MODULATION OF MOTONEURON ACTIVITY BY SEROTONIN

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LIST OF ABBREVIATIONS

5-HT	Serotonin
AHP	Afterhyperpolarization
AIS	Axon initial segment
Ca^{2+}	Calcium
Cd^{2+}	Cadmium
CsCl	Cesium chloride
GABA	γ-aminobutyric acid
I_{CAN}	Calcium activated non-selective cationic current
I_h	Hyperpolarization activated current
K^+	Potassium
mAHP	medium afterhyperpolarization
Na^+	Sodium
SK	small Ca^{2+}-activated potassium channel
TASK-1	TWIK-related acid-sensitive K^+ channel

1. LIST OF ARTICLES

The thesis is based on the following articles:

(1) Perrier JF, Hounsgaard J. Ca(2+)-activated nonselective cationic current (I(CAN)) in turtle motoneurons. J Neurophysiol. 1999;82(2):730-5. Epub 1999/08/13. PubMed PMID: 10444670.

(2) Perrier JF, Mejia-Gervacio S, Hounsgaard J. Facilitation of plateau potentials in turtle motoneurones by a pathway dependent on calcium and calmodulin. J Physiol. 2000;528 Pt 1:107-13.

(3) Perrier JF, Hounsgaard J. 5-HT2 receptors promote plateau potentials in turtle spinal motoneurons by facilitating an L-type calcium current. J Neurophysiol. 2003;89(2):954-9.

(4) Perrier JF, Alaburda A, Hounsgaard J. 5-HT1A receptors increase excitability of spinal motoneurons by inhibiting a TASK-1-like K+ current in the adult turtle. J Physiol. 2003;548(Pt 2):485-92.

(5) Grunnet M, Jespersen T, Perrier JF. 5-HT1A receptors modulate small-conductance Ca2+-activated K+ channels. J Neurosci Res. 2004;78(6):845-54.

(6) Perrier JF, Delgado-Lezama R. Synaptic release of serotonin induced by stimulation of the raphe nucleus promotes plateau potentials in spinal motoneurons of the adult turtle. J Neurosci. 2005;25(35):7993-9.

(7) Perrier JF, Tresch M. Recruitment of motor neu-ronal persistent inward currents shapes withdrawal reflexes in the frog. J Physiol. 2005 Jan 15;562(Pt 2):507-20.

(8) Perrier JF, Cotel F. Serotonin differentially modulates the intrinsic properties of spinal motoneurons from the adult turtle. J Physiol. 2008;586(5):1233-8.

(9) Cotel F, Exley R, Cragg SJ, Perrier JF. Serotonin spillover onto the axon initial segment of motoneurons induces central fatigue by inhibiting action potential initiation. Proc Natl Acad Sci U S A. 2013;110(12):4774-9.

(10) Perrier JF, Rasmussen HB, Christensen RK, Petersen AV. Modulation of the intrinsic properties of motoneurons by serotonin. Current pharmaceutical design. 2013;19(24):4371-84.

2. INTRODUCTION

The goal of the present work was to understand how the modulation of the intrinsic properties of a given group of neurons contributes to a well-defined physiological function. I chose the spinal cord as a model because, in contrast with other neuronal networks, it has well defined inputs (the primary afferent fibres innervating skin, joints and muscles) and outputs, the motoneurons. Motoneurons are the final common output of the central nervous system. Their cell bodies located in the brainstem and the ventro-lateral part of the spinal cord send axons that contact muscle fibres. Each time a motoneuron generates action potentials, it induces the contraction of the muscle fibres it innervates and therefore triggers a movement. For this reason it is possible to correlate the activity of individual motoneurons with motor

control, one of the most important functions of the central nervous system. Since serotonin is one of the major modulators in the spinal cord, I chose to study how it modulates the intrinsic properties of motoneurons.

It is thought that information in the brain is coded by action potentials in individual neurons. Two main factors contribute to shape firing patterns in neurons: the synaptic inputs and electrical currents flowing through voltage-gated ion channels expressed in the membrane. This latter factor provides a degree of freedom to individual cells allowing them to filter or amplify synaptic inputs, prolong firing or even allow firing in the absence of any input (11, 12). Hence, non-linearity provided by intrinsic properties increases the computational capabilities of neurons. In addition, voltage-gated ion channels can be regulated by the binding of neuromodulators such as dopamine, serotonin, noradrenaline, GABA or glutamate to metabotropic receptors (13, 14). The resulting modulation provides an even higher degree of flexibility to the central nervous system that enhances computational possibilities.

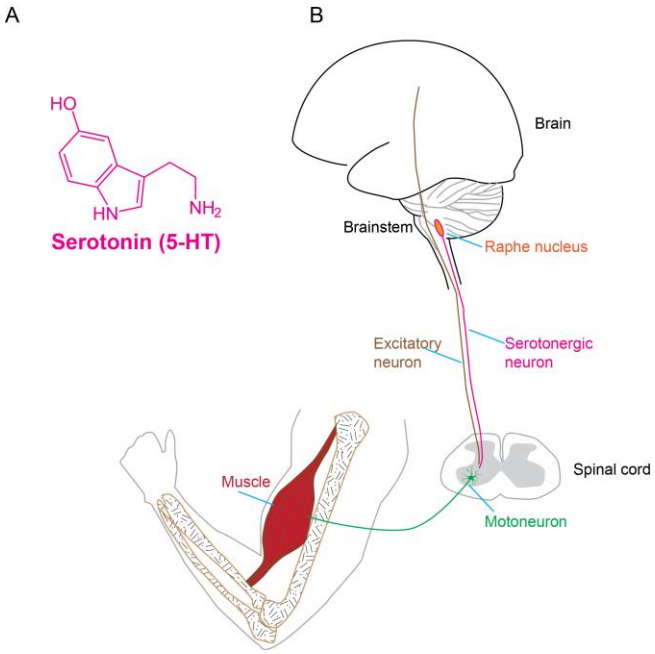


Figure 1: The serotonergic system
A, Serotonin structure. B, Organization of the serotonergic system. Motoneurons innervate muscle fibers belonging to a give muscle. During voluntary movements, motoneurons receive excitatory inputs from neurons originating from the brain. Neurons from the raphe project to the spinal cord and make synaptic contact on motoneurons. The release of 5-HT modulates the excitability of motoneurons.

SEROTONIN

Serotonin, also called 5-hydroxytryptamine (5-HT) is a biogenic amine that contributes to several functions of the central nervous system such as affective state, appetite regulation, sexual arousal pain modulation or motor control. 5-HT is synthetized by neurons from the raphe nuclei located around the midline in the brainstem. These neurons project to most areas of the central nervous system. A subgroup of raphe neurons belonging to the nuclei *raphe obscursus*, *raphe pallidus*, *raphe magnus* project to the spinal cord (15, 16). Serotonergic axons project both to dorsal and ventral areas and make synaptic contacts on motoneurons (17) (Figure 1).

Experiments performed in cats have shown that the activity of neurons from the raphe nuclei is positively correlated with the level of motor activity (18). During sleep, most serotonergic neurons are silent. In contrast, when the animal is in quiet waking state, neurons from the nucleus *raphe obscursus* and nucleus *raphe pallidus* fire at a regular low pace around 5 Hz. During active motor activities such as locomotion, the firing frequency increases in parallel with the intensity of the exercise (19), suggesting that the amount serotonin released correlates with the firing of motoneurons and thereby movements.

Several studies suggest direct effects of 5-HT on motoneurons. First of all, motoneurons express a range of serotonin receptors including the 5-HT_{1A}, 5-HT_{1B}, 5-HT_{2A}, 5-HT_{2B}, 5-HT_{2C} subtypes (reviewed in (10)). Second, application of 5-HT near the membrane of motoneurons increases their excitability through modulation of various types of conductances (20-22). 5-HT facilitates the hyperpolarization-activated cationic current (I_h) (23, 24) and the dihydropyridine sensitive non-inactivating L-type Ca^{2+} channels that can generate plateau potentials (25). 5-HT also inhibits leak K^+ conductances (26-28) and Ca^{2+}-activated K^+ conductances responsible for the medium afterhyperpolarization following action potentials (11, 28-32) (a systematic review of the literature is available in article (10)). Hence, it is a generally agreed that 5-HT promotes motoneuron activity. However, few other studies that are usually disregarded showed that serotonin also exerts inhibitory effects characterized by a hyperpolarization (28, 33) concomitant with an increase of membrane conductance (26). What is the explanation for this apparent contradiction?
Interestingly, *in vivo* studies showed that injection of the 5-HT precursor tryptophan in the blood or directly in the central nervous system accelerates the exhaustion occurring during motor activity (34, 35). Moreover, the time to exhaustion is decreased by serotonin receptor agonists and increased by serotonin antagonists (36). Even though none of these studies tested if the effect of serotonin affected motoneurons directly, they underlined the paradoxical effect of 5-HT on motor control. How can the same neuromodulator boost the activity of motoneurons and, thereby, muscle contraction and at the same time enhance exhaustion?

