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Dorsal and median raphe serotonergic system lesion does not alter the opiate withdrawal syndrome

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Abstract

Previous pharmacological studies have implicated serotonergic brain systems in opiate withdrawal. To test the hypothesis that serotonin (5-HT) has a critical role in the development of opiate withdrawal, we have employed a near-total brain 5-HT system lesion technique (90% depletion) using 5,7-dihydroxytryptamine combined with induction of opiate dependence by implantation of morphine pellets or by repeated injections of increasing doses of morphine. The effects of serotonergic neuron lesion were examined on spontaneous opiate withdrawal (changes in circadian locomotor activity) and naloxone-precipitated opiate withdrawal syndrome (the somatic aspect). The antiwithdrawal properties of clonidine, an α_2-adrenoceptor agonist currently used for clinical treatment for the somatic signs of opiate withdrawal, were tested also in the lesioned rats. Our findings show that serotonergic lesions in morphine-dependent rats did not alter either the spontaneous or the naloxone-induced withdrawal syndrome (with exception of jumping behavior). Moreover, clonidine alleviated the naloxone-induced withdrawal syndrome in lesioned as well as in sham-operated morphine-dependent rats. These results demonstrate that 5-HT systems are not directly responsible for the development of the somatic opiate withdrawal syndrome in morphine-dependent rats.

Keywords: Morphine; Naloxone; Withdrawal syndrome; Serotonin; 5,7-Dihydroxytryptamine; Clonidine

1. Introduction

"Opponent Process" theory postulates that dependence to opiates represents a novel homeostatic state resulting from neuroadaptive mechanisms that counteract the effects of opiates in the organism (Koob and Bloom, 1988; Solomon, 1980). The acute opiate withdrawal syndrome is assumed to be induced by these neuroadaptations, which no longer are counterbalanced by the presence of the drug (Koob and Bloom, 1988). The aversive component of morphine withdrawal can be evaluated by measuring the intensity of the place aversion conditioning triggered by injections of very low doses of naloxone (Schulteis et al., 1994). The administration of higher doses of naloxone or abstinence from morphine enables study of the somatic component of opiate dependence, which can be measured by rating various somatic and behavioral patterns during the acute abstinence syndrome (Schulteis et al., 1994). The spontaneous opiate abstinence usually is reflected by the disruption of the circadian cycle in rats (Stinus et al., 1998).

An acute injection of morphine increases serotonin (5-HT) release in the rat forebrain (Tao and Auerbach, 1995), which modulates dopaminergic activity within the nucleus accumbens (Spampinato et al., 1984). 5-HT transmission also is involved in the chronic effects of morphine. Increased brain 5-HT release is associated with the development of physical dependence to morphine (Javelle et al., 1997; Way et al., 1968). It has been shown that inhibition of 5-HT transmission, by partial lesion with 5,6-dihydroxytryptamine, attenuates morphine dependence (Ho et al., 1972). In this model, higher doses of naloxone were required to trigger the abstinence syndrome. Consistent with these results, Samanin et al. (1980) showed

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that drugs inhibiting central 5-HT function during the development of opiate dependence decrease the intensity of physical dependence and, more specifically, the jumping behavior displayed during acute opiate withdrawal (Cervo et al., 1983). Although these findings pointed to an involvement of serotonergic activity in opiate dependence, the conclusions were based mainly on the sole rating of jumping behavior. It is recognized nevertheless that the opiate withdrawal syndrome is a complex behavior that includes expression of numerous somatic manifestations, and jumping behavior alone is unlikely to account for the entire opiate-dependent state (Gellert and Holtzman, 1978).

In view of these results, the exact role of 5-HT systems in opiate withdrawal remains unclear. In order to answer this question, we have destroyed 5-HT neurons arising from the dorsal (DR) and median raphe (MR) nuclei by multiple injections of 5,7-dihydroxytryptamine (90% depletion). The effects of the lesion in morphine-dependent rats were examined both on the development of spontaneous opiate withdrawal by continuous evaluation of locomotor activity and the somatic component of opiate withdrawal by quantifying naloxone-induced opiate withdrawal symptoms. Finally, we tested the ability of clonidine to protect 5-HT-lesioned rats from an acute opiate withdrawal syndrome.

2. Materials and methods

2.1. Animals

A total of 123 male Sprague–Dawley rats (IFFA-CREDO, Lyon, France) weighing 220–240 g at the beginning of the experiments were used. Animals were housed by four in cages located in a thermoregulated room (22 °C) with a 12:12 h light–dark cycle (light from 8:00 a.m. to 8:00 p.m.). Food and water were available ad libitum. These conditions were maintained constant throughout the experiments. All manipulations and observations were made during the light phase of the circadian cycle.

