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# Differential modulation by monoamine membrane receptor agonists of reticulospinal input to lamina VIII feline spinal commissural interneurons

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## Abstract

Noradrenaline and serotonin have previously been demonstrated to facilitate the transmission between descending reticulospinal tracts fibres and commissural interneurons coordinating left–right hindlimb muscle activity. The aim of the present study was to investigate the contribution of subclasses of monoaminergic membrane receptors to this facilitation. The neurons were located in Rexed lamina VIII in midlumbar segments and identified by their projections to the contralateral gastrocnemius–soleus motor nuclei and by lack of projections rostral to the lumbosacral enlargement. The effects of ionophoretically applied membrane receptor agonists [phenylephrine (noradrenergic $\alpha_1$), clonidine (noradrenergic $\alpha_2$), 8-OH-DPAT (5-$HT_{1A}$, 5-$HT_7$), 2-me-5-HT (5-$HT_3$), 5-me-5-HT (5-$HT_2$) and $\alpha$-me-5-HT (5-$HT_2$)] were examined on extracellularly recorded spikes evoked monosynaptically by electric stimulation of descending reticulospinal fibres in the medial longitudinal fascicle. Application of $\alpha_1$ and 5-$HT_2$ agonists resulted in a facilitation of responses in all investigated neurons while application of $\alpha_2$, 5-$HT_{1A/7}$ and 5-$HT_3$ agonists resulted in a depression. These opposite modulatory effects of different agonists suggest that the facilitatory actions of noradrenaline and serotonin on responses of commissural interneurons reported previously following ionophoretic application are the net outcome of the activation of different subclasses of monoaminergic membrane receptors. As these receptors may be distributed predominantly, or even selectively, at either pre- or postsynaptic sites their differential modulatory actions could be compatible with a presynaptically induced depression and a postsynaptically evoked enhancement of synaptic transmission between reticulospinal neurons and commissural interneurons.

## Introduction

The coordination of activity of muscles on the two sides of the body is vital for the correct and meaningful performance of many motor tasks. In spinal neuronal networks involved in processing information required for this coordination, the importance of commissural interneurons has repeatedly been emphasized (see, e.g., Buchanan, 1982; Grillner & Wallen, 2002; Bannatyne *et al*., 2003; Butt & Kiehn, 2003; Krutki *et al*., 2003; Zhong *et al*., 2006). These neurons have direct excitatory and inhibitory actions on contralaterally located motoneurons and are elements of neuronal networks subserving centrally initiated movements, spinal reflexes and movements depending on intrinsic neuronal circuits (see, e.g., Kiehn, 2006; Jankowska & Stecina, 2007). Modulation of their activity, which could thus strongly affect motor output, was investigated in a number of studies. These have, for instance, demonstrated that descending monoaminergic neurons may strongly modify the activity of commissural interneurons and that at least some of the effects of serotonin (5-HT) and noradrenaline (NA) and their different receptor agonists on locomotion (see Schmidt & Jordan, 2000; Rossignol *et al*., 2001, for recent reviews) might be secondary to their actions on commissural interneurons. The same could be true for patterns of crossed reflexes (Aggelopoulos *et al*., 1996). We recently showed that ionophoretically applied 5-HT and NA have partly similar and partly opposite actions on commissural interneurons located in Rexed lamina VIII. Both potently facilitated responses of these neurons evoked by reticulospinal tract fibres but 5-HT facilitated while NA depressed responses evoked by group II afferents. Considering that responses evoked from different sources, monosynaptically by reticulospinal fibres and oligosynaptically from contralateral group II afferents, were modulated in the same direction, and that immunoreactive monoaminergic terminals were found in contact with intracellularly labelled commissural interneurons (Hammar *et al*., 2004), it appears that monoaminergic receptors located on commissural neurons could play an important part in the differential modulatory effects. Receptors located on the afferent fibres could also be important, especially for the depressive effects of noradrenaline, as the number of contacts immunoreactive for NA on commissural neurons was much smaller than that for 5-HT (Hammar *et al*., 2004).

Differential localization of various subclasses of monoaminergic membrane receptors on commissural interneurons could also be important for the modulatory actions. Some membrane receptors, such as 5-$HT_2$ and $\alpha_1$, seem to be located predominantly on soma or dendrites (Nicoll *et al*., 1990; Nicholas *et al*., 1996; Doly *et al*., 2004), others such as 5-$HT_{1A}$, 5-$HT_3$ and $\alpha_2$ predominantly on axon terminals (Olave & Maxwell, 2002; Maxwell *et al*., 2003; Dougherty *et al*., 2005), while at least the 5-$HT_7$ receptor was reported to be present on primary afferent terminals as well as on cell bodies (Doly *et al*., 2005).

The aim of the present study was to further investigate the differentiated effects of ionophoretically applied NA and 5-HT by comparing effects of a number of agonists to different subclasses of

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NA and 5-HT membrane receptors. The agonists were chosen on the basis of their previously described effects on spinal neurons and their solubility for ionophoretic application.

Some of the preliminary results have been presented previously (Hammar *et al*., 2006).

## Materials and methods

### *Preparation*

Nine deeply anaesthetized cats of both sexes, weighing 2.2–3.4 kg, were used. The animals were bred at the Experimental BioMedicine facility at Göteborg University. The experimental procedures were approved by Göteborg University Ethics Committee and followed NIH and EU guidelines of animal care.

