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Sleep Regulation After Reduction of Brain Serotonin: Effect of *p*-Chlorophenylalanine Combined with Sleep Deprivation in the Rat

Irene Tobler and Alexander A. Borbély

Institute of Pharmacology, University of Zürich, Zürich, Switzerland

Summary: Sleep was recorded in the rat after combined treatment with *p*-chlorophenylalanine (PCPA; 300 mg/kg) and 24-h sleep deprivation (SD) and then compared with sleep recorded after either treatment alone. PCPA alone reduced total sleep (TS), rapid eye movement sleep (REMS) per TS, as well as the power density of the EEG delta band (1.25–4.00 Hz) of non-REM sleep (NREMS). SD enhanced these sleep parameters and reduced the frequency of wake episodes. The combined treatment with PCPA and SD reduced TS and REMS/TS to a level similar to that induced by PCPA alone, and it increased delta activity to a level similar to that induced by SD alone. The frequency of wake episodes was reduced. It is concluded that essential aspects of sleep regulation are still functional during PCPA-induced insomnia. The sleep-inhibiting action of PCPA may be related to the hyperresponsiveness to stimuli rather than to the impairment of sleep regulation itself. **Key Words**: *p*-Chlorophenylalanine—Sleep deprivation—Sleep regulation.

p-Chlorophenylalanine (PCPA), the inhibitor of serotonin (5-HT) synthesis, induces a profound, long-lasting insomnia in the cat (1). This observation led to the recognition of the key role of the serotonergic system in sleep regulation. According to the "monoamine theory," 5-HT-containing neurons in the raphe nuclei are critically involved in the induction of non rapid eye movement sleep (NREMS) and in the priming of REM sleep (REMS) (2). Studies using PCPA or other 5-HT-depleting compounds have focused on changes of spontaneously occurring sleep. However, such effects on sleep could be indirect, since lowering the brain 5-HT level causes changes in a variety of waking behaviors as well (3). With the present experiments we addressed the problem of sleep regulation more specifically by augmenting the "sleep-pressure" during PCPA-induced insomnia. This was achieved by subjecting the animals to a 24-h sleep deprivation (SD) procedure,

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Address correspondence and reprint requests to I. Tobler, Pharmakologisches Institut, Universität Zürich, Gloriastrasse 32, CH-8006 Zürich, Switzerland.

which produces a massive increase in the slow wave sleep (SWS) fraction of NREMS (i.e., stages 3–4), as well as in REMS (4). The rise in the cerebral 5-HT level during recovery sleep suggested the involvement of serotonergic mechanisms in sleep rebound (5). On the other hand, lowering the 5-HT concentration by PCPA caused a partial insomnia with minimum sleep between 24 and 36 h following drug administration (6). This effect was causally related to the 5-HT depletion; it could be antagonized by raising the cerebral 5-HT level with administration of tryptophan. SWS and REMS were more affected by these manipulations than total sleep (TS) and NREMS. In the present study, we recorded sleep after the combined treatment with PCPA and SD; we then compared the effects with the results of either treatment alone.

METHODS

Adult male Sprague-Dawley-Ivanovas rats (SIV-50) weighing 280–320 g at the time of surgery were used. The animals were housed individually in transparent Plexiglas cages with unlimited access to food and water and were maintained on a 12-h light/12-h dark schedule (light from 0800 to 2000 h). Implantation of electrodes was performed under pentobarbital anesthesia (4,7). Recordings were started after a recovery and habituation period of 5–7 days. SD was carried out by placing the animal into a slowly rotating cylinder for 24 h (4). Cable recordings began 2 h after light onset and lasted for 5–6 h. The experiments were performed in two groups. In group 1, six rats were recorded on a control day and at 26 h after treatment with PCPA (*p*-chlorophenylalanine methylester, 300 mg/kg, i.p.; the dose is given for the acid). In group 2, nine rats were recorded on a control day and after 24 h of SD (starting 2 h after light onset). Three days were then allowed for recovery before the rats were injected with PCPA, deprived of sleep for 24 h (starting 2 h after PCPA injection), and recorded as in group 1. An additional group of four rats was recorded by telemetry to check for a possible interference of the cable leads with sleep after PCPA treatment and to test for a delayed REMS rebound during the 24-h recording period. The rats recorded by telemetry were subjected to the same treatment as described for group 2. The vigilance states recorded via cable leads were scored as waking, NREMS, and REMS for epochs of 10 s by visual inspection of the polygraphic electroencephalogram (EEG) and electromyogram (EMG) records (7). The 10-s criterion was also applied in determining the frequency of wake episodes. The EEG was recorded on analog magnetic tape, converted from analog to digital (conversion rate, 64 Hz), and subjected to Fourier analysis (8). The spectral power of the delta band (1.25–4.0 Hz) was expressed relative to the time spent in NREMS (total power/percentage NREMS). This provided a close estimate of delta activity in NREMS, since the delta activity values in waking and REMS are less by a factor of 10 (Borbély et al., in preparation; see also Fig. 2). The telemetric recording method and the automatic sleep state identification system have been described previously (9,10).