We investigated the modulation of intrinsic properties of motoneurons by 5-HT in a systematic manner with the aim of uncovering the mechanisms underlying the paradoxical effects of serotonin on motoneurons.

3. RESULTS AND DISCUSSION

SEROTONIN INCREASES THE EXCITABILITY OF MOTONEURONS

To study the effects of serotonin on motoneurons, we took advantage of the spinal cord slice preparation of the adult turtle developed by Hounsgaard (11). The preparation has the advantages of being resistant to anoxia and of being fully differentiated. This point is crucial because the expression of ion channels and receptors undergoes dramatic changes during development (37). We studied the intrinsic properties of motoneurons by performing intracellular recordings with sharp electrodes in the presence of blockers for fast synaptic transmission (see methods).

Serotonin inhibits TASK-1 like conductances

We investigated how 5-HT or agonists for serotonin receptors modulate the properties of motoneurons by adding agonists for serotonin receptors during recordings performed in current and voltage-clamp modes. In an initial study (4), we found that 5-HT

as well as an agonist for 5-HT_{1A} receptors added to the extracellular medium induced a depolarization and an increased input resistance of most motoneurons, but caused hyperpolarization and a decreased input resistance in the remaining smaller fraction of cells. We restricted our study to the excitatory effect by adding CsCl, a blocker of most voltage gated K^+ conductances (38), to the extracellular medium. Under this condition, addition of a 5-HT_{1A} receptor agonist inhibited a pH sensitive, voltage-insensitive current that reversed at a value compatible with K^+ (figure 2). Since the modulated current was blocked by anandamide, we concluded that 5-HT_{1A} receptors induced the inhibition of the TWIK-related acid-sensitive K^+ channel, TASK-1, a channel belonging to the two-pore domain K^+ channel family that contributes to the membrane leak conductance (39).

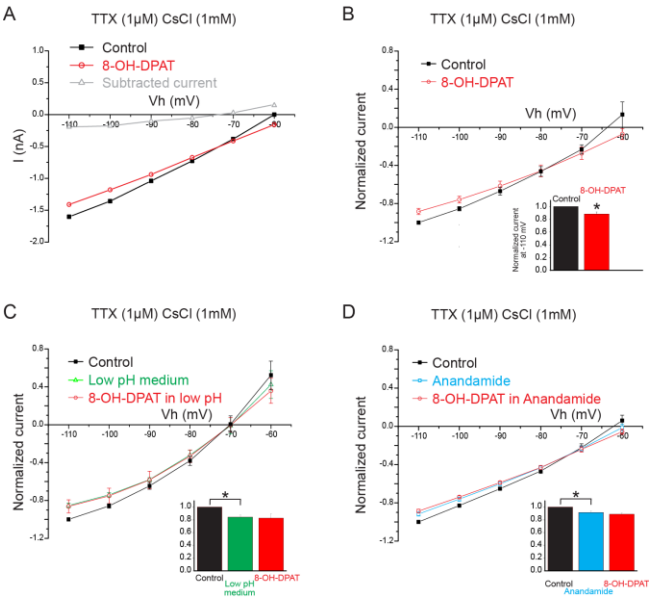


Figure 2: Activation of 5-HT_{1A} receptors inhibits a TASK-1-like channel

Experiments in voltage-clamp mode in the presence of tetrodotoxin (TTX, 1 µM) and CsCl (1 mM). A, I–V plot before (black) and after addition of the 5-HT_{1A} receptor agonist 8-OH-DPAT (10 µM; red). B, normalized and averaged I–V plot before (black) and after addition of 8-OH-DPAT (10 mM; red) (n = 4). Inset: normalized current measured at –110 mV. The inhibition was statistically significant. C, normalized and averaged I–V plot before (black) and after changing to medium with low pH (green) (n = 7). The inhibition was significant (*). Subsequent addition of 8-OH-DPAT (10 µM; red) had no additional effect. D, normalized and averaged IV plot before (black) and after addition of anandamide (blue) (n = 4). The inhibition was significant (*). Subsequent addition of 8-OH-DPAT (10 µM) had no additional effect (inset; red).

Serotonin inhibits the medium afterhyperpolarization following action potentials

Action potentials in motoneurons are followed by a fast afterhyperpolarization (AHP) mediated by delayed-rectifier K^+ channels and a slower AHP caused by the subsequent activation of small conductance calcium-activated K+ channels (SK) (40, 41). Because an even slower AHP was described in mammalian neurons (42), the SK-mediated AHP is usually called the medium afterhyperpolarization (mAHP).

The mAHP is modulated by 5-HT (28, 29) acting on 5-HT_{1A} receptors (30, 31). Since 5-HT inhibits Ca^{2+} current in motoneurons, the effect of 5-HT was ascribed to the inhibition of the Ca^{2+} influx responsible for the activation of SK channels (30, 32). Another possibility could be a modulation of SK channels through a specific pathway. We designed experiments to test this possibility (article (5)). We stably expressed the three different subtypes of SK channels in *Xenopus laevis* oocytes and activated them by intracellular injection of Cd^{2+} (see methods). Activation of 5-HT_{1A} receptors inhibited the three SK channels subtypes, suggesting a specific pathway between the receptors and the ion channels. To investigate the physiological relevance of this pathway, we characterized the mAHP present after action potentials in spinal motoneurons recorded in a slice preparation. In current clamp mode, we showed that 5-HT_{1A} receptor activation inhibited the mAHP. We analysed the underlying mechanism by performing voltage-clamp recording and found that 5-HT_{1A} receptors specifically inhibited the fraction of the AHP mediated by SK channels (Figure 3).

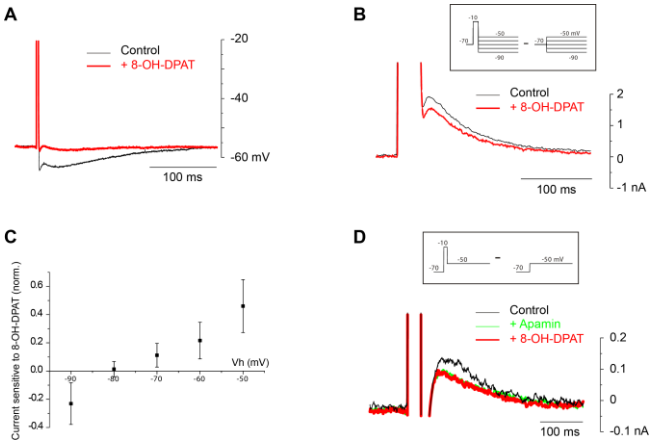


Figure 3: 5-HT_{1A} receptors inhibits the medium afterhyperpolarization in motoneurons

A, Action potential evoked by a brief depolarizing current pulse before (black) and after addition of the 5-HT_{1A} receptor agonist 8-OH-DPAT. B, motoneuron recorded in voltage-clamp mode. Inset: voltage clamp protocol. A depolarizing voltage step to -10 mV evoked an outward tail current (black) that was inhibited by 8-OH-DPAT (red). C, Mean IV plot of the normalized current sensitive to 8-OH-DPAT. D, Current recorded under control conditions (black), after addition of apamin (100 nM) (green), and after addition of 8-OH-DPAT (10 µM; red) in the presence of apamin. Note the absence of effect of 8-OH-DPAT. Inset: Voltage clamp protocol.

Serotonin promotes plateau potentials

A brief activation of spinal motoneurons can trigger prolonged firing in the absence of on-going stimulation. This sustained activity, which was first ascribed to a reverberating loop of excitatory neurons (43), is caused by the recruitment of the slowly activating voltage-gated L-type Ca^{2+} channels (11, 41).

When we initiated our project (article (1)), it was not known whether the Ca^{2+}-influx was the charge carrier for the plateau potential or merely the trigger for a non-inactivating Ca^{2+}-activated conductance generating the current underlying the plateau potential remained as shown in other types of neurons (44). The non selective calcium-activated cationic current (I_{CAN}) was a possible candidate because CAN channels, usually permeable for Na^+, K^+, and also Ca^{2+}, do not undergo voltage- or Ca^{2+}-dependent inactivation and thus provide a potential mechanism for maintaining depolarization and Ca^{2+} entry in the cell (45). Taking advantage of the ability of motoneurons to generate Ca^{2+} action potentials, we showed that a transient increase in Ca^{2+} concentration activates a CAN current. However, removal of the extracellular Na^+ ions, which produces a reversal of the CAN current at resting membrane potential, did not affect the ability of motoneurons to generate plateau potentials. We therefore concluded that plateau

potentials in spinal motoneurons are triggered and mediated by L-type Ca^{2+} channels.

