

INHIBITION OF REM SLEEP BY FLUOXETINE, A SPECIFIC INHIBITOR OF SEROTONIN UPTAKE*

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Summary—Fluoxetine, a specific inhibitor of serotonin uptake, suppressed REM sleep in cats. The onset of action was prompt and with doses of 2.5 mg/kg, (p.o.) the effect lasted a full 24 hr. After 2 or 3 weeks of daily dosing, the amount of REM sleep began to increase again. A small dose of fluoxetine added to a small dose of L-5-hydroxy-tryptophan caused a significant decrease in REM sleep whereas either treatment alone did not. Administered to *cerveau isolé* cats fluoxetine did not antagonize EEG desynchronization induced by the muscarinic stimulant arecoline, indicating the lack of a direct anticholinergic effect. These experiments indicate that REM sleep is suppressed when 5-HT accumulates at synapses as a consequence of fluoxetine administration. These data and a similar suppression of REM sleep that occurs when norepinephrine accumulates suggest that both NE and 5-HT can inhibit the cholinergic system that seems crucial for REM sleep. Non-REM sleep was usually increased in cats. In rats REM sleep was suppressed by fluoxetine but SWS did not increase.

Impairment of serotonergic mechanisms profoundly alters sleep patterns. Insomnia follows treatment with serotonin-depleting agents or destruction of serotonin-containing neurones in the median raphe (Jouvet, 1972). Depletion of monoamines by reserpine results in loss of slow wave sleep and bursts of pontogeniculo-orbital (PGO) spikes (Brooks and Gershon, 1977). The decrease in brain levels of serotonin (5-HT) that follows administration of *p*-chlorophenylalanine coincides with a decrease in slow-wave sleep (SWS). Administration of the serotonin precursor, 5-hydroxytryptophan (5-HTP), reinstates sleep that lasts only for the few hours during which 5-HT levels are restored. Early in the recovery from insomnia induced by *p*-chlorophenylalanine, cats display showers of PGO spikes (Jalfré, Ruch-Monachon and Haefely, 1974). These spikes also appeared in cats treated with the monoamine-depleting benzoquinolizine, RO-1284. Administering 5-HTP to these cats decreases the number of spikes, indicating suppression by a serotonergic mechanism.

The consequences of decreased levels of 5-HT are clear and reproducible, but attempts to examine the effect of increased availability of 5-HT have been frustrated by lack of specific agents. The effects of tryptophan are modest (Hartmann, 1977). Though 5-HTP at high doses may increase sleep, the effects cannot be ascribed to increased activity of serotonin neurons since decarboxylation of 5-HTP can occur in other neurons as well. Monoamine oxidase inhibitors, which decrease SWS and paradoxical sleep (REM)

elevate levels of catecholamines as well as 5-HT. Tricyclic antidepressants reduce REM sleep and usually increase SWS (Ritvo, Ornitz, LaFranchi and Walter, 1967) but these drugs generally inhibit re-uptake of both catecholamines and 5-HT. Although chlorimipramine itself selectively inhibits 5-HT uptake, its methylated metabolite inhibits norepinephrine (NE) uptake. Changes in sleep pattern after administration of this drug then become a consequence of an undetermined and mixed influence on both 5-HT and NE. Fluoxetine, (*dl*-*N*-methyl-3-phenyl-3- $[\alpha,\alpha,\alpha$ -trifluoro-*p*-tolyl)oxy] propylamine hydrochloride), and desmethyl fluoxetine are specific inhibitors of serotonin uptake that do not affect catecholamine uptake *in vivo* (Wong, Horng, Bymaster, Hauser and Molloy, 1974). In the present study fluoxetine was used to enhance serotonergic nerve function and was found to suppress REM and usually increase light or slow wave sleep.

METHODS

Sleep patterns were determined in male cats and rats carrying implanted electrodes. The animals were in sound-attenuated enclosures. One-minute segments of EEG were graded by the usual criteria (Slater, Jones and Moore, 1976) as awake, drowsy, light, light-to-deep slow-wave (SWS3), deep slow-wave (SWS4) and REM sleep in cats. Sleep patterns for each cat usually were reproducible from day to day over a period of a few weeks. The cats differed in age, time in the laboratory, temperament and, not surprisingly, in distribution of sleep stages. In rats, light sleep and both stages of slow-wave sleep were combined as SWS. Drugs were administered orally.

