4-21-2010

Social Interactions Determine Postural Network Sensitivity to 5-HT

Daniel Cattaert

Jean-Paul Delbecque

Donald H. Edwards
Georgia State University, dedwards@gsu.edu

Fadi A. Issa

Recommended Citation

Cattaert, D., Delbecque, J. P., Edwards, D.H., & Issa, F.A. (2010). Social interactions determine postural network sensitivity to 5-HT. *Journal of Neuroscience, 30*(16), 5603-5616. Available at: http://www.jneurosci.org/content/30/16/5603.full.pdf+html

Behavioral/Systems/Cognitive

Social Interactions Determine Postural Network Sensitivity to 5-HT

Daniel Cattaert,[1] Jean-Paul Delbecque,[1] Donald H. Edwards,[2] and Fadi A. Issa[2]

[1]Université de Bordeaux, Centre National de la Recherche Scientifique, Centre de Neurosciences Intégratives et Cognitives, Biologie Animale, 33405 Talence Cedex, France, and [2]Neuroscience Institute, Georgia State University, Atlanta, Georgia 30302-5030

The excitability of the leg postural circuit and its response to serotonin (5-HT) were studied *in vitro* in thoracic nervous system preparations of dominant and subordinate male crayfishes. We demonstrate that the level of spontaneous tonic activity of depressor and levator motoneurons (MNs) (which control downward and upward movements of the leg, respectively) and the amplitude of their resistance reflex are larger in dominants than in subordinates. Moreover, we show that serotonergic neuromodulation of the postural circuit also depends on social status. Depressor and levator MN tonic firing rates and resistance reflex amplitudes were significantly modified in the presence of 10 μM 5-HT in dominants but not in subordinates. Using intracellular recording from depressor MNs, we show that their input resistance was not significantly different in dominants and subordinates in control conditions. However, 5-HT produced a marked depolarization in dominants and a significantly weaker depolarization in subordinates. Moreover, in the presence of 5-HT, the amplitude of the resistance reflex and the input resistance of MNs increased in dominants and decreased in subordinates. The peak amplitude and the decay phase of unitary EPSPs triggered by sensory spikes were significantly increased by 5-HT in dominants but not in subordinates. These observations suggest that neural networks are more reactive in dominants than in subordinates, and this divergence is even reinforced by 5-HT modulation.

Introduction

Socially dominant and subordinate crayfish differ in a number of behavioral characteristics that result from changes in excitability of specific neural circuits (for a review, see Edwards and Spitzer, 2006). For example, posture is changed during the establishment of social status (Livingstone et al., 1980).

Serotonin (5-HT) has been implicated in the mechanisms that control status-dependent changes in neural circuits (Edwards and Spitzer, 2006). 5-HT was suggested to be involved in the postural control in dominant crayfish, because 5-HT injection caused animals to stand on the tips of their walking legs, with their claws open in front of them (Livingstone et al., 1980; Kravitz, 1988).

In crustacean legs, the coxo-basal chordotonal organ (CBCO) plays an important role in posture because it mediates monosynaptic resistance reflexes in the second leg joint (El Manira et al., 1991). In a previous analysis of the effects of 5-HT on the walking leg postural networks *in vitro*, it was shown that in some communal animals, 5-HT exerted an increase of the resistance reflex amplitude of depressor motoneurons (MNs), which are responsible for downward movements of the leg, and thereby controls the height of animal posture. By contrast, in other animals, no change (or a slight decrease) was observed (Le Bon-Jego et al., 2004). The finding that 5-HT had opposite effects on the reflex responses of different members of the same population was similar to the results of earlier experiments on the serotonergic modulation of the escape circuit mediated by lateral giant (LG) command fibers (Yeh et al., 1996, 1997; Teshiba et al., 2001). Those experiments demonstrated that 5-HT's neuromodulatory effect depended on the crayfish's social status. When applied to the ventral nerve cord of crayfish, 5-HT (50 μM) facilitated the LG response to sensory stimulation in dominant animals and inhibited it in subordinates (Yeh et al., 1996, 1997).

To test whether social status was also responsible for the duality of responses to 5-HT observed in the postural circuit, we applied 10 μM 5-HT on *in vitro* preparations of the walking leg systems dissected out from crayfish of known social status. We studied the properties of the postural network in dominant and subordinate animals, and demonstrated that the level of spontaneous tonic activity and the amplitude of the resistance reflex are larger in dominants than in subordinates. Moreover, we showed that serotonergic neuromodulation of the postural circuit also depends on social status, as in the case of the LG escape circuit (Yeh et al., 1996; Teshiba et al., 2001), thereby generalizing the effects of social status on 5-HT neuromodulation of different motor networks.

Materials and Methods

Experimental animals. Experiments were performed on male adult crayfish form I (*Procambarus clarkii*, $n = 39$) weighing 25–30 g. The animals were obtained locally. Communal animals were maintained indoors in large tanks containing 80–100 animals at 18–20°C and fed once a week

Received Jan. 22, 2010; revised March 6, 2010; accepted March 9, 2010.

This work was supported by Centre National de la Recherche Scientifique (CNRS), Agence Nationale pour la Recherche Grant NT05-1_41718 (to D.C.), and National Science Foundation Research Grants 0135162 and 0641326 (to D.H.E.). We thank Mr. Damien Perrot for his participation in pairing animals and data analysis.

Correspondence should be addressed to Daniel Cattaert, Université de Bordeaux, CNRS, Centre de Neurosciences Intégratives et Cognitives, Biologie Animale, Bâtiment B2, 33405 Talence Cedex, France. E-mail: d.cattaert@cnic.u-bordeaux.fr.

DOI:10.1523/JNEUROSCI.0367-10.2010

 0270-6474/10/305603-14$15.00/0

with shrimp pellets and carrots. Animals were maintained on a 12:12 h light:dark cycle.

Pair formation. Before pairing, crayfishes were isolated in individual aquaria (23 cm long × 15 cm wide × 17 cm high) for at least 2 weeks. Water was changed twice weekly and an air stone was used to provide constant aeration of water in the aquarium. After the isolation period, size-matched male crayfishes were paired in a testing aquarium (same size as the isolated aquaria). The animals were free to interact at all times during a 10 d pairing period. Two shelters were provided for each pair after the first 6 h of interaction. A digital camcorder (Panasonic) recorded the agonistic interactions (attacks, approaches, defensive and offensive tail flips, and retreats) for each pair during the first hour of interaction. Animal posture was also analyzed and an instantaneous score was calculated (0 = lowest, 5 = highest totally extended legs). This instantaneous postural score was then integrated over the observation time to obtain a posture index. For the remaining 9 d, the pairs were observed for 30 min in the morning (crayfish peak activity time) and the number and type of aggressive and submissive behaviors were noted. Dominance between two animals was determined based on the fraction of aggressive and submissive behavior each animal performed as described previously (Issa et al., 1999). Typically, after the 10 d pairing period, a crayfish was labeled "dominant" when it regularly presented a significantly larger number of approaches and attacks than the other, which was labeled "subordinate." Conversely, the subordinate animal presented a significantly larger number of retreats and defensive tail flips than the dominant. During the 30 min of observation after the hierarchy was formed, the number of retreats and tail flips of the dominant and the number of approaches and attacks of the subordinate were both close to zero. Moreover, when the two animals faced each other, the dominant adopted a high posture, while the subordinate displayed a lower posture. In the present report, we only used pairs in which the dominant systematically won all the fights during the last days of pairing. Only intermolt animals (not engaged in the molting process) that presented such clear social status differences (dominants and subordinates) were used in this study.

In vitro *preparation.* An *in vitro* preparation of the thoracic nervous system was used (Sillar and Skorupski, 1986; El Manira et al., 1991). Before dissection, each animal was chilled in ice water for 30 min. Then it was decapitated and the thorax and abdomen were pinned dorsal side up. A section of the ventral nerve cord containing the last three thoracic (T3–T5) and the first abdominal (A1) ganglia was dissected out with all the nerves of the two proximal segments of the left fifth leg (Fig. 1*A*). The coxo-basipodite chordotonal organ (CBCO), which monitors the movements of the second joint (coxo-basipodite), was also dissected out and kept intact. The distal end of its elastic strand was attached to an electromagnetic puller VT101 (Ling Dynamic Systems) controlled by a homemade function generator that allowed the application of sine-wave movements to the CBCO strand to mimic upward (during stretch) and downward (during release) movements of the leg.

The preparation was pinned dorsal side up on a Sylgard-lined Petri dish (Dow Corning). The nervous system was continuously superfused with oxygenated control saline containing the following (in mM): 195 NaCl, 5 KCl, 13 $CaCl_2$, 2 $MgCl_2$, and 3 HEPES (Sigma Chemical) with a pH of 7.65. The fourth and fifth ganglia were desheathed to improve the superfusion of the central neurons and to allow for intracellular recordings (Fig. 1*A*,*B*). In some experiments, a high divalent cation solution containing 34 mM $CaCl_2$ and 6.4 mM $MgCl_2$, with the sodium concentration reduced accordingly to preserve the osmolarity of the solution, was used to raise the spiking threshold of the interneurons.

5-HT application. The CNS was insulated from the CBCO by a Vaseline wall (Fig. 1*A*) to restrict the superfusion of 5-HT to the ganglia. At the same time, the CBCO was superfused with control saline. In the Vaseline well, the level of saline was kept as low as possible to allow fast change of saline. 5-HT was stepped to full concentration as rapidly as possible (within 1 min). Estimations of the rate of change were made by replacing 5-HT solutions with salt solutions and measuring conductance.

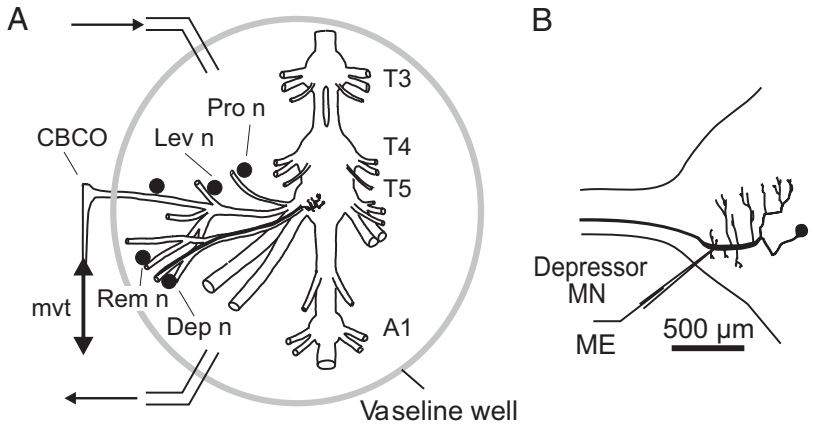

Figure 1. *In vitro* preparation for the study of the resistance reflex of the second leg joint of the crayfish. ***A***, The *in vitro* preparation of the crayfish thoracic locomotor system consists of thoracic ganglia 3–5 (T3, T4, T5) and the first abdominal ganglion (A1) dissected out together with motor nerves of the proximal muscles (Pro n, promotor; Rem n, remotor; Lev n, levator; Dep n, depressor) and the CBCO, a proprioceptor that encodes the vertical movements of the leg. A mechanical puller allowed us to mimic the vertical movements (mvt) of the leg by stretching and releasing the CBCO strand. The CNS was isolated from the CBCO by a Vaseline wall to superfuse only the ganglia with 5-HT (10 μM). Single or multiple intracellular recordings from motoneurons were performed within the neuropile with glass microelectrodes (ME). ***B***, Disposition of the glass microelectrode used for intracellular recording from depressor MNs. The recording microelectrode was placed in the main neurite.

The animal was chilled in ice water before and during the dissection. Therefore, *in vitro* preparations of the thoracic nervous system generally did not display spontaneous activity in the minutes following the dissection. However, after 20–40 min rest in oxygenated saline at 17°C, spontaneous activity generally recovered. In all experiments, nerve recordings were performed after 1 h of rest.