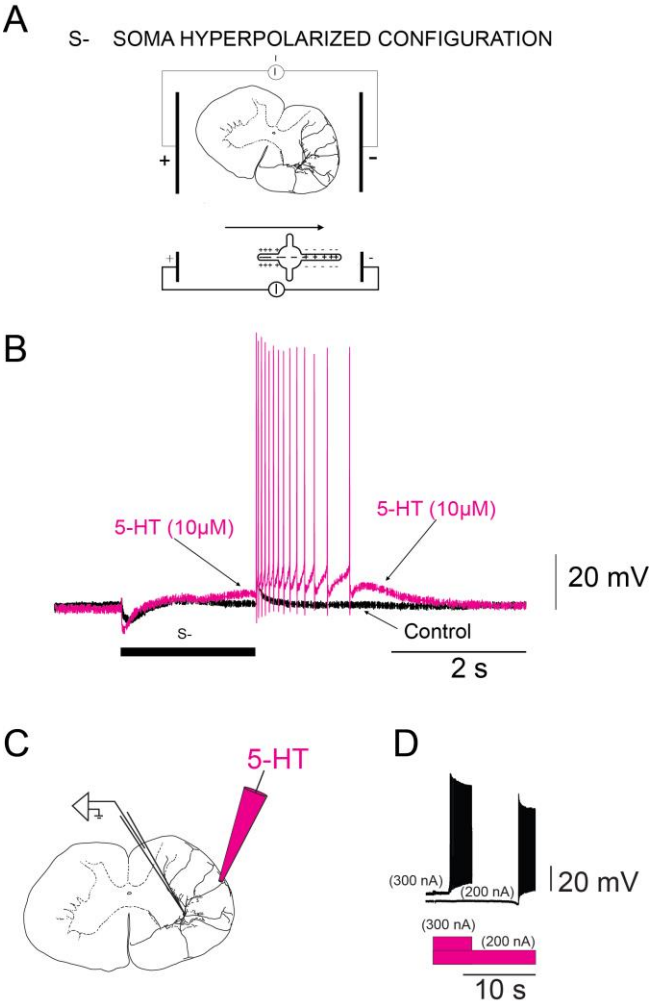


Figure 4: HT selectively facilitates plateau potentials in the dendritic compartment

A, Spinal cord slice positioned between the two silver electrodes used to apply an electrical field. With the cathode positioned laterally to the motoneuron recorded from, the medial dendrites and the soma were hyperpolarized (S- configuration), while the lateral dendrites were depolarized. B, bottom trace: control response induced by the S- field (black bar). Note the transient hyperpolarization. Top trace: response to the same field stimulation after addition of 5-HT (10 µM). Note the supplementary depolarization, the amplitude of which was strong enough to generate an afterdischarge when the field was stopped. C, electrode arrangement with a 5-HT microiontophoresis pipette near a distal dendrite and the recording electrode at the soma. D, Superimposed recordings from a motoneuron to release of 5-HT at 2 different intensities close to a dendrite (sweeps and stimulus bars labeled with iontophoresis current). Sweeps separated for clarity (the resting potential was the same throughout the experiment). With more 5-HT released the plateau potential was triggered faster.

5-HT has been known to promote plateau potentials in motoneurons for many years (25, 41). However, the mechanism by which monoamines facilitate plateau potentials remained hypothetical. It was not known whether plateau potentials were indirectly induced by the inhibition of an outward current such as a leak conductance or the current mediating the afterhyperpolarization following action potentials (11), or through a direct facilitation of the L-type Ca^{2+} current. We undertook a study to clarify the effects of 5-HT on spinal motoneurons and showed that 5-HT promotes plateau potentials by activating 5-HT_2 receptors, which specifically facilitate L-type Ca^{2+} channels (article (3)). By means of microiontophoresis application of 5-HT and by differential polarization of the dendrites, we showed that the effect induced by 5-HT_2 receptor activation occurs, at least partly, in the dendritic compartment of motoneurons (Figure 4).

L-type Ca^{2+} channels are inactivated by a Ca^{2+}-dependent process (46) that may involve calmodulin (47, 48). However, other studies showed that calmodulin facilitates the activity of L-type Ca^{2+} channels (47, 49). This raised the possibility that the level of cytosolic Ca^{2+} directly or via calmodulin is involved in the regulation of L-type Ca^{2+} channels in spinal motoneurons. The fact that 5-HT_2 receptors are coupled to G_q proteins, which induce the release of Ca^{2+} from intracellular stores (50), also suggests that increased intracellular Ca^{2+} concentration mediates the facilitation of plateau properties in response to serotonin receptor activation. We investigated this possibility in a dedicated study (article (2)). We found that a transient increase in the intracellular Ca^{2+} concentration triggered by Ca^{2+} spikes facilitated plateau potentials. In addition, chelating Ca^{2+} or antagonizing calmodulin inhibited plateau potentials. We therefore concluded that plateau potentials in spinal motoneurons are facilitated by activation of a calcium–calmodulin-dependent pathway.

In summary, we showed that 5-HT promotes the activity of motoneurons by acting on different receptors. The binding to 5-HT_{1A} receptors leads to the inhibition of a leak current mediated by TASK-1 channels, and to the inhibition of a Ca^{2+}-activated K^+ current mediated by SK channels. The activation of 5-HT_2 receptors induces the facilitation of L-type Ca^{2+} channels via an intracellular pathway involving Ca^{2+} and calmodulin (figure 5).

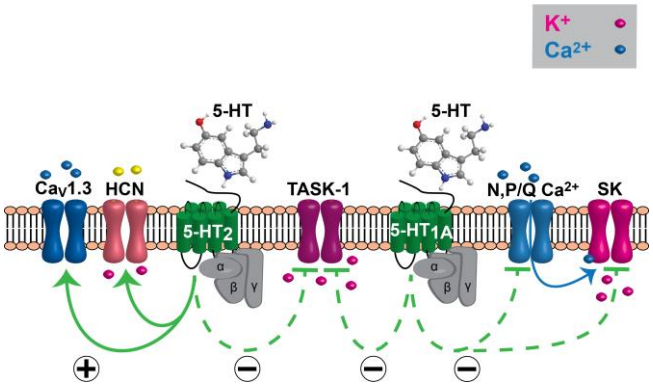


Figure 5: Summary of the serotonergic pathways leading to an increase of motoneuron excitability

The plain arrows indicate a facilitation of ion channels; the dotted lines correspond to an inhibition.

Synaptic release of serotonin modulates intrinsic properties of motoneurons

Pharmacological agents added to the extracellular medium are convenient tools for dissecting mechanisms for activation or inhibition of neurons. However, this approach is far from physiological condition since all the receptors from all the cells present

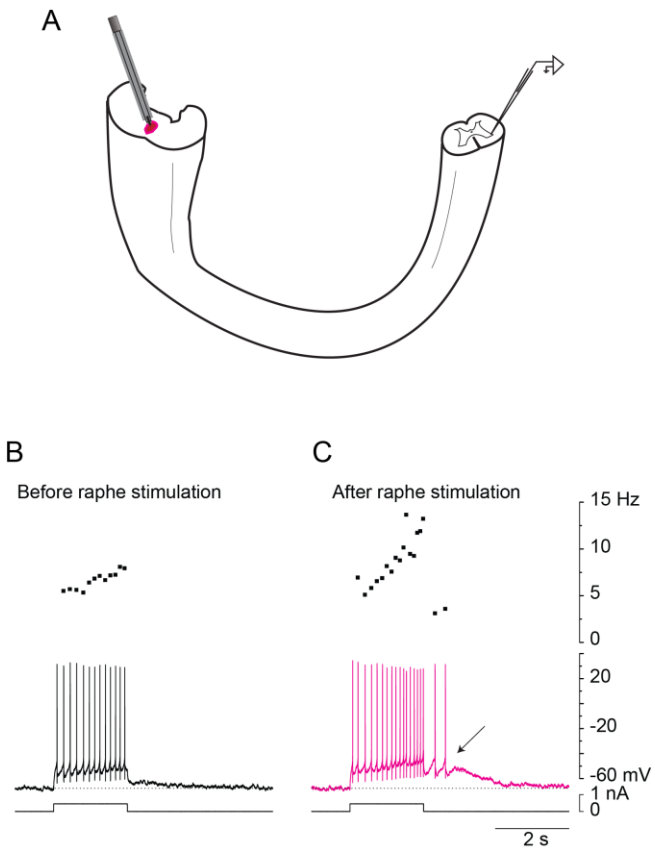
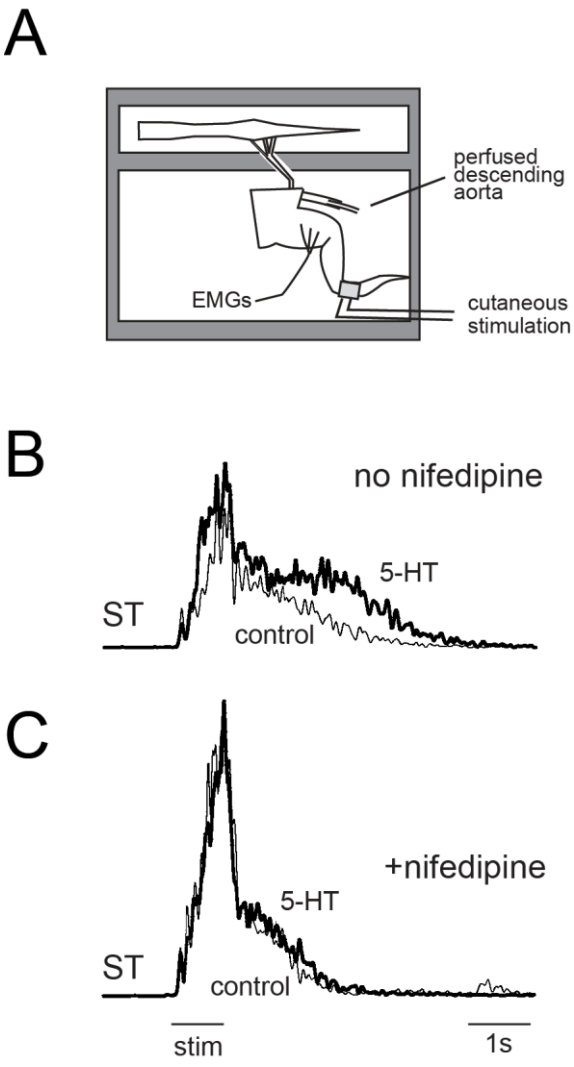


Figure 6: The plain arrows indicate a facilitation of ion channels; the dotted lines correspond to an inhibition.

