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# Activation of serotonin 5-HT$_1$-receptors decreased gripping-induced immobility episodes in *taiep* rats

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ABSTRACT

The *taiep* rat is a myelin mutant that shows a disorganized sleep–wake cycle and immobility episodes (IEs) when the animals are gripped at the base of the tail. During IEs electroencephalographic recordings show a rapid eye movement (REM) sleep-like pattern. These alterations are quite similar to those reported in narcolepsy–cataplexy. Pharmacologically, systemic administration of $\alpha_2$ adrenoceptor agonists increases gripping-induced IEs, whereas $\alpha_2$ antagonists decrease them. However prazosin, an $\alpha_1$ antagonist, increases gripping-induced IEs. In male 8-month-old *taiep* rats we have studied the effect of systemic administration of serotonergic autoreceptor agonists and antagonists on gripping-induced IEs. 8-Hydroxy-2-(di-*n*-propylamino) tetraline hydrobromide (8-OH-DPAT), a 5-HT$_{1A}$ agonist, and 3-trifluoromethylphenylpiperazine hydrochloride (TFMPP), a 5-HT$_{1B}$ agonist, produce a significant decrease in the frequency and mean duration of IEs. Systemic administration of spiperone and 1-(2-methoxyphenyl)-4[4-(2-phthalimido) butyl]piperazine hydrobromide (NAN-190), 5-HT$_1$ antagonists, increase IEs and their mean duration. When the specific serotonin antagonist *N*-[2[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-*N*-2-pyridinyl-cyclohexanecarboxamide maleate (WAY 100635, 100 μg/kg) was injected 15 min before 8-OH-DPAT, this specific antagonist reverses the effects caused by the 5-HT$_{1A}$ agonist. These results show that serotonergic 5-HT$_1$-receptors are involved in the susceptibility of gripping-induced IEs in *taiep* rats. Similar results have been reported in the food-elicited cataplexy test in narcoleptic dogs.



Serotonin receptors are classified into several subtypes from 5-HT$_1$ through 5-HT$_7$. The 5-HT$_1$-receptors are considered as presynaptic and modulate the synthesis and release of serotonin [2,15,24]. In the narcoleptic dog the systemic administration of 5-HT$_{1A}$ agonists caused a decrease in the frequency of immobility caused by the food-elicited cataplexy test (FECT) [20]. This effect was attributed to a decreased release of serotonin, which produced an increase in the awakening time. However, the administration of antagonists did not cause any significant effect [21]. It is well established that selective serotonin-reuptake inhibitors (SSRIs) decreased cataplexy in narcoleptic dogs and humans, suggesting a role of serotonin in cataplexy [20].

*Taiep* rats were described at the Institute of Physiology of the University of Puebla as a myelin mutant rat with a progressive motor syndrome characterized by tremor, ataxia, immobility, epilepsy, and paralysis [13]. The rat showed a hypomyelination followed by a progressive demyelination [9,18], caused by an accumulation of microtubules that disrupt the transporting mechanisms in the oligodendrocytes [9,22]. For *taiep* rats, we demonstrated that gripping-induced immobility episodes (IEs) are a dimorphic gender pattern, with males more susceptible than female *taiep* rats [7]. Gripping-induced IEs increased after systemic administration of $\alpha_2$ agonists and an $\alpha_1$ antagonist [10,6] and decreased after injection of $\alpha_2$ antagonists. These results are similar to the effects obtained in narcoleptic dogs [20].

Spontaneous and gripping-induced IEs showed a rapid eye movement (REM) sleep-like pattern with cortical desynchronization and a theta rhythm in the hippocampus [7]. Therefore IEs are an alteration of REM-sleep generation similar to narcolepsy–cataplexy in the canine model [20]. In the present experiments, we analyzed the effect of systemic injection of 5-HT$_1$ agonists and antagonists on gripping-induced IEs in 8-month-old male *taiep* rats.

