Pharmacology Biochemistry and Behavior, Vol. 47, pp. 283–287, 1994
Printed in the U.S.A.

0091-3057/94 $6.00 + .00

Relationships Between Arousal and Cognition-Enhancing Effects of Oxiracetam

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Received 22 March 1993

CAVOY, A., B. VAN GOLF-RACHT AND J. DELACOUR. *Relationships between arousal and cognition-enhancing effects of oxiracetam.* PHARMACOL BIOCHEM BEHAV 47(2) 283–287, 1994.—Cognition-enhancing effects of nootropic drugs are currently ascribed to an increase in arousal level. In order to test this hypothesis, we studied the effects of three doses of oxiracetam (25, 50 and 100 mg/kg IP) on a radial maze task and on slow wave sleep (SWS) latency in a familiar environment. The 25- and 100-mg/kg doses, but not the 50-mg/kg, significantly improved performance in the memory task. On the other hand, SWS latency was significantly increased by 50 and 100 mg/kg, with the effect of the 25-mg/kg dose going in the same direction but only approaching significance. These results give only a partial support to the "arousal factor hypothesis." Other factors are probably involved in the promnesic effects of oxiracetam.

Nootropic drug Oxiracetam Radial maze Arousal EEG Memory

OXIRACETAM (4-hydroxy-2-oxo-pyrrolidinoacetamide) is a nootropic drug of the piracetam type (10). It has a protective action against cerebrovascular pathologies (13) and improves performance in memory tasks of normal, young, or aged animals (2,16,17,28,30), memory-impaired animals (3,5,14, 20,23,24,29,31,32), and demented humans [(21); see however (11)].

Its action mode, as that of other nootropic drugs, is still ill-defined. At the molecular level, oxiracetam probably sets into play cholinergic (1,4,7,14,22,24,27) and glutaminergic (4,20,27) mechanisms, one of its action sites possibly being the septo-hippocampal system (4,19,25,27). At a global neurophysiological level, the most likely hypothesis is that oxiracetam enhances arousal, thus facilitating attention processes. However, since there is still no direct evidence in favor of this hypothesis, we tried testing it in the experiments reported here. In the first series of experiments, the doses at which oxiracetam improves performance of normal rats in the radial maze test were determined. In a second series, the effects of these doses on latency of slow wave sleep (SWS) in rats placed in a familiar environment were studied.

METHOD

Animals

Male Wistar rats (Iffa-Credo, l'Arbresle, France) weighing 230–250 g were housed in individual cages and maintained on a 12 : 12-h light/dark cycle with the light period starting at 0700. Ambient temperature was 22 ± 1°C.

Experiment 1

Twenty-four rats were trained in an elevated 12-arm radial maze, constructed of 13-mm whitewood. The arms (0.7 × 0.1 m) radiated from a central platform 0.45 m in diameter, with a small cup placed at the end of each. During testing, the apparatus remained in a constant position in a sound-attenuated room having a masking white noise situated at 72 dB above the human threshold. Illumination inside the testing room was provided by a 100-W lamp located 3 m above the apparatus. Several external constant cues were present around the maze, but there were no intramaze cues. Before testing, the subjects were submitted to five consecutive habituation sessions (one per day); during each session, the rats were placed for 10 min on the central platform and could explore the entire maze. During the habituation period, the rats had food and water ad lib in their home cages. At the end of this period, they were submitted to 48 h of water deprivation before the first training session, and then placed on a 23-h deprivation schedule throughout the training sessions, being allowed to drink for 10 min one hour after completion of each session. One training session took place each day and lasted until the rat made 12 choices or until 10 min had elapsed. The cup at the end of each arm contained 0.3 ml of water. The

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rats' choices were recorded. When a rat entered (engaged its four paws in) an already visited arm, an error was scored. All rats were trained for 20 sessions and the following measures were noted for each rat:

1. The number of errors per session.
2. The number of errors and sessions to criterion (i.e., two errors or less in each session for seven consecutive sessions with the allowance of three errors for one session). This criterion has been empirically determined, and in our conditions about 50% of normal Wistar male rats reach it within 20 sessions, which allows one to detect either facilitating or disturbing effects of the experimental treatments. When a rat failed to reach criterion by the 20th session, its error score was the total number of errors committed in the 20 sessions and its session score was 20.
3. The number of correct choices per session before the first error.
4. An index of sequential strategy per session. This strategy is characterized by the rat entering successively the adjacent arms, according to a constant direction, clockwise or counterclockwise. The index was the highest number of sequential choices in a given direction; for instance, if a rat entered successively three adjacent arms clockwise and four in the opposite direction, its index score for this index was 4.
5. The time spent for completing the session.