The anaesthesia was induced with sodium pentobarbital (40–44 mg/kg, i.p.) and maintained with intermittent doses of α-chloralose (Rhône-Poulenc Santé, France; doses of 5 mg/kg administered every 1–2 h, up to 50 mg/kg, i.v.). Following the initial surgical preparation the animals were paralysed with pancuronium bromide (Pavulon; Organon, Sweden; ∼0.2 mg/kg/h i.v.) and artificially ventilated during the recording session. Throughout the experiment additional doses of α-chloralose were given if any signs of increased blood pressure or heart rate appeared (both were monitored continuously) or if the pupils dilated. The blood pressure was measured via an intra-arterial line in the carotid artery (also used to infuse a bicarbonate buffer solution with 5% glucose, 1–2 mL/h/kg) and maintained at 100–130 mmHg. The end-tidal concentration of $CO_2$ was kept at ∼4% by adjusting the parameters of artificial ventilation and the body temperature was maintained at 37–38 °C by servo-controlled infrared lamps. At the conclusion of the experiments the animals were given a lethal dose of pentobarbital resulting in an ECG-verified cardiac arrest. Following this the brain was perfused with formalin for subsequent histological verification of the location of the stimulation sites (see below).

The surgical procedures involved a laminectomy exposing the eleventh to thirteenth thoracic (Th11–13) and third to seventh lumbar (L3–7) segments of the spinal cord and dissection of peripheral hindlimb nerves: ipsilateral quadriceps and sartorius and contralateral gastrocnemius–soleus, all mounted in subcutaneous cuff electrodes.

Opening the cranium over the cerebellum allowed the insertion of a tungsten electrode in the ipsilateral medial longitudinal fasciculus (MLF). The electrode (impedance 70–300 KΩ) was introduced at an angle of 30° (with the tip directed rostrally) at Horsley–Clarke co-ordinates P, 8–9; L, 0.8–1.2; and H, −5 mm but the final position of the electrode was determined by the records of descending volleys from the surface of the lateral funiculus at the Th11–13 level. The electrode was left at a location from which distinct descending volleys at a latency of 2.0–2.2 ms were evoked at stimulus thresholds of ≤20 μA. The final position of the tungsten electrode was marked by passing 0.4 mA constant current for 15 s and verified histologically on 100-μm-thick sections of the brain stem cut in the frontal plane using a freezing microtome, and counterstained with cresyl violet (see Fig. 1).

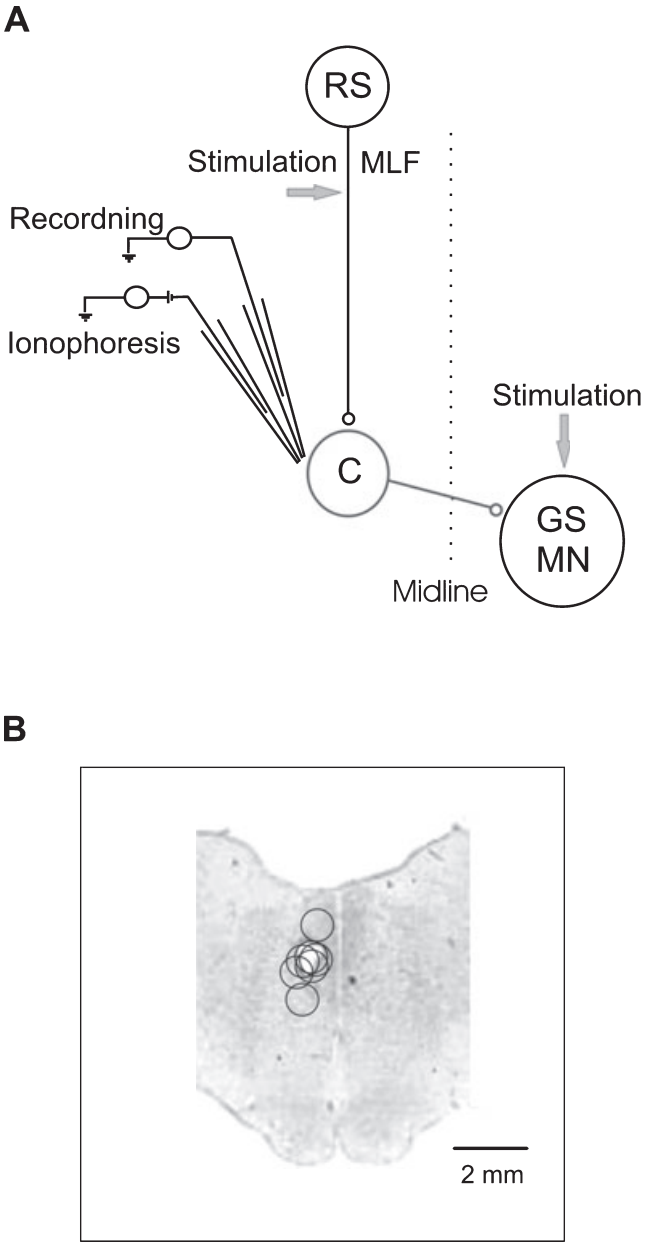


FIG. 1. A diagram of the setup and the locations of stimulation sites in the MLF. (A) A diagram of the experimental setup. The commissural neuron in lamina VIII (marked C) was activated by electrical stimulation of descending reticulospinal tract fibres (RS) within the medial longitudinal fasciculus (MLF). Its projection across the midline to the contralateral gastrocnemius motonucleus (GS MN) was verified by antidromic activation. The two micropipettes used for recording interneuronal responses and for drug ionophoresis were as indicated positioned close to each other and in close proximity to the neuron. (B) Transverse section of the medulla in the plane of the insertion of the electrodes. Circles indicate stimulation sites in the MLF. Scale bar as indicated.

