between doses (Fig. 2). Ten days after the last injection of 5HTP (30 mg/kg), PCPA treatment (100 mg/kg/day for 8 days) was initiated to maintain low EEG synchronization levels during milk drinking. On the fifth day of this PCPA treatment, a single injection of 5HTP (3mg/kg, 3 cats; 10 mg/kg, 3 cats; 30 mg/kg, 4 cats) was administered (Fig. 2).

DL-p-Chlorophenylalanine methyl ester (PCPA) and 5-hydroxy-L-tryptophan (5HTP) (Sigma Chemical Co.) were administered dissolved in sterile pyrogen free vehicle (Carbowax 4 g; benzyl alcohol 0.009 g; NaCl 0.008 g; citric acid 0.1 mg; H_2O c.b.p. 1 ml) at concentration of PCPA 100 mg/ml and 5HTP 30 mg/ml. The effect of the drug solvent on EEG parietal synchronization during milk drinking was tested in all cats. Injection of PCPA were performed at 15:00 hr. The day following the first injection and the day following the last

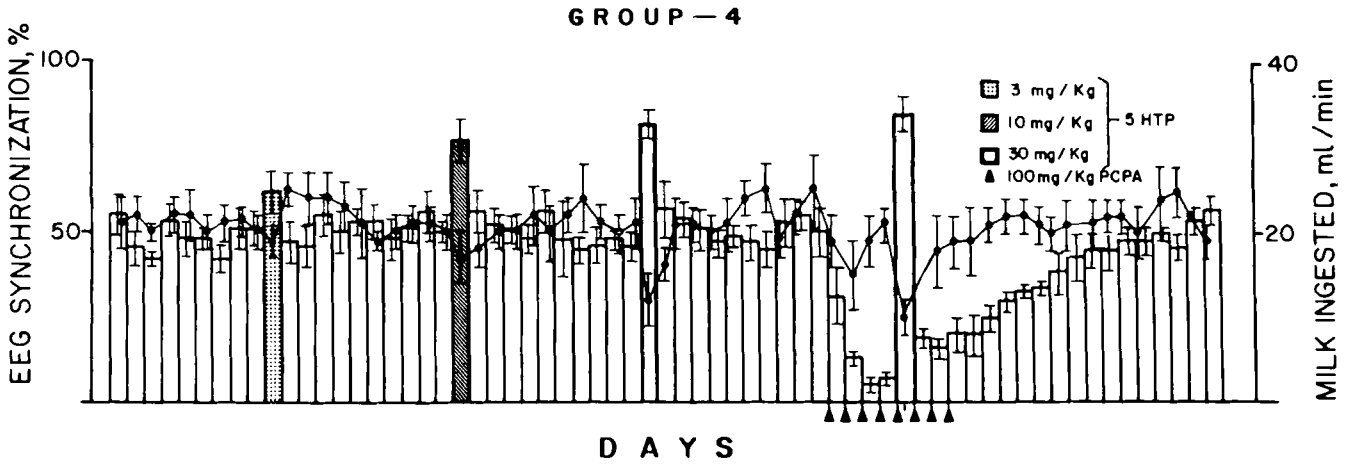

FIG. 2. Effect of 5HTP administration on parieto-occipital EEG synchronization during milk drinking. Bars represent the mean average daily percent of EEG synchronization for 10 cats, 4 of them injected with 5HTP (30 mg/kg) under PCPA treatment. The connecting line represents their average daily consumption of milk during the test period. Drugs and dosages are indicated in the figure.

injection were considered the first and last days of treatment. Injections of 5HTP were given 4 hr before testing. Values of EEG synchronization during control and treatment phases were compared by using the Mann-Whitney U-test [28]. This same test was used to compare the average milk volumes ingested during the various experimental phases.

RESULTS

EEG parieto-occipital synchronization, usually appearing in intermittent trains of high voltage (150 μv) and low frequency (6–10 c/s) was elicited by milk drinking in all cats. Duration of EEG synchronization during milk drinking periods achieved relatively constant levels as the cats became adapted to the experimental environment. Duration of EEG synchronization was relatively constant for individual cats but large interindividual variations were noted. Thus, duration of EEG synchronization in control conditions ranges from 40% to 85% of the time spent in milk drinking.