The experiments were carried out in accordance with the Declaration of Helsinki, the NIH Guide for the Care and Use of Laboratory Animals, the European Communities Council Directives (86/609/EEC, 24 November 1986) and the French Directives concerning the Use of Laboratory Animals (Decret 87-848, 19 October 1987). All efforts were made to minimize animal suffering and to reduce the number of animals used.

2.2. Drugs

The following compounds were used in this study: desmethylimipramine (Sigma, St. Louis, MO), 5,7-dihydroxytryptamine creatinine sulfate (5,7-DHT) (Sigma), morphine sulfate (Sanofi-Francopia, France), morphine pellets (National Institute on Drug Abuse, Bethesda, MD), naloxone hydrochloride (Research Biochemicals International, Natick, MA) and nomifensine maleate (RBI). All drugs were dissolved in NaCl isotonic solution (0.9% in water), except for 5,7-DHT, which had a vehicle consisting of NaCl isotonic solution (0.9% in water) containing 0.1 mg/ml of ascorbic acid. Naloxone hydrochloride, nomifensine and desmethylimipramine were injected subcutaneously (1 ml/kg). Morphine sulfate was injected intraperitoneally (1 ml/kg the first 4 days of treatment, then 2 ml/kg on subsequent days).

2.3. Surgery

Serotonergic innervation arising from the DR and MR were almost totally destroyed by multiple intracerebral injections of 5,7-DHT aimed at DR and MR 5-HT nuclei. Rats were anesthetized with chloral hydrate (270 mg/kg ip in isotonic saline, 1 ml/kg) and placed in a Kopf stereotaxic apparatus with the incisor bar set 5.0 mm above the interaural line. The 5,7-DHT solution contained 2 μg base in 1 μl (1 μg base = 2.099 μg 5,7-DHT creatinine sulfate). The rate of injection was 1 μl over 100 s. The following coordinates are expressed in millimeters, L for lateral from the midline, AP for anteroposterior from bregma and V for vertical from the skull surface. Twenty-four hours before each experiment, coordinates of injection sites were verified histologically on four animals of the same group. Four bilateral targets were chosen according to the distributional map of 5-HT-immunoreactive neurons in the rat brain (Bjorklund et al., 1984); 4 μg of 5,7-DHT were injected at each site: (I) two sites located in the ventral part of the periaqueductal gray matter (DR), $L = \pm 0.5$, $AP = -4.4$, $V = -6.3$ and $L = \pm 0.5$, $AP = -5.4$, $V = -6.2$; (II) the third site located just above the interpeduncular nucleus, $L = \pm 0.5$, $AP = -4.4$, $V = -8.7$; and (III) the fourth site located at the bottom part of the tectospinal tract (MR), $L = \pm 0.5$, $AP = -5.4$, $V = -9.2$ ($n = 190$). Sham-operated rats received vehicle injections ($n = 186$). Thirty minutes before the 5,7-DHT or vehicle injections, rats were pretreated with the noradrenaline (NA) reuptake inhibitor desmethylimipramine (10 mg/kg ip) (Beique et al., 1998) and the dopamine (DA) reuptake inhibitor nomifensine (10 mg/kg ip) (Dugast et al., 1994), to protect noradrenergic and dopaminergic neurons from the neurotoxic effects of 5,7-DHT. All experiments began after a 1-week postoperative recovery period.

2.4. Induction of opiate dependence

Two pharmacological approaches were employed to induce opiate dependence. For the spontaneous withdrawal paradigm, opiate dependence was induced by injection of escalating doses of morphine, whereas for opiate antagonist-triggered withdrawal, dependence was induced by subcutaneous implantation of morphine pellets.

2.4.1. Induction of morphine dependence by repeated injection of escalating doses of morphine

Morphine dependence was induced by intraperitoneal injections of morphine sulfate ($n=20$) twice a day for 10 days according to the following schedule: 5 mg/kg the first day, 10 mg/kg the second day and subsequent increases of 10 mg/kg/day to reach a dose of 90 mg/kg per injection on the 10th day. Injections were delivered at 8:00 a.m. and 8:00 p.m. The last injection (90 mg/kg) was given on the morning of the 11th day. Opiate abstinence was induced by interruption of the chronic morphine treatment.

2.4.2. Induction of morphine dependence by implantation of slow-release morphine pellets

Morphine dependence was induced by subcutaneous implantation (lower back) under a rapid deep anesthesia (halothane/air; induction 4% V/V for 10 s followed by 1.5% v/v for 30 s) of two slow-release morphine pellets (each morphine pellet contains 75 mg of morphine base, $n=2\times103$). Under these conditions, we have shown that full dependence to morphine is achieved 24 h after implantation of the morphine pellet and remains constant for 15 days (Gold et al., 1994).

