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PGO Waves and Insomnia in PCPA-Treated Rats

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MARKS, G. A. AND H. P. ROFFWARG. *PGO waves and insomnia in PCPA-treated rats.* PHARMACOL BIOCHEM BEHAV **31**(3) 509–513, 1988.—The serotonin-depleting drug, parachlorophenylalanine (PCPA), in a dosage of 300 mg/kg, was administered to rats in an effort to test the hypothesis that altered distribution of PGO waves following drug treatment may be responsible for the sleep disruption and consequent sleep loss that accompany decreased serotonin levels. Consistent with the hypothesis, we found that the greater the proportion of PGO waves that precede spontaneous arousals, the greater the reduction in slow wave sleep. However, inconsistent with the hypothesis, we found that the decrease in sleep did not result from an increase in the number of arousals. Further, though an increase in the proportion of waking waves always accompanied a rise in wake time, the two variables were negatively correlated. These data do not support a PGO wave/arousal hypothesis to account for the decrease in sleep following PCPA treatment in the rat. Rather, the findings tend to implicate an alteration in the mechanisms of arousal linked to serotonin depletion.

Sleep/waking cycle Parachlorophenylalanine PGO waves Rat

READILY discriminable waveforms, which may be recorded with macroelectrodes in several brain structures of the cat, dramatically appear preceding rapid eye movement (REM) sleep periods and continue to occur during the course of these periods (2). This activity has been called pontogeniculo-occipital (PGO) waves (7). In an investigation of PGO-type waves in the rat, their association with spontaneous awakenings was recognized (6). Additional observations during continuous 24-hour recording revealed that 54.5% of all awakenings from slow wave sleep (SWS) followed quickly after an appearance of PGO waves. The PGO waves in SWS that occurred before awakenings were similar in character, rate, and number to the PGO waves in SWS that heralded REM sleep onset (13).

The temporal association between PGO waves and spontaneous arousals in rats suggests that the neural processes that generate PGO activity may also induce arousal. Dement *et al.* (5) have proposed a hypothesis that attributes an arousing capability to PGO waves. This view is based mainly on results in cats obtained after chronic administration of the serotonin-depleting drug parachlorophenylalanine (PCPA) (9). This agent induces a severe insomnia as well as major alterations in the sleep-stage distribution of PGO waves (5,10). A great deal of evidence has been gathered that indicates that the serotonergic raphe system affects the temporal distribution of PGO waves (3). The relationship between serotonergic mechanisms and quantity of SWS provides the basis for Jouvet's hypothesis that the serotonergic raphe system induces SWS (8). In the cat, manipulations that deplete serotonin both decrease SWS amounts and disrupt PGO wave distribution.

Chronic administration of PCPA, which presumably causes serotonin to remain at very low levels in brain, also leads to persistent uncoupling of the customary PGO wave/REM-sleep relationship (4). It has been suggested that the insomnia following PCPA administration does not result from the inactivation of a sleep-inducing system but, alternatively, from arousals provoked by the altered PGO wave discharge (4). After several days, however, the proportion of time spent in sleep and waking moves in the direction of control levels (5). The apparent loss of the PGO waves' presumed arousal effect has been explained by habituation (4). Other interpretations also need to be considered, such as the possibility of differential development of receptor supersensitivity in CNS mechanisms that subserve induction of sleep and gating of PGO waves. It may be that the concentration of serotonin in brain most closely affects distribution of PGO waves and only secondarily affects sleep/wake stage amounts. It is not yet clear, however, whether alterations in PGO wave distribution with PCPA are causal to the changes observed in sleep/wake amounts.

Further, the argument may be advanced that, if the PGO wave/arousal hypothesis explains the insomnia induced by PCPA administration, the often-stated direct effect of serotonin depletion upon SWS-inducing mechanisms becomes questionable. Indeed, after PCPA treatment evidence of lowered threshold to arousal (17) as well as hyperreactivity to novel external stimuli (1) indicates an alteration in arousal mechanisms. The manner in which serotonin depletion reduces sleep time may throw further light on the underlying sleep/wake mechanisms affected by serotonin. If, after PCPA, the data reveal increased numbers of spontaneous arousals from sleep, longer average wake periods, and shorter average SWS periods, an alteration in arousal

threshold would be suggested, whereas if fewer SWS episodes are found, shifts in the mechanisms bearing on sleep induction would be more likely.