Recordings. Extracellular recordings from the motor nerves innervating the depressor levator, promotor, and remotor muscles and from the sensory nerve of the CBCO were made using stainless steel pin electrodes contacting the nerves and insulated with Vaseline (Fig. 1*A*). Intracellular recordings from depressor MNs (Fig. 1*B*) were performed with glass micropipettes (Clark Electromedical Instruments) filled with 3 M KCl (resistance, 10–20 MΩ) and connected to an Axoclamp 2B amplifier (Molecular Devices) used in the current-clamp mode. In crustacea, the somata of MNs lie outside of the neuropile (the region in which neurons form their synaptic contacts) (Fig. 1*B*) and are linked to the arbor of the neuron by a thin neurite, and so do not participate in the electrical activity of the neuron. For these reasons, intracellular recordings were made from the main neurite where EPSPs could be recorded (Fig. 1*B*).

Depressor MNs were identified following the procedure described in Hill and Cattaert (2008). The resting membrane potential of MNs was usually in the range of −78 to −65 mV. Stability of resting membrane potential over a long period of time (>4 h) was used as a criterion for evaluation of cell health during recordings. In crustacean MNs, soma and neurites do not actively convey spikes. Therefore, spike amplitude was generally small (<20 mV) at the recording site. Data were digitized and stored onto a computer hard disk through an appropriate interface (1401plus) and software (Spike2) from Cambridge Electronic Design.

Data analysis. Data were analyzed using the Spike2 analysis software. Spikes recorded from the CBCO nerve were discriminated according to their waveform based on a template matching protocol (wavemark). Templates were built automatically and corresponded to the mean duration of sensory spikes (~1.5 ms in duration). The sampling rate for CBCO nerve recording was set to 15 kHz, which resulted in templates containing 20–22 points. The procedure used two criteria to identify a spike: (1) >90% of the points should be in the confidence limits of the

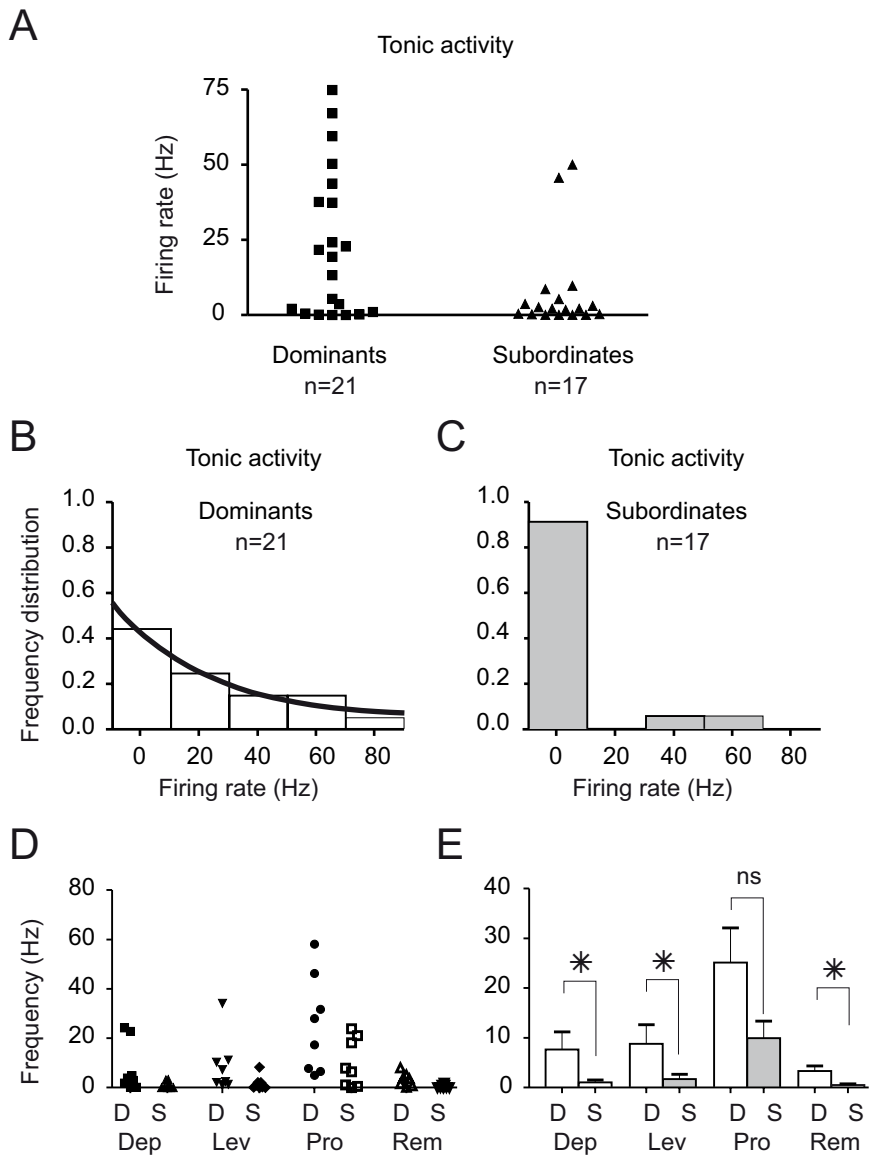

Figure 2. Tonic activity recorded *in vitro* from the depressor nerve of dominant and subordinate crayfishes. ***A***, Individual firing rates calculated from depressor global nerve activity from 21 dominant- and 17 subordinate-animal preparations. ***B***, ***C***, Frequency distribution of the depressor nerve firing rate in dominant-animal preparations (***B***) and subordinate-animal preparations (***C***). ***D***, ***E***, Individual firing rates of depressor (Dep), levator (Lev), promotor (Pro), and remotor (Rem) motor nerves from eight dominant (D) and eight subordinate (S) animal preparations (***D***) and statistical analysis of firing rates calculated over all dominants and all subordinates for depressor, levator, promotor, and remotor motor nerves (***E***). *$p < 0.05$, Mann–Whitney test. Vertical bars represent SEM. ns, Not significant.

template; and (2) the maximum amplitude change for a match was <5%. This procedure was applied off-line. After the completion of this protocol, each identified CBCO unit (spike shape) was assigned an arbitrary number. Subsequently, a spike-triggered average was performed for each CBCO unit, allowing us to observe in a given MN the occurrence of any postsynaptic events related to this unit. Statistical analyses were done with Prism (Graphpad Software). The results are given as mean values ± SEM.

Results

The level of tonic activity of leg MNs depends on the animal's social status

We performed systematic recordings of the depressor nerve activity in *in vitro* preparations from dominant and subordinate animals throughout the experiment. The total activity recorded from the depressor nerve was measured. This spontaneous activity was distributed among the 21 preparations from dominant animals (Fig. 2*A*, left). One was silent, four displayed a low level (<1 Hz) of tonic activity, and the other 16 preparations displayed spontaneous activity with firing rates ranging from 3 to 74.9 Hz. Among these, 10 were purely tonic, five displayed a tonic activity with slow weak rhythmic modulation (period 20–40 s), and one was slowly rhythmic (period 20–40 s). In all cases, the mean rate of activity was calculated over a period of 5 min to avoid problems of measurements due to pseudorhythmic activities. By contrast, in subordinate animals, the level of spontaneous activity was significantly lower (Fig. 2*A*, right) (Mann–Whitney test, $p = 0.019$), with four preparations silent, three displaying a low level (<1 Hz) of tonic activity, and nine displaying a moderate level of activity in the range 1–10 Hz. Only two preparations displayed tonic activity in the range 45–50 Hz. Only one subordinate-animal preparation displayed spontaneous rhythmic activity. These data show that spontaneous activity of the depressor nerve in subordinate and dominant-animal preparations differed significantly in their spontaneous firing rates and in the frequency distribution of firing rates (Fig. 2*B*,*C*). In the dominant-animal preparations, firing rates decreased monotonically from 0 to 80 Hz, following a decreasing exponential curve ($R^2 = 0.97$) (Fig. 2*B*), whereas in subordinate preparations, 10% displayed firing rates >20 Hz and 90% presented a low firing rate (<10 Hz) (Fig. 2*C*).

To test whether the effect of animal's social status was specific to depressor MN activity or not, the same analysis was also done in experiments in which the activity of proximal motor nerves to promotor, remotor, levator, and depressor muscles (Fig. 1) was recorded in the *in vitro* preparation (Fig. 2*D*,*E* and Table 1). In the 16 experiments analyzed (eight dominant and eight subordinate animals), the results confirm that the tonic activity of the depressor nerve in dominant animals (7.66 ± 3.51 spikes/s, mean ± SEM; $n = 8$) was significantly higher (Mann–Whitney test, $p = 0.0103$) than in subordinate animals (1.02 ± 0.51 spikes/s, mean ± SEM; $n = 8$). Moreover, in dominant animals, the tonic activities of the levator nerve (8.77 ± 3.89), promotor nerve (21.1 ± 6.95), and remotor nerve (3.30 ± 1.04) were also higher than in corresponding nerves in subordinate animals (levator, 1.71 ± 0.99; promotor, 9.91 ± 3.45; and remotor, 0.51 ± 0.25). The levator/remotor difference was significant (Mann–Whitney test, $p = 0.0148$ and 0.014, respectively), but the promoter/remoter difference, while close, failed to reach significance (Mann–Whitney test, $p = 0.0524$) (Fig. 2*E*). This lack of significance is likely due to the fact that promotor discharge was generally higher than in other motor nerves, with a larger variability both in dominant

Table 1. Statistical analysis of spontaneous activity (in hertz) in depressor, levator, promotor, and remotor nerves in dominant and subordinate crayfishes

	Dep		Lev		Pro		Rem	
	Dom	Sub	Dom	Sub	Dom	Sub	Dom	Sub
Mean	7.66	1.02	8.77	1.71	25.1	9.91	3.30	0.51
SE	3.51	0.61	3.89	0.99	6.95	3.45	1.04	0.25
No. of nerves	8	8	8	8	8	8	7	8
p	0.010		0.015		0.052		0.014	

For each motor nerve, the *p* value of the Mann–Whitney test indicates a significant difference between dominant and subordinate animals. Dep, Depressor; Lev, levator; Pro, promotor; Rem, remotor; Dom, dominant; Sub, subordinate.

(SEM = 6.95) and in subordinate animals (SEM = 3.45) (Fig. 2*D*). Moreover, like the depressor nerve activity over the 21 experiments (Fig. 2*A*), the variation in the frequencies of tonic promoter activity was also larger in the eight dominant animals than in the eight subordinate animals (Fig. 2*D*).

The large variation in the tonic firing rates of the depressor motor nerve in dominant animals could have been related to their social experience. To test this hypothesis, we quantified several behavior variables of dominant crayfish (rate of approaches, rate of attacks, fight duration, and posture index) and calculated their correlation with the corresponding *in vitro* firing rate of depressor MNs. No correlation was found between the *in vitro* depressor firing rate and the number of approaches and attacks per minute, the fight duration, or the posture index.