2.5. Activity recording

Locomotor activity was measured in activity cages ($35\times25\times25$ cm) whose door, floor and ceiling were made of wire mesh and whose side walls were made of 10 mm transparent Plexiglas (Imetronic, Pessac, France). Two infrared photoelectric cells were placed 14 cm apart and 3 cm above the floor so that each passage of the animal interrupted the beam and was recorded by a computer. A computer program (Imetronic) analyzed the total number of activity counts (total beam interruptions on both cells), which represented the total motor activity of the animal.

2.6. Opiate withdrawal syndrome

The opiate withdrawal syndrome was quantified in a circular enclosure (diameter 31 cm and 40 cm high), which was covered to prevent the rat from escaping. On the fourth day after implantation of morphine pellets, rats were injected with naloxone or saline and, 10 min later, they were enclosed and videotaped for 10 min (Sony camera and TV monitor, Panasonic video-recorder). A microphone just above the cage recorded sounds emitted by rats during opiate withdrawal.

The opiate withdrawal score was calculated using the scale described by Gellert and Holtzman (1978), in which two classes of signs were distinguished: graded signs (weight loss, jumping, abdominal contractions, wet dog shakes), which were quantified numerically, and checked signs (diarrhea, teeth chattering, swallowing, salivation, chromodacryorrhea, ptosis, abnormal posture, genital grooming, irritability) for which only its presence or absence was evaluated. Chromodacryorrhea is the secretion of so-called "bloody tears" from the harderian gland, which nearly circumscribes the eye within the bony orbit.

2.7. Measurement of tissue levels of biogenic amines

At the end of the experiments, when the acute effects of morphine abstinence had ceased (i.e., 1-month post-pellet implantation), lesion and sham-operated rats were killed by decapitation. Brains were removed rapidly and the cortex, striatum and hippocampus were dissected bilaterally at 4 °C. Dissected structures were immediately frozen on dry ice and stored at −80 °C until biochemical assays. DA, NA and 5-HT contents were measured in the dissected brain regions by HPLC coupled with electrochemical detection. Tissue samples were homogenized in 200 μl of 0.1 N $HClO_4$ and centrifuged at 11,000 rpm for 30 min at 4 °C. Aliquots of the supernatants (10–20 μl) were injected into the HPLC system after dilution with appropriate volumes of mobile phase. The mobile phase was as follows: 60 mM NaH_2PO_4, 0.1 mM disodium EDTA, 0.2 mM octane sulfonic acid, 7% methanol, adjusted to pH 3.9 with orthophosphoric acid and filtered through a 0.22-μm Millipore filter. This mobile phase was delivered at 1.2 ml/min (Pump 116, System Gold, Beckman, Paris) through a Chromasyl column (C8, 150×4.6 mm, 5 μm, Touzard and Matignon, Paris) protected by a Brownlee–Newgard precolumn (RP-8, 15×3.2 mm, 7 μm). A refrigerated injector (Injector 507, System Gold, Beckman, Paris) was used. NA, DA and 5-HT were detected by a coulometric system (Coulochem II, ESA, Paris) coupled to a dual electrode analytic cell (model 5011) and a conditioning cell (model 5021). The conditioning cell was set at +100 mV, the first electrode at +350 mV and the second at −270 mV. Results were expressed in pg/mg of tissue and each value was the mean ± S.E.M.

2.8. Experiment 1: 5-HT neuron lesion effects on spontaneous opiate withdrawal

Eight 5,7-DHT-lesioned and 12 sham-operated morphine-dependent rats were used. The experiment lasted 25 days. Animals were permanently housed in individual cages, which allowed continuous recording of locomotor activity. After a 2-day habituation period, locomotor activity was recorded for 4 days to provide a measure of each animal's baseline activity. The data first were averaged for each subject, then averaged for the group ($n=8$ or 12). Over the following 10 days, morphine dependence was induced by repeated injections of increasing doses of morphine (2/day, 8:00 a.m. and 8:00 p.m.). The last injection was carried out on the morning of the 11th day, then locomotor activity was continuously recorded during the first 9 days of abstinence. Temperature, dark–light cycle, and food and water availability were identical to those in the animal colony.

2.9. Experiment 2: 5-HT neuron lesion effects on the naloxone-induced acute withdrawal syndrome in morphine-dependent rats

The naloxone-induced opiate withdrawal syndrome was evaluated on 45 5,7-DHT-lesioned and on 36 sham-operated morphine-dependent rats (subcutaneous implantation of two morphine pellets). Each group was split into five subgroups, which received the following doses of naloxone: 0, 10, 50, 100 and 1000 μg/kg sc (Nal-0, Nal-10, Nal-50, Nal-100 and Nal-1000). On the fourth day following morphine pellet implantation, each rat received its respective naloxone injection and, 10 min later, its behavior was videotaped for 10 min.

