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Long-lasting recovery of locomotor function in chronic spinal rat following chronic combined pharmacological stimulation of serotonergic receptors with 8-OHDPAT and quipazine

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Abstract

In chronic spinal rats, long-term stimulation of 5-HT receptors with quipazine or 8-OHDPAT by means of daily injection, promotes robust locomotor recovery. The question of a possible potentiation between treatments when applied together was addressed. Daily injections of both 8-OHDPAT and quipazine, were performed for a month in spinal animals. Animals were placed on a treadmill and the bipedal hindlimb locomotion was tested. Motor performances (behavioural test) and locomotor parameters (EMG and kinematic) were analysed weekly during the treatment. Furthermore, the locomotor performances were evaluated during two supplemental months following the end of the treatment. Our results suggest that association of both agonists induced long-lasting positive effects on locomotor function. Motor performances were significantly better after combined injection of both drugs than when the agonists were used separately. But, the most significant and new result is that the locomotor scores did not decrease during the weeks that followed the end of the treatment. These results suggests a long-lasting and 5-HT-dependent reorganisation of spinal networks.

Keywords: Locomotion; Spinal rat; Locomotor recovery; EMG; Serotonin; Quipazine; 8-OHDPAT

A large amount of literature has stressed the involvement of serotonin in the control of automatic walking both in cat [3], and in neonatal rat [5,15,25,29]. As a consequence, various serotonergic strategies were developed to promote locomotor recovery in chronic spinal adult rats. Thus, locomotor function was continuously activated after sublesional thoracic transplantation of embryonic serotonergic neurons [19,27,31]. Comparatively, a single injection of either serotonin (subdural) or one of its agonists, quipazine (5-HT_2 agonist) only partially improved the efficacy and coordination of hindlimb movements [9] since, in those conditions, the quality of the recovered locomotor movement did not equal that of the movement recorded either in transplanted [25] or in intact animals. Recently, this difference has been attributed to differences in the duration of the serotonergic stimulation, which is long and continuous in transplanted animals (several months) and comparatively short and discrete in pharmacologically treated animals (a few minutes) [1,2]. As a matter of fact, the recovery appeared significantly improved when a single injection was replaced with chronic treatments. Interestingly, two different agonists were efficient in promoting locomotor recovery when used chronically: 8-OHDPAT (probably acting on 5-HT_{1A} and/or 5-HT_7 [13,14]) and quipazine (probably acting on 5-HT_2 receptors [28]). Thus, in this study we addressed two questions: (i) What are the consequences of chronic injection of 8-OHDPAT associated with quipazine ? (ii) If this treatment elicits some functional recovery, what happens in the weeks following the end of the treatment ?

The various protocols necessary to carry on these experiments were extensively detailed in previous publications [1,2]. Briefly, eight female Sprague–Dawley rats (OFA Iffa-

Abbreviations: 5-HT, serotonin; CPG, central pattern generator; d.p.o., days post-operative; EMG, electromyogram; TA, tibialis anterior muscle; VL, vastus lateralis muscle; s.c., sub-cutaneous; i.p., intraperitoneal; 8-OHDPAT, 8-hydroxy-(2-di-*N*-propylamino)-tetralin

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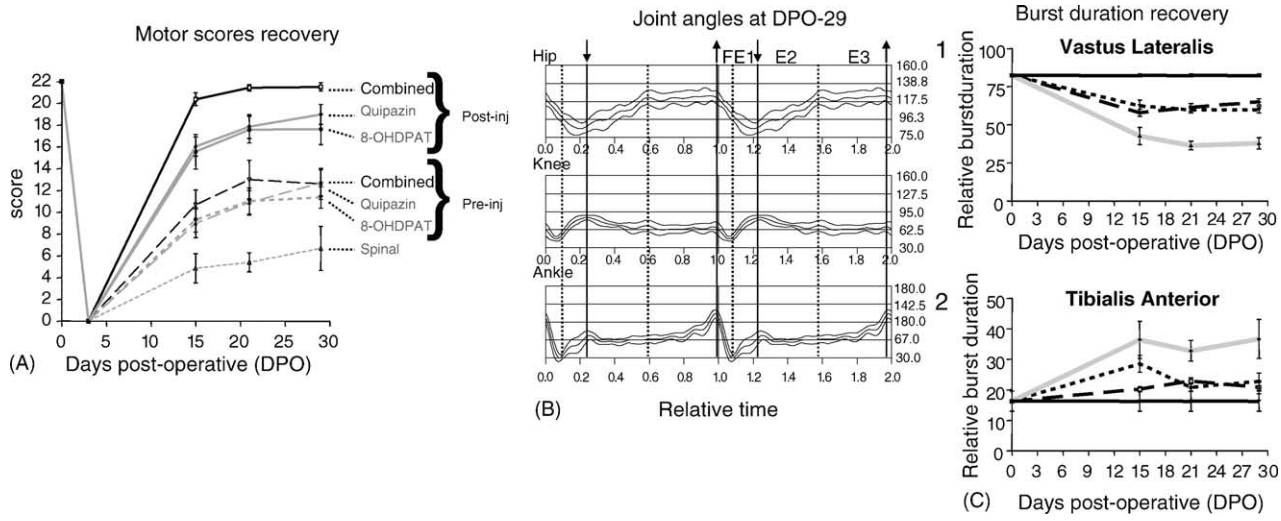