The effect of 5-HT on tonic activity of depressor MNs is social status dependent

When applied to *in vitro* preparations from dominant animals, 5-HT (10 μM) increased the depressor nerve firing rate to a plateau level within 4–5 min of perfusion (Fig. 3*A*). 5-HT exposure was maintained for 10 min, after which the preparation was washed with saline. However, the level of tonic firing remained largely above the control value for up to 40 min, gradually decreasing to reach a level generally lower than control after 50 min of wash. By comparison, when 10 μM 5-HT was applied to preparations from subordinate animals, the tonic discharge also increased in some preparations, but with two differences. First, no sustained plateau of activity was observed during the wash; the level of activity decreased as soon as 5-HT was washed from the bath (Fig. 3*B1*). Second, even after 1 h wash, the tonic activity was still above the control value and generally never went back to its control value even after 2 h wash. Note that in preparations that did not display

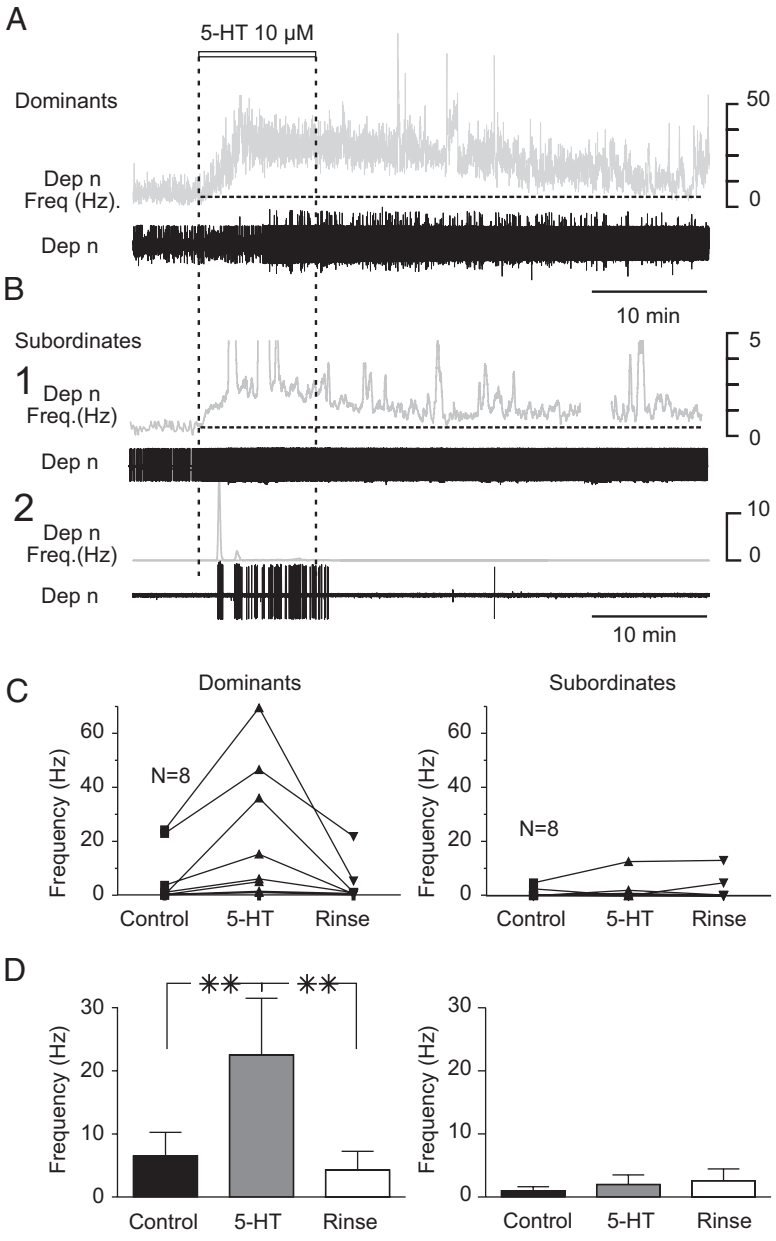

Figure 3. Effect of 10 μM 5-HT on depressor nerve tonic activity. ***A***, ***B***, Raw data of depressor nerve recording during 5-HT application (horizontal bar) in preparations from dominant (***A***) and subordinate (***B***) animals. The frequency (Freq) of the global depressor nerve (Dep n) activity (gray) is presented above each depressor nerve recording (black). Two recordings from subordinate-animal preparations are presented: one with a low level of tonic activity (***B1***) and one totally silent before 5-HT application (***B2***). ***C***, Mean firing rates of the depressor nerve measured from eight dominant animals (left) and eight subordinate animals (right) in control situation after 10 min of 5-HT and after a 50 min rinse. ***D***, Statistical analysis of the data presented in ***C***. Vertical bars represent SEM. $^{**}p < 0.01$.

Table 2. Statistical analysis of effect of 5-HT on spontaneous tonic activity (in hertz) in depressor, levator, promotor, and remotor nerves in dominant and subordinate crayfishes

	Dep		Lev		Pro		Rem	
	Control	5-HT	Control	5-HT	Control	5-HT	Control	5-HT
Dominant animals								
Mean	7.66	21.15	8.77	22.61	25.1	48.54	3.30	20.26
SE	3.51	8.77	3.89	8.06	6.95	17.54	1.04	9.67
No. of nerves	8	8	8	8	8	8	7	8
p	0.0078		0.0391		0.0039		0.0078	
Subordinate animals								
Mean	1.02	1.98	1.71	1.97	9.91	9.85	0.51	1.13
SE	0.61	1.53	0.99	0.71	3.45	3.59	0.25	0.83
No. of nerves	8	8	8	8	8	8	8	8
p	0.37		0.31		0.50		0.31	

For each motor nerve, the *p* value of the Wilcoxon signed rank test indicates a significant difference between control and 5-HT conditions. Dep, Depressor; Lev, levator; Pro, promotor; Rem, remotor; Dom, dominant; Sub, subordinate.

any spontaneous depressor activity (Fig. 3*B2*), the application of 10 μM 5-HT could result in a very low-frequency depressor activity (<0.2 Hz) limited to the duration of 5-HT application (Fig. 3*B2*). The results of all experiments performed from dominant ($n = 8$) and subordinate ($n = 8$) animals are presented in Figure 3*C* and Table 2. The application of 5-HT systematically produced a significant increase in the tonic discharge firing rate in dominant-animal preparations (Fig. 3*C*, left), whereas in subordinate-animal preparations, the effects of 5-HT were variable (Fig. 3*C*, right). 5-HT application produced small activity increases in some subordinate preparations, had no effect in others, and suppressed 3.15 Hz tonic activity in one preparation. Spontaneous activity was restored in that preparation after 30 min wash. Statistical analysis (Fig. 3*D*) confirmed that 5-HT (10 μM) elicited a significant increase of the depressor tonic discharge (Wilcoxon matched pairs test, $n = 8$, $p = 0.0039$) in dominant-animal preparations (Fig. 3*D*, left). This effect was reversed within 1 h after rinse (Wilcoxon matched pairs test, $n = 8$, $p = 0.0078$). By contrast, no significant increase of the depressor tonic discharge (Wilcoxon matched pairs test, $n = 8$, $p > 0.05$) was induced by 10 μM 5-HT in subordinate-animal preparations (Fig. 3*D*, right).

The effect of 5-HT on the depressor tonic activity (Fig. 3) was also observed in the other motor nerves analyzed (Table 2). In dominant animals, 5-HT induced a significant increase in promotor (from 25.10 ± 6.95 Hz to 48.54 ± 17.54 Hz, $p = 0.0039$, Wilcoxon matched pairs test), remotor (from 3.30 ± 1.04 Hz to 20.26 ± 9.67 Hz, $p = 0.0078$), levator (from 8.77 ± 3.89 Hz to 22.61 ± 8.06 Hz, $p = 0.039$), as well as depressor (from 7.66 ± 3.51 Hz to 21.15 ± 8.77 Hz, $p = 0.039$) motor nerves. By contrast, in subordinate animals, 5-HT did not induce any significant change in these motor nerves (Table 2).

The efficacy of the resistance reflex depends on social status

The CBCO strand is stretched during opening of the coxo-basal joint and released during closure, which corresponds to downward and upward movements of the leg, respectively. Among the 40 sensory neurons innervating the CBCO, 20 stretch-sensitive neurons code for leg depression and 20 release-sensitive neurons code for leg levitation. Because the axons of these two groups of CBCO neurons travel in the CBCO nerve, the application of sine-wave movements to the CBCO strand induced a sensory discharge during both stretch and release movements (Fig. 4*A*,*B*). This sensory information is conveyed to the ganglion where stretch-sensitive neurons directly excite levator MNs and release-sensitive neurons directly excite depressor MNs (El Manira et al., 1991) These monosynaptic connections are responsible for the resistance reflex. In this study, we have analyzed the effects of 5-HT and social status on this reflex.

Sinusoidal movements were applied to the CBCO strand, the activity of the depressor nerve was recorded, and the global firing rate (multiple units) of the depressor nerve activity was then calculated. The sinusoidal movement of the CBCO strand resulted in a resistance reflex response recorded in the depressor nerve (Fig. 4*A1*); each time the strand was released (corresponding to upward movement of the leg in an intact animal), the depressor nerve firing increased. In control situations, dominant-animal preparations displayed a significantly higher depressor nerve firing rate during sine-wave movements applied to the CBCO strand than subordinate-animal preparations (Mann–Whitney, $p = 0.046$). The resistance reflex response was obvious in dominant-animal preparations, which had significantly higher firing rates during release movements than during stretch movements (Fig. 4*A1*, histogram). In three of eight subordinate-animal preparations, the reflex response was not visible in the absence of 5-HT (Fig. 4*B1*). In addition, the frequency distribution of the resistance reflex firing rates was different in dominants and subordinates. In dominant-animal preparations, reflex firing rates ranged from 0.24 Hz to 115.56 Hz, with a linear decrease of occurrences from 46.2% of low (<15 Hz) firing rates to 8% of high (>105 Hz) firing rates. By contrast, in subordinate-animal preparations, 83% of the reflex firing rates were low (<15 Hz).

The effect of 5-HT on the efficacy of the resistance reflex is social status dependent

We found that 5-HT (10 μM) increased the resistance reflex in dominant-animal preparations (Fig. 4*A*). The mean firing rate of depressor nerve activity calculated over the whole movement cycle increased from 2.46 Hz in the control situation (Fig. 4*A1*) to 11.17 Hz (354% increase) 20 min after the beginning of perfusion of 10 μM 5-HT (Fig. 4*A2*). This increased reflex response was not due to an increase in the CBCO sensory activity, which stayed fairly consistent throughout the experiment. Although the CBCO discharge was slightly variable (Fig. 4*A1*,*A2*, CBCO nerve raw data recording and CBCO mean frequency), the number of spikes per movement cycle did not increase significantly (1192 ± 6.0 in control condition, 1212 ± 7.1 in the presence of 5-HT, $n =$ 10 cycles, $p > 0.05$, *t* test). In subordinate-animal preparations, application of 10 μM 5-HT increased the depressor reflex activity much less than in dominant-animal preparations. For example, the mean firing rate of depressor nerve activity increased from 3.49 Hz in the control situation (Fig. 4*B1*) to 5.01 Hz after 10 min

in the presence of 10 μM 5-HT, mainly because of increases in the resistance reflex activity (Fig. 4*B2*, large amplitude spikes).

A grouped analysis of the two factors (5-HT treatment and social status) indicates that the effect of 5-HT on resistance reflex response depends on social status [two-way ANOVA, $F = 14.35$, degrees of freedom (DF) = 13, $p = 0.0023$]. Separate analyses of 5-HT's effects on the responses of dominants and subordinates show that the effect of social status results from a 5-HT-induced increase in the reflex responses of dominants and no 5-HT-induced change in the reflex responses of subordinates. 5-HT (10 μM) consistently evoked a significant increase of the mean firing rate of the depressor reflex discharge in dominant-animal preparations over all experiments (Wilcoxon matched pairs test, $p = 0.0078$, $n = 7$) (Fig. 5*A*). Although 5-HT evoked an obvious increase of the reflex response of some subordinate-animal preparations (Fig. 4*B*), no significant change was observed over the entire set of subordinates (Wilcoxon matched pairs test, $p > 0.05$, $n = 7$) (Fig. 5*A*).

A similar analysis was made for the levator motor nerve resistance reflex discharge (Fig. 5*B*). A grouped analysis indicates that the effect of 5-HT on the levator resistance reflex response depends on social status (two-way ANOVA, $F = 5.90$, DFd = 14, $p = 0.029$). Here again, 5-HT (10 μM) consistently evoked a significant increase of the mean firing rate of the levator reflex discharge in dominant-animal preparations over all experiments (Wilcoxon matched pairs test, $p = 0.019$, $n = 8$), whereas in subordinate-animal preparations, no significant change was observed (Wilcoxon matched pairs test, $p > 0.05$, $n = 8$) (Fig. 5*B*).

