ELECTROPHYSIOLOGICAL PROPERTIES OF DORSAL RAPHE NUCLEUS NEURONS IN 5-HTT$^{+/+}$ AND 5-HTT$^{-/-}$ MICE

BEATRICE MIHAELA MACRI[*], *T.D. JACQUIN*[**], *MARIA-LUISA FLONTA*[*]

[*]Dept. of Animal Physiology and Biophysics, Faculty of Biology, University of Bucharest, 91–95, Splaiul Independenței, Bucharest, 050095, Romania
[**]UMR 677, INSERM-UPMC, NeuroPsychoPharmacologie, Faculté de Médecine Pitié-Salpêtrière, 91, Boulevard de l'Hôpital, 75634 Paris Cx 13, France

Abstract. A 5-HT transporter knockout (5-HTT$^{-/-}$) mouse is a model of whole-life treatment with selective serotonin reuptake inhibitors (SSRIs). In our study, whole-cell patch clamp recordings were performed on dorsal raphe nucleus (DRN) neurons in order to classify these cells depending on their biophysical features. Three distinct types of DRN neurons were recorded in brain stem slices from adult 5-HTT$^{+/+}$ and 5-HTT$^{-/-}$ mice, namely with a linear (type I) or rectifying (type II) I/V relationship, or a time dependent inward rectification (type III). Beside the I/V relationship, other electrophysiological characteristics of these neurons were analyzed (action potential duration and amplitude, membrane time constant of the voltage response to a 45–50 pA hyperpolarizing current pulse, afterhyperpolarisation duration and time constant, spike discharge frequency, current density). Action potential duration in type III neurons is shorter (3.8 ± 0.3 ms) than in type I and type II neurons (4.6 ± 0.6 ms, and 4.7 ± 0.8 ms respectively). Afterhyperpolarisation time constant is shorter in type III (1.2 ± 0.2 ms) than in type II neurons (2.4 ± 0.1 ms). For type I and type II, but not for type III neurons, a decrease of spike discharge frequency and an increase of inward current evoked by hyperpolarizing steps were recorded. Type I- and II-neurons are inhibited by 5-HT$_{1A}$ agonists being serotonergic neurons, while type III-neurons are not. These biophysical characteristics could explain the essential role played by 5-HT$_{1A}$ autoreceptors in the molecular mechanisms of depression.

Key words: dorsal raphe nucleus, 5-HTT mutation, spike discharge frequency, GIRK current density.

INTRODUCTION

Many studies suggest that the central serotonergic system is involved in the regulation of mood [15], notably in depressed patients [5, 14, 20]. Furthemore, the serotonin transporter (5-HTT) is the target of the most widely used antidepressants, i.e. the selective serotonin reuptake inhibitors (SSRIs) [8]. A 5-HT transporter knockout (5-HTT$^{-/-}$) mouse is considered as a model of whole-life treatment with selective serotonin reuptake inhibitors (SSRIs) [2]. In dorsal raphe nucleus, 5-HT$_{1A}$

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receptor immunoreactivity was found exclusively on neuronal cell bodies and dendrites, especially along extrasynaptic portions of their plasma membrane [11], and these receptors are also called *5-HT$_{1A}$* autoreceptors. In the hippocampal formation, both somas and dendrites of pyramidal and granule cells also displayed 5-HT$_{1A}$ immunoreactivity [11], and they are also called postsynaptic *5-HT$_{1A}$* receptors. Double immunohistochemical staining demonstrated that cells endowed with 5-HT$_{1A}$ autoreceptor are the serotonergic neurones [19]. In rat, dorsal and median raphe nuclei contain two distinct type of cells: 5-HT- (also called serotonergic) and non-5-HT (also called non-serotonergic)-containing neurons, with distinct projections, morphology, neurotransmitter-mediated effects and electrophysiological characteristics [1]. These differences may be important for understanding the etiology of neuropsychiatric disorders underlying dysfunction of the neurotransmission between raphe nucleus and hypocampus. Whole cell electrophysiological techniques in combination with fluorescence immunohistochemistry for 5-HT revealed in the dorsal raphe nucleus of rat that non-serotonergic neurons can be classified in three types of cells, while serotonergic neurons contain only the type I and II cells [1]. This classification is based on the biophysical characteristics of these neurons (action potential duration, amplitude and time constant, afterhyperpolarisation duration and time constant, spike discharge frequency, etc.).