### *Stimulation and recording*

The reticulospinal tract fibres were stimulated in the MLF using constant-current stimuli (0.2 ms, 50–200 μA) delivered via a 0.5-mm thin tungsten wire electrode insulated except for its tip and used as a cathode (see Jankowska *et al*., 2003). Either single or 2–3 stimuli 2.5 ms apart were applied at 3.3 Hz.

The motor nuclei within which axons of selected commissural interneurons terminated were located using a glass micropipette (tip diameter 2 μm) filled with 2 M NaCl. Once they were found, this was replaced with a tungsten electrode which was used to antidromically activate the commissural interneurons (by single pulses, 0.2 ms duration, 6–50 μA, allowing for <0.5 mm spread of current (Gustafsson & Jankowska, 1976).

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The peripheral nerves were stimulated with constant-voltage stimuli (0.2 ms duration). The stimulation intensity is expressed in multiples of threshold, T, for the most sensitive fibres in the nerve as determined by recording the incoming volleys from the cord dorsum. Group II muscle spindle afferents were stimulated at near maximum intensity for fast-conducting group II afferents (3–5 T; Jack, 1978).

Interneurons were recorded from extracellularly using glass micropipettes (tip diameter ~1.5 μm) filled with 2 M NaCl solution. For ionophoresis, a double micromanipulator was employed to which both the micropipette used for recording and a similar sized drug-containing micropipette were attached (see Fig. 1 and below).

### *Sampling*

The neurons were searched for in midlumbar (L4–5) segments of the spinal cord in areas where large field potentials were evoked following stimulation of the MLF, at depths between 2.8 and 4.5 mm from the spinal cord surface. Their commissural projections were identified by antidromic activation from the contralateral gastrocnemius–soleus motor nuclei in the caudal L7 segment, and by a collision between antidromic responses from the motor nuclei and synaptically evoked responses from the MLF (see, e.g., Jankowska *et al*., 2005). All neurons were monosynaptically excited by MLF stimuli (at latencies up to 1 ms from the first component of the reticular formation descending volley). The sample included only neurons with axonal projections within the lumbosacral enlargement and which were not antidromically activated by stimuli applied at the Th12–13 segments by two pairs of silver ball electrodes in contact with the left and right lateral funiculi at an intensity of 0.5–1 mA, duration 0.2 ms (Jankowska *et al*., 2003).

### *Ionophoresis*

The following membrane receptor agonists were used: phenylephrine ($\alpha_1$ receptor; 0.2 M; Sigma), clonidine ($\alpha_2$ receptor; 0.1 M; Sigma), 8-dihydroxy-dipropylaminotetraline (8-OH-DPAT; 5-$HT_{1A/7}$ receptor; 0.15 M; RBI), 2-methyl-5-HT (2-me-5-HT; 5-$HT_3$ receptor; 0.1 M; Sandoz), 5-methoxytryptamine HCl (5-me-T; 5-$HT_2$ receptor, RBI) and α-methyl-5-HT (α-me-5-HT; 5-$HT_2$ receptor; 0.1 M; RBI), all compounds being dissolved in $H_2O$, pH 4.5. They were applied in the close vicinity of single neurons by using a double-headed micromanipulator carrying two separate microdrives for the recording and the drug-containing micropipettes (Engberg *et al*., 1972). The recording micropipette was used when searching for and identifying commissural interneurons with the drug-containing pipette kept above the spinal cord. The latter was only inserted into the spinal cord and placed close to the recording pipette (with the tips of the two pipettes 5–10 μm apart) just prior to commencing the ionophoresis, and it was retracted once ionophoresis was completed (see Jankowska *et al*., 2000; Hammar & Jankowska, 2003). A 10-nA retaining current was used to reduce any possible leakage through the tip of the drug-containing micropipette whilst inserting it into the spinal cord. To ensure that the placement of the pipette carrying the drug did not alter the neuronal responses, control records were taken both before (control) and after (placement) placing the drug-containing pipette. To eject the pharmacological compounds a negative current of 20 nA was passed for a period of up to 3 min, during which the neuronal responses were recorded through the recording micropipette. The shape and amplitude of a small test current pulse applied through the drug-containing pipette were monitored throughout the ionophoresis to ensure that its resistance did not increase excessively, which would indicate blockage. The drug-containing pipette was withdrawn from the spinal cord after the ionophoresis was completed while the recording micropipette remained in position and neuronal responses were recorded for a recovery period of up to 25 min.