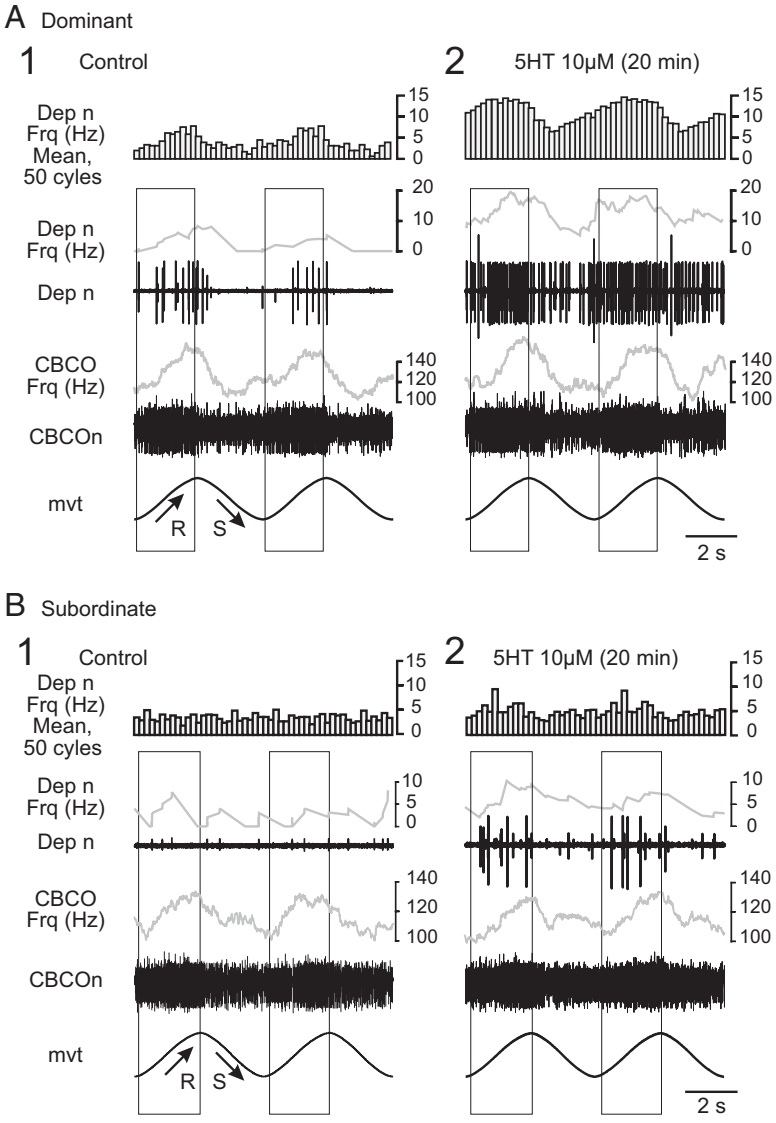

Figure 4. Effect of 10 μM 5-HT on the resistance reflex recorded from the depressor nerve. Raw data of the CBCO nerve (CBCOn) and the depressor nerve (Dep n) and the sine-wave movement (mvt) applied to the CBCO. The floating mean frequency (1 s) of the discharge is presented above each recording (gray lines). A histogram representing the distribution of the mean reflex discharge frequency (Frq) on two movement periods calculated over 50 cycles is presented on top. ***A***, Dominant-animal preparation in control situation (***A1***) and after 10 min in the presence of 10 μM 5-HT (***A2***). ***B***, Same as ***A*** in a subordinate-animal preparation.

These results indicate that social status affects the sensorymotor network involved in posture control and locomotion. Tonic firing rates are higher in dominants than subordinates, and 5-HT increased that activity in dominants but not in subordinates. Finally, 5-HT increased resistance reflex responses in dominant but not in subordinate preparations. In the next part of the study, we analyzed the mechanisms that were responsible for the differential effects of 5-HT in dominant- and subordinate-animal preparations.

The effects of 5-HT on depressor MN membrane potentials depend on social status

In a series of experiments performed in dominant- and subordinate-animal preparations, depressor MNs were intracellularly recorded in control conditions and during application of 10 μM 5-HT (Fig. 6). In two of eight dominant-animal preparations, depressor MNs were spontaneously firing in the control condition (Fig. 6*A1*). In these preparations, 5-HT induced a slow tonic depolarization starting ~1 min after application of 5-HT and reached a maximum of 2–4 mV in <5 min (Fig. 6*A1*). In the intracellularly recorded depressor MN presented in Figure 6*A1*, the depolarization reached 7.8 mV after 4 min in the presence of 10 μM 5-HT. Its tonic discharge firing rate increased from 0.14 Hz to 7.74 Hz after 4 min in the presence of 10 μM 5-HT. These results are in accordance with the increase of firing frequency observed in the depressor motor nerve (Fig. 3). After 1 h wash, the firing rate of the spontaneous tonic activity was back to its control value (data not shown). The membrane potential of depressor MNs in control situations in most of the depressor MNs was in the range of −78 to −72 mV (except for two spontaneously active depressor MNs in which it was ~−60 mV). To compare the effect of 5-HT on membrane potential of these depressor MNs, we adjusted their membrane potential to −78 mV in the control condition by injection of a continuous constant current (Fig. 6*A2*,*B2*). In these conditions, the application of 10 μM 5-HT induced a slow tonic depolarization in all depressor MNs re-

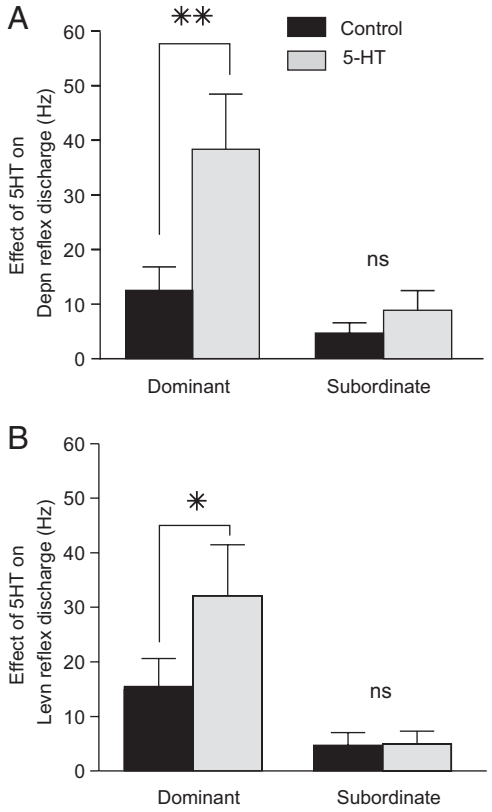

Figure 5. Statistical analysis of the effect of 5-HT on the resistance reflex recorded from the depressor and levator motor nerves. ***A***, Comparison of the effects of 10 μM 5-HT on depressor firing rate in dominant (left) and subordinate (right) animal preparations in control situation (black bars) and after 10 min in the presence of 10 μM 5-HT (gray bars). ***B***, Comparison of the effects of 10 μM 5-HT on levator firing rate in dominant (left) and subordinate (right) animal preparations in control situation (black bars) and after 10 min in the presence of 10 μM 5-HT (gray bars). ns, Not significant. *$p < 0.05$; **$p < 0.01$.

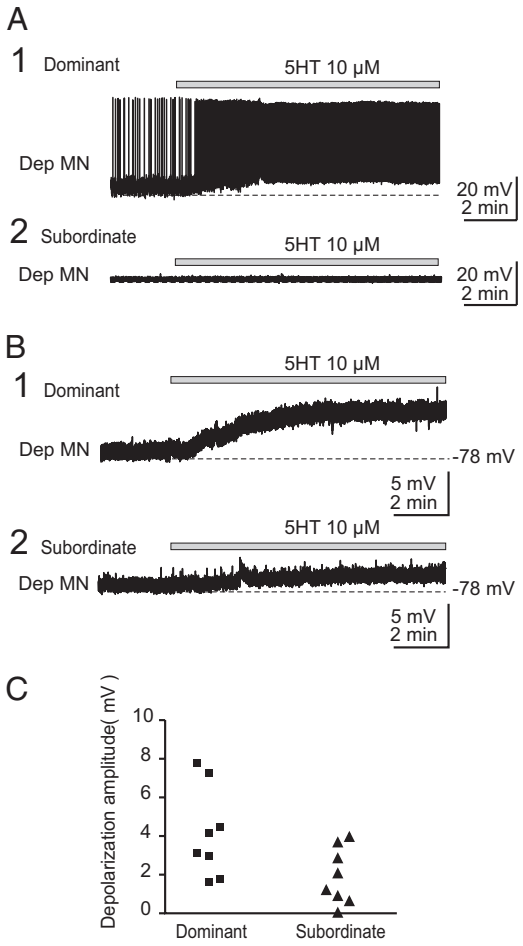

Figure 6. Effect of 10 μM 5-HT on the membrane potential of intracellularly recorded depressor MNs. ***A***, ***B***, Raw data of intracellular recordings from depressor (Dep) MNs during application of 10 μM 5-HT (horizontal bar) in dominant-animal preparations (***A1***, ***B1***) and in subordinate-animal preparations (***A2***, ***B2***). ***A***, Free-run membrane potential. ***B***, In other depressor MNs, the membrane potential was adjusted to −78 mV by negative-current injection before 5-HT application. ***C***, Effects of 5-HT on the membrane potential of depressor MNs from eight dominant-animal preparations (left) and eight subordinate-animal preparations (right).

corded from eight dominant-animal preparations and in seven of eight subordinate-animal preparations with the same kinetics, as in the example shown in Figure 6*A*. The amplitude of 5-HT-induced depolarization was, however, generally smaller in subordinate-animal preparations (in three recordings the amplitude of the depolarization was <1 mV) (Fig. 6*B*,*C*). The average depolarization induced by 10 μM 5-HT was 4.14 ± 0.82 mV (mean ± SEM, $n = 8$) in dominant-animal preparations and only 1.9 ± 0.52 mV (mean ± SEM, $n = 8$) in subordinate-animal preparations. This difference was significant (Mann–Whitney test, $p = 0.019$).

The effects of 5-HT on depressor MNs reflex response is social status dependent

When sine-wave movements were applied to the CBCO strand, most depressor MNs displayed a resistance reflex response consisting of a depolarization of membrane potential during the release movement and a repolarization during the stretch movement (Le Ray and Cattaert, 1997; Hill and Cattaert, 2008). The amplitude of reflex responses induced in depressor MNs was measured in dominant- and subordinate-animal preparations (Fig. 7). In control conditions, the amplitude of the resistance reflex response was generally larger in dominant-animal preparations (0.72 ± 0.41 mV; mean ± SEM, $n = 5$) (Fig. 7*A*) than in subordinate-animal preparations (0.34 ± 0.12 mV; mean ± SEM, $n = 5$) (Fig. 7*B*). Nevertheless, over all experiments, this difference was not significant (Mann–Whitney, $p > 0.05$). However, the effects of 5-HT on the amplitude of the resistance reflex response was opposite in dominant and subordinate preparations. For example, in the experiments reported in Figure 7*A*,*B*, 5-HT induced an increase of the resistance reflex response amplitude from 2.53 mV to 3.05 mV in the dominant-animal preparation (Fig. 7*A*), whereas it induced a decrease of the resistance reflex response from 0.56 mV to 0.36 mV in the subordinate-animal preparation (Fig. 7*B*). The effect of 5-HT was generally almost maximal after 4 min in the presence of 5-HT, and the evolution of the reflex response in dominant- and subordinate-animal preparations were mirror images (Fig. 7, compare *C* and *D*). Note that, in the dominant-animal preparation, the variability (SEM) of the reflex response decreased from 0.156 mV in the control situation to 0.079 in the presence of 5-HT (Fig. 7*C*). By contrast, in subordinate animals, the variability of the response (SEM = 0.032 mV) did not change significantly during the application of 5-HT (SEM = 0.038 mV) (Fig. 7*D*, error bars). Similar results were obtained in six dominant and five subordinate-animal preparations (Fig. 7*E*). Note that in subordinate-animal preparations, tonic depolarization of MNs and decrease of reflex response amplitude counteract each other and would be respon-

sible for the nonsignificant effect of 5-HT on the reflex discharges recorded from motor nerves (Fig. 5). Although the mean effect of 5-HT on reflex discharge is not significant, the balance between 5-HT-induced depolarization and reflex response amplitude would be positive enough to reach the threshold for spikes in some subordinate-animal experiments (Fig. 4*B2*).