Serotonin (5-HT) exerts a negative feed-back control on the activity of 5-HT synthetizing neurons through the stimulation of 5-HT$_{1A}$ autoreceptors. This effect is caused by 5-HT$_{1A}$-evoked hyperpolarization, leading to inhibition of 5-HT neurons firing. This hyperpolarization involves G_i proteins whose activation induces an increase in G protein-dependent inward rectifying potassium (GIRK) current [1, 6, 24] and a decrease in calcium conductance. The selective serotonin reuptake inhibitors (SSRIs) determine a reduced efficacy of 5-HT in inhibiting serotoninergic neurons of the dorsal raphe nucleus (DRN) because they trigger a functional desensitization of these receptors, either by 5-HT$_{1A}$ autoreceptors internalization [17], or by the uncoupling between 5-HT$_{1A}$ receptors and their G-proteins [16]. Disruption of the 5-HTT gene induces an increase in the 5-HT extracellular concentration because of lack of 5-HT reuptake. 5-HT$_{1A}$ autoreceptor tonic activation, which results from the 5-HT extracellular rate increase, causes a functional desensitization of these autoreceptors, as observed after chronic treatment with SSRIs [12].

However, no studies have described these subtypes of neurons in mice, or have assessed their implication in the multitude of adaptive mechanisms of 5-HT$_{1A}$ autoreceptors in response to the 5-HTT-gene disruption.

Using the whole cell patch-clamp technique on brain stem slices, we investigated the functional adaptative properties of somatodendritic 5-HT$_{1A}$ autoreceptors, in different neuron subtypes from wild-type (5-HTT$^{+/+}$) and knockout mice deficient in 5-HTT (5-HTT$^{-/-}$).

MATERIALS AND METHODS

CHEMICALS

NaCl, KCl, NaH_2PO_4, $MgCl_2 \cdot 6H_2O$, $CaCl_2 \cdot 2H_2O$, $NaHCO_3$, D-glucose, sucrose, 5-carboxamidotryptamine [5-CT], were purchased from Sigma-Aldrich Chimie, St Quentin Fallavier, France and N-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-N-(2-pyridinyl) cyclohexane carboxamide [WAY 100635] was purchased from Wyeth-Ayerst, Princeton, NJ, USA. D-glucose, sucrose were freshly added before recording.

ANIMALS

Experiments were performed using females and males of wild-type 5-HTT$^{+/+}$ and homozygous 5-HTT$^{-/-}$ genotype with C57Bl/6 genetic background obtained from heterozygous and homozygous breeding. Genotyping was performed as described by Bengel *et al*. [2]. Animals were used at 2 months of age. In females, although endogenous estrogen levels vary with estrus cycle, animals were used at all periods and data were pooled together. After weaning and sexing, males and females were housed in groups of 6 animals per cage and maintained under standard laboratory conditions (22 ± 1 °C, 60% relative humidity, 12–12 hours light-dark cycle, food and water *ad libitum*). Procedures involving animals and their care conformed to institutional guidelines and complied with national and international laws and policies (French Council Directive n° 87-848, 19 October 1987 of the Ministère de l'Agriculture et de la Forêt, Service Vétérinaire de la Santé et de la Protection Animale, permissions # 75-116 to M.H. and # 7192 to T.D.J., and European Communities Council Directive of 24 November 1986; 86/609/EEC).

RECORDING IN THE DORSAL RAPHE NUCLEUS

Whole-cell recordings in the DRN were obtained from 64 cells of wild-type mice (38 in females and 26 in males) and from 71 cells of 5-HTT$^{-/-}$ mice (39 in females and 32 in males) A total number of 135 animals were included in our research study. Cells were located throughout the rostro-caudal extent of the DRN (Fig. 1; according to [7], plates 66–72), mostly in the dorsal subdivision of the nucleus (proportions versus the total number of recorded cells in each group of mice varied from 84% to 100%).

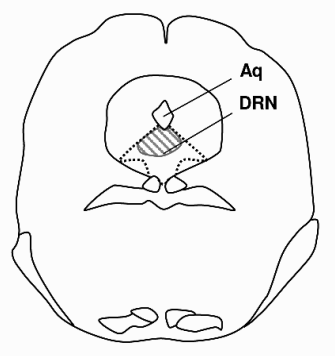

Fig. 1. Localization of the recorded neurons in the dorsal raphe nucleus (DRN). The hatched area shows the localization of the recorded neurons, mostly in the dorsal subdivision of the DRN. The dotted line indicates the limits of the DRN. Aq: aqueduct of Sylvius; plate n° 69 [7].