Cerveau isolé cats were prepared under ether anaesthesia. After making a coronal slot posterior to the

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bony tentorium and opening the dura, the brain stem was divided at the level of the junction of the inferior and superior colliculi with a modified nickel spatula inserted at a 46° or 50° angle. Stainless-steel 2 × 56 screws that reached, but did not penetrate, the dura served as surface leads. Bipolar insulated stainless-steel wire electrodes were placed in the lateral geniculate nucleus of the thalamus under stereotaxic control. The ether anaesthesia was stopped at least 1 hr before the experiment was begun. A dose of 0.1 mg/kg of atropine methiodide was injected to block peripheral cholinergic receptors.

When the pattern of slow-wave activity with intermittent "sleep spindles" was well established, 5 µg/kg of arecoline HCl, (i.v.) was injected. At 10-min intervals the dose was increased or decreased to determine how much arecoline was needed for induction of a desynchronized EEG. This threshold dose was determined again after intravenous injection of fluoxetine.

RESULTS

Cats—sleep pattern: 5-day trial

Three cats received fluoxetine on 5 consecutive days that were preceded or separated by days on which water was administered (Fig. 1). Since fluoxetine has a long duration of action (Parli and Hicks, 1974), EEG's were recorded for 22.5 hr. Each day the cats received drug or placebo at 8:45 a.m. Recording began at 9:00 a.m. and continued until 7:30 a.m. the next day, when the cats were exercised and observed outside the recording enclosure.

During the first 5-day course the three doses 1, 2.5 and 5 mg/kg all caused significant suppression of REM sleep; the two higher doses causing almost complete suppression (Fig. 1). While receiving 2.5 mg/kg of fluoxetine for 5 days, cat 82 had 8 min of REM sleep on one day, 4 min on two days and none on the two remaining days. During the period when cat 75 received 5 mg/kg, the percentage of time in REM sleep fell from 11.88 ± 0.88 to 0.18 ± 0.32 . Both light sleep and SWS3 increased in this cat, while the awake periods remained virtually unchanged. This cat had less than 1% of SWS4 during both control and drug treatment. Most of the lost REM sleep time for the cats receiving 1 or 2.5 mg/kg (#82 and 84) appeared as light sleep. While receiving only water, these two cats had 5.17 and 9.73% levels of SWS4 during the first 2½ hr of each day and a total of 1.13 and 4.22 for the 22.5 hr. During the first 3 days of fluoxetine treatment, SWS4 varied but was not detected in either cat at any time during the fourth and fifth day of drug treatment. Later in the week, SWS3 also decreased. The low level of REM sleep continued after cessation of drug administration returning to near control level in 9 days. Since the second 5 days of fluoxetine treatment was then begun, it was not known whether rebound would have occurred. In these and in subsequent experiments latency to SWS3 and REM sleep varied enormously

