Pharmacology, Biochemistry and Behavior 94 (2010) 503–509

Contents lists available at ScienceDirect

Pharmacology, Biochemistry and Behavior

journal homepage: www.elsevier.com/locate/pharmbiochembeh

Nicotine and fluoxetine induce arousing effects on sleep–wake cycle in antidepressive doses: A possible mechanism of antidepressant-like effects of nicotine

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ARTICLE INFO

Article history:
Received 9 February 2009
Received in revised form 30 October 2009
Accepted 11 November 2009
Available online 17 November 2009

Keywords:
Sleep
Sleep–wake cycle
REM sleep
Antidepressant
Depression
Nicotine
Fluoxetine
Selective serotonin uptake inhibitor

ABSTRACT

A number of studies have reported an association between smoking and depression, and several reports suggest that nicotine (NIC) may act as an antidepressant. The present study was designed to determine whether the effects of NIC on sleep–wake patterns in rats are similar to those of the antidepressant fluoxetine (FLX), a selective serotonin reuptake inhibitor. Male rats were chronically implanted with a standard set of electrodes for sleep recording. We compared the effects of antidepressive doses of NIC, FLX and the combination of both drugs on sleep–wake pattern in rats subjected to one day, one week and two weeks of administration, as well as after the withdrawal of the two-week treatment. The changes observed in our study in an 8-h sleep recording period during one day, one week and two weeks of NIC administration are very similar to those observed in the rats that received FLX, which led to a decrease in both slow wave sleep II and rapid eye movement (REM) sleep as a consequence of an increase in wakefulness. In addition, all treatments also induced a significant lengthening of REM sleep latency onset. These data suggest that the antidepressant-like action of NIC could be caused by its arousing properties.

1. Introduction

Both preclinical and clinical studies have suggested that nicotine (NIC) and related compounds may have therapeutic value for treating a wide range of neuropsychiatric disorders (Vazquez-Palacios and Bonilla-Jaime, 2004; Newhouse et al., 2004; Romanelli et al., 2007). Converging lines of evidence indicate a strong relationship between major depression, neuronal acetylcholine nicotinic receptors (nAChRs) and NIC (for review: see Bertrand, 2005; Quattrocki et al., 2000; Vazquez-Palacios and Bonilla-Jaime, 2004). The direct link between NIC and depression is suggested primarily by the fact that transdermal NIC patch treatment has improved mood in non-smoking depressed patients (Salin-Pascual et al., 1996). In addition, a growing number of findings in animal models of depression have recently shown that NIC and some nicotinic ligands also have antidepressant properties (Buckley et al., 2004; Ferguson et al., 2000; Semba et al., 1998; Nakamura and Tanaka, 2001; Tizabi et al., 1999; Vazquez-Palacios et al., 2004, 2005). It has been shown that most antidepressant drugs are associated with changes in sleep architecture, notably the delayed onset of rapid eye movement (REM) sleep and a reduced amount of REM sleep (Wilson and Argyropoulos, 2005). Because the majority of antidepressants, irrespective of their chemical classes, suppress REM sleep, it has been hypothesized that the improvement in symptoms of depression is related to sleep deprivation, especially the deprivation of REM sleep (Vogel et al., 1990; Thase, 1998; Giedke and Schwärzler, 2002; Berger et al., 2003). For instance, the effects on sleep of fluoxetine (FLX), a potent selective serotonin reuptake inhibitor (SSRI), have been studied extensively in both normal volunteers and depressed patients. Insomnia and other "activating" side effects occur in depressed patients treated with FLX (Beasley et al., 1992; Armitage and Sussman, 1997). This effective antidepressant medication is a potent suppressor of REM sleep (Kerkhofs et al., 1990; Gillin et al., 1997; Nicholson and Pascoe, 1988; Vasar et al., 1994). A similar result has been observed in animals (Pastel and Fernstrom, 1987; Bakalian and Fernstrom, 1990; Gao et al., 1992). Changes in sleep and, especially, REM sleep, in depressive patients have been attributed to an increased ratio of cholinergic to aminergic neurotransmission in critical central synapses (see Adrien, 2002). Given that these neurotransmitter systems are primarily involved in regulating sleep and wakefulness, it is believed that they represent common neurobiological substrates that underlie the impairment of the regulation of both mood and the sleep–wakefulness cycle (Adrien, 2002).

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doi:10.1016/j.pbb.2009.11.004

However, there is also contradictory evidence as to the role of NIC in sleep regulation, as early reports failed to detect any effect of NIC on sleep (George et al., 1964). In contrast, when administered intravenously (Domino and Yamamoto, 1965), subcutaneously (Jewet and Norton, 1966), or into the medial pontine reticular formation (Velazquez-Moctezuma et al., 1990), NIC actually increased REM sleep in cats. Similarly, research conducted with humans has yielded inconsistent results. Several studies have shown that transdermal NIC induced a decrease in total sleep time, sleep efficiency and REM sleep, as well as an increase in wakefulness (Gillin et al., 1994; Salin-Pascual et al., 1999; Vazquez et al., 1996; Page et al., 2006). In the present study, we compared the effects of antidepressive doses of NIC, FLX and the combination of both drugs on sleep–wake pattern in rats, according to the forced swim test (Vazquez-Palacios et al., 2004, 2005), with the objective of determining whether the effects of NIC are similar to those of FLX, a SSRI and currently the most widely-used antidepressant.