PREPARATION OF BRAINSTEM SLICES AND NEURONAL RECORDING

Mice were decapitated and the brains were rapidly removed and immersed in an ice-cold (≈4 °C) artificial cerebrospinal fluid (ACSF) of the following composition (in mM): NaCl 128, KCl 3.5, NaH_2PO_4 1.2, $MgCl_2$ 1.3, $CaCl_2$ 2, $NaHCO_3$ 25, D-glucose 11, sucrose 17, maintained at pH 7.4 by continuous bubbling with an O_2/CO_2 mixture (95%/5%). A block of tissue containing the DRN was affixed to the stage of a vibratome (TPI, series 1000) with cyanoacrylate glue, submerged in ice-cold and bubbled ACSF and cut into coronal sections of 250 µm thickness. Brain stem slices were collected in gassed ACSF (continuous bubbling with 95% O_2 / 5% CO_2 mixture, pH 7.4) and maintained at room temperature (22–25 °C). A slice was then transferred into a small recording chamber (2 ml) and covered with a nylon mesh, while continuously perfused with gassed ACSF (22–25 °C) at a constant flow rate of 3 ml/min. Recording electrodes (4–6 MΩ) were pulled from borosilicate glass tubes (Phymep, France) on a PP-830 micropipette puller (Narishige, Japan), filled with internal solution containing (in mM): KH_2PO_4 108, $CaCl_2$ 1, $MgCl_2$ 1, EGTA 11, Hepes 10, Mg-ATP 4, Na-GTP 0.4, adjusted to pH 7.4 with 36 mM KOH (final K^+ concentration 144 mM), and kept in ice until use. Electrodes were advanced towards DRN neurons under visual guidance, using an upright microscope (Nikon E600FN, Japan). Pipette voltage offset was neutralized prior to the formation of a gigaohm seal and was not further corrected. Patch-clamp recordings were performed in whole-cell configuration and signals were amplified with an Axopatch 200B amplifier (Axon Instruments, Union City, CA, USA) using fast current-clamp and voltage-clamp modes. Signals were low-pass filtered at 2 kHz, digitized at 15 kHz and analyzed with a computer using a Digidata 1200 interface coupled to the pCLAMP 9 software (Axon Instruments, Union City, CA, USA). Input resistance (R_m), series resistance (R_S) and membrane capacitance (C_m) were determined from current transients elicited by a 5 mV

depolarizing step from a holding potential of –60 mV. Criteria for cell inclusion [1, 4, 24] in the study were as follows: R_m > 100 MΩ and stable R_S (≤ 20 MΩ), C_m (30–55 pF) and spike amplitude (≥ 55 mV). It was also measured the membrane time constant of the voltage response to a 45–50 pA hyperpolarizing current pulse (hyperpolarization voltage time constant – HV time constant). The neurons were considered healthy when maintaining the same cellular characteristics, except drug-induced modifications. Liquid junction potential error [18] was calculated using pCLAMP 9.2 (–13.9 mV) and was corrected. In current-clamp mode, the average spike discharge frequency was regularly monitored and determined for at least 2 min at various times of recording. Starting from a holding potential of of –60 mV and using voltage-clamp mode, voltage steps from – 30 mV to –125 mV, with 5 mV increment and 1.2 s duration, were applied in order to hyperpolarize the neuronal membrane, which in turn activates the G-protein-dependent inwardly rectifying potassium current. Steady-state currents were plotted against voltage (I/V curves). Current density (pA/pF) was computed during the steady-state current induced by voltage steps down to –125 mV. Corresponding values are expressed as means ± S.E.M. with Prism 2.01 software (Graphpad, San Diego, CA, USA); *n* refers to the number of observations, versus the number of neurons tested. Comparisons between 2 groups of results were performed using paired or unpaired Student's *t*-test, as appropriate. In all cases, significance was assessed at the 0.05 (*) *p*-value level.

RESULTS

ELECTRORESPONSIVE PROPERTIES AND PUTATIVE UNDERLYING CURRENTS

When DRN neurons were voltage clamped close to their resting potential ($V_{holding}$ = –60 mV), increasing hyperpolarizing step voltage commands with duration of 1.2 s elicited an inward current of increasing amplitude in females and males in both 5-HTT$^{+/+}$ (*n* = 64) and 5-HTT$^{-/-}$ (*n* = 71) mice (Fig. 2).

DRN neurons could be grouped into three different types according to the profile of their evoked-inward current. Currents are evoked by applying 5 mV incremental voltage steps from –45 mV to –125 mV, from a holding potential of –60 mV. Type I and type II responses corresponded to a steady-state current that was maintained for the duration of the voltage command in successive current traces (Fig. 2A, C). Type I response was characterized by an I/V relationship that was linear (Fig. 2A, B) while the most prominent feature of the type II I/V plot was an inward rectification at relatively depolarized voltages (voltage dependent rectification, Fig. 2C, D). A distinct type III response (Fig. 2E, F) consisted of an

initial current (Instantaneous current) which was followed by a slow inward current reaching a steady-state level (Steady state current). In type III response, the instantaneous current (●) is smaller compared to the steady state current (▲) which reveals a time dependent rectification (Fig. 2F). Type I, II and III responses were also observed in current clamp recordings (not shown), as a steady state voltage response during hyperpolarizing current pulses with a linear (type I) or a rectifying (type II) I/V relationship, or as a depolarizing sag (type III).