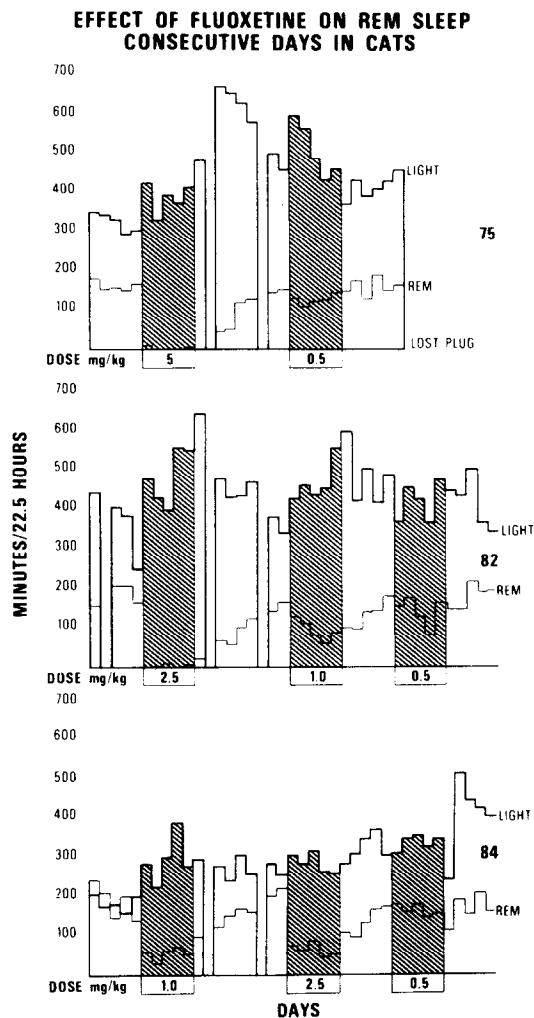


Fig. 1. Effect of fluoxetine on REM and light sleep in cats; EEG's were recorded for 22.5 hr each day. After a control period the cats received fluoxetine each morning for 5 days (cross-hatched interval). The clear areas indicate the days on which water was administered. Each bar equals one day. After the first drug period, there were 2 days of rest during which no recording was done. Slow-wave sleep and awake did not change. The dark line indicates minutes of light sleep and the thinner line, REM sleep. As indicated, cats 75 and 82 had days during the first drug period with no REM sleep at all.

between the cats during the control period. For any single cat, latency to SWS3 was usually about the same during control and drug periods, but the REM latency increased.

The second course of fluoxetine depressed REM sleep less than the first (Fig. 1). Cat 84 had more REM sleep while receiving 2.5 mg/kg than it had the previous week on 1 mg/kg. In contrast, cat 82 during the first drug trial had almost no REM sleep on 2.5 mg/kg of fluoxetine. The level of light sleep remained high but did not seem to change with changes in drug administration.

On the first day of the third week of drug treatment cat 75 lost its plug. Sleep patterns did not change

during this week in the other two cats (82 and 84) which were receiving 0.5 mg/kg of fluoxetine.

Long-term trial. Additional experiments were performed during which doses of fluoxetine were administered on a daily basis but EEG's recorded on only 2 days each week. This procedure was used to examine the effects of long-term administration of fluoxetine since several weeks of daily recording often loosened the plug on a cat's head. Cat 75, for example, was lost to further recording on the 30th day of the preceding study. In addition, allowing the cats to remain in their home cages enabled better observation of their appearance and behaviour to be made.

After the cats had been receiving drug treatment for a few days, it was noticed that their pupils were dilated, but still responsive to light. The degree of mydriasis seemed to be dose-related. By the fourth day of drug treatment the cats receiving the larger doses, which had been friendly for years, began to growl and hiss. They became distinctly unfriendly, but with careful handling it was possible to administer the drug in the usual way. The cats seemed to see clearly and did not seem to be hallucinating. They became less irritable toward the end of the second week of drug administration. After cessation of the drug treatment, the cats returned to their usual friendly behaviour in a week or two; those on the higher doses recovering more slowly. The severity of the behavioural change was dose-related being more severe and lasting longer in the cats receiving the highest dose. The cats treated with 0.5 mg/kg orally each day showed only modest irritability, which decreased and virtually disappeared even while they were still receiving the drug. During the first trial after 3 control sessions cats received fluoxetine (0.5, 1 or 2.5 mg/kg) on 8 consecutive days. Sleep patterns were recorded on day 1, 6, 8 and 10. As shown in Figure 2, a statistically significant (2-way analysis of variance) decrease occurred in REM sleep. Deep sleep (SWS4) also decreased but because of the variability and very low levels in some cats, this change was not significant at all dose levels nor was the change in SWS3. Light sleep time did increase, but the total sleep time stayed about the same.

In another experiment, drug administration continued for 19 or 31 days with 6 or 10 recording days during drug treatment and 4 or 8 recording days during the recovery phase. The changes in the EEG are illustrated graphically in Figure 3. Each point is the mean of the percentage of time that two cats receiving a given dose of fluoxetine spent in the various phases of sleep on one day of recording. Again, the decrease in REM sleep and the increase in light sleep can be seen. In the cats treated with 2.5 mg/kg this effect persisted without much change for 6 recording sessions, which covered 19 days of treatment. The question was raised as to how a serotonin receptor antagonist might affect the altered pattern of sleep. Two cats on the high dose (2.5 mg/kg) received 1 mg/kg of