2. Methods

In this study, sleep–wakefulness patterns in rats were monitored after one day, one week and two weeks of administration, as well as after the withdrawal of the two-week treatment with NIC, FLX and the combination of FLX plus NIC. Adult male Wistar rats (250–300 g at the beginning of the experiment) from our vivarium were chronically implanted with a standard set of electrodes for electroencephalogram (EEG) and electromyogram (EMG) recording under deep anesthesia [Ketamine (100 mg/ml): 0.25 ml plus xilacine (20 mg/ml): 0.05 ml plus acepromazine (0.2 mg/ml) 0.1 ml plus 0.6 ml of saline to obtain a 1 ml cocktail, ip] and aseptic conditions. Once anesthesized, the animals were injected with xylocaine (2%) in the dorsal part of the cranium to complete the local anesthesia. All electrodes were then soldered to the connectors of a plug that was permanently fixed to the skull with acrylic cement. At the end of the surgical procedure, each animal received an ip injection of 0.3 ml of penicillin and all appropriate antiseptic measures were taken to prevent infection. At that point, the animals were placed individually in transparent plastic cages (recording chambers) containing sawdust bedding. All animals were kept in the same sound-attenuated room and maintained on a 12-h alternating light–dark schedule (lights on 0900 h) and at a controlled temperature (23 ± 1 °C). Food and water were available *ad libitum* throughout the study. Following a post-surgical recovery period of at least 7 days, all animals were habituated for 3 days by being allowed to move freely around the recording chamber with their slip rings and cable-connectors attached. EEG and EMG were recorded continuously for 8 h during the light period (the sleep period in rats) of the 12-h light/12-h dark cycle, beginning at approximately 0900 h. Animals were randomly assigned to one of the following experimental groups ($n = 10$): saline control (CON), NIC, FLX and FLX + NIC. The same CON group was used for all treatments. Sleep recordings were obtained during 8 h after one day, one week and two weeks of administration, as well as after withdrawal from the two-week treatment period (7 days after completing the two-week treatment). In order to determine the possible effects on the sleep–wake cycle, four distinct states of vigilance were established, based on the visual scoring of records according to Takeuchi's (1970) criteria. The behavioral states of wakefulness (W), slow wave sleep I (SWS I), slow wave sleep II (SWS II) and REM sleep were scored in successive 10-s epochs. These sleep–wake measures provided the following dependent variables that were quantified for each 8-h recording session: total time of sleep–wakefulness stages; their frequency, duration and latencies; and sleep efficiency (percentage of total sleep time during the recording period) (Vazquez-Palacios and Velazquez-Moctezuma, 2000). Brief awakenings (less than 30 s) during SWS I, SWS II or REM sleep were counted as the total number of awakenings. Statistical analysis was conducted using Kruskal–Wallis analysis of variance (ANOVA) and significant sources of variance were identified using the Dunn post-hoc test. A level of $p \leq 0.05$ was considered significant in all tests.

2.1. Drugs

–(–) Nicotine bitartrate was dissolved in a saline solution, while FLX–HCL was dissolved in distilled H_2O. All drugs were administered subcutaneously in a volume equivalent to 0.2 ml. Doses were calculated on mg/kg of salt and prepared fresh each morning. The dose tested for each drug was as follows: NIC bitartrate at 0.4 mg/kg body weight/day (0.14 mg/kg body weight/day of nicotine base), and FLX–HCl at 5 mg/kg body weight/day (4.47 mg/kg body weight/day of FLX base). NIC was injected 10 min prior to sleep recording, while the FLX–HCl injections were given 30 min before the start of sleep recording. The combination of FLX + NIC was administered using the same doses and at the above mentioned times prior to sleep recording. The control rats received a 0.9% saline solution as the vehicle (same volume and route of administration). Both the NIC and FLX doses were selected based on reports in the literature and our own previous studies that had demonstrated antidepressive effectiveness in the forced swim test (Detke et al., 1995; Tizabi et al., 1999; Vazquez-Palacios et al., 2004, 2005).

Also relevant is the fact that no increase of general locomotor activity has been reported at these doses (Tizabi et al., 1999; Detke et al., 1995). All animals were treated in strict accordance with both the NIH Guidelines and Mexico's Official Norms (NOM-062-ZOO-1999) for the Care and Use of Laboratory Animals.