A, Scheme of the brainstem spinal cord preparation. A bipolar electrode was positioned at the ventral third of the midline of the brainstem section (i.e., at the level of the raphe nucleus) (see Materials and Methods). Intracellular recording of motoneurons was performed with a microelectrode positioned in the lateral part of the ventral horn of the spinal cord section. B, Control response to a depolarizing current pulse. C, After stimulation of the raphe nucleus, the same current protocol induced a train of action potentials occurring at a higher frequency and followed by an afterdepolarization with a superimposed afterdischarge (arrow), indicative of an underlying plateau potential. For B and C: bottom trace, current injected intracellularly; middle trace, membrane potential; top plot, instantaneous spike frequency.

in the preparation are simultaneously affected. After showing that 5-HT promotes the excitability of motoneurons, we investigated if similar results could be obtained under more physiological conditions. We first tested if the synaptic release of 5-HT also increases the excitability of motoneurons (article (6)). Since 5-HT is released from raphe spinal neurons, we developed an integrated preparation consisting of the brainstem left in continuity with the cervical spinal cord of the turtle (figure 6A). We demonstrated that synaptic release of 5-HT induced by stimulation of the raphe nucleus increases the excitability of motoneurons by decreasing the amplitude of the afterhyperpolarization following action potentials and by promoting plateau potentials (figure 6BC). The increase in excitability lasted several seconds. Pharmacological analysis showed that the facilitation of plateau potentials is due to activation of 5-HT_2 receptors specifically facilitating low threshold L-type calcium channels. This study demonstrates that the regulation of plateau potentials by 5-HT is a mechanism by which the brain can regulate the excitability of motoneurons. This form of short-term plasticity could be important for muscle tone. In support, it was shown that in rats with complete spinal transections, activation of 5-HT_{2C} receptors increases the ability of animals to perform weight-supported locomotion (51). The activation of 5-HT_2 receptors also upregulates the excitability of extensor motoneurons in acute spinal cats (52).



To Figure 7: In vitro frog spinal cord with attached hindlimb allows study of motor behaviors

A, Schematic diagram of the preparation. The spinal cord and innervated hindlimb were placed in separate chambers. Withdrawal reflexes were evoked by electrical stimulation of the foot. B, Mean EMG of semitendinosus muscle during withdrawal reflex in control Ringer solution (light trace) and after application of 5-HT to the spinal cord (dark trace). 5-HT increased the EMG activity. C, after application of the L-type Ca^{2+} channel blocker nifedipine, the potentiating effects of 5-HT were no longer observed.

further investigate the functional significance of the modulation of plateau potentials by 5-HT, I collaborated with Matthew Tresch (M.I.T.; Boston, USA) who had developed a preparation consisting in the in vitro spinal cord of an adult frog left attached with one leg. In this preparation, it is possible to record motoneurons intracellularly during movement of the leg monitored by electromyographic electrodes inserted in several muscles. We designed experiments aimed at determining if and how plateau potentials modulated by 5-HT contribute to a simple motor behaviour (article (7)). We found that plateau properties, following their potentiation by 5-HT, are recruited and contribute to the production of withdrawal behaviours. These properties confer a voltage-dependent prolongation to the duration of motor neuronal activity.

Consistent with this potentiation of motoneuronal plateau properties, 5-HT also increased the duration of evoked muscle activation (figure 7). These results demonstrated that plateau properties mediated by L-type Ca^{2+} channels in motoneurons can be recruited during the production of behavior and play a role in specifying the temporal details of motor output.

Serotonin decreases the excitability of motoneurons

The results presented so far show that 5-HT increases the excitability of motoneurons. These findings, which are in agreement with publications from several laboratories (25, 53-55), were not always reproducible. In several instances, we found that 5-HT applied to the extracellular medium had instead an inhibitory effect characterized by a decrease in the number of action potentials generated by intracellular current pulse injection (articles (3) and (8)). We investigated this discrepancy by applying 5-HT through microiontophoresis electrodes and found that activation of serotonergic receptors in somato-dendritic compartments promoted plateau potentials. In contrast, stimulation of receptors in an area located near the soma had an inhibitory effect. We found that the facilitation of plateau potentials was induced by 5-HT_2 receptor activation, while the inhibitory effect was caused by the activation of 5-HT_{1A} receptors. This observation shows that the effects of 5-HT are compartmentalized. Even though the results looked appealing, it was difficult to understand their physiological implications. How can the raphe spinal pathway activate one type of receptors without the other?

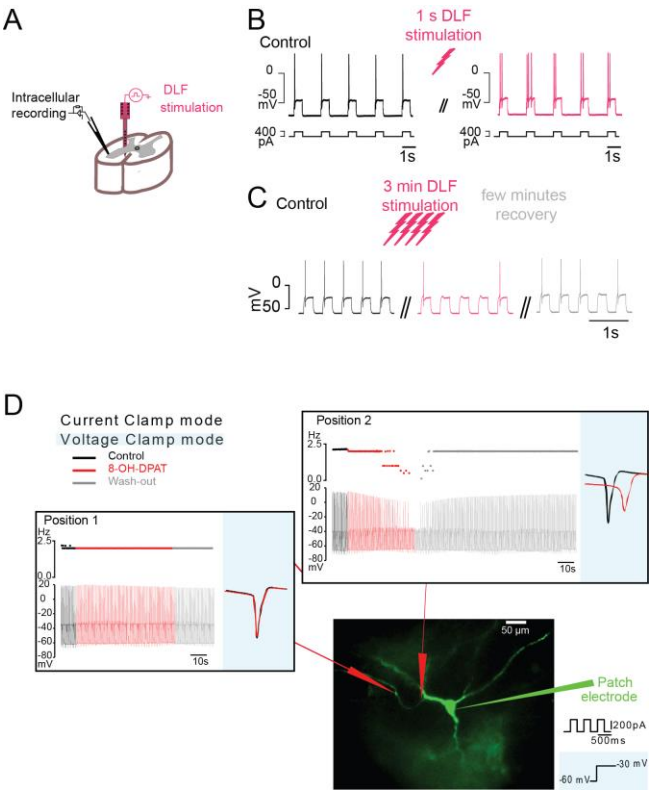


Figure 8: Dual modulation of MNs firing induced by stimulation of the raphe spinal pathway

A, Experimental setup. B, Intracellular recording of a motoneuron in control (black) and after a stimulation of the raphe spinal pathway during 1 s at 40 Hz (pink). C, I-clamp (white background) and V-clamp (blue background) recordings from a motoneuron during application of the 5-HT_{1A} receptor agonist 8-OH-DPAT (40 mM) at different spots on the membrane. The excitability was either tested by depolarizing current pulses (I-clamp; each pulse induced two spikes in control; black) or voltage pulses from −60 mV to −20 mV (V-clamp; each step induced a transient inward current). The release at various positions had no effect except at position 2, where there was a decrease in excitability.

We initiated a new study aimed at understanding the inhibitory function of 5-HT (article (9)). To figure out if both 5-HT_{1A} and 5-HT_2 receptors are activated during synaptic release of 5-HT, we induced stimulation of the raphe spinal pathway. As previously shown (article (6)), we found that a brief stimulation (1s) induced an increased excitability of motoneurons (figure 8A), while a prolonged stimulation (3 min.) decreased their excitability by activating 5-HT_{1A} receptors located at the initial segment (figure 8C). A detailed pharmacological and electrophysiological analysis showed that the activation of 5-HT_{1A} receptors at the axon initial segment (AIS) decreases action potential genesis by inhibiting the activity of Na+ channels. We showed by immunohistochemical staining that, in contrast to the somato-dendritic compartments, the AIS is devoid of serotonergic innervation. This suggests that the 5-HT_{1A} receptors are activated by 5-HT spillover. We tested this hypothesis by means of cyclic voltammetry. We monitored the variation in the extracellular concentration of 5-HT with a carbon fiber electrode positioned in the vicinity of motoneurons. We demonstrated that during prolonged stimulation of the raphe spinal pathway, the extracellular concentration of 5-HT increases sufficiently for activating 5-HT_{1A} receptors. Our results suggest that by decreasing the gain of motoneurons, 5-HT induces central fatigue.