Fig. 1. Motor performances of spinal-treated and non-treated animals (expressed as units of the locomotor rating scale) vs. time in days following spinal cord section. (A) Long-term consequences of 5-HT agonists on motor performances: black lines represent the locomotor behavioural scores of the spinal treated animals with 8-OHDPAT and quipazine injected at once. Scores were evaluated either 15 min before (lower curve) or 40 min after (upper curve) the injection. Grey lines are a recall of the results obtained during a separate treatment with the same agonists and during no treatment in the overall same conditions [1,2]. Pre-inj: pre-injection, post-inj: post-injection. (B) Angular displacement of the right hip, knee and ankle joint measured in an adult spinal rat after one month of combined treatment: The video was done 40 min after the 29th daily injection of both 8-OHDPAT and quipazine. This animal received a score of 22 in the locomotor behavioural test (highest score in this test), indicating that the recovered locomotor movement is similar to that measured in intact rats in the same treadmill conditions. The high quality of this movement is confirmed by the organization of the locomotor cycle as assessed by the fact that F, E1, E2, E3 phases of the locomotor cycle are clearly identified: flexion phase, E: extension phase. (C) Relative burst duration values of vastus lateralis (extensor, 1) or tibialis anterior (flexor, 2) in spinal rats treated with daily combined injections of 8-OHDPAT and quipazine: relative burst durations are expressed in percentage of the cycle duration. Time is expressed in days following spinal cord injury. In each graph, values from intact animals (thick line) and spinal-non-treated animals (thin line) are plotted together with values from spinal-treated animals obtained in pre-injection (thin broken line) and post-injection (thick broken line) conditions.

Credo) aged 3 months (weighing 200–250 g at the beginning of the experiment versus 250–300 g at the end) were used in this study. Implantation of chronic electromyographic electrodes and further surgeries were performed under general anaesthesia (equithesin; 0.3 ml/100 g, i.p. [4]). Electromyographic pairs of electrodes were chronically implanted in tibialis anterior (TA, flexor of the ankle) and in vastus lateralis (VL, extensor of the knee) muscles of both hindlimbs. Three weeks after implantation, hindlimb locomotor activity was analyzed in order to establish the "intact control" baseline (appearing as day post-operative 0 in Figs. 1A and 2A). This allowed further comparison with spinal treated animals recorded in exactly the same biomechanical condition, that is: (1) hindlimbs walking on a treadmill (10 cm/s) while forelimbs were kept on a platform and (2) tail pinch applied in animals of all experimental groups (intact, treated and non-treated rats). Thereafter, the spinal cord was transected at the T8–T9 segment level. By the first day post-operative (d.p.o. 1) and each following day during one month, spinal rats ($n = 8$) received a combined injection of 8-OHDPAT (8-OHDPAT, 0.3 ml, 0.125 mg/kg; sub-cutaneous—Sigma France) and quipazine (0.3 ml, 0.125 mg/kg; intraperitoneal, Sigma France). Different routes were used for each agonist, in accordance with pharmacological studies indicating that i.p. and s.c. were the most efficient ways to stimulate 5-HT_2 receptors with quipazine and 5-HT_{1A} receptors with 8-OHDPAT, respectively [17,18]. The quality of locomotor activity in hindlimbs was tested during treadmill locomotion by means of a behavioural test that has been extensively described and discussed in a previous article [2], and by analysis of the angle variations of the hip, knee and ankle (video kinematic analysis) [1]. Raw EMG data were analysed as described earlier [9]. The results were compared to those, already published [1], obtained in three parallel groups which have been experimented at the same time in exactly the same conditions: (1) spinal, but non-treated animals (injection of 0.3 ml of saline instead of agonists either s.c. ($n = 3$) or i.p. ($n = 2$)), (2) spinal animals treated with 8-OHDPAT alone (0.3 ml, 0.25 mg/kg/day; $n = 10$) and, (3) spinal animals treated with quipazine alone (0.3 ml, 0.25 mg/kg/day; $n = 10$). The corresponding data, taken from publication [1], are indicated by grey curves in Figs. 1A and 2A. A two-way ANOVA was used to compare each group of animals and estimate the effects of the treatments on the evolution of either the motor performances or the EMG pattern of activation. Furthermore an unpaired Student's *t*-test was used in order to compare data obtained at d.p.o. 29 to those obtained further after the ending of the treatment (d.p.o. 49, 75 and 95).

Though injections occurred daily, and in order to prevent a possible effect of training on the amplitude and time course of recovery, locomotor performances were tested only once a week at d.p.o. 3, 15, 21, and 29, and tests were limited to 10 min. In some animals, three additional locomotor tests were done at d.p.o. 49 ($n = 6$), 75 ($n = 4$) and 96 ($n = 4$) cor-