### *Analysis*

Series of responses evoked by 20 consecutive single or triple MLF stimuli were sampled every 15 s during ionophoresis and every 5 min during the subsequent recovery phase. The effects of the drug application were evaluated by comparing the number of responses to MLF stimuli obtained before, during and after ionophoresis. As commissural interneurons did not show any resting activity under our experimental conditions and none were activated by peripheral nerve stimulation, effects of the ionophoresed substances were tested only on responses evoked by stimulation of the MLF. Both peristimulus time histograms and cumulative sums (Jankowska *et al*., 1997) were created on-line and stored in parallel with the original data records. The stimulation intensity was adjusted individually for each neuron prior to collecting the control records, initially aiming for a response rate of ~50% which is optimal for detecting both increases and decreases of response probability due to drug effects. Three or four series of control records were taken and the average number of responses in these series was used as the control value. When the effect of a drug was persistently found to be either depressive or facilitatory, the stimulus intensity in the control records was increased or decreased, resulting in a corresponding increase or decrease in the probability of activation of the neurons in order to enable us to reveal even more potent effects. Hence, the average number of control responses was sometimes higher or lower than the initial target of 50%. Furthermore, as only monosynaptic responses were to be evaluated, we only counted responses evoked at latencies up to 1 ms from the first component of the descending volleys (Jankowska *et al*., 2003, 2005; to account for ~0.5-ms latencies of monosynaptic excitatory postsynaptic potentials evoked by reticulospinal fibres and a further delay of ~0.5 ms for the generation of the action potentials) and appearing within a time window of 1 ms from the earliest responses, considering that extracellularly recorded action potentials are usually somewhat asynchronous. The time window was set individually for each neuron. As control experiments in previous studies consistently failed to demonstrate effects of current passed through a micropipette filled with an HCl solution at pH 4.5 in order to exclude direct actions of $H^+$ ions (Bras *et al*., 1989; Hammar *et al*., 2002), these were omitted in the present series.

Data are expressed as means ± SEM. Statistical significance was calculated using the Wilcoxon signed-rank test, using Statview software.

## Results

A total of 57 commissural interneurons with monosynaptic input from reticulospinal tract fibres running in the MLF were analysed in the study. Stimulation of the MLF rather than of one of the nuclei in the mesencephalic or medullary reticular formation was used in order to activate a higher proportion of axons of reticulospinal neurons, and to activate these more synchronously, but similar effects on commissural interneurons have been reported to be evoked by stimuli applied at different locations within the reticular formation (Jankowska *et al*., 2003). Using intracellular records it has previously been demonstrated that interneurons in Rexed lamina VIII with axons crossing the midline constitute two neuronal subpopulations with

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monosynaptic input from either the reticulospinal tract or from group II muscle spindle afferents, and that the proportion of neurons with both these kinds of input is very small (Jankowska *et al*., 2005). In none of the neurons included in the present sample of neurons was any monosynaptic input from muscle afferents disclosed. However, for purposes of ionophoresis, only extracellular responses were recorded and hence weak input from muscle afferents, which would have been detected only with intracellular recordings, might have been missed.

### *Effects of noradrenergic subclass membrane receptor agonists*

Two noradrenergic membrane receptor agonists were chosen, phenylephrine ($\alpha_1$) and clonidine ($\alpha_2$). Both receptor types have been demonstrated to be present in the spinal cord of cats (Giroux *et al*., 1999) and to be involved in the modulation of the activity of other spinal neuronal populations (Hammar *et al*., 2002; Hammar & Jankowska, 2003; Harvey *et al*., 2006b).

### *Effects of phenylephrine ($\alpha_1$)*

The effects of phenylephrine were tested on 11 neurons. As illustrated in Fig. 2A the ionophoresis resulted in a rapid increase in the mean number of responses to 20 stimuli which was already significant after 1 min of ionophoresis (from 6.2 ± 1.2 to 12.7 ± 1.9; $P < 0.01$). In all but one neuron the increase in number of responses was seen after 15 s of ionophoresis (to 11.9 ± 1.8; $P = 0.01$) and remained elevated throughout the period of drug application. The recovery was slow and the number of responses had not decreased 10 min after the termination of the ionophoresis.

### *Effects of clonidine ($\alpha_2$)*

The effects of clonidine were investigated on 11 neurons. As shown in Fig. 2B, they were slower to develop than effects of phenylephrine and only six neurons showed a reduction in the number of responses after 1 min. However, all of the neurons showed a depression after 2 min of ionophoresis (from 14.9 ± 1.1 to 8.2 ± 1.5; $P < 0.01$). Although there was a tendency for the neurons to recover from the depressive effect of the drug this was a slow process and after 10 min the number of responses was still significantly reduced compared to the control level.

### *Effects of serotoninergic subclass membrane receptor agonists*

Four agonists were used in the present series of experiments: 8-OH-DPAT (5-$HT_{1A}$ and 5-$HT_7$ receptors), 2-me-5-HT (5-$HT_3$ receptors) and 5-me-T and α-me-5-HT (both 5-$HT_2$ receptors); all these receptor types are known to be present in the cat spinal cord (Helton *et al*., 1994; Giroux *et al*., 1999) and have previously been shown to mediate modulatory effects of monoamines on other spinal neurons (Hammar *et al*., 2002; Dougherty *et al*., 2005; Harvey *et al*., 2006a). The results are summarized in Fig. 3 and records from individual neurons are shown in Fig. 4.