4. CONCLUDING REMARKS

During movement, motoneurons receive descending and peripheral synaptic inputs that depolarize the membrane and induce the genesis of action potentials. If we assume that the release of 5-HT is positively correlated to motor activity as suggested by several studies (56), our results suggest that 5-HT during moderate physical activity facilitates muscle contraction by increasing the gain of motoneurons through modulation of several conductances. During more intense activity, more 5-HT is released and the reuptake mechanisms saturate. As a result, spillover now allows 5-HT to reach the AIS of motoneurons (figure 9). Here, activated 5-HT_{1A} receptors inhibit firing and, thereby, muscle contraction. This is the first identified cellular mechanism for central fatigue, a form of motor fatigue that does not depend on muscles (57, 58). Its function might be to secure rotation of motor units during prolonged contractions (59) and to prevent hyperactivity of muscles (60). The use of the same transmitter for both facilitating and inhibiting motoneurons makes the system particularly robust and reliable.

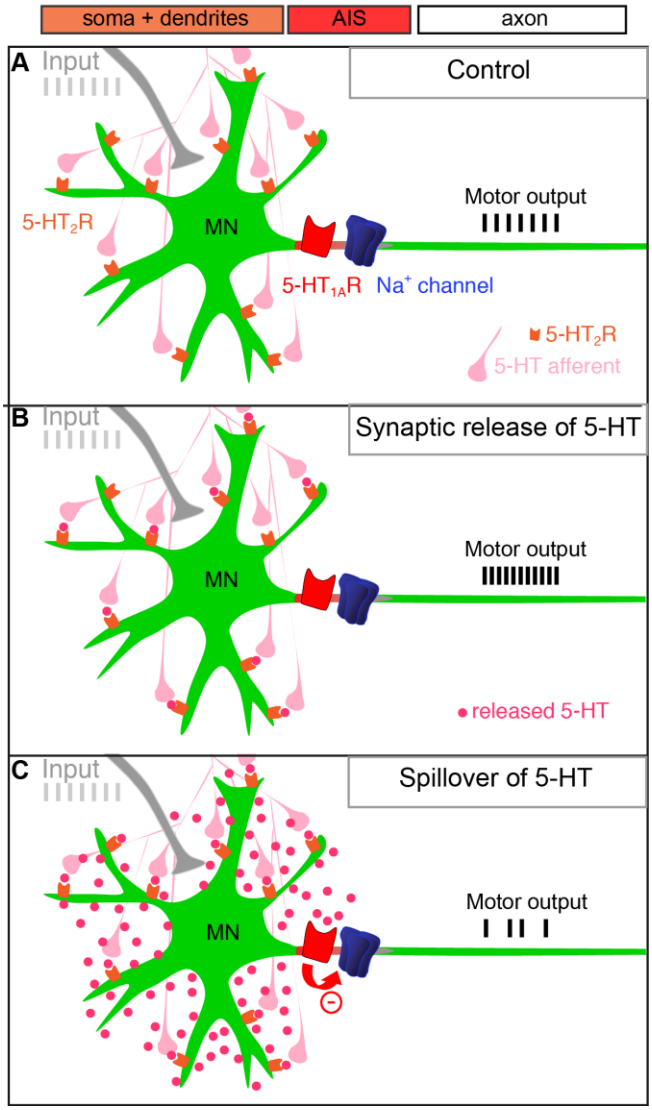


Figure 9: Spillover of serotonin activates 5-HT_{1A} receptors at the AIS and induces central fatigue

A, Scheme of a MN in control. During a motor exercise, 5-HT boutons located over the somatodendritic compartments (but not at the AIS) release 5-HT. B, The 5-HT_2 receptors that are synaptically activated facilitate the firing. C, When the level of 5-HT released increases, it induces spillover. 5-HT activates 5-HT1A receptors at the AIS, which, in turn, inhibit a Na^+ current. The 5-HT_{1A} receptors effect overcomes the excitatory effect induced by 5-HT_2 receptors. The motoneuron output is then decreased

5. METHODS

PREPARATIONS

Slice preparation from the spinal cord of the turtle

Most experiments presented in the dissertation were performed on the spinal cord of the adult turtle (*Chrysemys picta* and *Chrysemys scripta elegans*). After anesthesia induced by an intraperitoneal injection of 100mg sodium barbiturate, animals were killed by decapitation. Preparations were perfused in a solution containing (in mM): 120 NaCl; 5 KCl; 15 $NaHCO_3$; 2 MgCl2; 3 $CaCl_2$ and 20 glucose saturated with 98% O_2 and 2% CO_2 to obtain pH 7.6. Slices (1-2mm thick) were made from the lumbar enlargement of the spinal cord of the turtle.

Turtle brainstem spinal cord preparation

In a series of experiments, the portion of the central nervous system consisting of the caudal half of the brainstem and the cervical enlargement of the spinal cord of the turtle was placed in vitro without removing the surrounding bone. Most of the surrounding muscles were removed. Remaining parts were paralyzed with gallamine triesthiodide (20mg/ml; Sigma). The sides of rostral and caudal ends of the preparation were glued to a Plexiglas plate so that the cut surfaces of the spinal cord and the brainstem faced upwards. A catheter inserted in the subdural space at the level of the brainstem allowed continuous perfusion of ringer.

Spinal cord of the frog left attached with one hindlimb

A series of experiment was performed on the isolated adult frog (*Rana catesbiana*) spinal cord with attached hindlimb. After spinalization performed under anesthesia, hindlimbs muscles were implanted with bipolar EMG electrodes (*semitendinosus* (ST), *sartorius* (SA), *rectus internus* (RI), *vastus internus* (VI), *semimembranosus* (SM), *vastus externus* (VE), *biceps femoris* (BF), and *iliopsoas* (IP)). Frogs were perfused intracardially with ice cold oxygenated (95% O_2 and 5% CO_2) Ringer's solution (113mM NaCl, 2mM KCl, 20mM $NaHCO_3$, 5.5mM glucose, 1mM $MgCl_2$, 2mM $CaCl_2$, pH adjusted to 7.4-7.6). The spinal cord was exposed by ventral laminectomy. The 7-9th dorsal and ventral roots innervating the right hindlimb were dissected in continuity with the dorsal root ganglia and peripheral nerves in order to maintain the main sensory and motor innervation of the hindlimb. All other spinal nerves were cut, the left hindlimb excised, and the spinal cord removed from the spinal column. The spinal cord and hindlimb were placed in separated chambers. The descending aorta was catheterized in order to continuously perfuse the hindlimb with oxygenated Ringer's. Withdrawal reflexes, characterized by predominant activation of ST and BF were evoked by electrical stimulation (500-1000ms trains, 0.1-1.0ms pulses, 5-20V, 10-30Hz) applied across bipolar electrodes placed on the skin of the foot. Stimulation was applied at long, constant intervals (30-120s) to minimize habituation and potentiation of behavioral strength.

RECORDING TECHNIQUES

Recording with sharp electrodes

For all the preparations, intracellular recording of motoneurons in current clamp mode was performed with sharp electrode (30-60 MΩ) filled with either K-acetate (1M) or a mixture of K-Acetate and KCl. Recordings were made in current-clamp using an Axoclamp 2B amplifier (Axon Instruments).
Voltage-clamp recordings were performed in discontinuous service mode at a sample rate of 6-12 kHz, gain of 0.7-1.5 nA.mV-1 and low-pass filter of 0.1 kHz.

Recording with patch electrodes

The patch pipette solution (in mM: K-gluconate, 122; $MgCl_2$, 2.5; Mg-gloconate, 5.6; K-HEPES, 5; H-HEPES, 5; Na_2ATP, 5; EGTA, 1; biocytine, 2.5; K-OH to adjust the pH to 7.4) contained the fluorescent dye Alexa 488 (250 µM, Sigma), to stain the recorded cells and visualize their dendritic arborization. Electrodes had a resistance ranging from 5 to 18 MΩ that was automatically compensated. Neurons were recorded with a Multiclamp 700B amplifier (Molecular Devices, USA). Recordings were sampled at 10-50 kHz. Voltage clamp recordings were low-pass filtered at a cutoff frequency of 3 KHz (Bessel filter). Data were sampled with a 16-bit analog-to-digital converter (DIGIDATA 1322A, MDS) and displayed

by means of the Clampex 9.2 software (MDS). Fast glutamate and GABA synaptic transmission was blocked by CNQX (20-25 µM, Tocris), AP5 (50 µM, Tocris), Strychnine (10 µM, Sigma), SR 95531 hydrobromide (Gabazine; 10 µM, Tocris).