The effects of 5-HT on input resistance of depressor MNs is social status dependent

As was shown in communal animals, an increase of the resistance reflex response amplitude by 10 μM 5-HT in depressor MNs can be achieved by an increase of their input resistance (Le Bon-Jego et al., 2004). Therefore, here we tested this hypothesis by measuring the input resistance of depressor MNs in a control situation and in the presence of 10 μM 5-HT in eight dominant- and six subordinate-animal preparations (Fig. 8). In the presence of 10 μM 5-HT, the input resistance of depressor MNs significantly increased in dominant-animal preparations (Wilcoxon matched pairs test, $p = 0.03$, $n = 6$) from 4.04 ± 0.79 to 7.90 ± 3.2 MΩ (79.2% increase) (Fig. 8*A*). By contrast, in subordinate-animal preparations, the input resistance showed a tendency to decrease from 4.24 ± 0.74 (control) to 2.69 ± 0.43 MΩ (in the presence of 5-HT). However, this tendency was not significant (Wilcoxon matched pairs test, $p > 0.05$, $n = 6$). Over the six experiments performed in subordinate-animal preparations in which the input resistance of depressor MNs was measured, the application of 5-HT decreased it ($n = 4$) (Fig. 8*B*) or did not change it significantly ($n = 2$). At the same time, we measured the time constant of depressor MNs during the recovery after injecting a −1 nA hyperpolarizing current pulse (Fig. 8*A*,*B*). The curves were fitted with one exponential decay time function (Fig. 8*C*,*D*). These measurements agreed with the observations on input resistance. In dominant-animal preparations, the time constant increased in the presence of 10 μM 5-HT [from 18.69 to 31.78 ms in the recording of the depressor MN (Fig. 8*C*)], whereas in subordinate-animal preparations, the time constant decreased in four of the six depressor MNs analyzed (as did the input resistance). An example of a 5-HT-induced decrease of time constant of a depressor MN in a subordinate animal is presented in Figure 8*B* [from 17.8 to 14.5 ms in the recording of the depressor MN (Fig. 8*D*)]. It is important to note that the input resistances of dominant (4.04 ± 0.79 MΩ, $n = 8$) and subordinate (4.24 ± 0.74 MΩ, $n = 5$) depressor MNs measured in control conditions were not significantly different (Mann–Whitney test, $p > 0.05$), but their sensitivities to 10 μM 5-HT were significantly different (Fig. 8*E*). The input resistance of depressor MNs of dominant-animal preparations ($n = 6$) increased by 79.27% after 10 min in the presence of 10 μM 5-HT, and increased further to 109.21% above control after a 20 min rinse (Fig. 8*E*). By contrast, the input resistance of depressor MNs of subordinate-animal preparations ($n = 5$) decreased by 19.12% after 10 min in the presence of 10 μM 5-HT but recovered and even increased slightly (17.47%) after 20 min rinse (Fig. 8*E*). Statistical analysis (two-way ANOVA) of these data indicates that the effect of 5-HT is extremely dependent on social status ($F = 49.09$, $p < 0.0001$). After 10 min in the presence of 5-HT and after a 20 min rinse, the changes in input resistance were significantly different in dominants and subordinates [Bonferroni's post-test, $p < 0.001$ (Fig. 8*E*)]. These observations were confirmed by the statistical analysis (two-way ANOVA) of the effect of 10 μM 5-HT on membrane time constant in dominant and subordinate groups (Fig. 8*F*). The effect of social status on the differential sensitivity of membrane time constant to 5-HT was extremely significant ($F = 34.30$, $p < 0.0001$). After 10 min in the presence of 5-HT and after a 20 min rinse, the changes in time constant were signifi-

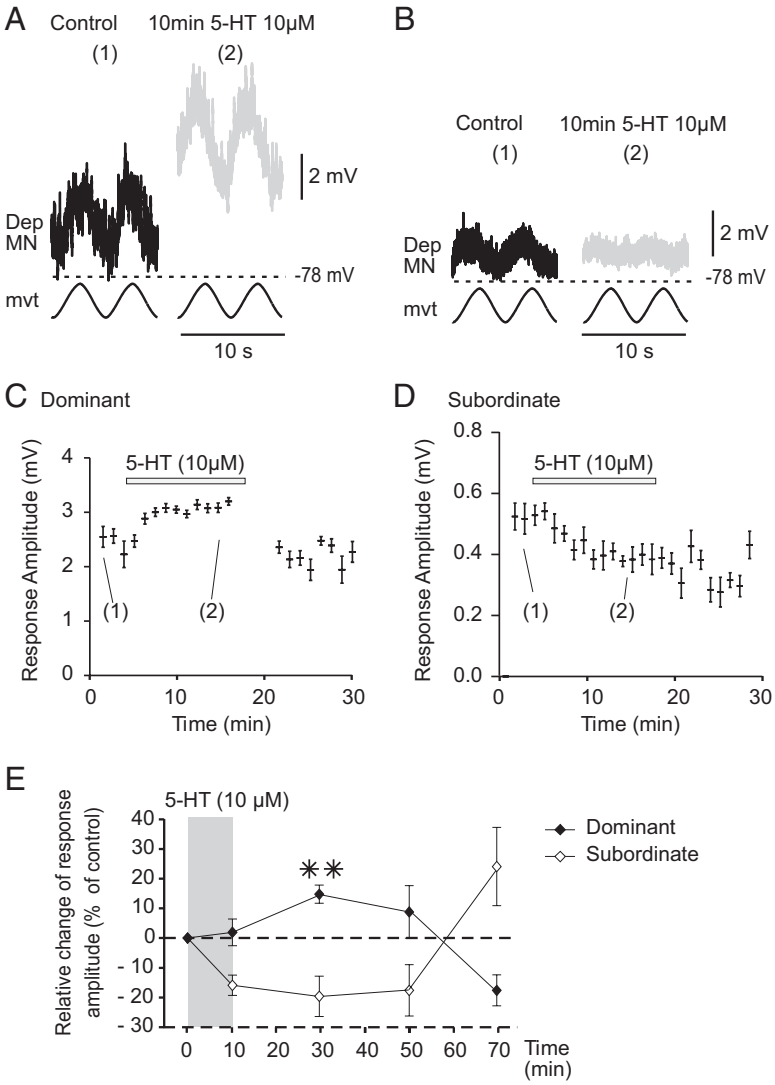

Figure 7. Effect of 10 μM 5-HT on the amplitude of the resistance reflex response of intracellularly recorded depressor MNs. ***A***, ***B***, Raw data of intracellular recordings from depressor (Dep) MNs during sine-wave movements (mvt) applied to the CBCO in control condition (black) and after 10 min in the presence of 10 μM 5-HT (gray) in a dominant (***A***) and in a subordinate (***B***) animal preparation. ***C***, ***D***, Time course of the effect of 10 μM 5-HT on the amplitude of the resistance reflex response in a dominant (***C***) and in a subordinate (***D***) animal preparation (each vertical bar represents the mean ± SEM calculated over 10 movement cycles). ***E***, Statistical analysis of the time course of the effects of 10 μM 5-HT on the amplitude of the resistance reflex response in dominants ($n = 5$) and subordinate ($n = 5$).

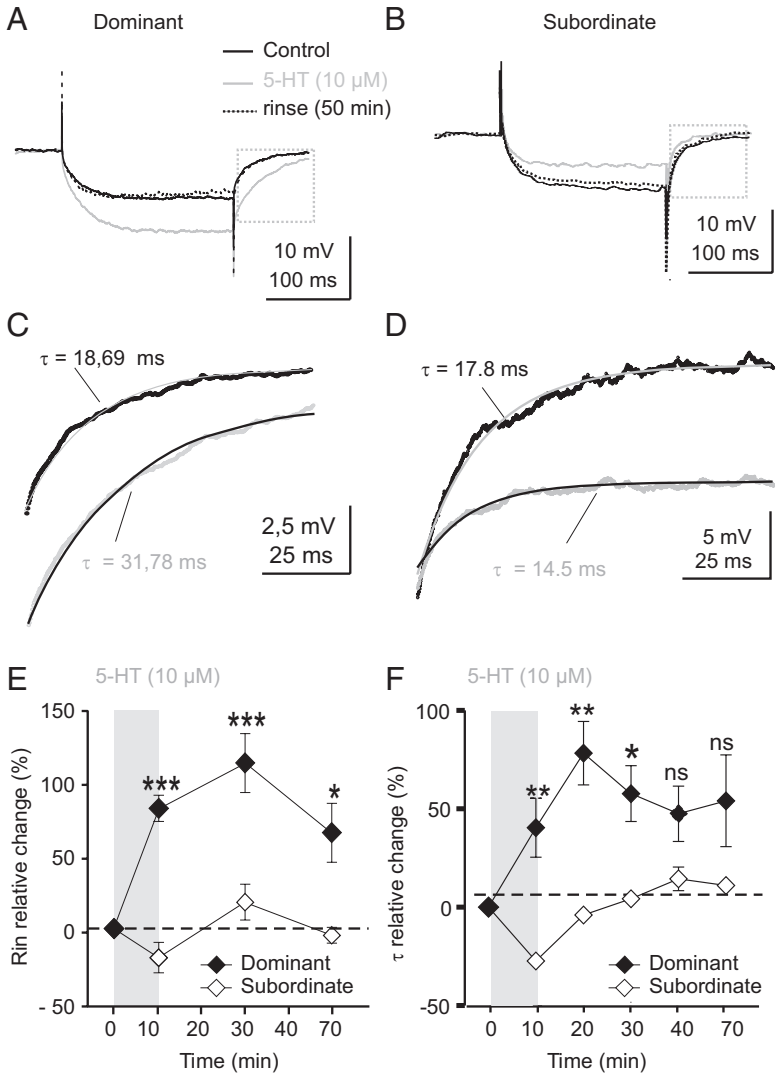

Figure 8. Effect of 10 μM 5-HT on the input resistance and membrane time constant of intracellularly recorded depressor MNs. ***A***, ***B***, Raw data of intracellular recordings from depressor during application of −1 nA current pulse in a dominant (***A***) and in a subordinate (***B***) animal preparation. Black trace, Control recording; gray trace, after 10 min in the presence of 10 μM 5-HT; dotted line, after 50 min rinse. ***C***, ***D***, Procedure to calculate the membrane time constant in control (black trace) and after 10 min in the presence of 10 μM 5-HT (gray trace). The traces taken from the recovery after −1 nA current pulse injection (see rectangles in ***A*** and ***B***) are fitted with one exponential decay curves. ***C***, Dominant-animal preparation; ***D***, subordinate-animal preparation. ***E***, Statistical analysis of the time course of the effects of 10 μM 5-HT on the input resistance of depressor MNs from dominants (◆ , $n = 5$) and subordinates (◇ , $n = 5$). ***F***, Same disposition for the membrane time constant. ns, Not significant. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

cantly different in dominants and subordinates [Bonferroni's post-test, $p < 0.01$ (Fig. 8*F*)].

The shape of EPSPs depends on social status

The shape of EPSPS were different in dominant- and subordinate-animal preparations (compare Figs. 9*A* and 11*A*). In control situations, the mean peak amplitude of EPSPs was 0.56 ± 0.069 mV over all unitary EPSPs ($n = 39$) recorded from depressor MNs in dominant-animal preparations and 0.29 ± 0.019 mV over all unitary EPSPs ($n = 28$) recorded from depressor MNs in subordinate-animal preparations. This difference was significant ($p = 0.002$, unpaired t test). Moreover, the decay phase was shorter in subordinates than in dominants. However, a direct fit to an exponential was not possible because the EPSP decay phase of dominants contained polysynaptic events (Le Bon-Jego et al., 2004), which did not allow an estimation of the decay time. To quantify the evolution of this late part of the EPSPs, the amplitude was measured 15 ms after the EPSP peak (Fig. 9*A*, vertical arrows). This late decay-phase amplitude of dominant animals (0.16 ± 0.02 mV, $n = 39$) was significantly larger ($p < 0.0001$, unpaired t test) than that of subordinate animals (0.04 ± 0.01, $n = 28$). This observation may have important functional consequences concerning the capabilities of EPSP summation in the resistance reflex response, which would be much smaller in subordinates (short decay time) than in dominants (large decay time). This is likely the reason why three of eight subordinate-animal preparations did not display any resistance reflex responses in the depressor motor nerve (Fig. 4*B1*).

The effects of 5-HT on synaptic transmission from CBCO terminals to depressor MNs is social status dependent

The social status-dependent effect of 10 μM 5-HT on the amplitude of the resistance reflex response recorded in depressor MNs was further analyzed by studying the evolution of unitary sensory-motor PSPs in depressor MNs. These measurements were done in the absence of movement applied to the CBCO strand to avoid summation effects altering the shape of individual EPSPs (Le Bon-Jego et al., 2004). During the entire experiment, each of the spikes recorded from the CBCO nerve were classified according to their shape, using the spike sorting analysis program provided by Spike2 software. Then each spike shape was used to perform spike trigger averaging of intracellularly recorded depressor MNs to identify unitary EPSPs and IPSPs in control situation, during application of 10 μM 5-HT, and during wash. The results of this analysis is presented in Figures 9 and 10 for EPSPs and IPSPs, respectively, recorded from depressor MNs of dominant-animal preparations, and in Figures 11 and 12 for EPSPs and IPSPs recorded from depressor MNs of subordinate-animal preparations.