### *Effects of 5-me-T and α-me-5-HT (5-$HT_2$)*

A total of 12 neurons were investigated with respect to effects of 5-$HT_2$ agonists, eight neurons with 5-me-T and four neurons with α-me-5-HT. Application of 5-me-T resulted in a fast and potent facilitation of responses evoked by MLF stimuli in all investigated neurons (with an increase from 7.0 ± 1.8 to 11.8 ± 1.7 after 1 min; $P < 0.05$). As summarized in Fig. 3A and illustrated in Fig. 4, the facilitatory effect of 5-me-T was short-lasting as all but one neuron

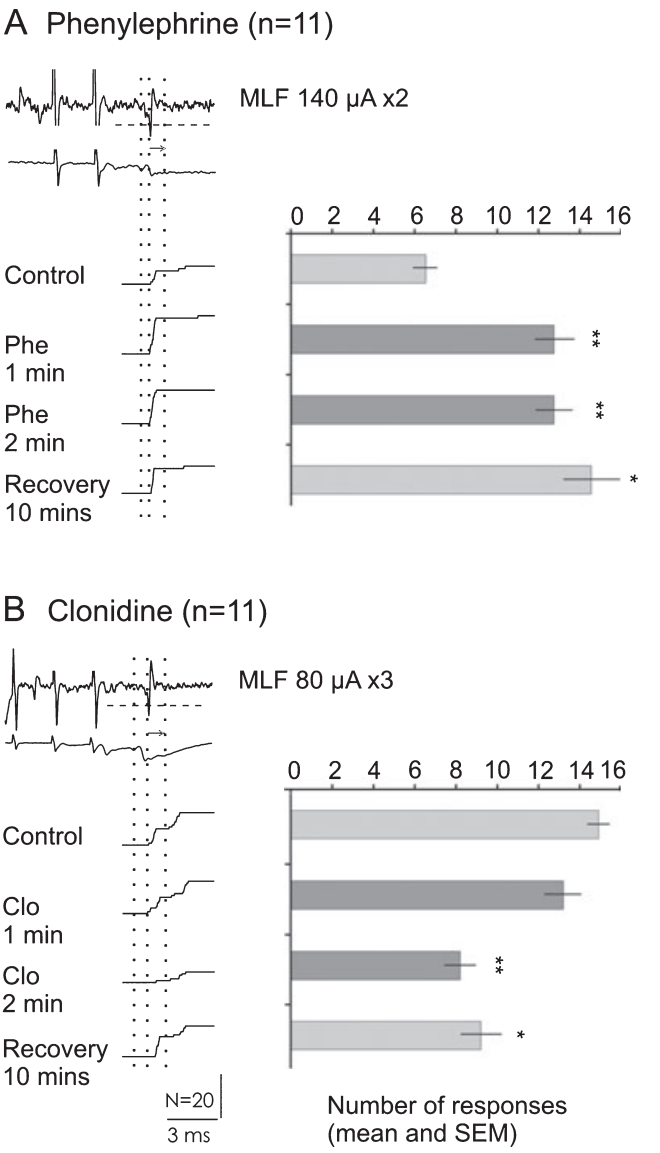


FIG. 2. Opposite effects of $\alpha_1$ and $\alpha_2$ receptor agonists on commissural interneurons monosynaptically activated by reticulospinal tract neurons. (A) Top pair of records are examples of extracellular records from an interneuron monosynaptically activated by reticular formation fibres and from the cord dorsum. Aligned with the interneuronal responses are cumulative sums of responses to 20 stimuli compiled from stimuli delivered before (control), during (1 and 2 min) and after (Recovery) ionophoresis of the $\alpha_1$ receptor agonist phenylephrine. The histograms to the right show the mean number of responses evoked by 20 stimuli (abscissa) in the whole sample of 11 commissural interneurons. Light and dark grey bars indicate the data before and after (light) or during (dark) the ionophoresis. *$P = 0.05$–$0.01$, **$P < 0.01$ with respect to the control. (B) The same format as in A but for an interneuron on which effects of the $\alpha_2$ receptor agonist clonidine were tested. The dotted vertical lines in A and B indicate (1) the onset of the descending volley, (2) the minimal latency in the control records and (3) the end of the time window of 1 ms within which the numbers of spikes were measured. The time window is also indicated by an arrow. The dotted horizontal lines indicate the discrimination levels; only spikes crossing these lines were used for construction of cumulative sums. Time calibration as indicated.

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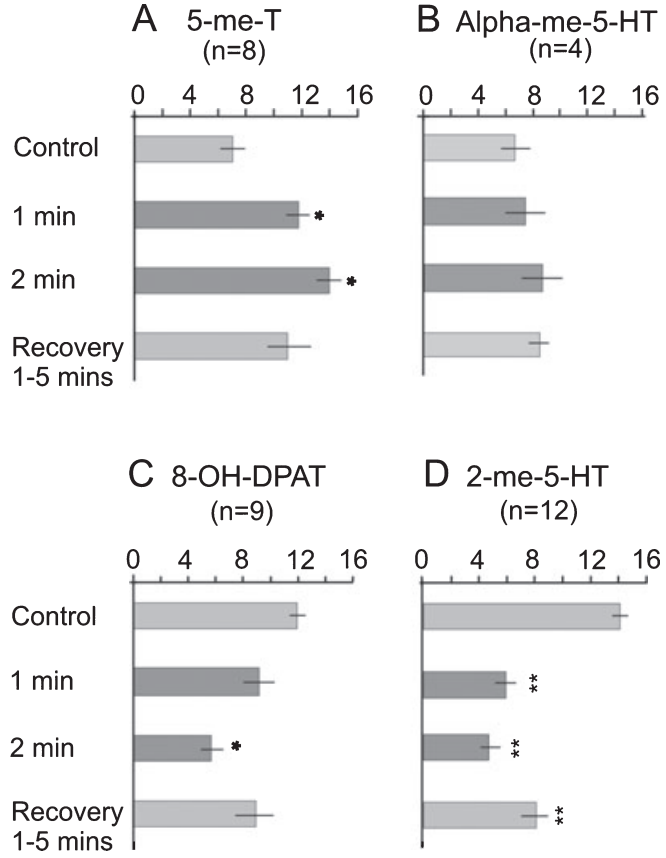