Recording of Xenopus oocytes

Currents were measured by using the two-electrode voltage-clamp technique. Oocytes were impaled with a current electrode and a voltage clamp electrode pulled from borosilicate glass capillaries on a horizontal patch electrode puller (Zeitz Instruments, Munich, Germany). Both electrodes had a tip resistance of 0.5–2.5 MΩ when filled with 1 M KCl and were connected to a two-electrode voltage-clamp amplifier (Dagan CA-1B). During the experiments, oocytes were placed in a chamber connected to a continuous flow system (6 ml/min). Oocytes were voltage clamped at -5 mV. SK channels were activated by $CdCl_2$ (9.2 nl, 100 mM) injected intracellularly with a Nanoject injector. Cd^{2+} is as efficient as injection of Ca^{2+} in activating SK channels expressed in oocytes (Grunnet et al., 2002). Cd^{2+} was used because it minimizes the activation of Ca2+-activated Cl^- currents (Grunnet et al., 2002).

EMG recording in the frog preparation

For quantification of behavior, recorded EMG activity was rectified and digitally filtered (10Hz low pass Butterworth filter). Onsets and offsets of individual muscle activations following cutaneous stimulation were identified visually using custom analyses written in Matlab (Mathworks). Any responses with multiple bursts of muscle activity were excluded from analysis. The duration of response for a muscle on a particular trial was taken as the time between its onset and offset. The activity of each muscle was also integrated from onset to offset to obtain a measure of the total intensity of the evoked response. For the analyses described below, we used the duration and intensity of ST activity to characterize the behavior, since this muscle was most reliably activated between different frogs and between different trials. Durations of intracellular depolarizations following stimulation were also identified visually for each trial using custom software. For all statistical tests we used the conservative significance level of $\alpha = 0.01$, in order to minimize type I errors.

Voltammetry

A carbon-fiber electrode was placed in the lateral part of the ventral horn of a slice (1500 µm thick). Evoked extracellular 5-HT signals were detected using fast-scan cyclic voltammetry (FCV) with a Millar voltammeter (Julian Millar, Barts and the London School of Medicine and Dentistry, UK) and 10 µm-diameter carbon fiber microelectrodes (tip length 50 -100 µm, fabricated in house). The scanning voltage was a triangular waveform from -700 mV to +1300 mV to -700 mV vs. Ag/AgCl at a scan rate of 800 V/sec and scan frequency of 8 Hz. Between scans, the electrode was switched out of comparison of the peak potentials for oxidation and dual reduction currents against those seen in 5-HT calibration solutions (500 nM 5-HT in experimental media). These were typically +600 mV for the oxidation current and -60 mV and -560 mV respectively for the dual reduction currents (vs. Ag/AgCl).

STIMULATION

Stimulation with bipolar electrodes

The nucleus raphe inferior in the brainstem spinal cord preparation, or the dorsolateral funiculus in the spinal cord preparation were stimulated by means of electrical chocks applied with a bipolar electrode positioned on the surface of the tissue.

Stimulation applied with an electrical field

Selective depolarization or hyperpolarization of part of the dendritic tree of neurons from slice preparations was performed by applying an electrical field through the slice. This was achieved by means of two silver electrodes positioned laterally to the slice. The electric field was applied in the lateral direction of motoneuronal dendrites. With this orientation of the electric field, maximal polarization is obtained in the distal compartments of lateral and medial dendrites while an indifferent point with no change in membrane potential is located proximally in lateral dendrites (61, 62). With the cathode lateral to the ventral horn recorded from, the lateral dendrites are depolarized while the medial dendrites and the soma are hyperpolarized. This configuration, that allows a selective depolarization of part of the dendritic compartment, is termed a soma-hyperpolarizing field (S-). When the recording electrode was withdrawn from a motoneuron the extracellular potentials induced by the fields applied during intracellular recording were measured. Offline, the transmembrane potential was obtained as the difference between intra and extracellular potentials for each intensity of the electric field applied.

PHARMACOLOGY

For most of the experiments, the effects of drugs were tested by superfusion of the whole preparation (slices, spinal cord attached with one leg). For the brainstem spinal cord, drugs were applied at the cut end of the spinal cord by means of a local superfusion system consisting in an inlet and an outlet. The continuous perfusion of the spinal cord with ringer prevented drugs to penetrate the tissue deeply.

For all the experiments performed on slice preparation, the synaptic potentials were inhibited by blocking fast synaptic receptors with a mixture of CNQX (25µM), Strychnine (10µM), DL-AP5 (50µM) or DL-AP7 (25µM).

Microiontophoresis

Focal pharmacology was peformed with microiontophoresis technique. Micropipettes filled with 150 mM serotonin hydrochloride, pH 4-4.5 were positioned either close to the cell body or close to a dendrite of a motoneuron recorded in a slice preparation.

Other drugs used

(+/)-1-[2,5]-dimethoxy-4-iodophenyl-2-aminopropane (DOI; 10µM); (+/-)-2-Dipropylamino-8-hydroxy-1,2,3,4-tetrahydronaphthalene hydrobromide (8-OH-DPAT; 10µM); (RS)-1-aminoindan-1, 5-dicarboxylic acid (ADA; 20 µM); (S)-alpha-methyl-4-carboxyphenylglycine (MCPG; 0.5 mM); 1-(2-methoxyphenyl)-4-(4-phthalimidobutyl) piperazine HBr (NAN-190; 12.5-25 µM); Arachidonylethanolamide (Anandamide; 10µM); atropine (0.1 µM); BAPTA-AM (20-100 µM); BayK8644 (1-2 µM); Cesium chloride (CsCl; 1mM); cis-(+/-)-1-aminocyclopentane-1,3-dicarboxylic acid (cis-ACPD; 40µM); Cobalt (2mM); Hepes sodium salt (N-[2-hydroxyethyl]piperazine-N'-[2-ethanesulfonic acid]); kynurenic acid (0.5 mM); Lidocaine N-Ethyl Bromide (QX314; 0.1M); Muscarine (20 µM); N-(6-Aminohexyl)-5-chloro-1-napthalenesulfonamide, HCl (W-7; 50-200 µM); N-[2-[4-(2-Methoxyphenyl)-1-piperazinyl]ethyl]-N-2-pyridinylcyclohexanecarboxamide (WAY-100635; 10µM); Nifedipine (10-40 µM); N-methyl-D-glucamine chloride (NMDG); pindobind-5-HT_{1A} (10 µM); SB 206553 hydrochloride (10µM); Serotonin (10µM); tetra-ethyl-ammonium (TEA; 10 mM);

tetrodotoxin (TTX; 1-2 µM); Trifluoperazine (250-500µM); ZD7288 (100µM); ω-conotoxin (1-2 µM).

IMMUNOHISTOCHEMISTRY

Neurons were filled with biocytin during their recording with patch. Slices were fixed in paraformaldehyde (1%; 30 minutes at 4°C) and then washed three times in Phosphate-buffered saline (PBS). After 1 h of incubation in a blocking solution (0.1 % triton X100, 1 % human serum albumin), recorded neurons were revealed staining with streptavidin coupled to Alexa 488 (1:200; sigma). Motoneurons were identified by immunohistochemical staining with a polyclonal antibody directed against Choline Acetyl Transferase (ChAT, 1:50, host goat; Chemicon).

PanNaV

High densities of sodium ion channels were detected with a monoclonal antibody directed against all isoforms of voltage-sensitive sodium channels (PanNaV, 1:50, host mouse; Sigma). The primary antibodies were added to the solution and let for incubation during 6 days at 4°C. After washout, the slices were incubated with the secondary antibodies (Donkey anti goat 647; 1:500; Molecular Probes) during 4 hours at room temperature and then mounted on slides using vectashield medium to protect the fluorescence (Vector Laboratories, California, USA). Large ChAT positive cells present in the lateral part of the ventral horn were considered as motoneurons.

Serotonin

Slices were incubated with a primary antibody anti-5-HT, 1:100, Chemicon for 6-7 days at 4°C after fixation and blockage steps. A secondary antibody (anti-rat Alexa 488, 1:200; Invitrogen; anti-mouse Cy5, 1:500; Chemicon) was added to the bath for 2 hours at room temperature. Pictures were obtained with a confocal microscope (Leica TCS SP2).

MOLECULAR BIOLOGY

Expression of SK channels in Xenopus oocytes

To obtain robust expression in Xenopus laevis oocytes, cDNAs encoding for h5-HT_{1A} and hSK1, rSK2, and rSK3 were subcloned into the oocyte and mammalian expression vector pXOOM, which contains the 5' and 3' untranslated regions from the Xenopus laevis β-globin as well as a poly-A segment. For in vitro transcription, the plasmids were linearized downstream of the poly-A segment, and mRNA was synthesized from the T7 RNA polymerase promoter by using the mCAP mRNA capping kit (Stratagene, La Jolla, CA). mRNAs were phenol/chloroform extracted, ethanol precipitated, and dissolved in TE buffer to a concentration of approximately 1 µg/µl. The integrity of the transcripts was confirmed by agarose gel electrophoresis, and the mRNA was stored at –80°C until injection.

Oocyte treatment

Oocytes were kept in Kulori medium (90 mM NaCl, 1 mM KCl, 1 mM MgCl2, 1 mM CaCl2, 5 mM HEPES, pH 7.4) for 24 hr at 19°C before injection of 50 nl mRNA (approximately 50 ng) with a Nanoject microinjector (Drummond). For coexpression of SK channels and 5-HT_{1A} receptors, molar ratios between 1:1 and 1:5 were applied. Oocytes were kept at 19°C in Kulori medium for 2–7 days before measurements were performed.