Forebrain 5-HT was determined according to a modified method of Curzon and Green (11) in three additional groups of five rats: (1) untreated controls, (2) 32 h after treatment with PCPA (300 mg/kg, i.p.), and (3) 32 h after PCPA (300 mg/kg) administered concomitantly with the onset of a 24-h SD period.

RESULTS

The present results for PCPA alone and SD alone are in agreement with previous findings obtained by telemetric recording techniques (4,6). Figure 1 shows the mean level of sleep parameters for the three experimental conditions. The administration of PCPA alone reduced the level of TS, REMS/TS, and delta activity in NREMS, and caused a nonsignificant increase in the frequency of wake episodes. SD alone enhanced TS, REMS/TS, and particularly the delta activity in NREMS, and reduced the frequency of wake episodes. The combined treatment with PCPA and SD reduced TS and REMS/TS to the same low level as PCPA alone. The situation was quite different for the delta activity, where the values after the combined treatment reached the same high level as after SD alone. A significant reduction in the frequency of wake episodes was seen after the combined treatment; it was, however, less prominent than after SD alone.

The telemetric recordings essentially confirmed the results obtained with cable recordings. The following mean values, expressed as a percentage of the control day (SEM in parentheses; $n = 3-4$), were obtained for the first 6 h of the light phase: SD alone—TS, 99.9 (2.5); (REMS/TS) × 100, 153.0 (15.7); PCPA + SD—TS, 75.5 (8.0); (REMS/TS) × 100, 73.9 (19.8). Because of the small sample size, no statistical analysis was performed. Inspection of the records obtained for the entire light phase and the consecutive 12-h dark phase revealed no evidence of a delayed rebound of the sleep parameters.

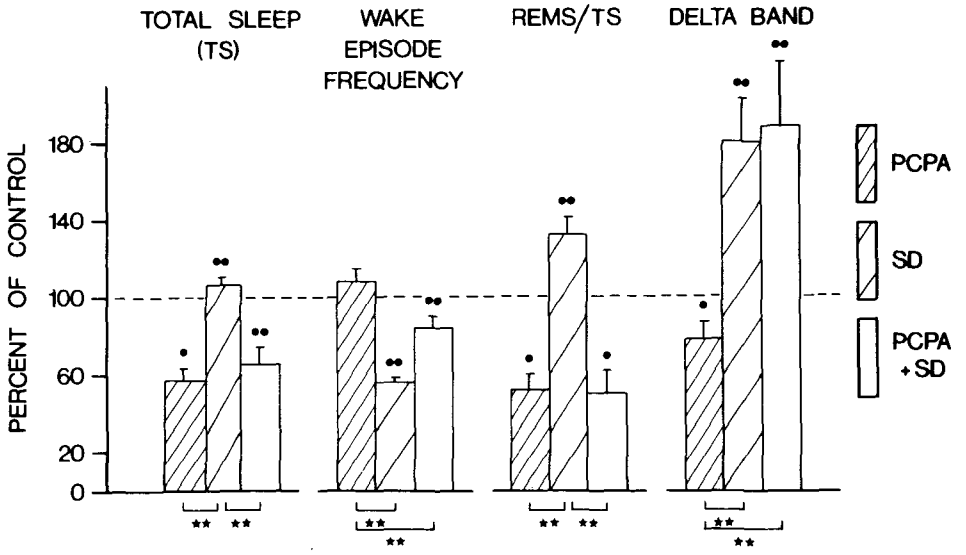


FIG. 1. Effect of treatment with PCPA, sleep deprivation (SD), and a combined treatment (PCPA and SD) on sleep. Mean values with SEM expressed as a percentage of the control day (100%, interrupted horizontal line). Control-day values for group 1 (PCPA; $n = 9$) and group 2 (SD and PCPA + SD; $n = 6$), respectively: TS, 76.8 and 76.2%; (REMS/TS) × 100, 21.1 and 18.9%; wake episode frequency per hour, 21.6 and 21.0. The power density of the delta band in NREMS was expressed in arbitrary units. Dots indicate significant differences from control, asterisks significant differences between the values indicated by brackets (• and *, $p < 0.05$; •• and **, $p < 0.01$; Wilcoxon paired and unpaired signed rank test, two-tailed).