It is also possible that redistribution of PGO wave activity, by evoking arousal responses, may interact with altered arousal mechanisms. Following PCPA treatment, the initial appearances of PGO waves in waking are accompanied by orienting responses, responses similar to those elicited by external stimuli (4,5). If PGO wave activity during SWS are producing arousals, then following PCPA treatment an increased number of arousals from SWS, an increased proportion of PGO waves preceding these arousals, and an increased number of arousals preceded by PGO wave should also be observed. Additionally, if an increase in PGO wave activity during waking prolongs wake periods, the increase in wake PGO waves would be expected to be proportional to the increase in wake time.

In summary, the rationale for this study derives from earlier work in this laboratory concerned with the distribution of PGO waves in the rat with respect to sleep/wake stage (13). We observed that a significant percentage of PGO waves in SWS momentarily preceded spontaneous awakenings. This normative association between PGO waves and spontaneous awakenings in rat (6) permits a test of the relationship of PGO redistribution and sleep loss after serotonin depletion.

METHOD

The subjects were male Sprague-Dawley albino rats (Holtzman), weighing between 370 and 482 g at the time of surgery. Under sodium pentobarbital anesthesia, animals were implanted with a standard array of bipolar electrodes for chronic sleep recording. In addition to electrodes for the recording of the electroencephalogram, nuchal electromyogram and electro-oculogram, a stainless steel bipolar electrode (250 μm diameter) was stereotaxically implanted either in the dorsal pontine tegmentum or deep anterior lobe of the cerebellum for the recording of PGO waves. The surgical procedure and the loci from which PGO waves are best obtained have been presented in detail elsewhere (12).

The development of criteria for and quantification of PGO waves in the rat have also been described (2). The method is individualized for each animal and is based upon establishing a minimum amplitude criterion for a recurring waveform, exhibiting constant polarity, a duration between 60 and 120 msec, and appearance in every REM sleep period. At least 80 percent of these waves must either occur in REM sleep or in the two minutes of SWS preceding REM sleep onsets and spontaneous awakenings. These criteria were met in the four animals used in this study.

Once the defining characteristics of the PGO wave were established for each animal in an initial test recording, they were used throughout all subsequent experimental conditions. Quantification of recordings was achieved by dividing the 24-hour tracings into 2,880 epochs of 30 seconds each. An epoch was characterized in two ways: first, in regard to sleep stage (using a standard scoring procedure) and, independently, in regard to the number of PGO waves contained within it.

The distribution of PGO waves was determined by computing their frequency within five mutually exclusive stage categories: 1) Awake (AW); 2) REM sleep (REM); 3) SWS, in the two-minute period before REM sleep onset (R-2); 4) SWS, in the two-minute period before spontaneous awakening (AW-2) (the awakening is defined as an AW epoch preceded by one or more consecutive epochs of SWS); and 5) the remainder of SWS. Values for the absolute frequency, rate per minute, and percent frequency of PGO waves were computed for each category.

TABLE 1

CONTROL VS. PARA-CHLOROPHENYLALANINE (PCPA) STAGE AMOUNTS (MIN)

	Vehicle Baseline			PCPA (300 mg/kg)		
Subject	AW	SW	REM	AW	SW	REM
RS-23	525.0	755.0	137.3	736.5	574.5	62.4
RS-37	726.5	574.0	134.6	905.0	465.2	59.1
RS-41	654.5	671.5	110.7	1021.5	375.5	37.0
RS-44	854.5	468.2	111.2	1120.5	276.9	35.9
Mean*	690.0	617.1	123.4	945.8	423.0	48.6
±SEM	±97.3	±87.6	±10.2	±116.6	±89.7	±10.0

*All respective stage means differ significantly across conditions ($p<0.05$).