FIG. 3. Effects of 5-HT membrane receptor agonists on commissural interneurons monosynaptically activated by reticulospinal tract neurons. The bars show changes in the number of spikes evoked during a series of 20 consecutive stimuli during ionophoresis of (A) 5-me-T and (B) α-me-5-HT (both 5-$HT_2$ receptor agonists), (C) 8-OH-DPAT (5-$HT_{1A}$ and 5-$HT_7$ receptor agonist) and (D) 2-me-5-HT (5-$HT_3$ receptor agonist). The mean numbers of responses (± SEM) are plotted for periods before (control; light grey), during (1 and 2 min; dark grey) or after (recovery 1–5 min; light grey) ionophoresis at the times indicated. *$P$ = 0.05–0.01, **$P$ < 0.01 compared to control levels.

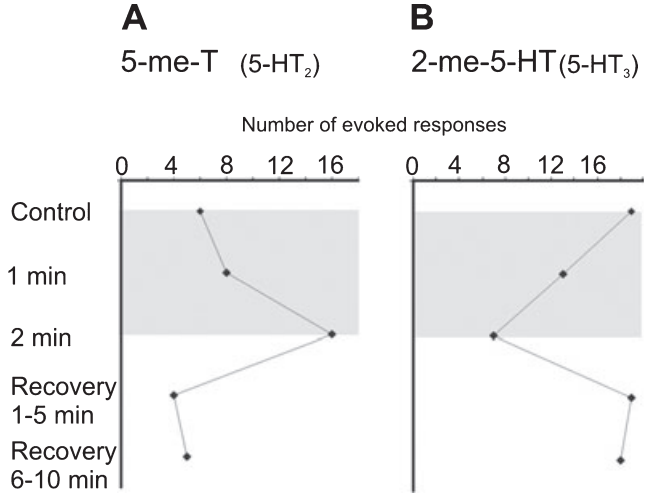


FIG. 4. Time course for the facilitation and depression of two individual neurons following ionophoresis of 5-HT agonists. (A) Time course for the changes in the number of responses evoked by a series of 20 MLF stimuli in a neuron before (control) during (1min, 2 min) and after (recovery) application of the 5-$HT_2$ receptor agonist 5-me-T. The grey area indicates the period of drug ionophoresis. (B) Effect on the number of responses in another neuron before, during and after ionophoresis of the 5-$HT_3$ receptor agonist 2-me-5-HT.

showed a reduction in the number of responses a few minutes after the end of the ionophoresis. In contrast, the effects of α-me-5-HT (see Fig. 3B) were much weaker and the effects on the number of evoked responses were less stable. Although there was an increase in the mean number of responses of three of the four investigated neurons after 2 min (from 8.6 ± 1.1 to 11.7 ± 1.2; not significant) this change was small and slow to develop, and one neuron remained unaffected throughout the ionophoresis.

### *Effects of 8-OH-DPAT (5-$HT_{1A}$, 5-$HT_7$)*

A total of nine neurons were investigated for effects of 8-OH-DPAT. Of these, responses of eight neurons were potently depressed with the number of responses evoked by MLF stimuli reduced from 11.9 ± 1.3 to 5.7 ± 1.9 ($P$ = 0.05) after 2 min of ionophoresis (see Figs 3C and 4). The onset was slow and only in four of the neurons were the responses reduced by >80% after 1 min while for the remaining neurons it took a further minute for the depression to become manifest. The recovery was, in contrast, quite rapid and the number of responses had increased in all but one neuron 5 min after the ionophoresis ended.

### *Effects of 2-me-5-HT (5-$HT_3$)*

The effects of the 5-$HT_3$ receptor agonist 2-me-5-HT were investigated in 12 neurons. The depressive effects on responses during ionophoresis were fast, as shown in Fig. 3D and illustrated with records from a single neuron in Fig. 4. In seven neurons a depression to <80% of the number of responses in the control records was observed after only 15 s of ionophoresis and after 1 min all neurons with the exception of one were clearly depressed (from 14.1 ± 1.1 to 5.9 ± 1.5; $P$ < 0.01). The depression was stable and increased during the continuation of ionophoresis. Following the end of ionophoresis the number of responses slowly increased but 5 min after the end of ionophoresis it was still significantly reduced compared to control values.

## Discussion

Potent modulatory effects of monoamines (serotonin and noradrenaline) on activation of spinal neurons in reflex pathways have previously been demonstrated (Bras *et al*., 1989; Jankowska *et al*., 2000; Hammar *et al*., 2002, 2004; Hammar & Jankowska, 2003; Dougherty *et al*., 2005) and the role of these modulatory effects in reflex pathways and in locomotor networks has been reviewed recently (Schmidt & Jordan, 2000; Rossignol *et al*., 2001). Less attention has been paid to the modulation of transmission from descending tract fibres, in particular reticulospinal tract fibres, which play an important part in a variety of motor reactions, from vestibular and neck reflexes (see Wilson & Peterson, 1978) and centrally initiated locomotion (see Jordan, 1991; Grillner *et al*., 1995; Deliagina *et al*., 2002; Noga *et al*., 2003) to voluntary movements in association with corticospinal neurons (see Lundberg, 1979; Jankowska *et al*., 2006).