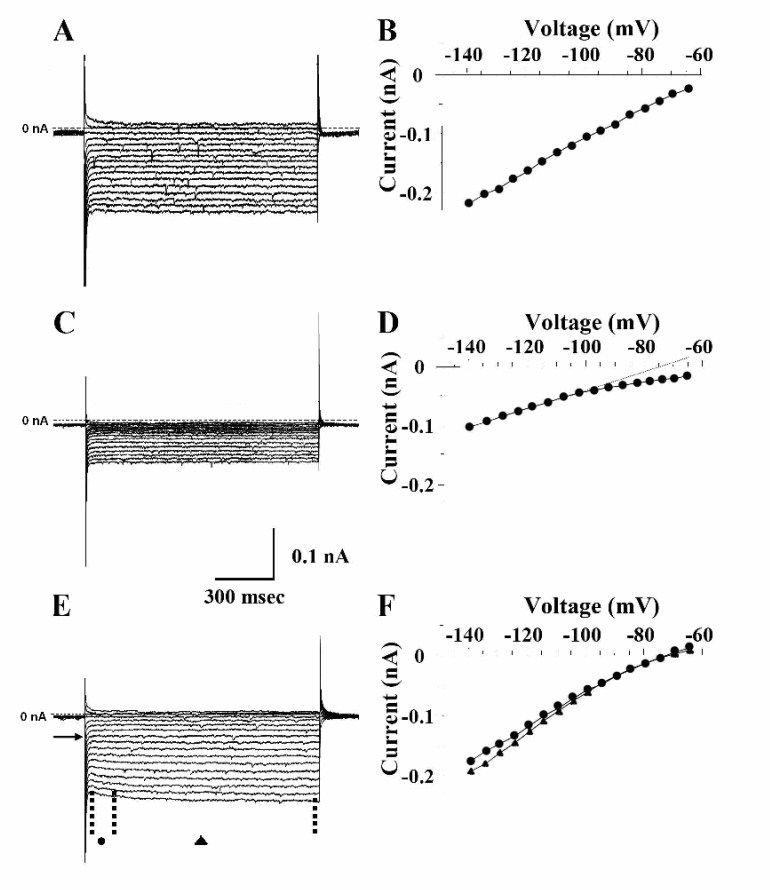

Fig. 2. Selected recordings of inward currents for type I, II and III neurons of the dorsal raphe nucleus. A, C, E. Currents evoked in three different cellular types by applying 5 mV incremental voltage steps from –45 mV to –125 mV, from a holding potential of –60 mV. B, D, F. Current /voltage curves for neurons represented in A, C and E. In plots B, D and F, a correction of –13.9 mV was applied for the liquid junction potential error [18]. The symbol for the instantaneous current is (●) and for the steady state current is (▲).

The majority of neurons recorded in the DRN of 5-HTT$^{+/+}$ (n = 63/64) and 5-HTT$^{-/-}$ (n = 70/71) mice exhibited spontaneous regular firing in current clamp recordings. Other neurons were silent. The electrophysiological characteristics of spontaneously active type I, II and III neurons of the DRN are provided in Table 1. These characteristics were not significantly different between females and males or between wild-type versus 5-HTT$^{-/-}$ mice. Also, these characteristics were not significantly different among type I, II and III neurons except for spike duration at threshold (p = 0.0417) and time constant of spike after-hyperpolarization (AHP tau, p = 0.0358). Spike duration in type III neurons (3.8 ms) was significantly shorter than in type I (4.6 ms, p = 0.0230)- and type II (4.7 ms, p = 0.0275)-neurons while AHP tau was significantly shorter in type III neurons (1.2 ± 1.2 ms) than in type II (2.4 ± 6.1 ms, p = 0.0112) neurons.

Table 1

Biophysical characteristics of type I, II and III neurons recorded from DRN.