3. Results

3.1. One day of treatment

Our results indicate that NIC induces sleep–wake changes similar to those found in the FLX group in that it increases wakefulness and decreases both SWS II and REM sleep. Sleep time decreased in all experimental treatments as indicated by total sleep time, and sleep was also less efficient (Fig. 1). With regard to REM sleep latency onset, a significant increment was observed under all treatments (Fig. 2). Fig. 1 shows the effects of all treatments on the total time of the different sleep stages. In the 8-h recording sessions, sleep–wake patterns after one day of NIC administration were characterized by a significant increment in the duration of W [+78%] with a consequent significant decrease in the total time of both SWS II [−50%] and REM sleep [−63.15%] (Fig. 1A). These changes, induced by one day of NIC treatment, led to decreased sleep efficiency (Table 1). The greater amount of time spent in W resulted from an increase in the average duration of each episode (Table 1), while the reduction in both SWS II [−52.18%] and REM sleep [−69.84%] occurred due to a significant reduction in the number of episodes. NIC also induced a significant lengthening of REM sleep latency onset (Fig. 2A).

One day of FLX administration induced effects in the sleep–wake architecture similar to those of NIC during the entire recording period (Fig. 1A). Overall amounts of W increased following of one day of FLX treatment [+56.69%] with a concomitant decrease in SWS II [−33.83%] and REM sleep [−61.47%]. These changes led to decreased sleep efficiency (Table 1). One day of FLX administration increased the total duration of W through an increase in the duration of each episode (Table 1), though in this case the number of episodes remained unchanged. In contrast to W, the duration of SWS II diminished due to a reduction in the number of episodes, though the duration of each single episode increased. Overall amounts of REM sleep also decreased via a reduced number of episodes, but the average duration of each REM sleep episode remained unchanged (Table 1). With regard to REM sleep latency onset, a significant