Figure 2 illustrates the delta band activity for two rats. The peaks correspond to NREMS, whereas the low values represent either waking or REMS. Typically, a decreasing trend was seen across the recording period (6). A low level of delta activity was present after treatment with PCPA alone (PCPA + 1 day). After the 24-h SD period, the power density of the delta band was markedly enhanced as compared with the control period. Regular intervals between the delta peaks were present in the REMS rebound period (12). The bottom record in Fig. 2 illustrates the massive enhancement of delta activity after the combined treatment. However, in comparison to SD alone, the delta bouts were longer.

Figure 3 shows plots of two rats recorded by telemetry. In confirmation of previous results (4), SD increased the percentage of REMS and enhanced the SWS fraction of NREMS, which is evident from the low level of the ZCR values. The ZCR curve also reflects the regular alternation between synchronized and desynchronized EEG periods. The bottom records in Fig. 3 obtained for the combined treatment with PCPA and SD show, on the one hand, the high level of daytime waking and motor activity, a typical effect of PCPA. On the other hand,

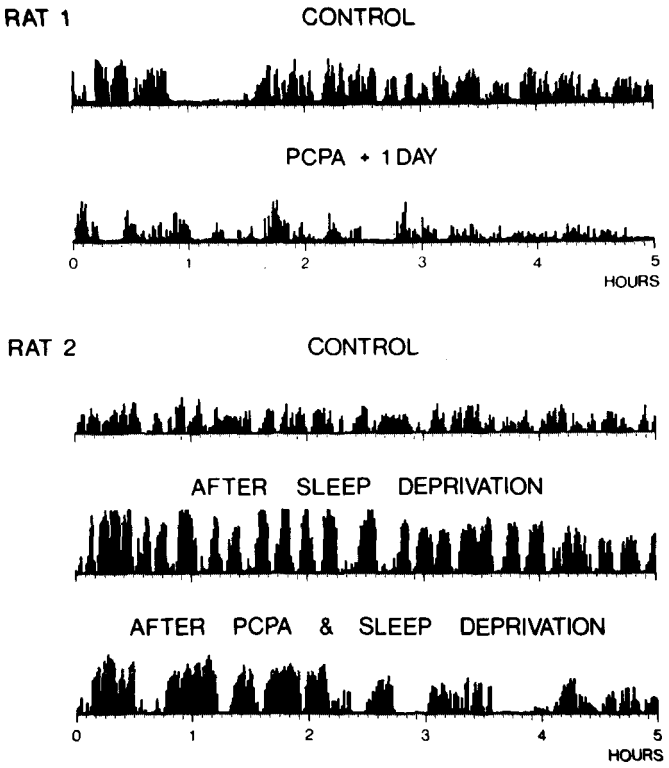


FIG. 2. Effect of PCPA, sleep deprivation (SD), and PCPA + SD on the power density of the delta band (1.25–4.0 Hz). Records of two rats plotted in arbitrary units for successive 1-min epochs starting 2 h after light onset. Rat 1: control day and a period starting 26 h after PCPA treatment (PCPA + 1 day); rat 2: control day, the period following 24-h SD and the period following the combined treatment with PCPA and SD.

the periodic cycles of the ZCR curve of rat 1 represent typical changes seen after SD. In this animal, REMS/TS was somewhat higher after the combined treatment than under control conditions (REMS/TS × 100, 18.4 vs. 16.3), suggesting that the REMS-inhibiting effect of PCPA can also be partly antagonized by SD. However, the effect was neither seen in the other animal illustrated in Fig. 3 (rat 2) nor was it present in the experiments performed by cable recording (Fig. 1).