	Type I 5-HTT$^{+/+}$ (n = 20) 5-HTT$^{-/-}$ (n = 21)	**Type II** 5-HTT$^{+/+}$ (n = 26) 5-HTT$^{-/-}$ (n = 31)	**Type III** 5-HTT$^{+/+}$ (n = 17) 5-HTT$^{-/-}$ (n = 18)
Resting membrane potential (mV)	–54.6 ± 0.5	–54.9 ± 0.8	–55.3 ± 0.3
Input resistance (MΩ)	580.9 ± 11.4	427.7 ± 51.0	516.6 ± 50.7
HV time constant (ms)	13.9 ± 0.5	16.7 ± 0.9	19.5 ± 0.5
Spike threshold (mV)	–43.6 ± 0.1	–44.6 ± 0.6	–44.78± 0.4
Spike amplitude (mV)	66.4 ± 0.2	70.9 ± 0.1	71.7 ± 0.3
Spike duration (ms) [a, b]	**4.6 ± 0.6**	**4.7 ± 0.8**	**3.8 ± 0.3**
AHP amplitude (mV)	19.9 ± 0.1	20.1 ± 0.6	21.4 ± 0.5
AHP duration (ms)	119.5 ± 10.1	127.3 ± 13.6	128.2 ± 9.4
AHP time constant (ms) [b]	1.6 ± 0.7	**2.4 ± 0.1**	**1.2 ± 0.2**
Current density (pA/pF)	3.8 ± 0.5	4.2 ± 0.2	4.5 ± 0.1
Spike discharge frequency (Hz)	9.14 ± 0.8	9.9 ± 0.6	9.7 ± 0.4

Values indicate mean ± SE; numbers in parentheses indicate total number of cells studied. [a]: values are significantly different between type I and type III neurons, [b]: values are significantly different between type II and type III neurons. These distinguishing characteristics are further highlighted by bold print.

ELECTROPHYSIOLOGICAL CHARACTERISTICS OF DORSAL RAPHE NUCLEUS NEURONS IN RESPONSE TO 5-HT$_{1A}$-RECEPTOR AGONISTS

In the presence of 5-CT (5-carboxamidotryptamine), an agonist of 5-HT$_{1A}$ autoreceptor, the regular firing (spike discharge frequency) of DRN neurons was inhibited. Different responses were exhibited by type I, II and III raphe neurons (see Table 2 and Figures 3 and 4).