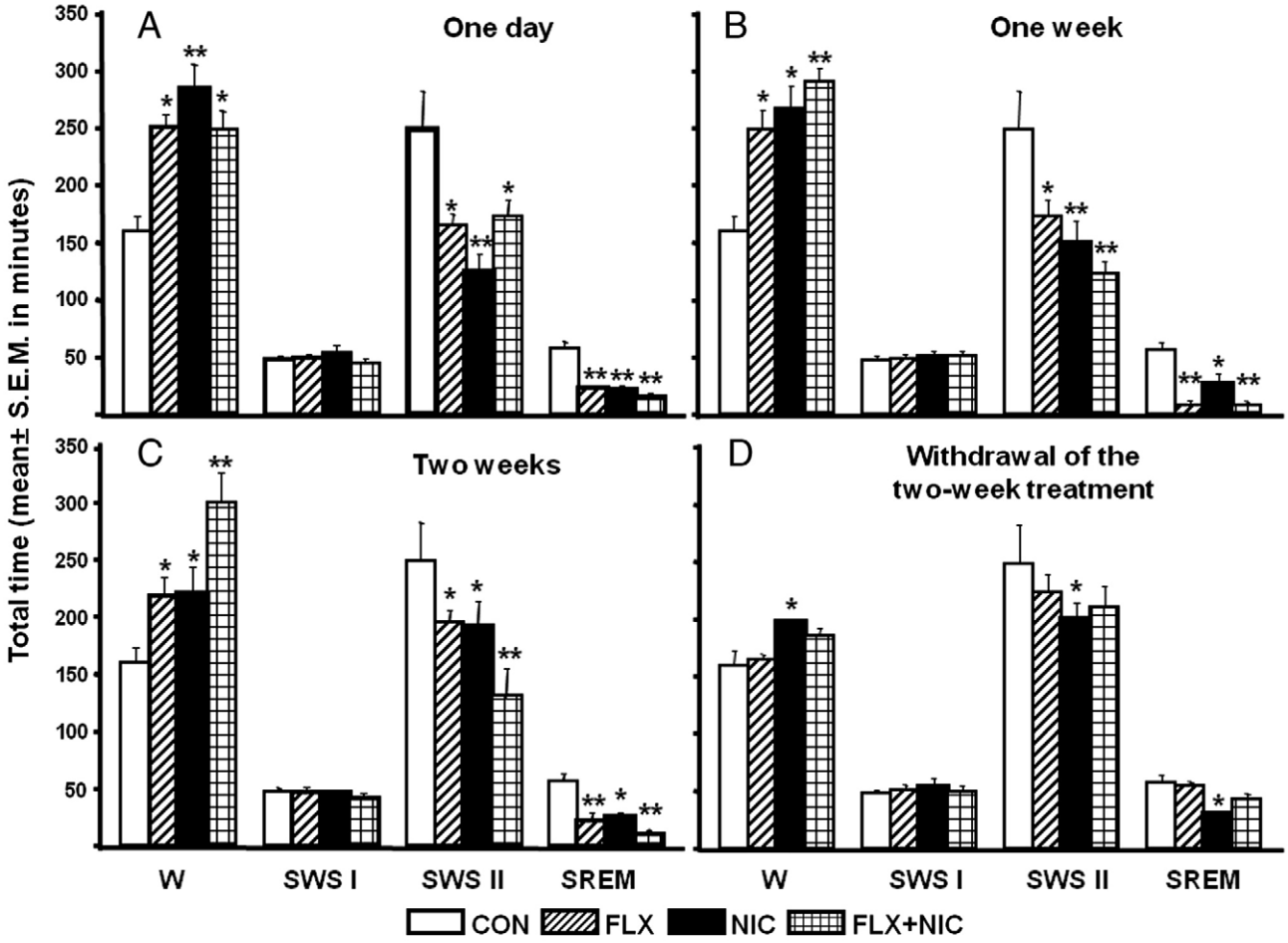

Fig. 1. Effects of nicotine (NIC; 0.4 mg/kg/day sc), fluoxetine (FLX; 5 mg/kg/day sc), and the combination of both drugs (FLX + NIC) administered one day (A), one week (B), two weeks (C) and 7 days after the end of two-week treatment (D), on the total time of each vigilance stage: wakefulness (W), slow wave sleep I (SWS I), slow wave sleep II (SWS II), and REM sleep (SREM). Control (CON), fluoxetine (FLX), nicotine (NIC), and fluoxetine plus nicotine (FLX + NIC). For each group $n = 10$; bars represent the mean values (± S.E.M.) in minutes. Kruskal–Wallis ANOVA followed by the Dunn test. *$p<0.05$; **$p<0.01$ vs. Control (CON).

increment [+52.17 min and +49.72%] was observed under one day of FLX administration (Fig. 2A).

Time spent in W was also greater [+46.89%] after one day of the combined FLX + NIC administration, which led to a decrease in both SWS II [−30.74%] and REM sleep [−74.58%] (Fig. 1A). These alterations led to a decreased sleep efficiency similar to that observed in the FLX group. The increased time spent in W was due to a 45% rise in the number of episodes of that type (Table 1). The reduction of SWS II was due to a decrease in the number of episodes [−46.26%] despite the increased duration of each one [+47.23%]. Similarly, reductions in the time spent in REM sleep occurred due to a significant decrease in the number of episodes [−77.16%]. FLX + NIC administration also induced a significant increase in REM sleep latency onset [+85.3 min and 81.3%] (Fig. 2A).

3.2. One week of treatment

Fig. 1B displays the effects of all one-week treatments on the amount of the different vigilance states in the rats. Statistical analysis showed that one week of treatment with NIC in intact rats decreased total REM sleep duration [−54.21%] via a marked reduction in the number of episodes [−69.37%] (Table 1). The total duration of SWS II also declined significantly [−39.86%] because of a lower number of episodes [−46.25%], while the total duration of W increased [66.76%] due to a significant rise of the number of episodes [+52%], which led to a reduction in sleep efficiency (Table 1). NIC administration also induced a significant extension of REM sleep latency onset [+76.65 min and +73.06%] (Fig. 2B).

One week of treatment with FLX also induced comparable effects to those of NIC in sleep–wake architecture (Fig. 1B), as it resulted in greater W duration [+56.33%]. In addition, a reduction in both SWS II [−30.33%] and REM sleep [−85.39%] produced a decline in sleep efficiency. The increase of W time was due to a slight rise in the duration of each episode. whereas the reductions in SWS II and REM sleep were due to a lower number of episodes (Table 1). FLX treatment induced a significant lengthening of REM sleep latency onset (Fig. 2B).

Overall amounts of W also increased after one week of the combined FLX + NIC treatment [+81.8%] (Fig. 1B). In contrast to the NIC and FLX regimens, this effect was due to a significant increase in the average duration of each episode [+63.45%]. The increase of W induced by one week of FLX + NIC treatment occurred through a greater decrease in both SWS II [−50.33%] and REM sleep [−86.13%]. With regard to the reduction of SWS II, this effect was due to a significant reduction in the number of episodes [−52.11%] (Table 1). FLX + NIC treatment also induced a significant lengthening of REM sleep latency onset [+142.36 min and +135.69%] (Fig. 2B).

3.3. Two weeks of treatment

The sleep–wake pattern after two weeks of NIC treatment was characterized by a significant increase in the duration of W [+37.58%]