2.10. Experiment 3: 5-HT neuron lesion effects on the ability of clonidine to protect morphine-dependent rats from the naloxone-induced acute withdrawal syndrome

Twelve 5,7-DHT- and 10 sham-operated morphine-dependent animals (2 morphine pellets) were used to rate the naloxone-induced somatic opiate withdrawal syndrome. Each 5,7-DHT and sham-operated rat received the following pharmacological treatments in random order: saline (Nal-0), naloxone 1000 μg/kg sc (Nal-1000), naloxone + clonidine 50 μg/kg ip (Nal-1000 + Clo-50), naloxone + clonidine 200 μg/kg ip (Nal-1000 + Clo-200), saline + clonidine 50 μg/kg ip (Nal-0 + Clo-50) and saline + clonidine 200 μg/kg ip (Nal-0 + Clo-200). Rats were tested at 4, 6, 8, 10, 12 and 14 days after morphine pellet implantation. Clonidine and naloxone were injected 70 and 10 min, respectively, before videotape recording for 10 min.

2.11. Statistical analysis

2.11.1. Tissue levels of biogenic amines

Differences between 5,7-DHT-lesioned and sham-operated groups were tested using a two-tailed Student's t test.

2.11.2. Locomotor activity

A first ANOVA was made in order to analyze the baseline locomotor activity. It was a two-way ANOVA with a between-subjects factor of 5,7-DHT lesion and a within-subjects factor of time of day. Then, a global ANOVA was performed on the 9 days following the last morphine injection (i.e., the spontaneous withdrawal period), taking into account three factors: one between-subjects factor of 5,7-DHT lesion and two within-subjects factors of day and time of day. Post-hoc analyses consisted of comparisons of simple main effects using the Bonferroni correction to maintain a constant P value of $P < .05$ for all comparisons.

2.11.3. Withdrawal syndrome

Frequency of graded signs or the percent of weight loss were analyzed using the Kruskal–Wallis nonparametric rank test with the naloxone dose as the independent variable. The Mann–Whitney U test was used for the two-way comparisons (sham- vs. 5-HT-lesioned) and the Chi-square test for the checked signs.

3. Results

3.1. Biochemical verification of 5-HT lesion

Results are presented in Table 1. Bilateral multiple injections of 5,7-DHT into the DR and MR nuclei and in the vicinity of the interpeduncular nucleus, where all the ascending serotonergic ascending fibers are localized, produced an almost total lesion of ascending serotonergic innervation. Almost complete depletion of 5-HT was observed in the cortex (−88%, $P < .001$), in the hippocampus (−86%, $P < .001$) and in the striatum (−99.6%, $P < .001$). NA was not affected in either hippocampus (+1%, NS) or cortex (+10%, NS). There was no modification of DA in the striatum (−11%, NS).

3.2. Experiment 1: 5-HT neuron lesion effects on spontaneous opiate withdrawal

The general locomotor activity of the 5,7-DHT-lesioned and sham-operated rats during the spontaneous withdrawal is presented in Fig. 1. The first part of the graph represents the baseline locomotor activity of both 5,7-DHT and sham-operated rats during one light–dark cycle (data averaged over 4 days). Both groups developed biphasic low activity during the light phase and a high level of activity during the dark phase. A two-way ANOVA

Table 1
Effects of 5,7-DHT-induced lesion on monoamine levels in various brain areas

	NA	DA	5-HT
Striatum			
Sham	n.d.	10198 ± 1319	256 ± 19
5,7-DHT	n.d.	9050 ± 508 −11%	1 ± 1 −99.6%*
Cortex			
Sham	201 ± 22	n.d.	127 ± 13
5,7-DHT	224 ± 17 +10%	n.d.	16 ± 4 −88%*
Hippocampus			
Sham	190 ± 18	n.d.	165 ± 8
5,7-DHT	193 ± 11 +1%	n.d.	24 ± 12 −86%*

For all groups, each value, expressed in pg/mg of tissue, represents the mean ± S.E.M. In addition, for 5,7-DHT-lesioned rats, monoamine levels were also expressed in percent of control values. Assays were carried out at the end of each experiment.

* $P < .001$ vs. sham-operated animals (two-tailed Student's t test); n.d. = not determined.