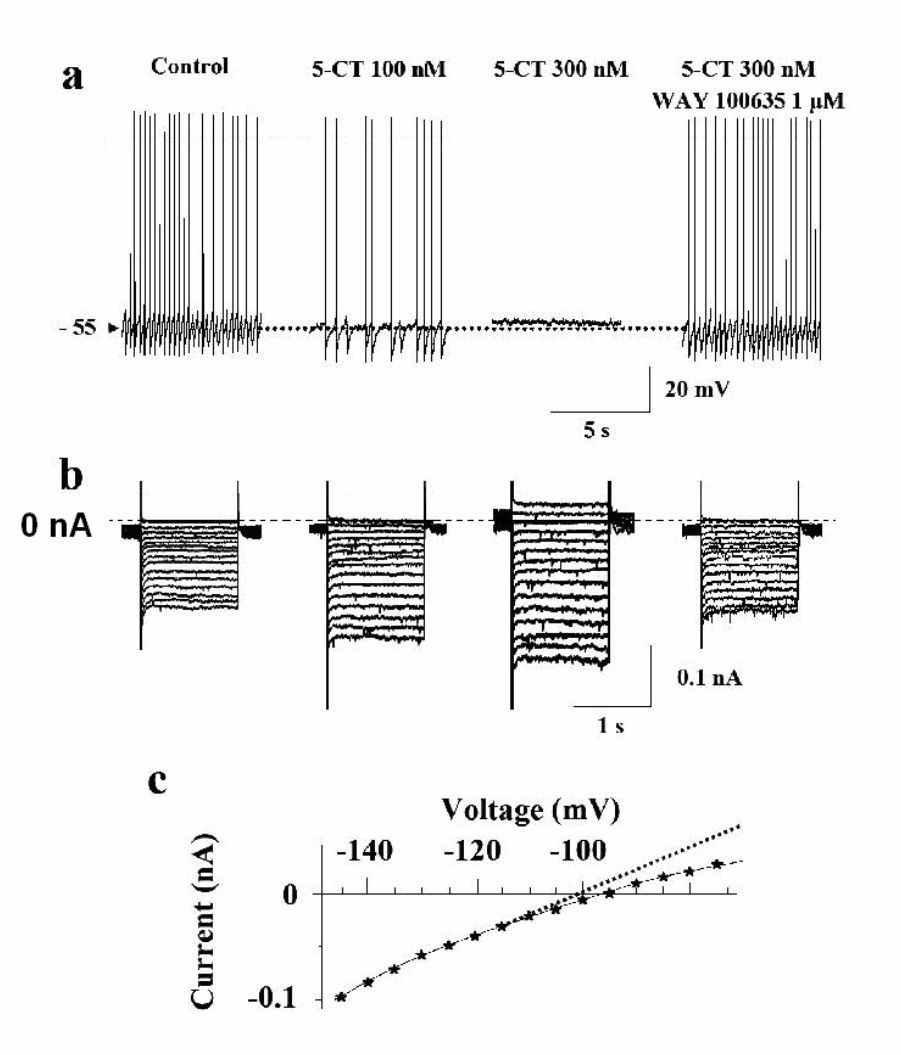

Fig. 3. Example of a typical response to a 5-HT1A-receptor agonist (5-carboxamidotryptamine) perfusion for a type I neuron from dorsal raphe nucleus. a. Discharge frequency reduction in response to increased concentrations of 5-CT (100 nM, 300 nM) and frequency recovery under WAY 100635 (1 μM) perfusion. b. Increase of GIRK current amplitude corresponding to the agonist/antagonist perfusion from a. Currents in panel b were elicited under the same conditions as those in Figure 2c. Current/voltage curve for the GIRK current obtained as a difference between 5-CT 300 nM perfusion and control conditions. In this plot, a correction of –13.9 mV was applied for the liquid junction potential error [18].

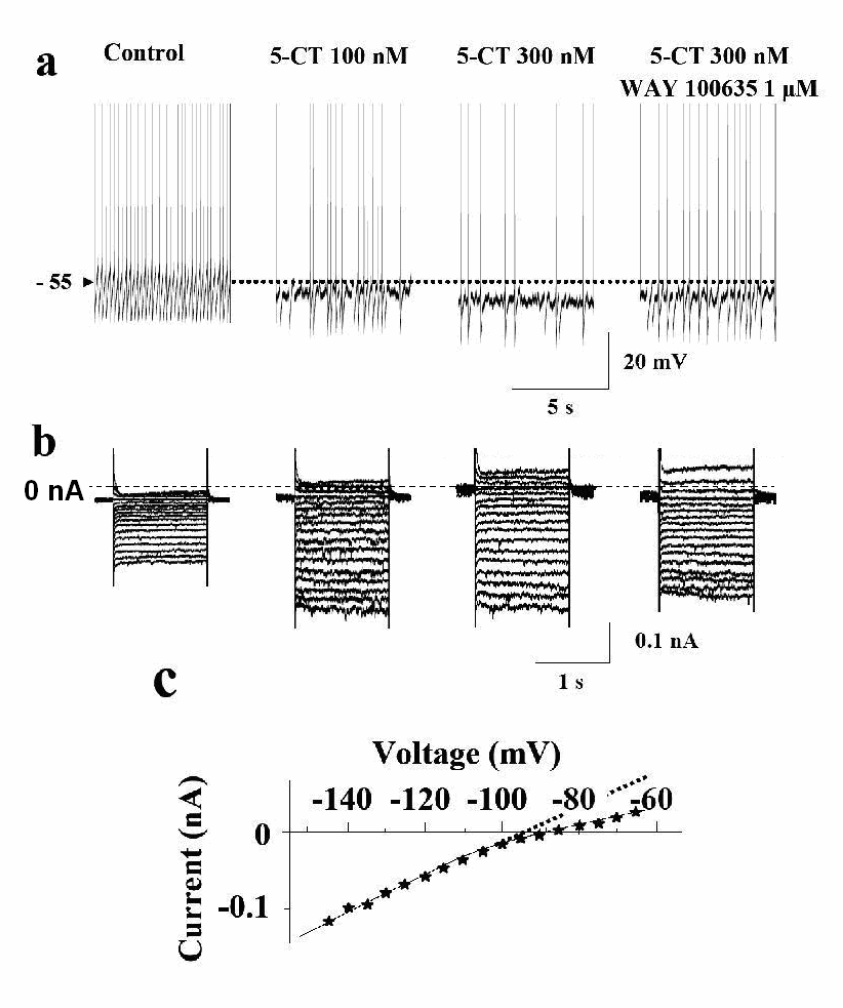

Fig. 4. Example of a typical response to a 5-HT1A-receptor agonist (5-carboxamidotryptamine) perfusion for a type II neuron from dorsal raphe nucleus. a. Discharge frequency reduction in response to increased concentrations of 5-CT (100 nM, 300 nM) and frequency recovery under WAY 100635 (1 μM) perfusion. b. Modification of GIRK current amplitude corresponding to the agonist/antagonist perfusion from a. Currents in panel b were elicited under the same conditions as those in Figure 2c. Current/voltage curve for the GIRK current obtained as a difference between 5-CT 300 nM perfusion and control conditions. In this plot, a correction of –13.9 mV was applied for the liquid junction potential error [18].