The experiments consisted of a vehicle-baseline condition and a drug manipulation. The subjects served as their own controls. Every rat received an IP injection of sterile normal saline. Twenty-four hours after an injection, a 24-hour recording was obtained (vehicle-baseline condition). At the termination of the recording, a solution of 300 mg/kg PCPA methylester HCl (K & K) and sterile normal saline, which was equal in volume to the baseline injection, was similarly administered. After a 24-hour waiting period, a second 24-hour recording was obtained (drug condition).

The quantities of sleep and the PGO wave distributions were compared in terms of sleep stage and stage categories, respectively, between the postvehicle and postPCPA recordings. All statistical tests were two-tail. Significance was defined as the chance of error with a probability less than 5 in 100. Differences in means were tested by correlated t-tests, degrees of freedom equal to two. Pearson product-moment correlation coefficients were determined to be different from zero by t-tests (r=.95).

RESULTS

Amounts of Sleep/Waking Stages

In the period 24 to 48 hours after administration of 300 mg/kg PCPA, the time spent in all stages differed significantly from the 24-hour control period. Calculated as mean percentages of control values ±SEM, AW was 138±6.8, SWS was 68±6.2, and REM sleep was 39±3.4 (see Table 1).

The increase in AW time in the drug condition was due to longer average wake periods [mean duration (min): 2.8 in control vs. 3.8 following PCPA]. The mean number of spontaneous awakenings during SWS remained fairly constant in the vehicle and drug phases (246 vs. 251). Though shorter SWS periods [mean duration (min): 2.1 vs. 1.6] were the main component of the decrease in SWS, a minor decrease in the number of these episodes was also observed. Mean REM sleep durations were almost identical [mean duration (min): 2.3 vs. 2.3]. Correspondingly, the decrease in REM sleep time in the PCPA condition resulted from a significantly reduced number of REM sleep periods (mean number: 53.8 vs. 21.5).

TABLE 2

CONTROL VS. PARA-CHLOROPHENYLALANINE (PCPA) WAVE DISTRIBUTION*

	Vehicle Baseline					PCPA (300 mg/kg)					Totals	
	R-2	AW-2	REM	SW	AW	R-2	AW-2	REM	SW	AW	BL	PCPA
(a)	180 ±57	336 ±78	2132 ±937	177 ±79	166 ±35	93 ±40	305 ±105	324 ±44	115 ±72	513 ±272	2992 ±1128	1349 ±469
(b)	1.62 ±0.43	1.20 ±0.28	16.7 ±6.54	0.69 ±0.17	0.26 ±0.08	1.57 ±0.47	1.17 ±0.37	6.98 ±1.14	0.87 ±0.29	0.63 ±0.39		
(c)	6.27 ±0.78	13.18 ±3.46	67.94 ±5.42	6.19 ±2.18	6.44 ±0.82	6.13 ±1.40	23.68 ±4.17	31.85 ±7.77	7.29 ±2.27	31.06 ±7.08		
(d)	106.6 ±6.1	277.1 ±14.9	123.4 ±10.2	231.8 ±62.1	690.0 ±97.3	51.6 ±11.4	263.3 ±20.8	48.6 ±10.0	106.8 ±35.4	945.8 ±116.6		

*Mean ± SEM on the measures of (a) absolute frequency, (b) rate per minute, (c) percentage total frequency, and (d) time in minutes for four rats.

See text for description of sleep categories.

BL—baseline condition.

PGO Wave Distribution

As previously reported by this laboratory (13), in the period 24 to 48 hours after 300 mg/kg of PCPA, the relationship between PGO waves and sleep stage in the rat was altered. The mean absolute number of PGO waves increased from 166 to 513 in the AW category though total PGO wave number decreased from 2992 to 1348. There were corresponding increases (2.5-fold) during the PCPA condition in regard to the mean rate of PGO waves per minute in AW and, significantly, in the AW percentage of total waves. The mean percentage of total PGO waves occurring in the AW-2 category also increased significantly though mean rate per minute remained the same due to the fall in total output. After drug administration, a significant decrease was found in the mean REM sleep percentage of total waves. No change was observed in the mean R-2 percentage (see Table 2). The association of PGO waves and spontaneous awakenings from SWS did not appear to be affected by PCPA: 54.5 percent of spontaneous awakenings in the vehicle control condition were preceded by waves and 48.8 percent during the drug condition.