In dominant-animal preparations in the presence of 5-HT (10 min), the sensory EPSPs shapes displayed two changes illustrated in the recording of the seven unitary EPSPs recorded from a given depressor MN (Fig. 9). First, the peak amplitude of some EPSPs increased (Fig. 9*A*). This increase continued after a 50 min wash (Fig. 9*A*) and was significant (Mann–Whitney test, $p < 0.0001$ for units 5, 6, 9, 16, 17; $p < 0.05$ for unit 15) for six of the seven unitary EPSPs (Fig. 9*B*). For the remaining unitary EPSP (unit 1), a nonsignificant increase was observed ($p > 0.05$, Mann–Whitney test) (Fig. 9*B*). The average peak amplitude over all seven unitary EPSPs was significantly increased ($p = 0.0021$, paired t test) (Fig. 9*B*). Second, the decay time of some EPSPs increased (Fig. 9*A*), likely due to the presence of polysynaptic EPSPs. Note that, as was the case for the peak amplitude, the decay time also

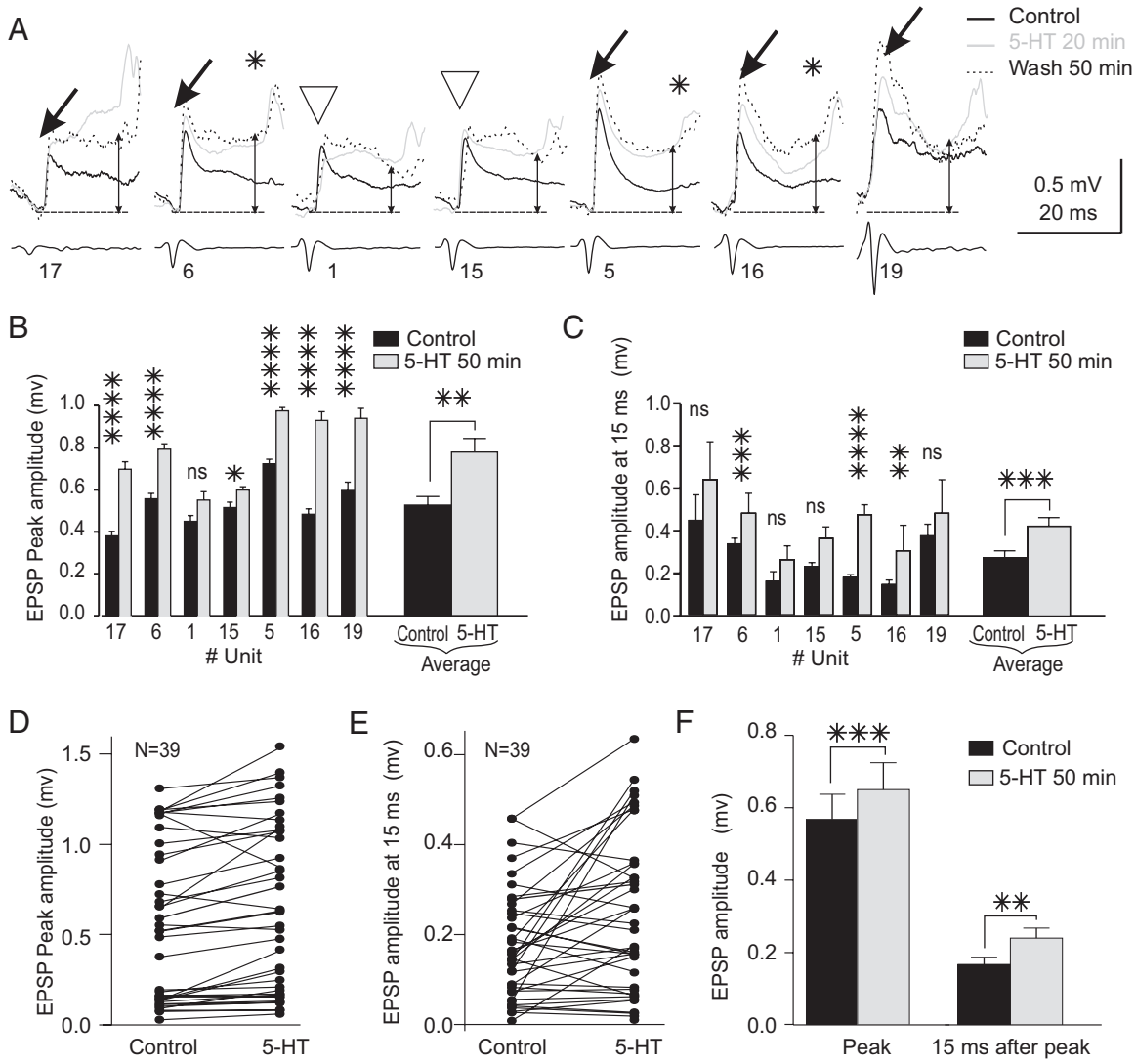

Figure 9. Effect of 10 μM 5-HT on the shape of EPSPs recorded from depressor MNs in dominant-animal preparations. ***A***, Seven EPSPs were obtained in the same intracellularly recorded depressor MN by spike-trigger averaging from templates of CBCO spikes (see Materials and Methods) in control situation (black trace), 20 min after 10 μM 5-HT application (gray trace), and after a 50 min wash (dotted line). Each corresponding presynaptic sensory spike is presented below each EPSP trace. In 5-HT condition, asterisks indicate marked increase in the shape of the EPSP repolarizing phase, arrows indicate marked increase in the peak amplitude of EPSPs, and open triangles indicate an absence of effect on the peak amplitude of the EPSP. ***B***, Statistical analysis of the peak amplitude of each EPSP in control condition (black bars) and after a 50 min wash post-5-HT application (gray bars). The two wider bars on the right represent the mean peak amplitude of the seven EPSPs. ***C***, Same disposition as in ***B*** for the amplitude of the EPSP measured 15 ms after the peak. ***A***, ***B***, and ***C*** are from the same experiment. In a subordinate-animal preparation, five EPSPs were identified from this depressor MN (same disposition as in ***A***). ***D***, ***E***, Over all experiments, effects of 5-HT on EPSP peak amplitude ($n = 39$ EPSPs) (***D***) and on EPSP amplitude measured 15 min after the peak ($n = 39$ EPSPs) (***E***). ***F***, Statistical analysis comparing the means using paired *t* test. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; ****$p < 0.0001$.

continued to increase even after a 50 min wash. To quantify the evolution of this late part of the EPSPs, the amplitude was measured 15 ms after the EPSP peak (Fig. 9*A*). These late-decay-phase EPSPs significantly increased (Mann–Whitney test, $p < 0.0001$, $p < 0.001$, and $p < 0.01$) in three of the seven unitary EPSPs (Fig. 9*C*). The average increase of the amplitude over the seven unitary EPSPs was nevertheless significant ($p = 0.0008$, paired *t* test) (Fig. 9*C*). This analysis was repeated in four experiments (a total of 39 EPSPs were analyzed). The effects of 5-HT on peak amplitude (Fig. 9*D*) and late decay phase (15 ms after EPSP peak) (Fig. 9*E*) were measured after a 50 min wash and compared with control values. The peak amplitude of 34 of 39 EPSPs was increased by 5-HT (Fig. 9*D*), and the late decay-phase amplitude was also increased by 5-HT in 22 of 39 EPSPs (Fig. 9*E*). The average increase of the peak amplitude was highly significant ($p = 0.0003$, paired *t* test) (Fig. 9*F*), as was the average increase of the late decay phase ($p = 0.0015$, paired *t* test) (Fig. 9*F*), although this effect was more variable among individual unitary EPSPs (Fig. 9*E*). In the presence of a high-divalent cation solution, the effect of 10 μM 5-HT on peak EPSPs did not change significantly in dominant-animal preparations, but the slope of the decay time was decreased to the control value and the polysynaptic components of unitary EPSPs were suppressed (data not shown). This result indicates that, in dominant animals, the increased duration of EPSP decay phase was essentially due to polysynaptic pathways activated by 5-HT. These results are very similar to previous findings in communal animals (Le Bon-Jego et al., 2004).

In addition, the peak amplitude of unitary sensory IPSPs followed a very similar change in dominant animals: their amplitude increased in the presence of 5-HT and during wash (Fig. 10*A*). In the experiment presented in Figure 10*A–C*, the peak amplitude increase was highly significant for the four identified

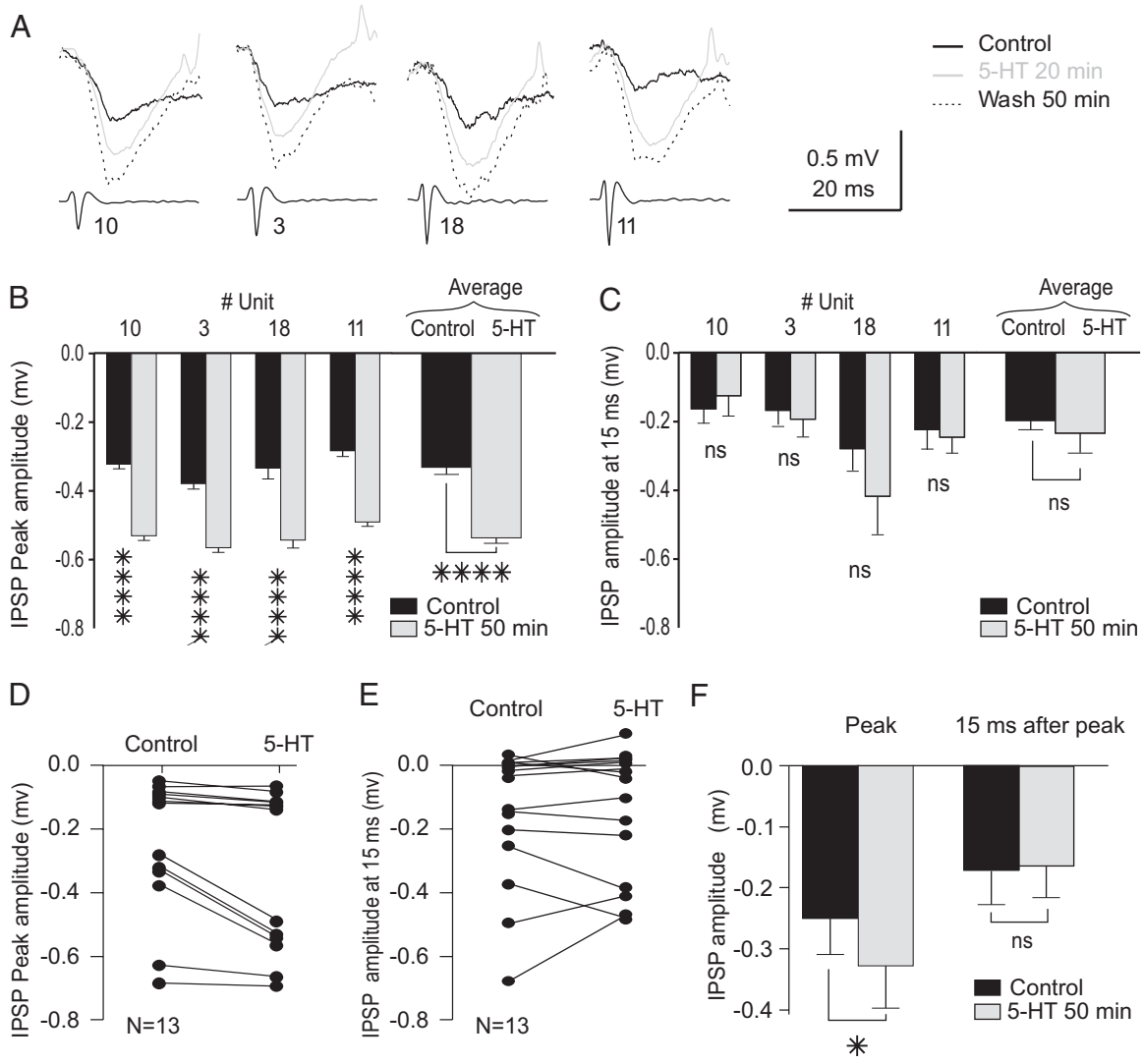

Figure 10. Effect of 10 μM 5-HT on the shape of IPSPs recorded from depressor MNs in dominant-animal preparations. Same disposition as in Figure 9.