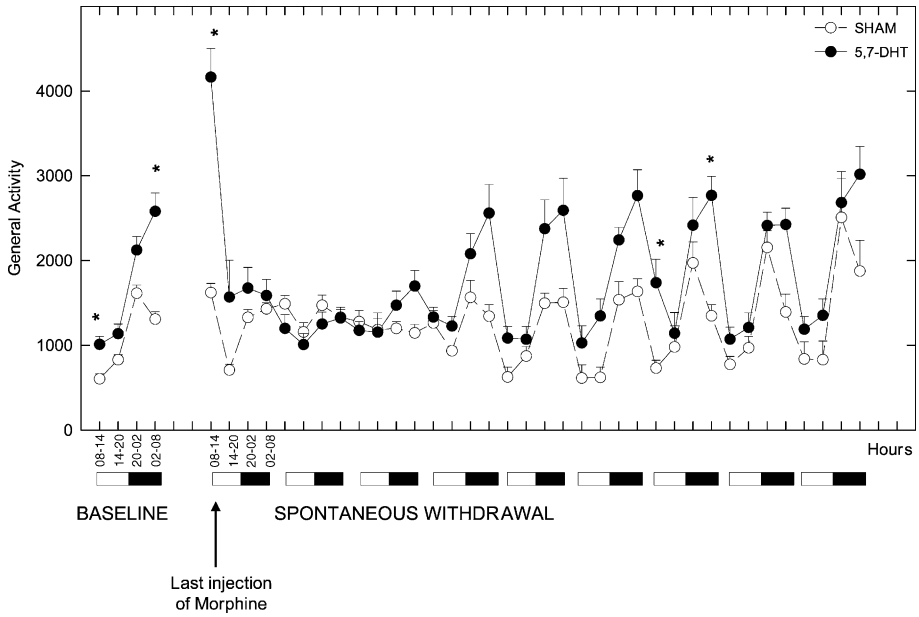

Fig. 1. 5-HT neuron lesion effects on the time course of total locomotor activity (total photocell activity counts) recorded in rectangular activity cages during 9 days following the last morphine injection. The first block represents the baseline activity of each group (mean of 4 consecutive days recording before the beginning of morphine treatment) in 5,7-DHT-lesioned rats ($n=8$, black dots, solid lines) and in sham-operated rats ($n=12$, white dots, dotted lines). The second block represents the motor activity recorded during the first 9 days of morphine abstinence. Morphine dependence was induced by injections of increasing doses of morphine (from 10 to 90 mg/kg ip) for 10 days. The last injection of morphine (arrow) was administered on the morning of the 11th day, which corresponds to the first day of abstinence. The number of counts is shown at 6-h intervals: 0800–1400 and 1400–2000 h for the light phase (white rectangles), 2000–0200 and 0200–0800 h for the dark phase (black rectangles) of each day. Mean general activity ± S.E.M. Post-hoc t test comparisons, sham vs. 5,7-DHT-lesioned group: $*P<.05$.

(lesion as a between-subjects factor, and time of day as a within-subjects factor) indicated that overall activity in the 5,7-DHT-lesioned rats was higher than that of the sham-operated rat group [$F(18,1)=37.53$, $P<.001$]. ANOVA further indicated that this hyperactivity was dependent on the time of day [$F(54,3)=10.28$, $P<.001$]. Post-hoc comparisons indicated that lesioned rats were hyperactive at the 0800–1400 h interval (sham = 606 ± 60 counts, 5,7-DHT-lesioned = 1010 ± 96 counts, $P<.05$) and at the 0200–0800 h interval (sham = 1309 ± 86 counts and 5,7-DHT-lesioned = 2580 ± 216 counts, $P<.05$).

Circadian locomotor activity was not recorded during the chronic morphine treatment of rats. Then, recording started again just after the last morphine injection and lasted for the following 9 days. Thus, the second part of the graph represents the activity during spontaneous opiate withdrawal.

For each group, statistical comparison of each of the 9 abstinence days to baseline activity showed two different phases. During the first phase, we observed a disruption of circadian activity immediately after the last injection of morphine. This phase persisted for 4 days in the sham-operated rats (vs. baseline, ANOVA, Days 1–3, $P<.001$; Day 4, $P<.01$) and for 3 days in the 5,7-DHT-lesioned rats (vs. baseline, ANOVA, Days 1–3, $P<.001$). In the second phase (Days 4–9), animals of both groups returned to baseline circadian activity. Sham-operated rats expressed circadian locomotor activity similar to baseline on Days 5–7 (vs. baseline, ANOVA, Day × Time interaction, NS). For the 5,7-DHT-lesioned group, a return to baseline activity was observed on Day 4 and persisted until Day 9 (ANOVA, Day × Time interaction, NS).

3.3. Experiment 2: 5-HT neuron lesion effects on the naloxone-induced acute withdrawal syndrome in morphine-dependent rats

The results are presented in Fig. 2a: the checked signs and the graded signs. In the sham-operated group, injections of increasing doses of naloxone induced the expression of somatic withdrawal symptoms: salivation, vocalization, diarrhea (χ^2, $P<.01$); jumping (Kruskal–Wallis, $P<.01$); mastication and weight loss (Kruskal–Wallis, $P<.001$). These rats exhibited the maximum intensity of the withdrawal syndrome for Nal-1000. For the overall withdrawal score of the sham-operated rats, maximal values were recorded at Nal-1000 (Gellert–Holtzman score, Kruskal–Wallis, $P<.001$, Fig. 2b).