Correlations Between PGO Wave Distribution and Sleep Stage Amounts

Selected relationships between PGO wave distribution following PCPA and the time spent in particular stages were examined by means of Pearson product-moment correlations. PGO wave distribution was measured by the percent frequency of PGO waves in a category. Effects on sleep attributed to PCPA were derived by calculating the percentage of each stage's control quantity.

To measure the relationship between the quantity of preawakening PGO waves and the reduction in SWS amounts observed after PCPA, a correlation coefficient was computed for the percentage of PGO waves preceding SWS arousals under PCPA (AW-2) in relation to the percent of PCPA/control SWS time. A significant relationship ($r=-.96$) existed between these variables; that is, the greater the percentage of PGO waves preceding spontaneous awakenings, the greater the reduction in SWS.

A similar but lower correlation was found between the AW-2 PGO percentage and the reduction in REM sleep time ($r=-.85$). This relationship, however, is not independent of the high correlation between SWS and REM sleep amounts ($r=.95$). When a measure of REM sleep time that is less dependent on SWS performance was used in the correlation, such as REM sleep's percentage of total sleep time, the correlation not only fell but changed in sign ($r=.38$).

The relationship between the number of PGO waves in the wake state and the increased amount of wakefulness observed after PCPA was further analyzed. A correlation coefficient was computed for the percentage of waves in waking after PCPA in relation to the wake time under PCPA expressed as a percentage of baseline. A negative though not significant relationship was detected in the data ($r=-.47$). In individual animals, a tendency was noted for small increases in AW waves to accompany large increases in AW time, and for large increases in AW waves to accompany small increases in AW time.

One other relationship was pursued to assess the hypothesized normative tendency for arousal to be associated with PGO waves. For this purpose, the baseline percentage of total waves situated in the AW-2 intervals was computed. Significant correlation coefficients were obtained for this variable in reference to the reduction in SWS amount ($r=-.97$) as well as to REM sleep amount ($r=-.98$). Again, however, the baseline percentage of AW-2 waves did not correlate independently with REM sleep reductions when the latter were measured by REM sleep percentage of total sleep time ($r=.05$).

DISCUSSION

In support of earlier findings by Mouret *et al.* (14), we found that PCPA causes a significant reduction in both SWS and REM sleep time in the rat. The size of the effects reported with our dosages of 300 mg/kg (rather than the 500 mg/kg used in the other study) were smaller within the study period but similar to those found by Rechtschaffen *et al.* (16) who used 500 mg/kg.

The lack of consistent results in the earlier studies is also reflected within our own data. For example, though mean

SWS time decreased to 68.1 percent of control, the range was 55.9 to 81.1 percent among the individual animals. Due to the variability in results, we were unable to develop a consistent PCPA dose/response relationship for sleep loss (unpublished data). On the other hand, dose-dependent depletion of brain serotonin has been found after PCPA administration in the rat. Koe and Weissman (9) report that 24 hours after a single IP injection of PCPA methylester to rats at doses of 32, 100 and 316 mg/kg, mean (±SEM) brain serotonin levels were 87±7, 38±4 and 18±2 percent, respectively, of control level. The unpredictable nature of dosage upon sleep loss coupled with the predictability of dosage upon brain serotonin concentration strengthens the view that the time spent in sleep may not depend wholly on serotonin. It is necessary to point out however, that the variability in sleep amounts reported in this study is possibly due to variable amounts of serotonin depletion.

In the cat, insomnia is a consistent finding after administration of serotonin-depleting drugs or after destruction of the midline raphe nuclei (8). In addition to species differences in the effects produced by serotonin depletion, species differences have also been found when serotonergic mechanisms are potentiated. To illustrate, fluoxetine, a specific inhibitor of serotonin reuptake, presumably increases serotonin levels at receptor sites (21). This agent is reported to suppress REM sleep and, usually, increases SWS in cats, whereas in rats, SWS is not increased (18). Even destruction of the raphe nuclei in the rat seems to have little long-term effect on sleep quantity (15). In appraising the available evidence, we do not believe that it favors the existence of a simple, serotonergic-dependent, sleep-induction mechanism in the rat.