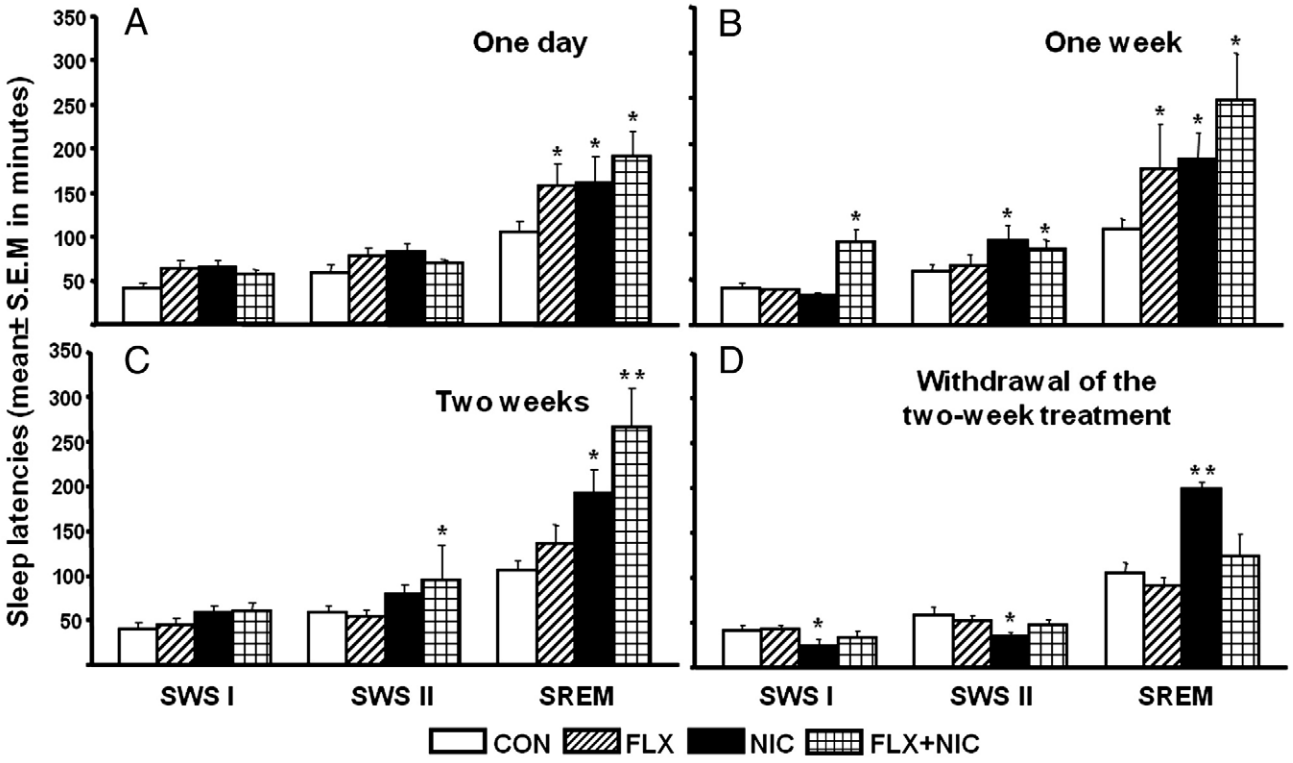

Fig. 2. Effects of nicotine (NIC; 0.4 mg/kg/day sc), fluoxetine (FLX; 5 mg/kg/day sc), and the combination of both drugs (FLX + NIC) administered one day (A), one week (B), two weeks (C) and 7 days after the end of two-week treatment (D), on sleep latencies: wakefulness (W), slow wave sleep I (SWS I), slow wave sleep II (SWS II), and REM sleep (SREM). Control (CON), fluoxetine (FLX), nicotine (NIC), and fluoxetine plus nicotine (FLX + NIC). For each group $n = 10$; bars represent the mean values (± S.E.M.) in minutes. Kruskal–Wallis ANOVA followed by the Dunn test. *$p<0.05$; **$p<0.01$ vs. Control (CON).

with a consequent significant decrease in the total time of both SWS II [−23.21%] and REM sleep [−56.99%] (Fig.1C), changes that led to a decreased sleep efficiency [−18.74%] (Table 2). Two weeks of NIC administration increased total W duration via an increase in the number of episodes [+28%] (Table 2). SWS II duration diminished due to a reduction in the number of episodes [37.32%], despite the increased duration of each one [+51.3%]. NIC treatment also induced a significant increase in REM sleep latency onset [+86.21] (Fig. 2C). Two weeks of treatment with FLX induced effects comparable to those of NIC in sleep–wake architecture (Fig. 1C), and resulted in an increased duration of W [+36.3%]. In addition, a reduction in both SWS II [−30.33%] and REM sleep [−85.39%] produced a decline in sleep efficiency [−16.44%]. The increased W time was due to a significant increase in the number of episodes [+51.3%], whereas the reduction in SWS II and REM sleep was caused by a reduction in the number of episodes [−39.78% and −64.12%, respectively] (Table 2). Overall amounts of W also increased after administration of the combination of FLX + NIC [+88%] (Fig. 1C), an effect brought about by

Table 1
Comparison of the difference between baseline and post-treatment values of several sleep parameters assessed in animals after one day and after one week of nicotine (NIC), fluoxetine (FLX) or fluoxetine plus nicotine (F + N) treatment. Values are presented as mean ± S.E.M. ($n = 10$). *$p<0.05$; **$p<0.01$ vs. CON.