6. ABSTRACT

Serotonin is a major neuromodulator in the central nervous system involved in most physiological functions including appetite regulation, sexual arousal, sleep regulation and motor control. The activity of neurons from the raphe spinal tract, which release serotonin on motoneurons, is positively correlated with motor behaviour.

During moderate physical activity, serotonin is released from synaptic terminals onto the dendrites and cell bodies of motoneurons. Serotonin increases the excitability of motoneurons and thereby facilitate muscle contraction by acting on several parallel intracellular pathways. By activating 5-HT_{1A} receptors, serotonin inhibits TWIK-related acid-sensitive potassium channels and small conductance calcium-activated potassium channels. In parallel, serotonin binds to 5-HT_2 receptors, which promotes the low-threshold L-type Ca^{2+} channels. During intense physical activity, more serotonin is released. The reuptake systems saturate and serotonin spills over to reach extrasynaptic 5-HT_{1A} receptors located on the axon initial segment of motoneurons. This in turn induces the inhibition of the Na^+ channels responsible for the initiation of action potentials. Fewer nerve impulses are generated and muscle contraction becomes weaker. By decreasing the gain of motoneurons, serotonin triggers central fatigue.

7. REFERENCES

1. Perrier JF, Hounsgaard J. Ca(2+)-activated non-selective cationic current (I(CAN)) in turtle motoneurons. J Neurophysiol. 1999;82(2):730-5. Epub 1999/08/13. PubMed PMID: 10444670.

2. Perrier JF, Mejia-Gervacio S, Hounsgaard J. Facilitation of plateau potentials in turtle motoneurones by a pathway dependent on calcium and calmodulin. J Physiol. 2000;528 Pt 1:107-13. Epub 2000/10/06. doi: PHY_1060 [pii]. PubMed PMID: 11018109; PMCID: 2270105.

3. Perrier JF, Hounsgaard J. 5-HT2 receptors promote plateau potentials in turtle spinal motoneurons by facilitating an L-type calcium current. J Neurophysiol. 2003;89(2):954-9. Epub 2003/02/08. doi: 10.1152/jn.00753.2002. PubMed PMID: 12574471.

4. Perrier JF, Alaburda A, Hounsgaard J. 5-HT1A receptors increase excitability of spinal motoneurons by inhibiting a TASK-1-like K+ current in the adult turtle. J Physiol. 2003;548(Pt 2):485-92. Epub 2003/03/11. doi: 10.1113/jphysiol.2002.037952 2002.037952 [pii]. PubMed PMID: 12626670; PMCID: 2342869.

5. Grunnet M, Jespersen T, Perrier JF. 5-HT1A receptors modulate small-conductance Ca2+-activated K+ channels. J Neurosci Res. 2004;78(6):845-54. Epub 2004/11/03. doi: 10.1002/jnr.20318. PubMed PMID: 15521063.

6. Perrier JF, Delgado-Lezama R. Synaptic release of serotonin induced by stimulation of the raphe nucleus promotes plateau potentials in spinal motoneurons of the adult turtle. J Neurosci. 2005;25(35):7993-9. Epub 2005/09/02. doi: 25/35/7993 [pii]10.1523/JNEUROSCI.1957-05.2005. PubMed PMID: 16135756.

7. Perrier JF, Tresch MC. Recruitment of motor neuronal persistent inward currents shapes withdrawal reflexes in the frog. J Physiol. 2005;562(Pt 2):507-20. Epub 2004/11/06. doi: jphysiol.2004.072769 [pii] 10.1113/jphysiol.2004.072769. PubMed PMID: 15528248; PMCID: 1665506.

8. Perrier JF, Cotel F. Serotonin differentially modulates the intrinsic properties of spinal motoneurons from the adult turtle. J Physiol. 2008;586(5):1233-8. Epub 2007/12/22. doi: jphysiol.2007.145706 [pii] 10.1113/jphysiol.2007.145706. PubMed PMID: 18096602; PMCID: 2375675.

9. Cotel F, Exley R, Cragg SJ, Perrier JF. Serotonin spillover onto the axon initial segment of motoneurons induces central fatigue by inhibiting action potential initiation. Proc Natl Acad Sci U S A. 2013;110(12):4774-9. Epub 2013/03/15. doi: 10.1073/pnas.1216150110. PubMed PMID: 23487756.

10. Perrier JF, Rasmussen HB, Christensen RK, Petersen AV. Modulation of the intrinsic properties of motoneurons by serotonin. Current pharmaceutical design. 2013;19(24):4371-84. Epub 2013/01/31. PubMed PMID: 23360270.

11. Hounsgaard J, Mintz I. Calcium conductance and firing properties of spinal motoneurones in the turtle. J Physiol. 1988;398:591-603. Epub 1988/04/01. PubMed PMID: 2455804; PMCID: 1191789.

12. London M, Hausser M. Dendritic computation. Annu Rev Neurosci. 2005;28:503-32. Epub 2005/07/22. doi: 10.1146/annurev.neuro.28.061604.135703. PubMed PMID: 16033324.

13. Delgado-Lezama R, Perrier JF, Nedergaard S, Svirskis G, Hounsgaard J. Metabotropic synaptic regulation of intrinsic response properties of turtle spinal motoneurones. J Physiol. 1997;504 (Pt 1):97-102. Epub 1997/11/14. PubMed PMID: 9350621; PMCID: 1159939.

14. Svirskis G, Hounsgaard J. Transmitter regulation of plateau properties in turtle motoneurons. J Neurophysiol. 1998;79(1):45-50. Epub 1998/02/21. PubMed PMID: 9425175.

15. Bowker RM, Westlund KN, Coulter JD. Origins of serotonergic projections to the spinal cord in rat: an immunocytochemical-retrograde transport study. Brain Res. 1981;226(1-2):187-99. Epub 1981/12/07. doi: 0006-8993(81)91092-1 [pii]. PubMed PMID: 7028211.

16. Bowker RM, Westlund KN, Coulter JD. Serotonergic projections to the spinal cord from the midbrain in the rat: an immunocytochemical and retrograde transport study. Neurosci Lett. 1981;24(3):221-6. Epub 1981/07/17. PubMed PMID: 6168977.

17. Alvarez FJ, Pearson JC, Harrington D, Dewey D, Torbeck L, Fyffe RE. Distribution of 5-hydroxytryptamine-immunoreactive boutons on alpha-motoneurons in the lumbar spinal cord of adult cats. J Comp Neurol. 1998;393(1):69-83. Epub 1998/03/31. doi: 10.1002/(SICI)10969861(19980330)393:1<69::AID-CNE7>3.0.CO;2-O [pii]. PubMed PMID: 9520102.

18. Jacobs BL, Fornal CA. Serotonin and motor activity. Curr Opin Neurobiol. 1997;7(6):820-5. Epub 1998/02/18. doi: S0959-4388(97)80141-9 [pii]. PubMed PMID: 9464975.

19. Veasey SC, Fornal CA, Metzler CW, Jacobs BL. Response of serotonergic caudal raphe neurons in relation to specific motor activities in freely moving cats. J Neurosci. 1995;15(7 Pt 2):5346-59. Epub 1995/07/01. PubMed PMID: 7623157.

20. McCall RB, Aghajanian GK. Serotonergic facilitation of facial motoneuron excitation. Brain Res. 1979;169(1):11-27. PubMed PMID: 156574.

21. White SR, Neuman RS. Facilitation of spinal motoneurone excitability by 5-hydroxytryptamine and noradrenaline. Brain Res. 1980;188(1):119-27. PubMed PMID: 7370747.

22. Katakura N, Chandler SH. An iontophoretic analysis of the pharmacologic mechanisms responsible for trigeminal motoneuronal discharge during masticatory-like activity in the guinea pig. J Neurophysiol. 1990;63(2):356-69. PubMed PMID: 1968966.

23. Takahashi T, Berger AJ. Direct excitation of rat spinal motoneurones by serotonin. J Physiol. 1990;423:63-76. Epub 1990/04/01. PubMed PMID: 1696989; PMCID: 1189746.

24. Kjaerulff O, Kiehn O. 5-HT modulation of multiple inward rectifiers in motoneurons in intact preparations of the neonatal rat spinal cord. J Neurophysiol. 2001;85(2):580-93. Epub 2001/02/13. PubMed PMID: 11160495.

25. Hounsgaard J, Kiehn O. Serotonin-induced bistability of turtle motoneurones caused by a nifedipine-sensitive calcium plateau potential. J Physiol. 1989;414:265-82. Epub 1989/07/01. PubMed PMID: 2607432; PMCID: 1189141.

26. Wang MY, Dun NJ. 5-Hydroxytryptamine responses in neonate rat motoneurones in vitro. J Physiol. 1990;430:87-103. Epub 1990/11/01. PubMed PMID: 2150862; PMCID: 1181729.

27. Ziskind-Conhaim L, Seebach BS, Gao BX. Changes in serotonin-induced potentials during spinal cord development. J Neurophysiol. 1993;69(4):1338-49. Epub 1993/04/01. PubMed PMID: 8388043.