Alternatively, our data in this study are consistent with an interpretation more in line with the operation of serotonin-dependent arousal or sleep maintenance mechanisms (these are not distinguishable in this context). Consistent with such processes, decreased SWS time following PCPA did not result from fewer SWS episodes but, rather, from decreased SWS episode length. Further, the increases in wake time were accompanied by increased AW period lengths. An expected increase in the number of awakenings, given circumstances of increased arousability, was not observed. However, it must be pointed out that certain natural relationships among the variables may place constraints on the possible magnitude of their variations. For example, inasmuch as a SWS episode can be associated with only a single awakening, a large increase in the number of SWS arousals requires an increase in the number of SWS episodes. Following PCPA, however, the number of entries into SWS did not change much from baseline, though once in SWS, animals were more likely to wake up after a shorter period of time and to stay awake longer.

Returning to the issue of whether the redistribution of PGO waves following PCPA contributes to the decrease in SWS, we find the data to be somewhat conflicting. In support of the PGO wave/arousal hypothesis, a significant correlation was found after PCPA between the percentage of total PGO waves preceding spontaneous arousals from SWS and the magnitude of SWS reduction. This result can be considered consistent with the notion that PGO waves awaken the animal from SWS, and it is also consistent with shorter SWS periods and decreased SWS time after PCPA. Incompatible with the hypothesis, however, is the data revealing that only one-half of all arousals in *either condition* were preceded by PGO waves. In other words, postdrug PGO waves do not seem to account for more awakenings than postvehicle waves.

We also found that the increase in total wake time after PCPA was due to longer AW periods accompanied by an increased proportion of waking waves. This result can be considered consistent with the notion that PGO waves maintain wakefulness. However, though increases in PGO waves during waking always accompanied increases in wake time, closer examination revealed that the *greater* the percentage of AW waves the *less* the increase in wake time under PCPA conditions. This finding is also inconsistent with the PGO/arousal concept.

It is important to emphasize that the CNS state most influenced by PCPA is REM sleep. There is no support in the data that PGO waves have an arousal effect in this sleep stage. All measures of PGO wave output are reduced in REM sleep following PCPA, and REM time appears to suffer due to fewer REM periods not to shorter period lengths. This finding argues for serotonin depletion affecting the mechanism of REM sleep induction. The relationship between REM sleep mechanisms and serotonin, however, must be a complex one inasmuch as potentiation as well as inhibition of serotonin mechanisms reduce REM sleep time (18).

It would seem that neither the hypotheses of serotonergic sleep-induction nor of PGO wave/arousal adequately account for sleep loss following PCPA in the rat. Other workers have also questioned the adequacy of these hypotheses to explain the data in the cat (11, 19, 20). Nonetheless, it remains very curious that, following PCPA administration, the proportion of PGO waves appearing prior to spontaneous arousals is highly correlated with the amount of sleep loss. Beyond that, the baseline proportion of PGO waves in this category is highly predictive of the subsequent effect of drug ($r=-.97$). It may be that a high proportion of PGO waves preceding spontaneous arousals in SWS reflects poor control over the way this REM sleep-related phasic discharge is distributed. In other words, insofar as PGO wave distribution is under the control of a serotonergic gating mechanism, a high proportion of PGO waves in the AW-2 intervals may connote a weak serotonergic system, one that may be more susceptible to the effects of PCPA serotonin depletion.

In conclusion, acute depletion of serotonin by single administration of PCPA can produce independent effects upon both the *time* spent in sleep/wake stages and the *distribution* of PGO wave activity. Reductions in SWS and REM sleep and concomitant increases in wake time appear to result from altered arousal and altered REM sleep-induction mechanisms. Increases in the proportion of PGO waves in waking and also in SWS just before spontaneous arousals signal that the PGO wave gating mechanism has been modified as a result of PCPA treatment. In the rat, the extent to which PGO waves appear in SWS momentarily before arousals may mirror the general status of the serotonergic mechanisms in brain. Perhaps this measure can be used to predict the magnitude of serotonin depletion effects on behaviors unrelated to sleep that also exhibit a dependence upon the concentration of brain serotonin.

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