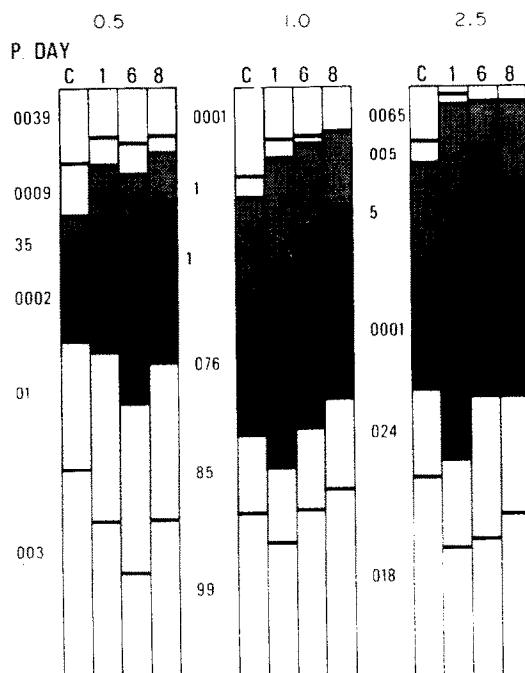


Fig. 2. Effect of fluoxetine on sleep pattern. Cats received fluoxetine on 8 consecutive days. Sleep patterns were recorded three times before drug administration and on day 1, 6 and 8 of drug treatment. Each bar graph represents the mean of results for 2 cats. The number at the top indicates dose in mg/kg, (p.o.). Column C is the mean for 3 control days. Columns labelled 1, 6 or 8 are from recordings of the corresponding day of drug treatment. The stages from the top down are REM, sleep, SWS4, SWS3, light, drowsy and awake. The length of each segment indicates the portion of time spent in that stage of sleep. Total sleep time, indicated by the solid segments and the segments above it, did not change in any consistent way; REM sleep (the top clear area) and SWS4, (the next lightly stippled area) decreased. The *P*-values beside the bar graph indicate that the changes in REM sleep were significant ($P < 0.01$ by analysis of variance) but that changes in SWS4 occurring after 1 mg/kg were not.

methysergide on the 19th day. Both became agitated; they slept much less than before and REM sleep was completely absent. The day after this trial each of the cats appeared ill and all drugs were stopped. These two cats recovered slowly; return to the pre-drug pattern of sleep and behaviour took about two weeks. It was quite clear that methysergide, an agent known to block the effects of 5-HT on peripheral tissue receptors, did not restore a sleep pattern resembling the control.

The cats receiving 0.5 or 1.0 mg/kg of fluoxetine continued for a total of 31 days. Suppression of REM sleep had decreased by the fourth week of recording, and light sleep remained high. When the drug treatment was stopped in these cats, recovery occurred over a shorter period of time than with the larger dose. The amount of REM sleep did not increase over baseline. This absence of a REM-rebound may be a consequence of the long half-life of fluoxetine and its biologically active metabolite, desmethylfluoxetine (Parli *et al.*, 1974).

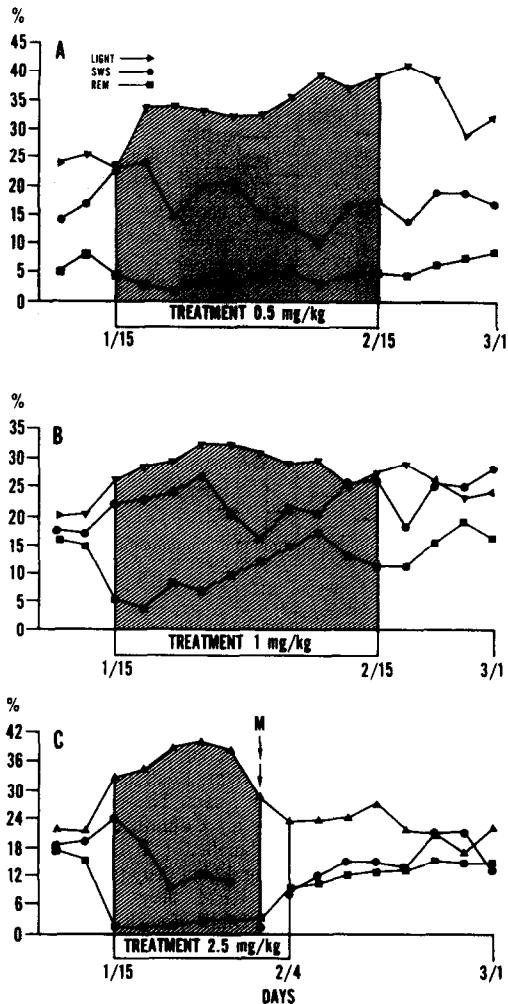


Fig. 3. Each vertical set of points indicates the mean percentage of sleep stage on one day; EEG's were recorded twice each week but drug was administered every day; M] indicates the administration of 1 mg/kg. (p.o.) of methysergide which blocked SWS and REM sleep.