IPSPs ($p < 0.0001$, Mann–Whitney test), as was the average increase of these four units ($p < 0.0001$, paired t test) (Fig. 10*B*). However, unlike for EPSPs, the decay phase of individual unitary IPSPs was not significantly modified by 5-HT ($p > 0.05$, Mann–Whitney test), nor was the average over all IPSPs ($p > 0.05$, paired t test) (Fig. 10*C*). IPSPs were identified in three experiments. The peak amplitude of 12 of these 13 unitary IPSPs was increased by 5-HT (Fig. 10*D*), whereas the late decay phase was decreased for eight of 13 unitary IPSPs and increased for the remaining five unitary IPSPs (Fig. 10*E*). The average increase of the peak amplitude over all IPSPs was significant ($p = 0.0188$, paired t test), whereas the absence of significant effect on the decay phase was confirmed over the 13 identified IPSPs ($p > 0.05$, paired t test) (Fig. 10*F*, right).

By contrast, in subordinate-animal preparations, very few changes were observed. A typical example of unitary EPSPs identified in a depressor MN in a subordinate-animal preparation is presented in Figure 11*A–C*. Among the eight unitary EPSPs identified in this experiment, seven presented no significant change in peak amplitude after 5-HT application ($p > 0.05$; Mann–Whitney test) and one presented a significant decrease (unit 15; $p = 0.0188$, Mann–Whitney test) (Fig. 11*B*). The average effect of 5-HT on the peak amplitude over the eight units was not significant ($p > 0.05$, paired t test) (Fig. 11*B*). The EPSP decay phase was not significantly changed by 5-HT ($p > 0.05$, Mann–Whitney test) (Fig. 11*C*) in seven of the eight unitary EPSPs identified in this experiment. One unitary EPSP (unit 3) presented a significant decrease ($p < 0.05$, Mann–Whitney test) (Fig. 11*C*). The average effect of 5-HT over the eight unitary EPSPs was not significant ($p > 0.05$, paired t test) (Fig. 11*C*).

All unitary EPSPs identified in depressor MNs from subordinate-animal preparations were similarly analyzed. The peak amplitude of 15 of the 28 EPSPs was increased after 5-HT application (Fig. 11*D*). However, the average effect over all EPSPs increase was not significant ($p > 0.05$, paired t test) (Fig. 11*F*). Similarly, the late decay-phase amplitude was not significantly modified by 5-HT ($p > 0.05$, paired t test) (Fig. 11*F*), reflecting the fact that the late decay-phase amplitudes were very variable among individual unitary EPSPs (Fig. 11*E*). The reduction of the decay phase was observed in the largest EPSPs recorded from MNs in subordinate-animal preparations (Fig. 11*E*). This result could explain why the amplitude of the reflex response is smaller in these MNs (Fig. 7*B*,*D*,*E*), if we consider that these EPSPs with long decay phase contributed most to the reflex response amplitude.

Very few IPSPs were identified in the depressor MNs from subordinate-animal preparations. Only four IPSPs were identi-

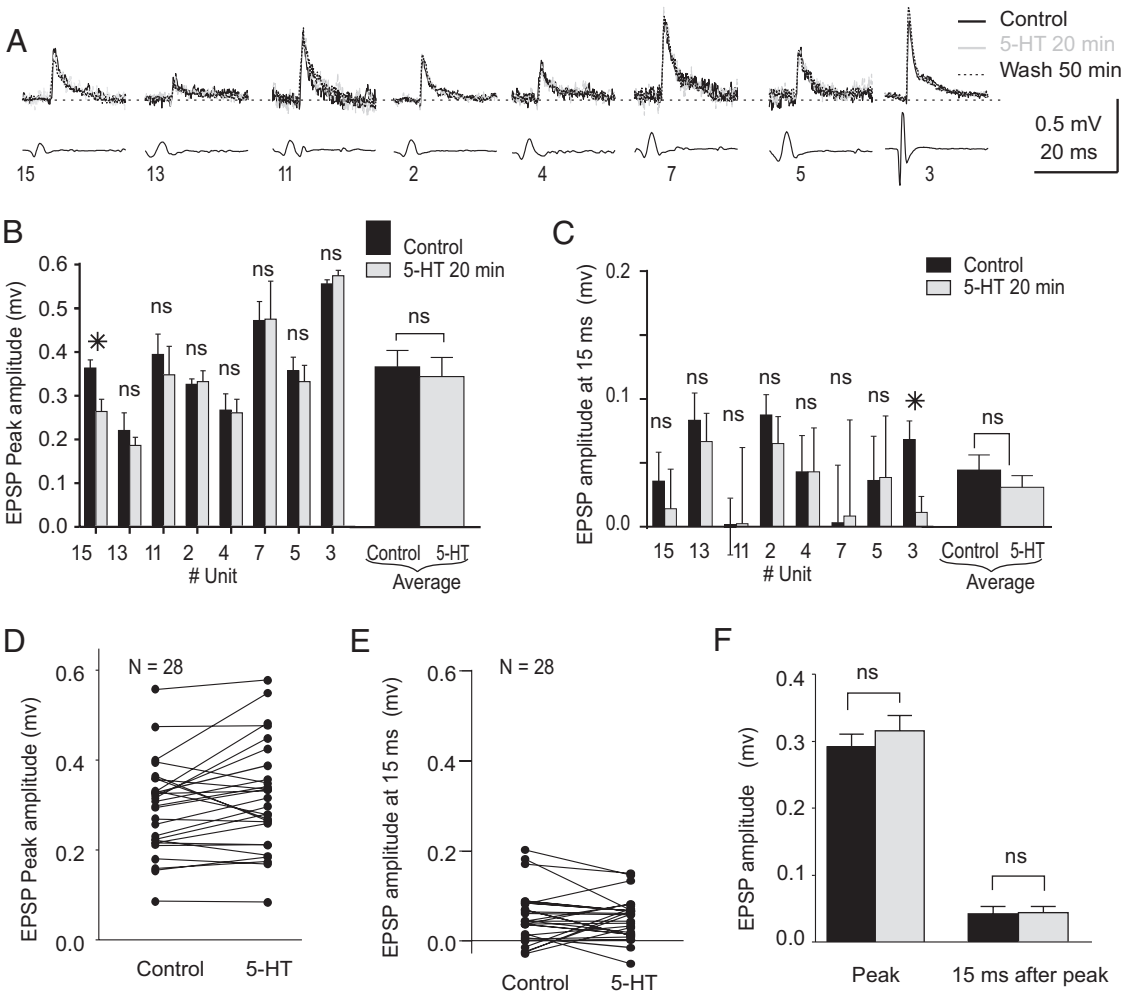

Figure 11. Effect of 10 μM 5-HT on the shape of EPSPs recorded from depressor MNs in subordinate-animal preparations. Same disposition as in Figure 9.

fied in one experiment (Fig. 12). As was the case for EPSP, in subordinate-animal preparations, the average peak amplitude of the unitary IPSPs did not display any significant changes after 5-HT application ($p > 0.05$, paired t test), although the amplitude of one unitary IPSPs (unit 10) was significantly reduced after 5-HT application ($p < 0.01$, Mann Whitney test) (Fig. 12*B*). Similarly, the average late decay-phase amplitude did not display any significant change after 5-HT application ($p > 0.05$, paired t test), although two presented a significant decrease ($p < 0.01$, Mann–Whitney test) (Fig. 12*C*) after 5-HT application.

Discussion

During the formation of a social hierarchy by crayfish, several behavior features are modified, including the excitabilities of different escape tail flip behaviors (Krasne et al., 1997; Herberholz et al., 2001) and the response to unexpected unilateral touch of the abdomen (Song et al., 2006). Analysis of the neural responses that mediate escapes revealed status-dependent differences in the stimulus thresholds of the LG and medial giant command neurons and the nongiant circuits that trigger the different types of escape. Moreover, serotonergic neuromodulation of the LG escape circuit also depends on the social status (Yeh et al., 1996; Teshiba et al., 2001). In the present study, we demonstrated that both excitability and 5-HT modulation of the walking leg postural circuit depends on social status too, thereby generalizing the effects of social interactions on motor systems.

Basal tonic activity and resistance reflex intensity depend on social status

Walking leg MNs in dominant-animal preparations demonstrated a higher spontaneous tonic activity than in subordinate animals. What are the physiological mechanisms mediating this increased tonic activity? We did not find any significant difference in resting membrane potential of depressor MNs, although they displayed a tendency to be more depolarized in dominant-animal preparations than in subordinate-animal preparations. The input resistance of these MNs was not significantly different in both status groups either. It is therefore possible that the difference in depressor MNs' tonic discharge [and other MN activity as well (Fig. 2*D*,*E*)] was due to the interneuronal drive being different in the two social phenotypes.

It may also be due to a higher sensitivity to sensory inputs. Indeed, we demonstrated that reflex discharge was higher in dominant-animal preparations than in subordinate-animal preparations. However, no significant difference was found in the level of sensory discharge in both groups. This observation indicates that CBCO-depressor MNs sensory-motor pathways are more effective in dominant than in subordinate crayfish. This increased efficacy of the resistance reflex was not associated with a relative increase in the input resistance of MNs in dominant animals compared with subordinate animals. The shape of unitary EPSPs triggered by sensory spikes from CBCO is significantly

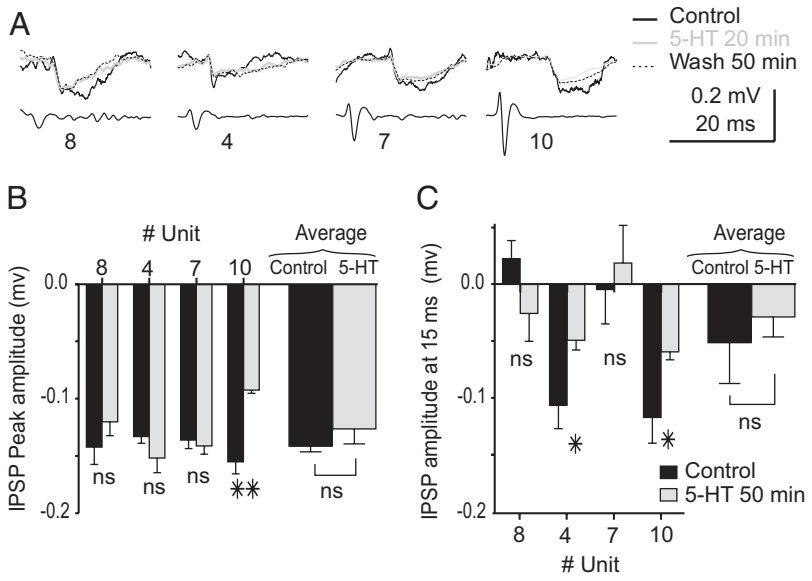

Figure 12. Effect of 10 μM 5-HT on the shape of IPSPs recorded from depressor MNs in subordinate-animal preparations. ***A***, Four unitary IPSPs were identified over the four experiments analyzed (same disposition as in Fig. 9*A*). ***B***, ***C***, Statistical analysis of the peak amplitude (***B***) and the amplitude of the EPSP measured 15 ms after the peak (***C***) of each IPSP in control condition (black bars) and after a 50 min wash post-5-HT application (gray bars) (same disposition as in Fig. 9, *B*, *C*).

different in dominant and subordinate animals, with EPSP decay phase being highly significantly shorter in subordinates (compare Figs. 9*A* and 11*A*).

The effects of 5-HT on walking leg postural circuits depend on social status.

The application of 5-HT on walking leg postural circuits induced clearly different effects in subordinate and dominant animals (Figs. 3–12). Moreover, the excitatory effects were highly significant only in dominant animals (Figs. 3*D*, 6*A*, 8*E*,*F*, and 9*F*,*G*), whereas 5-HT had no significant effects in subordinate animals.