*Taiep* rats were supplied by our animal house facilities. Animals were under a 12 h:12 h light:dark cycle (lights on at 0700), at 21 ± 2 °C and 30–45% relative humidity, with free access to water and rodent food pellets (Zeigler, USA). Rats were tested in acrylic cages at 0800, when a peak in susceptibility of gripping-induced

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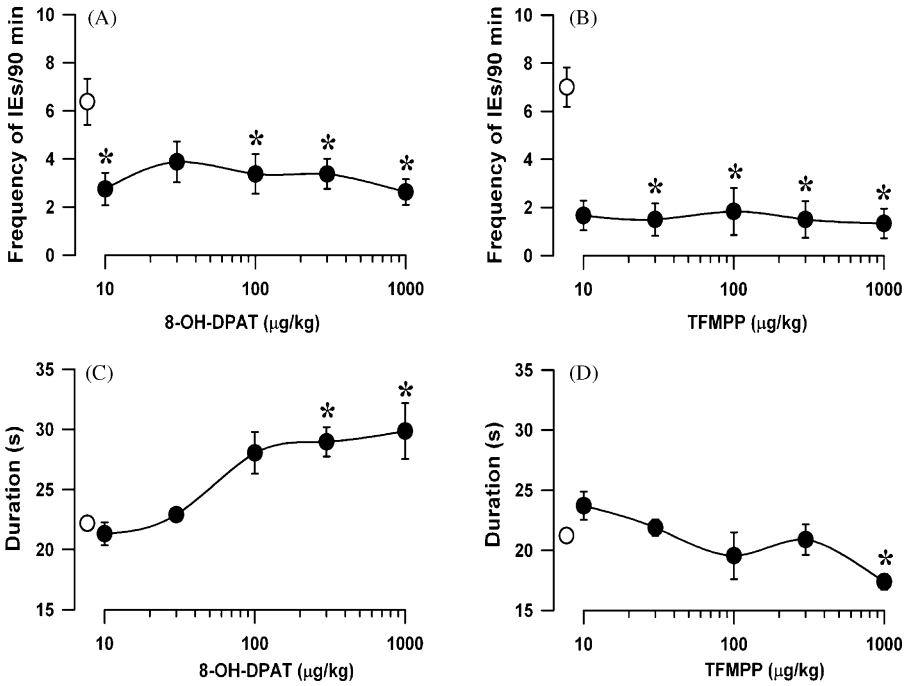


**Fig. 1.** Effects of serotonergic presynaptic activation on gripping-induced immobility episodes in male *taiep* rats. Values are expressed as means ± S.E. (A) 8-OH-DPAT between 10 and 1000 μg/kg caused a significant decrease in the frequency of gripping-induced IEs ($\chi^2$ test = 27, d.f. = 5, $P < 0.001$, followed by the Dunn test $P < 0.05$). (B) TFMPP also produced a significant decrease in gripping-induced IEs ($\chi^2$ test = 31.4, d.f. = 5, $P < 0.001$, followed by the Dunn test $P < 0.05$). (C) The 8-OH-DPAT increase in the mean duration with the 300 and 1000 μg/kg dosages is different from control group ($\chi^2$ test = 15.2, d.f. = 5, $P < 0.01$, followed by the Dunn test $P < 0.05$). (D) TFMPP decreased the mean duration and was significant at 1000 μg/kg ($\chi^2$ test = 14.2, d.f. = 5, $P < 0.01$, followed by the Dunn test $P < 0.05$). Open circles correspond to sterile water injection, which are control values for each group.

IEs occurs [7]. The IEs were caused by gripping the base of the rat's tail for 10 s every 5 min. If an immobility occurs the elapsed time was measured using a chronograph, if not the animal is put into the observation box [7]. The frequency, mean duration of the IEs, and the latency to the first immobility were recorded. The agonists were tested 8-hydroxy-2-(di-*n*-propylamino) tetraline hydrobromide (8-OH-DPAT) and 3-trifluoromethylphenylpiperazine hydrochloride (TFMPP), with a range of doses between 10 and 1000 μg/kg.