	One day of treatment				One week of treatment			
	CON	NIC	FLX	F + N	CON	NIC	FLX	F + N
Wake								
Duration (min)	10.7 ± 0.8	15.8 ± 1.3*	16.0 ± 1.5*	13.6 ± 0.9	12.6 ± 1.6	14.3 ± 1.5	16.3 ± 1.6*	17.5 ± 1.4*
Number of episodes	15.2 ± 1.0	15.8 ± 1.0	18.0 ± 0.8	18.4 ± 0.9*	15.2 ± 1.0	18.0 ± 1.1*	17.1 ± 1.4	17.0 ± 1.0
Number of awakenings	2.6 ± 1.6	10.0 ± 2.5**	11.2 ± 2.6**	12.5 ± 3.5**	1.9 ± 0.4	12 ± 3.6**	14.2 ± 4.6**	16.5 ± 4.4**
Sleep								
Total time (min)	314.5 ± 10.7	235.3 ± 12.7**	196.2 ± 21.7**	231.3 ± 16.1*	335.4 ± 9.3	230.7 ± 16.5*	206.6 ± 26.3**	190.1 ± 12.1**
Sleep efficiency (%)	66.8 ± 2.74	49.0 ± 2.6*	40.8 ± 4.5**	48.2 ± 3.3*	69.8 ± 1.9	48.0 ± 3.4*	44.6 ± 4.3*	39.6 ± 2.5*
Slow wave sleep I								
Duration (min)	2.1 ± 0.1	2.8 ± 0.1**	2.9 ± 0.2**	2.6 ± 0.1*	2.0 ± 0.1	3.0 ± 0.1*	2.6 ± 0.2*	2.7 ± 0.1*
Number of episodes	22.7 ± 1.3	17.6 ± 1.4*	17.8 ± 0.9*	17.5 ± 1.3*	19.0 ± 1.4	16.1 ± 1.2	19.0 ± 1.0	18.3 ± 1.1
Slow wave sleep II								
Duration (min)	6.8 ± 0.4	9.7 ± 0.5	9.5 ± 0.9*	11.2 ± 0.8*	7.9 ± 0.4	12.5 ± 1.4*	8.8 ± 1.0	8.6 ± 0.5*
Number of episodes	32.1 ± 1.7	18.4 ± 0.8**	22.4 ± 1.8*	17.8 ± 1.6**	28.0 ± 1.9	14.4 ± 1.3**	17.3 ± 1.5*	15.7 ± 1.7**
REM sleep								
Duration (min)	2.2 ± 0.1	2.3 ± 0.2	2.4 ± 0.2	2.4 ± 0.4	2.9 ± 0.2	2.3 ± 0.2	1.8 ± 0.1	2.4 ± 0.3
Number of episodes	25.7 ± 1.6	9.5 ± 0.9*	7.8 ± 1.8**	5.8 ± 1.8**	25.0 ± 2.6	3.6 ± 1.6*	7.8 ± 1.8*	2.77 ± 1.0**

Table 2
Comparison of the difference between baseline and post-treatment values of several sleep parameters assessed in animals after two weeks and one week after the withdrawal of treatment with nicotine (NIC), fluoxetine (FLX) or fluoxetine plus nicotine (F+N) treatment. Values are presented as mean ± S.E.M. ($n=10$). *$p<0.05$; **$p<0.01$ vs. CON.

	Two weeks of treatment				Withdrawal of treatment			
	CON	NIC	FLX	F+N	CON	NIC	FLX	F+N
Wake								
Duration (min)	13.0±1.3	13.0±1.5	13.7±1.9	19.4±2.6**	12.9±1.3	12.4±1.5	14.4±2.8	13.9±2.3
Number of episodes	12.0±1.1	18.0±0.9*	16.8±1.5*	16.0±1.0	12.0±1.1	14.4±1.4	18.6±2.7*	17.0±2.5
Number of awakenings	3.2±0.8	17.0±2.5**	18.8±2.6**	19.5±5.5**	2.6±1.6	10.6±4.8**	14.5±2.9**	17.5±3.5**
Sleep								
Total time (min)	335.0±8.2	262.8±17.0*	260.6±23.7*	180.3±26.5*	335.0±8.2	320.2±3.6	287.1±3.1*	287.1±3.1*
Sleep efficiency (%)	69.8±1.7	54.7±3.5	54.3±5.0	37.5±5.5*	69.8±1.7	65.7±0.8*	59.1±0.5*	61.3±1.1*
Slow wave sleep I								
Duration (min)	2.0±0.1	2.5±0.1*	2.5±0.1*	2.6±0.1*	2.0±0.1	2.1±0.1	2.7±0.1*	2.5±0.1*
Number of episodes	18.2±1.3	18.5±2.0	18.1±1.5	15.7±1.8*	18.2±1.3	22.0±2.5	19.7±2.4	19.5±2.4
Slow wave sleep II								
Duration (min)	7.1±0.3	11.1±1.4**	10.4±1.3*	9.9±0.8*	8.0±0.3	8.0±0.8	8.5±0.7	8.3±0.8
Number of episodes	29.8±1.5	19.0±2.1**	20.1±2.8*	13.0±1.9**	27.8±1.5	28.2±1.9	24.33±1.4*	25.3±1.5*
REM sleep								
Duration (min)	2.7±0.2	2.2±0.2	1.9±0.2	2.4±0.4	2.7±0.2	2.4±0.1	2.2±0.1	2.33±0.1
Number of episodes	24.7±1.6	9.22±3.1**	12.3±2.4*	3.4±1.0**	24.7±1.6	23.0±1.6	14.0±0.5**	18.2±2.7