The 5,7-DHT-lesioned rats also exhibited increased somatic withdrawal symptoms: salivation, vocalization, diarrhea (χ^2, $P<.001$); jumping (Kruskal–Wallis, $P<.05$); mastication and weight loss (Kruskal–Wallis, $P<.001$) (Fig. 2a). The lesioned rats exhibited the maximum acute withdrawal syndrome for Nal-1000. In addition, when overall opiate withdrawal score was considered, the Gel-

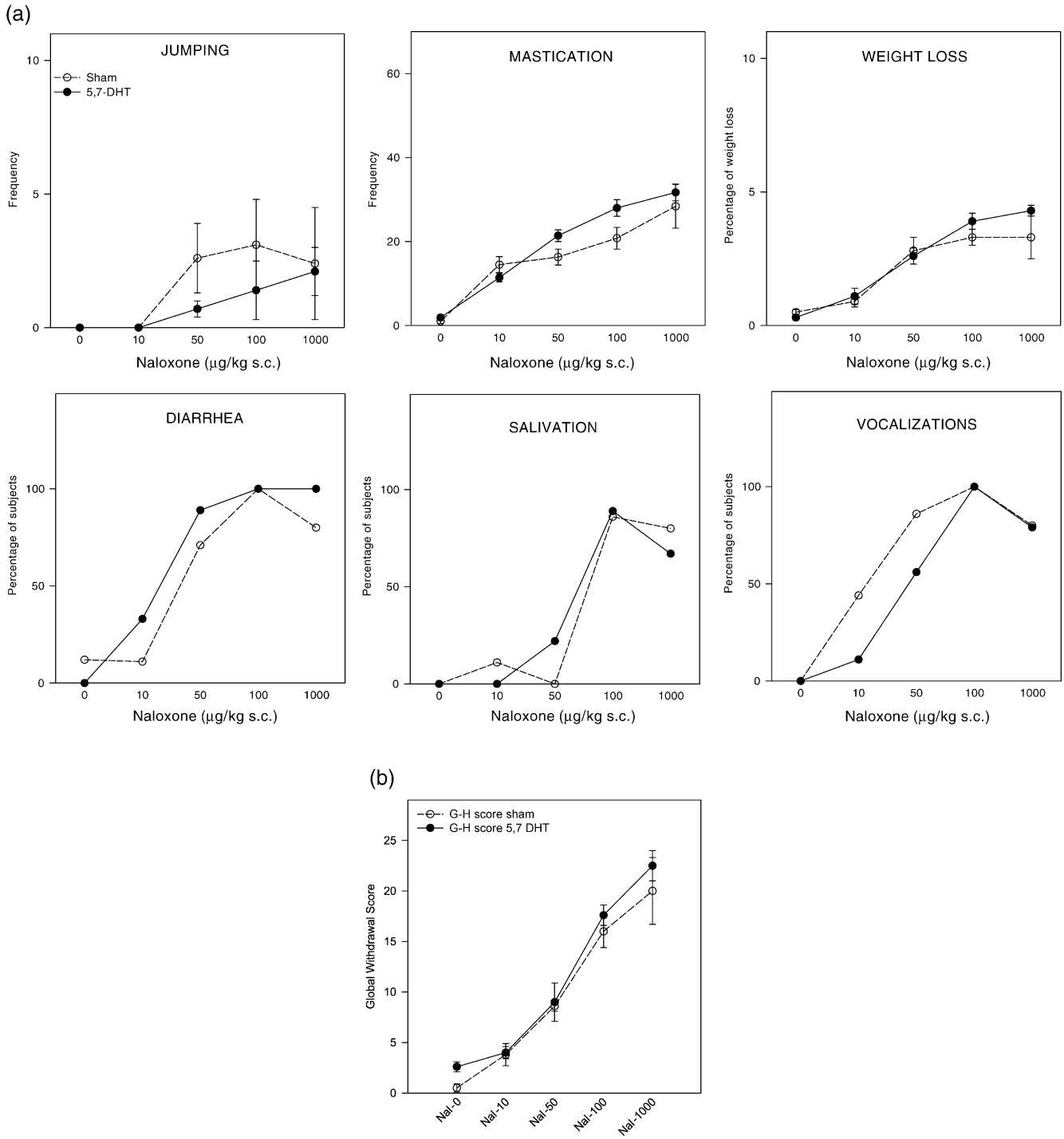

Fig. 2. (a) 5-HT neuron lesion effects on naloxone-induced withdrawal-graded signs (jumping, mastication and weight loss) and checked signs (diarrhea, salivation and vocalizations). Morphine dependence was induced by subcutaneous implantation of two morphine pellets. Opiate withdrawal was precipitated by naloxone 0, 10, 50, 100 and 1000 μg/kg sc. Mean ± S.E.M. of the frequency of graded signs and percentage of subjects expressing the checked signs are represented for 5,7-DHT-lesioned rats (black dots and solid lines; $n = 9$ for each dose) and sham-operated (white dots and dotted lines; $n = 5-9$). Weight loss is expressed in percentage of initial body weight. (b) 5-HT neuron lesion effects on the overall naloxone-induced opiate withdrawal syndrome. All rats were implanted subcutaneously with two morphine pellets. The following doses of naloxone were tested: 0, 10, 50, 100 and 1000 μg/kg sc in 5,7-DHT-lesioned rats ($n = 9$ for each naloxone dose) and in sham-operated rats ($n = 5-9$). Opiate withdrawal signs were quantified both by the Gellert and Holtzman rating scale (G–H score) in 5,7-DHT-lesioned rats (black dots and solid lines) and in sham-operated rats (white dots and dotted lines). Mean global score ± S.E.M. Mann–Whitney analysis, sham-operated vs. 5,7-DHT-lesioned rats.

lert–Holtzman score peaked at Nal-1000 (Kruskal–Wallis, $P < .05$, Fig. 2b).

Regardless of the incidence of somatic abstinence signs and the dose of naloxone (Nal-0 to Nal-1000), there was no