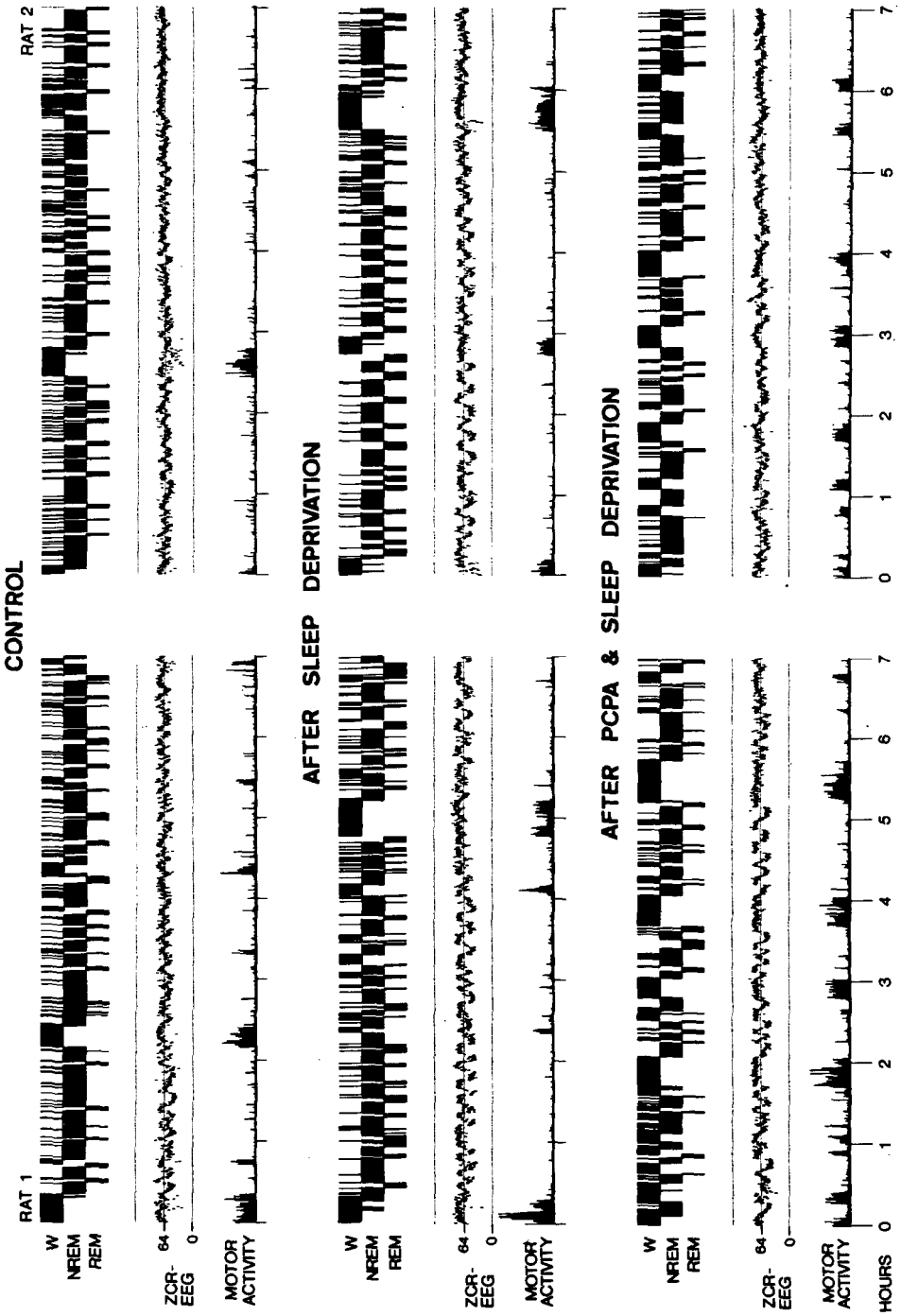
The reduction of cerebral 5-HT by PCPA was similar to that observed in a previous study (13). The following concentrations were measured in the forebrain (the mean values with SEM in parentheses are expressed as the percentage of the mean control level corresponding to 391.3 nmol/g tissue): PCPA alone, 43.4 (2.8); PCPA + SD, 38.5 (2.5).

DISCUSSION

The present study was based on the finding that SD and PCPA influence sleep in opposite directions (4,6). The results of this study confirmed that PCPA reduces TS time, the REMS fraction of TS, and delta activity in NREMS, whereas SD enhances these sleep parameters (Fig. 1). The experiment with the combined treatment showed that the low level of TS and REMS in the 5-HT-depleted animal could not be raised by increasing the "sleep pressure" through enforced 24-h waking. These results stand in marked contrast to the effect of the combined treatment on delta activity and the frequency of wake episodes, which were not only restored to the control level, but assumed values that were at a level similar to that after SD alone. Essential aspects of sleep regulation are therefore obviously intact at a time when PCPA-induced insomnia is at a maximum. It is perhaps not surprising that the SWS-regulating mechanisms proved to be particularly insensitive to 5-HT depletion, as in a previous study the typical daily distribution of this sleep substate was maintained throughout the 6 experimental days following PCPA administration (6). The highest daily level of SWS occurs during the first part of the light phase, at a time when the sleep periods of the rat are longest and the frequency of wake episodes lowest (4,14). Also, in the present study, delta activity and the frequency of wake episodes changed in opposite directions during the three treatments (Fig. 1). The level of delta activity may therefore be an indicator of the predominance of sleep-maintaining processes.