Figures 1 and 2 show the time course of the changes of EEG synchronization during the entire experimental period. As shown in Fig. 1, PCPA single injections resulted in an abrupt decrease in EEG synchronization values. This decrease was dose dependent, since 50 mg/kg PCPA only reduced EEG synchronization duration to about $^2/_3$ of the control values while 100 and 150 mg/kg PCPA reduced EEG synchronization to less than $^1/_4$ of the control values. The effects were already apparent 16 hr after PCPA, but the maximal effect was noted between 48 and 72 hr after treatment (Fig. 1). Recovery of EEG synchronization to control levels was achieved within one week of the PCPA injections regardless the dose level employed. As shown in Fig. 1 (middle and low panels) a series of PCPA injections also significantly reduced EEG parietal synchronization (the 10 control means preceding PCPA vs. the 4 mean values obtained after PCPA in group 2 or vs. the 8 experimental values in group 3; Mann Whitney U, $p<0.01$). PCPA, either single or repeated injections, induced alterations in the spontaneous behavior of cats. Thus, during control observations, cats adopted a quiet posture after a brief period of exploration, groomed and often appeared drowsy. By contrast, following PCPA cats appeared restless, and orienting behavior and mewing were consistently displayed during the 15 min adaptation period. PCPA did not significantly alter the volume of milk ingested by cats during the experimental sessions, which ranged between 15 to 45 ml/min (Fig. 1). Administration of 5HTP 10 or 30 mg/kg (four hours before testing) to drug naive cats significantly increased the duration of EEG parietal synchronization during milk drinking (10 individual values obtained the day before 5HTP vs. 10 individual values obtained the day of 5HTP injection; Mann-Whitney U, $p<0.05$). The effect induced by 5HTP was transient since duration of EEG synchronization returned to the control levels within 24 hours (Fig. 2). Administration of 3, 10 or 30 mg/kg of 5HTP to cats with very low values of EEG synchronization due to chronic treatment with PCPA, restored the levels of EEG synchronization during milk drinking to their control values (Figs. 2 and 3). The effect of 5HTP was transitory since low values of EEG synchronization reappeared 24 hr after 5HTP, provided PCPA treatment was continued (Figs. 2 and 3). Solvent administration, either as single injections or in series of injections, did not affect EEG parietal synchronization duration during milk drinking.

Miosis, drowsiness and reduction of motor activity with ataxia at the higher doses were some of the alterations induced by 5HTP injections. A clear reduction of the volume of ingested milk also occurred following 10 or 30 mg/kg of 5HTP. 5HTP at the dose levels employed induced delta-like waves in the EEG. However, during milk drinking, parietal EEG synchronization, 6–10 c/s, 150 μv, predominated over delta activity.

DISCUSSION

Previous experimental data indicate that EEG parieto-occipital synchronization is a reliable correlate of the consummatory phenomena involved in behaviors which have been labeled as "relaxation" [1, 3–5, 12, 26, 29, 31]. The present results show that PCPA inhibits EEG parieto-occipital synchronization in a dose dependent manner. In contrast, administration of 5HTP, the serotonin precursor, restores the levels of EEG parieto-occipital synchronization in PCPA treated cats.

Changes in the occurrence or vigour of a given behavior or specific EEG rhythm by PCPA or 5HTP treatments are usually interpreted as presumptive evidence that serotonin is