The 5-HT neuromodulation has also proved to depend on social status in other systems, such as the LG escape circuit, where application of 50 μM 5-HT caused facilitation of synaptic transmission to LG in dominants and depression in subordinates (Yeh et al., 1996, 1997). As was reported for socially isolated crayfish, the effect of applied 5-HT on LG escape circuits depends on the rate of 5-HT's application, as well as on its concentration and the animal's social status (Teshiba et al., 2001). Both a low concentration (5 μM) applied quickly (full concentration achieved in $<$2 min) and a high concentration (50 μM) applied slowly (full concentration in 25 min) facilitated LG's response in social isolates, whereas the same high concentration applied quickly was inhibitory. In our experiments on the walking leg motor neurons from dominant animals, 5-HT (10 μM) was applied quickly, and its facilitating effects are like those of 5 μM 5-HT on LG responsiveness in socially isolated animals.

Socially dominant animals across phyla demonstrate their dominance through higher, more prominent postures and a more active engagement with their social and environmental surroundings. Conversely, subordinates tend to display lower, less visible postures and to retreat from the same stimuli that elicit confrontation in dominants (Darwin, 1873; Wilson, 1975). Here we have demonstrated that these behavioral differences in crayfish (Edwards et al., 2003) are reflected in the tonic activity levels, reflex responsiveness, and sensitivity to applied serotonin of the leg depressor and levator motor neurons that determine the animal's posture. These and other recent results show that the effect of social dominance status on the nervous system is widespread, affecting circuits that mediate behavioral switches (Krasne et al., 1997; Herberholz et al., 2001), postural circuits, including leg motor neurons that determine posture, and the different effects of neuromodulators like 5-HT that modulate the excitability of neurons throughout the nervous system (Yeh et al., 1997; Spitzer et al., 2005).

Analysis of 5-HT's effects on LG concluded that they occur through at least two distinct competitive pathways (Lee et al., 2008). In isolated crayfish, the pathway leading to facilitation involves pKA signaling whereas non-cAMP/PKA signaling pathways mediate inhibition (Lee et al., 2008). Moreover, experiments with 5-HT receptor agonists suggested that the different effects of 5-HT on LG excitability resulted from a difference in the balance of 5-HT receptors in dominant and subordinate animals (Yeh et al., 1997). Facilitation at mammalian and *Aplysia* synapses appears to be mediated by receptors that recruit cAMP/PKA or PKC signaling, similar to 5-HT4 and 5-HT2 receptors, respectively, in mammals (Nishimura and Akasu, 1989; Hori et al., 1996; Li and Zhuo, 1998; Cai et al., 2002; Shay et al., 2005; Rygh et al., 2006; Huang and Kandel, 2007). It is possible that similar competing pathways exist in the walking leg postural circuit of dominant animals. Moreover, as in LG, it is possible that differences in the expression of 5-HT-receptors account for the different effects of 5-HT on depressor motor neurons in dominant and subordinate animals.

Multiple mechanisms involved in 5-HT's effects on walking leg postural circuits

In the present work, we demonstrated that 5-HT (10 μM) produced a significant increase of the resistance reflex amplitude recorded in depressor MNs from dominant but not from subordinate animals (Fig. 7*A*). This increase was produced by multiple effects that act cooperatively. The application of 5-HT (10 μM) induced the following: (1) a tonic depolarization in depressor MNs (Fig. 6), (2) a significant increase of the input resistance of depressor MNs (Fig. 8), and (3) a change in the shape of unitary EPSPs triggered by CBCO sensory units; their amplitude and their decay time increased while polysynaptic EPSPs were recruited (Fig. 9). The systematic involvement of polysynaptic pathways in the presence of 5-HT would explain the decrease of variability of the reflex response compared with control condition in which polysynaptic pathways are more randomly active (Fig. 7*C*).

Multiple facilitatory effects of low concentration of 5-HT (5 μM) were also shown to influence the LG escape circuit of socially isolated crayfish (Antonsen and Edwards, 2007), consisting mainly of an increase of LG input resistance and an increase of electrical synaptic conductance. As was already shown for the walking leg postural circuit, the increased input resistance and increased EPSP decay time induced by 5-HT both contribute substantially to the increased resistance reflex and the depolarizing response recorded in depressor MNs (Le Bon-Jego et al., 2004). The tonic depolarization of the depressor MNs would also enhance their reflex response as the muscarinic component of the sensory synaptic inputs was activated (Le

Bon-Jego et al., 2006). This muscarinic current is voltage dependent and activated by the cholinergic CBCO–MN synapse. It increases the amplitude of the resistance reflex response when the membrane potential reaches its threshold of activation (Le Bon-Jego et al., 2006). Note that activation of the voltage-dependent muscarinic current was prevented by a constant negative current injection that counteracted the 5-HT-induced tonic depolarization; without current injection, the muscarinic currents would add to the 5-HT-induced increase in the resistance reflex response. In dominant crayfish, the 5-HT-induced tonic depolarization is large enough to reach the threshold (−60 mV) for the muscarinic component of the sensorymotor synapse. The fact that depolarization was always much smaller (when present) in subordinate crayfish than in dominant animals would also contribute to the absence of increase of the resistance reflex response amplitude observed in subordinate-animal preparations.

Relationship to behavior

We have found that both tonic motorneuron activity and postural resistance reflexes are greater in dominant than in subordinate preparations, and are enhanced by 5-HT in dominant but not in subordinate preparations. What are the behavioral consequences of these differences? The higher tonic activity suggests that dominants maintain a higher postural tone than subordinates, and that this tone and reflex gain are increased by the release of 5-HT. Dominant crayfish have been associated with characteristic elevated postures and subordinate crayfish with lowered postures (Livingstone et al., 1980; Kravitz, 1988); however, careful observations indicate that such postures are adopted mainly when crayfish face each other (Van der Velden et al., 2008). Such encounters are likely to trigger descending commands to the thoracic ganglia to activate or modulate postural circuits. The pair of 5-HT neurons in the first abdominal ganglion (A1) are among the potential targets of such descending responses. The A1 5-HT cells are tonically active, they can be excited by local mechanosensory stimuli, they project to the thoracic ganglia that contain the postural circuits (Beltz and Kravitz, 1983; Beltz and Kravitz, 1987; Beltz, 1999), and they modulate the reflex responses of the depressor motorneurons (Issa, 2008). These hypotheses can be applied to other circuits that are involved in other patterns of behavior displayed by dominant and subordinate animals, including the different responses to an unexpected touch (Song et al., 2006).

References

Antonsen BL, Edwards DH (2007) Mechanisms of serotonergic facilitation of a command neuron. J Neurophysiol 98:3494–3504.

Beltz BS (1999) Distribution and functional anatomy of amine-containing neurons in decapod crustaceans. Microsc Res Tech 44:105–120.

Beltz BS, Kravitz EA (1983) Mapping of serotonin-like immunoreactivity in the lobster nervous system. J Neurosci 3:585–602.

Beltz BS, Kravitz EA (1987) Physiological identification, morphological analysis, and development of identified serotonin-proctolin containing neurons in the lobster ventral nerve cord. J Neurosci 7:533–546.

Cai X, Flores-Hernandez J, Feng J, Yan Z (2002) Activity-dependent bidirectional regulation of GABA(A) receptor channels by the 5-HT(4) receptor-mediated signalling in rat prefrontal cortical pyramidal neurons. J Physiol 540:743–759.

Darwin C (1873) The expression of the emotions in man and animals. New York: Appleton.

Edwards DH, Spitzer N (2006) Social dominance and serotonin receptor genes in crayfish. Curr Top Dev Biol 74:177–199.

Edwards DH, Issa FA, Herberholz J (2003) The neural basis of dominance hierarchy formation in crayfish. Microsc Res Tech 60:369–376.

El Manira A, Cattaert D, Clarac F (1991) Monosynaptic connections mediate resistance reflex in crayfish (*Procambarus clarkii*) walking legs. J Comp Physiol A Neuroethol Sens Neural Behav Physiol 168:337–349.

Herberholz J, Issa FA, Edwards DH (2001) Patterns of neural circuit activation and behavior during dominance hierarchy formation in freely behaving crayfish. J Neurosci 21:2759–2767.

Hill AA, Cattaert D (2008) Recruitment in a heterogeneous population of motor neurons that innervates the depressor muscle of the crayfish walking leg muscle. J Exp Biol 211:613–629.

Hori Y, Endo K, Takahashi T (1996) Long-lasting synaptic facilitation induced by serotonin in superficial dorsal horn neurones of the rat spinal cord. J Physiol 492:867–876.

Huang YY, Kandel ER (2007) 5-Hydroxytryptamine induces a protein kinase A/mitogen-activated protein kinase-mediated and macromolecular synthesis-dependent late phase of long-term potentiation in the amygdala. J Neurosci 27:3111–3119.

Issa FA (2008) Effect of social experience on the behavior and neurophysiology of crayfish. PhD dissertation, Georgia State University.

Issa FA, Adamson DJ, Edwards DH (1999) Dominance hierarchy formation in juvenile crayfish *Procambarus clarkii*. J Exp Biol 202:3497–3506.

Krasne FB, Shamsian A, Kulkarni R (1997) Altered excitability of the crayfish lateral giant escape reflex during agonistic encounters. J Neurosci 17:709–716.

Kravitz EA (1988) Hormonal control of behavior: amines and the biasing of behavioral output in lobsters. Science 241:1775–1781.

Le Bon-Jego M, Cattaert D, Pearlstein E (2004) Serotonin enhances the resistance reflex of the locomotor network of the crayfish through multiple modulatory effects that act cooperatively. J Neurosci 24:398–411.

Le Bon-Jego M, Masante-Roca I, Cattaert D (2006) State-dependent regulation of sensory-motor transmission: role of muscarinic receptors in sensory-motor integration in the crayfish walking system. Eur J Neurosci 23:1283–1300.

Lee SH, Taylor K, Krasne FB (2008) Reciprocal stimulation of decay between serotonergic facilitation and depression of synaptic transmission. J Neurophysiol 100:1113–1126.

Le Ray D, Cattaert D (1997) Neural mechanisms of reflex reversal in coxobasipodite depressor motor neurons of the crayfish. J Neurophysiol 77:1963–1978.

Li P, Zhuo M (1998) Silent glutamatergic synapses and nociception in mammalian spinal cord. Nature 393:695–698.

Livingstone MS, Harris-Warrick RM, Kravitz EA (1980) Serotonin and octopamine produce opposite postures in lobsters. Science 208:76–79.

Nishimura T, Akasu T (1989) 5-Hydroxytryptamine produces presynaptic facilitation of cholinergic transmission in rabbit parasympathetic ganglia. J Auton Nerv Syst 26:251–260.

Rygh LJ, Suzuki R, Rahman W, Wong Y, Vonsy JL, Sandhu H, Webber M, Hunt S, Dickenson AH (2006) Local and descending circuits regulate long-term potentiation and zif268 expression in spinal neurons. Eur J Neurosci 24:761–772.

Shay BL, Sawchuk M, Machacek DW, Hochman S (2005) Serotonin 5-HT2 receptors induce a long-lasting facilitation of spinal reflexes independent of ionotropic receptor activity. J Neurophysiol 94:2867–2877.

Sillar KT, Skorupski P (1986) Central input to primary afferent neurons in crayfish, *Pacifastacus leniusculus*, is correlated with rhythmic motor output of thoracic ganglia. J Neurophysiol 55:678–688.

Song CK, Herberholz J, Edwards DH (2006) The effects of social experience on the behavioral response to unexpected touch in crayfish. J Exp Biol 209:1355–1363.

Spitzer N, Antonsen BL, Edwards DH (2005) Immunocytochemical mapping and quantification of expression of a putative type 1 serotonin receptor in the crayfish nervous system. J Comp Neurol 484:261–282.

Teshiba T, Shamsian A, Yashar B, Yeh SR, Edwards DH, Krasne FB (2001) Dual and opposing modulatory effects of serotonin on crayfish lateral giant escape command neurons. J Neurosci 21:4523–4529.

Van der Velden J, Zheng Y, Patullo BW, Macmillan DL (2008) Crayfish recognize the faces of fight opponents. PLoS One 3:e1695.

Wilson EO (1975) Sociobiology: the new synthesis. Cambridge, MA: Belknap.

Yeh SR, Fricke RA, Edwards DH (1996) The effect of social experience on serotonergic modulation of the escape circuit of crayfish. Science 271:366–369.

Yeh SR, Musolf BE, Edwards DH (1997) Neuronal adaptations to changes in the social dominance status of crayfish. J Neurosci 17:697–708.