In a 5-hr experiment, 9 cats received water one day and 1 mg/kg of fluoxetine on the next day. The percentage of SWS increased from 38.0 ± 3.85 (SE) to 59.52 ± 4.67 , a difference significant at $P < 0.01$. Among the 9 cats, 69 periods of REM sleep with a

median duration of 5.7 min occurred after control medication, and 35 periods with median duration of 6 min, after fluoxetine. Counting the number of PGO spikes, occurring during REM sleep periods that exceeded 3 min, did not reveal any obvious difference in density. Fluoxetine decreased the number of REM periods but did not affect the duration or the PGO density during REM sleep. The PGO spikes occurring in 3 cats during 3 min were counted, beginning 1 min after the onset of REM sleep periods of sufficient length. After control treatment, 125.9 ± 6.37 (SE) spikes occurred in 20 periods of REM sleep and, after 1 mg/kg of fluoxetine, 131.44 ± 4.55 , during 9 periods.

Co-administration of fluoxetine and 5-HTP. One group of 6 cats previously used in these and other sleep experiments received a placebo oral dose of water at 8:30 a.m. on Tuesday and Wednesday on each of 3 weeks that were separated by 1 week without treatment. On Thursdays and Fridays, 2 cats received fluoxetine, 2 received 5-HTP and 2 received both medications according to a cross-over design. Recording sessions lasted 5 hr. On the first drug day, 10 mg/kg of 5-HTP was administered either alone or in combination with fluoxetine to 4 cats. All these cats vomited and the sleep data from that day were not included. It was then found that doses of 2.5 mg/kg or 5.0 mg/kg of 5-HTP caused vomiting but 1 mg/kg did not. For the remaining 5 treatment days, the dose of 5-HTP was 0.5 mg/kg orally. The dose of fluoxetine was 0.5 mg/kg orally.

An analysis of variance revealed significant differences only in the amount of REM sleep (Table 1). The decreased REM sleep in cats treated with fluoxetine alone was not significant at the 95% level (Duncan Multiple Range Test). However, the cats that received both fluoxetine and 5-HTP had significantly less REM sleep than controls or cats treated with 5-HTP alone. This joint action suggests that the change in REM sleep was indeed a consequence of increased 5-HT at serotonergic synapses.

Cerveau isolé. Electroencephalograms recorded from surface leads in normal cats vary in pattern. For several hours after transection of the brain stem at the ponto-mesencephalic junction, a single characteristic record predominates. The basic form consists

Table 1. Effect of 5-hydroxytryptophan and fluoxetine on the sleep of cats

	n	AWK	SWS	REM
Control	18	29.42 ± 6.78	36.52 ± 6.48	14.70 ± 5.53^a
5-HTP	6	28.32 ± 4.28	38.25 ± 5.10	15.20 ± 5.21^a
Fluoxetine	6	25.41 ± 14.98	48.29 ± 15.36	$8.74 \pm 5.08^{a,b}$
5-HTP + Fluoxetine	6	28.09 ± 10.10	44.24 ± 14.51	5.70 ± 3.19^b

^a All 6 cats received water by gavage on 2 consecutive days. The next 2 days, they received 5-HTP 0.5 mg/kg, fluoxetine 0.5 mg/kg or both 5-HTP and fluoxetine in random order. A week without treatment separated each trial.