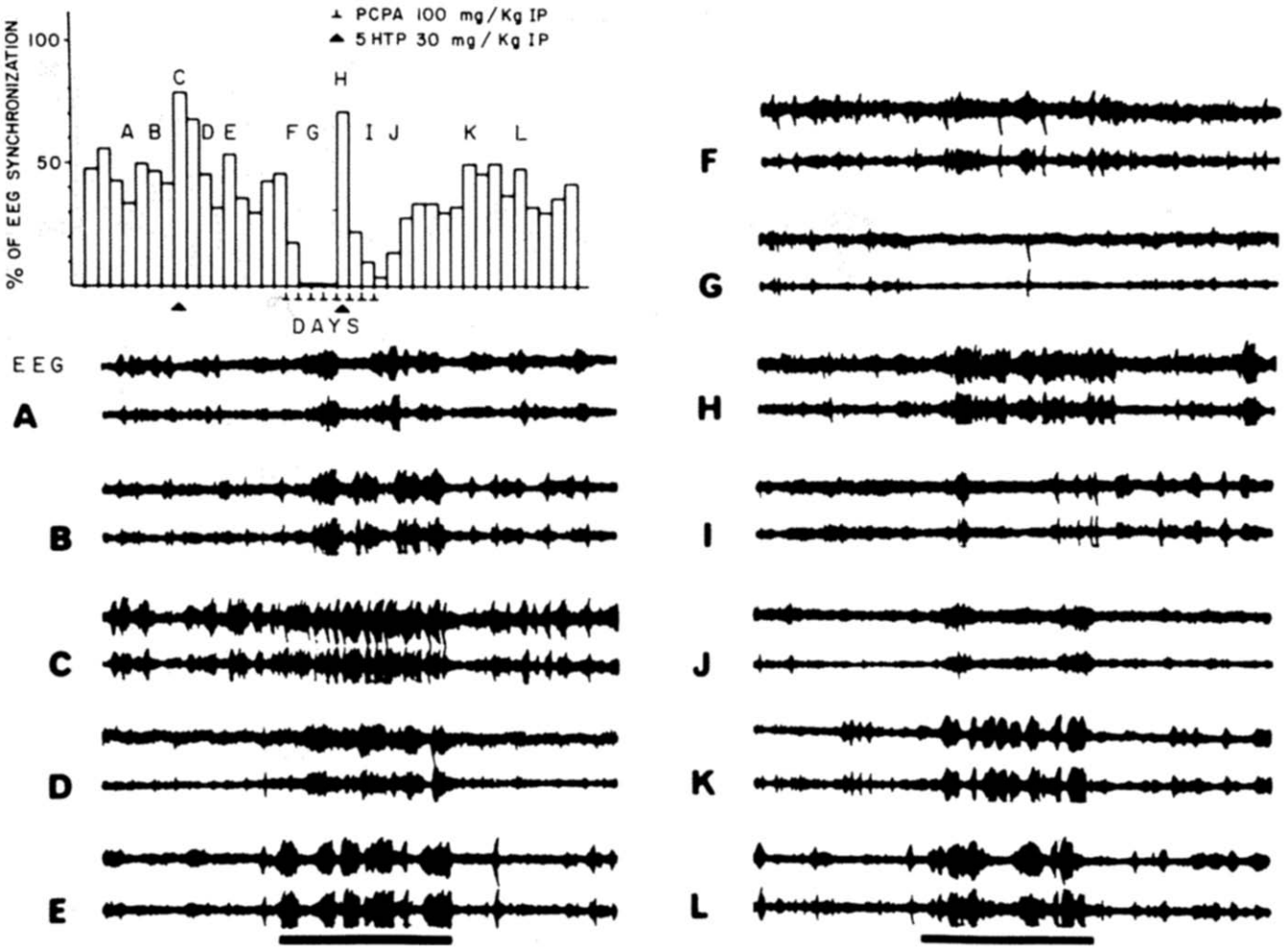

FIG. 3. Effect of PCPA and 5HTP on parieto-occipital EEG synchronization during milk drinking in the cat. Graph (upper left) shows daily values of EEG synchronization during a 40 day period. Letters on bars indicate days from which EEG records were selected for illustrations. EEG records were recorded from both parieto-occipital cortices, right cortex (upper trace), left cortex (lower trace). Black bars at bottom of figure indicate period of milk drinking (one min). Drugs and dosages are indicated in the upper graph.

involved in the regulation of this behavioral or EEG pattern [2, 6–8, 14, 15, 17, 22, 25, 30, 32, 33]. The temporal course of the EEG changes induced by PCPA or 5HTP related well with the action of these drugs on brain serotonin levels [6, 16, 17, 25], thus suggesting that this neurotransmitter is involved in the production of the EEG changes characteristic of milk drinking.

Low frequency stimulation of brainstem serotonergic structures induces EEG synchronization in curarized cats and freely moving rats [18,19]. Therefore, it is possible that the decrease in serotonin induced by PCPA prevented the development of EEG parieto-occipital synchronization by interfering with the activity of a serotonergic "synchronizing system." On the other hand, activation of the serotonergic system by electrical stimulation induces widespread EEG synchronization [18,19], rather than well circumscribed parieto-occipital synchronization observed during milk drinking. It is possible, however, that under physiological conditions the serotonergic brainstem mechanism modulates the electrical activity of limited cortical areas. This is supported by the observation that stimulation of the dorsal raphe induces localized release of serotonin from the parietal cortex [9].

Several workers have reported an inverse relationship between the serotonergic and the noradrenergic brain systems [10, 11, 13, 24, 27]. Experimental manipulations resulting in serotonin depletion induce behavioral changes such as insomnia, aggressiveness and hyperirritability [2, 6, 7, 14, 24, 30, 34] that can be diminished by drugs decreasing the content or availability of noradrenaline in the cerebral areas [24,34]. These behavioral alterations presumably mediated by noradrenaline [27,34], might interfere with the development of EEG parietal synchronization since this electrophysiological pattern appears only in relaxed animals [4]. Conversely, the facilitatory effect of 5HTP on EEG synchronization, observed both in intact and PCPA treated cats, could be related to the sedated condition produced by a decrease in noradrenergic activity associated with a rise in serotonin brain levels [27]. Further studies, however, are required to establish the participation of the noradrenergic system in the development of EEG parietal synchronization during milk drinking.

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