For type I neurons, the perfusion of increased concentrations of 5-CT determined a reduction in the discharge frequency from 8.7 ± 0.5 Hz to 3.5 ± 0.1 Hz ($p < 0.01$). This reduction of spike frequency, associated with a membrane hyperpolarization, was correlated with an increase of the GIRK current amplitude, described in terms of current density variation from 3.6 ± 0.5 pA/pF to 4.3 ± 0.2 pA/pF ($p < 0.05$). Figure 3 shows the traces recorded for a typical type I neuron (see the I/V curve) from the dorsal raphe nucleus. Concentrations of 100 nM and 300 nM 5-CT, reduce and respectively inhibit the spike discharge of this neurone, and the perfusion of 1 μM WAY 100635 restore the initial discharge (Fig 3a). Similarly, 100 nM and 300 nM 5-CT increase the amplitude of the GIRK current from 0.12 nA to 0.2 nA (Fig. 3b), and 1 μM WAY 100635 generates a final amplitude of 0.13 nA. The I/V curve was plotted by extracting the GIRK current in control conditions from the GIRK current at the maximal agonist concentration (300 nM 5-CT) which induces the complete reduction of the spike discharge (Fig. 3c).

A typical type II neuron is represented in Figure 4, where the discharge frequency is reduced by 300 nM 5-CT and recovered by adding 1 μM WAY100635 (Fig. 4a), and the amplitude of the GIRK current is increased by 300 nM 5-CT (from 0.15 nA to 0.3 nA) and reduced by 1 μM WAY 100635 (at 0.21 nA) (Fig. 4b). The I/V curve is plotted similarly as for type I neurons (Fig. 4c).

In type I and II neurons, perfusion of 5-HT_{1A}-receptor agonists, such as 5-carboxamidotryptamine, reduces the spike discharge frequency (from 8–10 Hz to 2–3 Hz) or in some cases abolishes it completely (0 Hz). The number of neurons that completely abolished their firing is larger for type II ($n = 4/6$) than for type I ($n = 1/6$) cells.

Data for type III neurons are presented only in Table 1, without showing any trace recording.

For all three types of neurons we have analyzed two electrophysiological parameters (current density and spike discharge frequency), during the perfusion of 300 nM 5-CT and in control conditions (Table 2). Thus, three types of responses at 5-CT perfusion can be distinguished: (I) responsive neurons, for which the current density increases and the spike discharge frequency decreases in the presence of 5-HT_{1A}-receptor agonists (type I and type II neurons); (II) non-responsive neurons, where none of these electrophysiogical parameters were modified by 5-HT_{1A}-receptor agonists perfusion; and (III) atypical neurons, where either the current density increased without any modification of the spike discharge frequency, or the spike discharge frequency decreased without any change in the current density. The third type of response was not considered in Table 2, because it is rather rare and its molecular basis is poorly understood. In principle, the responsive neurons (type I and type II) can be considered as being serotonergic cells and the non-responsive neurons (type III) as non-serotonergic. In the dorsal raphe nucleus, the proportion of serotonergic neurons (12/21) is higher than the non-serotonergic cells (9/21). In wild-type mice, the number of type I neurons (4/8) is higher than type II (2/8) or type III (2/8). In contrast, for mutant 5-HTT mice the number of type III neurons

(7/13) becomes significantly higher than type I (2/13) or type II (4/13) neurons. In conclusion, the serotonin transporter mutation induces an increase of the non-serotonergic neurons fraction (7/13) in comparison with the fraction in wild-type mice (2/8).

Table 2

Biophysical characteristics of type I, II and III neurons recorded from DRN that were perfused with 5-carboxamidotryptamine (300 nM).

	Type I 5-HTT$^{+/+}$ (n = 4) 5-HTT$^{-/-}$ (n = 2)		**Type II** 5-HTT$^{+/+}$ (n = 2) 5-HTT$^{-/-}$ (n = 4)		**Type III** 5-HTT$^{+/+}$ (n = 2) 5-HTT$^{-/-}$ (n = 7)	
	Control	**5-CT**	**Control**	**5-CT**	**Control**	**5-CT**
Current density (pA/pF)	3.6 ± 0.5	**4.3 ± 0.2***	5.1 ± 0.3	**6.7 ± 0.5***	4.1 ± 0.2	4.0 ± 0.5
Spike discharge frequency (Hz)	8.7 ± 0.5	**3.5 ± 0.1****	10.1 ± 0.2	**2.9 ± 0.3*****	9.8 ± 0.7	9.7 ± 0.6

Values indicate mean ± SE; numbers in parentheses indicate total number of cells studied. Data are statistically significant, * $p < 0.05$; ** $p < 0.01$, *** $p < 0.001$.

In control conditions, the current density for type I neurons (3.6 ± 0.5 pA/pF, n = 6) is smaller than for type II neurons (5.1 ± 0.3 pA/pF, n = 6), $p < 0.05$, or for type III neurons (4.1 ± 0.2 pA/pF, n = 9), $p = 0.09$. The spike discharge frequency is also smaller for type I (8.7 ± 0.5 Hz) than for type II (10.1 ± 0.2 Hz) neurons, $p < 0.05$, or for type III (9.8 ± 0.7 Hz), $p = 0.11$.