^b In preparing the results for analysis, a mean was computed for control days and treatment days for each cat each week. Thus, the data in the table are the means of the 2-day means which were computed. The letter superscripts (a,b) indicate the results of a Duncan Multiple Range Test at 0.05 level of probability. Values with the same letter are not different from each.

of 4–6 Hz waves interrupted periodically by spindles of 10–12 Hz activity of higher voltage. When decerebrate cats are kept alive for several days or weeks other wave forms will emerge (Jouvet, 1972). During the first few hours, however, the pattern of slow activity interrupted by sleep spindles usually continues without change. After intravenous injection of muscarinic cholinergic stimulants such as physostigmine or arecoline, the EEG changes to one of low voltage high frequency. The threshold dose for desynchronization by arecoline of the EEG in the *cerveau isolé* cat varies between 2.5–50 $\mu\text{g}/\text{kg}$. Doses of atropine as small as 0.1 mg/kg, (i.v.), will cause a substantial increase in this dose and may block the effect entirely (Rathbun and Slater, 1963). The response is, therefore, a sensitive test for central anticholinergic activity of the muscarinic type.

Three of 4 cats, in which the brain was divided at the ponto-mesencephalic junction, showed well-defined slow wave activity with intermittent spindling in the surface EEG. In these 3 cats, doses of arecoline of 10 or 20 $\mu\text{g}/\text{kg}$ (i.v.) converted the synchronized EEG to a desynchronized pattern. When these cats were treated with 1 and then 3 mg/kg of fluoxetine, (i.v.), the threshold dose of arecoline did not change.

Rat-Sleep Pattern

Four rats received various doses of fluoxetine during a series of range finding experiments. On the first two consecutive days they received water and on the next two, either water, or 2.5, 5 or 10 mg/kg of fluoxetine; EEG's were recorded for 7.5 hr. The consistent observation from these trials was a decreased amount of REM sleep in rats treated with 5 or 10 mg/kg of fluoxetine (Table 2). Although one of the 2 rats treated with 10 mg/kg was awake for most of the first

drug day and had a 38% reduction of SWS on the second day, the other rat had increased SWS (17 and 16%) on the same two days. Rats with 2.5 mg/kg did not show a change in sleep pattern. Fluoxetine treatment did not increase SWS in these rats.

DISCUSSION

Fluoxetine selectively blocks uptake of serotonin by isolated synaptosomes (Wong *et al.*, 1974). In the brain, re-uptake is a major factor in terminating the action of serotonin. Interference with this process should increase the amount of serotonin at synaptic clefts. The reduction of serotonin turnover in fluoxetine-treated rats provides biochemical evidence that this has occurred. The decreased firing rate of raphe neurones confirms this neurophysiologically (Clemens, Sawyer and Cerimele, 1977). Potentiation of ACTH secretion induced by 5-HTP adds a neuroendocrine parameter to indicate again that fluoxetine, by inhibiting neuronal re-uptake of 5-HT, enhances serotonergic mechanisms (Fuller, Snoddy and Molloy, 1976).

Fluoxetine is completely specific for blocking 5-HT uptake *in vivo* without affecting norepinephrine uptake at well-tolerated doses. For example, fluoxetine in rats (Fuller, Perry and Molloy, 1974) and mice (Fuller, Perry, Snoddy and Molloy, 1974) prevents the depletion of 5-HT by *p*-chloroamphetamine but does not affect norepinephrine depletion by 6-hydroxydopamine. This specificity is also confirmed by a simple unpublished blood pressure experiment in which fluoxetine had little effect on the pressor response to tyramine or norepinephrine whereas nisoxetine, like the tricyclic antidepressants, blocked the effect of tyramine and increased the pressor effect of norepinephrine. The administration of fluoxetine can be used as a tool for studying the consequences of increased 5-HT at synaptic clefts.

Tricyclic antidepressants which suppress REM sleep and increase SWS, are relatively non-specific inhibitors of monoamine uptake usually inhibiting NE uptake more than 5-HT. Nisoxetine is a NE uptake inhibitor chemically related to fluoxetine but without activity on 5-HT uptake at concentrations achieved after reasonable doses. Experiments with fluoxetine and nisoxetine should help in the understanding of how NE and 5-HT affect sleep. Nisoxetine, like the tricyclic antidepressant, and fluoxetine clearly inhibited REM sleep (Slater *et al.*, 1976).