From the beginning of training, the rats were divided into four groups (n = 6), three experimental groups receiving IP injections of 25, 50 and 100 mg/kg of oxiracetam, respectively, 30 min before each session and a control group (C) receiving the same volume (0.1 ml/100 g) of the vehicle (physiological saline).

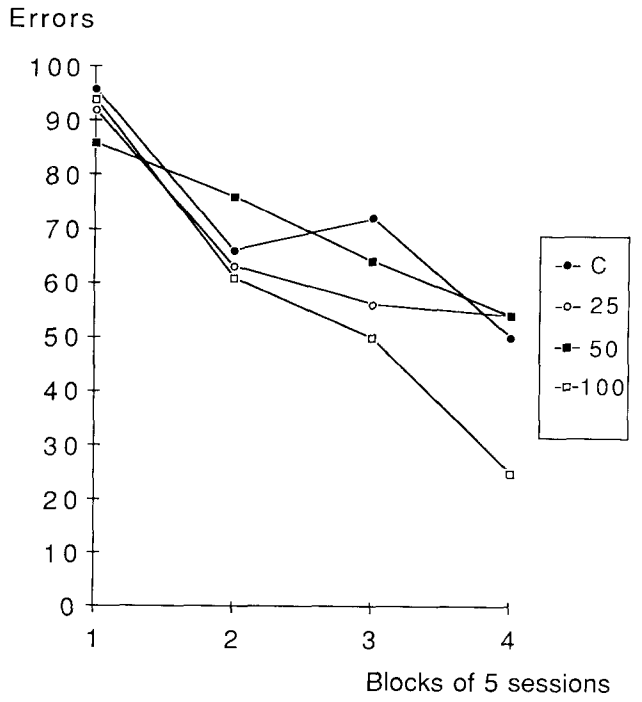

FIG. 1. Learning curve (radial maze test). Each point represents a block of five sessions. C = control group; 25, 50, and 100 = experimental groups receiving, respectively, 25, 50, and 100 mg/kg of oxiracetam.

TABLE 1
BEHAVIORAL DATA (RADIAL MAZE TEST)

	C	25	50	100
Total number of errors B4	8.33 (0.99)	9 (1.98)	6.5 (0.85)	4.17* (0.54)*
Sequential index B4	13.33 (2.80)	15.83 (6.02)	15.00 (2.84)	15.67 (8.33)
Time(s) B4	137.80 (6.93)	148.40 (11.74)	145.70 (8.69)	145.93 (5.31)
Choices before 1st error B4	37.83 (2.02)	40.83 (3.44)	43.67 (2.87)	49.83 (3.79)
Errors to criterion	40.17 (3.96)	24.83* (8.78)	45 (3.09)	21.33* (4.57)
Sessions to criterion	15.83 (2.04)	7.5* (2.84)	18.5 (1.5)	7.0* (1.97)

Mean and SEM of the following variables. C = control group; 25, 50, and 100 = experimental groups receiving, respectively, 25, 50, and 100 mg/kg of oxiracetam; B4 = last block of five sessions. *Significant difference ($p < 0.05$) between an experimental group and the control group.

Experiment 2

Twenty-nine rats were divided into three groups (ns = 9, 10, 10) ascribed to the study of the effects of 25, 50, and 100 mg/kg of oxiracetam, respectively. Under Pentothal® anesthesia (thiopental sodique, Abbott France, Rungis; 70 mg/kg IP) silver electrodes were chronically implanted in all rats over the frontal and somatic cortices and in nape muscles (sphenius and rhomboidus) according to standard techniques (9). After a recovery period of at least one week, the following procedure was applied to each group: All rats were first submitted to two 3-h habituation sessions in recording boxes (30 L × 30 W × 50 H cm) located in a sound-attenuated room with a masking white noise 72 dB above human threshold. The rats were then submitted, under the same conditions, to two recording sessions separated by an interval of one week. For half of the rats of a given group, the beginning of the first recording session was immediately preceded by an injection of the dose of oxiracetam ascribed to that group; for the other half, by an injection of the vehicle. For the second session, the nature (drug vs. vehicle) of the injection was reversed for each rat: If, for instance, it has received 25 mg/kg of oxiracetam just before the first session, it received the vehicle before the second and vice versa. Injections were made as in experiment 1.