While the SWS fraction of NREMS was clearly enhanced by the combined treatment, the REMS fraction of TS was reduced. This result may indicate that the regulation of REMS was truly impaired by the 5-HT depletion. However, the occurrence of the regular cycles of synchronized and desynchronized EEG, which are typically seen after SD (Fig. 3, rat 1), shows that some aspects of a REMS rebound may still be present after treatment with PCPA. The reduction of REMS may therefore be a consequence of the curtailment of TS time rather than a sign of the disruption of REMS regulating mechanisms. These arguments lead to the major question of the present study: What are the mechanisms responsible for the PCPA-induced insomnia and why is SD ineffective in restoring sleep?

PCPA not only inhibits sleep, but also affects a variety of waking behaviors (see



ref. 3). These include enhancement of sexual activity (15), agressivity (16), and daytime motor activity (17), and increased responsiveness to noxious (18) or neutral environmental stimuli (19). It is possible that insomnia is at least partly due to a general hyperresponsiveness to stimuli that prevents the animal from shifting to a deactivated drowsy state prior to sleep onset. PCPA-treated rats in fact show frequent immobile wake periods with desynchronized EEG, a state not commonly seen in untreated animals (4) (see refs. 20 and 21 for similar observations in the cat).

If the impairment of deactivating processes is the major cause of insomnia, one would expect sleep to be restored not only by raising the cerebral 5-HT level (see refs. 4 and 22 for studies in the rat), but also by damping the general hyperresponsiveness through nonserotonergic mechanisms. The feasibility of the latter approach is illustrated by a study in which chlorpromazine (5 mg/kg) restored NREMS to baseline level and increased REMS significantly in the PCPA-treated cat (23). On the other side, SD seems to enhance mainly processes within sleep rather than promoting deactivation in the waking animal and prolonging sleep time. This may explain why, in comparison to PCPA alone, the combined treatment did not significantly shorten the *time* spent in waking, although it reduced significantly the *frequency* of wake episodes (Fig. 1).

The results of the present study suggest that sleep-regulating mechanisms are masked, but not disrupted, in PCPA-treated rats. On the basis of this hypothesis one would expect that the inhibition of sleep by PCPA would activate similar compensatory mechanisms as forced wakefulness obtained through nonpharmacological means. A rebound of SWS and REMS was in fact observed 5–6 days following the injection of PCPA (6), and a REMS rebound was reported 6 and 8 days following the administration of 5,7-dihydroxytryptamine, a 5-HT-depleting neurotoxin (24). Moreover, a compensatory response to PCPA-induced insomnia may contribute to the striking early recovery of the normal rest and sleep pattern at a time when 5-HT depletion is still severe (6,13,25). Finally, the observation that tryptophan administered to the PCPA-treated rat caused a preferential increase of SWS and REMS, the sleep states that had been most strongly suppressed by PCPA (6), may also be interpreted in terms of a rebound mechanism. By causing a transitory increase in the level of brain 5-HT, tryptophan may unmask the "sleep pressure," due to the preceding insomnia, whereas in the untreated animal it has little or no effect on sleep or motor activity (refs. 26–28, and own unpublished data).

The present observations may appear to be inconsistent with a basic tenet of the "monoamine theory," which postulates a central role of the serotonergic system in the regulation of NREMS. It should be pointed out, however, that most previous experiments have focused on the spontaneous sleep–wake cycle rather than on dynamic aspects of sleep regulation as revealed by the SD paradigm.

FIG. 3. Effect of sleep deprivation (SD) and PCPA + SD on vigilance states, EEG zero crossings, and motor activity in two rats recorded by telemetry. Vigilance states (W, waking; NREM, non-REM sleep; REM, REM sleep); EEG zero-crossing values (ZCR-EEG) per 10-s periods provide a measure of the predominant EEG frequency; motor activity plotted in arbitrary units. Records start with light onset.

Moreover, SWS, presumably the "high-intensity" fraction of NREMS (4), has generally not been discriminated in earlier studies. Thus, while the monoamine theory in its original form may require modifications, experiments exploring more specifically the regulatory mechanisms of sleep will be necessary to delineate the role of the serotonergic system.

In conclusion, the present results suggest that regulatory processes *within* sleep are still functional in 5-HT-deficient animals, whereas the deactivating process mediating sleep onset is impaired.

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