statistically significant difference between the lesioned- and sham-operated group Gellert–Holtzman scores (Mann–Whitney, NS).

Calculation of naloxone ED_{50} indicated comparable efficiency of naloxone in both sham and lesioned rats. For the Gellert–Holtzman score, naloxone $ED_{50} = 50$ μg/kg in sham and 55 μg/kg in lesioned rats.

3.4. Experiment 3: 5-HT neuron lesion effects on the ability of clonidine to protect morphine-dependent rats from naloxone-induced acute withdrawal syndrome

The behavioral effects of clonidine alone will be considered first. In both sham-operated and 5,7-DHT-lesioned, morphine-dependent rats, clonidine alone (50 or 200 μg/kg ip) did not affect behavior (Nal-0 + Clo-0 vs. Nal-0 + Clo-50 and Nal-0 + Clo-0 vs. Nal-0 + Clo-200, Wilcoxon, NS for both groups; Fig. 3). However, when sham-operated or 5,7-DHT-lesioned rats were injected with NAL-1000, the Gellert–Holtzman score was increased dramatically indicating the appearance of a full opiate withdrawal syndrome (Nal-0 + Clo-0 vs. Nal-1000 + Clo-0, Wilcoxon, $T = -2.80$, $P < .01$ and $T = 3.06$, $P < .01$, respectively).

When sham-operated animals received a clonidine injection prior to naloxone, opiate withdrawal was significantly attenuated since the Gellert–Holtzman scores of the Nal-1000 + Clo-50 and Nal-1000 + Clo-200 groups were reduced.

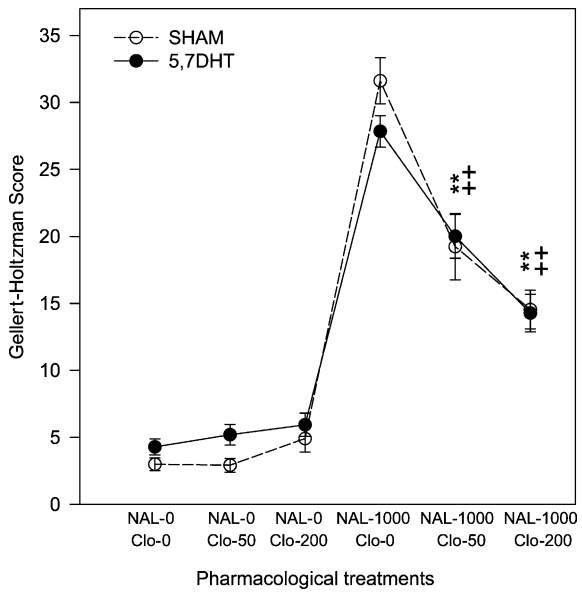

Fig. 3. 5-HT neuron lesion effects on clonidine protection of the naloxone-induced opiate withdrawal syndrome. All rats were made dependent by the subcutaneous implantation of two morphine pellets. Opiate withdrawal signs were quantified by the Gellert and Holtzman rating scale in sham-operated ($n = 10$, white dots, dotted lines) and 5,7-DHT-lesioned rats ($n = 12$, black dots, solid lines) for each pharmacological treatment. Mean global score ± S.E.M. Wilcoxon analysis (Nal-1000 + Clo-0 vs. Nal-1000 + Clo-50 and Nal-1000 + Clo-0 vs. Nal-1000 + Clo-200): 5,7-DHT-lesioned rats, $^{++}P < .01$ and sham-operated rats, $^{**}P < .01$.