EEG and EMG were recorded on a standard EEG polygraph (Alvar, Paris) and on a magnetic tape recorder (Sony). Recordings from the polygraph were analyzed visually, thus allowing verification of the qualitative aspects of the EEG—for instance, possible abnormal wave morphologies or artefacts—and measurement of latency of the appearance of SWS (i.e., the time between the injection and the onset of the first SWS episode lasting at least 15 s). From magnetic tape recordings, power spectrums (2–49 Hz, one hertz step) of EEG data

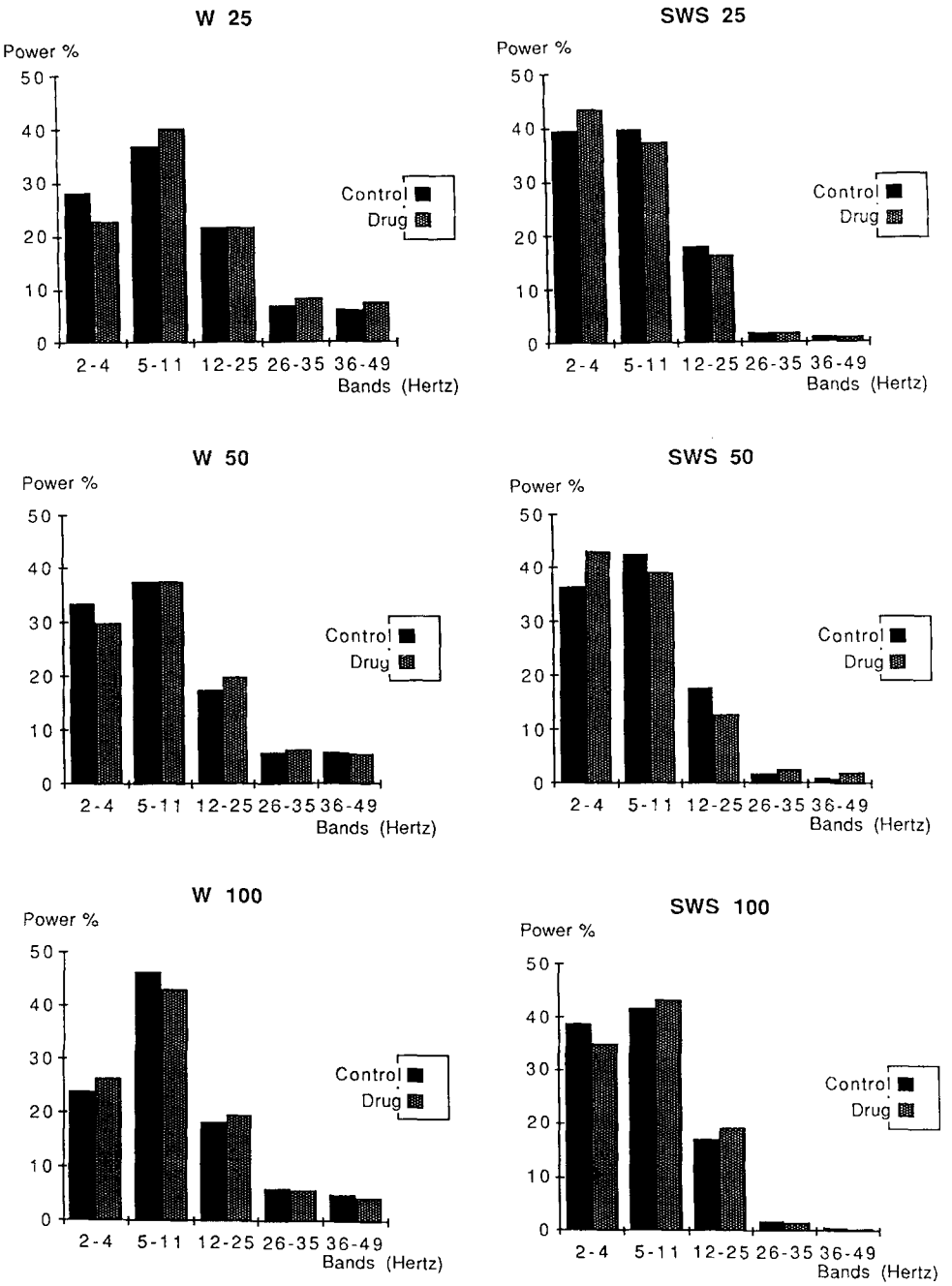

FIG. 2. Power spectrums of EEG. Relative power (percentage of total power) of each frequency band of EEG recorded during wakefulness (W) or slow wave sleep (SWS), after control (saline) or injection of 25, 50, or 100 mg/kg of oxiracetam.

sampled during wakefulness (W) and SWS were computed on a special purpose computer (Deltamed, Paris).