During 5-HT$_{1A}$-receptor agonists perfusion, (a) for type I neurons, the current density significantly increases (from 3.6 ± 0.5 pA/pF to 4.3 ± 0.2 pA/pF, $p < 0.05$) and the spike discharge significantly decreases (from 8.7 ± 0.5 Hz to 3.5 ± 0.1 Hz, $p < 0.01$); (b) for type II neurons, the current density significantly increases (from 5.1 ± 0.3 pA/pF to 6.7 ± 0.5 pA/pF, $p < 0.05$) and the spike discharge significantly decreases (from 10.1 ± 0.2 Hz to 2.9 ± 0.3 Hz, $p < 0.001$); (c) for type III neurons, neither the current density nor the firing rate were changed (Table 2).

DISCUSSION

To our knowledge, this is the first study that characterizes the types of neurons from dorsal raphe nucleus in mice, previous studies being focused only on rat investigation [1].

Our results proved that membrane responses to hyperpolarizing voltage steps exhibited three types of current profile in DRN neurons of 5-HTT$^{+/+}$ and 5-HTT$^{-/-}$ mice. A steady state current was maintained for the duration of the voltage command in type I and type II neurons with an I/V relationship that was linear or

inwardly rectifying, respectively. The type III response consisted of a slow inward relaxation at the beginning of hyperpolarizing voltage steps. These three types of cells recall those described previously in the DRN of rat [1, 4, 24] and many of their electrophysiological characteristics are within the range reported previously: resting membrane potential: –66 mV [10], input resistance: 150–400 MΩ [3], membrane time constant of the voltage response to a 45–50 pA hyperpolarizing current pulse: 21.4–33.5 ms [10], threshold, amplitude and duration of action potential: –54.9 mV, 61.4 mV, and 2.0 ms, respectively [10], amplitude and duration of spike after hyperpolarization: 10–20 mV and 200–800 ms, respectively [22], as well as time constant of the initial phase of the AHP: 1.8–7.3 ms [10].

In our mice preparations, the majority of cells from the recorded brain slices exhibited a spontaneous activity. Somatodendritic 5-HT_{1A} autoreceptors regulate serotonergic neuronal firing [6]. In some rat slice preparations, most DRN neurons are found silent and require superfusion of the α_1-adrenergic agonist phenylephrine to induce firing [1, 22], spontaneously active putative serotonergic neurons can also be found *in vitro* in various proportions in the DRN of rats [18, 22] and mice [21]. Such discrepancy between spontaneously active and silent neurons in the DRN *in vitro* can be due to extracellular Ca^{2+} concentration, anesthesia, pH, and also species differences, since spontaneous firing frequency in the DRN was found larger in mice than in rats *in vivo* [6, 19, 22].

An increase in G-protein inward rectified potassium current by 5-HT_{1A}-receptor stimulation has been described previously in various regions of the dorsal raphe nucleus in the rat [1, 24]. In our preparation, 5-HT_{1A} agonists inhibited type I- and II-, but not type III-neurons. These data are consistent with previous results indicating that neurons exhibiting slow time dependent rectification are not sensitive to 5-HT_{1A} agonists [9]. Furthermore, cells in our preparation were significantly differentiated based on large (type I- and type II-neurons) or short (type III neurons) spike duration. This is of importance knowing that large spike duration is required, together with 5-HT_{1A} agonist-induced inhibition, to characterize putative 5-HT containing neurons, while neurons with short spike duration are not [13]. Hence, type I- and II-neurons which are inhibited by 5-HT_{1A} agonists in our preparation can be putative 5-HT containing neurons while type III- or 5-HT_{1A} agonist non-sensitive type I- and II-neurons can not.

The biophysical pattern of raphe nucleus neurons is considered for two genotypes of C57Bl/6 mice: 5-$HTT^{+/+}$ and 5-$HTT^{-/-}$. Thus, 5-HTT mutation does not modify any of the described biophysical properties for the dorsal raphe nucleus neurons. Despite the lack of correlation between serotonergic/non-serotonergic neurons and their biophysical characteristics, our study has a great physiological importance. Two important electrophysiological features of the dorsal raphe neurons in mice are evidenced for the first time by our study: (1) the possibility of cellular classification considering a specific electrical pattern; and (2) the association of different types of neurons with their response to 5-HT_{1A}-receptor agonists. The cells that are sensitive to 5-HT_{1A} agonists can be indirectly considered as serotonergic neurones [10], as it is the case for type I and II neurons.

Future immunohistochemical assays are necessary for a complete characterization of dorsal raphe nucleus neurons in mice.

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