Cats showed an unequivocal loss of REM sleep after receiving fluoxetine. The effect of a marginally effective dose of fluoxetine (0.5 mg/kg orally) was made statistically significant by the co-administration of a small non-emetic dose of 5-HTP (0.5 mg/kg). This demonstrates a serotonergic suppression of REM sleep and of the PGO spikes characteristic of this stage of sleep. Jalfre *et al.* (1974) suppressed PGO spikes with doses of 5-HTP in cats pretreated with *p*CPA. During the 24 hr following large, emetic doses

Table 2. Effect of fluoxetine on sleep pattern of rats

Dose (mg/kg)	n	Percentage of sleep state (7.5 hr)		
		AWK	SWS	REM
0	4	45.8	45.6	8.61
0		47.7	44.1	7.67
0	2	43.6	52.1	4.4
2.5		55.1*	40.9	4.0
0	2	46.4	42.5	11.1
2.5		45.1	44.3	10.6
0	4	42.2	46.9	10.9
5		47.0	48.0	5.0*
0	2 × 2†	39.9	48.3	11.7
5		45.3	48.2	6.5*
0	2	48.6	43.4	8.0
10		67.8	30.8	1.5

* Over a period of 3 months, 4 rats received various doses of fluoxetine on 2 consecutive days that followed 2 days on which they had received water by gavage. For statistical analysis, it was judged necessary to divide the data into compatible sets. The first number of each pair is the mean of the observations on Tuesday and Wednesday and the second number, the mean for Thursday and Friday. Differences marked with an asterisk were significant by an analysis of variance.

† The same 2 rats run for 2 weeks.

of 5-HTP or tryptophan. Ursin (1976) did not find a decrease in the time cats spent in stage REM sleep but she did report an increase in latency to REM sleep after both amino acids. These studies are difficult to interpret because of the vomiting, but they do seem to confirm the suppression of REM sleep and PGO spikes by 5-HT.

In the present study when cats were treated for several weeks with fluoxetine, REM sleep began to return and PGO spikes were seen during both wakefulness and during other stages of sleep. This emergence of REM sleep and PGO spikes in the presence of fluoxetine suggests that 5-HT modulates rather than controls the electroencephalographic signs. Cholinergic mechanisms are probably the final common pathway through which REM-related phenomenon are expressed (Sitaram, Mendelson, Wyatt and Gillin, 1977). Hobson and McCarley (1976) have suggested that this cholinergic process can be inhibited by either serotonergic or noradrenergic neurones. Since tricyclic antidepressants (which interfere with monoamine uptake), nisoxetine (a relatively specific inhibitor of norepinephrine uptake chemically similar to fluoxetine) and fluoxetine itself (a specific inhibitor of 5-HT uptake), all decrease the amount of REM sleep, dual monoamine mechanisms for suppression of REM sleep seems an attractive hypothesis.

In some of the present experiments, fluoxetine increased the amount of SWS on the first day but not on later days. Light sleep, which in this laboratory refers to an EEG pattern of mixed slow activity (4–6 Hz), occasional spindles (8–12 Hz) and some (less than one-third) fast activity, was usually increased in cats. This stage marks the border between wakefulness and asleep, between conscious and unconscious. In this sense, the present data fit with Jouvet's (1972) suggestion that increases in 5-HT are concerned with the initiation of sleep and the present experiments fit the monoamine theory of sleep, but the suppression of REM sleep has been more striking than any increase in SWS.

During the course of these experiments two unexpected findings were encountered. The present authors are at a loss to explain why cats receiving fluoxetine for several days began to hiss and growl or why this behaviour decreased with continued treatment. The subjects who received fluoxetine in a Phase I clinical trial (Lemberger, unpublished data) have not described any change in mood nor have observers noted any change in affect.

The mydriasis that occurred in cats treated with fluoxetine was also puzzling. There seems to be no neuroanatomical basis for mydriasis as a consequence of activation of serotonergic pathways. Pupillary dilation often is a sign of anticholinergic activity. This seemed an unlikely explanation since the EEG pattern of high-voltage slow-wave activity that occurs in cats treated with atropine or scopolamine was not seen. Fluoxetine did not affect the threshold dose of arecoline that induced EEG desynchronization in the

cerveau isolé cat. Since small doses of atropine sulphate (0.1 mg/kg) either elevate the threshold dose or completely block arecoline-induced desynchronization, it was concluded that fluoxetine does not act as a central anticholinergic blocking drug. Unpublished data of Dr James Aiken on several isolated smooth muscle systems indicate that fluoxetine is not a cholinergic blocking agent peripherally. If fluoxetine suppresses REM sleep and PGO spikes through serotonergic inhibition of a cholinergic pathway, the mydriasis may also be a consequence of an analogous mechanism.

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