a significant increase in the average duration of each episode [+80.22%] (Table 2). The increase of W induced by two weeks of FLX+NIC treatment occurred through a decrease of both SWS II [−47.66%; $F(3,36)=8.5$, $p<0.03$; Dunn $p<0.01$] and REM sleep [−82.8%] (Fig. 1C). Concerning the reduction of SWS II, this effect was due to a significant reduction in the number of episodes [−62.67%]. FLX+NIC treatment also induced a significant lengthening of REM sleep latency onset [+142.36 min and +135.69%] (Fig. 2C).

3.4. Withdrawal of treatment

After withdrawal of all treatments (7 days after the last injection of each substance), only NIC induced residual effects on sleep–wake patterns, as the withdrawal of that treatment was characterized by a significant increase in the duration of W [+37.58%] with a consequent significant decrease in the total time of both SWS II [−23.21%] and REM sleep [−56.99%] (Fig. 1D), changes that resulted in a decreased sleep efficiency [−18.74%] (Table 2). NIC treatment also induced a significant increase in REM sleep latency onset [+86.21 min and +82.17%] (Fig. 2D).

4. Discussion

When compared to the control animals (CON), all treatments introduced changes in the sleep–wake architecture of intact rats. The changes observed in our study in an 8-h sleep recording period during one day, one week and two weeks of NIC treatment are very similar to those observed in the rats that received FLX, in that both drugs led to a reduction in sleep efficiency as a consequence of a decrease in the amount of both SWS II and REM sleep and an increase in W. These effects were similar in magnitude for all groups. One important finding of this study, then, is the marked effect on REM sleep. The systemic administration of NIC, FLX and FLX+NIC decreased the amount of REM sleep and increased REM sleep latency onset, a well-known effect of classical antidepressants (Wilson and Argyropoulos, 2005). Reductions in the amount of REM sleep and increases in REM sleep latency onset were seen after the application of antidepressants (Mayers and Baldwin, 2005; Wilson and Argyropoulos, 2005). The increase in W can be explained by an activational influence on the pontine brain stem (Hobson et al., 1998). Accordingly, it has been shown that FLX and other SSRIs have alerting effects on sleep (Beasley et al., 1992; Maudhuit et al., 1994; Dorsey et al., 1996).

In the present study, the suppression of REM sleep coupled with an increased REM latency induced by FLX confirms previous findings from studies with normal volunteers (Nicholson and Pascoe, 1988; Vasar et al., 1994), patients with depression (Von Bardeleben et al., 1989; Trivedi et al., 1999), and rats (Pastel and Fernstrom, 1987; Bakalian and Fernstrom, 1990). Recent studies have corroborated the finding that in rats both the systemic injection and direct infusion of FLX into the laterodorsal tegmental nucleus (LDT) or the medial pontine reticular formation (mRTF) significantly reduced REM sleep and the number of REM periods, whereas REM sleep latency onset was augmented (Monti and Jantos, 2005). In addition, FLX resulted in a decrease in sleep efficiency. Clearly, the serotonin (5-HT) reuptake inhibition of FLX is expected to increase 5-HT availability at all postsynaptic 5-HT receptors (Kreiss and Lucki, 1995; Rutter et al., 1994); thus, FLX may induce the suppression of REM sleep by inhibiting the brainstem structures involved in promoting and inducing it (Monti and Jantos, 2005). Overall, these data would explain the FLX-induced increment in REM sleep latency onset and the decrease in REM sleep duration, a mechanism that would also explain the elevated level of arousal expressed in an overall increase of the duration of W.