The results reported here show that the monoaminergic facilitation of excitation of lamina VIII commissural interneurons by reticulospinal tract neurons (Hammar *et al*., 2004) may be the result of the activation of several different subclasses of monoaminergic membrane receptors. As ionophoretic application of $\alpha_1$ and 5-$HT_2$ receptor agonists resulted in a facilitation of the activation of these interneurons

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while $\alpha_2$ and 5-$HT_{1A}$, 5-$HT_7$ and 5-$HT_3$ receptor agonists potently depressed it, the overall effects of 5-HT and NA might reflect the sum of opposite modulatory effects, facilitation and depression. The results also indicate that several subclasses of monoaminergic membrane receptors may contribute in parallel to these opposite effects.

Considering that both 5-HT and NA membrane receptors have been reported to be located either on spinal neurons or on axon terminals of presynaptic fibres that provide input to them (see Introduction), the reported modulatory effects could have been mediated by receptors distributed on the commissural interneurons or on terminals of reticulospinal tract fibres. The depressive effects of the 5-$HT_3$ receptor agonist 2-me-5-HT on feline dorsal horn interneurons activated by group II afferents (Dougherty *et al*., 2005) were not associated with the demonstration of 5-$HT_3$ membrane receptors on these neurons. On the basis of this finding, as well as the previous demonstration that the 5-$HT_3$ receptors are located on presynaptic axon terminals (Maxwell *et al*., 2003), the depressive effect of 2-me-5-HT was attributed to presynaptic inhibition of transmission from group II muscle afferents in contact with the neurons. By generalizing this conclusion, depressive effects of both 2-me-5-HT and the $\alpha_2$ receptor agonist clonidine on synaptic transmission between reticulospinal neurons and commissural interneurons might therefore be due to presynaptic actions, even though the presence of monoaminergic receptors on descending reticulospinal tract axons has not yet been demonstrated. Indications that the facilitatory effects of 5-$HT_2$ and $\alpha_1$ agonists are due to postsynaptic actions on the interneurons are much stronger in view of the previous evidence for the presence of both noradrenergic and serotoninergic contacts on the soma and dendrites of commissural interneurons (Hammar *et al*., 2004). A presynaptically induced depression together with a postsynaptic enhancement of synaptic actions of reticulospinal neurons on commissural interneurons might thus be an important part of the modulation of the activation of these neurons.

It should be noted that the investigated population of commissural interneurons with monosynaptic input from the reticulospinal tract includes both excitatory glutamatergic and inhibitory glycinergic neurons (Bannatyne *et al*., 2003). No attempts to distinguish between neurons containing either of the two transmitters were made in the present study but it would be unlikely that the sample included neurons of only one kind. Similar effects of the tested agonists on all of the investigated neurons suggest therefore that both inhibitory and excitatory commissural interneurons express the same subset of receptors. Previously investigated actions of serotonin and noradrenaline on a sample of lamina VIII commissural interneurons with input from primary afferents likewise failed to indicate major differences in the modulatory actions on these neurons (Hammar *et al*., 2004), even though these should also include both excitatory and inhibitory neurons (Arya *et al*., 1991; Bajwa *et al*., 1992). However, a change in the balance between actions of excitatory and inhibitory commissural interneurons induced by monoamines could have a major impact on the motor output and on the selection of different neuronal networks (Aggelopoulos *et al*., 1996). How this occurs is still unresolved but differential distribution of various monoamine receptors on soma and dendrites of the interneurons, or presynaptically on terminals of reticulospinal fibres, might allow a more selective modulation of responses of these neurons which in turn would determine the way they respond.

### *Functional consequences at the level of commissural interneurons*

The differential distribution of various subclasses of monoaminergic membrane receptors might play a particularly important role in the case of neurons in which contacts made by primary afferents and descending tract neurons, including reticulospinal neurons and monoaminergic neurons releasing 5-HT or NA, are unevenly distributed, and there is accumulating evidence for this. It has for instance been demonstrated in the case of synaptic contacts formed by various types of peripheral afferents and descending tract fibres at different distances from the soma: see, e.g., Burke & Glenn (1996) for Ia afferents, Weber *et al*. (2007) for unspecific afferents and Lawrence *et al*. (1985) for corticospinal contacts. It may also be reflected in differences in contacts made on dendrites extending in opposite directions (see Savtchenko *et al*., 2001) as well as in differences in the relative concentration of monoamines in various laminae of the spinal cord (Noga *et al*., 2004). Provided that a differential distribution of afferent contacts is coupled to a spatially differential localization of subclasses of monoaminergic membrane receptors with sometimes opposing effects this would allow selective monoaminergic modulation of synaptic transmission directly related to the source of synaptic input, where certain sources of input might be coupled to only certain subclasses of receptors. Furthermore, if 5-HT- and NA-releasing neurons located in different brainstem nuclei and terminating at different sites within the spinal cord are activated under different circumstances, this would enable a highly differentiated and dynamic monoaminergic modulation of the excitability of spinal neurons.