However, the withdrawal syndrome was not abolished (Nal-0 + Clo-0 vs. Nal-1000 + Clo-50 and Nal-0 + Clo-0 vs. Nal-1000 + Clo-200, Wilcoxon, $P < .01$). Similar results were obtained with the 5,7-DHT-lesioned rats, since clonidine prior to naloxone also reduced the Gellert–Holtzman score (Nal-1000 + Clo-0 vs. Nal-1000 + Clo-50, $T = -2.67$, $P < .01$; Nal-1000 + Clo-0 vs. Nal-1000 + Clo-200, $T = -3.06$, $P < .01$), but did not abolish the opiate withdrawal syndrome (Nal-0 + Clo-0 vs. Nal-1000 + Clo-50 and Nal-0 + Clo-0 vs. Nal-1000 + Clo-200, Wilcoxon, $P < .01$).

The naloxone-induced withdrawal syndrome and its attenuation by clonidine were similar in the lesioned and sham-operated groups (Mann–Whitney, NS). This result was confirmed by the ED_{50} determination of clonidine: sham = 29 μg/kg, lesioned = 46 μg/kg.

4. Discussion

In the present study, we tested the hypothesis that brain serotonergic systems are critical for manifestations of spontaneous and precipitated opiate withdrawal. Multiple intracerebral injections of 5,7-DHT induced a reliable specific and nearly total (90%) depletion of ascending 5-HT forebrain innervation.

The present results have shown that spontaneous opiate withdrawal, as measured by disruption of circadian locomotor activity observed during the first days of opiate abstinence, was not affected by almost total lesion of ascending 5-HT innervation. Moreover, 5,7-DHT lesion did not alter the development of the naloxone-induced somatic opiate withdrawal syndrome.

The 5,7-DHT lesion effects were investigated on spontaneous opiate abstinence. In 5-HT-lesioned rats as observed in sham-operated rats, normal circadian locomotor activity was completely disrupted during the first 3 days of opiate abstinence. Moreover, destruction of the ascending 5-HT pathway led to locomotor hyperactivity in both the light and dark periods of the circadian cycle (Asin and Fibiger, 1983; Wirtshafter et al., 1986). This locomotor hyperactivity is thought to result from the loss of hippocampal 5-HT neurotransmission (Jacobs et al., 1975). In accordance with Stinus et al. (1998), we observed increased locomotor activity in sham-operated rats several days after the beginning of spontaneous opiate abstinence.

With respect to the precipitated somatic symptoms of opiate withdrawal, rats with an almost complete 5-HT lesion still displayed the full somatic naloxone-induced opiate withdrawal syndrome. The maximum intensity of the abstinence syndrome for both lesioned and sham-operated rats was observed for naloxone 1000 μg/kg. Moreover, for all doses of naloxone tested, the incidence of acute withdrawal signs did not differ between the lesioned and sham-operated rats. Although the difference was not statistically significant, the 5,7-DHT-lesioned rats tended to jump less than the control rats, which is in agreement with previous studies

(Ho et al., 1972; Way et al., 1968). Our data showed that the acute somatic withdrawal syndrome was unaffected by 5,7-DHT lesion.

Early studies showed that clonidine was of value in the treatment of opiate withdrawal (Gold et al., 1978), although it has undesirable side-effects on mood and the cardiovascular system. Thus, in a second part of the study, the influence of 5,7-DHT lesion on the antiwithdrawal action of clonidine was examined. We found that clonidine still attenuated the somatic syndrome in both 5-HT-lesioned and sham-operated rats, as reflected by the decrease of the Gellert–Holtzman score. These results are in line with other reports that the protective effects of clonidine on withdrawal signs such as diarrhea, ptosis or "wet dog shakes" are independent of 5-HT neurotransmission (Romandini et al., 1984; Samanin et al., 1980). Although lesion of 5-HT neurons did considerably potentiate the protection afforded by clonidine on naloxone-induced place aversion (Caille et al., 1999), it did not enhance action on the somatic withdrawal symptoms.

Although the somatic and motivational aspects of the opiate withdrawal syndrome cannot be completely dissociated, our results support previous work indicating that they are mediated by different neurobiological processes and can be elicited independently of each other (Maldonado et al., 1992; Stinus et al., 1990). Indeed, Higgins et al (1991) have shown that 5-HT_3 antagonists block naloxone-precipitated conditioned place aversion whereas we have no effects of the 5,7-DHT-induced lesion on the global somatic opiate withdrawal.

In conclusion, the present study shows that drastic lesion of 5-HT neurons does not block the development of both the spontaneous and naloxone-triggered morphine withdrawal syndrome in rats.

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