28. Hsiao CF, Trueblood PR, Levine MS, Chandler SH. Multiple effects of serotonin on membrane properties of trigeminal motoneurons in vitro. J Neurophysiol. 1997; 77(6):2910-24. Epub 1997/06/01. PubMed PMID: 9212246.

29. Berger AJ, Bayliss DA, Viana F. Modulation of neonatal rat hypoglossal motoneuron excitability by serotonin. Neurosci Lett. 1992;143(1-2):164-8. Epub 1992/08/31. PubMed PMID: 1436663.

30. Bayliss DA, Umemiya M, Berger AJ. Inhibition of N- and P-type calcium currents and the after-hyperpolarization in rat motoneurones by serotonin. J Physiol. 1995;485 (Pt 3):635-47. Epub 1995/06/15. PubMed PMID: 7562606; PMCID: 1158033.

31. Wikstrom M, Hill R, Hellgren J, Grillner S. The action of 5-HT on calcium-dependent potassium channels and on the spinal locomotor network in lamprey is mediated by 5-HT1A-like receptors. Brain Res. 1995;678(1-2):191-9. Epub 1995/04/24. doi: 0006-8993(95)00183-Q [pii]. PubMed PMID: 7620887.

32. Talley EM, Sadr NN, Bayliss DA. Postnatal development of serotonergic innervation, 5-HT1A receptor expression, and 5-HT responses in rat motoneurons. J Neurosci. 1997; 17(11):4473-85. Epub 1997/06/01. PubMed PMID: 9151764.

33. Phillis JW, Tebecis AK, York DH. Depression of spinal motoneurones by noradrenaline, 5-hydroxytryptamine and histamine. Eur J Pharmacol. 1968;4(4):471-5. Epub 1968/11/01. PubMed PMID: 4302133.

34. Farris JW, Hinchcliff KW, McKeever KH, Lamb DR, Thompson DL. Effect of tryptophan and of glucose on exercise capacity of horses. J Appl Physiol. 1998;85(3):807-16. Epub 1998/09/08. PubMed PMID: 9729551.

35. Soares DD, Lima NR, Coimbra CC, Marubayashi U. Evidence that tryptophan reduces mechanical efficiency and running performance in rats. Pharmacol Biochem Behav. 2003;74(2):357-62. Epub 2002/12/14. doi: S0091305702010031 [pii]. PubMed PMID: 12479955.

36. Bailey SP, Davis JM, Ahlborn EN. Neuroendocrine and substrate responses to altered brain 5-HT activity during prolonged exercise to fatigue. J Appl Physiol. 1993;74(6):3006-12. Epub 1993/06/01. PubMed PMID: 8366000.

37. Perrier JF, Hounsgaard J. Development and regulation of response properties in spinal cord motoneurons. Brain Res Bull. 2000;53(5):529-35. Epub 2001/02/13. doi: S0361-9230(00)00386-5 [pii]. PubMed PMID: 11165788.

38. Hille B. Ion Channels of Excitable Membranes. Third ed: Sinauer Associates; 2001. 814 p.

39. Patel AJ, Honore E. Properties and modulation of mammalian 2P domain K+ channels. Trends Neurosci. 2001;24(6):339-46. PubMed PMID: 11356506.

40. Zhang L, Krnjevic K. Apamin depresses selectively the after-hyperpolarization of cat spinal motoneurons. Neurosci Lett. 1987;74(1):58-62. Epub 1987/02/10. PubMed PMID: 2436107.

41. Hounsgaard J, Kiehn O, Mintz I. Response properties of motoneurones in a slice preparation of the turtle spinal cord. J Physiol. 1988;398:575-89. Epub 1988/04/01. PubMed PMID: 2455803; PMCID: 1191788.

42. Sah P. Ca(2+)-activated K+ currents in neurones: types, physiological roles and modulation. Trends Neurosci. 1996;19(4):150-4. Epub 1996/04/01. PubMed PMID: 8658599.

43. Hultborn H, Wigstrom H, Wangberg B. Prolonged activation of soleus motoneurones following a conditioning train in soleus Ia afferents - A case for a reverberating loop? Neurosci Lett. 1975;1(3):147-52. PubMed PMID: 19604777.

44. Rekling JC, Funk GD, Bayliss DA, Dong XW, Feldman JL. Synaptic control of motoneuronal excitability. Physiol Rev. 2000;80(2):767-852. Epub 2000/04/04. PubMed PMID: 10747207.

45. Partridge LD, Muller TH, Swandulla D. Calcium-activated non-selective channels in the nervous system. Brain Res Brain Res Rev. 1994;19(3):319-25. PubMed PMID: 7820135.

46. Eckert R, Chad JE. Inactivation of Ca channels. Prog Biophys Mol Biol. 1984;44(3):215-67. PubMed PMID: 6095365.

47. Ehlers MD, Augustine GJ. Cell signalling. Calmodulin at the channel gate. Nature. 1999;399(6732):105, 7-8. doi: 10.1038/20076. PubMed PMID: 10335837.

48. Peterson BZ, DeMaria CD, Adelman JP, Yue DT. Calmodulin is the Ca2+ sensor for Ca2+ -dependent inactivation of L-type calcium channels. Neuron. 1999;22(3):549-58. PubMed PMID: 10197534.

49. Zuhlke RD, Pitt GS, Deisseroth K, Tsien RW, Reuter H. Calmodulin supports both inactivation and facilitation of L-type calcium channels. Nature. 1999;399(6732):159-62. doi: 10.1038/20200. PubMed PMID: 10335846.

50. Barnes NM, Sharp T. A review of central 5-HT receptors and their function. Neuropharmacology. 1999;38(8):1083-152. Epub 1999/08/26. PubMed PMID: 10462127.

51. Kim D, Murray M, Simansky KJ. The serotonergic 5-HT(2C) agonist m-chlorophenylpiperazine increases weight-supported locomotion without development of tolerance in rats with spinal transections. Exp Neurol. 2001;169(2):496-500. Epub 2001/05/19. doi: 10.1006/exnr.2001.7660 S0014-4886(01)97660-5 [pii]. PubMed PMID: 11358463.

52. Miller JF, Paul KD, Lee RH, Rymer WZ, Heckman CJ. Restoration of extensor excitability in the acute spinal cat by the 5-HT2 agonist DOI. J Neurophysiol. 1996;75(2):620-8. Epub 1996/02/01. PubMed PMID: 8714639.

53. Conway BA, Hultborn H, Kiehn O, Mintz I. Plateau potentials in alpha-motoneurones induced by intravenous injection of L-dopa and clonidine in the spinal cat. J Physiol. 1988;405:369-84. Epub 1988/11/01. PubMed PMID: 3255795; PMCID: 1190980.

54. Hounsgaard J, Hultborn H, Jespersen B, Kiehn O. Bistability of alpha-motoneurones in the decerebrate cat and in the acute spinal cat after intravenous 5-hydroxytryptophan. J Physiol. 1988;405:345-67. Epub 1988/11/01. PubMed PMID: 3267153; PMCID: 1190979.

55. Bennett DJ, Li Y, Siu M. Plateau potentials in sacrocaudal motoneurons of chronic spinal rats, recorded in vitro. J Neurophysiol. 2001;86(4):1955-71. PubMed PMID: 11600653.

56. Jacobs BL, Fornal CA. 5-HT and motor control: a hypothesis. Trends Neurosci. 1993;16(9):346-52. Epub 1993/09/01. PubMed PMID: 7694403.

57. Reid C. The Mechanism of Voluntary Muscular Fatigue. Br Med J. 1927;2(3481):545-6. Epub 1927/09/24. PubMed PMID: 20773416; PMCID: 2524887.

58. Butler JE, Taylor JL, Gandevia SC. Responses of human motoneurons to corticospinal stimulation during maximal voluntary contractions and ischemia. J Neurosci. 2003;23(32):10224-30. Epub 2003/11/14. doi: 23/32/10224 [pii]. PubMed PMID: 14614080.

59. Bawa P, Pang MY, Olesen KA, Calancie B. Rotation of motoneurons during prolonged isometric contractions in humans. J Neurophysiol. 2006;96(3):1135-40. Epub 2006/06/16. doi: 01063.2005 [pii]10.1152/jn.01063.2005. PubMed PMID: 16775202.

60. Gandevia SC. Spinal and supraspinal factors in human muscle fatigue. Physiol Rev. 2001;81(4):1725-89. Epub 2001/10/03. PubMed PMID: 11581501.

61. Hounsgaard J, Kiehn O. Calcium spikes and calcium plateaux evoked by differential polarization in dendrites of turtle motoneurones in vitro. J Physiol. 1993;468:245-59. Epub 1993/08/01. PubMed PMID: 8254508; PMCID: 1143824.

62. Svirskis G, Gutman A, Hounsgaard J. Detection of a membrane shunt by DC field polarization during intracellular and whole cell recording. J Neurophysiol. 1997;77(2):579-86. Epub 1997/02/01. PubMed PMID: 9065831.