### *Functional consequences for interlimb coordination*

The importance of the differential effects of monoamines evoked by different subclass of membrane receptors on commissural interneurons may be seen in the light of effects of various 5-HT and NA agonists and antagonists on bilateral movements. The effects of monoamines on spinal rhythmic activity involving the two spinal halves have been extensively investigated in neonatal *in vitro* preparations (see, e.g., Cazalets *et al*., 1990, 1992; Kiehn *et al*., 1999; Butt & Kiehn, 2003; Gabbay & Lev-Tov, 2004) as well as in feline or rodent *in vivo* preparations (Jankowska *et al*., 1967; Barbeau & Rossignol, 1990, 1991; Jankowska & Noga, 1990; Schmidt & Jordan, 2000; Guertin & Steuer, 2005) and the importance of monoamines in the normal animal as well as in restoring locomotor-like activity following a spinal transection has been emphasized (Barbeau & Rossignol, 1990; Chau *et al*., 1998; Schmidt & Jordan, 2000; Rossignol *et al*., 2001) although the results were not always the same in different species or at different developmental stages. For instance, while 5-HT applied in the neonatal rodent *in vitro* preparation initiates locomotion (see for example Cazalets *et al*., 1990; Kiehn & Kjaerulff, 1996; Nishimaru *et al*., 2000; Madriaga *et al*., 2004), it fails to do so in the adult acute spinal cat (Barbeau & Rossignol, 1990). Likewise, although the facilitatory actions mediated by 5-$HT_2$ membrane receptors on feline commissural interneurons are in agreement with the rhythm-inducing effects on fictive locomotion mediated by 5-$HT_2$ in the neonatal rodent preparation (Cazalets *et al*., 1992; Madriaga *et al*., 2004; Liu & Jordan, 2005), the strong facilitatory effect of α-me-5-HT was not reproduced. The effects of α-me-5-HT were, on the contrary, slower and weaker than those of 5-me-T. This might reflect a differential subclass membrane receptor affinity (Barnes & Sharp, 1999) of these two substances in the cat but also effects on different kinds of neurons or species-related differences. With respect to any actions evoked by 5-$HT_7$ membrane receptors we used 8-OH-DPAT, which has affinity for 5-$HT_7$ as well as 5-$HT_{1A}$ membrane receptors (see Barnes & Sharp, 1999). As its effects were inhibitory they appear to be opposite to the reported effects on locomotor-like activity by 5-$HT_7$ receptors in neonatal rodents (Madriaga *et al*., 2004; Liu & Jordan, 2005);

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however, the possibility cannot be excluded that any facilitatory effects mediated by 5-$HT_7$ receptors were obscured by a more potent inhibition mediated by 5-$HT_{1A}$ receptors in the feline spinal cord. Such observations might also indicate that different subpopulations of commissural interneurons are differently modulated by various 5-HT-subclass membrane receptor agonists. Our data are for monosynaptically evoked actions of reticulospinal neurons on a particular subpopulation of commissural interneurons while practically all the data derived from neonatal preparations are based on effects on big conglomerates of neurons and often of unspecified origin. The differences in effects might also indicate that initiation of the locomotor-like activity on which they were tested critically depends on subpopulations of neurons other than that of commissural interneurons with input from reticulospinal neurons.

Regarding different reported effects of NA, those evoked via $\alpha_2$ membrane receptors are of particular interest. While the $\alpha_2$ receptor agonist clonidine assists treadmill walking in spinalized cats (Forssberg & Grillner, 1973; Barbeau *et al*., 1987; Barbeau & Rossignol, 1991; Chau *et al*., 1998; Marcoux & Rossignol, 2000), activation of commissural interneurons by reticulospinal neurons has now been found to be depressed by clonidine. This result thus suggests that locomotion induced by clonidine may depend on effects on other neurons than commissural interneurons with monosynaptic input from reticulospinal neurons. Another possibility might be that, as suggested by Barbeau & Norman (2003), a decrease in release of neurotransmitter via $\alpha_2$ receptors present on descending terminals occurs in the intact or partially lesioned preparation but not in chronic spinal preparations in which effects of clonidine are only mediated postsynaptically. If so, in the intact preparation both facilitatory (postsynaptic) and inhibitory (presynaptic) actions of clonidine might be evoked on commissural interneurons but the latter might dominate. The facilitation of activation of commissural interneurons with input from reticulospinal tract neurons by noradrenaline (Hammar *et al*., 2004) suggests in addition that in the preparation with the spinal cord-intact activation of commissural interneurons via reticulospinal neurons (see Jankowska *et al*., 2003; Matsuyama *et al*., 2004) may be primarily mediated by $\alpha_1$ receptors. $\alpha_1$ Receptors might thus be especially important during the initiation of motor tasks requiring bilateral coordination of movements, where the timing of motor activity on either side of the body would be essential. This would apply to any movement requiring bilateral activation via reticulospinal neurons and be related not only to locomotion.

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## Abbreviations

2-me-5-HT, 2-methyl-5-HT; 5-HT, 5-hydroxytryptamine or serotonin; 5-me-T, 5-methoxytryptamine HCl; 8-OH-DPAT, 8-dihydroxy-dipropylaminotetraline; α-me-5-HT, α-methyl-5-HT; MLF, medial longitudinal fasciculus; NA, noradrenaline.

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