The REM sleep suppressant effect induced by an acute dose of NIC is similar to that reported in rats (Salin-Pascual et al., 1999) and normal volunteers (Gillin et al., 1994; Davila et al., 1994; Vazquez et al., 1996; Page et al., 2006), but different from the effect produced when it is injected directly into the brain stem. As mentioned above, a microinjection of NIC into mRTF increases REM sleep in cats (Velazquez-Moctezuma et al., 1990). This discrepancy may be related to species differences, route of administration, dosage, duration of treatment or the different nAChR subtypes involved. Diversity in the receptor function is evident among the different subtypes, with variability in the degree of membrane ion channel activation that occurs in response to NIC binding (Dajas-Bailador and Wonnacott, 2004; Picciotto et al., 2000). Moreover, the degree of receptor desensitization, which occurs with the continued presence of NIC, also varies among different receptor subtypes (Wooltorton et al., 2003; Alkondon and Albuquerque, 2005). In rats, it has been shown that the effects of NIC on sleep can be prevented by pretreatment with the nicotinic-receptor antagonist mecamylamine (Salin-Pascual et al., 1999), which suggests that NIC initiates its action on sleep by binding

to nAChRs. While the sleep–wake effects of FLX may be explained by its effect on the serotonergic system, those that result from NIC administration may also be due to an enhancement of the activity of several systems of neurotransmitters that may play a role in the arousal process (for example, acetylcholine, dopamine and 5-HT) (Dani and Bertrand, 2007). However, with respect to arousing properties (Hubbard and Gohd, 1975; McNamara et al., 1990), NIC has been linked to wake-promoting systems (Boutrel and Koob, 2004; Lena et al., 2004). The authors and other researchers have suggested that such actions allow acetylcholine and nicotine to enhance wakefulness by inhibiting sleep-promoting systems while at the same time facilitating other wake-promoting systems (Saint-Mleux et al., 2004; Boutrel and Koob, 2004; Lena et al., 2004). Since these neurotransmitter systems are primarily involved in the regulation of sleep and wakefulness, it is believed that they represent common neurobiological substrates that underlie impairments in regulating mood and sleep–wakefulness patterns (Adrien, 2002). Further studies are necessary to directly establish the precise mechanism through which NIC increases the state of wakefulness.

On the other hand, there is evidence for a bidirectional relationship in the interaction of the nicotinic and serotonergic systems (for review, see Seth et al., 2002). For example, nicotinic receptors are expressed on the cell bodies of the 5-HT raphe neurons, and nicotine has been shown to increase 5-HT release on hippocampus, dorsal raphe nucleus and hypothalamus (Sher, 2004; Mihailescu et al., 1998; Seth et al., 2002). The NIC-induced 5-HT release was much higher during the decrease in firing rates, indicating that NIC might influence the 5-HT1A autoreceptors of the 5-HT neurons. Because NIC alters serotonergic transmission, it has been suggested that both acute and chronic NIC treatments modulate the expression of 5-HT1A receptors located in certain cortical and limbic regions (Kenny et al., 2001) implicated in the etiology of depression (Savitz et al., 2009) and REM sleep (Monti and Monti, 2000). All these effects lead us to suggest that NIC-induced 5-HT release may alter the postsynaptic sensitivity to 5-HT (Kenny et al., 2001; Rasmussen and Czachura, 1997) by desensitizing the 5-HT1A autoreceptors (Chaput et al., 1990; Le Poul et al., 1995, 2000) and that this mechanism could be mediating the sleep–wake and mood effects associated with NIC. It is now known that most treatments currently employed as antidepressants improve serotonergic transmission (for example, FLX) (Blier and de Montigny, 1999; Lesch, 2000). Both FLX and NIC enhance 5-HT transmission across 5-HT synapses (Kreiss and Lucki, 1995; Rutter et al., 1994; Mihailescu et al., 1998), but through different mechanisms. In earlier behavioral studies we have suggested that the possible mechanism through which NIC exerts its antidepressant-like effects could be related to the stimulation of neuronal nicotinic receptors in the serotonergic system (Vazquez-Palacios et al., 2004, 2005). These antidepressant-like effects of NIC treatment were equivalent in both intact rats and in an animal model of depression (Vazquez-Palacios et al., 2004, 2005).

However, the lack of synergy observed when FLX was co-administered with NIC (FLX + NIC group) may be related to pharmacological profile differences, dosage, or the involvement of different mechanisms. The potencies and rates with which NIC induces the persistent functional inactivation of diverse nAChR subtypes may also be related to the sequences and degrees of NIC's effects on nAChRs and their subsequent effects on the sleep–wake cycle (Wooltorton et al., 2003; Alkondon and Albuquerque, 2005).

In summary, the results of the present study indicate that one day, one week and two weeks of systemic administration of FLX and NIC increased wakefulness and reduced both SWS II and REM sleep in rats. Moreover, these treatments also induced increases in REM sleep onset latency. These findings suggest that the antidepressant action of NIC could be mediated by both its effects on REM sleep and its arousing properties. Because NIC has been suggested as a potential treatment for depression, findings of that substance have similar properties to FLX on architecture of sleep, may be relevant to its potential as an antidepressive agent.

Acknowledgments

This study was supported by the *Consejo Nacional de Ciencia y Tecnologia* (CONACyT-2115-35133). GVP was the recipient of a scholarship from CONACyT (CONACyT-95162), and the present study was part of his research for his Doctoral Degree in Experimental Biology (C/PFPN-2002-35-32).

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