The antagonists tested were spiperone hydrochloride and 1-(2-methoxyphenyl)-4[4-(2-phthalimido) butyl]piperazine hydrobromide (NAN-190) with doses between 10 and 1000 μg/kg. The specific serotonin antagonist *N*-[2[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-*N*-2-pyridinyl-cyclohexanecarboxamide maleate (WAY 100635) at 100 μg/kg was used to counteract the effects produced by the 8-OH-DPAT. All drugs were purchased from Sigma–Aldrich (St Louis, MO, USA). The drugs were freshly dissolved in sterile water, are expressed as a free drug, and the dosage volume adjusted to 1 mL/kg weight of rat. Animals received an intraperitoneal injection of sterile water as a control and the drugs in an increasing dose scheme every 48 h. Behavioral analysis was made by two observers, one of them blind to the drug tested. Analysis of the data was done with a $\chi^2$ test, followed by a Dunn test with $P < 0.05$ considered statistically significant [27].

Before each gripping-induced IE, we measured the behavioral state of the rats with 0 indicating somnolence; 1, quiet rat; 2, low activity such as sniffing and horizontal head movements; 3, regular activity as grooming, scratching, and locomotion; and 4, high activity indicating displacement and other motor activities such as grooming, scratching, or even jumping behavior [10]. Somnolence was characterized by closed eyes, no movement, and regular breathing.

All procedures described in this study were in accordance with the "*Guide for the Care and Use of Laboratory Animals*" of the Mexican Council for Animal Care as approved by the BUAP Animal Care Committee and are also in accordance with guide for the care and use of laboratory animals [5].

Systemic administration of 8-OH-DPAT, a specific agonist of 5-$HT_{1A}$-serotonin receptors, caused a statistically significant decrease of gripping-induced IEs between 30 and 1000 μg/kg ($\chi^2$ test = 27, d.f. = 5, $P < 0.001$, followed by the Dunn test $P < 0.05$) and an increase in the mean duration with the higher doses 300 and 1000 μg/kg ($\chi^2$ test = 15.2, d.f. = 5, $P < 0.01$, followed by the Dunn test $P < 0.05$; see Fig. 1). Also the latency to first Immobility episodes increased from 11.2 ± 4.4 min in the control session to 43.1 ± 24 min with the highest dose ($P < 0.01$).

Similar effects were obtained with the systemic administration of TFMPP, a specific 5-$HT_{1B}$ agonist, with a significant decrease of gripping-induced IEs between 30 and 1000 μg/kg ($\chi^2$ test = 18.8, d.f. = 5, $P < 0.02$, followed by the Dunn test $P < 0.05$) and with the mean duration being significant at 1000 μg/kg ($\chi^2$ test = 14.2, d.f. = 5, $P < 0.01$, followed by the Dunn test $P < 0.05$; see Fig. 1). The latency showed that the first IEs also increased from 11.6 ± 2.1 min in control subjects to 57.5 ± 36.8 min with the highest dose ($P < 0.001$). Behaviorally, 8-OH-DPAT produced motor activation particularly with the highest dose and TFMPP caused some decrease of the motor activity with all doses tested (data not shown).

To analyze with some detail the participation of presynaptic serotonin receptors on gripping-induced IEs, we evaluated the effects of the antagonist WAY 100635, a specific antagonist of 5-$HT_{1A}$-receptors. Systemic administration of WAY 100635 at 100 μg/kg did not cause any significant effect in the frequency of IEs compared to control session. However, 8-OH-DPAT injection of 300 and 1000 μg/kg produced a statistically significant decrease of gripping-induced IEs, as in previous series, however this effect was totally reversed 15 min after injection of WAY 100635 before 8-OH-DPAT at both doses (ANOVA $P < 0.02$, see Table 1).