Statistics

Tests of statistical significance of between-group differences were based on the H test of the Kruskal–Wallis analysis of variance; 2 × 2 post hoc comparisons were performed according to Conover (6). Within-group differences were evaluated by the Student's t test for paired samples. The significance threshold was 0.05 if not otherwise specified.

RESULTS

Behavioral Data

Evolution of learning performances is shown in Fig. 1. Significant differences appeared in the following measures (Table 1):

TABLE 2
SWS LATENCY

	25	50	100
Saline	1446	1800	1935
	(89)	(263)	(239)
Oxiracetam	1727	2340*	2670*
	(137)	(253)	(272)

Mean and SEM of latency (in s) of the first episode of slow wave sleep (SWS) (i.e., the time between the injection and the onset of the first SWS episode lasting at least 15 s after saline or after 25, 50, or 100 mg/kg of oxiracetam. *Significant difference within the same group.

1. The total number of errors for the last five sessions (H = 8.655, df = 3, $p < 0.05$) is significantly inferior in the 100-mg/kg group compared to groups 50 and C.
2. The number of errors to criterion (H = 10.442, df = 3, $p < 0.02$) is significantly inferior in groups 100 and 25 compared to groups 50 and C.
3. The number of sessions to criterion (H = 12.041, df = 3, $p < 0.01$) is significantly inferior in group 100 and 25 compared to groups 50 and C.

In other words, 25- and 100-mg/kg doses significantly improved learning performances in the radial maze, but the drug had no effect on the time for completing a session, the index of sequential strategy, and the number of correct choices before the first error.

EEG Data

Oxiracetam did not produce abnormalities in the morphology or amplitude of EEG waves in either W or SWS, and no effect of the drug on the relative power of the frequency bands in W as well as in SWS was disclosed (Fig. 2). However, the drug significantly increased the latency of the first SWS episode at 50- and 100-mg/kg doses (Table 2), and a tendency for a similar effect was seen at the lower dose ($0.05 < p < 0.10$). In other words, oxiracetam facilitated the persistence of awake state in a familiar environment.

DISCUSSION

Our behavioral results confirm the promnesic effects of oxiracetam already observed in a number of tasks in normal animals. However, Magnani et al. (14), at doses identical or very similar to ours (30 and 100 mg/kg), found no effect of the drug on performances of normal rats in the radial maze test. This discrepancy may be explained by our experimental conditions which made the test more sensitive: We used a 12-arm maze, while Magnani et al. had an 8-arm one; moreover, we administered the drug from the beginning of training (i.e., for 20 consecutive days), whereas Magnani et al. studied its acute effects on rats that had mastered the task.

Improvement of the performances of normal rats in the radial maze test has already been reported for pramiracetam (18) and aniracetam (15). In these experiments, the test had both reference and working memory components, and in the case of Murray and Fibiger (18), the drug facilitated reference but not working memory. In our procedure, the radial maze was used only for measuring working memory; even so, the test may have some reference memory components such as sequential strategy. However, the drug had no effect on the sequential index.

Indications from the EEG data are also clear-cut. Oxiracetam increased the latency of SWS; in other words, it facilitated the persistance of the wakefulness state in a familiar environment. This effect is significant at 50- and 100-mg/kg doses and approaches significance at 25 mg/kg, which is in agreement with data on other nootropic drugs, even though it was not associated with modifications of the relative power of any frequency bands either in W or in SWS, as observed for other drugs (8,12,26,34,35). The increase in arousal is in agreement with the cholinergic action of oxiracetam, since cholinergic systems of the brain stem and basal telencephalon have a facilitatory influence on thalamo-cortical arousal (33).

Although our behavioral and EEG data are globally concordant and seem to confirm the "arousal" hypothesis, their relationships are complex. Only the 100-mg/kg dose had significant effects on performances in the radial maze and on SWS latency. The 50-mg/kg dose had no effect on the memory task, although it significantly increased SWS latency; reciprocally, the 25-mg/kg dose, while significantly improving performances in the radial maze, only had a tendency to facilitate wakefulness. Moreover, the fact that the time for completing a session was unaffected by the drug provides evidence against the arousal hypothesis. Finally, a complete verification of the arousal hypothesis would require a direct determination of the correlation between learning performances in the radial maze and SWS latency in the same group of rats.

In conclusion, the promnesic effects of oxiracetam could be due to an increase in arousal state, but depending on the dose, other factors may also be set into play.

ACKNOWLEDGEMENTS

This work was supported by a DRET grant (91/105) to J.D.

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